ESTACIÓN BIOLÓGICA
DE DOÑANA

UNIVERSIDAD DE
SEVILLA

DINÁMICA DE UNA POBLACIÓN EN CRECIMIENTO DE AGUILA CALZADA EN EL PARQUE NACIONAL DE DOÑANA: HETEROGENEIDAD DE HABITAT Y AJUSTE INDIVIDUAL

Memoria presentada por Eva Belén Casado para optar al título de Doctor en Biología por la Universidad de Sevilla

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A mi madre, a mi padre
a Jaito y a Vera

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1. Introducción general

1.1 Dinámica poblacional

La dinámica poblacional es el estudio del tamaño de las poblaciones, de su variación en el tiempo y en el espacio, así como de los procesos biológicos y ambientales que condicionan dichas variaciones. El estudio de la dinámica de una población comprende el estudio de su comportamiento social, dispersión, tamaño, movimientos, reproducción y mortalidad (Newton 1979).

Existen evidencias de que el tamaño de las poblaciones de aves territoriales se encuentra limitado de alguna forma (Newton 1979). Las evidencias son: 1- La estabilidad de la población reproductora, tanto en tamaño como en distribución, a lo largo de largos periodos de tiempo siempre y cuando la población estudiada se encuentre en un medio ambiente estable y sin presiones humanas ante las que responder. La estabilidad del tamaño poblacional se alcanza bien mediante la propia producción de jóvenes si es semejante a la tasa de mortalidad adulta, o bien mediante un aporte de individuos provenientes de otras poblaciones. 2- La existencia de un grupo de adultos no reproductores (aquellos que no regentan un territorio) pero capaces de criar en el momento en que encuentren un territorio disponible. 3- El restablecimiento de una población, tras haber sido eliminada por acción del hombre, con un tamaño y distribución similar a la anterior. 4- La distribución espacial regular de los nidos en una población donde la disponibilidad de los mismos no está restringida. Esto indica que los individuos de especies territoriales tienden a asentarse en territorios lo más alejado posible de sus vecinos reduciendo así la interferencia entre ellas.

Estos cuatro argumentos evidencian que la densidad de las poblaciones está limitada, y que este control se produce a través de la competición por los territorios de cría. Los territorios varían en su calidad, o lo que es lo mismo, varían en las oportunidades que ofrecen para el éxito reproductivo.

DENSIDAD POBLACIONAL

El tamaño de una población reproductora en relación al área que ocupa se denomina densidad poblacional. Su estudio requiere el conteo de todos los individuos de una especie dada en un área de estudio concreta a lo largo del tiempo, y cuanto más se prolongue en el tiempo mayor será el valor de la información para poder analizar aspectos previamente referidos (estabilidad de la población en el tiempo, restablecimiento de una población, utilización del mismo territorio por diferentes individuos). Los principales factores naturales que condicionan la densidad poblacional en especies territoriales son el **alimento** y la disponibilidad de lugares **de nidificación** (Newton 1979).

El alimento condiciona la densidad poblacional en aves territoriales en tanto que cuanto mayor suele ser la especie estudiada mayor es el **tamaño** de las especies sobre las que preda y éstas se encuentran a su vez en menores densidades; una mayor **disponibilidad** de alimento en un medio ambiente o área más productivos, así como en un periodo más continuo, permite una mayor densidad de predadores. La densidad poblacional presenta además de la variabilidad espacial, variabilidad estacional o anual por variaciones estacionales o anuales en la disponibilidad de presas, como por ejemplo es el caso de algunos pequeños roedores que sufren explosiones cíclicas en su fecundidad.

La no **disponibilidad** de lugares adecuados para la nidificación conduce a la no ocupación por una especie de áreas por otra parte adecuadas. De hecho, cuando en algún área se han instalado nidos artificiales, se ha logrado la ocupación de la misma por una especie antes inexistente como reproductora.

Otros factores naturales que pueden influir en las variaciones en densidad poblacional son la mortalidad registrada en los cuarteles de invierno en aves migratorias (Newton 1979, Newton 2007) y la emigración o la mortalidad adulta en las áreas de cría (Ferrer & Bisson 2003, Penteriani & Ferrer 2004). Entre los factores no naturales encontramos la persecución humana o el uso de pesticidas.

La tendencia a criar en altas o bajas densidades no está genéticamente fijada en cada individuo, puesto que poblaciones con distintas densidades suelen encontrarse adyacentes y no reproductivamente aisladas. Además, individuos nacidos en áreas de alta densidad han sido encontrados como reproductores en áreas de baja densidad y viceversa (Newton 1979).

A su vez, la densidad poblacional condiciona los parámetros demográficos en cualquier ser vivo porque afecta a la disponibilidad de recursos (Fowler 1987; Francis *et al.* 1992; Sedinger *et al.* 1998). El comportamiento territorial o territorialidad, es el mecanismo que ajusta el tamaño de los territorios, y por tanto la densidad poblacional, en función de su disponibilidad de recursos (Lack 1966, Newton 1979). Este mecanismo actúa al menos de dos formas; por un lado, cuantos menos recursos ofrezca un área mayor será el territorio que necesite una pareja reproductora para cubrir sus necesidades. Y por otro lado, la presencia de otras parejas reproductoras en el área hará que una nueva pareja para asentarse, deba expulsar a una de las ya existentes o por el contrario tenga que desplazarse a otra área.

FECUNDIDAD

La fecundidad es uno de los principales parámetros reproductivos que condicionan la dinámica poblacional (Newton 1991), junto con el tamaño de la población, la mortalidad juvenil, la adulta, la edad de la primera reproducción (Ferrer *et al.* 2004) o el sex-ratio (Ferrer *et al.* 2009).

Para el estudio de la fecundidad en aves, se requiere conocer el número de parejas territoriales (que regentan un territorio) o de parejas reproductoras (que inician la reproducción con la puesta de huevos), así como el número de pollos que logran criar hasta que abandonan el nido. El éxito reproductivo o fecundidad, puede expresarse como el número de jóvenes volados del nido por pareja territorial, o por pareja reproductora o por pareja que logra criar al menos un pollo. Se ha considerado que la mejor medida es el número de pollos volados por pareja territorial, puesto que tiene en cuenta todo tipo de causas que conducen al fallo en la reproducción incluyendo la no-puesta de huevos (Newton 1979).

Los seres vivos tienden a maximizar su fecundidad y con ello la tasa de propagación de sus genes en las siguientes generaciones (Darwin1859). En un ambiente heterogéneo se espera que los animales maximicen su tasa reproductiva mediante su distribución espacial (Pulliam 1996). Bajo el modelo de selección de hábitat llamado "Distribución Libre Ideal", los individuos son libres de de asentarse donde quieran y se distribuyen de tal forma que su tasa reproductiva sea igual en todos los hábitats (Fretwell & Lucas 1970; Bernstein *et al.* 1991). Sin embargo, hay evidencias de que el éxito reproductivo de las aves difieren entre hábitats a múltiples escalas (e.g., Donovan *et al.* 1995; Dias 1996; Donovan *et al.* 1997; Huhta *et al.* 1998; Tewksbury *et al.* 1998). Una posible explicación proviene del modelo de selección de hábitat "Distribución Despótica Ideal" (Fretwell & Lucas 1970; Bernstein *et al.* 1991). Bajo este modelo, los individuos dominantes seleccionan preferentemente los mejores hábitats, forzando a los subordinados a asentarse en hábitats más pobres, y los parámetros reproductivos variarán concordantemente (Andrén 1990; Ferrer & Donázar 1996, Huhta *et al.* 1998, Ferrer *et al.* 2006).

