UNIVERSIDADE DO ALGARVE

FACULDADE DE CIÊNCIAS DO MAR E DO AMBIENTE

ECOLOGIA E DEMOGRAFIA DE UMA POPULAÇÃO DE ÁGUIA DE BONELLI *AQUILA FASCIATA* EM MEIO FLORESTAL

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(Tese para a obtenção do grau de doutor no ramo de Ecologia,
especialidade de Ecologia das Populações)

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2008

Ao futuro dos meus filhos

AGRADECIMENTOS

Correndo o sério risco de esquecer alguém entre as inúmeras pessoas que tornaram possível a realização desta tese, quero agradecer sinceramente:

- pela colaboração voluntária no trabalho de campo ao longo de 17 anos de recolha de dados, a Carlos Noivo, Francisco Faria, Glória Faria, Paulo Cabrita, Pedro Portela, Jaime Miguel, João Ferreira, João Ministro, José Raiado Pereira, Miguel Caldeira Pais, Nuno Ferrand e Rita Alcazar, com um especial agradecimento a Fernando Grade pelo extraordinário esforço e dedicação na realização do censo de pombos rurais;

- pela realização da morosa e entediante identificação e quantificação dos itens alimentares, a Rita Inácio e João Ferreira; pela cedência das informações sobre a columbofilia de competição no Algarve, a Daniel Santos; pelo contributo no controlo da qualidade das determinações de mercúrio, a Valentina Costa e Humberto Lopes; pela viabilização do estudo sobre a tricomoníase, a Leonor Cancela, Pedro Melo, Pedro Rosal Gonçalves e Ursula Höfle;

- pela hospitalidade recebida, sem a qual teria sido impossível trabalhar numa tão grande área, a Branca Franqueiro, Carlos Costa, Carlos Noivo, Esmeralda Antunes, Fernando Almeida, Isabel e Sebastião Pernes, Lena Espinha, Margarida Machado, Manuel João Pinto, Pedro Beja e Pedro Portela; pelo apoio logístico na cedência de instalações ou outro, a João Lagido e João Nunes, ex-directores do Parque Natural do Sudoeste Alentejano e Costa Vicentina, João Fialho e a Eleutério, José António Simões e Víctor Pina, respectivamente responsável e guardas do Centro Cinegético de Silves;

- pelo especial apoio e incentivo na Faculdade de Ciências do Mar e do Ambiente da Universidade do Algarve, aos meus colegas Margarida Machado e João Reis, e a Adelino Canário, Leonor Cancela, Luís Cancela da Fonseca e Rui Santos; a

Adelino Canário devo, em especial, a oportunidade de desenvolver esta tese, ao me ter convidado em 1991 para técnico superior da Universidade do Algarve;

- pela revisão de manuscritos, a Andreas Helbig, Erkki Korpimaki, Eva Casado, Francisco Moreira, Joan Real, José Tella, Martina Carrete, Maria Filomena Magalhães, Santi Mañosa, Simon Thirgood e Steve Redpath; pelo aconselhamento científico, a Joan Real, Nuno Onofre, Rui Borralho e Santi Mañosa e em especial aos meus orientadores Jorge Palmeirim, Luís Cancela da Fonseca e Pedro Beja;

- pelo apoio financeiro, ao Parque Natural da Ria Formosa, na pessoa do seu ex-director Nuno Lecoq, e do ex-Grupo de Trabalho do Algarve nas pessoas de Fernando Pessoa e José António Cavaco; a investigação foi também parcialmente suportada pelo projecto PRAXIS/BIA/132/96, financiado pela Fundação para a Ciência e Tecnologia.

- por último, à Rosana Peixoto pela compreensão, apoio e carinho sempre presentes ao longo dos anos.

RESUMO

A presente tese tem como objecto a população de *Aquila fasciata* do Sudoeste Serrano português, caracterizada pela nidificação exclusivamente arborícola, encontrar-se em acentuada expansão e apresentar marcada divergência genética em relação às populações ibéricas vizinhas. Pretendeu-se contribuir para uma base científica de uma estratégia de conservação, tendo como objectivos específicos: a) conhecer a ecologia alimentar da população, b) estudar a selecção de habitat de uma presa fundamental, o coelho-bravo; c) avaliar os níveis de contaminação ambiental da população; d) desenvolver métodos expeditos de sexagem de juvenis; e) rastrear agentes infecciosos potencialmente letais e f) avaliar metodologias de análise demográfica.

No respeitante à dieta da população, constatou-se um elevado consumo de presas domésticas, sobretudo pombos rurais, em especial no início da época reprodutiva. Ao longo da reprodução, o coelho-bravo, a perdiz, o gaio e outras aves selvagens adquirem progressivamente maior importância. A selecção entre pombos e coelhos é inversamente denso-dependente, sendo o pombo rural uma presa complementar aos coelhos e perdizes e não unicamente uma presa alternativa em situação de escassez destes. O estudo da selecção de habitat do coelho-bravo mostrou que prefere estevais de densidade intemédia, por fornecerem simultaneamente abrigo contra predadores e recursos alimentares nos estratos herbáceo e arbustivo. O estudo dos níveis de mercúrio na população evidenciou uma forte variação espacial, indiciando uma fonte poluidora em Sines. Desenvolveram-se dois métodos expeditos de sexagem morfométrica, validados por métodos moleculares, os quais permitem comprovar a sexagem em aves subnutridas. Esta ocorrência está associada à elevada prevalência de tricomoníase, potencialmente letal em juvenis nidícolas, devido à frequente ingestão de pombos rurais infectados. A análise dos parâmetros demográficos mostrou não ser possível inferir qualquer padrão de fecundidade denso-dependente na população estudada. Considerar apenas os dois mecanismos usualmente utilizados para explicar a fecundidade denso-dependente em populações naturais pode não ser o mais adequado.

PALAVRAS-CHAVE: *Aquila fasciata*, arborícola, coelho-bravo, pombo rural, mercúrio, sexagem, tricomoníase, denso-dependência.

ABSTRACT

The present thesis targets the population of *Aquila fasciata* of the Southwest Portugal uplands, an exclusively tree-nesting, steadily expanding population, and genetically distinct from neighbouring populations in Iberia. The thesis is intended to be a contribution to a scientifically based conservation strategy. The specific objectives were: a) to study the feeding ecology of the population and b) the habitat selection of a key prey, the wild rabbit; c) to check the levels of environmental contamination in the population; d) to develop and test simple field biometric sexing protocols; e) to screen the nestlings for lethal infectious agents, and f) to evaluate some methods commonly used in demographic analyses.

Eagle diet analysis showed a high consumption of rural pigeons, especially at the onset of breeding. Along the breeding season, rabbits, partridges, jays and other wild bird species become increasingly important in diet. Selection between pigeons and rabbits is inversely density-dependent, and the rural pigeon is a complement to rabbits and partridges, rather than a mere alternative prey. The wild rabbit prefers medium density gum cistus scrub, presumably because it provides both shelter against predators and food resources at the herbaceous and shrub layers. Mercury levels in the population showed a strong spatial variation, and indicate a putative contamination source at the Sines industrial complex. Two simple biometric field sexing methods were developed and validated by molecular genetics, which allows the confirmation of sex in emaciated nestlings. This condition is associated with a high prevalence of potentially lethal tricomonosis, caused by the high frequency of nestling feeding with infected rural pigeons. The analysis of demographic parameters did not allow to infer any pattern of density-dependent fecundity. Hence, considering the two mechanisms commonly used to explain density-dependent fecundity in natural populations alone may not be the most adequate procedure.

KEY WORDS: *Aquila fasciata*, tree-nesting, wild rabbit, domestic pigeon, mercury, sexing, trichomonosis, density-dependence.

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PRÓLOGO

A ideia da presente tese surge em certo momento de uma investigação de longo-termo sobre a população de *Aquila fasciata* das serras do Sudoeste português, desenvolvida de forma progressiva, mas quase ininterrupta, no contexto de projectos e apoios financeiros diversos. Salientam-se, como determinantes, o apoio inicial do Parque Natural da Ria Formosa e, mais tarde, o da Fundação para a Ciência e Tecnologia (PRAXIS PCNA/C/BIA/132/96) que permitiu um importante salto qualitativo na investigação.

O estudo da ecologia e demografia de espécies lôngevas e de reprodução lenta, como a águia de Bonelli, só pode produzir resultados sólidos e fiáveis numa base de continuidade e por um período suficientemente longo, sobretudo se pretendemos que possam vir a orientar, numa base científica, a sua conservação na prática. Essa continuidade é impossível de garantir à partida e muito difícil de manter, devido ao custo elevado dessa investigação, às dificuldades logísticas inerentes à dimensão e dificuldades de acesso na área de trabalho e, sobretudo, à imponderabilidade existente na obtenção consecutiva dos necessários financiamentos.

A esta continuidade não é alheia a estabilidade laboral na Universidade do Algarve, o apoio constante de diversos membros do corpo docente da actual Faculdade de Ciências do Mar e Ambiente, bem como a compreensão dos seus sucessivos Conselhos Directivos. Esta não é portanto uma tese tradicional, na medida em que não esteve na origem da investigação desenvolvida, mas é sim um corolário dela e dos artigos científicos *peer-reviewed*, entretanto publicados, alguns mesmo antes do início oficial da tese.

CAPÍTULO 1. INTRODUÇÃO GERAL

1.1. Enquadramento

Taxonomia e distribuição global

A águia de Bonelli só foi descoberta para a ciência em 1819 pelo naturalista italiano Franco Andrea Bonelli e descrita em 1822 como *Aquila fasciata* pelo ornitologista francês Louis Jean Pierre Vieillot. Foi posteriormente integrada no género *Hieraaetus* como *Hieraaetus fasciatus* (Vieillot, 1822) mas estudos filogenéticos recentes (e.g. Helbig et al. 2005; Lerner & Mindell 2005) demonstraram que as espécies correntemente integradas nos géneros *Hieraaetus* e *Aquila* não formam grupos monofiléticos distintos. Assim, foi proposta a reintegração da espécie no género *Aquila* (Sangster et al. 2005), retomando-se a denominação original de *Aquila fasciata* Viellot, 1822, ultimamente adoptada por diversos autores (e.g. Carrascal & Seoane 2008; Soutullo et al. 2008).

Aquila fasciata é uma águia sedentária de médio porte, com uma distribuição altamente disjunta através do Paleárctico, desde a região Mediterrânica até ao Sudeste asiático. As principais áreas de ocorrência são a Península Ibérica e Marrocos no extremo ocidental da distribuição e o subcontinente Indiano e Sul da China no seu extremo oriental (Cramp and Simmons 1980; Ferguson-Lees and Christie 2001). No arquipélago das Pequenas Sundas (Leste da Indonésia e Timor-Leste) ocorre a forma *renschi*, morfologicamente diferente de *fasciata* e que, embora considerada uma subespécie desta, poderá tratar-se de uma espécie distinta (Ferguson-Lees and Christie 2001; Mebs & Schmidt 2006). No Sul da Ásia, a espécie encontra-se aparentemente em situação de conservação favorável (Bildstein et al. 1998), enquanto que na Europa se verifica uma regressão em todos os países desde a década de 1980 (Real et al. 1996).

Situação populacional na Europa

A população europeia foi recentemente estimada em 938-1039 casais (Ontiveros et al. 2004), mais de 80% dos quais na Península Ibérica. Em Espanha, os últimos censos permitiram estimar uma população de 733-786 casais, 80% dos quais em quatro comunidades autónomas: Andaluzia (44%), Extremadura (12,3 %), Comunidade Valenciana (12,3%) e Castilla-La Mancha (11,6%) (Del Moral, 2006). Em Portugal as estimativas mais recentes apontam para uma população de 99-107 casais, 56-58% dos quais a sul do Tejo e os restantes distribuídos pelas bacias do Douro e Tejo Internacionais e Estremadura (L. Palma et al., dados não publicados).

A semelhança desta estimativa à dos 85-106 casais estimados 22 anos antes (Palma et al. 1996) deve-se ao facto dos declínios verificados no NE (-8 casais min., ≥ 26%) e, à excepção do SE subestepário, na peneplanície e serras alentejanas (-7 casais min., ≥ 50%), ter sido numericamente compensado por incrementos verificados noutras regiões: por um lado, o aumento populacional verificado no SO Serrano e SE subestepário (+23 casais, 48%) e, por outro, pelo menos mais 13 casais (≥ 62%) no interior centro e Estremadura, neste caso presumivelmente devidos, em grande parte, a uma melhoria da prospecção da espécie do terreno. Assim, o balanço global no país, entre 1996 e 2008, corresponderá a um aumento real de pelo menos 7 casais. Na Península Ibérica, a frequência de ocorrência da águia de Bonelli decresce de Sul para Norte, com um máximo nas latitudes médias meridionais (Carrascal & Seoane 2008).

Depois de um período de declínio na década de 1980, em que se perderam 25% dos casais em Espanha e 15% em Portugal (Arroyo et al. 1995; Palma et al. 1996), a espécie parece encontrar-se em recuperação, embora a situação actual seja objecto de controvérsia (Del Moral 2006; Cadahía et al. 2008). Nas últimas duas décadas o declínio ultrapassou os 20% em duas gerações (Carrascal & Seoane 2008). Enquanto as sub-populações do Sul e Este da Península parecem estar estáveis ou em aumento, as do planalto central, NE de Portugal e Norte de Espanha parecem manter-se em declínio (Ontiveros et al. 2004; Soutullo et al. 2008).

As causas mais importantes apontadas para este drástico declínio são a perseguição directa, e a colisão e electrocussão em linhas eléctricas, sendo os adultos afectados sobretudo pela primeira e os não reprodutores pela segunda. Estas diferenças estão associadas a diferenças na distribuição espacial das classes de idade: a perseguição é a principal causa de morte nas áreas de nidificação e a electrocussão nas áreas de não reprodução (Real et al. 2001; Carrete et al. 2002).

Habitat e condicionantes da distribuição

Diversos estudos realizados em Espanha (e.g. Muñoz et al. 2005; Carrascal & Seoane 2008) revelaram que o padrão de distribuição das populações rupícolas de águia de Bonelli é explicado por um número limitado de variáveis – topografia, clima, vegetação, relações interspecíficas e factores antrópicos. As áreas mais favoráveis à espécie são de baixa altitude, acidentadas e com elevada proporção anual de dias de sol (i.e. níveis altos de radiação solar). Enquanto que as formações vegetais esparsas (sobretudo matagais) têm um efeito positivo, a altitude, coberto agrícola e florestas decíduas têm o efeito inverso. Em Portugal, Inácio et al. (1999) confirmam a tendência da espécie para ocorrer com maior probabilidade em áreas com elevada irregularidade topográfica, onde são reduzidas a rede viária, a ocupação por resinosas e a precipitação em Fevereiro (média e nº de dias com pluviosidade intensa).

O declive tem explicado uma grande parte da distribuição da espécie, a diversas escalas (Sánchez-Zapata et al 1996; Ontiveros 1999; Muñoz et al. 2005; Carrascal & Seoane 2008). Em parte, esta preferência está provavelmente relacionada com a disponibilidade de escarpas rochosas, o factor mais limitante para a reprodução da espécie em grande parte da região mediterrânica, nomeadamente no norte de Portugal, onde toda a população é rupícola e em Espanha, onde apenas 4% da população espanhola nidifica em árvores (Del Moral 2006). O declive, junto com baixa precipitação e as temperaturas estivais elevadas são igualmente bons indicadores da distribuição da perdiz-vermelha, uma das uma das espécies-presa fundamentais em Espanha (Carrascal & Lobo 2003).

No entanto, a associação a áreas declivosas não se verifica de forma tão estreita no Sul de Portugal, pelo menos em termos da dependência de escarpas rochosas, pois nesta região predomina largamente a nidificação em árvore (Palma 1994), localizando-se uma parte importante dos territórios de reprodução em áreas de relevo suave (L. Palma et al., dados não publicados). No resto da região mediterrânica, só se encontra uma proporção elevada de nidificação arborícola em Chipre (Iezekiel et al. 2004) e em algumas populações norte-africanas (Bergier & Naurois 1985) mas esta tipologia é frequente nas populações asiáticas (e.g. Zheng 1987).

Segundo Carrascal & Seoane (2008), a distribuição e densidade da águia de Bonelli em Espanha é explicada pela topografia e pelo clima, enquanto a estrutura da paisagem e a disponibilidade trófica (principalmente a perdiz) têm um papel relevante mas secundário. Os padrões de grande escala são quase coincidentes com os que estão presentes à escala local, revelando que os processos locais se traduzem frequentemente em padrões de grande escala. Na Península Ibérica, a espécie torna-se pouco frequente ou escassa ligeiramente acima dos 600 m e não ocorre para lá dos 1500 m (Ontiveros et al. 2004).

O potencial impacto negativo das altas temperaturas estivais na reprodução é evitado pelo facto de esta ser a espécie de reprodução mais precoce entre todas as águias mediterrânicas (Cramp & Simmons 1980), ao mesmo tempo que este facto lhe permite reduzir a competição de outras espécies mais tardias (Muñoz et al. 2005).

Embora a competição com a águia-real (*Aquila chrysaetos*) tenha sido frequentemente considerada um factor limitante para a ocorrência da espécie, os estudos mais recentes demonstraram que a abundância relativa daquela espécie pouco influi na distribuição ou abundância da águia de Bonelli, quer à escala regional (Carrascal & Seoane 2008), quer à escala local (Carrete et al. 2002; López-López et al. 2004). A grande escala, a distribuição das duas espécies pode inclusivamente estar positivamente correlacionada por ambas preferirem as mesmas características básicas de habitat.

As variáveis que descrevem o nível de pressão humana, à excepção da actividade agrícola, têm igualmente um papel secundário nos padrões de distribuição da espécie

(Carrascal & Seoane 2008), já que esta demonstra um grau relativamente elevado de tolerância à presença humana (Gil-Sánchez et al.1996; Carrete et al. 2002).

Dinâmica populacional

A fragmentação espacial das áreas favoráveis para a águia de Bonelli sugere uma dinâmica metapopulacional sobreposta a uma dinâmica de *source-sink* (Muñoz et al. 2005). Assim, um acréscimo de territórios favoráveis desocupados pode promover a dinâmica metapopulacional, em detrimento da dinâmica de *source-sink*, causando um declínio populacional nas áreas de *sink*. Como a disponibilidade de territórios óptimos desocupados depende sobretudo da mortalidade adulta, esta poderá ser o factor-chave no balanço entre os dois tipos de dinâmica espacial da espécie (Muñoz et al. 2005). Em áreas favoráveis, um acréscimo na mortalidade adulta não resulta no declínio da população local, pois os adultos serão substituídos por subadultos. Assim, quando o declínio é detectado nas áreas de baixa densidade, a causa pode estar a actuar nas de alta densidade. Por esta razão, o status favorável da espécie nas áreas meridionais da Península Ibérica pode ser ilusório, podendo estar a ocorrer uma onda de rarefacção de NO para SE, já notado nos limites da distribuição, em áreas de *sink* ou desfavoráveis, mas cuja origem se situa nas áreas produtivas (Muñoz et al. 2005).

Ao contrário dos anteriores modelos demográficos fechados (Real & Mañosa 1997; Carrete et al. 2002), os modelos metapopulacionais que incorporam estrutura espacial e estocasticidade, mais realistas, evidenciam a sobrevivência pré-adulta, mais do que a sobrevivência dos adultos, como o parâmetro com maior impacto na taxa de crescimento da população (Soutullo et al. 2008).

Ecologia alimentar

Ao contrário de outras rapinas mediterrânicas (Delibes & Hiraldo 1981) em que o coelho-bravo (*Oryctolagus cuniculus*) é a presa básica, as aves têm uma maior relevância na dieta da águia de Bonelli, que pode mesmo reproduzir-se com êxito na quase ausência de coelhos (Simeon & Wilhelm 1988). A espécie está adaptada a predar sobre a presa mais abundante no território, modificar radicalmente a dieta conforme a estação do ano e manter o desempenho reprodutor de modo relativamente independente da disponibilidade de presas. Uma dieta ecléctica permite que a distribuição, abundância ou produtividade da águia de Bonelli não estejam limitadas pela abundância das suas presas básicas (Ontiveros & Pleguezelos 2000). Esta adaptabilidade reside na baixa razão de aspecto (*aspect ratio*) da morfologia alar da espécie (Parellada et al. 1984; Kirmse 1998) que lhe confere agilidade e rapidez de voo. Do ponto de vista trófico, a águia de Bonelli pode ser considerada um *especialista facultativo*, preferindo o coelho quando este é relativamente abundante mas mudando para outras presas quando é demasiado escasso (Moleón et al. 2007).

Além da perdiz-vermelha, os pombos (*Columba* sp.), sobretudo os pombos domésticos, constituem uma das presas alternativas mais importantes na dieta de *Aquila fasciata*. No entanto, estas presas domésticas são o principal reservatório de um protozoário flagelado patogénico que afecta com alta prevalência os juvenis nidícolas (Real et al. 2000). Apesar disso, apenas uma pequena proporção dos juvenis afectados desenvolvem tricomoníase clínica, em especial lesões letais, o que pode indicar que, ou a maioria das estirpes do parasita são não-patogénicas, ou os juvenis detêm alguma imunidade. No entanto, embora grande parte da população esteja exposta ao protozoário, as crias dos territórios com maior proporção de pombos domésticos têm uma maior probabilidade de exposição a uma estirpe virulenta (Real et al. 2000). De qualquer forma, a tricomoníase constitui uma das principais causas de mortalidade nidícola nas populações com elevada proporção de pombos domésticos na dieta, em parte devido à escassez de presas selvagens (Real et al. 2000).

Estrutura genética

Do ponto de vista genético, a análise do DNA mitocondrial ao nível da região de controlo (mtCR) indica um nível reduzido de diversidade. A maior parte da variância encontrada em populações do Mediterrâneo ocidental é intra-populacional, sem detecção de estruturação genética inter-populacional, como reflexo não só do pequeno número de polimorfismos encontrados, como do facto de todos os polimorfismos consistirem em *transições*, muito mais frequentes que as *transversões* no genoma mitocondrial (Cadahía et al. 2007). Isto está de acordo com os anteriores resultados da sequenciação do gene citocromo b mitocondrial e da variabilidade ao nível das proteínas, embora se verifique uma ligeira diferença entre as populações ocidentais e orientais da região mediterrânica (Cardia et al. 2000; Cardia et al. 2002).

De acordo com os estudos anteriores não há evidência de restrição de fluxo genético na parte ocidental da bacia mediterrânica, indicando uma população relativamente panmítica na Península Ibérica. Por seu lado, a reduzida diversidade genética detectada poderá indicar a ocorrência de anteriores *bottlenecks*, durante a última glaciação ou recentemente de origem antrópica (Cadahía et al. 2007).

Ao contrário dos resultados dos estudos anteriores, a análise de 17 microssatélites polimórficos (cf. Mira et al. 2005) em quatro populações adjacentes do sudoeste ibérico (bacia do Douro internacional, Extremadura, Cadiz e Sudoeste Serrano português), revelaram níveis moderados de riqueza alélica e de heterozigotia, à excepção da população do Sudoeste Serrano que demonstrou níveis muito reduzidos de diversidade genética (Mira 2006). A pequena diversidade genética desta população concorda com a evidência histórica de se ter mantido com um número muito pequeno de efectivos durante um período prolongado (dados não publicados).

Verificou-se também a existência de estruturação espacial, com divergências significativas da composição genética entre as populações estudadas. As três primeiras populações aparentam proximidade genética, provavelmente intercambiando indivíduos com alguma frequência. Inversamente, os valores elevados de diferenciação genética da população do Sudoeste Serrano de Portugal indicam ausência ou frequência muito limitada de imigração, sugerindo um certo isolamento reprodutor (Mira 2006). Este isolamento poderá ter origem eco-etológica, através da perpetuação da tradição da nidificação arborícola por impregnação (*imprinting*). Diferentes preferências de habitat e tipologia de nidificação poderão estar na origem de uma redução da probabilidade de intercâmbio génico com as populações vizinhas e contribuir para um relativo isolamento. Baseada nos elevados valores de diferenciação genética, Mira (2006) recomenda que a subpopulação arborícola do Sudoeste de Portugal seja considerada, no contexto da conservação da espécie na Península Ibérica, uma unidade operacional de gestão.

O desenvolvimento de barreiras reprodutoras entre subpopulações, de carácter etologico-dependente, tem sido observado noutras espécies, em particular no decurso da difícil reinstalação da população arborícola de falcão-peregrino *Falco peregrinus* das florestas de planície da Europa central (Kirmse 2008), totalmente extinta em 1972 em consequência de contaminação por agro-químicos (Kirmse 2001). A marcação, no decurso do projecto, de todos os juvenis libertados, previamente impregnados à nidificação em árvore por *hacking*, bem como de todos os juvenis nascidos em liberdade na Alemanha oriental, em todos os tipos de suporte de nidificação, revelou que a reinstalação da subpopulação arborícola se processa quase exclusivamente a partir dos juvenis libertados, não havendo imigração de indivíduos provenientes de subpopulações vizinhas, nascidos noutros tipos de plataformas de nidificação, resultando portanto numa subpopulação isolada (Kirmse et al. 2006; Kirmse 2008).

1.2. Justificação e objectivos

A descoberta de uma população coesa de *Aquila fasciata* nas serras do Cercal, bacia do rio Mira, Monchique e Caldeirão, praticamente desconhecida e, além disso, de nidificação quase exclusivamente arborícola, foi o principal incentivo para o início da investigação iniciada em 1991-1992. Antes de 1991, à excepção de dois casais que então ainda nidificavam em substrato rochoso, tanto a existência dessa população serrana, como até a ocorrência de nidificação arborícola em Portugal, eram factos completamente insuspeitos (cf. Palma 1985; Rufino et al. 1985). Aliás, nessa altura, a nidificação desta espécie em árvore na Europa era conhecida apenas como uma ocorrência anedótica (e.g. Cabot et al. 1978; Billet 1991). O reconhecimento da existência da única população europeia a nidificar quase exclusivamente em árvore, peculiariedade ecológica só descrita, na região mediterrânica, para alguns países norte-africanos (Bergier & Naurois 1985), conferia ao seu estudo uma relevância especial. Na realidade, esta população é constituída por dois núcleos populacionais, um serrano, de ambiente florestal e objecto dos artigos desta tese, e outro ocupando a região subestepária de peneplanície do Sudeste alentejano/Nordeste algarvio que lhe fica adjacente.

O objectivo central da investigação desenvolvida desde 1991 foi a aquisição de um conhecimento multidisciplinar, abrangente e integrado sobre o núcleo populacional arborícola de águia de Bonelli do Sudoeste Serrano português, posteriormente estendido ao núcleo populacional da peneplanície adjacente, de forma a poder constituir-se como base científica para o delineamento de uma estratégia de conservação desta população de características únicas na Europa e raras na região mediterrânica. Assim, desenvolveram-se estudos sobre diversos aspectos da população – ecologia trófica, abundância das presas fundamentais, selecção de habitat, distribuição e expansão populacional, parâmetros reprodutores, mortalidade adulta, recrutamento, emancipação e dispersão juvenil, patologia, ecotoxicologia, genética populacional – que continuam na actualidade. Nestes estudos têm participado diversos investigadores e estudantes da Universidade do Algarve, Universidade de Évora, Faculdade de Ciências da Universidade do Porto e Faculdade de Ciências da Universidade de Lisboa.

O desenvolvimento da investigação de um espectro tão amplo de temas é, naturalmente, um processo progressivo e continuado em que se reunem, passo a passo, as condições objectivas para viabilizar cada um dos estudos sectoriais. Alguns deles estão ainda numa fase inicial, como a selecção de habitat, outros necessitam de aprofundamento, como a epidemiologia da tricomoníase ou a genética populacional. Por outro lado, algumas matérias centrais, com a evolução demográfica da população e o seu processo de expansão geográfica, embora detenham um conjunto robusto de dados, reunidos de forma sistemática e contínua ao longo dos ultimos 17 anos, não foram ainda devidamente tratadas.

É assim, como parcela integrante deste *rationale* de *continuum* de investigação de longo-termo, que se insere a presente tese, constituída por um conjunto de seis artigos, publicados entre 2000 e 2008 em revistas internacionais e num livro das respectivas especialidades, e que pretendem responder aos seguintes objectivos específicos:

a) conhecer a ecologia alimentar da águia de Bonelli na área de estudo em função do habitat e da disponibilidade dos recursos tróficos, bem como as preferências ecológicas de uma das suas presas fundamentais – o coelho-bravo;

b) avaliar os níveis de contaminação ambiental susceptíveis de interferência negativa no sucesso reprodutor;

c) desenvolver e testar métodos de sexagem dos juvenis, de aplicabilidade expedita no terreno;

d) rastrear os agentes infecciosos responsáveis pela elevada prevalência de patologias letais nos juvenis nidícolas;

e) avaliar criticamente metodologias utilizadas no estudo da dinâmica populacional

1.3. Área de estudo

O núcleo populacional de águia de Bonelli objecto desta tese ocupa uma unidade natural relativamente homogénea que poderemos intitular de *Sudoeste Serrano* e que coincide com a unidade biogeográfica denominada *Superdistrito Serrano-Monchiquense* (Rivas-Martinez et al. 1990, Costa et al. 1998). Abrange de Norte para Sul as serras de Grândola, Cercal, Vigia, colinas da bacia do Mira e Monchique (incluindo as serras do

"Espinhaço de Cão" e "Silves") e para Este a cadeia montanhosa do Caldeirão. Em termos litológicos, predominam os xistos e grauvaques do Carbónico Marinho (Complexo Xisto-Grauváquico) dissecados por uma densa rede hidrográfica de morfologia dendrítica. O relevo desta vasta região, que abrange cerca de 4800 km^2, é bastante homogéneo, constituindo uma paisagem singular, relativamente monótona, descrita por Chodat (1909) como aparentando montículos de toupeiras (*taupinières*), sobretudo evidente ao longo da Serra do Caldeirão. Exceptuam-se o reduzido maciço eruptivo da Fóia/Picota (Monchique) e a Serra do Cercal que apresentam natureza litológica e geomorfológia distintas.

Em termos climáticos quase toda a região se integra no andar termomediterrânico sub-húmido a húmido, atingindo as zonas mais elevadas o andar mesomediterrânico húmido. A temperatura média anual ronda os 16ºC, podendo atingir os 15ºC, como valor mínimo e os 19ºC como valor máximo. A precipitação média anual é de 650 mm, atingindo o seu máximo, 1300 mm, no topo da Serra de Monchique. Os sobreirais sub-húmidos e húmidos com *Quercus canariensis* em algumas áreas (*Myrto-Quercetum suberis* e *Sanguisorbo-Quercetum suberis* na nomenclatura fitossociológica) constituem a vegetação potencial dominante, respectivamente nos andares termomediterrânico e mesomediterrânico (Rivas-Martinez et al. 1990, Costa et al. 1998, Seng & Deil 1999). Actualmente, a vegetação climácica restringe-se a pequenas áreas de declive mais acentuado em umbrias e fundos de vertente, menos afectadas no passado pelas actividades agrícolas e portanto em solos mais profundos e férteis, e cuja localização em áreas de menor insolação permite uma recuperação mais vigorosa da vegetação.