La incorporación de nuevas parejas reproductoras puede ocasionar el descenso de la fecundidad media de la población. Dependiendo del modelo de selección de hábitat que emplee una especie, variará el mecanismo por el que fluctúe la fecundidad de una población en función de su densidad. El descenso en la fecundidad se puede producir bien porque un mayor número de parejas territoriales supone un incremento en las interferencias entre ellas, o bien porque las nuevas parejas se ven relegadas a territorios de peor calidad. Estos dos mecanismos para generar fecundidad denso-dependiente se han formulado tradicionalmente como Ajuste Individual el primero (Lack 1954, 1966, Fretwell & Lucas 1970, Haller 1996, Both 1998, Fernandez *et al.* 1998), y Heterogeneidad de Hábitat el segundo (Andrewartha & Birch 1954, Pulliam & Danielson 1991, Dhondt *et al.* 1992, Ferrer & Donázar 1996, Rodenhouse *et al.* 1997, Krüger & Lindström 2001, Horne & Fielding 2002). Ambos mecanismos pueden actuar al mismo tiempo en diferentes escalas (Rodenhouse *et al.* 2003).

REDUCCIÓN DE NIDADA

Algunos individuos, pertenecientes a cualquier grupo animal, producen un número de crías que posteriormente no son capaces de mantener hasta su independencia. Por ejemplo, algunas aves realizan la puesta de huevos en un momento en que no pueden predecir la disponibilidad futura de los recursos necesarios, de modo que el tamaño de puesta puede ser optimista (Lack 1947, Mock & Forbes 1995). Esto puede conducir al fallecimiento de una o más crías lo que recibe el nombre de reducción de nidada. A menudo se ha registrado mortalidad en el nido por agresiones entre las crías sin intermediar falta de recursos (Simmons 1988). La varianza en la frecuencia de reducción de nidada dentro de una población influye en la fecundidad de dicha población.

SEX RATIO

El sex ratio es la proporción de sexos que se registra en una población, tanto en el momento del nacimiento (sex ratio primario) como el resultante en el momento de la independencia de los individuos tras el periodo de cuidado parental (sex ratio secundario).

En las especies con dimorfismo sexual en el tamaño corporal tiene especial interés el estudio del sex ratio. El sexo de cualquier individuo viene determinado genéticamente y en la mayor parte de los animales la proporción del sex ratio secundario es 1:1 (Newton 1979). La explicación para esta igualdad la dio por primera vez Fisher (1930), y la basaba en la acción de la selección natural sobre los padres y los sexos de las crías que producían. Si suponemos que

la mayoría de las crías que se producen en un momento dado son hembras, es probable que un padre que produzca solo hijos deje más descendientes que un padre que produzca solo hijas o una mezcla de hijos e hijas, ya que los machos son escasos en la población. Así, cualquier rasgo que conduzca a la producción de hijos será perpetuado por la selección hasta que haya un exceso de machos en la población, momento en que la tendencia a producir hijas será favorecida. Por lo tanto, siempre que el sex ratio se desvíe de la unidad la selección tenderá a restaurarla y el único sex ratio estable a largo plazo es el 1:1. Esto no significa que todos los padres tengan el mismo número de hijos que de hijas, sino que hay las mismas probabilidades de producir uno u otro sexo y así se producirá similar número en toda la población.

Fisher argumentó que en realidad lo que sería igual no era el número de hembras y machos sino la inversión parental. Cuando los sexos son del mismo tamaño los padres generarán el mismo número de hijos que de hijas. Pero cuando los sexos difieren en el tamaño, la misma inversión puede ir dirigida a criar más crías del sexo menor o menos crías del sexo de mayor tamaño. También hay que considerar la mortalidad durante el periodo de cuidado parental pues puede ser mayor en un sexo que en otro. Entonces el sex ratio primario debería estar sesgado a favor del sexo que sufre mayor mortalidad para compensarla y que al final quede un sex ratio igualado. Cualquier mortalidad diferencial que pudiese ocurrir después de la independencia de los jóvenes no tienen relevancia para el sex ratio primario (Newton 1979).

La proporción de sexos en una nidada o camada condiciona la fecundidad del territorio mientras que el sex ratio de una población condiciona su dinámica poblacional.

1.2 El águila calzada

El águila calzada (*Hieraaetus pennatus*, Gmelin 1788) cría desde el Suroeste de Europa y Noroeste de África, hasta Asia central, Noreste de Mongolia y el Lago Baikal, pasando por Europa del Este, Asia Menor y el Caúcaso. También cría en Provincia del Cabo (Sudáfrica) y quizá en Namibia (del Hoyo *et al.* 1994).

Es un accipitriforme de mediano tamaño, con comportamiento territorial y marcado dimorfismo sexual (machos:hembras, 709:975 g- del Hoyo *et al.* 1994- 685:984 g –datos propios- n= 22 y n= 29 respectivamente). El plumaje no es uniforme entre todos los individuos, dándose una fase oscura y otra clara, pasando por varias intermedias. El 13,8% de los 87 adultos capturados en el Parque Nacional de Doñana pertenecieron a fase oscura (datos propios). Los juveniles al igual que los adultos son polimórficos, y apenas se puede distinguir la edad de los individuos por el plumaje.

La especie presenta carácter migrador. Tras la época reproductora, a lo largo del mes de septiembre, migra a través del Estrecho de Gibraltar hacia sus cuarteles de invierno en el África sub-sahariana. Aquellos individuos que fallan en la reproducción pueden iniciar la migración antes, y de hecho se han registrado individuos que abandonaron el área de cría desde mediados de agosto, aunque la mayor parte lo hizo en la segunda quincena de septiembre (datos propios). Los individuos van regresando a las áreas de cría entre marzo-abril y un mes más tarde de su llegada las hembras realizan la puesta de los huevos; normalmente 2 aunque a veces también 1 y ocasionalmente 3. En la población estudiada, los individuos regresaron de África entre el 22 de febrero y el 11 de abril, aunque la mayoría de las llegadas se concentraron entre el 8 y 28 de marzo. Las primeras puestas se registraron el 31 de marzo y la última el 1 de junio (datos similares se han encontrado en Murcia -Martínez *et al.* 2006a), aunque la mayor parte se realizó en la primera quincena de abril (datos propios). Debido a que el periodo de puesta se prolongó durante 2 meses, las primeras eclosiones coincidieron con las últimas puestas. Tras 37-40 días de incubación eclosionan los huevos. A pesar de que el águila calzada es un especie migradora, algunos ejemplares (30-50, aunque en ascenso) invernan en el Parque Nacional de Doñana (García *et al.* 2000).

El águila calzada nidifica sobre árboles en bosques con claros, a menudo en bosques abiertos (Sánchez-Zapata & Calvo 1999, Suárez-Seoane *et al.* 2000) y selecciona unos nidos ante otros que resultan menos adecuados (Suárez-Seoane *et al.* 2000, Martínez *et al.* 2006b). Caza un amplio rango de presas y tamaños (Martínez *et al.* 2004, Martínez & Calvo 2005), aunque el conejo (*Oryctolagus cuniculus*) es probablemente su presa principal (Veiga 1986).