O Sudoeste Serrano passou por profundas transformações durante o século XX. Na segunda metade do século XIX podia estimar-se a superfície coberta pelos matagais em 80-90% (Feio 1983). Praticava-se sobretudo a cultura itinerante através do fogo, utilizado também para o rejuvenescimento dos pastos para os rebanhos de cabras. No início do século XX, as serras encontravam-se ainda abundantemente revestidas de vegetação, sendo mais arborizada a Serra de Monchique do que a Serra do Caldeirão (Dionísio 1927). O matagal de estevas *Cistus ladanifer* (esteval) dominava a paisagem, encontrando-se algumas vertentes das serras ocidentais cobertas de matagal arborescente dominado pelo medronheiro *Arbutus unedo* (medronhal). Os sobreiros e azinheiras também abundavam na região (Feio 1983).

Nas primeiras décadas do século XX, com o aparecimento dos adubos químicos inicia-se a desmatação, generalizada após 1929 através dos subsídios da "campanha do trigo". Como resultado, restava em 1949 apenas 10-30% do coberto arbustivo, conforme as áreas, em toda a região serrana. As escassas áreas que se mantiveram arborizadas nesse período cobriam apenas cerca de 10% da região, em grande parte no Caldeirão central e nas vertentes ocidentais do maciço xistoso de Monchique (Guerreiro 1954, Feio 1983). Em consequência do esgotamento dos solos, teve início em meados do século XX o abandono progressivo da cultura cerealífera e o consequente êxodo rural generalizado, voltando as grandes extensões contínuas de matagal a partir do início da década de 1960 (Acácio et al. 2008).

Nas serras ocidentais – Serra do Cercal, colinas da bacia do Mira e Serra da Vigia, e Serra de Monchique/Espinhaço de Cão – surge então uma nova metamorfose da paisagem com a rápida expansão do eucaliptal para produção de pasta para papel.

Durante as décadas de 1970-1980, extensas plantações de *Eucalyptus globulus* substituíram os cultivos de cereal e a vegetação natural restante (Krohmer & Deil 2003). Assim, ca. 70% da Serra do Cercal, 25% do Concelho de Odemira e 50% da Serra de Monchique encontram-se cobertos por eucaliptais (Alhinho et al. 2002). Finalmente, nas décadas de 1980-1990, entraram em acção, de forma consecutiva, diversos programas comunitários de apoio à arborização, que resultaram num total de 13 459 ha de plantações puras de diversas espécies, só na região do Algarve, sobretudo quercíneas autóctones e coníferas (Louro 1999).

1.4. Métodos gerais

A águia de Bonelli é uma rapina de difícil observação, apesar do seu porte considerável e do comportamento activo. A habitual discrição e reduzida conspicuidade da espécie é bem conhecida dos ornitólogos (e.g. Del Moral 2006) e, aliada à grande dimensão dos territórios, dificulta notavelmente a sua detecção. Como tal, torna-se necessário repetir várias vezes a prospecção de uma área para confirmar a sua presença e estatuto de ocorrência e, em especial, a sua ausência.

A distribuição da população estudada foi estabelecida através da prospecção sistemática do terreno, seguindo um processo por etapas:

1) Localização dos territórios de reprodução por detecção de aves adultas a partir de pontos dominantes do terreno, com o auxílio de binóculos 10x e telescópio monocular ou binocular. A partir de uma fase inicial, esta prospecção passa a ser dirigida para áreas reconhecidas pelo observador como de habitat potencialmente idóneo para a nidificação ("imagem de busca") e é auxiliada pela

grande territorialidade da espécie. Assim, quanto mais distantes de áreas de nidificação conhecidas se encontram as zonas de aparente habitat favorável, maior a probabilidade de constituirem novas zonas de nidificação. Da mesma forma, pelo facto da águia de Bonelli ser um predador de topo também dentro da sua própria guilda, a ocorrência frequente, numa determinada zona, de rapinas diurnas de médio porte como nidificantes, tais como *Aquila pennata*, *Buteo buteo*, *Milvus* spp. ou outras espécies de porte similar, indica com bastante segurança a ausência de *Aquila fasciata* como reprodutora.

2) Detectados os territórios de reprodução, a pesquisa de ninhos foi efectuada a pé ou em veículo, através da observação de comportamentos dos adultos que indiquem a existência de ninhos nas zona prospectada. Regra geral, os ninhos desta espécie encontram-se em locais bastantes discretos, sendo porém detectáveis com relativa facilidade a partir do momento em que entram no campo de visão do observador, dado o seu grande tamanho (podendo atingir com frequência 1,8 a 2m de diâmetro).

A monitorização da reprodução foi realizada por observação do conteúdo dos ninhos a partir do exterior, com o auxílio de telescópio, ou através de escalada das árvores de suporte com o auxílio de equipamento especializado. A idade das crias foi estabelecida por comparação visual com desenhos do desenvolvimento da plumagem (e.g. Blondel 1969) ou por medição directa das rémiges e rectrizes (Mañosa et al. 1995). Durante as visitas aos ninhos foram sistematicamente recolhidas do ninho e imediações todas as regurgitações e restos alimentares encontrados, à excepção dos que ainda não tinham sido inteiramente consumidos, bem como as penas de muda dos adultos.

As manipulações dos juvenis nidícolas foram efectuadas no solo, após retirada dos juvenis do ninho e durante o mínimo tempo necessário, após o qual foram devolvidos ao ninho em segurança. A manipulação incluíu a recolha de dados morfométricos, sangue e penas em crescimento, amostras para rastreio de patologias, bem como a anilhagem com anilhas metálicas e de PVC alfanuméricas, estas últimas segundo códigos estabelecidos internacionalmente e coordenados pela Universidade de Barcelona. Entre 1993 e 1996 foi efectuada também a marcação com placas alares com códigos de cor igualmente sob a mesma coordenação.

Todos os dados foram devidamente registados em blocos de campo e transpostos posteriormente para bases de dados. Em todos os procedimentos de monitorização e recolha de material foram respeitadas as regras de minimização da perturbação sobre adultos e crias, particularmente nos períodos mais sensíveis da pré-postura, postura, incubação e pré-voo dos juvenis. Da mesma forma, a manipulação dos juvenis foi efectuada de acordo com os procedimentos de minimização do stress.

1.5. Referências

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1.6. Estrutura da Tese

A presente tese é composta pelos seguintes 6 artigos publicados:

Höfle, U., J.M. Blanco, L. Palma & P. Melo 2000. Trichomoniasis in Bonelli's Eagle (*Hieraaetus fasciatus*) Nestlings in South-west Portugal. Pp. 45-52 in J.T. Lumeij, J.D. Remple, P.T. Redig, M. Lierz and J.E. Cooper [EDS.], Raptor Biomedicine III. Zoological Education Network, Inc. Lake Worth (FL), U.S.A.

Palma, L., S. Mira, P. Cardia, P. Beja, T. Guillemaud, N. Ferrand, M.L. Cancela & L.C. da Fonseca 2001. Sexing Bonelli's eagle nestlings: morphometrics versus molecular techniques. Journal of Raptor Research 35: 187-193.

Palma, L. P. Beja, P.C. Tavares & L.R. Monteiro 2005. Spatial variation of mercury levels in nesting Bonelli's eagle from Southwest Portugal: effects of diet composition and prey contamination. Environmental Pollution 134: 549-557.

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

Beja, P., M. Pais & L. Palma 2007. Rabbit *Oryctolagus cuniculus* habitats in Mediterranean scrubland: the role of scrub structure and composition. Wildlife Biology 13: 28-37.

Beja, P. & L. Palma 2008. Limitations of methods to test density-dependent fecundity hypothesis. Journal of Animal Ecology 77: 335-340.

Capítulo 2. Rabbit *Oryctolagus cuniculus* habitats in Mediterranean scrubland: the role of scrub structure and composition

Wildlife Biology 13: 28-37 (2007)

Rabbit *Oryctolagus cuniculus* habitats in Mediterranean scrubland: the role of scrub structure and composition

Pedro Beja, Miguel Pais & Luís Palma

Beja, P., Pais, M. & Palma, L. 2007: Rabbit *Oryctolagus cuniculus* habitats in Mediterranean scrubland: the role of scrub structure and composition. - Wildl. Biol. 13: 28-37.

Although scrub cover is generally regarded as an important habitat component of the European wild rabbit *Oryctolagus cuniculus*, little is known about the species' responses to variation in scrub structure and composition. Such information is required for conservation management of rabbits in Mediterranean scrublands of the Iberian Peninsula (southwestern Europe), where the species is both a key resource for a range of endangered predators and a popular game species. To address this issue, we estimated the effects of variables characterising scrub vegetation on the occurrence and number of rabbit latrines in 60 250-m transects distributed in a 5,000-ha scrubland-dominated landscape in southwestern Portugal. The strongest effect was that of cover by the shrub *Cistus ladanifer*, which was positively related to both the occurrence and relative abundance of rabbits. Conversely, rabbits showed negative responses to cover by *Chamaespartium tridentatum*, ericoid and other broad-leaved shrubs. Rabbits favoured scrubs with a low density of woody vegetation at the ground level, but with a dense overhead cover. The herbaceous layer was also influential with positive effects of cover by grasses under the scrub and by forbs at the edge of the scrub. Taken together, our results suggest that conservation management of rabbit habitats in southwestern Portugal should strive to maintain scrub patches with a combination of favourable characteristics, including dense overhead cover but open access at the ground level, a developed herbaceous layer and woody species providing feeding opportunities during the summer period.

Key words: Cistus ladanifer, conservation, habitat management, Iberia, lagomorphs, Portugal, rabbit

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Received 30 April 2005, accepted 22 December 2005

Associate Editor: Miguel Delibes

In the European Mediterranean region, major conservation challenges have emerged in recent decades from the pervasive trend for the abandonment of agricultural land and its subsequent occupation by scrubland and woodland (González Bernáldez 1991, Krohmer & Deil 2003, Romero-Calcerrada & Perry 2004). This has resulted in habitat losses for species associated with early-successional vegetation and edge habitats, which had benefited from the mosaic structure created by the traditional management of Mediterranean landscapes (Farina 1997, Sánchez-Zapata & Calvo 1999, Moreira et al. 2005). One such species is the European wild rabbit *Oryctolagus cuniculus*, which has declined since the 1950s due essentially to diseases, excessive hunting and changes in traditional land uses (Blanco & Villafuerte 1993, Calvete et al. 2004).

The rabbit is native to the Iberian Peninsula where a considerable effort is devoted to its conservation management. This is primarily because the rabbit is an important prey for a wide range of avian and mammalian predators of conservation concern (Delibes & Hiraldo 1981), and a key resource for globally threatened species such as the Spanish imperial eagle *Aquila adalberti* and the Iberian lynx *Lynx pardinus* (González et al. 1990, Palma et al. 1999). The rabbit is also a popular game species, providing an important source of income in rural areas. Therefore, a detailed understanding of rabbit habitat requirements has been actively sought in recent years in order to develop management prescriptions to enhance its populations (Moreno & Villafuerte 1995, Moreno et al. 1996, Martins et al. 2003, Calvete et al. 2004).

Favourable rabbit habitats are judged to include herbaceous patches providing adequate food resources, combined with some kind of scrubland cover or warrens offering protection against predators (Rogers & Myers 1979, Palomares & Delibes 1997). Given these general ecological conditions, rabbits can occur over a wide variety of habitat configurations, ranging from open grasslands, where protection is provided primarily by warrens, to areas dominated by scrubland interspersed with small herbaceous patches (Lombardi et al. 2003). Habitat management strategies vary accordingly; efforts focusing to a large extent on reducing predation risk in open habitats, frequently by providing artificial warrens, whereas increasing food resources is usually the main goal in closed habitats (Moreno & Villafuerte 1995).

In landscapes resulting from the abandonment of traditional land uses, clearing scrubland patches and eventually sowing nutritious herbs are usually the main management prescriptions (Moreno & Villafuerte 1995). Recommendations tend to ignore the scrubland component, implicitly assuming that scrub cover is plentiful and so unlikely to limit rabbits. This view, however, may not be correct, as different kinds of scrub may vary widely in the habitat conditions they offer to rabbits. For instance, variation in stem density may affect the potential of scrubland to provide concealment and escape routes against predators (Villafuerte & Moreno 1997). Also, rabbits consume woody plants during some periods of the year, and so the temporal and spatial availability of important food resources may be influenced by the distribution, abundance and nutritional value of different shrub species (Martins et al. 2002). It is likely, therefore, that a greater consideration should be given to the scrub component for effectively managing rabbit habitats. However, taking this approach is difficult at present, due to a very poor understanding of the ways in which rabbits may be influenced by scrub attributes.

Our present study addressed this issue, quantifying the influences of scrub structure and composition on the distribution and abundance of rabbits, in a scrubland dominated landscape of southwestern Portugal. Our results were then used to evaluate the importance of the scrub component when managing rabbit habitats for conservation in this region, and to suggest Mediterranean scrubland management guidelines favouring rabbits.

Methods

Study area

A study area representative of the vast scrublands occupying former arable land in the mountain ranges of southwestern Portugal was selected at the foothills of the Monchique massif (902 m; Fig. 1). This is a rugged landscape situated at 100-300 m a.s.l. It is crossed by the entrenched valleys of the Seixe River and its numerous small tributaries. Mean temperature is 16°C and the average annual rainfall is about 750 mm; only 6-7% of the annual rain falls during June-September, the hottest period of the year, causing a water deficit at the end of the dry season. The potential vegetation on the schist-siliceous soil prevalent in the region is a mixed oak forest dominated by cork oak *Quercus suber* (Rivas-Martinez et al. 1990, Seng & Deil 1999), but natural vegetation was largely cleared for cereal cropping from the 1930s up to the 1960s, and presently occur at various stages of recovery from past agricultural disturbance (Krohmer &

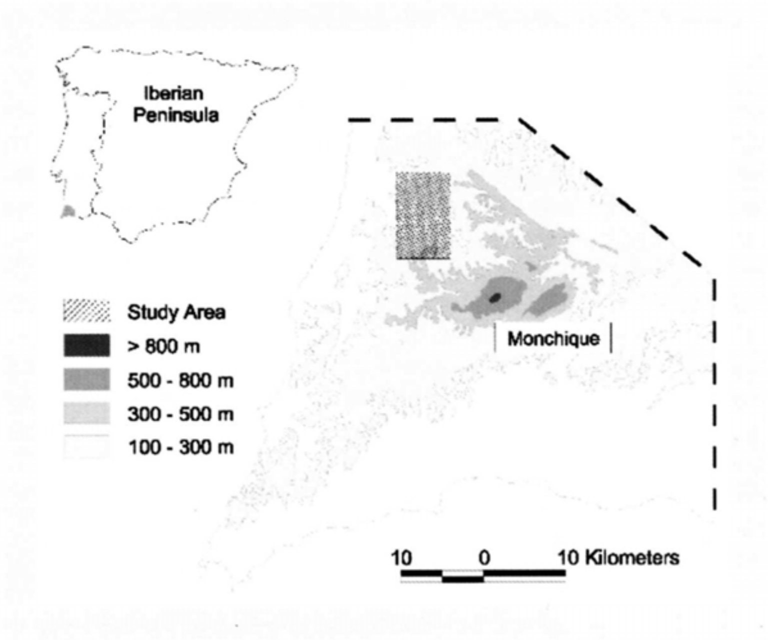

Figure 1. Study area in southwestern Portugal from which rabbits were sampled during June-August 1997.

Deil 2003). Evergreen sclerophyllous shrubs dominate the landscape with a shrub composed by *Cistus ladanifer* occupying vast dry areas with strongly eroded soils. Depending on soil, moisture and disturbance conditions, there is also a variety of more diverse shrub types, the most representative being gorse-heaths composed of several species of *Ulex, Genista, Erica* and Cistaceae (Rivas-Martinez et al. 1990, Seng & Deil 1999). The most developed vegetation is largely confined to north-facing slopes, including cork oak woods and a tall and dense thicket dominated by *Arbutus unedo* and *Erica arborea*. During the 1970s and 1980s there was large-scale afforestation with eucalyptus *Eucalyptus globulus* which replaced abandoned cereal fields and natural vegetation that had been spared from past human activities (Krohmer & Deil 2003). Agricultural activities are now extremely reduced, and human occupation is restricted to a few isolated houses and sparsely cultivated valleys. Hunting pressure on rabbits is apparently low and localised, and there is no game management.

Study design and rabbit survey

The study was carried out in an area of about 5,000 ha in which a systematic rabbit survey recorded the presence of 30 discrete sites occupied by rabbits (see below). The effects of scrub vegetation structure and composition on rabbit distribution were then estimated by comparing habitat conditions at sites occupied by rabbits with an equal number of random sites within the study area from which the species was absent. Variation in habitat conditions within rabbit sites was used to estimate the factors affecting rabbit abundance.

Rabbit distribution was surveyed during June-August 1997, i.e. after the end of the breeding season (Gonçalves et al. 2002), by systematically searching for activity signs (footprints, faecal pellets, scratches and direct observation of animals) along the network of dirt roads and firebreaks crossing the study area. These tracks provided the main discontinuities in otherwise densely vegetated terrain where rabbit signs appeared largely absent and systematic surveys were virtually impossible. Warrens were not used in surveys, as the thin and rocky soils largely prevented rabbit digging. The distribution of signs was noted on 1:25,000 maps, and discrete rabbit sites were identified as clusters of signs separated from the nearest cluster by at least 300 m with no evidence of rabbit activity in-between. The 300-m distance was chosen because movements by rabbits in similar habitats tend to be shorter than this (e.g. Gibb 1993), though dispersing animals may eventually travel much longer distances (e.g. Moreno et al. 2004). Nevertheless, the lack of any rabbit sign in-between sites suggested that movements among sites separated by this distance were at most sporadic, and so they could be taken as independent locations. As this assumption could not be tested, eventual spatial dependencies among sampling sites were controlled for statistically in the data analysis (see below).

After the survey, 30 random points were distributed across the network of dirt roads and firebreaks crossing the study area, at a minimum distance of 300 m from the nearest site occupied by rabbits. At each rabbit site, the relative abundance of rabbits was indexed from a latrine count along a 250-m transect, counting as a latrine any group of > 20 pellets within a circle with a 10-cm radius. Latrines are special communal sites where rabbits deposit faecal pellets for social reasons, in addition to depositing faeces apparently at random throughout their range (Sneddon 1991), and they have been widely used to index rabbit abundances (e.g. Iborra & Lumaret 1997). This indirect method was used because the actual observation of individuals in the study area was not feasible due to dense vegetation cover.

Vegetation structure and composition

The scrub bordering dirt roads along the 250-m transects set at rabbit sites and at random sites, was characterised from 11 variables quantifying the woody vegetation vertical structure and the herbaceous layer (Table 1). In each transect, vegetation variables were estimated at six sampling points, located at 50-m intervals on alternating sides of the road. At each point, the overall woody cover and the proportion of different scrub types was estimated at four 0.25-m vertical strata (0-0.25 m - 0.75-1.0 m), using a vegetation profile board (Hays et al. 1981). We recognised six shrub types: 1) ericoid shrubs (heather *Calluna vulgaris* and *Erica* spp.); 2) thorny shrubs (e.g. *Ulex* spp. and *Genista* spp.); 3) *Cistus ladanifer*; 4) other broad-leaved evergreen shrubs (e.g. *Arbutus unedo, Quercus* spp. and Cistaceae except *C. ladanifer*); 5) *Chamaespartium tridentatum*; and 6) *Dittrichia revoluta*. The herbaceous layer was quantified in terms of percentage cover made up by grasses (Gramineae) and forbs (non-grassy and herbaceous species, e.g. legumes and composites), both at the edge of the scrub and under the scrub at 5 m from the edge. The same observer carried out all measurements, thereby minimising methodological variations. Vegetation variables were averaged across the four strata and the six points measured in each transect, except where indicated otherwise.

Data analysis

The angular transformation was used for proportional data to reduce the weight of a few large values in subsequent statistical analysis and to overcome the unity sum constraint. The interrelationships among vegetation variables were then evaluated using Principal Components Analysis (PCA; Legendre & Legendre 1998). Generalised linear modelling was used to examine the effects of vegetation variables on the distribution and abundance of rabbits (GLM; McCullagh & Nelder 1989). The occurrence data were modelled with logistic regression, whereas models for latrine counts were based on Poisson regression corrected for overdispersion. Model development involved a preliminary selection of significant variables using likelihood-ratio statistics, testing both the linear and unimodal effects of each vegetation variable.

Table 1. Scrubland vegetation structure and composition at sites used (N = 30) and non-used (N = 30) by rabbits in southwestern Portugal during June-August 1997. The distance was measured to the nearest site occupied by rabbits.

	Mean ± SD (Min-Max)	
Variables	Used sites	Non-used sites
Scrub cover (%)	62.7 ± 13.7 (35.2-82.9)	70.4 ± 14.7 (39.8-98.1)
Scrub composition (%)		
Ericoid shrubs	13.0 ± 14.1 (0.0-61.7)	25.5 ± 19.4 (0.8-81.1)
Thorny shrubs	5.1 ± 6.65 (0.0-26.4)	6.0 ± 8.69 (0.0-36.8)
Cistus ladanifer	63.4 ± 27.2 (0.0-100.0)	37.5 ± 30.3 (0.0-90.7)
Other broad-leaved shrubs	10.3 ± 17.3 (0.0-75.7)	22.2 ± 26.0 (0.0-77.9)
Chamaespartium tridentatum	3.6 ± 6.33 (0.0-22.3)	5.7 ± 8.10 (0.0-35.8)
Dittrichia revoluta	4.6 ± 7.14 (0.0-22.3)	3.2 ± 4.26 (0.0-12.4)
Herbaceous cover (%)		
Grasses, edge	45.2 ± 21.9 (10.0-83.3)	37.0 ± 20.5 (6.7-73.3)
Grasses, interior	41.0 ± 25.7 (0.0-86.7)	26.2 ± 22.6 (0.0-83.3)
Forbs, edge	9.8 ± 8.71 (0.0-33.3)	4.4 ± 5.49 (0.0-16.7)
Forbs, interior	4.1 ± 5.85 (0.0-23.3)	1.8 ± 2.59 (0.0-10.0)
Distance (km)	0.9 ± 0.58 (0.3-2.2)	0.6 ± 0.24 (0.3-1.2)

Combinations of significant variables and their interaction terms were selected using stepwise procedures, and the Akaike Information Criteria (AIC) was used to select the best multivariate models (Burnham & Anderson 1998). Percentage reduction in deviance between the null model (model fitted to the intercept only) and each model tested was taken to indicate the amount of variability explained by the models. In the modelling procedure, the log-transformed distance from each sampling point to the nearest site occupied by rabbits was used to test for spatial structure in rabbit distribution. The marginal effects of vegetation variables in models including the spatial term were then tested, thereby accounting for potential confounding effects of spatial autocorrelation (Legendre & Legendre 1998). Throughout the study, the significance level was set at $P < 0.05$. Analyses were made using S-Plus 2000 (MathSoft 1999).

Table 2. Loadings of vegetation variables on the first four axes (PC 1-PC 4) extracted by PCA, and the proportion of variance accounted for by each axis, for 60 rabbit habitat sampling sites in southwestern Portugal during June-August 1997. Values in italics indicate loadings >|0.40|.

Variables	PC1	PC2	PC3	PC4
Scrub layer				
C. ladanifer	_0.74_	0.20	_-0.59_	-0.15
C. tridentatum	-0.34	_0.54_	0.08	_-0.54_
Ericoid shrubs	_-0.61_	0.17	0.39	-0.25
Thorny shrubs	0.30	0.10	0.36	_0.67_
Broad-leaved shrubs	_-0.58_	-0.18	0.32	0.36
Dittrichia revoluta	0.24	-0.32	_0.44_	-0.25
Scrub cover	_-0.62_	0.07	-0.07	-0.09
Herbaceous layer				
Grasses, edge	_0.53_	_-0.49_	_0.52_	-0.27
Grasses, interior	_0.71_	0.27	_0.41_	-0.19
Forbs, edge	_0.66_	_0.61_	0.04	0.00
Forbs, interior	0.41	_0.72_	0.11	0.14
Eigenvalue	3.29	1.72	1.39	1.15
% Var.	29.9	15.6	12.6	10.5

Results

The PCA for vegetation variables highlighted a dominant gradient primarily related to the proportional cover by *C. ladanifer* (Table 2). The first PC accounted for 29.9% of the variance in the data, contrasting scrubland dominated by *C. ladanifer* with scrubland covered primarily by ericoid and other broad-leaved shrubs. *C. ladanifer* scrubland tended to be less dense and to have a higher soil coverage by grasses and forbs, both within and at the edge of the scrub, than ericoid and other broad-leaved shrubs. The second PC accounted for 15.6% in variance and it was primarily related with the herbaceous layer, contrasting areas dominated by either forbs or grasses. The third PC (12.6%) contrasted *C. ladanifer* scrubland with long-term fallows, dominated by *Dittrichia revoluta* and with a well-developed grassy layer. The fourth PC (10.2%) depicted a weak gradient of increasing cover made up by thorny shrubs and decreasing cover made up by *Chamaespartium tridentatum*.

The strongest influence on rabbit distribution was that of *C. ladanifer* (Table 3), with the highest estimated probabilities of occurrence recorded for scrub-

Table 3. Summary of univariate Generalised Linear Models for the effects of vegetation and spatial structure on rabbit occurrence (logistic regression) and relative abundance (Poisson regression) in southwestern Portugal during June-August 1997. Signs indicate the directions of the association between the dependent and the explanatory variables, and % DEV is the percentage of explained deviance. Significant variables ($P < 0.05$) are given in italics, and * indicate variables that remained significant ($P < 0.05$) after accounting for spatial structure in rabbit distribution.

	Occurrence models (N = 60)			Abundance models (N = 30)		
		% Dev	P-value		% Dev	P-value
Scrub layer						
Scrub density	(-)	_5.8_	_0.027_	(-)	1.5	0.520
Ericoid shrubs	(-)	_11.6_	_0.002*_	(-)	11.6	0.062
Thorny shrubs	(-)	0.0	0.955	(-)	7.3	0.149
Cistus ladanifer	(+)	_13.3_	_<0.001*_	_(+)_	_23.8_	_0.007*_
Broad-leaved shrubs	(-)	_4.8_	_0.046_	(-)	6.1	0.198
Chamaespartium tridentatum	(-)	1.5	0.182	(-)	7.0	0.157
Dittrichia revoluta	(+)	0.5	0.505	(-)	1.8	0.482
Herbaceous layer						
Grasses, edge	(+)	3.0	0.113	(-)	2.3	0.421
Grasses, interior	(+)	_6.5_	_0.020*_	(-)	4.0	0.297
Forbs, edge	(+)	_10.0_	_0.004*_	(+)	0.1	0.868
Forbs, interior	_(+)_	3.6	0.084	(-)	0.1	0.883
Distance	(-)	_8.0_	_0.010_	(-)	3.0	0.374

land dominated by this species (Fig. 2). However, there was also a negative response to scrub density, suggesting that rabbits were favoured by more open scrub. Ericoid shrubs showed a negative effect with very low estimated probabilities of rabbit occurrence (< 0.4) noted for scrubland with > 25% cover made up by these shrubs (see Fig. 2). A similar pattern, though not as strong, was found for broad-leaved shrubs. The herbaceous layer was also influential with a strong positive effect of cover made up by forbs at the scrub edge, and less so by grasses under the scrub (see Fig. 2). There was no evidence for unimodal responses of rabbits to any vegetation variable. After accounting for the significant effect of spatial structure, the linear effects of *C. ladanifer*, ericoid shrubs, edge forbs and interior grasses remained significant. The best AIC model included only the significant effects of *C. ladanifer* and edge forbs, reducing the total deviance by 18.3%. No significant improvement could be obtained by adding the spatial term to this model (P = 0.098).

Results of a similar analysis based on woody vegetation variables broken by vertical strata yielded broadly similar results (Table 4). The occurrence of rabbits was favoured by a scrub dominated at all levels by *C. ladanifer* and with reduced cover made up by ericoid shrubs. The broad-leaved shrubs also had an overall tendency to negatively affect the occurrence of rabbits, but this was significant only for the second strata. At the basal level, but not for other strata, there were negative effects of scrub density and cover made up by *Chamaespartium tridentatum*. After accounting for spatial structure in rabbit distribution, the effects of broad-leaved shrubs and *Chamaespartium tridentatum* lost significance. The best AIC model included the basal cover made up by *C. ladanifer* and edge forbs, reducing the deviance by 19.3%. Adding the spatial term to this model did not improve its performance (P = 0.125).

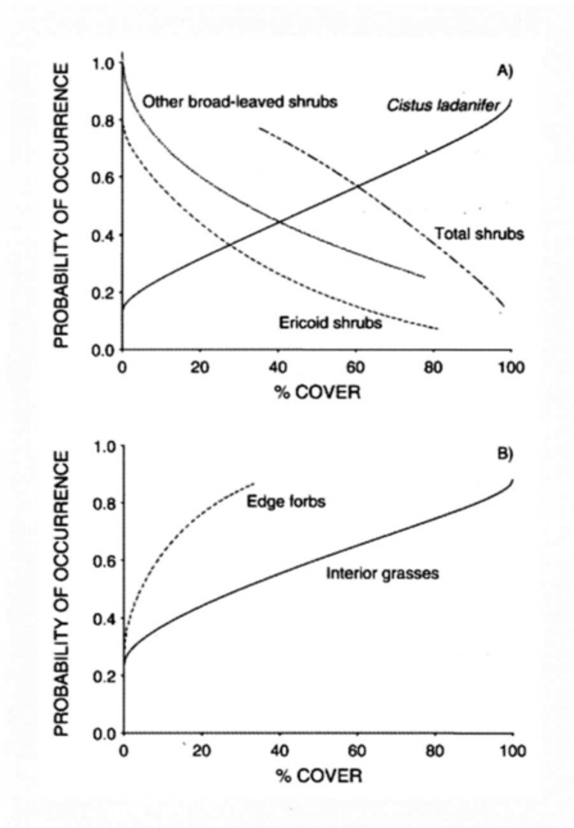

Figure 2. Logistic regression models for the effects of variables characterising the scrub (A) and herbaceous (B) layers on the probability of occurrence of rabbits in Mediterranean scrublands of southwestern Portugal during June-August 1997. The curve depicted for each variable was restricted to the range of observed values.

Table 4. Summary of univariate Generalised Linear Models for the effects of scrub vegetation divided by vertical strata on rabbit occurrence (logistic regression) and relative abundance (Poisson regression) in southwestern Portugal during June-August 1997. Signs indicate the directions of the association between the dependent and significant (P < 0.05; in italics) and nearly significant (0.05 < P < 0.1) explanatory variables, and * indicate variables that remained significant (P < 0.05) after accounting for spatial structure in rabbit distribution.

	Scrub vegetation strata (m)			
Variables	0-0.25	0.25-0.50	0.50-0.75	0.75-1.0
Occurrence				
Scrub density	(-) *0.026**	(-) 0.051	(-) 0.094	(-) 0.098
Ericoid shrubs	(-) *0.019*	(-) *0.005**	(-) *0.002**	(-) *0.002**
Cistus ladanifer	(+) *0.001**	(+) *<0.001**	(+) *0.001**	(+) *0.003**
Broad-leaved shrubs		(-) *0.048*	(+) 0.088	(+) 0.072
Chamaespartium tridentatum	(-) *0.030*			
Abundance				
Scrub density				(+) *0.037**
Ericoid shrubs	(-) 0.067	(-) *0.044**	(-) 0.084	(-) 0.072
Cistus ladanifer	(+) *0.005**	(+) *0.007**	(+) *0.017**	(+) 0.054

The proportion of *C. ladanifer* was the only variable significantly related to variation in rabbit abundance (see Table 2), irrespective of spatial structure, with the highest latrine counts recorded for almost pure stands (> 80%) of this species (Fig. 3). The other variables did not show significant relationships with the latrine counts, though there was a tendency for an inverse relationship with ericoid shrubs. When vegetation variables were broken by vertical strata, the results for *C. ladanifer* remained similar, and the negative effects of the ericoid shrubs became significant at the second level; overall scrub density showed a positive relationship at top level (see Table 3). The best AIC model included the positive effects of *C. ladanifer* and interior grasses, reducing the total deviance by 31.4%. Adding the spatial term to this model did not improve its performance (P = 0.685).