En estudios comparativos sobre reducción de nidada en rapaces, se considera al águila calzada como especie que muestra agresión facultativa (Edwards & Collopy 1983, Simmons 1988).

Nadie interesado exclusivamente en los principios generales de regulación de poblaciones escogería para trabajar aves de presa, pues comparadas con la mayor parte de las aves, las rapaces habitualmente nidifican en bajas densidades, y a menudo en lugares remotos e inaccesibles. Todo esto hace que los tamaños muestrales para realizar análisis estadísticos sean desesperanzadoramente pequeños (Newton 1979). No obstante, la población de águila calzada en el Parque Nacional de Doñana es en la mayor parte de sus ambientes, fácil de monitorizar debido a la baja cobertura forestal. Otras características de la especie facilitan sus seguimiento y por tanto la determinación anual del número de parejas territoriales y que inician la reproducción, como su comportamiento conspicuo durante el periodo de formación de pareja antes de realizar la puesta, la ocupación continuada año tras año de un mismo nido y/o territorio, el pequeño tamaño del territorio en el cual puede ocupar un nuevo nido y la rápida identificación de sus nidos puesto que, salvo el Ratonero (*Buteo buteo*), es la única ave de presa diurna que forra el nido con material verde que renueva asiduamente.

Por otro lado, la población reproductora de águila calzada en el Parque Nacional de Doñana ha incrementado rápidamente en los últimos 15 años (presente memoria), por lo que su tamaño poblacional no resulta escaso. De hecho, este continuado crecimiento de la especie en el Parque Nacional de Doñana es lo que incitó su estudio, puesto que permitía analizar la evolución de la fecundidad de la población en relación a sus cambios en la densidad. El tamaño poblacional del águila calzada en gran parte de su distribución, incluida la Península Ibérica, es desconocido puesto que no se realizan censos regulares; no obstante se estima que se encuentran estables (del Hoyo *et al.* 1994). Parece que está experimentando un crecimiento generalizado de sus poblaciones en Europa Occidental (Carlon 1996). La misma tendencia se refleja en algunas provincias de la Península Ibérica (Cataluña, Extremadura y Murcia) aunque en Madrid se registró un descenso (Bosch 2003, Martí & Del Moral 2003). De las poblaciones africanas solo sabemos que no ha descendido en Burkina Faso, Mali y Níger (Thiollay 2006). En cualquier caso, no es una especie amenazada (del Hoyo *et al.* 1994).

A pesar de que la especie es abundante y relativamente sencilla de monitorizar comparada con otras rapaces, hasta recientemente no se han iniciado trabajos que nos den a conocer otros aspectos diferentes de su biología reproductiva y distribución, como son los factores que influyen en la ocupación de un territorio (Martínez *et al.* 2006b, Casado *et al.* 2008), la selección de hábitat (Suárez-Seoane *et al.* 2000) o aspectos bioquímicos (Casado *et al.* 2002).

1.3 Parque Nacional de Doñana. Área de estudio

Se escogió como objeto de estudio la población reproductora de águila calzada del Parque Nacional de Doñana, porque en el Parque no se encuentran, o lo hacen de forma muy limitada, otros factores externos a la propia población que pudieran influir en la relación densidad-fecundidad (Newton 1979): 1- No ha cambiado drásticamente el uso del terreno y por tanto el paisaje, en el Parque Nacional de Doñana en el periodo de estudio. 2- La especie no es perseguida por cazadores o expoliada dentro de los límites de este espacio protegido. 3- Se encuentran ausentes del Parque tendidos eléctricos o el uso de pesticidas con fines agrícolas, que suponen amenazas para la supervivencia del águila calzada.

Desde luego no ignoramos que los individuos se desplazan fuera de los límites del Parque Nacional. Sin embargo, el seguimiento de algunos ejemplares mediante telemetría (datos propios) mostró que los desplazamientos en la época reproductora se dirigieron sobretodo a la marisma que en verano se encuentra totalmente seca. El único agente externo que pudo afectar a la fecundidad o mortalidad de la población estudiada fue la rotura de una balsa de decantación de residuos de una mina de Aznalcollar, acaecida en 1998. Hasta ahora no se ha demostrado que la contaminación que sufrió el Parque Nacional de Doñana y su entorno debido al famoso vertido tóxico se reflejase en las siguientes épocas reproductoras en un menor éxito reproductor de los adultos de águila calzada. El análisis de 3 huevos infértiles de águila calzada recolectados entre 1999-2000 arrojó una concentración de PCBs en los mismos entre 0,010- 1,5 µg/g f.w. Si bien estos valores son elevados, no se asocian a fallo reproductivo (Gómara & González 2005). Entre 1998 y 2000 se pudo producir algún fallecimiento de adultos causado directamente por envenenamiento, pero esto no se pudo determinar en ninguno de los cadáveres encontrados en dicho periodo. En definitiva, aunque desconocemos el grado en el que el vertido tóxico pudo afectar a la mortalidad o éxito reproductor de la especie, parece que fue bajo.

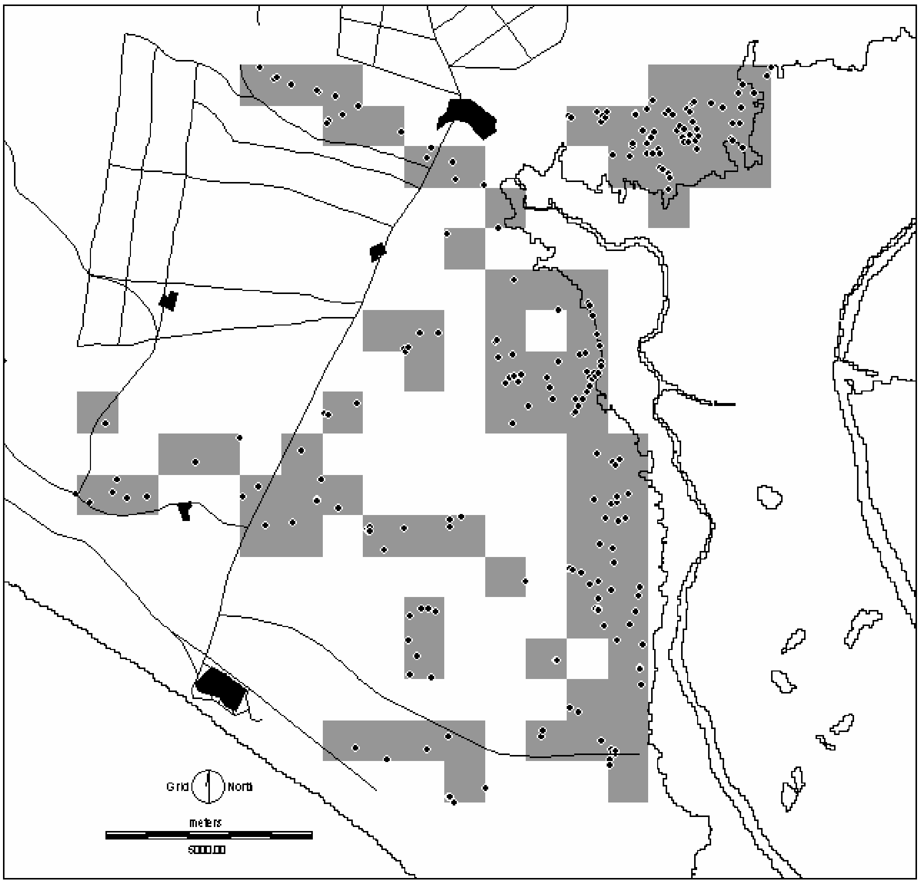
El segundo y principal motivo por el que se seleccionó la población de águila calzada en el Parque Nacional de Doñana como objeto de estudio, fue que ella se conocía su tamaño y fecundidad desde que se asentó la primera pareja reproductora. Esta larga serie temporal de datos permitía analizar la fecundidad en diferentes densidades de población.