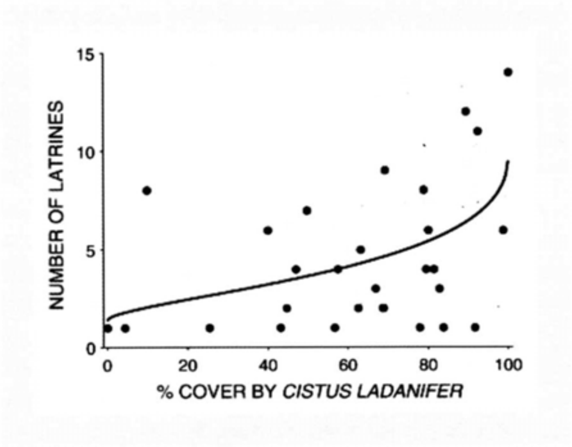

Figure 3. Poisson regression model for the effects of percentage cover made up by *C. ladanifer* on the number of rabbit latrines in Mediterranean scrublands of southwestern Portugal during June-August 1997; sites from which rabbits were absent were excluded.

Discussion

Our study supported the view that the distribution and abundance of rabbits can be influenced to a large extent by scrubland composition and vertical structure. In the uplands of southwestern Portugal, rabbits seemed to be favoured by scrubland dominated by *C. ladanifer*, with relatively sparse woody vegetation at the ground level but with dense overhead cover, and with abundant herbaceous vegetation both at the edge and within scrub patches. The negative responses recorded for ericoid shrubs, other broad-leaved shrubs and *Chamaespartium tridentatum* suggested that rabbits avoided most scrub types available within the region, including the *Arbutus unedo* - *Erica arborea* thicket and several heathlands with *Calluna vulgaris, Erica australis* or *Erica umbellata* (Rivas-Martinez et al. 1990, Seng & Deil 1999).

Although our study had some limitations and potential shortcomings, it is unlikely that these affected significantly the patterns described above. A major limitation was the relatively small size of the study area (5,000 ha), which could have caused the results to be site-specific. However, the study area was much comparable to other landscapes in southern Portugal, particularly in the southwestern uplands where agricultural abandonment resulted in extensive cover made up by dense scrubland, including vast expanses dominated by *C. ladanifer* (e.g. Krohmer & Deil 2003). Also, the main habitat associations uncovered herein have been recorded in more extensive rabbit surveys carried out within the region (P. Beja, L. Palma & M. Pais, unpubl. data), thus suggesting that the patterns observed might be valid over a much wider region. These surveys also suggested that despite the rather limited temporal scope of our present study, the patterns described may be valid across seasons and over the years. These views should be confirmed by future studies analysing larger areas over longer time frames. Another potential problem of our study was the relatively small distance among sampling points which could have introduced confounding effects due to spatial autocorrelation in environmental attributes or rabbit population processes (Legendre & Legendre 1998). This was unlikely to be a major problem, however, as the introduction of a spatial term (distance to the nearest site occupied by rabbits) in the habitat models did not change the statistical significance of habitat variables in the vast majority of cases. Explicitly incorporating spatial terms in ecological models is considered a far better approach to deal with spatial autocorrelation than trying to eliminate spatial dependencies through sampling design (Legendre & Legendre 1998).

Several non-exclusive hypotheses may be put forward to explain the observed association of rabbits with *C. ladanifer* shrub. One possibility is that this shrub might provide a critical food resource, at least during some periods of the year. At first, this seems unlikely, as *C. ladanifer* is usually considered a poor forage due to high leaf lignin, low nutritional value and the presence of toxic flavonoids and terpens (e.g. Gómez-Castro et al. 1978, Sosa et al. 2004), making it little used by rabbits when more profitable foods are available (Martins et al. 2002). However, *C. ladanifer* is extensively consumed by the red deer *Cervus elaphus* in periods of nutritional constraint (Rodríguez 1978a, Bugalho & Milne 2003), and this may also be the case for the rabbit. In Mediterranean climates, the main period of food stress for rabbits is the summer when very dry and hot conditions result in a minimal standing crop of grasses and forbs, and a low nutritional quality of natural forages (Myers & Poole 1963, Alves & Moreno 1996). Under these circumstances, rabbits can not meet their daily water and protein requirements by feeding solely on the herbaceous layer (Alves & Moreno 1996). They must supplement their diet with a number of alternative foods, including the leaves and seeds of scrubs (Cooke 1982). Although the summer leaves of *C. ladanifer* have a minimal nutritional value (Gómez-Castro et al. 1978), the capsules (seeds plus woody walls) produced by this plant are nutritionally very profitable, as they are rich in raw protein and fat (Rodríguez 1978b). This resource is available throughout the dry season, and it may comprise up to 45% of red deer summer diet in habitats similar to that in southwestern Portugal (Rodríguez 1978a). Clearly, further data should be gathered to evaluate whether capsules are a preferred food resource influencing the association of rabbits with *C. ladanifer* scrubland.

Another possibility is that rabbits were influenced by the herbaceous layer, which seemed to be more developed in *C. ladanifer* stands than elsewhere. Indeed, there was a relatively high cover of grasses under *C. ladanifer* which was probably favoured by the lower woody cover recorded in this scrub. The presence of grasses within scrubland may be particularly valuable, allowing rabbits to feed during the day on a preferred resource (Bhadresa 1987) when their activities outside protective cover tend to be severely restricted due to predation risk (Moreno et al. 1996). This view was supported by the positive response of rabbits to grass cover within scrubland, and the lack of a significant relationship recorded for edge grasses.

The value of different scrub types in providing protection against predators is another potential determinant of rabbit distribution and abundance. This may be particularly important in the study area where the thin and rocky soils largely prevented rabbits from digging warrens. Scrub is generally regarded as offering effective protection against avian predators, but increases the risk of predation by stalking mammalian predators (Moreno et al. 1996). Dense scrub may be particularly unsafe because it can hinder the visual perception of approaching predators and obstruct escape movements (Villafuerte & Moreno 1997). This may justify the observed negative responses to scrub cover at the ground level, and the positive relationships with overhead cover. The structure of *C. ladanifer* shrub, dense at the higher level but relatively sparse at ground level, tends to provide this kind of cover, thus probably representing a good compromise in terms of protection from both aerial and terrestrial predators.

Whatever the ultimate causes, the observed association between rabbits and *C. ladanifer* suggests that efforts to increase rabbit populations need to recognise the influential role played by the composition and structure of scrubland vegetation. For instance, the common practice of creating small grassland patches in scrubland landscapes to improve rabbit habitats (Moreno & Villafuerte 1995), would likely be more effective if the preferred scrub patches are not removed and the clearings are opened in their vicinity. Also, favourable scrub patches, with an optimal vertical structure and high herbaceous cover, may be actively created and maintained across the landscape through rotational burning or cutting (e.g. Patón et al. 1998, Tárrega et al. 2001). To design adequate conservation management prescriptions, the most favourable scrubland vegetation needs to be identified in each case as it will probably differ among regions in relation to local habitat conditions. For instance in southern Spain, *C. ladanifer* shrub appears to be little used by rabbits in the Sierra Morena (M. Delibes, pers. comm.), whereas in the Province of Cadiz a positive association was recorded between rabbits and the shrub *Pistacia lentiscus* (Fa et al. 1999). In general, we hypothesise that the preferred scrub for rabbits should have a combination of favourable characteristics, including a developed herbaceous layer, dense overhead cover but open access at the basal level and at least some woody species providing feeding opportunities during the summer period. This view should be confirmed in future studies, preferably through the experimental manipulation of scrub characteristics.

Acknowledgements - our study was supported by the Instituto da Conservação da Natureza and by the European Union (Project LIFE B4-3200/94/767). We thank the contribution from Manuel João Pinto to plant identification and vegetation characterisation, and the advice from Paulo Célio relative to aspects of rabbit biology. Nuno Pedroso, Teresa Sales-Luis, Teresa Silva, Susana Almeida, Pedro Vaz, Susana Coelho, Maria Carlos Reis, Luisa Chaves and Pedro Portela helped during the field work. Gary White, Rui Borralho, Francisco Moreira, Filomena Magalhães, José Carlos Brito, Miguel Araújo and two anonymous referees provided valuable comments to early drafts of the manuscript.

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Capítulo 3. Why do raptors take domestic prey? The case of Bonelli's eagles and pigeons

Journal of Applied Ecology 43: 1075-1086 (2006)

Journal of Applied Ecology 2006
43, 1075–1086

Why do raptors take domestic prey? The case of Bonelli's eagles and pigeons

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Summary

1. Predator conservation management requires detailed understanding of the ecological circumstances associated with predation, especially that on economically valuable prey. We examined the mechanisms behind Bonelli's eagle *Hieraaetus fasciatus* predation on prey of domestic origin, using dietary data from 22 pairs breeding in south-west Portugal (1992–2001) together with information on landscape composition and prey availability.

2. Numerically, 42·7% (37·7% in biomass) of eagle prey comprised domestic species, about 70% of which were rural pigeons *Columba livia* and the remainder were racing pigeons *Columba livia* and domestic fowl *Gallus gallus*. Rabbits *Oryctolagus cuniculus*, red-legged partridges *Alectoris rufa* and jays *Garrulus glandarius* were the most frequent wild prey (43·1%; 50·8% in biomass). This dietary pattern was remarkably stable over a decade, but within each year the intake of pigeons almost halved over the course of the breeding season.

3. Landscape composition significantly affected the dietary proportion of wild and domestic prey items. This was particularly evident in territories dominated by eucalyptus *Eucalyptus globulus* plantations, where there was reduced consumption of rural pigeons and partridges, an increased intake of minor avian prey items and greater diversity in the diet overall.

4. Bonelli's eagles showed type II functional responses while preying on the most important wild (rabbit) and domestic (rural pigeon) prey, although the former was much stronger. Eagle predation on rabbits declined with increasing abundance of pigeons, and vice versa, but there was no switching in the traditional sense, as selection between these two species was inversely frequency dependent.

5. *Synthesis and applications*. Predation by Bonelli's eagle on domestic pigeons results from a combination of high vulnerability of the pigeons to eagles and a shortage of key wild prey such as rabbits and partridges, especially during the early breeding season. Given the relatively low economic value of rural pigeons and their importance in the diet of Bonelli's eagles, they could probably be used as a conservation tool to enhance food resources in breeding territories and to deflect predation from more valuable prey such as partridges and racing pigeons.

Key-words: conservation, functional response, *Hieraaetus fasciatus*, Mediterranean, partridge, pigeon, predation, rabbit, switching

Journal of Applied Ecology (2006) **43**, 1075–1086
doi: 10.1111/j.1365-2664.2006.01213.x

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Introduction

Predators feeding on poultry, livestock and game have long been killed by humans, often resulting in population declines and even extermination (Thirgood *et al.* 2000; Treves & Karanth 2003; Graham, Beckerman & Thirgood 2005). Recently, many of these predators have recovered as a consequence of increased tolerance and protection (Treves & Karanth 2003; Valkama *et al.* 2005). However, population recovery involves major challenges when protected predators expanding into human-dominated landscapes come into contact with species that are hunted, harvested or farmed for human consumption or recreation (Meriggi & Lovari 1996; Landa *et al.* 1999; Stahl *et al.* 2001). Such food resources may actually contribute to supporting increased predator densities (Kenward, Marcström & Karlbom 1981; Selås 1997; Redpath & Thirgood 1999), thereby creating dilemmas between the protection of predators and the mitigation of predation damage (Thirgood *et al.* 2000; Woodroffe *et al.* 2005). These management conflicts need to be addressed if population recoveries of protected predators are to be sustained (Graham, Beckerman & Thirgood 2005).

Birds of prey are often perceived as damaging by hunters, gamekeepers, farmers and pigeon fanciers (Shawyer, Clarke & Dixon 2000; Thirgood *et al.* 2000; Valkama *et al.* 2005). Conflicts involving predation on economically valuable game species have been thoroughly investigated, showing that persecution remains a threat for many predatory species (Villafuerte, Viñuela & Blanco 1998; Thirgood *et al.* 2000; Carrete *et al.* 2002). Much less is known of cases involving predation on domestic prey, although they may also be a significant source of conflict (Stahl, Ruette & Gros 2002; Avery & Cummings 2004). For instance, domestic pigeons *Columba livia* L. are often consumed by recovering species such as peregrine falcons *Falco peregrinus* L., resulting in complaints that raptor attacks are becoming unacceptably high (Shawyer, Clarke & Dixon 2000; Henderson, Parrott & Moore 2004).

High predation rates on domestic prey can usually be explained by three main predatory mechanisms, each of which has different implications for the mitigation of predator damages. An alternative prey hypothesis (APH; *sensu* Angelstam, Lindström & Widén 1984) is assumed, often implicitly, when predators switch to domestic prey as wild prey becomes scarce (Meriggi & Lovari 1996). In these circumstances, the restocking of wild prey may alleviate predation on domestic species. This solution may be ineffective if predators take domestic prey primarily because of their high vulnerability, irrespective of wild prey abundance. The vulnerable prey hypothesis (VPH) calls for management strategies reducing the exposure of domestic prey, using, for instance, deterrents or fencing (Shawyer, Clarke & Dixon 2000; Kenward *et al.* 2001; Treves & Karanth 2003). Finally, the problem animal hypothesis (PAH) is proposed when predation involves a behavioural feeding specialization by a few individuals, in which case the removal of problematic animals is often advocated (Stahl *et al.* 2001; Treves & Karanth 2003).

Testing these hypotheses requires information on the responses of predators to variation in the densities and relative frequencies of wild and domestic prey. Under the APH, predation on domestic prey may be low until the abundance of wild prey declines below a minimum threshold. Switching (*sensu* Murdoch 1969) should then occur, implying a frequency-dependent response to fluctuating prey densities, with the predator eating disproportionately more of the most abundant prey item. In this case, predators are usually expected to show a Holling type III (sigmoid) functional response to fluctuations in prey density (Akre & Johnson 1979). In contrast, under the VPH the functional response to fluctuations in domestic prey density would show a type II (convex) curve, characterized by a rapid increase in the number of prey taken per predator at low prey densities, indicating the presence of highly vulnerable or rewarding prey. The PAH requires that predation on domestic prey occurs only locally and largely independently of prey abundance. In practice, these predicted responses to fluctuations in prey abundance are likely to be more complex, as predators often feed on several different wild and domestic prey types, and their abundance and profitability may change across habitats and over time. Assessing the mechanisms involved in a particular instance of predation on a domestic prey thus requires a good understanding of the predator–prey interactions and the ecological context in which they take place (Graham, Beckerman & Thirgood 2005).

Factors influencing predation on domestic prey were examined by analysing Bonelli's eagle *Hieraaetus fasciatus* Vieillot predation patterns in south-west Portugal (1992–2001). Bonelli's eagle is an endangered species showing a marked decline in Mediterranean Europe since the early 1980s (Real & Mañosa 1997). Bonelli's eagles feed on a range of vertebrates, with rabbits *Oryctolagus cuniculus* L., red-legged partridges *Alectoris rufa* L. and pigeons *Columba* spp. often being the main prey (Real 1991; Valkama *et al.* 2005). As a consequence, persecution by hunters and pigeon fanciers represents a major threat for some Bonelli's eagle populations (Real *et al.* 2001; Carrete *et al.* 2002). In the study area, rabbits and partridges are scarce, while rural pigeons are relatively abundant in small villages and isolated farmhouses. Preliminary evidence showed that eagles consume pigeons very frequently (Palma *et al.* 2005) and there was the possibility that the pigeons acted as an alternative resource to wild prey. This creates a management dilemma (Petty, Lurz & Rushton 2003) as pigeons provide an important food resource while also representing a potential cause of conflict with pigeon fanciers. A closer examination of the factors affecting predation on domestic pigeons used information on variation across breeding territories in diet composition, land uses and the abundance of wild and domestic prey. The aim was to assess: (i) the extent of predation on domestic prey; (ii) variation in diet across years and over the breeding season; (iii) the influence of habitat on predation patterns; (iv) the functional responses to variation in wild and domestic prey abundance; and (v) the eventual occurrence of switching between wild and domestic prey. This information was then used to infer the predatory mechanisms involved in this predator–prey system, and to suggest management solutions that might contribute to the conservation of these eagles while mitigating potential conflict with humans in south-west Portugal and elsewhere.

Methods

STUDY AREA

This study focused on a dense breeding nucleus of 25 Bonelli's eagle pairs occupying about 3000 km^2 in the uplands of south-west Portugal (Fig. 1). This is an area of low mountains and rolling hills mostly under 650 m a.s.l. in altitude. The climate is Mediterranean, with a dry and hot summer season with little precipitation. The land is predominantly covered by open to dense cork oak *Quercus suber* L. woodland and extensive scrubland often dominated by the gum cistus *Cistus ladanifer* L. In the western half of the area, eucalyptus *Eucalyptus globulus* Labill. plantations for paper pulp production cover up to 50% of the land. Human settlement is currently low and local; most of the former scattered dwellings are abandoned and agriculture is residual.

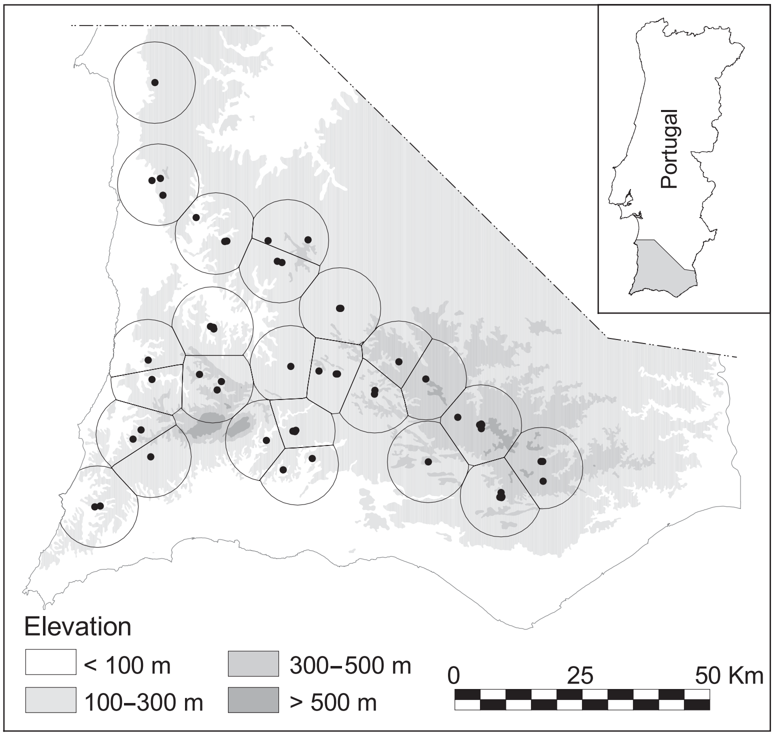

Fig. 1. Location in south-west Portugal of alternative nests of Bonelli's eagle pairs (1992–2001) and the approximate territory boundaries estimated using Dirichlet tessellation.

EAGLE DIET

The diets of 22 pairs of eagles surveyed in 1992–2001 were described from prey identified in remains ($n = 747$) and regurgitated pellets ($n = 1039$) collected during the breeding season (> 85% in March–May) at active nests and surrounding perches, together with uncollected fresh prey observed at nests ($n = 199$). Information was obtained for 9·6 ± 4·8 (2–17) pairs per year, with variation as a result of population growth, breeding failures, unknown nest locations and reduced field effort in 1992–94 and 2001. Nests were checked 5·5 ± 2·2 (1–11) times year^{-1}. The species, age and gender of each individual prey were estimated whenever possible using published identification keys and a reference collection. Metallic rings provided a minimum estimate of racing pigeons in the diet, as rural pigeons were seldom ringed within the study area.

To reduce biases normally associated with dietary studies (Real 1996; Marchesi, Pedrini & Sergio 2002) and to increase sample sizes, data from pellets, remains and direct observations were combined (Ontiveros & Pleguezuelos 2000; Penteriani, Gallardo & Roche 2002; Ontiveros, Pleguezuelos & Caro 2005). For each pair and sampling occasion, the number of individuals of a given prey species represented in the sample was taken only from the data source yielding the largest estimate, thereby avoiding duplications among data sources. In the case of two siblings, the estimated number of prey items represented in pellets was halved, to avoid duplication of the same prey in two pellets (Real 1996). The biomass of each prey species was estimated by multiplying the number of individuals by its estimated mean weight, using data from the study area, bibliographic sources (Real 1987; Cardona & Esteban 2002) and information from racing pigeon fanciers (D. Santos, personal communication). The number of individuals of each prey species taken by an average eagle pair during the breeding season was estimated following Petty, Lurz & Rushton (2003), considering one nestling per pair, an overall daily consumption of 750 g food per pair plus one nestling (Real 1987), a mean incubation period of 39 days (Arroyo, Ferreiro & Garza 1995) and a mean nestling period of 63 days (Real & Mañosa 1998).

HABITAT AND PREY AVAILABILITY

Variation in diet, landscape composition and prey availability among territories was used to estimate the factors influencing eagle predation patterns. Given the lack of detailed information on the actual breeding territories, habitat and prey data were quantified within approximate territory boundaries estimated using Dirichlet tessellation (Doncaster & Woodroffe 1993). For each pair, the mean geographical location of nests was the Dirichlet centre, and boundaries were constrained to be at a maximum of 8 km from the centre (Fig. 1). These criteria were based on home range data from other studies (Mure 2003) and on nearly 800 haphazard observations at > 500 m from active nests through the 10-year study period, suggesting that each Bonelli's eagle pair maintained an exclusive foraging territory, which only rarely extended beyond 8 km from the active nest (L. Palma, unpublished data). Although this was a relatively crude approach, each range boundary encompassed > 90% of observations of individually recognized elements of the respective breeding pair.

Landscape composition was quantified using digital land cover cartography from 1995 (www.dgrf.min-agricultura.pt/ifn/mapas.htm, accessed 1 April 2005), considering six categories: (i) native Mediterranean forests dominated by cork oaks and strawberry trees *Arbutus unedo* L., (ii) eucalyptus plantations, (iii) pine (mostly *Pinus pinaster* Ait.) plantations, (iv) scrubland, (v) farmland and (vi) urban areas (Table 1). Landscape diversity was computed using the Shannon index.

Rabbit abundance was estimated in September–October 1997 from latrine counts in 296 250-m line transects walked along dirt roads and firebreaks crossing eagle territories and neighbouring areas (Palma, Beja & Rodrigues 1999). Latrine counts were used (Iborra & Lumaret 1997; Beja, Palma & Pais 2007) as direct observation of rabbits was not feasible because of dense vegetation cover. One transect was walked per 1-km^2 grid square, with five random squares surveyed in each of 65 5-km^2 grid squares distributed in a checkerboard pattern. To increase comparability among sites, each transect was located in the most favourable shrubland habitats available within each square (Beja, Palma & Pais 2007). The mean latrine count per breeding territory was used to index relative rabbit abundance. Only 14 territories for which there were at least 10 line transects (15·2 ± 4·2, 10–23) were used in further analyses because information could not be obtained for the entire study area. A comprehensive rural pigeon survey was carried out from September 1994 to December 1995, encompassing the approximate breeding territories of 18 eagle pairs known at the time. Pigeon lofts were mapped by visiting all small villages and isolated farmhouses, where the number of pigeons was estimated for each loft either through enquiries or direct counts. Point estimates of pigeon abundance were interpolated into a 250-m raster grid using kernel density analysis with a 5-km searching radius, and these grid values were then used to estimate average rural pigeon densities within each breeding territory. The abundance of racing pigeons crossing the study area was estimated from information provided by homing pigeon fanciers on the periods and frequency of races and training flights, and the approximate numbers of pigeons involved, their origin and geographical distribution (D. Santos, personal communication).

The prey data only referred to a restricted period, whereas the dietary information spanned 10 years. This was recognized as a shortcoming in the data but was unavoidable. It was not possible to repeat the surveys and a long diet study would be needed to characterize a large number of pairs. This shortcoming was unlikely to affect the results of this study seriously as unsystematic observations suggested that regional trends in prey abundance remained essentially the same. In particular, there were no marked changes in land cover and human activities that might have changed the distribution of rabbit and pigeon abundances extensively. Also, no rabbit crashes as a result of viral haemorrhagic disease similar to that reported elsewhere (Fa, Sharples & Bell 1999) or severe myxomatosis outbreaks were observed. Moreover, surveys were carried out approximately half-way through the study period, when most dietary information was collected.

DATA ANALYSIS

Preliminary to data analysis, the angular transformation (arcsine $\sqrt{p}$) was used to reduce the influence of extreme values and to overcome problems associated with the unity sum constraint of proportional data (Zar 1996). Multicollinearity among habitat variables was investigated using principal component analysis (PCA), thereby describing the main gradients in land-use variability (Legendre & Legendre 1998). A varimax rotation was performed on the principal components with eigenvalues > 1 to enhance their interpretability (Legendre & Legendre 1998). Seasonal and annual trends in dietary proportions were examined using linear regression, including quadratic terms to check for non-linear trends (Legendre & Legendre 1998). Relationships between spatial variation in prey consumption and land-use variables were examined likewise. In temporal analyses, data from all eagle pairs were pooled per time period, whereas in spatial analyses

Table 1. Average (± SD) landscape composition and summary results of a principal component analysis with varimax rotation describing the dominant land-use gradients across Bonelli's eagle territories in south-west Portugal (1992–2001). The proportion of explained variation and the scores of each variable are provided for the first two rotated axis (PC)

Land cover types	Mean (%)	± SD	Minimum–maximum	PC1 (38·1%)	PC2 (30·1%)
Scrubland	40·1	14·2	16·8–62·5	0·84	
Mediterranean forests	26·3	12·6	6·5–46·4		–0·82
Farmland	16·7	15·7	2·7–55·6	–0·92	
Eucalyptus plantations	14·4	12·2	0·0–43·8		0·79
Pine plantations	2·1	2·7	0·0–7·5		0·63
Urban	0·4	0·4	0·0–1·4	–0·78	

data were pooled per breeding pair. Only cases for which there were at least 25 prey items were used, because dietary proportions stabilize around this threshold (Ontiveros, Pleguezuelos & Caro 2005).

Functional responses were investigated by modelling the relationships between the average number of a prey type eaten per breeding pair and the abundance of either pigeons or rabbits. In each case, functions representing linear (type I), convex (type II) and sigmoidal (type III) curves were fitted to the data (Patterson, Benjamin & Messier 1998) and the best model was selected based on second-order Aikaike information criterion (AICc) because of small sample sizes (Burnham & Anderson 1998). Type II and III responses were modelled using the generalized Michaelis–Menton function (Real 1977). The effects of pigeons and rabbits on the consumption of other prey were investigated using linear regression and partial correlation analysis (Zar 1996).

Prey switching was examined by relating the ratio of pigeons to rabbits eaten (e_1/e_2) to their availability ratio (A_1/A_2) using the equation $e_1/e_2 = (V \times A_1/A_2)^b$ (Greenwood & Elton 1979). The parameter V is a measure of frequency-independent selection, whereas b is a measure of frequency dependence: $b > 1$ provides evidence for switching, with the predator eating disproportionately more of the most abundant prey, $b < 1$ reflects disproportionate predation on the less common prey and $b = 1$ indicates frequency-independent selection. As A_1 and A_2 were expressed in distinct units and there was no absolute estimate of rabbit densities, this analysis should be regarded as approximate and the parameter V should not be interpreted. Nevertheless, the shape of the curve relating e_1/e_2 to A_1/A_2 is meaningful, providing the best approach to evaluating the presence of switching between the two prey species.

Results

HABITAT AND PREY ABUNDANCE

Bonelli's eagle territories were dominated by scrubland and oak forests, together accounting for almost 70% of landscape composition (Table 1). Eucalyptus plantations and farmland were also important, whereas land cover by pine plantations and urban areas was negligible. The PCA with varimax rotation revealed a dominant gradient largely reflecting agricultural abandonment that accounted for 38·1% of land cover variability, ranging from territories with a significant proportion of farmland and urban areas to those dominated by scrubland. The second rotated PCA axis (30·8%) represented the dominant forest types, contrasting territories dominated by Mediterranean oak forests with those that were predominantly eucalyptus plantations.

There was strong variation among territories in the abundance of rabbits and pigeons (Fig. 2). Rabbits occurred in only 27% of the 296 transects surveyed, and they tended to be fairly scarce where they occurred (4·2 latrines 250 m^{-1} (4·3, 1–24, $n = 80$). This resulted in a 50-fold variation in rabbit abundance among eagle territories, with densities up to 1–5 latrines 250 m^{-1} along the southern and western edges, whereas rabbits were scarce elsewhere (< 0·5 latrines 250 m^{-1}). A total of 436 pigeon lofts was identified across the region, with on average 25·5 ± 37·7 (2–550) pigeons per loft. Pigeons were more evenly distributed than rabbits, although there was about a 10-fold variation among territories, from about 0·47 to 4·71 pigeons km^{-2} (2·4 ± 1·2 pigeons km^{-2}). There was no correlation between rabbit and pigeon abundance ($r = -0{\cdot}37$, $P = 0{\cdot}262$)

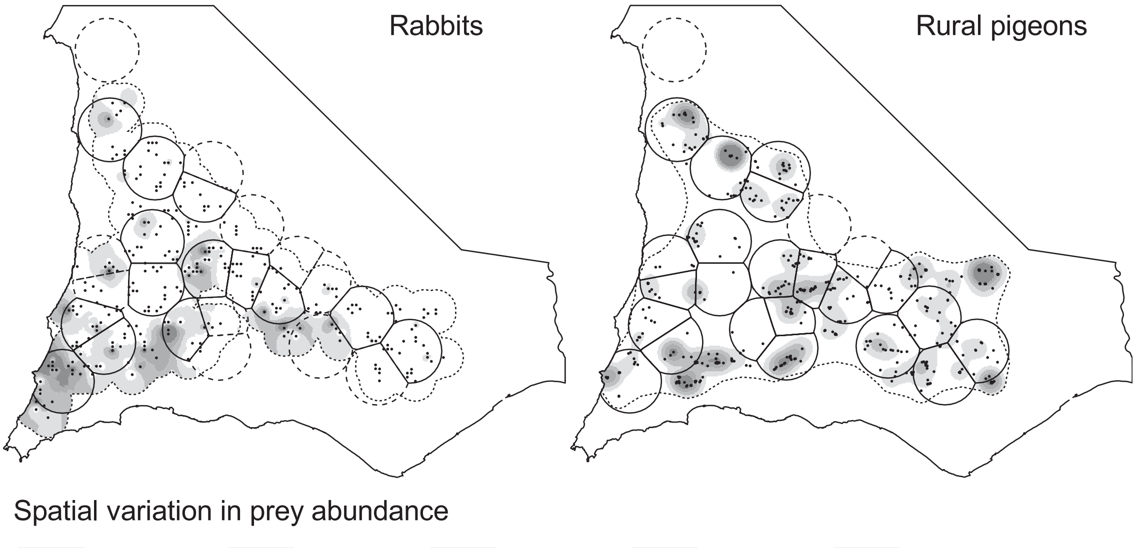

Fig. 2. Interpolated maps of spatial variation in prey abundances across Bonelli's eagles territories in south-west Portugal. Values are SD from the mean, to allow comparisons between maps. Dots are rabbit sampling sites and pigeon lofts. Dotted lines define the interpolation limits. Hatched lines indicate territories with no information on prey abundance.

Table 2. Diet of Bonelli's eagles and prey consumption per pair during the breeding season in south-west Portugal (1992–2001)

Prey categories	*n*	% Numbers	% Biomass	Consumption per pair, mean ± SD (minimum–maximum)
Domestic birds				
Rural pigeon	451	30·1	26·0	48·0 ± 14·6 (17–77)
Racing pigeon	136	9·1	7·2	14·7 ± 7·7 (0–37)
Domestic fowl	52	3·5	4·5	5·4 ± 8·6 (0–37)
Wild birds				
Red-legged partridge	258	17·2	14·9	28·7 ± 11·9 (6–59)
Jay	112	7·5	2·7	12·0 ± 9·2 (0–28)
Other birds	198	13·4	11·4	24·2 ± 18·3 (2–68)
Mammals				
Rabbit	276	18·4	33·2	30·4 ± 11·8 (16–58)
Hare	3	0·2	< 0·1	0·2 ± 0·6 (0–2)
Reptiles				
Ocellated lizard	11	0·7	< 0·1	1·1 ± 1·9 (0–6)

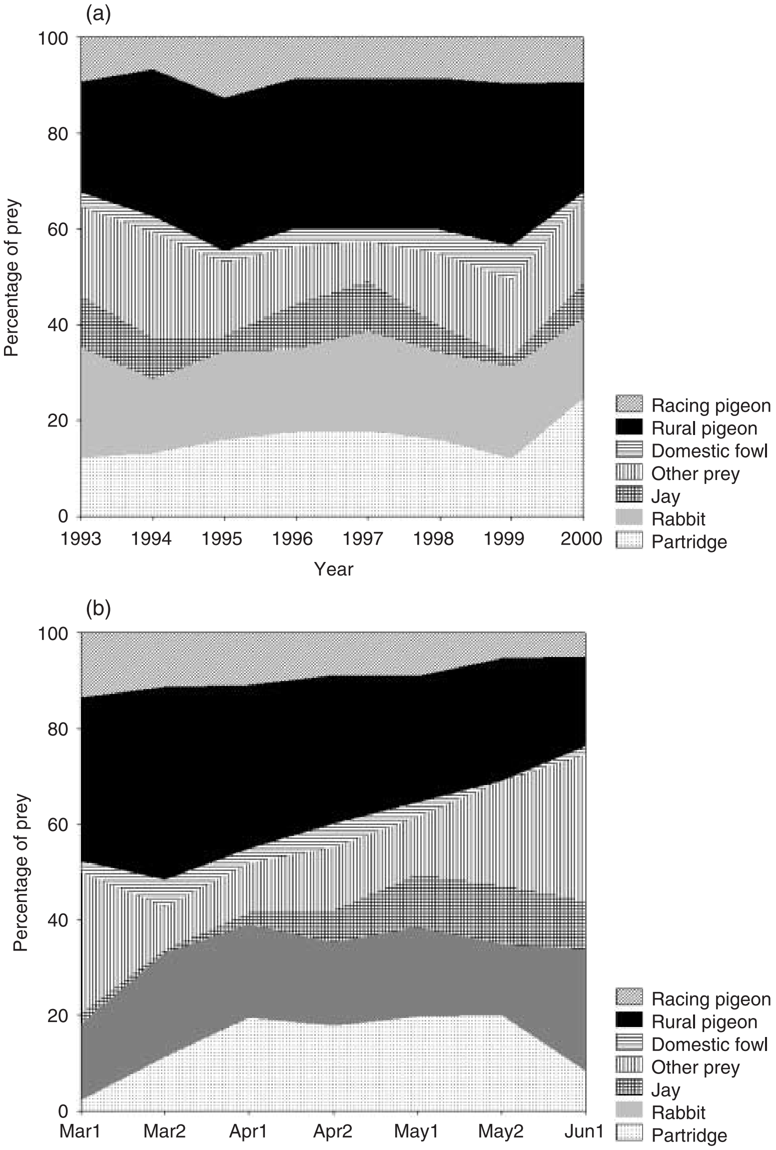

Fig. 3. Variation of Bonelli's eagle diet in south-west Portugal (a) over the years 1993–2000 and (b) across the breeding season 1 March–15 June.

across the 11 territories for which there was information on both prey.

DIET COMPOSITION

Dietary analysis yielded a minimum estimate of 1497 individuals of 32 prey species (see Table S1 in the supplementary material). Numerically, prey of domestic origin comprised 42·7% of the diet (37·7% in biomass), consumed in every year and by all eagle pairs (Table 2). About 70% of these prey items were rural pigeons, but racing pigeons and domestic fowl were also consumed frequently. Considering the estimates of prey intake (Table 2), prey densities and territory sizes, the average proportion of the rural pigeons available per eagle pair that were taken through the breeding season was 17·8 ± 12·6% (5·7–57·8%). Rabbits, red-legged partridges and jays *Garrulus glandarius* L. were the most frequent wild prey, numerically representing 43·1% (50·8% biomass) of the eagle diet (Table 2).

Of the 114 racing pigeons aged through metallic rings, 31·6% were 1 year old or under, 51·8% 2–3 years old and 16·7% more than 4 years old. The remains of freshly killed domestic fowl observed at nests were all from juvenile birds ($n = 12$) whereas 86·2% of the rabbits were adults ($n = 29$). A majority of partridges killed by eagles were males (65·7%; $n = 35$).

TEMPORAL VARIATIONS IN DIET COMPOSITION

Variation in diet between years was analysed for 1993–2000, i.e. when annual samples were sufficiently large (prey per year 185·4 ± 93·1, 65–352). The relative importance of the most frequently consumed items remained largely the same over the study period (Kendall's coefficient of concordance, $W = 0{\cdot}86$, $P < 0{\cdot}001$), with no evidence for consistent trends in the consumption of any prey species (Pearson correlations $-0{\cdot}41 < r < 0{\cdot}57$, $P > 0{\cdot}14$; Fig. 3a). The numeric proportion of rural pigeons was fairly stable (23·1–33·8%), as was that of rabbits (15·4–23·1%) and racing pigeons (6·6–12·6%). The largest variation was recorded for relatively less important prey such as jays (2·0–10·8%) and domestic fowl (1·2–7·1%).

Prey were assigned to 2-week periods from 1 March to 15 June (prey per 2 weeks 210·8 ± 177·6, 44–504) to investigate variations during the breeding season (Fig. 3b). Although there was concordance in diet composition over time (Kendall's $W = 0{\cdot}73$, $P = 0{\cdot}001$), most prey species showed distinctive temporal trends. There were marked declines in the dietary importance of rural ($r = -0{\cdot}91$, $P < 0{\cdot}01$) and racing pigeons ($r = -0{\cdot}96$, $P < 0{\cdot}001$), which halved their overall numeric contribution from a maximum of about 50% in March to a minimum of 23·7% in June. This was largely compensated for by increases in jays ($r = 0{\cdot}91$, $P < 0{\cdot}05$) and a wide group of birds represented in 'other' prey ($r = 0{\cdot}92$, $P < 0{\cdot}01$), from a minimum in the second half

Table 3. Explained variance (R^2), significance levels and directions of association (–, negative; +, positive; ∩, unimodal) for significant relationships between Bonelli's eagle diet and land uses across 20 breeding territories in south-west Portugal

Prey categories	Oak forest	Eucalyptus forest	Scrubland	Farmland	Urban
Domestic prey					
Rural pigeon		–^{a}0·40**			
Racing pigeon	∩0·41**				
Wild prey					
Rabbit					∩0·45**
Partridge	+^{a}0·38**	–^{a}0·33**	∩0·35*	–0·22*	–^{a}0·22*
Jay				∩0·45**	
Other birds		+^{a}0·61***			
Diversity		+^{a}0·33**		∩0·37*	

*$P < 0{\cdot}05$; **$P < 0{\cdot}01$; ***$P < 0{\cdot}001$.
a, Quadratic term.

of March (0·8% and 8·9%) up to a maximum in June (10·2% and 30·5%). Patterns for other prey species were more complex. A unimodal pattern was found for partridges (multiple $r = 0{\cdot}96$, $P = 0{\cdot}01$) that increased from about 2% in early March up to a maximum at around 20% in April–May and declined thereafter. The consumption of rabbits did not show any significant trend through the breeding season.

LANDSCAPE EFFECTS ON DIET COMPOSITION

Land use explained 22–61% of variation in the dietary proportions of both domestic and wild prey (Table 3). The consumption of rural pigeons declined steadily with increasing eucalyptus cover, while racing pigeons showed unimodal responses to Mediterranean oak forests. The intake of domestic fowl was apparently not affected by landscape composition. Partridge consumption increased with oak forests, declined with eucalyptus cover, farmland and urban areas, and responded unimodally to scrubland. The other prey category and diet diversity increased markedly with eucalyptus plantation cover. Diet diversity and the intake of jays showed unimodal responses to farmland. The unimodal response to urban areas was the only landscape effect recorded for rabbits.

FUNCTIONAL RESPONSES AND PREY SWITCHING

A type II functional curve significantly explained half the variation in rabbit consumption (Fig. 4a), while there was no support for alternative response types (ΔAICc > 2·5). The abundance of rabbits also affected the consumption of other prey, with less rural and racing pigeons eaten in territories with more rabbits (Fig. 5a). The inverse relationship between rabbit abundances and partridge consumption was marginally significant ($P < 0{\cdot}051$). When controlling for the effects of rural pigeon densities using partial correlations, rabbit abundances still showed significant inverse relationships with rural ($r = -0{\cdot}63$, $P = 0{\cdot}050$) and racing pigeons ($r = -0{\cdot}75$, $P = 0{\cdot}013$) and approached significance with partridges ($r = -0{\cdot}57$, $P = 0{\cdot}085$). Although the functional response curve accounted for < 25% in the consumption of rural pigeons (Fig. 4b), the type II model still performed better than the two alternative models (ΔAICc > 2·5). The number of rabbits eaten was inversely related to pigeon densities (Fig. 5b), even after controlling for rabbit abundances using partial correlations ($r = -0{\cdot}81$, $P = 0{\cdot}004$). Shannon diet diversity computed at the species level was unrelated to the abundance of both rabbits ($r = 0{\cdot}33$, $P = 0{\cdot}317$) and pigeons ($r = -0{\cdot}27$, $P = 0{\cdot}280$).

The ratio of pigeons to rabbits consumed per eagle pair increased significantly with the ratio of pigeon to rabbit abundances (Fig. 6). The frequency-dependent parameter b was significantly smaller than unity (95% confidence interval 0·09–0·42), suggesting that prey selection was inversely frequency dependent. This indicated that at low pigeon to rabbit ratios eagles captured more pigeons than might be expected, whereas more rabbits than expected were taken in breeding territories with high pigeon to rabbit ratios. There was therefore no evidence of Bonelli's eagles switching to pigeons when the abundance of rabbits declined in relation to that of pigeons. Instead, the less abundant prey always tended to be consumed more than expected.

Discussion

THE DIETARY ROLE OF DOMESTIC VS. WILD PREY

Domestic prey played a major role in the diet of Bonelli's eagles in south-west Portugal. This resulted primarily from the high consumption of rural pigeons, which was the single most important prey item, although racing pigeons and domestic fowl were also taken frequently. These were major dietary components for a very large proportion of this eagle population, and their importance was consistently high over a decade. Domestic prey was particularly important early in the breeding season when it accounted for more than half the prey eaten. Overall, however, wild prey such as rabbits, partridges and jays were a major dietary component of most eagle pairs. These species, together with several minor avian prey such as cattle egrets *Bubulcus ibis* L., thrushes *Turdus* spp. and woodpeckers *Picus viridis* L., became increasingly important later in the breeding season.

The importance of pigeons in south-west Portugal, particularly that of rural pigeons, was higher than that reported for most Bonelli's eagle populations (39·2% vs. 19·2 ± 12·8%, 1·5–39·0%, $n = 14$; Valkama *et al.* 2005). Although some studies found a relatively high consumption of woodpigeons *Columba palumbus* L. (Real 1991; Ontiveros, Pleguezuelos & Caro 2005), the overall dietary contribution of pigeons was still lower than that observed in south-west Portugal. Only in the diet of peregrine falcons and sometimes in goshawks

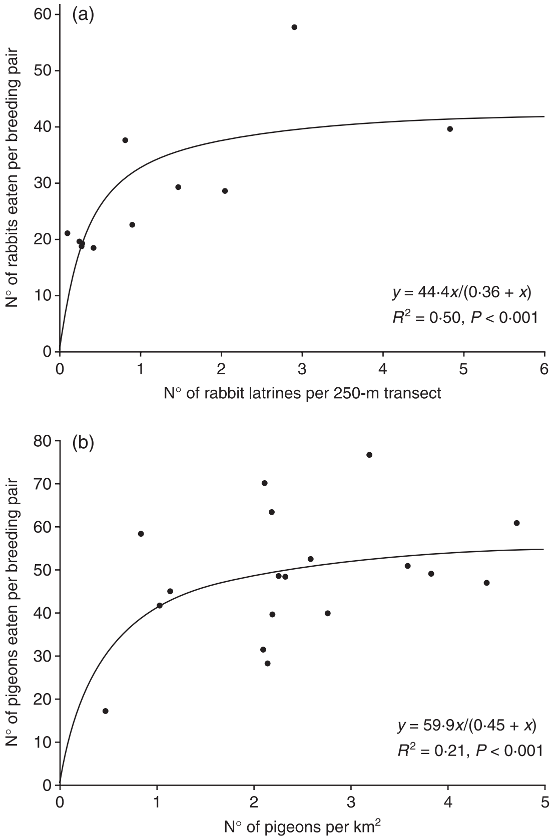

Fig. 4. Functional responses of Bonelli's eagles to variation among territories in the abundance of (a) rabbits ($n = 11$) and (b) rural domestic pigeons ($n = 18$).

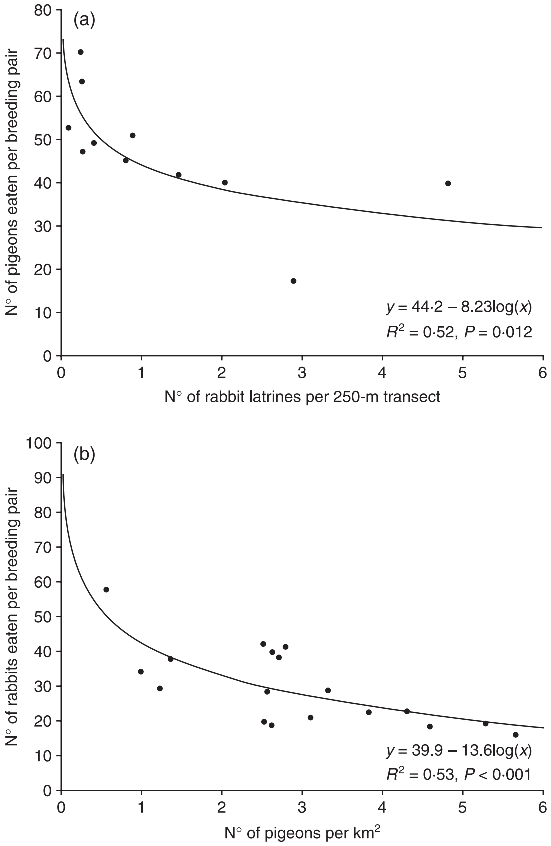

Fig. 5. Relationships between (a) rabbit abundance and rural pigeon intake, and (b) rural pigeon abundance and rabbit intake, across Bonelli's eagle territories in south-west Portugal.

Accipiter gentilis L. have similarly high frequencies of rural and racing pigeon predation been reported (Shawyer, Clarke & Dixon 2000; Henderson, Parrott & Moore 2004; Valkama *et al.* 2005). Bonelli's eagle is thus one of the raptors showing the highest potential conflict with pigeon fanciers.

When only wild prey is considered, the dietary patterns observed in south-west Portugal shared many similarities with those reported elsewhere for breeding Bonelli's eagles. These eagles frequently feed on rabbits (Real 1991; Valkama *et al.* 2005), although this prey is generally less important than in many other raptors in the Iberian Peninsula (Delibes & Hiraldo 1981). Conversely, the importance of avian prey tends to be higher than in other Mediterranean eagles, with most studies reporting that partridges, pigeons and corvids are the most frequently eaten birds (Real 1991; Ontiveros & Pleguezuelos 2000; Gil-Sánchez *et al.* 2004; Iezekiel, Bakaloudis & Vlachos 2004).

The dietary importance of wild prey increased through the breeding season, along with a decline in the intake of both rural and racing pigeons. The early peak consumption of pigeons and its subsequent replacement by other prey has been noted elsewhere (Real 1991; Iezekiel, Bakaloudis & Vlachos 2004), and it is often interpreted as resulting from temporal increases in the availability of highly profitable prey such as rabbits and juvenile birds. A similar reasoning may justify the patterns observed in south-west Portugal, where Bonelli's eagles lay their eggs in January–February (L. Palma, unpublished data), when most wild prey species normally taken by these eagles probably occur in lower numbers. These prey then become increasingly available through the breeding season, with different species peaking at different times according to their phenology and behaviour. For instance, the consumption of (mainly male) partridges peaked during the courting period in April–May, when the birds may be more vulnerable to predators. Increases in the consumption of jays and other birds through the season may reflect the progressive emergence of easily captured fledglings. The highest rabbit densities also tended to occur in late spring, but their numbers in the study area were generally low and their consumption was fairly stable through the eagle's breeding season.

WHY DO EAGLES TAKE DOMESTIC PREY?

In this study, none of the hypotheses usually invoked to explain the incidence of high predation rates on domestic prey could be fully supported. Nevertheless, the hypothesis of a feeding specialization by a few individuals (PAH) could be clearly dismissed, as predation on domestic prey was widespread and was not a feature restricted to a few eagle pairs. In contrast, the two other hypotheses gained some partial support, suggesting

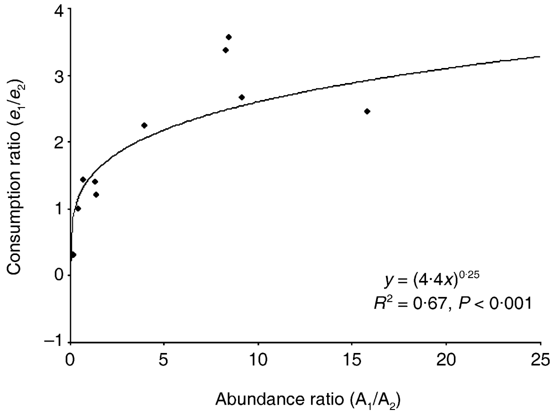

Fig. 6. Frequency-dependent selection curve for Bonelli's eagles preying on rural pigeons (1) and rabbits (2), relating the consumption (e_1/e_2) and availability (A_1/A_2) ratios.

that the heavy predation by Bonelli's eagles on domestic prey recorded in south-west Portugal might result from both a shortage of wild prey, in accordance with APH, and a positive selection of domestic prey, following the VPH.

Compared with other areas, there was a shortage in south-west Portugal of those wild prey species commonly favoured by the Bonelli's eagle, such as rabbits and partridges (Gil-Sánchez 1998). Rabbits were particularly scarce, as they were largely absent from most of the region and their abundance was reduced where they occurred (Palma, Beja & Rodrigues 1999; Beja, Palma & Pais 2007; this study). Unsystematic observations suggested that partridges also occurred at low densities across the region (L. Palma, unpublished data). In contrast, rural pigeons were very abundant through the year. Information from homing pigeon associations suggested that racing pigeons were also very abundant, with at least 10–15 thousand pigeons crossing the study area each week in January–April. Strayed or lost pigeons could thus be commonly found within the study area (L. Palma, unpublished data), presumably providing easy and rewarding prey for raptors (Shawyer, Clarke & Dixon 2000; Henderson, Parrott & Moore 2004).

Breeding territories with the lowest abundance of both wild and domestic prey were probably those with high eucalyptus cover, where there was reduced consumption of rural pigeons and partridges, along with increases in both the intake of minor prey items and diet diversity. Considering the predictions of optimal foraging theory (Stephens & Krebs 1986) and the findings of empirical studies on other birds of prey (Lindén & Wikman 1983; Marchesi, Sergio & Pedrini 2002), these dietary responses are consistent with a shift to low-ranked prey and an associated widening of niche breadth in a situation where there is reduced availability of the most profitable prey. Previous studies and personal observations suggest that both rabbits and partridges avoid eucalyptus landscapes (Palma, Beja & Rodrigues 1999; L. Palma, unpublished data), whereas rural pigeons were scarce because of the effects of marked rural depopulation. A less obvious widening of niche breadth may have occurred in territories with intermediate farmland cover, where diet diversity increased along with a high consumption of jays. This pattern might also be explained by the lower abundance of preferred wild and domestic prey. Indeed, partridges were consumed most frequently in breeding territories dominated by native oak forest and intermediate scrubland cover, where they appeared most abundant (L. Palma, unpublished data), and less with increasing cover by farmland and urban areas. In contrast, rural pigeons were presumably most abundant in farmland, although this was not evident in terms of dietary responses. Rabbits were not responsive to this gradient, probably because land-use influences were confounded by climatic gradients affecting rabbit abundances across the region (L. Palma, P. Beja & M. Pais, unpublished data).

The dietary responses of eagles to variation in the abundance of the most important prey of wild (rabbit) and domestic (rural pigeon) origin also suggested that domestic prey could compensate for the shortage of wild prey, although there was no switching in the traditional sense (Murdoch 1969). Rabbits were clearly the primary prey item, with their consumption following a pronounced type II response curve and the highest intake of both rural and racing pigeons occurring at the lowest rabbit densities. However, eagles also showed a type II functional response to rural pigeons, although much weaker than in the case of rabbits and not the type III sigmoid curve that might be expected if there was switching (Akre & Johnson 1979; Patterson, Benjamin & Messier 1998). Furthermore, there was a negative relationship between the consumption of rabbits and the abundance of rural pigeons, as well as evidence that selection between these two prey was inversely frequency dependent, with pigeons consumed more often than expected at high rabbit densities and the same occurring for rabbits at high pigeon densities. This may tentatively be related to the critical dietary role of pigeons early in the breeding season, when there seems to be low abundance of most wild prey, including rabbits. In these circumstances, many rural pigeons may be taken in areas where the abundance of rabbits will increase following their spring reproductive peak. Later in the season, rabbits may be taken more often than expected, as they could be more profitable to Bonelli's eagles than pigeons. Testing this hypothesis would require more detailed information on the seasonal variation in the abundance of wild and domestic prey and their relative profitability to the predator.

Taken together, our results suggest that rural pigeons should be regarded as complementary prey items to rabbits and partridges, rather than an alternative prey that is consumed only where there is a shortage of these primary wild prey. Conversely, racing pigeons were probably taken opportunistically because of their high vulnerability. Where the most profitable wild and domestic prey were scarce, the eagles widened their niche breadth and consumed a range of relatively minor avian prey. This is therefore a complex multispecies system that supports the view that predator conflicts cannot be addressed as a single pairwise predator–prey interaction (Graham, Beckerman & Thirgood 2005).

MANAGEMENT IMPLICATIONS

Conflicts with pigeon fanciers are considered a major threat for the conservation of some Bonelli's eagle populations (Real *et al.* 2001; Carrete *et al.* 2002). The situation is probably not yet a serious one in south-west Portugal, despite eagles generally showing high predation rates on domestic pigeons. Enquiries to rural pigeon breeders ($n = 326$) revealed that they generally attributed a low value to pigeons, regarding them simply as pets and home-grown food (93·3%), with only 6·7% of those censused making use of pigeons for trade, racing or recreational shooting (L. Palma, unpublished data). Most rural pigeon breeders disregarded eagles as a significant threat as they were generally unaware of their presence, except possibly where predation was concentrated at particular lofts. In the case of racing pigeons, Bonelli's eagles killed a very small proportion of the huge numbers released regularly, indicating that they do not represent a real threat to pigeon races. Overall, there is therefore a relatively benign situation, justifying the lack of active persecution that is apparent elsewhere (Real *et al.* 2001). However, given the dietary importance of domestic pigeons for these eagles, management options reducing the potential conflicts with pigeon breeders should not be disregarded, as it is possible that less-tolerant attitudes may develop in the near future.

Rearing pigeons in specially designed lofts might be an effective means of reducing potential conflicts with pigeon breeders while maintaining an adequate food supply for Bonelli's eagles. Lofts could be established by conservation organizations in territories with depleted food resources and in areas where persecution might be a problem. Pigeons provided at these 'conservation' lofts might act as diversionary food to reduce predatory pressure upon both game and racing pigeons (Carrete *et al.* 2002), similar to the lofts with low-value pigeons that have been used elsewhere to deflect predation by peregrine falcons (Shawyer, Clarke & Dixon 2000). In south-west Portugal, this management approach could help to offset a possible medium-term decline in traditional pigeon rearing associated with rural abandonment, with negative effects on the eagle's food resources. It might also help to reduce predation on valuable game species, such as partridges, given the development of small game hunting in this region. Providing health-screened pigeons could also reduce the infection of eagles by the protozoan *Trichomonas gallinae* Rivolta, which is a significant cause of nestling mortality in this and other Bonelli's eagle populations (Höfle *et al.* 2000; Real, Mañosa & Muñoz 2000). These applications of rural pigeons as a conservation tool require testing in south-west Portugal and elsewhere, to evaluate their potential benefit to eagle breeding success, disease prevalence and conflicts with people.

Acknowledgements

We are grateful to José Raiado, Carlos Noivo, Pedro Portela, Rita Alcazar, Francisco Faria, Paulo Cabrita and João Ferreira for field assistance, Rita Inácio for collaboration in dietary analysis, Daniel Santos for information on pigeon racing, and Fernando Grade for the census of rural pigeons. We thank Simon Thirgood and two anonymous referees for critical review of the manuscript. This study was supported by the Portuguese Institute for Nature Conservation and the Portuguese Science Foundation (PRAXIS/BIA/132/96).

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Received 17 November 2005; final copy received 24 May 2006
Editor: Simon Thirgood

Supplementary material

The following supplementary material is available as part of the online article (full text) from http://w.w.w.blackwell-synergy.com.

Table S1. Detailed results of Bonelli's eagle diet analysis.

Table S1. Detailed results of diet analysis of Bonelli's eagles in the uplands of SW Portugal in 1992-2001.

Prey item		**%Years (n=10)**	**%Pairs (n=22)**	**N**	**%N (n=1497)**	**Weight**	**%B**
Rural pigeon	*Columba livia*	100.0	100.0	451	30.1	400.0	26.0
Rabbit	*Oryctolagus cuniculus*	90.0	100.0	276	18.4	835.0	33.2
Red-legged partridge	*Alectoris rufa*	90.0	95.5	258	17.2	400.0	14.9
Racing pigeon	*Columba livia*	90.0	90.9	136	9.1	365.0	7.2
Jay	*Garrulus glandarius*	100.0	81.8	112	7.5	170.0	2.7
Domestic fowl (juvenile)	*Gallus gallus*	80.0	63.6	52	3.5	600.0	4.5
Cattle egret	*Bubulcus ibis*	80.0	40.9	35	2.3	350.0	1.8
Blackbird	*Turdus merula*	70.0	50.0	34	2.3	100.0	0.5
Yellow-legged gull	*Larus cachinnans*	90.0	50.0	26	1.7	1075.0	4.0
Tawny owl	*Strix aluco*	60.0	45.5	21	1.4	400.0	1.2
Unidentified bird	-	70.0	50.0	21	1.4	-	-
Green woodpecker	*Picus viridis*	80.0	40.9	19	1.3	200.0	0.5
Ocellated lizard	*Timon lepidus*	50.0	31.8	11	0.7	60.0	<0.1
Crow	*Corvus corone*	50.0	13.6	6	0.4	525.0	0.5
Woodpigeon	*Columba palumbus*	50.0	22.7	5	0.3	480.0	0.3
Mistle thrush	*Turdus viscivorus*	30.0	18.2	5	0.3	115.0	<0.1
Kestrel	*Falco tinnunculus*	20.0	9.1	4	0.3	200.0	0.1
Hare (juvenile)	*Lepus granatensis*	10.0	13.6	3	0.2	1000.0	1.0
Azure-winged magpie	*Cyanopica cookii*	20.0	9.1	3	0.2	70.0	<0.1
Mallard	*Anas platyrhynchos*	20.0	4.5	3	0.2	1000.0	0.4
Robin	*Erithacus rubecula*	20.0	9.1	2	0.1	18.0	<0.1
Cuckoo	*Cuculus canorus*	20.0	9.1	2	0.1	115.0	<0.1
Grey heron	*Ardea cinerea*	20.0	4.5	2	0.1	1550.0	0.4
Little grebe	*Tachybaptus ruficollis*	10.0	4.5	1	0.1	163.0	<0.1
Grey parrot	*Psittacus erithacus*	10.0	4.5	1	0.1	450.0	<0.1
Pheasant	*Phasianus colchicus*	10.0	4.5	1	0.1	1000.0	0.1
Nightingale	*Luscinia megarhynchos*	10.0	4.5	1	0.1	20.0	<0.1
Cirl bunting	*Emberiza cirlus*	10.0	4.5	1	0.1	25.0	<0.1
Great-spotted woodpecker	*Dendrocopos major*	10.0	4.5	1	0.1	85.0	<0.1
Raven	*Corvus corax*	10.0	4.5	1	0.1	1100.0	0.2
Goldfinch	*Carduelis carduelis*	10.0	4.5	1	0.1	15.0	<0.1
Little owl	*Athene noctua*	10.0	4.5	1	0.1	175.0	<0.1
Common sandpiper	*Actitis hipoleucos*	10.0	4.5	1	0.1	55.0	<0.1

Capítulo 4. Spatial variation of mercury levels in nesting Bonelli’s eagles from Southwest Portugal: effects of diet composition and prey contamination

Environmental Pollution 134: 549-557 (2005)

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Spatial variation of mercury levels in nesting Bonelli's eagles from Southwest Portugal: effects of diet composition and prey contamination

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Received 25 January 2004; accepted 21 May 2004

The effects of diet composition and prey contamination added up to determine the spatial variation of Hg levels in breeding Bonelli's eagles.

Abstract

Mercury (Hg) was determined in adult Bonelli's eagles (*Hieraaetus fasciatus*) and their avian prey, from samples of feathers collected between 1992 and 2001 at the nesting sites of 21 pairs in Southwest Portugal. Eagle Hg levels showed great variation, reflecting primarily differences in diet composition and food chain biomagnification. Concentrations were positively correlated with the dietary proportion of insectivorous and omnivorous birds (e.g. egrets, corvids and thrushes), with very low levels for pairs feeding mainly on herbivores (e.g. rabbits, pigeons and partridges). Differences in prey contamination among breeding territories added to dietary effects in determining variation of Hg levels in eagles, shaping a spatial pattern that was largely consistent with a source of contamination in a coal-burning power-plant lying upwind of the study area. Despite this presumed contamination, Hg levels seemed to be of little concern to this eagle population, though there might be subtle deleterious effects on the reproductive output of a few pairs. This study emphasizes the need to account for dietary effects when biomonitoring Hg contamination using birds of prey.