Actualmente no deberíamos hablar de Parque Nacional de Doñana puesto que éste, junto al Parque Natural y al Entorno han sido rebautizados como Espacio Protegido de Doñana. No obstante, el área de estudio es la comprendida dentro de los límites del antiguo Parque Nacional de Doñana y parece más apropiado seguir utilizando esta terminología en el marco de la presente tesis.

Utilizando como fuente la página web de la Junta de Andalucía (www.juntadeandalucia.es/medioambiente), el Parque Nacional de Doñana abarca una superficie de 50.720 ha. repartidas entre Huelva (42.573 ha., Almonte e Hinojos) y Sevilla (8.247 ha., Aznalcázar). Las primeras 34.625 ha. del Parque Nacional fueron declaradas como tal en 1969, y su superficie no alcanzó las dimensiones actuales hasta 1978. Posteriormente (1989) se declaró el Parque Natural de Doñana y, como ya he mencionado anteriormente, en 2007 el Parque Nacional junto con el Parque Natural y la Zona de Protección del Parque Nacional fueron declaradas Espacio Protegido de Doñana. El área de estudio de la presente tesis (Figura 1) se circunscribe a la provincia de Huelva y, dentro de esta área, se eliminó de los muestreos el extremo sur que se corresponde a la zona llamada Marismillas, extenso bosque de *Pinus pinea* asentado sobre dunas estabilizadas. En el año 2000, el área de estudio albergaba el 75% de la población de águila calzada del Parque Nacional de Doñana, la cual fue estimada en unas 80 parejas reproductoras (Archivos de la Estación Biológica de Doñana).

El Parque Nacional de Doñana incluye cinco sistemas terrestres (Rogers & Myers 1980). 1- Marisma; estacional y de origen deltaico. 2- Sistemas dunares formados por arenas del cuaternario. Se distinguen dunas móviles en cuya base aparecen bosques de *P.pinea*, dunas parcialmente estabilizadas y dunas totalmente estabilizadas y en paralelo, en Marismillas. 3- Pastizal; que aparece en el ecotono entre el resto de los sistemas y que permanece relativamente húmedo a lo largo de todo el año. 4- Matorral. Se distingue entre el que se forma en niveles inferiores y que se inunda en invierno, constituido principalmente por *Erica scoparia*, y el que se instala en niveles algo más elevados y dominado por *Halimium halimifolium*. En este sistema terrestre es donde aparecen bosques de alcornoque (*Quercus suber*) y lagunas temporales. 5- Las Navas. Constituido por un suelo llano de dunas estabilizadas sobre las que se asientan grandes extensiones de pinar, y en las partes más altas se dispersan algunos ejemplares de enebro (*Juniperus phoenicea*). Los tipos de vegetación terrestre que se encuentran en Doñana son por orden de extensión (www.juntadeandalucia.es/medioambiente): 1- Matorral, 2- Pinares (densos, dispersos o corrales), 3- Suelo desnudo, 4- Dunas, 5- Sabinar/Enebral, 6- Playas, 7- Eucaliptal (*Eucaliptus cameldulensis*), 8- Pastizal y 9- Vegetación de ribera.

Figura 1. *Área de estudio y localización de los territorios ocupados por águila calzada entre 1983 y 2000 (n = 77) señalados por los cuadrados grises de 1km de lado; los puntos negros corresponden a los nidos. Las líneas negras representan carreteras y carriles, mientras que los polígonos negros representan áreas urbanas*



1.4 Objetivo y estructura de la tesis

El objetivo de la presente tesis es intentar comprender la dinámica de la población de águila calzada del Parque Nacional de Doñana, es decir, cómo varía su tamaño y en respuesta a qué factores. Para ello me he centrado en el estudio de su tamaño y su reproducción, que es uno de los principales factores que influye en las variaciones de tamaño de una población junto con la mortalidad, dispersión y otros movimientos y comportamiento social. El análisis de las variaciones espacio-temporales en el tamaño y reproducción de la población de águila calzada del Parque Nacional de Doñana, me ha permitido averiguar qué mecanismos actúan en la regulación denso-dependiente de la población así como la influencia que la mortalidad en nido (reducción de nidada) y la proporción de sexos en el momento de la eclosión (sex-ratio primario) tienen sobre el tamaño poblacional. El resultado final es una composición sobre cómo ha podido ir creciendo la población de águila calzada del Parque Nacional de Doñana y cómo se ha ido regulando a sí misma.

Para abordar dicho objetivo inicialmente tuve que desarrollar aspectos metodológicos que se requerirían posteriormente para testar diferentes hipótesis. Así, esta tesis se estructura en siete capítulos o apartados que se podrían agrupar en dos bloques. El primer bloque comprendería los capítulos 2, 3, 4 y 5 reseñados en el índice, en los que se desarrollan metodologías de trabajo que se utilizan posteriormente. El segundo bloque comprende los capítulos 6, 7 y 8, en los que se analiza la estructura poblacional del águila calzada en el Parque Nacional de Doñana y su repercusión en la dinámica de dicha población.

En el **Capítulo 2** se analizan las concentraciones en plasma de algunos parámetros sanguíneos relacionados con la condición física de los individuos. Los resultados obtenidos sirvieron para poder estimar la condición física de adultos y pollos de la especie y relacionarla en siguientes capítulos con la fecundidad. En el **Capítulo 3** se obtienen fórmulas mediante análisis discriminante, para determinar el sexo de adultos y pollos de águila calzada en función de variables biométricas. Si bien posteriormente no se tuvieron que emplear dichas fórmulas para la elaboración de los siguientes capítulos, los resultados obtenidos son muy prácticos cuando no se pueden recurrir a técnicas moleculares. El **Capítulo 4** surgió de la necesidad de determinar un criterio robusto que sirviese para discernir entre los dos mecanismos que conducen a la regulación denso dependiente de una población (heterogeneidad de hábitat o ajuste individual). Hasta entonces se había utilizado asiduamente la relación entre fecundidad media de la población estudiada y el coeficiente de variación de dicha fecundidad. Análisis preliminares con mis propios datos me sugirieron que esta relación no era un criterio definitivo, pues podía surgir como consecuencia de un artefacto estadístico. Pensamos entonces en que se deberían encontrar diferencias sustanciales en la distribución de la fecundidad entre una población regulada mediante heterogeneidad de hábitat y otra regulada mediante ajuste individual. La utilidad del criterio se evaluó comparando las distribuciones de la fecundidad, obtenidas mediante la simulación de las condiciones que existirían en una población teórica regulada por uno u otro mecanismo. El **Capítulo 5** complementa al capítulo 4 y surge como respuesta al debate suscitado por su publicación. En el capítulo 5 se detalla el razonamiento biológico del método encontrado para discernir claramente entre ambos mecanismos reguladores, el sesgo en la distribución de la fecundidad o skewness. A partir del **Capítulo 6** se aborda el estudio directo sobre la población del águila calzada del Parque

Nacional de Doñana. En este capítulo empleo el sesgo en la fecundidad de la población con el fin de averiguar qué mecanismo es el que puede estar condicionando su dinámica. Mientras que en los dos capítulos siguientes se analiza el efecto que dos aspectos de la reproducción como son la frecuencia de reducción de nidada (**Capítulo 7**) y el sex-ratio primario encontrado en la población (**Capítulo 8**) tienen sobre la dinámica de una población regulada mediante heterogeneidad de hábitat.