Keywords: Biomagnification; Biomonitoring; Birds of prey; Jay; Partridge; Pigeon

1. Introduction

As top predators, birds of prey are exposed to an array of persistent environmental contaminants that biomagnifies through food webs, especially organochlorine pesticides, polychlorinated biphenyls (PCBs), and mercury (Hg). Accumulation of these chemicals has been particularly well documented for aquatic food webs, where species such as sea eagles (*Haliaetus* spp.) and ospreys (*Pandion haliaetus*) have shown poor breeding and enhanced mortality in association with high pollutant burdens (Helander et al., 1982; Wiemeyer et al., 1984, 1988). Although much less documented, population declines attributed to environmental contaminations have also been shown for species feeding on terrestrial food chains such as the sparrowhawk (*Accipiter nisus*) (Newton et al., 1993). Because of this vulnerability to a variety of contaminants, birds of prey have been used extensively as biomonitors of environmental quality (Berg et al., 1966; Lindberg and Odsjö, 1983; DesGranges et al., 1998; Mañosa et al., 2003).

Besides their high trophic status, many birds of prey are territorial, non-migratory and long-lived, and so pollutant burdens recorded in body soft tissues, bones,

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0269-7491/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envpol.2004.05.017

feathers and eggs are likely to reflect chemical contamination within their extended home ranges. This view underlies most biomonitoring programs, which assume, often implicitly, that spatial or temporal variations in pollutant burdens are coupled with comparable spatial or temporal trends in environmental contamination. Although this assumption may sometimes be warranted for birds of prey (e.g. DesGranges et al., 1998), there are at least some circumstances in which it may fall short of reality. A major source of potential shortcomings is related to diet composition, which may elicit variation in pollutant burdens among individuals of the same species collected at different locations or at different times, irrespective of corresponding variation in environmental contamination. For instance, some studies have linked local peak contamination levels in bald eagles (*Haliaetus leucocephalus*), golden eagles (*Aquila chrysaetos*) and peregrine falcons (*Falco peregrinus*) with a high consumption of aquatic birds such as waders and seabirds (Lindberg and Odsjö, 1983; Parrish et al., 1983; Furness et al., 1989; Anthony et al., 1999). Similar effects for species feeding exclusively on terrestrial prey are scarce, though recent evidence suggests that they may also occur (Mañosa et al., 2003). Clearly, there is a need to evaluate in more detail the effects of diet composition on the pollutant burdens of birds of prey feeding on terrestrial food chains, and how these may confound the interpretation of spatial or temporal patterns in environmental contamination.

The present study addresses these issues, by analyzing the relationships between diet composition, prey contamination, and spatial variation of Hg levels in feathers of Bonelli's eagles (*Hieraaetus fasciatus*). These are medium-sized eagles, whose numbers and range have declined markedly in Europe, where they are restricted to the Mediterranean region (Rocamora, 1994). Bonelli's eagles feed primarily on terrestrial birds and mammals, showing significant geographical variation in diet composition depending on local habitat conditions (Real, 1991). The study was carried out in the uplands of southwestern Portugal, where a dense Bonelli's eagle population of great conservation significance lies downwind of a coal-burning power-plant. Because of this, there were concerns that these eagles could be exposed to an important source of Hg contamination, with potential negative repercussions upon their reproductive output and health condition. This justified a closer examination of factors underlying spatial variation in Hg burdens in the eagles and their prey.

2. Materials and methods

2.1. Study area

Data were collected as part of a long-term study on the Bonelli's eagle in the uplands of Algarve and western Alentejo (southern Portugal), from 21 out of 25 eagle territories occupying about 3000 km^2 in a rough triangle linking the mountains of Cercal (341 m), Monchique (902 m) and Caldeirão (589 m) (Fig. 1). The hilly landscape is predominantly covered by cork oak (*Quercus suber*) woods, dense Mediterranean scrub and eucalyptus (*Eucalyptus globulus*) plantations, with sparse human occupation. Bonelli's eagles breed primarily in large cork oaks, eucalyptus and pine trees (*Pinus* spp.), and feed on domestic doves (*Columba livia*), red-legged partridges (*Alectoris rufa*), jays (*Garrulus glandarius*), rabbits (*Oryctolagus cuniculus*), and many other secondary prey (Palma, 1994; L. Palma, unpublished data). The main potential source of Hg contamination is a coal-burning power-plant located at Sines, on the northwest corner of the study area (Freitas et al., 1999). No additional sources of Hg contamination, either telluric or agricultural, were identified within the study area.

2.2. Sampling procedures

2.2.1. Feather samples

From 1992 to 2001, shed feathers of adult Bonelli's eagles and feathers from avian prey remains were collected from nests and neighbouring tree perches to measure Hg levels. Active nests were visited three times during each breeding season, between the end of incubation and shortly after nest abandonment (March–July), and feathers of each species were collected in separate labelled plastic bags and stored in a freezer at −20 °C. Eagle feather samples were obtained on only 2.3 ± 1.1 SD (1–4) years per breeding pair, because the location of some nests was unknown in early years of the study, some pairs did not breed every year and shed feathers were occasionally absent. Most eagle feathers were probably from females, as they tend to spend far more time near nests than males (Blondel et al., 1969; Morvan and Dobchies, 1987; L. Palma, unpublished data), and because the matching between sampling and moulting periods was closer for females than for males (L. Palma, unpublished data). Feathers were used as monitoring units because Hg in feathers reflects body Hg burden (Furness et al., 1986; Thompson et al., 1990) and it is almost entirely in the mono-methylated form (Thompson and Furness, 1989a,b). Furthermore, feathers have been widely used to monitor Hg levels in freshwater, marine and terrestrial bird species (Furness, 1993), including birds of prey (Dauwe et al., 2003). Only body feathers were analysed, since they provide more representative samples for estimating whole-bird Hg content than flight feathers (Furness et al., 1986).

2.2.2. Diet composition

The diet of eagles in each individual breeding territory was analysed from prey remains collected during the visits to active nests and surrounding perches. Although

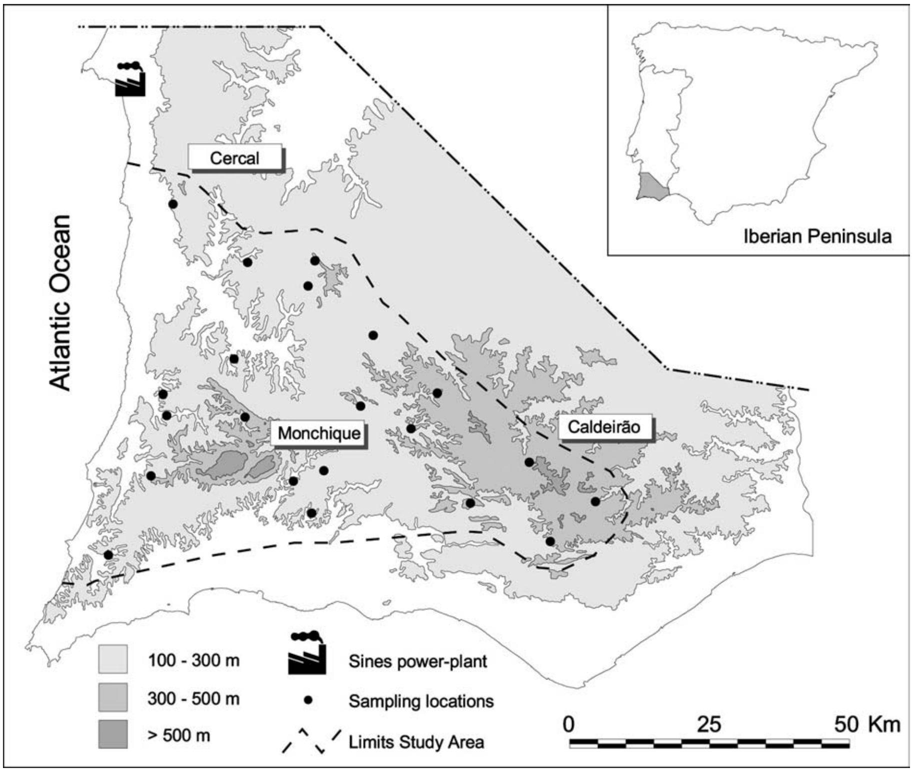

Fig. 1. Locations in Southwest Portugal where feather samples of Bonelli's eagles and their main avian prey were collected for the analysis of Hg contamination (1992–2001). Main mountain ranges are indicated.

remains correspond primarily to prey consumed by nestlings, they likely reflect also the diet of adults, which regularly eat part of the prey delivered to the nests (Blondel et al., 1969; Morvan and Dobchies, 1987; L. Palma, unpublished data). Remains were identified with the help of keys to bird feathers and a reference collection, and the minimum number of individuals in any sample was estimated from the highest number of identical bones of each prey type. This method tends to underestimate the consumption of small prey yielding few remains, while overestimating large prey or prey with a large proportion of rejected parts, such as bird feathers (Real, 1996). However, the method may be considered useful in comparative studies like this one, which aim to detect variation in the relative consumption of different prey, and not estimating the absolute diet composition. Because Hg contamination is strongly dependent on trophic position (Dietz et al., 2000), prey items were categorised according to whether they feed predominantly on plants or animals. Species such as rabbits, partridges, wildfowl and seed-eating passerines were classified as primary consumers, whereas species such as egrets, gulls, birds of prey, corvids and other insectivore passerines were classified as secondary consumers.

2.2.3. Mercury determinations

Feather samples were analysed for total Hg concentration by Cold Vapour Atomic Absorption Spectroscopy (CV-AAS). Samples were digested in a water bath at 70 °C for 6 h by the addition of concentrated H_2SO_4. After this period 5% $KMnO_4$ was added and the solution kept at 70 °C for two more hours. The $KMnO_4$ in excess was reduced with 20% $NH_2OH.HCl$. All reagents used throughout the work were of analytical grade. The glassware was previously decontaminated by immersion in an HNO_3 1:5 solution and then washed with deionized water. Reproducibility was checked by performing successive measurements with the same sample. Relative standard deviations in the range 3–5% were found. Accuracy of the method was within 10% and was monitored analysing reference materials: tuna muscle 350 (International Atomic Energy Agency, Monaco) and RM50 (USA National Bureau of Standards for Biological Material). Minimum detection limits (MDL) of 0.01 μg Hg/g digested sample were quantified using the Kaiser–Currie method (Gibbons and Coleman, 2001). Interferences due to matrix and the pre-treatment were assessed by the method of standard additions before the wet mineralization procedure. Recoveries of added Hg were close to 100%. Hg concentration is given on a wet weight basis.

2.2.4. Statistical analysis

Mean Hg concentrations were computed for feather samples collected from each Bonelli's eagle pair in any given year. The overall Hg concentration corresponding to each breeding pair was then quantified as the mean of concentrations estimated in different years. Samples from avian prey were treated likewise. Before statistical analysis, nondetected measurements were replaced by half the detection limit (Gibbons and Coleman, 2001), and Hg concentrations were log-transformed to approach normality and homogenising variances (Zar, 1996). The arcsine transformation was used likewise for percentage data quantifying diet composition. Differences in Hg levels between species at matching locations were compared using paired-samples *t*-tests (Zar, 1996). Significance levels were corrected for multiple comparisons using the sequential Bonferroni technique (Rice, 1989). Pearson correlations and regression analyses were used to evaluate the relationships between eagle Hg levels, diet composition and avian prey contamination (Zar, 1996).

Spatial patterns in Hg levels for eagles and their main prey were mapped by interpolating to a continuous grid, the concentrations recorded at sampling locations, using inverse-distance weighing (Legendre and Legendre, 1998). Residuals of the regression equation relating eagle Hg levels to diet composition were also mapped, to illustrate the spatial contamination patterns after statistically accounting for dietary effects. In distance weighing, the extinction rule was $1/r^2$ (r is the distance between grid and sampling points), producing a smooth surface and avoiding the need to introduce an artificial cut-off distance (Legendre and Legendre, 1998).

3. Results

3.1. Diet

Eagle diets were described from an average 24.6 ± 15.2 SD (5–64) prey remains identified per eagle pair (Table 1). Almost half the overall remains were pigeons, over 95% of which were identified as domestic pigeons. Other important prey items were red-legged partridges, rabbits and corvids, about 75% of which were jays. Only 17.5% of prey remains corresponded to species categorised as secondary consumers, though their relative importance in the diet varied markedly among breeding pairs, from about 2.1% to 44.4%. Over 95% of individual prey identified corresponded to terrestrial species, with only gulls and mallards (*Anas platyrhynchos*) feeding regularly on aquatic food chains.

3.2. Eagle and avian prey Hg levels

Bonelli's eagles, red-legged partridges, domestic pigeons and jays showed some marked differences in their Hg concentrations (Table 2). Eagles showed much higher Hg concentrations than both partridges ($t_{18} = 7.513$, $P < 0.001$) and pigeons ($t_{19} = 9.822$, $P < 0.001$), but they had similar levels to those of jays ($t_{10} = 1.630$, $P > 0.1$). Likewise, levels in jays were much higher than in partridges ($t_9 = 7.434$, $P < 0.001$) and pigeons ($t_{10} = 8.195$, $P < 0.001$). Concentrations of Hg in partridges and pigeons were virtually identical ($t_{16} = 0.111$, $P > 0.9$).

Table 1
Composition of Bonelli's eagle diet in Southwest Portugal (1992–2001), as assessed from the remains of 541 identified preys recovered from the nests of 21 breeding pairs

Prey items		N	%
Birds			
Cattle egret	*Bubulcus ibis*	11	2.0
Gulls	*Larus* spp.	17	3.1
Red-legged partridge	*Alectoris rufa*	92	17.0
Domestic fowl	*Gallus gallus*	20	3.7
Pigeons	*Columba* spp.	256	47.3
Corvids	Corvidae	43	7.9
Thrushes	Turdidae	12	2.2
Other birds	Mainly Anatidae, Picidae and Strigidae	23	4.3
Mammals			
Rabbit	*Oryctolagus cuniculus*	67	12.4
Hare	*Lepus granatensis*	1	0.2

N = number of individual prey items; % = percentage of total prey recovered.

3.3. Effects of diet and prey contamination

Relationships between eagle Hg levels and diet compositions were assessed using the 17 breeding pairs for which there were more than 10 prey remains. Hg concentrations were negatively correlated with the dietary proportion of pigeons ($r = -0.529$, $P < 0.05$), but not with those of partridges ($r = 0.233$, $P > 0.4$), jays ($r = 0.381$, $P > 0.1$) and rabbits ($r = 0.003$, $P > 0.9$). Analyses for other prey items were not made because they occurred too infrequently in eagle's diet. A strong positive correlation was found for prey categorised as secondary consumers ($r = 0.813$, $P < 0.001$), reflecting the strong influence of prey trophic position on eagle Hg levels (Fig. 2).

Concentrations of Hg in eagles were correlated with those in jays ($r = 0.634$, $P < 0.05$, $N = 11$), but not with

Table 2
Means, standard deviations and ranges of Hg concentrations (μg g^{-1} wet weight) in feather samples of Bonelli's eagles and their main avian prey collected in Southwest Portugal (1992–2001)

Species	N	Mean	Standard deviation	Range
Bonelli's eagle	21	1.94	1.54	0.25–5.42
Domestic pigeon	20	0.13	0.17	<MDL–0.70
Red-legged partridge	18	0.11	0.11	<MDL–0.46
Jay	11	1.58	0.71	0.83–3.41

N = number of breeding pairs from which samples were collected; MDL = Minimum Detection Limit.

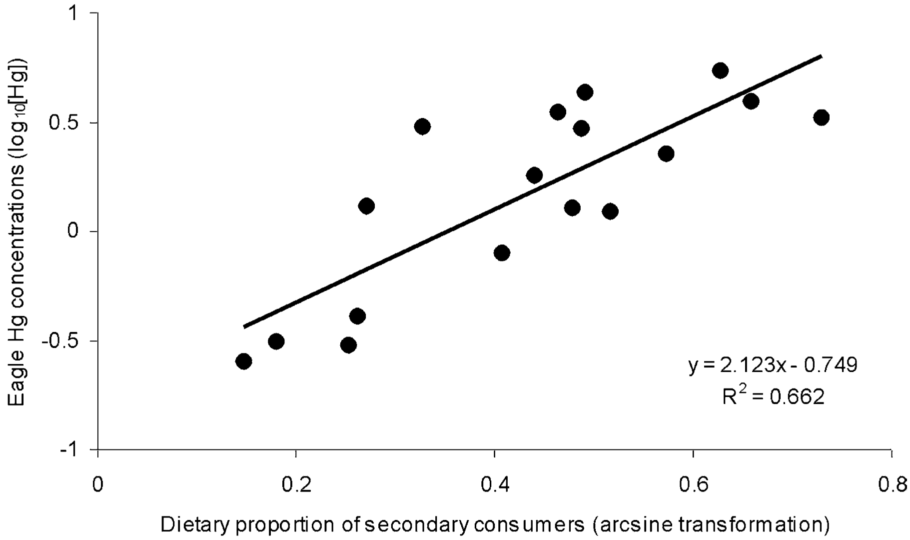

Fig. 2. Mean Hg concentrations (log-transformed; *y*) recorded in 17 Bonelli's eagle pairs breeding in Southwest Portugal (1992–2001), as a function of the dietary proportion (arcsine-transformed; *x*) of secondary consumers.

those in either partridges ($r = -0.064$, $P > 0.7$, $N = 19$) or pigeons ($r = 0.190$, $P > 0.4$, $N = 20$). In a multiple regression accounting for both the effects of diet composition and prey contamination, variation in Hg levels in eagles could be explained to a very large extent by the positive effects of the dietary proportion of secondary consumers and the concentration of Hg in jays (Table 3). Hg concentrations in pigeons and partridges never showed significant effects in similar multiple regressions relating eagle Hg levels with diet composition and prey contamination.

3.4. Spatial patterns

Hg levels in Bonelli's eagles tended to decline eastwards from the relatively high values recorded along the western portion of the coastal mountain ranges of Cercal and Monchique to the low values found in the eastern Caldeirão uplands (Fig. 3). However, high values were also found in one pair breeding along the north-eastern edge of the study area, and in two pairs breeding southeast of Monchique. The residuals of the regression equation between eagle Hg levels and the dietary proportion of secondary consumers (Fig. 2) were used to illustrate the spatial distribution of eagle contamination after correcting for dietary variation (Fig. 3). The emerging spatial pattern underlined the contrast between the western and eastern part of the study area, with the highest Hg levels concentrating around Monchique and the lowest in Caldeirão. High contamination values were also found in two pairs breeding on the north-eastern border of the study area.

For the three avian prey species there were differences in detail for the spatial patterns of Hg contamination, though they all showed a trend for higher values in the western part of the study area (Fig. 3). Furthermore, the highest Hg levels in both pigeons and jays were recorded in the mountain of Cercal, in the sampling site closest to the industrial complex of Sines (Fig. 3). There were, however, exceptions to the west–east gradient of declining Hg levels, with some high values also recorded at the eastern end of Caldeirão for both pigeons and partridges.

4. Discussion

The Hg levels found in feathers of Bonelli's eagles breeding in the uplands of south-western Portugal showed great variation, which seemed to reflect primarily differences in diet composition and food chain biomagnification. The highest concentrations were recorded in pairs incorporating a high proportion of secondary consumers in their diet, whereas much lower values were found for eagles feeding almost exclusively on herbivores such as rabbits, pigeons and partridges. Comparable effects of trophic chain length have been

Table 3
Multiple linear regression relating Hg concentrations in 10 Bonelli's eagle pairs breeding in Southwest Portugal, to prey contamination and diet composition ($R^2 = 0.954$, $F_{2,7} = 35.676$, $P < 0.001$)

Variables	Regression coefficients	*t*	*P*
Intercept	−0.678	−4.181	<0.01
Concentration of Hg in jays (log-transformed)	1.716	4.577	<0.01
Dietary proportion of secondary consumers (arcsine-transformed)	1.461	4.302	<0.01

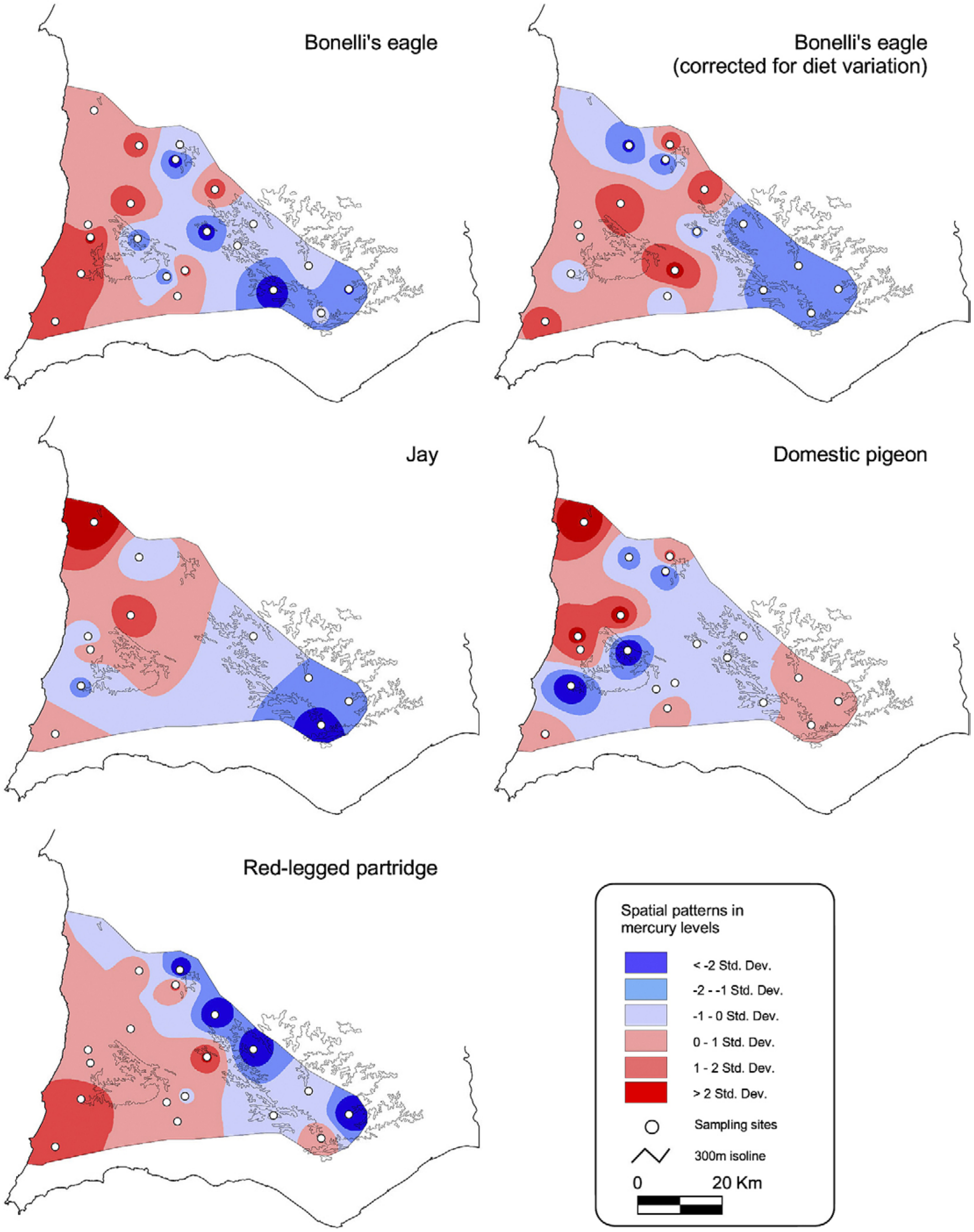

Fig. 3. Spatial distribution of Hg concentrations measured in Bonelli's eagles and their main avian prey. Values are given as standard deviations from the mean, to increase comparability among maps. Eagle data corrected for diet composition are the residuals of the linear regression depicted in Fig. 2, between eagle Hg levels and the dietary proportion of secondary consumers.

noted mainly in marine and freshwater systems (e.g. Elliot et al., 1996; Anthony et al., 1999), with comparable data generally lacking for terrestrial food webs. In a study involving organochlorine contaminants in goshawk (*Accipiter gentilis*) eggs, however, Mañosa et al. (2003) also documented the highest concentrations in association with a higher consumption of passerine birds relative to that of rabbits. The paucity of data for terrestrial chains is probably related to their shorter length in relation to that of aquatic ones, which lessens the potential for Hg biomagnification along the food web (Dietz et al., 2000). Nevertheless, this study strongly suggests that food web length in terrestrial systems may also be a major source of variation in Hg contamination for top predators such as the Bonelli's eagle, which can feed at multiple trophic levels.

After statistically accounting for dietary effects, Hg concentrations in eagles also reflected the contamination level in some of their prey. Indeed, there was a strong correlation between Hg concentrations in feathers of eagles and jays, though no such relationship was apparent for pigeons and partridges. The general pattern for other secondary consumers might have been similar to that recorded for jays, though the shortage of feather samples precluded the testing of this hypothesis. Lack of relationship between eagle and pigeon contamination was unexpected, as these are the staple food of eagles. However, Hg levels were very low in herbivorous prey, which suggests that eagles acquire most of their burden through the intake of secondary consumers. Additional information on the sources of variability in prey contamination is needed to gain a better understanding of the mechanisms leading to Hg accumulation in the eagles.

The strong relationship between eagle Hg levels, diet composition and contamination of prey collected from nests, suggests that concentrations found in shed body feathers probably resulted primarily from exposure during the breeding season. In the study area, adult eagles are largely resident within the breeding territories, starting to visit the nests in November, long before the shed feathers could be found, and remaining in the surroundings at least until juvenile emancipation in August–September (L. Palma, unpublished data). The shed body feathers analysed were generally collected in the late nestling and early fledging periods ($>85\%$ in April–June), corresponding to the post-nuptial moult, which may extend until early autumn (Parellada, 1984; L. Palma, unpublished data). These shed feathers grew during the previous moulting season, thus receiving Hg that had been stored in body tissues over the preceding months (Furness et al., 1986; Furness, 1993; Dauwe et al., 2003). Hg probably accumulated in the adult eagles mainly while foraging within their extended breeding ranges, thus integrating contamination from areas lying in general within 10 km from the nests (L. Palma, unpublished data). This supports the assumption that variation among pairs in the concentrations recorded in shed feathers should reflect at least partly the broad scale spatial trends in environmental contamination, once the dietary effects are accounted for.

Regional Hg concentration trends in prey species and in Bonelli's eagles, after correcting for dietary variation, broadly agree with the hypothesis of a contamination source in the industrial complex of Sines, presumably associated with the coal-burning power-plant, though there were differences among species. Reasons for these differences are unclear, but they may probably be attributed to local factors and sampling variability, which likely added to the large scale contamination trend in influencing the spatial patterns observed. These local factors are impossible to assess with the data collected, but they may result from variation among eagle breeding territories concerning the diets or feeding habitats of the prey species captured. Despite these confounding factors, there was a general trend for higher Hg levels in the western uplands of Cercal and Monchique, which lie immediately downwind of the industrial complex and are thus probably more likely to be contaminated from airborne pollutants than the eastern Caldeirão mountains. Furthermore, precipitation along the coastal uplands, particularly in Monchique, is in general much higher than further inland, which may favour the removal from the atmosphere and local wet deposition of Hg emitted in combustion facilities (Carpi, 1997). This view is also supported by the distribution in lichens of pollutants presumably originating from the Sines coal-powered electric plant, namely Hg, sulphur and selenium, which tended to show higher concentrations in the western uplands than in the east (Freitas et al., 1999). Although comparable patterns were not readily apparent in a similar study using mosses (Figueira et al., 2002), these results call for a more detailed examination of the distribution and biological effects of contaminants emitted from Sines up to several tens of kilometres from the source. This is particularly important in the case of Hg, which biomagnify through food chains and may negatively affect endangered top predators such as the Bonelli's eagle.

Although the mean Hg contamination recorded in eagles can be considered generally low, the highest levels detected might be of concern regarding eventual adverse impacts on the breeding productivity of some individual pairs (Berg et al., 1966; Lindberg and Odsjö, 1983; Parrish et al., 1983; Movalli, 2000). Establishing a benchmark for critical Hg concentrations in feathers is difficult, however, because Hg bonded to keratin and sequestered in feathers no longer represents a risk to the bird (Furness, 1993), and its levels may be uncorrelated with concentrations in eggs (e.g. DesGranges et al., 1998). Nevertheless, Hg concentrations in eagle feathers reported in this study, were correlated with those found in a small sample of addled eggs ($N = 13$) collected from 10 breeding territories in a concurrent study (Blanco, 2001). There was a strong linear relationship between Hg levels in feathers and eggs from individual pairs ($R^2 = 0.772$, $F_{1,8} = 27.078$, $P < 0.001$), with feather levels of 4.1 $\mu g\ g^{-1}$ corresponding to eggs containing the benchmark of 1.0 $\mu g\ g^{-1}$ (wet weight). This concentration may be the lowest associated with deformities of particularly sensitive embryos, though it is unlikely to affect more than a small percentage of eggs (Heinz and Hoffman, 2003). In this study, only two out of 21 Bonelli's eagle pairs (9.5%) showed feather levels in excess of this threshold (4.3–5.4 $\mu g\ g^{-1}$), and may thus be considered moderately susceptible to reproduction impairment due to Hg contamination. For the overall breeding population, however, it is unlikely that Hg contamination can negatively affect the reproductive output.

Results from this study add to a body of evidence derived primarily from aquatic food webs, suggesting that diet variation may have major confounding effects in studies biomonitoring environmental contamination using birds of prey (Anthony et al., 1999; Mañosa et al., 2003). To overcome potential shortcomings, some authors recommended that bird species with narrow and inflexible diets should be used in contamination studies, rather than generalist feeders (Monteiro and Furness, 1995). However, true dietary specialists are probably hard to find, and so the critical assumption of constant diets across space and time may frequently be unwarranted. A detailed knowledge of diet variation and the statistical control of dietary influences, as in this study, may thus be generally required to derive meaningful trends in Hg environmental contamination from the corresponding spatial or temporal variation in concentrations recorded in birds of prey.

Acknowledgements

This study was funded by the Portuguese Science Foundation (PRAXIS/BIA/132/96). Pedro Beja was supported by a grant from Agência de Inovação. Bonelli's eagle nests were visited under permission from the Portuguese Nature Conservation Agency (Instituto da Conservação da Natureza). We thank Valentina Costa and Humberto Lopes for contributing to quality control of mercury determinations, and the collaboration of Rita Inácio in diet analysis. We thank M.F. Magalhães, S. Mañosa and two anonymous referees for the critical reading of the manuscript and suggestions.