Los capítulos se presentan en el formato típico de los artículos científicos, con las siguientes secciones: Resumen, Introducción, Material y Métodos, Resultados, Discusión, Agradecimientos y Referencias utilizadas. Se encuentran escritos en inglés, de modo que al inicio de cada capítulo se ha traducido al español el Resumen. El formato de las citas puede no ser concordante entre los capítulos.

1.5 Referencias

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2. Plasma chemistry in the Booted eagle (*Hieraaetus pennatus*) during breeding season

Referencia: CASADO, E., J. BALBONTIN & M. FERRER. 2002. Plasma chemistry in the booted eagle (*Hieraaetus pennatus*) during breeding season. *Comparative Biochemistry and Physiology,* A.131: 233-241.

Resumen

La mayoría de los trabajos que examinan la química sanguínea en rapaces, se han realizado con muestras procedentes de individuos que permanecían en cautividad. En este estudio describimos los valores típicos de algunos indicadores de la condición física de los individuos, encontrados en el plasma de águilas calzadas *Hieraaetus pennatus*, pertenecientes a la población del Parque Nacional de Doñana. Los valores encontrados se comparan con los de otras rapaces. La concentración media en plasma de creatinina, ácido úrico y urea fue menor en adultos que en pollos de águila calzada, mientras que los de glucosa, DAT y AAT fueron menores en pollos que en adultos. Los niveles medios en plasma de creatín quinasa, glucosa, AAT, ácido úrico y urea se vieron afectados por la interacción entre sexo y edad de los individuos. Las hembras adultas mostraron niveles de creatín quinasa, ácido úrico y urea significativamente inferiores a los encontrados en adultos machos y en pollos. Los adultos machos tuvieron el nivel de AAT significativamente más alto que el de los otros grupos. Los niveles más bajos de glucosa y los más altos de ácido úrico se encontraron en los pollos hembra. Pensamos que las diferencias en los parámetros sanguíneos se pueden explicar por las diferencias en el tamaño de las especies, de los individuos (debido tanto a la condición física como al dimorfismo sexual) y en la dieta.

Palabras clave: Edad, parámetros sanguíneos, Águila calzada, época reproductora, Parque Nacional de Doñana, rapaces en libertad, sexo, interacción sexo-edad, condición nutricional.

Abstract

Most studies that have examined raptor plasma chemistry have been conducted on birds living in captivity. In this study, we describe typical plasma chemistry values indicators of body condition in free living Booted eagles, *Hieraaetus pennatus*, from Doñana National Park (Spain). Values are compared with those of other raptors. Mean concentrations of creatinine, uric acid and urea were lower in adults than in nestlings, while glucose, DAT and AAT were lower in nestlings than in adults. Interactions of age/sex affected plasma mean levels of creatine kinase, glucose, AAT, uric acid and urea. Adult females showed significantly lower levels of creatine kinase, uric acid and urea than adult males and nestlings. Adult males had significantly higher levels of AAT than the other groups. The lowest levels of glucose and the highest levels of uric acid were found in nestling females. We think the differences in blood parameters can be explained by differences in size of species, of individuals (because of both body condition and sexual dimorphism) and diet.

Keywords: Age-related differences, blood parameters, Booted eagle, breeding season, Doñana National Park, free-living raptor, gender, interaction age-sex, nutritional condition.

Introduction

Studies of avian plasma chemistry are of value to ecologists and veterinarians, especially for the management of threatened species. An increasing number of studies of avian plasma chemistry have examined aspects of the biochemistry and physiology of birds, including the influence of age (Viñuela *et al.*, 1991; Dobado-Berrios *et al.*, 1998; Ferrer & Dobado-Berrios, 1998), sex (Gee *et al.*, 1981; Ferrer & Dobado-Berrios, 1998), fasting (García-Rodriguez *et al.*, 1987a) and circadian rhythms (García-Rodriguez *et al.*, 1987b). Most of these studies have been done on captive birds (for example Balasch *et al.*, 1976; Gee *et al.*, 1981; Ferrer *et al.*, 1987; Lumeij & Remple, 1991; Polo, 1995). Knowledge of what constitutes typical plasma chemistry values is essential for interpreting the results of such studies. However, studies on typical concentrations of blood constituents of free-living raptors are rare (Viñuela *et al.*, 1991; Dobado-Berrios & Ferrer, 1997; Ferrer & Dobado-Berrios, 1998). The description of typical concentrations of blood parameters in wild populations provides an improved understanding of the physiological condition of free living parents and offspring (Gee *et al.*, 1981; Ferrer, 1992).

In this study, we describe concentrations of selected chemical parameters in plasma of free living Booted eagles (*Hieraaetus pennatus*) of known age and gender.

This paper includes eleven chemical parameters (see "material and methods") that have been shown to be related to nutritional condition (Rosskopf *et al.*, 1982; Ferrer, *et al.*, 1987; Dawson & Bortolloti, 1997; Dobado-Berrios *et al.*, 1998; Ferrer & Dobado-Berrios, 1998; Alonso, 2000).

Materials and methods

We sampled a free living population of Booted eagles at Doñana National Park (South-western Spain, 37º N, 6º 30´ W), between 1996 and 2000. Nests were visited when young were between 35 and 45 days old, an age at which their bones were completely grown in length. The exact ages in days of the nestlings were known due to previous visits to the nests. Adults were captured during the nestling period using a net and an irrecoverable Eagle owl as a lure. All samples were collected between 9:00 and 16:00 CST to minimise variation in blood chemicals caused by the circadian rhythm (García-Rodriguez *et al.*, 1987b; Ferrer, 1990).

The brachial vein of each bird was lanced with a hypodermic needle and 2 ml of blood was collected in a heparinized tube. Blood was carefully placed into tubes containing lithium heparin to prevent coagulation, and avoiding formation of bubbles. Blood was centrifuged (10 min; 907.2 g), and the plasma was stored at –80ºC until it was analysed for amylase (AMY), cholesterol (CHO), creatinine (CRE), creatine kinase (CK), glucose (GLU), aspartate aminotransferase (DAT), alanine aminotransferase (AAT), total protein (PRO), triglycerides (TG), urea (URE) and uric acid (UA), using a portable autoanalyser (Reflotron II, with the reagents recommended by Boehringer-Mannheim Inc.).

The cellular fraction was used to sex the eagles following Ellegren (1996). We used primers 2945F, cfR and 3224R to amplify the W chromosome gene following one of Ellegren´s recommendations. Analyses were carried out at Doñana Biological Station.

We analysed the differences in blood parameters mean concentrations among gender, age classes and their interactions by means of two-way ANOVA with type I error, introducing age and sex as fixed factors and the chemical values as dependent variables, in a simple block design. We transformed dependent variables in ranks to normalised them. We used Newman-Keuls post-hoc test to check the significant differences among groups (adult male, adult female, nestling male and nestling female).

Statistics were conducted by STATISTICA Statsoft Inc. Differences were considered significant at $P<0.05$. All tests were two-tailed.

Results

A total of 143 young, 55 adults and 1 bird of unknown age were caught, banded and sampled. None were injured as a result of our activities. Sixty-three nestlings and twenty-eight adults were females while fifty-two nestlings and twenty-four adults were males. We couldn't identify the gender of 28 nestlings and 3 adults. In some cases, it was not possible to analyse all chemical variables; therefore sample size is not the same for all blood components.

Table 1 shows the plasma descriptive parameters by age. Because of age differences in some parameters, we did not pool these data to present a table by sex group.

Levels of glucose, AAT, uric acid and urea were affected by age and interaction age-sex. Creatinine and DAT plasma levels only were affected by age, while creatine kinase plasma level was affected only by interaction age-sex (Table 2). We did not find any difference within gender group, according to Ferrer & Dobado-Berrios (1998).

Adult males and females significantly differed in mean concentration of creatine kinase (F= 7.04, $P<0.01$), AAT (F= 6.14, $P<0.05$), uric acid (F= 4.31, $P<0.05$) and urea (F= 11.51, $P<0.001$) levels, having females lower mean concentrations. Adults blood levels of glucose (F= 14.33, $P<0.001$), DAT (F= 7.50, $P<0.01$) and AAT (F= 16.62, $P<0.005$) were higher than those in nestlings. Conversely, the concentrations of creatinine (F= 10.30, $P<0.005$), uric acid (F= 24.05, $P<0.005$) and urea (F= 4.92, $P<0.05$) in adults were lower than in nestlings. Nestlings had higher creatine kinase levels in blood than adult females (F= 7.04, $P<0.01$), but similar to adult males. There was a tendency in triglycerides blood concentration to be higher in nestlings than in adults (F= 3.75, $P=0.05$). Nestling females had the lowest mean levels of glucose, but the difference was significant only with adults (F= 4.38, $P<0.05$). Nestling females also, showed the highest levels of uric acid in plasma (F= 4.32, $P<0.05$).

Table 1. *Descriptive chemical values in plasma of Booted eagle (mean ± SD, and range) by age class*

	ADULTS			CHICKS		
	n	$\bar{X} \pm SD$	Range	n	$\bar{X} \pm SD$	Range
AMY U/l	35	638.51 ± 404.30	117-1736	124	636.06 ± 275.27	115-1330
CHOmg/dl	44	203.80 ± 38.47	112-280	129	211.1 ± 63	96-440
CK U/l	43	548.13 ± 568.31	57.6-2523	117	728.17 ± 688.89	12-2665
CRE mg/dl	26	0.5 ± 0.3	0.1-1.5	126	1.07 ± 1.08	0.14-7.56
GLUmg/dl	44	241.53 ± 108.34	1-357	131	207.01 ± 84.62	1-394
DAT U/l	21	120.89 ± 57.39	12.6-286	90	86.82 ± 29.9	15.50-180
AAT U/l	26	13.27 ± 4.96	2.53-22.10	93	9.15 ± 5.02	3.17-30.60
PRO g/dl	23	$25x10^{-4} \pm 10^{-4}$	10^{-4}-$33x10^{-4}$	79	$24x10^{-4} \pm 11x10^{-4}$	10^{-4}-$5x10^{-3}$
TG mg/dl	40	105.99 ± 66.44	39.4-347	116	127.86 ± 88.98	21-632
UA mg/dl	52	8.66 ± 4.70	0.28-20	134	12.92 ± 6.54	0.2-33.47
URE mg/dl	42	17.74 ± 14.71	4.7-79	124	20.9 ± 10.8	4.8-74.6

Following to Balasch *et al.* (1976), Gee *et al.* (1981), Ferrer *et al.* (1987), Dobado-Berrios *et al.* (1998), and Ferrer & Dobado-Berrios (1998), adults of Booted eagle had a mean concentrations of amylase and urea higher and those of AAT, cholesterol, DAT, glucose, total protein, triglycerides and uric acid lower than other captive raptors. Depending on species and references, the differences varies from slightly (AAT, amylase, DAT, uric acid) to strong (five times higher level of triglycerides in Spanish imperial eagle, or half level of urea in Egyptian vulture than in Booted eagle). The blood concentration of total protein shows great differences among species, with some of them (Spanish imperial eagle, Eagle owl and Bonelli's) having twice the mean level than free-living Booted eagles, while other species (Andean condor, Eastern imperial eagle, Peregrine falcon) show a much higher mean level. Mean blood creatinine concentration was similar in Booted eagle than in other raptors.

Free-living Egyptian vulture showed similar levels of creatinine and triglycerides than Booted eagle, higher level of cholesterol and protein, and lower level of AAT, DAT and urea.

Studies about plasma chemical values of raptors nestlings (Ferrer, 1992, Dobado-Berrios *et al.*, 1998; Ferrer & Dobado-Berrios, 1998), found higher levels of amylase (three times more in Spanish imperial eagle), cholesterol, creatinine, glucose and total protein; while those of urea and uric acid were lower than in nestlings of Booted eagle. AAT and DAT concentrations were higher in captive nestlings than in Booted eagle nestlings, but were lower in free-living nestlings of Egyptian vulture than those of Booted eagle. Triglycerides level was higher or lower than in Booted eagle according to reference.

Table 2. *Two-way ANOVA with gender, age and their interaction as factors affecting blood parameters. Values of F and P shown correspond to sex in first line, age in second line and the interaction between both in third line. Mean and SD correspond to rank-transformed parameters*

	ADULTS				NESTLINGS					
	Male		Female		Male		Female			
	n	$\bar{X}$ ± SD	n	$\bar{X}$ ± SD	n	$\bar{X}$ ± SD	n	$\bar{X}$ ± SD	F	P
AMY U/l	15	89.87 ± 57.03	18	63.39 ± 51.81	43	75.76 ± 39.39	56	84.77 ± 48.03	0.000	0.984
									0.329	0.567
									3.485	0.642
CHO mg/dl	19	95.34 ± 32.09	22	74.02 ± 42.32	46	85.37 ± 57.08	57	83.61 ± 51.01	0.769	0.382
									0.004	0.948
									1.122	0.291
CK U/l	17	96.68 ± 32.63	24	58.25 ± 41.04	40	89.13 ± 44.43	53	92.31 ± 41.29	1.803	0.182
									4.609	0.037
									7.043	0.009**
CRE mg/dl	13	48.54 ± 33.34	11	46.68 ± 22.79	40	76.83 ± 43.94	58	79.32 ± 43.50	0.314	0.577
									10.303	0.002***
									0.053	0.819
GLU mg/dl	19	102.03 ± 56.79	22	116.48 ± 55.09	46	88.88 ± 45.66	57	66.85 ± 41.05	2.340	0.128
									14.331	0.00***
									4.376	0.038*
DAT U/l	7	74.86 ± 49.69	12	80.08 ± 30.10	32	49.34 ± 27.20	36	59.68 ± 31.20	2.575	0.112
									7.504	0.008**
									0.093	0.761
AAT U/l	10	16.28 ± 5.88	14	11.30 ± 3.31	33	8.92 ± 3.05	38	9.25 ± 5.41	0.803	0.373
									16.621	0.00***
									6.137	0.015*
PRO g/dl	10	0.062 ± 0.032	12	0.049 ± 0.026	24	0.05 ± 0.029	39	0.051 ± 0.032	0.119	0.732
									0.264	0.609
									0.910	0.343
TG mg/dl	18	70.39 ± 44.84	19	61.21 ± 38.29	36	81 ± 48.69	52	84.53 ± 46.64	0.010	0.921
									3.745	0.055
									0.495	0.483
UA mg/dl	23	81.76 ± 42.17	26	50.64 ± 34.98	49	105.46 ± 51.16	57	111.08 ± 59.58	0.487	0.486
									24.047	0.00***
									4.315	0.040*
URE mg/dl	19	86.82 ± 57.89	20	46.13 ± 37.52	44	75.15 ± 47.34	55	93.18 ± 42.20	0.064	0.802
									4.916	0.028*
									11.514	0.00***