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Capítulo 5. Sexing Bonelli's eagle nestlings: morphometrics versus molecular techniques

Journal of Raptor Research 35: 187-193 (2001)

J. Raptor Res. 35(3):187–193

SEXING BONELLI'S EAGLE NESTLINGS: MORPHOMETRICS VERSUS MOLECULAR TECHNIQUES

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ABSTRACT.—We report details of two PCR-based molecular sexing techniques for the Bonelli's Eagle (*Hieraaetus fasciatus*) and evaluate the reliability of morphometric measurements to predict the sex of nestlings in the field. Blood samples taken from 63 nestlings in southwest Portugal (1994–99) were analyzed using the intron polymorphism method (M1), and 56 of these were also analyzed with the single-strand conformation polymorphism approach (M2). Contamination or poor preservation of samples precluded one sex determination with M1 and six others with M2. Sexing by both methods was concordant for 98.0% of samples. Linear discriminant analysis was used to determine whether any single variable or combination could provide reliable sex determinations, using 10 body measurements from 43 nestlings aged 35–50 d, sexed unambiguously by both molecular methods. Models were evaluated by cross-validation of the original data and from the classification of an external sample ($N = 12$). Females were significantly larger than males. The greatest separation between sexes occurred in body mass, but differences were also noted in tarsus diameter and the lengths of the hind claw, foot, culmen, and forewing; no differences were detected in the lengths of tarsus, fore claw, seventh primary, and central tail feather. A discriminant model including body mass, hind claw length, and age provided the maximum separation between sexes and it correctly sexed 96% of the nestlings. A model including tarsus diameter, hind claw, and age showed similar accuracy. Both models were satisfactory in determining the sex of nestling Bonelli's Eagles between the ages of 35–50 d in the field, but combination with molecular techniques may be preferable in studies requiring absolute precision for every individual handled.

KEY WORDS: *Bonelli's Eagle*; Hieraaetus fasciatus; *CHD1 gene*; *sex determination*; *Portugal.*

Determinación del sexo en pollos de *Hieraetus fasciatus:* medidas morfométricas versus técnicas moleculares

RESÚMEN.—Exponemos detalles de dos métodos moleculares para la determinación de sexo de águila-azor perdicera (*Hieraaetus fasciatus*) y evaluamos la validez de algunas medidas morfométricas para sexar pollos en el campo. Se analizaron muestras de sangre de 63 pollos del suroeste de Portugal (1994–99) mediante el "intron polymorphism method" (M1) y 56 de ellas también por el "single-strand conformation polymorphism method" (M2). La determinación del sexo no fue posible por el método M1 en

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una muestra y por el M2 en otras seis, a causa de la contaminación y deterioro de las muestras. Los sexados obtenidos por los dos métodos moleculares fueron concordantes en el 98.0% de las muestras. Evaluamos qué variables o combinaciones de variables permiten la máxima separación entre los sexos, utilizando un análisis discriminante linear en diez medidas externas, tomadas en los 43 individuos con edades entre 35–50 días, en que ambos métodos moleculares han producido resultados coincidentes. Los modelos fueron evaluados por validación cruzada de los datos originales y por la clasificación de una muestra adicional de validación ($N = 12$). Las hembras fueron significantemente mayores que los machos en peso, diámetro del tarso, garra posterior, pata, pico y antebrazo; no se encontraron diferencias en la longitud del tarso, garra anterior, séptima primaria y pluma central de la cola. El modelo que incluye el peso, garra posterior y edad, clasificó correctamente el sexo de 96% de individuos. Un modelo que incluye el diámetro del tarso, garra posterior y edad, obtuvo semejante grado de exactitud. Los dos modelos permiten la correcta clasificación del género de los pollos de águila perdicera con 35–50 días de edad, pero la combinación con métodos moleculares es preferible para una determinación absolutamente precisa del sexo de todas las aves.

[Traducción de autores]

In recent years, a number of methods have been proposed for sexing birds using DNA markers (e.g., Ellegren and Shelton 1997, Griffiths et al. 1998, Cortés et al. 1999). This has prompted a renewed scientific interest in a number of topics requiring the rigorous determination of sex and thus facilitating the investigation of species showing only slight, if any, sexual dimorphism. These techniques have been particularly helpful in addressing questions related to avian offspring sex ratios (Ellegren and Shelton 1997), owing to difficulties in sexing nestlings by conventional methods (Clutton-Brock 1986).

Raptors tend to be sexually dimorphic in size and external morphometry is commonly used in sex identification (e.g., Ferrer and de le Court 1992). In many instances, however, there is considerable overlap between males and females in external characteristics and significant rates of misclassification may occur (Morrison and Maltbie 1999). Additional errors are likely if physical condition affects body measurements, eventually causing diseased or undernourished birds to diverge from the pattern typical for their sex. Therefore, it is important that studies using external morphological characters for sex determination are able to document, in detail, the error rates associated with the use of these methods. Reliable sex determination using molecular techniques provides the means to assess the validity of the methods based on external morphology and to identify the morphological characteristics most adequate for sex determination (King and Griffiths 1994, Morrison and Maltbie 1999).

The Bonelli's Eagle (*Hieraaetus fasciatus*) is a medium-sized raptor, whose numbers and range have declined markedly in Europe, where it is restricted to the Mediterranean region (Rocamora 1994). Adult females are significantly larger than males (Parellada, 1984), and this may also be the case for nestlings (Mañosa et al. 1995). However, it is not known whether size alone is reliable to determine sex in Bonelli's Eagle nestlings. The objectives of this study were to adapt distinct molecular techniques to identify the sex of Bonelli's Eagles following Griffiths et al. (1998) and Cortés et al. (1999) and to derive and evaluate methods for using external morphometric measurements to determine accurately the sex of nestlings in the field.

MATERIALS AND METHODS

Field Sampling. Data were collected as part of a long-term study on the Bonelli's Eagle in the uplands of Algarve and western Alentejo in southern Portugal. This is a hilly landscape covered primarily by cork oak (*Quercus suber*) woods, dense Mediterranean scrub and eucalyptus (*Eucalyptus globulus*) plantations, with sparse human occupation. Bonelli's Eagles breed primarily in large cork oaks, eucalyptus, and pine trees (*Pinus pinaster*) (Palma 1994). We have monitored this population regularly since 1993, checking breeding performance and ringing the nestlings each year. During 1994–99, external morphometric measurements and blood samples were taken from 63 nestlings to determine sex. To minimize the risk of premature fledging (Grier and Fyfe 1987), sampling was limited to nestlings <50 d of age.

Molecular Methods. Blood samples were collected from the brachial vein of Bonelli's Eagle nestlings. One drop of blood (± 50 μl) from each nestling was conserved in "Queen's" lysis buffer at 4°C, and later used for the extraction of genomic DNA as described by Seutin et al. (1991). A second portion (1–2 ml) of blood was conserved in a Lithium-heparinized sterile tube and maintained at 4°C, and subsequently separated in serum and red cell fractions by centrifugation. The red cell fraction was conserved in a glycerol solution at −20°C, and was used for both the analysis of allozyme variability (Cardia et al. 2000) and DNA extraction with a QIAamp Tissue Kit (QIAGEN, Gmbh, Hilden, Germany). The two DNA samples, each one analyzed in a different laboratory with a distinct molecular method, were then used in a double blind scheme for sex determination. Both molecular methods are based on the PCR amplification of a portion of the CHD1 gene (Ellegren and Sheldon 1997, Griffiths et al. 1998), which is known to be located in a region of the sex chromosomes that is not affected by recombination (Fridolfsson et al. 1998). Molecular sexing techniques were tested on blood samples taken from four captive adult Bonelli's Eagles (2 M, 2 F). The first DNA sample was analyzed with the intron polymorphism method (M1), which is based on the amplification of the second intron of the CHD1 gene, the size of which is different between males and females in most bird species (Griffiths et al. 1998). The primers used for this method were chosen so that they would amplify the same region as primers P2 and P8 described by Griffiths et al. (1998). They were designed from an alignment of 16 CHD1 sequences of birds (CHD1-Z and -W denoting the copy of the Z and W chromosomes; accession numbers: FAY12939, FAY12942, ECY12940-1, AF006661-2, AF006659-60, AF060701, AF128256, AF128255, AF128254 and AF077936-7) and mammals (L10410, AF006513) found in the Genebank database (http://www.ncbi.nlm.nih.gov/) in order to ensure the success of amplification in the Bonelli's Eagle. The primers were located in the two exons bordering the target intron and corresponded to the regions of highest homology between the aligned sequences (from 5′ to 3′, numbers correspond to the numbering of the *M. musculus* sequence): CCAAGRATGAGAAACTGTGC (3375–3395) and TCTGCATCRCTAAATCCTTT (3760–3740). Radioactive PCR reactions contained about 50 ng DNA, 1X reaction buffer supplied by the manufacturer, 1.5 mM $MgCl_2$, 100 μM dATP, 60 μM each of the other dNTPs, 10 μM each primer, 0.15 unit of *Taq* polymerase (Gibco BRL, Life Technologies Inc., Gaithersburg, MD U.S.A.) and 0.16 μl [α-^{35}S]dATP (12.5 mCi/ml, 1250 Ci/mmol). The amplification was performed in a Stratagene Robocycler (Stratagene Cloning Systems, La Jolla, CA U.S.A.) and cycles consisted of 1.5 min at 94°C, then 30 sec at 94°C, 45 sec at 48°C, 45 sec at 72°C, 30 times, and finally 5 min at 72°C. PCR products were denatured and run for 4 hr at 1700 V in a denaturing acrylamide gel (5% acrylamide) in 1XTBE buffer.

The second DNA sample was analyzed with a molecular method (M2) based on the amplification of a portion of an exon of the CHD1 gene. The detection of differences between males and females is achieved by a single-strand conformation polymorphism (SSCP) approach (Cortés et al. 1999) using the previously described primers P2 and P3 (Griffiths and Tiwari 1995). The PCR reactions contained about 50 ng of template DNA, 1X reaction buffer supplied by the manufacturer, 2 mM $MgCl_2$, 60 μM of each dNTP, 10 μM each primer, and 0.15 unit of *Taq* polymerase (Promega, Madison, WI U.S.A.). The initial denaturating period of 94°C for 2 min was followed by 35 cycles of 50 sec at 92°C, 1 min at 45°C, and 1 min at 72°C. The program was completed by a final step of 1 min at 72°C. All PCR reactions were performed in a Stratagene Robocycler. PCR products were denatured for 30 sec at 96°C and subsequently cooled to 0°C in ice. Sample electrophoresis was performed in a 14% nondenaturating polyacrylamide gel (29:1 Acrylamide/Bisacrylamide) in 1X TBE buffer at 14°C for 15 hr at 200V and DNA bands were visualized by silver staining.

Morphometric Measurements. The following measurements were taken following Ferrer and de le Court (1992) and Mañosa et al. (1995): body mass, measured with a dynamometer to the nearest 50 g; tarsus length, from the back of the tarsal joint to the front of the folded central toe; antero-posterior tarsus diameter at the middle point of the leg; culmen length from bill tip to the distal edge of the nostril; lengths of the hind and central fore claws, measured dorsally from the base to the tip of the claw; foot length, measured ventrally with the foot resting on a flat surface, from the base of the central fore claw to the base of the hind claw; and forewing length, from the front of the folded wrist to the proximal extremity of the ulna. These measurements were taken with calipers to the nearest 0.1 mm (1 mm in the case of the forewing length). The lengths of the stretched seventh primary and central tail feather were measured with a metal ruler to the nearest 1 mm from the tip of the feather to the skin insertion point, and they were used to estimate the age of nestlings (Mañosa et al. 1995). For a few birds only some of the body measurements were recorded.

Data Analysis. In the analysis we used the nestlings that were sexed by both molecular techniques (M1 and M2) and that showed consistent results among methods. When repeated biometric recordings were taken on the same individual over the breeding season, we only included in the analysis the last taken measurement. We discarded five birds that were only measured before they were 35 d old. This age was considered an adequate cutoff, because the size of most body structures tends to level off at about this age (Mañosa et al. 1995), and because handling of nestlings younger than this is uncommon in most field studies.

Univariate *F*-tests (Zar 1996) were used to test each measurement for significant differences between males and females. Significance of the tests was assessed after correction for multiple comparisons using the sequential Bonferroni technique (Rice 1989). Significant measurements ($P < 0.05$) were then used in linear discriminant analysis (Huberty 1994) to identify the variables or combinations of variables providing the maximum separation between sexes. Selection of variables was carried out with a forward stepwise procedure, but we forced the estimated age of nestlings (in d) in the equation to account for the eventual variation in body measurements due to growth. We used a cross-validation procedure to assess the predictive power of the discriminant functions, in which each individual was classified using a function derived from the total sample less the individual being classified (Huberty 1994). We also used the discriminant functions to corroborate gender for individuals sexed by only one of the two molecular techniques (external sample). All statistical analyses were conducted with SPSS v9.0 (SPSS Inc. 1998).

RESULTS

Molecular Sexing. The sexes of all four captive adult Bonelli's Eagles were correctly identified by

Table 1. A comparison of means of external measurements between 35–50 d-old male and female nestling Bonelli's Eagles from southern Portugal (1994–99) sexed using molecular techniques. Measurements are given in g or mm. Asterisks represent significant differences for multiple comparisons based on sequential Bonferroni's correction for 10 simultaneous tests and table-wise error rate of 0.05.

	FEMALES				Males					
VARIABLES	*N*	Mean	SD	Range	*N*	Mean	SD	Range	*F*	*P*
Body mass[a]	25	2112	132	1850–2400	15	1660	97	1500–1800	132.96	<0.001*
Tarsus length	27	113.4	5.6	100.9–121.0	16	110.2	5.8	104.0–129.0	3.26	0.078
Tarsus diameter	27	12.6	1.5	10.6–17.6	16	11.3	1.4	9.0–14.6	8.09	0.007*
Fore claw length	27	27.1	1.4	22.1–29.6	16	26.0	1.6	24.7–30.6	5.28	0.027
Hind claw length	27	34.5	1.3	31.7–36.5	15	31.7	1.2	29.8–33.4	45.89	<0.001*
Foot length	19	125.3	5.1	111.5–135.3	12	116.8	4.5	111.4–129.0	22.23	<0.001*
Primary length	27	190.2	23.2	127.0–239.0	16	197.6	18.2	157.0–231.0	1.20	0.280
Tail length	26	141.0	18.3	102.6–167.0	16	146.9	13.8	121.0–165.0	1.25	0.271
Culmen length	27	25.2	1.3	21.8–28.2	16	23.6	0.9	22.0–25.1	19.32	<0.001*
Forewing length	25	188.0	8.9	159.5–210.0	16	177.9	4.8	169.0–185.0	17.22	<0.001*

[a] An exceedingly light female (1300 g) was considered an outlier due to disease and undernourishment, and thus it was excluded from this analysis.

both molecular sexing methods. The two adult females presented a heterozygous phenotype, corresponding to the amplification of a portion of the CHD1 gene from both the Z and W chromosomes, whereas the two males were homozygous, corresponding to the amplification of both copies from chromosome Z. For males, the M1 method yielded a single PCR product of about 380 bp as expected from the location of the primers used for PCR amplification, and the females presented an additional product of about 385 bp, suggesting that intron 2 of the W and Z chromosome differ by an insertion/deletion of a few base pairs. The M2 method yielded one PCR product of about 110 bp for both sexes, but females always displayed two bands while males showed a single band.

A total of 62 of 63 samples analyzed with M1, and 50 of 56 samples analyzed with M2, allowed a clear identification of sex for nestling Bonelli's Eagles. The only exception with M1 was a sample yielding a migration pattern of the PCR product totally different from the others, which was interpreted as the result of contamination. PCR amplification of six samples analyzed with M2 produced very few copies of the desired DNA fragment; those samples showed no bands in the SSCP gel and were therefore not sexed. From the 49 samples that could be sexed by both methods, results were congruent in all but one case (98.0%). This was a bird identified as female by M1 and male by M2.

Morphometric Analyses. Given the results of molecular sexing, 16 males and 27 females older than 35 d of age were available for morphometric analyses. In this sample, the mean age estimated for males ($\bar{x}$ = 45.6 ± 2.8 d, ± SD, range = 40–49 d) and females ($\bar{x}$ = 44.4 ± 3.6 d, range = 37–50 d) were similar ($F_{1,41}$ = 1.183, P = 0.283), thus any difference between sexes in mean body measurements could not be attributed to variation in age.

Except for an exceedingly light female (1300 g), the ranges of body mass for both sexes were nonoverlapping, with males averaging about 80% of the female weights (Table 1). The only female mismatch was considered an outlier and it was not used in further analysis involving weight, because it was a diseased and undernourished bird. Body mass alone provided a useful criteria for the determination of gender in Bonelli's Eagle nestlings, assigning to the correct sex about 92% of the weighed individuals, in both cross and external validation (Table 2). Although the diameter of the female tarsus and the lengths of the hind claw, foot, culmen, and forewing were also larger than that of males, these measurements presented considerable overlap between the sexes (Table 1), making them unreliable for sex determination (Table 2).

Body mass (BM) and hind claw length (HCL) were retained in the stepwise discriminant analysis together with age, which was forced into the equation. The resulting linear function ($D_1 = 25.049 - 0.007BM - 0.702HCL + 0.283age$) assigned all but

Table 2. Accuracy of sexing nestling Bonelli's Eagles (35–50 d), obtained from discriminant analysis using single measurements or linear combinations of morphometric variables, as assessed by cross-validation and by the classification of an external sample.

		CASES CORRECTLY SEPARATED							
		CROSS-VALIDATION				EXTERNAL SAMPLE			
	WILK'S	FEMALES		MALES		FEMALES		MALES	
VARIABLE	LAMBDA	%	*N*	%	*N*	%	*N*	%	*N*
Body mass	0.222	88.0	(25)	100.0	(15)	80.0	(5)	100.0	(8)
Tarsus diameter	0.835	59.3	(27)	75.0	(16)	100.0	(4)	87.5	(8)
Hind claw length	0.466	85.2	(27)	100.0	(15)	100.0	(4)	100.0	(8)
Foot length	0.566	78.9	(19)	91.7	(12)	100.0	(4)	100.0	(5)
Culmen length	0.680	74.1	(27)	81.3	(16)	100.0	(4)	87.5	(8)
Forewing length	0.694	72.0	(25)	81.3	(16)	50.0	(4)	100.0	(6)
Linear discriminant functions									
Function D_1	0.122	92.0	(25)	100.0	(14)	100.0	(4)	100.0	(7)
Function D_2	0.253	96.3	(27)	100.0	(15)	100.0	(4)	87.5	(8)

Table 3. Comparisons of the frequency distributions of discriminant scores between male and female nestling Bonelli's Eagles (35–50 d), from southern Portugal (1994–99), along the axis defined by the discriminant functions D_1 and D_2.

DISCRIMINANT SCORES	NO. OF FEMALES	%	NO. OF MALES	%
D1				
<−3.0	6	24.0	0	0.0
]−3.0, −2.0]	8	32.0	0	0.0
]−2.0, −1.0]	5	20.0	0	0.0
]−1.0, 0.0]	4	16.0	0	0.0
]0.0, 1.0]	2	8.0	0	0.0
]1.0, 2.0]	0	0.0	0	0.0
]2.0, 3.0]	0	0.0	3	21.4
]3.0, 4.0]	0	0.0	8	57.1
>4.0	0	0.0	3	21.4
Total	25		14	
D2				
<−2.0	7	25.9	0	0.0
]−2.0, −1.0]	7	25.9	0	0.0
]−1.0, 0.0]	11	40.7	0	0.0
]0.0, 1.0]	1	3.7	2	13.3
]1.0, 2.0]	1	3.7	6	40.0
]2.0, 3.0]	0	0.0	3	20.0
>3.0	0	0.0	4	26.7
Total	27		15	

two individuals to the correct sex (overall success = 96%), where values of $D_1 > 0$ identified males and values < 0 identified females (Table 2). In some circumstances data on body mass may not be available (e.g., carcasses); therefore, we repeated the stepwise discriminant analysis excluding this variable. The resulting discriminant function ($D_2 = 25.624 - 1.072\text{HCL} - 0.239\text{DT} + 0.295\text{age}$) included claw length, tarsus diameter (DT), and age and it classified all but one bird correctly in both cross- and external-validation (overall success = 96%). The frequency distribution of discriminant scores indicated that in most cases males were well separated from females by the linear combinations of variables D_1, although not as well by D_2 (Table 3). The separation was much smaller when the effects of age were not accounted for, particularly in the case of D_2. The single sample for which there was disagreement among molecular techniques was clearly classified as a female irrespective of the morphometric criterion used, thus supporting the results of M1.

DISCUSSION

Molecular Sexing. The lower success in sexing nestlings with M2 (one putative error and six blanks) than with M1 (one blank) could be partially attributed to the lower quality of the samples used, which had been frozen and thawed several times before DNA extraction and amplification. If good-quality samples were used, a higher success rate could have been achieved, probably similar to that obtained by M1. However, the difference in success rate between the two methods may also have resulted from the techniques themselves, thus indicating that M1 may be more robust than M2. For one sample, the M2 method apparently gave a false result (a male instead of a female pattern). One explanation for this could be that the M2 method is expected to allow the detection of single point mutations, while M1 can only detect differences in length of about 5–10 bp between homologous fragments. This difference in sensitivity to detect sequence variation could explain the difficulty in the interpretation of results and the discrepancy between methods, as M2 would produce an unknown migration profile for each variant, including potential false female or false male patterns. Therefore, M2 is apparently less robust than M1 because of the lower success rate of interpretable migration profiles and probably is also less reliable than M1 according to the false result obtained. An important factor is the amount of effort (quantity of products, money, and time) necessary for each method. The results can be obtained within 24 hr using M2 and 48 hr with M1, and the cost of the products is higher for the latter method (e.g., radioactive labeling, large sequencing gel, and autoradiography exposure). Thus, M2 is globally quicker and less costly but also less efficient. This balance strengthens the need for a simple morphological way to determine the sex of Bonelli's Eagle nestlings.

Morphometrics. Our results clearly demonstrated a marked sexual size dimorphism for most external body measurements in Bonelli's Eagle nestlings from age 35–50 d. The main exceptions were the lengths of the seventh primary and the central tail feather, which were remarkably similar between males and females at any given age. These two measurements have been used in the age estimation of nestlings (Mañosa et al. 1995) on the assumption that feather growth shows small variations between sexes, as is typical of other raptor species (Poole 1989, Sodhi 1992). Although this issue was not addressed directly in this study, our results do support this assumption, and thus the aging method proposed by Mañosa et al. (1995).

Body mass, either alone or combined with other variables, provided the most consistent cue for sexing the nestlings. However, the use of this parameter should be regarded with some caution, for mass is highly variable, even within a 24-hr period, and depends on growth rate, degree of hydration, amount and time of the most recent meal, among other factors. Nevertheless, our results suggest that the differences in mass between sexes tend to prevail over the background of natural variability that may be present. There was a single individual that could not be classified by any discriminant function including mass, and this was an extremely lightweighted female, with a severe infection caused by the protozoan parasite *Trichomonas gallinae* (trichomoniasis). This disease induces large, fibrous lesions in the oesophagus and oropharynx preventing birds from swallowing food, and eventually leading to death by starvation (Höfle et al. 2000). Care should thus be taken when sexing nestlings on body mass criteria, if signs of severe trichomoniasis and emaciation are apparent. This same bird could, however, be sexed with the linear discriminant function D_2, suggesting that even diseased birds can be sexed on the basis of morphometric criteria. In these circumstances, however, molecular sexing techniques are likely to provide more reliable results.

The estimated age of nestlings was included in both discriminant functions, though this variable did not improve the correct assignment of sex of the sampled individuals. However, when age was forced into the equations, the separation between groups along the discriminant axis was always increased (expressed by the Wilk's lambda). Although the growth of most body structures levels off at about 35 d, they tend to continue growing at slow rates almost until fledging (Mañosa et al. 1995), making the difference between the two sexes more evident when nestlings of the same estimated ages are compared. Therefore, by considering the age of nestlings, we achieved more robust discriminant functions, providing more confidence to the classifications obtained with these models.

In conclusion, the results of this study demonstrated that external morphometry may be used for the determination of sex in nestling Bonelli's Eagles from 35–50 d. The discriminant measurements needed to use our method are easy to obtain in the field, allowing an immediate and about 96% accurate determination of sex. Difficulties may arise, however, in the case of undernourished or diseased birds, for which our equation D_1 should not be used. In general, we recommend that both discriminant equations should be computed for each bird to assess the internal coherence of the sexing results. Whenever possible and logistically feasible, molecular sexing should be used along with morphometrics to reduce the overall error rates, particularly in those instances where it is essential to know, with absolute precision, the sex of every individual handled.

ACKNOWLEDGMENTS

This work was supported partially by a grant from the Portuguese Science Foundation (PRAXIS/BIA/132/96). Nestlings were handled under permission from the Portuguese Nature Conservation Agency (Instituto da Conservação da Natureza). T. Guillemaud was the recipient of a postdoctoral fellowship (PRAXIS XXI/BDP/4470/96) from the Portuguese Science Foundation. P. Cardia was supported by an ICETA grant. We thank the critical reading of the manuscript by A. Helbig, F. Moreira, J. Real, E. Casado, and an anonymous referee.

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Received 30 December 2000; accepted 15 May 2001

Capítulo 6. Trichomoniasis in Bonelli's eagle (*Hieraaetus fasciatus*) nestlings in South-west Portugal

Pp. 45-52 in J.T. Lumeij, J.D. Remple, P.T. Redig, M. Lierz and J.E. Cooper [EDS.] 2000, Raptor Biomedicine III. Zoological Education Network, Inc. Lake Worth (FL), U.S.A.

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CHAPTER 6

Trichomoniasis in Bonelli's Eagle (*Hieraaetus fasciatus*) Nestlings in South-west Portugal

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SUMMARY

Since 1993 the biology and reproduction of a population of Bonelli's Eagles (*Hieraaetus fasciatus*) in south-west Portugal have been studied. This population has a productivity of 0.9 young per pair per year and has been expanding. Every year several nestlings are seen with oropharyngeal lesions consistent with those observed in trichomoniasis in birds of prey. In spring 1997 a preliminary study of the condition was initiated to determine the cause and contributing factors. The present study included health checks and sampling for microbiology, virology and parasitology of sixteen nestlings, determination and sampling of prey species from remains in nests and necropsies of dead nestlings. Necropsies were performed on addled eggs, and samples were taken of the contents for microbiology and virology. The remaining contents were remitted for toxicology.

Domestic pigeons (*Columba livia*) are known to form a large part of the diet of all breeding pairs. In 1997 *Trichomonas* sp. was detected in direct oropharyngeal smears of four Bonneli's Eagle nestlings, but cultures for *Trichomonas* sp. were negative. *Salmonella choleraesuis* was isolated from the liver of one dead nestling, and *Candida albicans* was isolated from the oropharynx and crop of three live nestlings. Cultures of organs of dead birds and leucocytes of live birds were negative for virus. The levels of mercury and organochlorines in the egg contents were low. After modification of the culturing and transport medium, *Trichomonas gallinae* could be cultured from six out of ten nestlings with oropharyngeal lesions, while direct microscopic examination yielded only one positive case. From these findings it seems likely that *Trichomonas* sp., possibly in combination with *Candida* sp., is the cause of the lesions observed. Further studies are needed in order to determine if predisposing factors exist, and to study the population of domestic pigeons as possible reservoir of infection.

INTRODUCTION

One of the two largest and densest populations, currently numbering 22 to 23 pairs, of Bonelli's Eagles in Portugal is found in the south-west region of the

TABLE 6.1. Breeding success and losses of nestlings in a Bonelli's Eagle population in south-west Portugal

Year	Number of pairs closely monitored	Number of nestlings hatched	Number of nestlings lost	Number of nestlings lost (or presumably lost) by disease	Percentage of nestlings lost by disease	Percentage of nestlings lost by disease
1992	5	9	4	(1)	11.1	25
1993	8	13	5	(2)	15.4	40
1994	14	22	5	1+(2)	13.6	60
1995	16	19	6	1+(1)	10.5	33.3
1996	16	14	4	1	7.1	25
1997	18	23	8	5	21.7	62.5
Total	77	100	32	8+(6)	—	—
Mean					13.2	41

country (Génsbol, 1995; Palma *et al.*, 1996). The population was hardly known until a study of the endangered species found in the region was carried out in 1992/93 (Palma, 1993). The population is of special interest, not least because all but one of the breeding pairs nest in trees, unlike Bonelli's Eagles elsewhere in Europe (Palma, 1994). Accordingly, during the past four years close monitoring of the population and an intensive study of its ecology, demography and genetics (Palma, unpublished, 1995) has been conducted.

Prior to 1997 interesting additional information in relation to this paper was compiled. At least 30% of the diet of the eagles consisted of domestic pigeon (*Columba livia*) (Palma, unpublished, 1995). Mortality of nestlings in the presence of large, fibrous oropharyngeal lesions was observed.

From 1993 to 1997, in 77 closely monitored pairs/breeding attempts, 32 out of 100 nestlings died in the nest. Eight of these died from severe oropharyngeal lesions and another six were found dead and are presumed to have succumbed from pathological conditions (Table 6.1).

Presumably some fledged young could have also died after leaving the nest, as they had developing lesions when observed before fledging.

As domestic pigeons are known to be a reservoir for *Trichomonas* sp. a correlation with the diet was suspected. Apart from *Trichomonas*, other parasites such as *Capillaria* sp. or the yeast, *Candida albicans*, had to be considered as etiologic agents (Cooper, 1969). Other microorganisms that may be transmitted to the nestlings by consumption of domestic pigeons may also play a role in the observed mortality, such as for example herpes- and paramyxovirus infections (Gerlach, 1994).

To determine the incidence and importance of different pathologies in the nestlings of this population a study on the health status of the nestlings was initiated in 1997.

The main objective was to isolate and identify the etiologic agent responsible for any observed pathology, and to identify factors that may predispose some nestlings to disease.

MATERIALS AND METHODS

Sampling procedures

Health checks were carried out on sixteen Bonelli's Eagle nestlings from ten nests. Samples were taken for microbiology, virology and parasitology.

Samples from cloaca, oesophagus, trachea and conjunctiva were taken with sterile cotton swabs and kept refrigerated (4°C) in transport medium (Amies®, Eurotubo, Deltalab, Barcelona, Spain) for a maximum of 48 hours until cultivation.

Swabs of the oropharyngeal region, including crop and oesophagus as well as any visible lesion, were taken from all nestlings with sterile cotton swabs that had been soaked in culture medium for *Trichomonas* spp. (2.95g lyophilized CPLM broth for *Trichomonas* spp., Biocheck S.A., Barcelona, Spain; dissolved in 100ml Ringers Lactate solution, autoclaved, and supplemented with 0.01g mycostatin, 0.061g penicilline and 0.1g streptomycin at a pH of 6.02). The swabs were transferred to sterile tubes containing 5 ml of the described medium. This culture medium has been seen to work well with *Trichomonas* strains obtained from pigeons and birds of prey (Muñoz, 1995), but because of the failure of the observed *Trichomonas* spp. to grow in the medium in the 1997 breeding season, the medium was slightly modified and the transport methods ameliorated. During the 1998 breeding season the swabs taken from the oropharynx, oesophagus and crop were cultivated in CPLM broth for *Trichomonas* spp. (Biolife s.r.l., Milano, Italy). The broth (35.7g lyophilyzed CPLM medium in 1000ml Ringer's Lactate) was autoclaved, transferred to sterile tubes and supplemented with 0.25ml inactivated (30min at 56°C) rabbit serum (Sigma-Aldrich Co. Ltd., Poole, UK) and 0.5ml antibiotic/antimycotic solution (100x, Sigma-Aldrich Co. Ltd., Irvine, UK) for each 5ml of CPLM broth. The inoculated tubes were maintained at approximately 36°C during transport to the laboratory by use of a device for the thawing of semen straws for artificial insemination.