* significant for *P*<0.05 ** significant for *P*<0.01 *** significant for *P*<0.005

Discussion

The main objective of this study was to establish the typical range of values of several plasma chemical parameters for free-living Booted eagles, and to determine the amount of variation in these parameters attributable to sex, age or their interaction. Furthermore, we compared these values in Booted eagle with those of other raptors obtained from the literature (Tables 3a, b).

The information obtained from literature reflects a high variance in plasma levels. We must be careful with comparisons among species and take into account the ranges and mean values differences among authors for the same species.

We compared values of adults from a free-living population of Booted eagle with those of captive birds because most studies have been conducted on captive birds, which may explains in some degree the difference found in range of concentrations. Compared to free living birds, captive birds have different diets, food supply and physical activity. These factors affect blood parameters (Dobado-Berrios *et al.*, 1998; Alonso, 2000 and references therein). In fact, four captive Booted eagles (Ferrer *et al.*, 1987) had slightly higher concentrations of cholesterol, glucose and total protein, but lower urea levels than our free-living population, probably due to a high food supply under captive condition. Care must be taken in using values from captive birds to interpret plasma chemistry of wild individuals. However, the differences between free-living Egyptian vulture and Booted eagle, could reflect a better nutritional condition of Egyptian vulture.

According to some authors, glucose levels may depend on body mass (Polo, 1995; Totzke & Bairlein, 1998), which could explain why free-living nestlings of large species (Golden eagle, Spanish imperial eagle) have lower concentrations than smaller ones (Booted eagle, Egyptian vulture). The higher levels in other raptors than in Booted eagle of amylase, creatinine, glucose, liver enzymes (AAT, DAT), ingested proteins and triglycerides (low values are indicative of malnutrition), and higher levels in Booted eagle than other raptors of urea and uric acid, seems to indicate a worse physical condition of Booted eagle.

Age had a significant effect on concentrations of creatinine, glucose, DAT, AAT (enzymes related with liver function), urea and uric acid in Booted eagle. Mean levels of urea and uric acid, as well that of creatinine, decreased with age, with adult females having the lowest

concentrations, suggesting adults were in better physical condition than young (Rosskopf & Van de Water 1982). The remaining blood parameters values increased with age. According to Ferrer & Dobado-Berrios (1998), glucose values in blood are related to metabolic rate and mass, so an increase with age is expected. Also, glucose is source of energy, reflecting that there is a nutritional condition gradient being adult females the best fed and nestling females the worst fed. This fact is consistent with urea and uric acid levels, which showed an opposite tendency: adult females had the lowest plasma concentration, meanwhile nestling females had the highest one. Mean urea level was nearly the double than uric acid both in chicks and adults. High urea and uric acid blood concentrations are indicators of bad nutritional condition. Alternatively the relative contribution of urea to total nitrogen excretion may have declined after young Booted eagles leave the nest. Since the water volume required for excreting urea is much larger than that needed to eliminate uric acid, the physiological change that we report herein may contribute to weight economy which is important for flight. Lower plasma creatinine level in nestling than in adult class, point to worse nestling nutritional condition than adult similar to Rosskopf & Van de Water (1982), who suggested that creatinine values decline with food supply. However, a higher plasma creatinine level could reflect increased protein turnover caused by rapid nestling growth.

High levels in AAT and DAT have been associated with pathological changes in avian liver (Rosskopf & Van de Water, 1982; Brugere-Picoux *et al.*, 1987); but there are no data about typical values for these enzymes in Booted eagles. Blood parameters determined in free-living nestlings range widely, probably reflecting the wide range of environment conditions to which different species are exposed.

It has been suggested that creatine kinase levels in plasma are inversely related to weight (Polo, 1995, Ferrer & Dobado-Berrios 1998), i.e to a lower metabolic rate. This could explain why adult females had the lowest creatine kinase levels. Nestlings showed less sexual dimorphism in size than adults and we failed to find any significant differences in creatine kinase between genders.

We, as well other raptors studies, have not detected differences in cholesterol and protein plasma level, both being parameters affected by the qualitative composition of diet.

In short, blood parameters indicate higher food stress in nestling females than in nestling males, higher food stress in nestlings than in adults, and within adults females are in better nutritional condition than males. Differences in adult class could be the result of differences in sex roles during reproduction: males undertake nearly all of the hunting duties for the pair until chicks are two weeks old (pers. obs.). Male parents are more active than females during reproduction and this could lead to physical wastage. On the other hand, differences within nestling class could be explained by marked sexual size dimorphism Booted eagle. Nestling females are bigger than nestling males, which implies a different metabolic rate by different growing pattern, thus nestling female food requirements are larger than those of nestling males. Furthermore, differences could reflect a differential parental investment towards the cheapest nestling (males). Further studies are needed to check this hypothesis.

Table 3a. *Values of blood parameters in adults raptors*

Auth.	**Param.**	**Andean condor** *Vultur gryphus*		**Griffon vulture** *Gyps fulvus*		**Eastern imperial eagle** *Aquila heliaca*		**Spanish imperial eagle** *Aquila adalberti*		**Eagle owl** Bubo bubo		**Bonelli's eagle** Hieraaetus fasciatus	
		X±SD	Range	X±SD	Range	X±SD	Range	X±SD	Range	X±SD	Range	X±SD	Range
(2)	AMY	442±120	326-681										
(5)								15.6±14.9	0-149.6				
(2)	CHO	184±30	141-228										
(3)				217±9	130-257			213	213	226±17	168-280	214±9	196-225
(5)								229±11	183-272				
(2)	CRE	0.8±0.1	0.6-1										
(5)								0.52±0.04	0.42-0.81				
(1)	GLU	219±43.7		209.3±24.2		180							
(2)		337±43	278-395										
(3)				267±8	233-316			323±15	308-338	369±21	327-445	353±17	307-389
(5)								354±9	319-410				
(5)	DAT							288+102	125-1182				
(5)	AAT							29+8	12-98				
(1)	PROT	2.97±0.79		2.96±0.61		2.41							
(2)		3700±200	3500-4200										
(3)				4±0.21	2.9-5.5			3.3	3.3	3.8±0.32	2.8-4.2	4.2±0.26	3.8-4.7
(5)								3.08±0.12	2.69-3.99				
(2)	TG	109±32	75-171										
(5)								519±95	50-891				
(2)	UA	8.9±2.4	4.5-11.3										
(5)								10.23±1.1	5.14-16.12				
(1)	URE	13.8±0.8		14.9±1.6		11.5							
(3)				9.5±2.8	0-26			10	10	9.7±1.1	4-18	5.7±2.7	3-11
(5)								9±1	5-13				