Blood samples were taken with sterile syringes and 23g needles and transferred to sterile tubes containing lithium heparin and kept at 4°C until processing. Blood was centrifuged, the buffy coat was separated and leucocytes were separated by centrifugation with histopaque and washing with phosphate buffered saline (PBS). Leucocytes were frozen at -76°C in freezing medium DMSO (Sigma-Aldrich Co. Ltd., Poole, UK).

Post mortem examination of dead nestlings and addled eggs

Full *post mortem* examinations were carried out on two nestlings from the target population. One nestling had severe oropharyngeal lesions on clinical examination, twelve days before its death. A third nestling was from another population in the north of Portugal where similar problems had been observed. Tissue samples were fixed in 10% neutral buffered formalin and H&E stained slides were prepared and examined microscopically. Although the three nestlings were moderately to markedly autolysed, samples for microbiology and virology were taken from internal organs (liver, lung, spleen, kidney, brain and intestine) and from the lesions in the oropharynx and oesophagus.

Six addled eggs were necropsied and samples for microbiology were taken from the albumen prior to opening the egg, from the yolk sac and from the internal organs of the embryo in the case of fertile eggs. In fertile eggs, half the embryo was fixed in 10% neutral buffered formalin, while the other half of the embryo and the rest of the contents were homogenised and distributed in sterile tubes and frozen at –20°C. In infertile eggs the whole contents was homogenised, distributed in sterile tubes and frozen. Half of the homogenate of each egg was remitted to The Laboratory for Terrestrial Ecology (Monks Wood, Abbots Ripton, Huntingdon, Cambridgeshire PE17 2LS, U.K.) for toxicology. Due to the advanced autolysis of most of the embryos, histopathological studies were carried out only on one embryo and no virological analyses were performed.

Bacteriology and mycology

The swabs taken from the nestlings were streaked out on sheep-blood agar, selective media for gram-negative bacteria (MacConkey agar) and fungi (Sabouraud -Dextrose- agar) and dipped into a *Salmonella* enrichment broth (Rappaport-Vasialidis or Selenium- broth).

Isolated cultures were differentiated by colonial morphology and colour, gram stain, and their growth on additional selective media (Brilliant-green and XLD-agar, Baird Parker-agar etc.) and their biochemical characteristics (e.g. lactose, sucrose fermentation, oxidase, catalase, urease, decarboxylation of lysine, production of hydrogen sulphide, citrate utilization, production of indole). Particular attention was paid to gram-negative bacteria, especially Enterobacteriaceae that form part of the normal flora or may be pathogenic, fungi and yeasts (*Aspergillus* spp., *Candida* spp., *Cryptococcus neoformans*).

Presumptive identification of *Salmonella* sp. was made by the above mentioned characteristics and by the use of RapId-OnE (IDS, Atlanta, U.S.A.) strips for Enterobacteriaceae. Serotyping of *Salmonella* spp. was carried out by the Instituto Regional de Salud Publica (Talavera de la Reina, Spain).

Presumptive diagnosis of pathogenic yeasts was confirmed by the use of RapId-Yeast® (IDS, Atlanta, U.S.A.) biochemical identification strips.

Virology

Leucocytes of live nestlings were thawed, centrifuged on a micro-centrifuge and the supernatant was rejected. The leucocytes were washed twice in PBS and finally diluted to a concentration of $2x10^6$ leuco-

TABLE 6.2. Results of examination of Bonelli's Eagle nestlings from south-west Portugal for the presence of oropharyngeal/oesophageal lesions and of the analysis carried out in order to detect the etiologic agent(s) during the 1997 and 1998 breeding seasons.

Year	Number of nestlings examined	Number of nestlings with lesions	Number of small lesions	Number of large lesions	*T. gallinae* obs. direct microscopy	*T. gallinae* cultured	*Capillaria* observed	*Candida* spp. isolated
1997	16	14	10	4	4	0	0	3
In %	100%	87.5%	62.5%	25%	25%	—	—	18.75%
1998	12	10	5	5	1	6	0	6
In %	100%	71.4%	35.7%	35.7%	7.14%	42.9%	—	42.9%

cytes/ml. Samples of internal organs of dead nestlings were homogenized, suspended in 4ml of Basal Medium Eagle (BME, supplemented with 2% foetal calf serum (both Sigma-Aldrich Co. Ltd., Irvine, UK) and enrofloxacine), sonicated and centrifuged (5 min at 3000 rpm). 0.2 ml of the supernatant of the leucocyte suspensions and of the supernatant of organ suspensions, respectively were inoculated in chicken embryo fibroblast cultures that had been prepared from specific pathogen free eggs at ten days of incubation. The cultures were observed daily for cytopathogenic effects, subcultured on day five after infection and passaged twice (Mayr *et al.*, 1977).

Additionally, haemaglutination-inhibition assays were carried out with serum of all nestlings in order to determine the presence of neutralizing antibodies against Paramyxovirus 1 (Mayr *et al.*, 1977).

The virological analyses were performed at the Institut fuer Gefluegelkrankheiten of the Veterinary Faculty of the Justus Liebig-University of Giessen, Germany.

RESULTS

The results of clinical examinations, microbiological and parasitological analysis of the nestlings examined throughout the 1997 and 1998 breeding season are summarised (Table 6.2). In seven of the birds in which small lesions were observed during the 1997 breeding season, no pathogen could be isolated from the lesions, the oropharynx, oesophagus or crop which may be due to the failure of *Trichomonas* spp. to multiply in the culture medium. In 1998 both *Candida* spp. and *Trichomonas gallinae* were isolated from the observed lesions from two birds, which were severe in one case. Of the remaining eight birds with lesions, *Trichomonas* spp. and *Candida* spp. were isolated from four birds each. Although *Candida albicans* in pure culture was isolated on most occasions, *Candida rugosa* and *Candida crusei* were isolated in one nestling each. Other potential pathogens isolated include: *Aspergillus niger* (one bird), *Klebsiella pneumonia* (three birds), and *Salmonella* spp. (three birds). None of the nestlings, however, seemed to have systemic or respiratory disease. No correlation could be established between either nutritional state or the presence of additional potential pathogens, and the severity of the lesions. Nestlings that had severe lesions in the oropharynx, oesophagus or crop were in bad nutritional state, possibly due to the impairment of proper food intake.

All three of the nestlings, of which one was from a northern population, that underwent a *post mortem* examination, had severe fibrous lesions in the oesophagus, and in the oropharynx. *Trichomonas gallinae* could be identified readily on impression smears from the borders of the lesions of two birds. In the bird from the northern population *Candida albicans* was isolated from the lesion. In the bird, that had died twelve days after clinical examination *Salmonella choleraesuis* was isolated from the liver. The brother of this bird had died two weeks earlier, but was not examined due to advanced mummification of the carcass.

Virology

Results are only available for nestlings from the 1997 breeding season.

Cell cultures were negative for cytopathogenic effects in all nestlings throughout three passages. In none of the nestlings neutralising antibodies against Paramyxovirus 1 could be detected by the use of haemaglutination inhibition tests.

TABLE 6.3. Results of necropsies of six addled eggs of Bonelli's Eagle from south-west Portugal

Reference	Contents	Age of embryo in days	Macroscopic Lesions	Microorganisms Isolated
Hfa 1/97	Embryo	29	Brachygnathism	*Haemophilus* spp.
Hfa 2/97	Embryo	15	None	*Escherichia fergusonii*
Hfa 3/97	Infertile	-	-	*Escherichia coli*
Hfa 4/97	Embryo	37	None	*Escherichia coli*
Hfa 5/97	Embryo	39	None	Sterile
Hfa 15/97	Embryo	35	Hepatomegalia	Sterile

The results of *post mortem* examination and microbiology of six addled eggs are summarised in Table 6.3. Histopathologic slides had been prepared only from embryo HFA 15/97 because on macroscopic examination it appeared less autolytic than the other embryos, and because hepatomegaly was observed. Nevertheless due the extensive autolytic changes no conclusive observations could be recorded.

Two eggs contained nearly full term (37 and 39-day-old) embryos. These eggs had been abandoned after the nest had collapsed.

A summary of concentrations of some organochlorines, total PCBs and mercury in the egg contents is given in Table 6.4. Organochlorine and mercury contamination of the eggs was low, although high mercury concentrations (up to 10.92 ppm) were found in moulted covert feathers of some adults of this population in preliminary examinations carried out at the University of Azores (Monteiro, pers. comm.).

Diet

Approximately one third of the prey species observed at nests throughout the 1997 breeding season consisted of domestic pigeon (*Columba livia*).

DISCUSSION

During the analyses three potential pathogens were isolated from a number of Bonelli's Eagle nestlings. Domestic pigeons are potential reservoirs for these pathogens. Two of these, *Candida* spp. and *Trichomonas gallinae*, were isolated from lesions in the upper digestive tract of the nestlings, and resulted in mortality in three birds.

That *Trichomonas gallinae* could not be isolated in 1997 although it had been detected on direct microscopy, is most probably due to sub-optimal culture media and transport conditions during the 1997 breeding season.

Trichomonas spp. are very sensitive to temperature changes, and osmotic pressure. They also require a constant nutrient source for constant replication. The sub-optimal conditions with the medium and methods primarily used may have slowed the growth and replication of *Trichomonas* and have favoured overgrowth by faster growing bacteria and fungi.

The culture medium used in 1998 had a pH of 6.5 which is more suitable for the growth of *Trichomonas* spp. Enrichment with rabbit serum provided more nutrients and bacterial and especially fungal growth were prevented by a ten-fold increase in antibiotic and antifungal drug concentrations. One of the major problems had been the maintenance of a constant culturing temperature of about 36°C during the field work that in some cases took several days. This was overcome by the use of a portable automotive device for thawing semen straws. This device helped to maintain a constant temperature by means of a water bath. Using the changed culture medium and methods, culturing proved successful also in cases where no *Trichomonas* spp. could be observed on direct microscopy.

Pathologic changes caused by *Trichomonas* spp. in captive and free-ranging birds of prey are well known (Ward, 1986; Redig, 1987; Pokras *et al.*, 1993). Trichomoniasis is seen in species in which pigeons form a habitual part of the diet, such as falconiformes and Northern Goshawks (*Accipiter gentilis gentilis*) (Samour *et al.*, 1995; Cooper and Petty, 1988). Pigeons and doves are also considered the reservoir for trichomoniasis in nocturnal raptors (Delogu, *et al.*, 1997).

Cooper and Petty (1988) expressed their concern about the possible influence of nestling mortality due to trichomoniasis on the population dynamics of a breeding population of reintroduced Northern Goshawks. They stated that limited genetic variability may have rendered the population more suscepti-

TABLE 6.4. Concentration of HCB, a-HCH, g-HCH, p,p-DDE, HEOD, p,p-TDE, p,p-DDT, Total PCBs and mercury in terms of wet weight of the contents of addled Bonelli's Eagle eggs from south-west Portugal of the 1997 breeding season.

Reference	HCB (µg/g)	a-HCH (µg/g)	g-HCH (µg/g)	p,p-DDE (µg/g)	HEOD (µg/g)	p,p-TDE (µg/g)	p,p-DDT (µg/g)	Tot PCBs (µg/g)	Mercury (µg/g)
Hfa 1/97	nd	nd	nd	1.010	0.030	nd	nd	2.064	nd
Hfa 2/97	nd	nd	nd	0.182	0.021	nd	nd	1.228	0.189
Hfa 3/97	nd	nd	nd	1.61	0.067	nd	nd	3.154	0.298
Hfa 4/97	0.022	nd	nd	0.973	0.049	nd	nd	9.797	0.044
Hfa 15/97	0.018	nd	nd	1.973	0.193	nd	nd	15.018	0.343
Conc. effects[1]	1,5[2]	>10	>10	15-20[1,2]	1	?	1	25	3.25[3]

[1] (Blus, *et al*, 1996; O'Hara and Rice, 1996; Heinz, 1996)

[2] (Jarman, *et al*, 1996)

[3] causing embryo and hatchling mortality

ble to the infection in comparison to Peregrine Falcons (*Falco peregrinus*) which although feeding mainly on pigeons seem to be more resistant to the disease.

Boal *et al*. (1998) described an epiphenomenon involving trichomoniasis in urban Cooper's Hawks (*Accipiter cooperii*) in Tucson, Arizona, wherein urban hawks feeding on wild columbiformes (predominantly doves) experienced very high rates of nestling mortality, while extraurban hawks had little occurrence of the disease. Here the problem was clearly related to exploitation of an abundant, but deadly prey source without any likelihood of limited genetic variability playing a role.

Pepler and Oettle (1992) reported an outbreak of trichomoniasis among raptors in relation to outbreaks of the same disease among different dove and pigeon species on the Cape peninsula (South Africa), and also discussed the possible effect of the disease on the reproductive success of specific species.

Lesions and mortality related to infections with *Trichomonas gallinae* in Bonelli's Eagles were first observed in 1994 (Muñoz, 1995) in a population of Bonelli's Eagles in Catalunya, Spain. Like the population in the present study, these Bonelli's Eagles depended on domestic pigeons for more than one third of their diet (Real, 1990; Palma, 1995). This is in contrast to a population of Bonelli's Eagles in Central Spain that still relies mainly on its main original prey species such as Red-legged Patridges (*Alectoris rufa*) and rabbits (*Oryctolagus cuniculus*) and in which no lesions were found in eight nestlings examined in 1997 by two of the authors (Hoefle and Blanco, respectively).

The findings of the present study and those of the aforementioned studies are consistent as they show that nestlings were primarily affected by the disease (this may be biased as the adults were not examined); that pigeons or doves were considered to be the reservoir for the parasite; and that the mortality caused by the disease negatively affected reproductive success of the affected populations. In some of the cases the problem seemed to be limited to a region or a time period, which might indicate that highly virulent strains of *Trichomonas gallinae* may be responsible for these outbreaks.

None of the cited authors detected *Candida* spp. in raptors infected with the parasite, which may be partly due to the reason that it has not been sought. Although the isolation of *Candida* spp. from samples obtained under field conditions has to be considered carefully, as it may be an environmental contaminant, the isolation of *Candida albicans* or other *Candida* spp. in pure culture suggests a relation of this potential pathogen to the observed lesions. It is difficult to say, however, whether it should be considered a secondary or primary pathogen in the development of severe trichomoniasis, as both pathogens have been isolated alone and in combination from nestlings with small and severe lesions.

Kaleta *et al*. (1990) hypothesised that herpesvirus could be transmitted to young pigeons via trichomonad protozoa. This pathogen has not been identified in our or any of the other mentioned studies on trichomoniasis, but it could be an additional factor with a predisposing effect on the immune status of the birds, or even a primary pathogen in the upper digestive tract.

Further *in vitro* and *in vivo* studies of the *Trichomonas* strains isolated during the 1998 breeding period will hopefully give further insights on their virulence, and particularities. This is of particular interest to the epidemiology and pathogenesis of the disease in these nestlings and also in relation to future strategies for control through management of local pigeon lofts, with a view to promoting use of adequate treatment protocols to avoid underdosing due to possible drug resistance as it has been reported by Franssen and Lumeij (1992).

Further studies should also include analysis of vitamin A and lead levels in the nestlings, as the presence of opportunistic yeasts may be indicative of impaired immune function or loss of mucous membrane integrity (Bauck, 1994).

ACKNOWLEDGEMENTS

This project was partially supported by Project PRAXIS/PCNA/BIA/132/96 – The conservation of Bonelli's Eagle (*Hieraaetus fasciatus*) in the Mediterranean context: Ecology, Demography and Genetics of the Portuguese Populations.

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Capítulo 7. Limitations of methods to test density-dependent fecundity hypothesis

Journal of Animal Ecology 77: 335-340 (2008)

Journal of Animal Ecology 2007 doi: 10.1111/j.1365-2656.2007.01312.x

Limitations of methods to test density-dependent fecundity hypothesis

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Summary

1. Two main hypotheses are usually invoked to explain density dependence in fecundity: the habitat heterogeneity hypothesis (HHH) and the individual adjustment hypothesis (IAH). Although simple methods have been proposed to discriminate between the two hypotheses, their adequacy was tested for only a limited set of real and model populations.

2. In a computer simulation study based on a stochastic territory-based approach, Ferrer, Newton & Casado (2006, *Journal of Animal Ecology*, **75**, 111–117) argued that a strong negative relationship between mean fecundity and its skewness in stable or increasing populations provides critical support for HHH, as this relationship should be lacking under IAH. A negative relationship between mean fecundity and its coefficient of variation (CV) was predicted under both hypotheses, although with a lower slope under IAH.

3. We used a comparable simulation approach, with model populations parameterized from an increasing Bonelli's eagle *Hieraaetus fasciatus* population (1992–2006), to show that both HHH and IAH can produce indistinguishable relationships between mean fecundity and both its CV and its skewness.

4. Strong negative correlations between the mean and both its CV and its skewness can emerge as statistical artifacts under biologically plausible assumptions, and so they may be largely inadequate to infer mechanisms underlying density dependence in demographic parameters.

Key-words: density dependence, habitat heterogeneity, intraspecific competition, population ecology, statistical artefact.

Introduction

Understanding the mechanisms underlying density dependence in demographic parameters is a prominent theme in population ecology (Ferrer & Donázar 1996; Rodenhouse, Sherry & Holmes 1997; Krüger & Lindström 2001; Kokko, Harris & Wanless 2004; Gunnarsson *et al.* 2005; Carrete *et al.* 2006a,b,c). These mechanisms are often inferred by testing the predictions of alternative hypothesis against data from observational studies (Ferrer & Donázar 1996; Ferrer, Newton & Casado 2006), although this is prone to potential shortcomings because different ecological processes may generate similar patterns (Cale, Henebry & Yeakley 1989; Tyre, Possingham & Lindenmayer 2001; Ward *et al.* 2007). However, taking this approach is largely unavoidable in populations that are not amenable to experimental manipulation, making it necessary to check the adequacy of extant criteria to discriminate between regulatory mechanisms.

In a recent study, Ferrer *et al.* (2006) proposed a simple method to differentiate between two main hypotheses explaining density dependence in fecundity: the habitat heterogeneity hypothesis (HHH) and the individual adjustment hypothesis (IAH) (Dhondt, Kempenaers & Adriansen 1992; Both 1998). The first hypothesis derives from the model of Ideal Preemptive Distribution of territorial animals (Fretwell & Lucas 1970; Pulliam & Danielson 1991; Rodenhouse *et al.* 1997), which leads to density dependence due to increased usage of poor-quality habitats at high population densities (Dhondt *et al.* 1992; Ferrer & Donáza 1996; Krüger & Lindström 2001; Kokko *et al.* 2004; Gunnarson *et al.* 2005). Under HHH, fecundity in the best habitat patches may remain high irrespective of population density, but per capita breeding success declines with population size due to low performance in poor habitats (Ferrer & Donázar 1996; Krüger & Lindström 2001; Kokko *et al.* 2004; Gunnarson *et al.* 2005). As a corollary, it was predicted that heterogeneity in breeding performance among individuals should increase with declining population fecundity, due to increased occupation of poor sites (Ferrer &

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Donázar 1996; Ferrer *et al.* 2006). In contrast, IAH assumes a relatively uniform decline in breeding success across the population with increasing density, due to increased interference and aggression among individuals (Ferrer & Donázar 1996; Fernandez, Azkona & Donázar 1998; Ferrer *et al.* 2006). Under this hypothesis, mean fecundity should vary inversely with population size, as under HHH, but heterogeneity among individuals should be largely independent of both population size and mean fecundity (Ferrer & Donázar 1996; Ferrer *et al.* 2006).

Considering these predictions, Ferrer & Donázar (1996) suggested that observation in a growing population of an inverse relationship between mean fecundity and its coefficient of variation (CV) would provide a critical support for HHH, as no such relationship should occur under IAH. Although this test was used subsequently in several studies (Blanco *et al.* 1998; Krüger & Lindström 2001; Penteriani *et al.* 2003; Sergio & Newton 2003; Carrete *et al.* 2006a,c), it was shown recently that a negative correlation between mean fecundity and its CV could occur under both hypotheses (Ferrer *et al.* 2006). On the basis of an individual-based simulation study, Ferrer *et al.* (2006) argued that skewness can be used instead of the CV as the adequate parameter that shows strong relationships with mean fecundity only in populations under HHH (Ferrer *et al.* 2006). Although the results of this study appeared unambiguous their conclusions are contingent upon model assumptions, and so they may lack generality. For instance, simulations assumed that brood sizes were distributed normally, with constant variance, although long-lived species with low fecundity often show left-skewed fecundity distributions, with variance tending to increase along with mean fecundity (Moreno *et al.* 2003). This makes it necessary to evaluate whether the test proposed by Ferrer *et al.* (2006) is robust to changes in model assumptions.

This study used the simulation approach of Ferrer *et al.* (2006) to investigate circumstances under which the methods outlined above may fail to discriminate between the two regulatory hypotheses. First, the methods were applied to real data from an increasing population of Bonelli's eagles *Hieraaetus fasciatus* Vieillot in southern Portugal (1992–2006), showing the kind of inferences that might be drawn from the analyses. Then, a computer simulation approach tested whether results similar to that of the natural population could actually be assigned unequivocally to a single density-dependency mechanism. Simulation conditions were equivalent to that of Ferrer *et al.* (2006) to allow comparability between studies, although assumptions regarding the distribution of brood sizes were changed and model populations were parameterized from Bonelli's eagle data. Results from the study were used to discuss the information required to test density-dependent fecundity hypothesis.

Methods

BONELLI'S EAGLE DATA

The Bonelli's eagle is a medium-sized raptor showing marked declines in numbers and range in Mediterranean Europe (Real & Mañosa 1997). As part of a long-term study in southern Portugal (e.g. Palma *et al.* 2006), comprehensive information was obtained on population dynamics and reproduction, the details of which will be presented elsewhere. Here we considered those aspects directly relevant to the methods outlined above and to parameterize model populations. Population sizes and fecundity data were gathered when the population increased from 18 to 30 breeding territories (1992–2006), occupying an increasing area from about 3000–4000 km^2 (Palma *et al.* 2006; L. Palma & M. Pais unpublished data). The region is hilly and dominated (> 95%) by oak forests and scrubland, eucalyptus plantations and extensive farmland. The breeding population was monitored each year, thoroughly surveying all the area during courtship, nest site selection and breeding periods (e.g. Krüger & Lindström 2001; Ferrer *et al.* 2006). Breeding data, including the number of fledged juveniles, were gathered by visiting each active nest-site 5·5 ± 2·2 (one to 11) times per season. Fecundity information was obtained from 21·1 ± 5·7 (eight to 30) pairs per year (316 breeding events). The overall mean fecundity was 0·75 ± 0·79 fledged juveniles per pair, showing a markedly left-skewed distribution of brood sizes: 0 juveniles = 46·2%; 1 = 32·3%; 2 = 21·5% (L. Palma & M. Pais unpublished data).

COMPUTER SIMULATION AND MODEL ASSUMPTIONS

The study followed the approach of Ferrer *et al.* (2006) with modifications, using a stochastic territory-based simulation to model population dynamics and variation in fecundity. Briefly, at each time step (1 year) the number of offspring produced in each territory was simulated through random sampling from a specified frequency distribution of brood sizes. The figures obtained for all active territories in each year were then averaged to provide an estimate of the annual population fecundity, and the corresponding CV and skewness were computed. Simulations were run for periods of 10 and 30 years, with 5000 replicates per population. Models were developed and run using Microsoft Excel spreadsheets (e.g. Brett 2004).

The main difference in relation to Ferrer *et al.* (2006) was that empirical distributions of brood sizes were used in simulations instead of the normal distribution. The number of offspring per territory simulated at each time step was thus a bootstrap sample from an observed frequency distribution, from which sample estimates for all parameters of interest were computed (Efron & Tibshirani 1993). This procedure was selected because the observed distribution of brood sizes involved a small number of discrete categories (0, 1 and 2), frequently left-skewed, with strict lower and upper bounds, which is not approached adequately by a continuous, symmetric and unbounded theoretical distribution such as the normal. Distributional patterns comparable to that of Bonelli's eagles are common in other long-lived species used in recent works testing density-dependent fecundity in wild populations (e.g. Kokko *et al.* 2004; Carrete *et al.* 2006a,c; Ferrer *et al.* 2006). In these conditions, using the normal curve is unwarranted and can cause simulation problems, such as predicted fecundities below zero. Discarding such values would result in mean values of simulated fecundities higher than the mean of the normal distribution from which they were simulated. In Ferrer *et al.* (2006) it is unclear how this problem was tackled. Forcing the normal distribution is also unnecessary, as neither HHH nor IAH assume any underlying frequency distribution. The criteria tested by Ferrer *et al.* (2006) involve different expectations on how the shapes of fecundity frequency distributions should change with increasing population fecundity, and not on the characteristics of such distributions at any given time. Our approach thus allows simulations to be closer to actual observations, instead of assuming any arbitrary, theoretical distribution. Furthermore, it allows both mean fecundities and variances to vary without any prior expectation, thereby providing simulation conditions closer to natural populations (Moreno *et al.* 2003).

SIMULATIONS UNDER HABITAT HETEROGENEITY AND INDIVIDUAL ADJUSTMENT HYPOTHESES

Simulation conditions for HHH and IAH were as similar as possible to that adopted by Ferrer *et al.* (2006), although assuming the breeding parameters of Bonelli's eagles in southern Portugal. Simulations were run for populations growing over time from 10 to 30 pairs, and for populations stable at both 15 and 30 pairs. Under HHH, populations included a group of high-quality territories with mean fecundity (± SD) of 1·23 ± 0·74, and a group of poor-quality territories with mean fecundity of 0·28 ± 0·53. Breeding parameters for good and poor territories were derived from brood size distributions observed in 1992–2006 for the 10 eagle territories with the highest (juveniles: 0 = 17·9%; 1 = 41·1%; 2 = 41·1%; $n = 95$ breeding events) and the lowest (juveniles: 0 = 76·0%; 1 = 20·0%; 2 = 4·0%; $n = 75$ breeding events) fecundities, respectively. Good-quality territories were assumed to remain so from low to high population densities, hence implying that fecundity declines with increasing population size resulted from the progressive occupation of poor territories. In increasing populations, it was assumed that good territories were occupied first, while poor territories were occupied only when there were no good territories available. In stable populations the proportions of good and poor territories were assumed to remain constant over time. Simulations for increasing and stable populations were run considering a ratio between good : poor territories of both 1 : 2 and 2 : 1.

Under IAH, fecundity declines in increasing populations were assumed to be similar across all territories. Simulations thus started with a brood size distribution comparable to that of good territories under HHH, with the frequency of each class changing linearly thereafter until reaching a final value corresponding to poor territories. For stable populations, simulations were run assuming each year the global brood size distribution recorded for the entire Bonelli's eagle population in 1992–2006.

STATISTICAL ANALYSES

Relationships between population sizes, mean fecundities, coefficients of variation and skewness were evaluated using Pearson correlations, screening the data beforehand with scatterplots to detect the presence of outliers or influential points (Legendre & Legendre 1998). Data screening was conducted in each case using the simulation closest to the mean of all replicates. In simulations, 95% confidence intervals of coefficients of correlation were computed based on percentiles of the corresponding bootstrap distributions (Efron & Tibshirani 1993).

Results

BONELLI'S EAGLE POPULATION

During the 15-year study, mean fecundity of the Bonelli's eagle population declined from 1·12 ± 0·64 to 0·70 ± 0·75 fledged juveniles per pair (Fig. 1), showing an inverse relationship with population density ($r = -0{\cdot}61$, $P < 0{\cdot}05$). The tendency for increasing over time was not significant for the CV ($r = 0{\cdot}42$, $P > 0{\cdot}10$), but it was nearly significant for skewness ($r = 0{\cdot}49$, $P < 0{\cdot}10$) (Fig. 1). Nevertheless, both these parameters showed a very strong negative relationship with mean fecundity (Fig. 2).

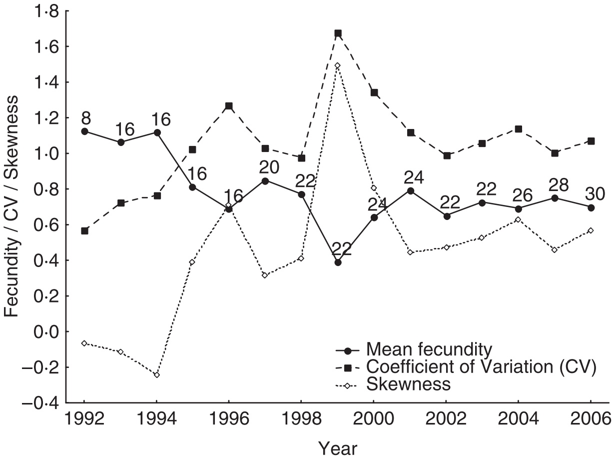

Fig. 1. Temporal variation in mean fecundity, coefficient of variation (CV) and skewness in an increasing Bonelli's eagle population in south-western Portugal (1992–2006). The number of pairs monitored each year are given above the mean fecundity line.

MODEL POPULATIONS

Strong negative relationships between mean fecundity and both its CV and its skewness were found in simulations under both HHH and IAH (Table 1). Furthermore, these results were consistent for all simulation conditions, including increasing and stable populations, different population sizes, short and long simulation periods and high and low ratios of good to poor territories (Table 1). Analysis of scatterplots suggested that these relations were very consistent, and not a consequence of odd outliers or influential points, holding over the entire range of fecundities tested (Fig. 3). Overall, in no case did the relationships between mean fecundity and the metrics used show consistent differences between HHH and IAH in simulated populations.

Discussion

This study showed that strong negative relationships between mean fecundity and both its CV and its skewness can be produced by model populations simulated under both HHH and IAH. This suggests that these parameters may not be adequate to investigate the mechanisms generating density-dependent declines in fecundity, contrary to the contention of previous studies (Ferrer & Donázar 1996; Ferrer *et al.* 2006).

The limitations of the CV criteria have been reported previously by Ferrer *et al.* (2006), who also found negative relationships between mean fecundity and its CV in model populations simulated under the two regulatory mechanisms.

Table 1. Mean correlations and 95% confidence intervals, between mean fecundity and coefficient of variation (CV) and skewness, in model populations simulated under different hypothesis of density-dependent fecundity [habitat heterogeneity hypothesis (HHH) vs. individual adjustment hypothesis (IAH)], population trends (increasing vs. stable), number of breeding pairs (Pairs), fecundities of good and poor territories (Fecundity) and ratios of good: poor-quality territories (Good : poor)

					CV		Skewness	
Hypothesis	Pairs	Fecundity	Years	Good : poor	*r*	95% CI	*r*	95% CI
HHH, increasing	10–30	1·23–0·28	30	10 : 20	–0·956	(–0·980, –0·913)	–0·984	(–0·999, –0·886)
HHH, increasing	10–30	1·23–0·28	30	20 : 10	–0·928	(–0·970, –0·851)	–0·909	(–0·990, –0·702)
IAH, increasing	10–30	1·23–0·28	30	–	–0·949	(–0·977, –0·881)	–0·978	(–0·997, –0·929)
HHH, stable	30	1·23–0·28	30	10 : 20	–0·953	(–0·977, –0·914)	–0·996	(–0·999, –0·988)
HHH, stable	30	1·23–0·28	10	10 : 20	–0·953	(–0·977, –0·914)	–0·996	(–0·999, –0·988)
HHH, stable	30	1·23–0·28	30	20 : 10	–0·931	(–0·969, –0·868)	–0·999	(–1·000, –0·997)
HHH, stable	30	1·23–0·28	10	20 : 10	–0·931	(–0·969, –0·864)	–0·999	(–1·000, –0·997)
HHH, stable	15	1·23–0·28	30	5 : 10	–0·935	(–0·970, –0·878)	–0·987	(–0·998, –0·939)
HHH, stable	15	1·23–0·28	10	5 : 10	–0·936	(–0·971, –0·874)	–0·987	(–0·998, –0·936)
IAH, stable	30	0·75	30	–	–0·958	(–0·978, –0·932)	–0·999	(–1·000, –0·996)
IAH, stable	30	0·75	10	–	–0·957	(–0·989, –0·900)	–0·999	(–1·000, –0·997)
IAH, stable	15	0·75	30	–	–0·945	(–0·975, –0·897)	–0·994	(–0·999, –0·969)
IAH, stable	15	0·75	10	–	–0·953	(–0·990, –0·849)	–0·997	(–1·000, –0·988)

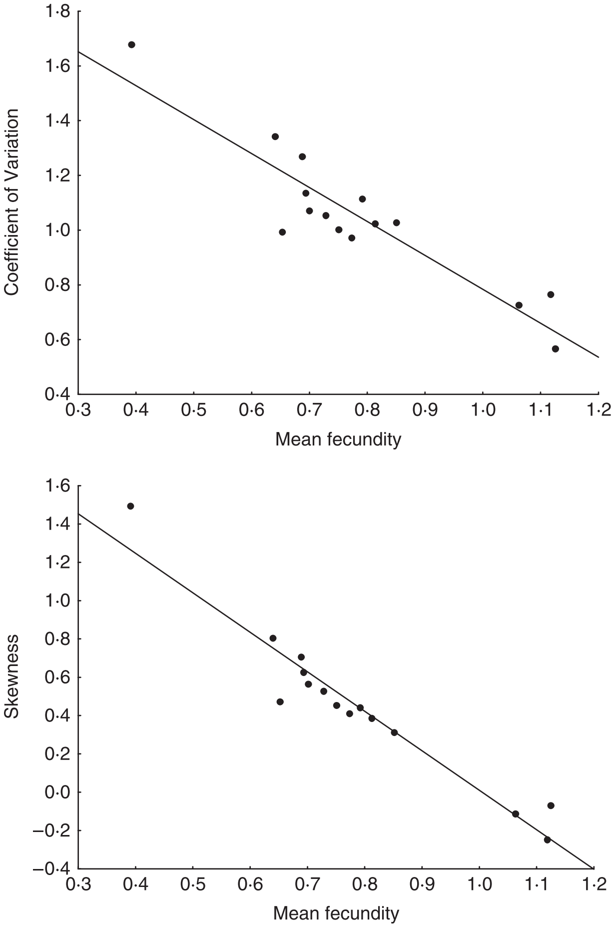

Fig. 2. Linear regressions between coefficient of variation and mean fecundity and between skewness and mean fecundity, in an increasing Bonelli's eagle population in south-west Portugal (1992–2006).

They suggested that this resulted from spurious correlations in populations simulated under IAH, due to the low number of brood-size categories, limited by fixed boundaries (Ferrer *et al.* 2006). More generally, however, it should be noted that spurious correlations might be expected because this is a case of correlation between two non-independent variables of the form X vs. Y/X. Simulations studies have shown that regressions of this form are almost always negative, producing spurious coefficients of determination > 0·50 (Brett 2004). This, together with our results and those of Ferrer *et al.* (2006), suggests that the relationship between mean fecundity and its CV should not be used to differentiate between density-dependent fecundity hypotheses. By and large, caution should be taken when interpreting results of previous studies relying on this relationship to draw inferences about population processes (e.g. Ferrer & Donázar 1996; Blanco *et al.* 1998; Krüger & Lindström 2001; Penteriani *et al.* 2003; Sergio & Newton 2003; Carrete *et al.* 2006a,c).

The skewness criteria also proved inadequate to unravel the mechanisms producing density-dependent variation in fecundity, in marked contrast with the results of Ferrer *et al.* (2006). This was related probably to differences in brood-size frequency distributions used to simulate fecundity of model populations. Breeding outputs in the study of Ferrer *et al.* (2006) were drawn randomly from normal distributions with a constant variance despite changes in mean fecundity, whereas simulations in our study were based on empirical distributions of brood sizes. These empirical distributions were close to Poisson distributions, which probably caused the observed negative relationships between mean fecundity and skewness. Indeed, a Poisson distribution with mean μ has the positive skewness $\gamma = 1/\sqrt{\mu}$, implying that $\gamma \rightarrow 0$ when $\mu \rightarrow \infty$ (Kreyszig 1970), without the need to invoke any underlying biological mechanism. This suggests that in many long-lived species with low fecundity, as those most commonly used as models to test HHH (e.g. Kokko *et al.* 2004; Carrete *et al.*

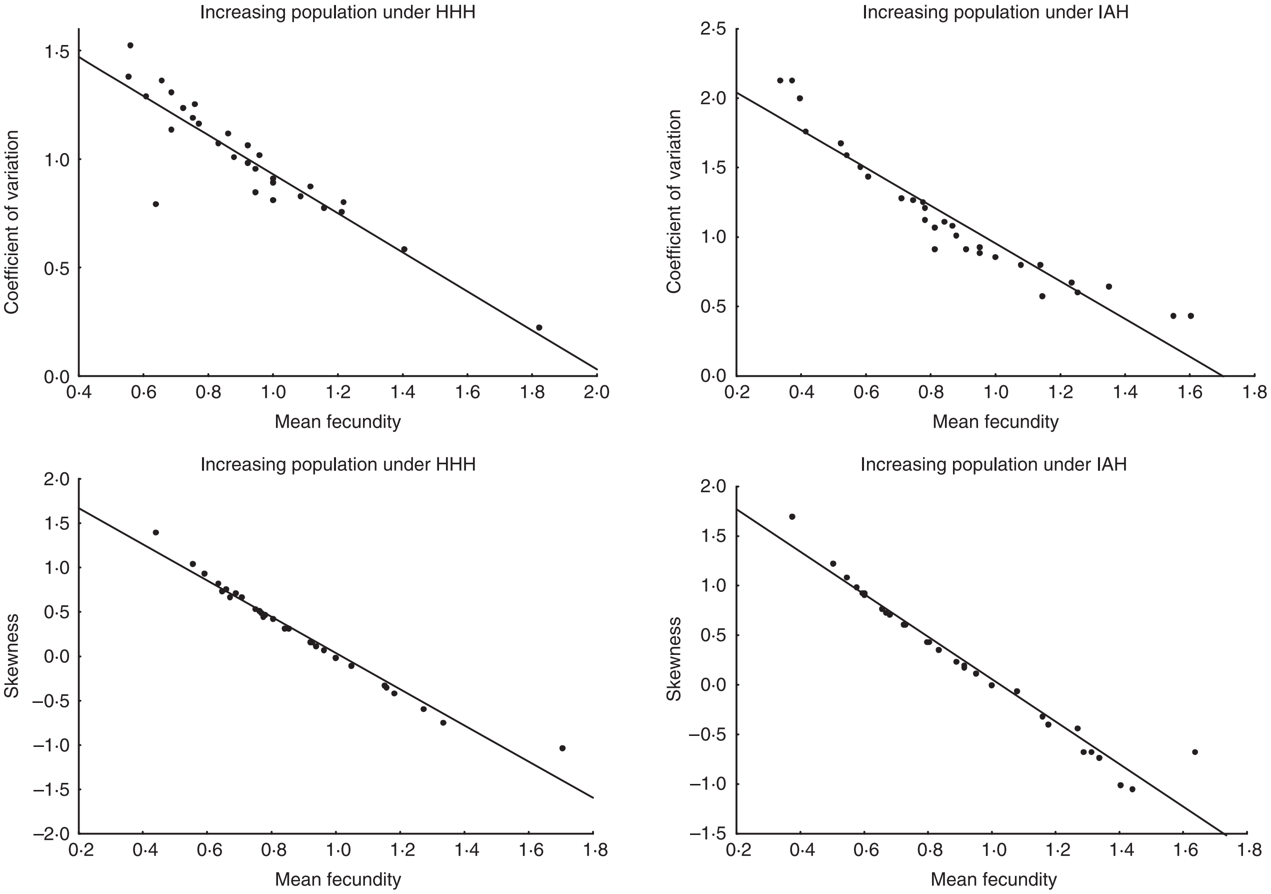

Fig. 3. Linear regressions between coefficient of variation and mean fecundity and between skewness and mean fecundity, in increasing simulated populations according to habitat heterogeneity (30 years; 30 pairs; 10 good: 20 poor territories) and individual adjustment (30 years; 30 pairs) hypothesis.

2006a,c; Ferrer *et al.* 2006), the inverse relationship between the mean and its skewness may be inevitable due to left-skewed and lower-bounded fecundity distributions. The approach by Ferrer *et al.* (2006) thus seems inadequate under at least some plausible ecological circumstances, thereby suggesting that care should be taken when using it to evaluate mechanisms generating density-dependent fecundity.

Given these limitations, the patterns observed in the Bonelli's eagle population of southern Portugal could not be used to infer any mechanism of density-dependent fecundity. Although the strong negative relationships between mean fecundity and both its CV and its skewness would be strongly supportive of HHH following previous criteria (Ferrer & Donázar 1996; Ferrer *et al.* 2006), the simulation study showed clearly that similar relationships could have emerged from a population under IAH. Recognizing the potential for spurious correlations may help to reconcile apparently contradictory observations recorded in other studies. For instance, Carrete *et al.* (2006c) found strong negative relationships between mean fecundity and its coefficient variation for two Bonelli's eagle and two golden eagle *Aquila chrysaetos* populations, although additional data suggested that habitat heterogeneity was unimportant in driving breeding success. Indeed, variation in productivity among territories appeared to be driven primarily by parental age and local breeding density of both species (Carrete *et al.* 2006c).

Results from our study suggest that there is at present no simple and reliable criterion to differentiate between HHH and IAH in natural populations. Moreover, it may be inadequate to approach density-dependent fecundity by considering only two mutually exclusive mechanisms, as density dependence may result from additional factors such as changes in age structure (Carrete *et al.* 2006c) or mating systems (Carrete *et al.* 2006b), and multiple mechanisms may operate concurrently (Rodenhouse *et al.* 2003; Carrete *et al.* 2006a,b). Therefore, understanding mechanisms of density-dependent fecundity probably requires detailed, long-term studies combining demographic and ecological data, documenting at least the following aspects (Ferrer & Donázar 1996; Rodenhouse *et al.* 2003; Kokko *et al.* 2004; Carrete *et al.* 2006a,b,c; Ferrer *et al.* 2006): (i) the relationship between population density and fecundity, controlling statistically for potentially confounding factors such as habitat degradation, resource depletion, climate change and human impacts; (ii) the spatial patterns of fecundity variation over time, identifying whether some sites are consistently better than others; (iii) the spatial patterns of site occupancy, evaluating whether the best sites are the first and most frequently occupied sites; and (iv) the factors determining site quality, using statistical modelling to evaluate the relative role of heterogeneity in ecological conditions, interference among conspecifics and potential competitors, mating systems and age structure. Although taking this combination of approaches may be difficult in most cases, recent studies have demonstrated its feasibility and usefulness in disentangling complex sets of factors interacting to determine density-dependent changes in fecundity (e.g. Carrete *et al.* 2006a,b,c). It is unlikely that a simpler and generally applicable alternative can be provided by any synthetic metric.

Acknowledgements

We are grateful to Miguel Pais, José Raiado, Carlos Noivo, Pedro Portela, Rita Alcazar, Francisco Faria, Paulo Cabrita and João Ferreira for collaboration in field work. We thank Steve Redpath, Erkki Korpimaki, Martina Carrete, Maria Filomena Magalhães, José Tella and two anonymous referees for comments on the manuscript. The field study was supported by the Institute for Nature Conservation and the Portuguese Science Foundation (PRAXIS/BIA/132/96).

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Received 26 November 2006; accepted 13 August 2007
Handling Editor: Ken Norris

Capítulo 8. Conclusões

Selecção de habitat do coelho-bravo

O estudo realizado sobre a selecção de habitat do coelho-bravo, uma das presas fundamentais da águia de Bonelli na Península Ibérica, demonstra que, nas serras do sudoeste de Portugal, a espécie parece ser favorecida por mato dominado por *Cistus ladanifer* (esteval), com vegetação lenhosa pouco densa ao nível do solo mas com cobertura densa no nível superior, e com uma abundante cobertura herbácea tanto nas margens como no interior das manchas de mato. Ao contrário, a maioria dos outros tipos de coberto arbustivo da região, incluindo todos os matos de arbustos ericoides (e.g. *Erica australis*, *Erica umbellata* e *Calluna vulgaris*), incluindo o matagal arborescente de *Arbutus unedo/Erica arborea* (medronhal) são evitados pela espécie. Estes padrões genéricos são aplicáveis a toda a região de terras altas do Sudoeste do país (Sudoeste Serrano) e provavelmente a outras paisagens do Sul do país dominadas por extensas áreas de esteval.

Diversas hipóteses não-exclusivas poderão explicar a associação observada entre o coelho-bravo e a esteva. Por um lado, as cápsulas desta planta, em particular, são ricas em conteúdo proteico e lipídico e poderão fornecer um recurso crítico, pelo menos durante o verão, quando a disponibilidade de plantas herbáceas e o valor nutricional das pastagens atingem o seu mínimo. De facto, alguns estudos recentes confirmaram um elevado consumo de esteva pelo coelho-bravo.

A associação com o esteval pode dever-se igualmente ao estrato herbáceo, cuja cobertura é maior sob a esteva do que nas restantes formações arbustivas, provavelmente devido à menor densidade lenhosa do esteval. Isto permitirá aos coelhos alimentarem-se no interior do mato durante o dia, evitando o maior risco de predação existente no exterior.

Finalmente, a preferência marcada pelo esteval poderá dever-se às suas características favoráveis como factor de protecção anti-predatória. Numa área em que a natureza do solo não permite a escavação de túneis, o coberto arbustivo constitui a única protecção contra os predadores, mas um mato demasiado denso pode tornar-se inseguro por obstruir a percepção visual da aproximação de mamíferos carnívoros e obstruir a fuga, como sugerem as respostas à densidade do coberto observadas, negativa ao nível do solo e positiva em relação à camada superior. A estrutura do esteval, denso ao nível superior e relativamente esparso no nível inferior fornece um compromisso adequado de protecção simultânea contra predadores alados e terrestres.

Em termos de gestão do coberto arbustivo para manutenção e reforço das populações de coelho como presa preferencial da águia de Bonelli na área de estudo, estas constatações têm grande relevância. Em geral, as formações arbustivas mais favoráveis serão as que aliam, a um coberto denso na camada superior mas permeável ao nível basal, um estrato herbáceo relativamente desenvolvido e uma espécie arbustiva que ofereça recursos alimentares durante a época crítica do verão.

Estudo da dieta

O estudo da dieta revelou que, no período estudado, as presas domésticas desempenhavam um papel fundamental na dieta das águias de Bonelli no sudoeste de Portugal, principalmente em resultado do elevado consumo de pombos rurais. No entanto, pombos correios e aves de capoeira também foram consumidos com frequência. Os pombos, em particular os pombos rurais, são mais importantes na dieta desta população que na maioria das populações europeias de águia de Bonelli. As presas domésticas são particularmente importantes no início da época de reprodução quando representam mais de metade da dieta.

No entanto, em termos gerais, as presas selvagens, tais como coelhos, perdizes e gaios, constituem uma componente relevante da dieta na maioria dos territórios. Em conjunto com diversas outras espécies de aves selvagens, aquelas espécies tornam-se progressivamente mais representadas na dieta para o final da época de reprodução, enquanto que se verifica simultaneamente um decréscimo na utilização de pombos, tanto rurais como correios. Esta alteração é explicável pelo aumento progressivo da disponibilidade de presas de elevada proficuidade, como coelhos e aves jovens conforme evolui a época de reprodução das várias espécies.

Neste estudo, nenhuma das hipóteses usualmente invocadas para explicar taxas elevadas de predação sobre espécies domésticas pôde ser inteiramente suportada. A hipótese de especialização alimentar (PAH - *problem animal hypothesis*) pode ser claramente rejeitada, pois a predação sobre espécies domésticas é generalizada e não restrita a alguns casais. Ao contrário, as outras duas hipóteses ganham suporte parcial, sugerindo que a elevada predação sobre espécies domésticas no sudoeste de Portugal pode resultar da escassez das presas selvagens (usualmente preferidas pela espécie na Europa, como coelhos e perdizes) de acordo com a hipótese APH (*alternative prey hypothesis*), e de uma selecção positiva de presas domésticas, segundo a hipótese VPH (*vulnerable prey hypothesis*). Ao contrário da marcada escassez de coelhos e das baixas densidades de perdizes que se verificam na área de estudo no período analisado, os pombos rurais eram muito abundantes ao longo de todo o ano, tal como os pombos correio entre Janeiro e Abril.

Os territórios com elevada cobertura de eucaliptal são os que apresentam menor abundância de presas, tanto selvagens como domésticas. Nestes, o reduzido consumo de perdizes e pombos é compensado por um incremento na utilização de presas secundárias, resultando num aumento da diversidade trófica. De facto, tanto os coelhos como as perdizes evitam os eucaliptais, ao mesmo tempo que o abandono rural que lhes está associado provoca uma acentuada escassez de pombos rurais.

Embora as presas domésticas compensem a reduzida abundância das principais presas selvagens, não se verifica uma mudança (*switching*) no sentido tradicional. Claramente, os coelhos são uma presa primária, seguindo uma pronunciada resposta funcional de tipo II, com o consumo mais elevado de pombos rurais e correios correspondendo às menores densidades de coelho-bravo.

No entanto, as águias também apresentam uma resposta funcional de tipo II em relação aos pombos rurais, embora bastante menos intensa que no caso dos coelhos, e não a curva sigmoide expectável se ocorresse *switching*. Existe uma correlação negativa entre o consumo de coelhos e a abundância de pombos rurais, assim como evidência da selecção entre o pombo doméstico e o coelho-bravo ser inversamente denso-dependente. Assim, os pombos foram predados mais frequentemente do que o esperado com densidades elevadas de coelho, o mesmo ocorrendo para os coelhos com altas densidades de pombos. Isto poderá dever-se ao papel crítico dos pombos domésticos no início da reprodução, quando as presas selvagens aparentam ser menos abundantes.

Assim, os pombos rurais devem ser encarados como presas complementares aos coelhos e perdizes, em vez de uma simples presa alternativa, só consumida em situações de escassez das presas selvagens primárias. Por seu lado, os pombos correios são provavelmente capturados oportunisticamente devido à sua elevada vulnerabilidade.

É provável que a composição da dieta deste núcleo populacional tenha sofrido alterações marcadas desde a realização deste estudo, na medida em que se verificaram, desde então, alterações significativas na disponibilidade, pelo menos, de duas das presas fundamentais. De facto verificou-se um declínio de 74% no número de pombos rurais entre 1994 e 2008 (A. Dias, dados não publicados) e um aumento significativo na abundância da perdiz, na sequência da conversão generalizada do regime cinegético da área de estudo, de regime livre para zonas de caça especial (associativa, turística e municipal) desde a realização do estudo da dieta. A confirmar-se uma alteração significativa na dieta da população, este facto poderá levar a uma diferente perspectiva da ecologia trófica desta população.

Ecotoxicologia

As penas das presas aladas utilizadas no estudo da dieta foram também utilizadas para analisar os níveis de contaminação por mercúrio, à semelhança das penas de mudas dos adultos de águia de Bonelli. Nestas últimas, a grande variação dos níveis de mercúrio detectados parece reflectir primariamente as diferenças na dieta individual dos adultos e a bioacumulação através da cadeia trófica. As maiores concentrações apareceram nos casais cuja dieta contém uma elevada proporção de consumidores secundários, enquanto que níveis muito mais baixos encontram-se em territórios onde a dieta se baseia quase exclusivamente em espécies herbívoras, como coelhos, pombos e perdizes. Existe uma forte correlação entre as concentrações de Hg nas águias e nos gaios, o que não se verificou em relação aos pombos e perdizes. Este facto dever-se-á aos níveis muito baixos de Hg nas presas herbívoras, sugerindo que as águias ingerem o mercúrio sobretudo através de consumidores secundários.

Desde que sejam tomados em consideração os efeitos da dieta, a variação das concentrações de Hg nos diversos casais deverá reflectir pelo menos parcialmente o padrão espacial genérico de contaminação ambiental. Este padrão concorda em geral com a hipótese de uma fonte contaminadora no complexo industrial de Sines, presumivelmente associada à central térmica a carvão. Há uma tendência geral para níveis mais altos na zona montanhosa ocidental imediatamente a sotavento do complexo industrial (Serras do Cercal e Monchique) como tal provavelmente mais susceptíveis à contaminação por poluentes aéreos do que as serras orientais (Caldeirão). Por outro lado, a precipitação é em geral bastante mais alta nas serras costeiras ocidentais do que mais para o interior, o que pode potenciar a remoção e deposição do mercúrio atmosférico. Embora, em geral, os níveis encontrados nas águias se possam considerar baixos, os valores mais altos são susceptíveis de impactos adversos na fecundidade. Os valores de Hg nas penas analisadas estavam correlacionados com os níveis encontrados em 13 ovos inviáveis, colectados em 10 dos territórios estudados, tendo-se verificado uma forte relação linear entre os níveis de Hg nas penas e ovos. Porém, apenas 2 (9,5%) dos 21 casais estudados apresentavam concentrações de Hg nas penas acima do valor correspondente ao limite mínimo de concentração nos ovos que está associado a deformidades embrionárias.

Sexagem de juvenis

As análises demográficas e genéticas de uma população dependem de uma correcta sexagem dos indíviduos, que apresenta dificuldades no caso de aves não-adultas. O estudo realizado permitiu validar, por métodos moleculares, protocolos de sexagem morfométrica, aplicáveis no terreno.

O menor sucesso do método de sexagem molecular M2 (1 presumível erro e 6 brancos) em relação ao método M1 (1 branco), pode ser parcialmente atribuído à inferior qualidade das amostras analisadas, que foram congeladas e descongeladas repetidas vezes antes da extracção e amplificação do ADN. No entanto, a diferença observada também se pode dever às próprias técnicas, indicando que M2 será menos robusto que M1 devido a uma menor taxa de perfis de migração interpretáveis e provavelmente menos fiável também, de acordo com o falso resultado obtido. M2 é globalmente mais rápido e menos dispendioso mas menos eficiente que M1.

Em relação à sexagem morfométrica, este estudo demonstrou claramente a existência de um dimorfismo sexual marcado na maioria das medidas corporais externas das crias de águia de Bonelli de 35-50 dias de idade. O peso, tanto isoladamente como em combinação com outras variáveis, forneceu a chave de sexagem mais consistente. Este parâmetro deve ser utilizado com precaução porque a massa corporal é altamente variável, dependendo de uma série de factores, mas a diferença entre os sexos tende a prevalecer sobre o fundo de variabilidade natural. Apenas um indivíduo não foi correctamente sexado por nenhuma das funções discriminantes que incluíam a massa, mas tratou-se de uma fêmea anormalmente leve, devido a uma infecção grave por *Trichomonas gallinae*. No entanto, este indivíduo foi correctamente sexado pela função discriminante D2, sugerindo que mesmo indivíduos doentes podem ser sexados com base em critérios morfométricos. Os resultados do estudo demonstram que a morfometria externa pode ser usada para sexar crias de águia de Bonelli de 35-50 dias. Ambas as equações discriminantes devem ser calculadas em cada indivíduo, excepto no caso de aves subnutridas ou doentes, em que a função D1 não deve ser usada. Sempre que possível, a sexagem molecular deve ser usada em paralelo com a morfométrica, de forma a reduzir a margem de erro.

Patologias

A partir das análises clínicas efectuadas a crias de águia de Bonelli foram isolados três potenciais patógenos, dois dos quais em lesões presentes no tracto digestivo superior que resultaram na morte de três aves. O acompanhamento da mortalidade nidícola ao longo do período posterior à realização deste estudo revelou que entre 1992 e 2006, a mortalidade média devido à tricomoníase foi de 7,26%/ano (± 6,10; 0 - 21,74) das crias nascidas. No entanto, durante o mesmo período, as mortes por tricomoníase representaram em média 38,1% (± 26,07; 0 – 83,33) da mortalidade nidícola total anual (dados pessoais não publicados).

Os pombos domésticos constituem reservatórios potenciais destes agentes patogénicos. Em particular, *Trichomonas* spp. causam alterações patológicas bem conhecidas em aves de rapina que consomem habitualmente pombos domésticos. Lesões e mortalidade relacionadas com *Trichomonas gallinae* em águias de Bonelli foram inicialmente observadas em 1994 em populações da Catalunha que, tal como na presente população estudada, também dependem do pombo doméstico em mais de 30% da dieta. Em contraste com a anterior situação, em populações cujas presas fundamentais são espécies selvagens como a perdiz-vermelha *Alectoris rufa* e o coelho-bravo *Oryctolagus cuniculus*, não foram encontradas lesões.

A mortalidade causada pela doença afecta negativamente o sucesso reprodutor. O facto de, em alguns casos, o problema parecer limitado a uma região ou a um período, pode indicar que os surtos infecciosos podem dever-se a estirpes virulentas de *T. gallinae*. O isolamento de *Candida albicans* ou outras *Candida* spp. em culturas puras sugere a relação deste potencial patógeno com as lesões observadas, embora não seja possível assegurar se se trata de um patógeno secundário ou primário no desenvolvimento de casos graves de tricomoníase, pois ambos os patógenos foram isolados tanto sozinhos como em combinação, tanto em crias que apresentavam lesões ligeiras como graves.

Demografia

No que respeita aos aspectos demográficos, o estudo mostrou que fortes correlações negativas entre a fecundidade média e os respectivos coeficiente de variação (CV) e declive (*skewness*) podem ser produzidos por populações modelo simuladas, tanto sob a hipóteses HHH (*habitat heterogeneity hypothesis*), como a IAH (*individual adjustment hypothesis*). Isto sugere que estes parâmetros podem não ser os adequados para investigar mecanismos geradores de declínios de fecundidade denso-dependentes, contrariamente ao defendido em estudos anteriores. Face a estas limitações, do observado na população de águia de Bonelli do Sul de Portugal, não se pode inferir qualquer padrão de fecundidade denso-dependente.

O reconhecimento da eventualidade de correlações ilegítimas, pode ajudar a reconciliar observações aparentemente contraditórias registadas noutros estudos. Os resultados do presente estudo sugerem que não existe actualmente nenhum critério fiável para diferenciar HHH de IAH em populações naturais. Para além disso, pode ser inadequado abordar a fecundidade denso-dependente considerando unicamente dois mecanismos que se excluem mutuamente, pois a denso-dependência pode resultar de outros factores e podem existir mecanismos múltiplos a operar em simultâneo.

Assim, a compreensão dos mecanismos de fecundidade denso-dependente requer provavelmente estudos detalhados de longo termo que combinem dados demográficos e ecológicos e que documentem pelo menos: *i.* a relação entre a densidade populacional e a fecundidade, controlando estatisticamente em relação a factores potencialmente perturbadores; *ii.* os padrões espaciais da variação da fecundidade ao longo do tempo; *iii.* os padrões espaciais de ocupação espacial e *iv.* os factores que determinam a qualidade do sítio.

Implicações de conservação e perspectivas de investigação

No período estudado, o pombo doméstico de origem rural e o coelho-bravo constituiram as duas presas mais importantes na dieta da população de águia de Bonelli do Sudoeste Serrano. O pombo rural constitui uma presa complementar ao coelho e à perdiz, desempenhando um papel crítico no início da época de reprodução quando as presas selvagens são menos abundantes. No entanto, a importância relativa das presas fundamentais – pombo doméstico, coelho e perdiz – poderá ter-se alterado nos últimos anos, em resultado do forte declínio verificado nas populações de pombo rural, em associação com o despovoamento humano, e do incremento populacional da perdiz, associado às alterações do estatuto de regime cinegético da região. A drástica diminuição da disponibilidade de pombos rurais poderá estar na origem da tendência de atraso fenológico e correspondente decréscimo da produtividade verificadas na população ao longo do período de estudo (dados pessoais não publicados).

Porém, investir na recuperação da população de pombos rurais, embora tecnicamente simples em princípio, não parece realizável no actual contexto socio-económico, a não ser pontualmente em situações particularmente justificáveis. É o caso de territórios com elevada cobertura de eucaliptal, onde a resultante escassez das presas básicas da espécie, revelado pelo incremento das “outras presas” e da diversidade trófica na dieta, poderá conduzir a situações de baixa produtividade crónica. Por seu turno, uma menor disponibilidade de pombos rurais poderá resultar numa tendência de diminuição da prevalência da tricomoníase, tal como parece verificar-se nos últimos anos (dados pessoais não publicados).

Actualmente, aparenta ser mais viável investir em medidas de apoio à gestão cinegética, visando o incremento e a manutenção das populações de perdiz e coelho. Em particular, seria particularmente importante difundir práticas correctas de condução do esteval no contexto da gestão florestal e cinegética, devido à sua relevância para o coelho-bravo por razões tróficas e estratégia anti-predatória. Por seu lado, estas medidas deverão ser acompanhadas de medidas específicas de integração da conservação da espécie na actividade venatória.

No âmbito da investigação de longo-termo sobre a população de águia de Bonelli do Sudoeste Serrano, muito está por fazer nos diversos campos de conhecimento. Caberá em primeiro lugar ressaltar a importância do estudo aprofundado da dinâmica populacional, sobretudo no que respeita ao processo de expansão da população e às suas perspectivas futuras. A continuação e aprofundamento da investigação sobre a genética populacional poderá permitir a verificação de pressupostos relativos à origem, dinâmica e cronologia da expansão demográfica, estabelecer os limites geográficos da população e esclarecer o nível real do seu aparente isolamento em relação às populações vizinhas, com implicações a nível de opções estratégicas de conservação. Seria ainda interessante verificar, através da comparação biométrica interpopulacional, se o suposto isolamento populacional se traduz em diferenciação morfológica da população, como tem sido suspeitado a partir da morfometria realizada em adultos recentemente capturados.

Releva também a necessidade do estudo multi-escala da selecção de habitat desta população, dada a sua ecologia peculiar, estudo que deu os primeiros passos recentemente com a análise das preferências ao nível do micro-habitat de nidificação e cuja compreensão abrangente tem importantes implicações ao nível da conservação, tanto do habitat actualmente ocupado, como em termos da previsão dos habitats e áreas que poderão ser colonizadas ao longo do processo expansivo. A estes aspectos caberá associar-se a continuação dos estudos em curso sobre os movimentos dos adultos territoriais e sua utilização do espaço, através de telemetria de satélite, e suas implicações de conservação.

Um outro campo importante de investigação, ainda incipiente, cujas lacunas seria importante colmatar, é o da epidemiologia da tricomoníase, sobretudo no seio do seu reservatório principal – o pombo doméstico – em relação à transmissibilidade e influência de factores ambientais na sobrevivência do parasita, diversidade genética e virulência das estirpes e interacções com outros patógenos; ou ainda, relativamente ao desenvolvimento de imunidade no hospedeiro, suas relações com os níveis de contaminação com substâncias potencialmente imunossupressoras e prevalência da patologia.