(1) Balasch *et al.*, 1976
(2) Gee *et al.*, 1981
(3) Ferrer *et al.*, 1987
(4) Dobado-Berrios *et al.*, 1998 (4) *=free living birds
(5) Ferrer & Dobado-Berrios, 1998

Table 3a. *Continued.*

Auth.	**Param.**	**Egyptian vulture** *Neophron percnopterus*		**Black kite** *Milvus migrans*		**Buzzard** *Buteo buteo*		**Booted Eagle** *Hieraaetus pennatus*		**Peregrine Falcon** *Falco peregrinus*		**Kestrel** *Falco tinnunculus*	
		X±SD	Range	X±SD	Range	X±SD	Range	X±SD	Range	X±SD	Range	X±SD	Range
(1)	CHO												
(3)				338±25	221-443	226±13	134-313	226±34	131-287				
(4)		330.8±23	296.1-361.5										
(4)*		257.7±38.5	200-319.2										
(4)	CRE	0.35±0.05	0.30-0.40										
(4)*		0.27±0.06	0.20-0.40										
(1)	GLU			215.7±47.2		322.6±62							
(2)										316±18	290-337		
(3)				350±19	260-418	362±19	277-504	292±7	274-305			392±23	326-475
(4)		282.6±28.8	252-324										
(4)*		270±36	183.6-313.2										
(4)	DAT	128±52	82-226										
(4)*		75±13	56-99										
(4)	AAT	16±12	7-30										
(4)*		4±2	1-6										
(1)	PROT			2.69±0.68		2.96±0.8							
(2)										3300±500	2700-4100		
(3)				3.7±0.10	3.1-4.1	3.8±0.12	3.3-4.9	3.8±0.32	2.8-4.2				
(4)		4.3±0.7	3.1-5.2										
(4)*		3.2±4	2.5-3.7										
(4)	TG	108±50	44-186										
(4)*		110±27	72-158										
(2)	UA									11.4±2.3	8.9-14		
(1)	URE			11.2±1.8		7±0							
(3)				7.2±1.4	5-11	22.5±3.5	6-36	8±11	5-10				
(4)		7.8±4.2	1.8-13.2										
(4)*		6±3	1.8-12										

Table 3b. *Values of blood parameters in free-living nestlings*

Author.	**Param.**	**Golden eagle** *Aquila chrysaetos*		**Spanish imperial eagle** *Aquila adalberti*		**Egyptian vulture** *Neophron percnopterus*	
		X±SD	Range	X±SD	Range	X±SD	Range
(4)	AMY			1994±89	1238-4384		
(3)*	CHO					230.8±42.3	150-338.5
(4)				208±4	127-295		
(3)*	CRE					0.34±0.14	0.20-1.40
(4)				0.38±0.01	0.27-0.71		
(1)	GLU	254±12.7					
(3)*						225±32.4	140.4-304.2
(4)				243±6	93-317		
(3)*	DAT					57±17	26-107
(4)				175±8	102-383		
(3)*	AAT					3±1	0-6
(4)				19±2	7-91		
(1)	PROT	1.83±0.23					
(3)*						2.9±0.4	2.1-4
(4)				3.47±0.06	2.17-4.59		
(3)*	TG					138±35	57-239
(4)				108±7	46-268		
(2)	UA			9.42±4.78	3.47-18.35		
(4)				11.01±0.74	3.47-36.30		
(1)	URE	14±2.5					
(2)				12.33±4.36	8-21		
(3)*						9±4.2	1.8-16.8
(4)				23±3	8-115		

(1) Balasch *et al.*, 1976
(2) Ferrer, 1992
(3) Dobado-Berrios *et al.*, 1998 (3) *=free living birds
(4) Ferrer & Dobado-Berrios, 1998

Aknowledgements

We thank to C. Alonso for critical review of the manuscript. Cristina F. Vega helped us with blood analysis by Reflotron. The comments of three anonymous referees greatly improved the original manuscript.

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3. Sex determination in Booted eagles using molecular procedures and discriminant function analysis

Referencia: BALBONTIN, J., M. FERRER & E. CASADO. 2001. Sex determination of Booted eagles using molecular procedures and discriminant function. *Journal of Raptor Research* 35: 20-23.

Resumen

En este artículo se ha estudiado la población reproductora de águila calzada (*Hieraaetus pennatus*) del Parque Nacional de Doñana, con el fin de obtener un método para distinguir el sexo de los individuos basado en análisis discriminantes. Debido a las diferencias de tamaño que se dan entre clases de edad y sexo de las águilas, se han desarrollado dos funciones discriminantes para sendos grupos de edad. El sexo de los adultos se determina con una función discriminante que clasifica correctamente el 100% de los individuos, y que utiliza el antebrazo y el peso como variables predictoras. El sexo de los pollos se determina con una función discriminante que clasifica correctamente el 98,8% de los individuos, utilizando como variables predictoras el antebrazo, la cola, el pico y el tarso.

Palabras clave: águila calzada, *Hieraaetus pennatus*, determinación del sexo, morfometría, sexado molecular.

Abstract

We studied a breeding population of Booted eagles (*Hieraaetus pennatus*) in Doñana National park (south western Spain) to develop a method of determining the sex of an individual based on the use of discriminant functions. Because there are size differences between age classes and sexes of eagles, we developed two different discriminant functions for each age group. Our discriminant function method approached 100% accuracy in correctly aging individuals using forearm length and body mass as predictor variables. Sex of young eagles was also determined with 98.8% accuracy using forearm, tail, bill, and tarsus lengths.

Key words: Booted eagle, *Hieraaetus pennatus*, sex determination, morphometrics, molecular sexing.

Introduction

Easy and reliable methods to identify the sex of individuals are useful for the study of many aspects of avian biology, including foraging ecology (Anderson & Norberg 1981), behaviour, evolutionary ecology and genetics (Clutton-Brock 1986), survivorship (Newton *et al.* 1983), and dispersion and conservation genetics (Griffith & Tiwari 1995). Sex determination is also important in conservation programs that concern the reintroduction of endangered birds when a fixed sex ratio is preferred. Recently, Ellegren (1996) proposed molecular methods to sex birds based on chromosome differences but few studies have used this information to develop additional methods to sex birds based on biometric data. Field methods to sex raptors have several advantages over molecular techniques that require time and/or money. Despite the fact that the majority of raptors are highly dimorphic in size, which should allow for the development of sexing methods based on morphometric data, only a few species have been utilized (Bortolotti 1984a, 1984b, Garcelon *et al.* 1985, Edwards & Kochert 1987, Ferrer & De Le Court 1992). The majority of these studies have been based on live individuals and museum skins. In most cases, both adults and immatures have been studied at museums or in private collections and few studies have been based on wild individuals. The objective of this study was to assess the differences between young and adult Booted eagles (*Hieraaetus pennatus*) and to develop predictive discriminant models to determine the sexes of adults and immatures of the species.

Methods

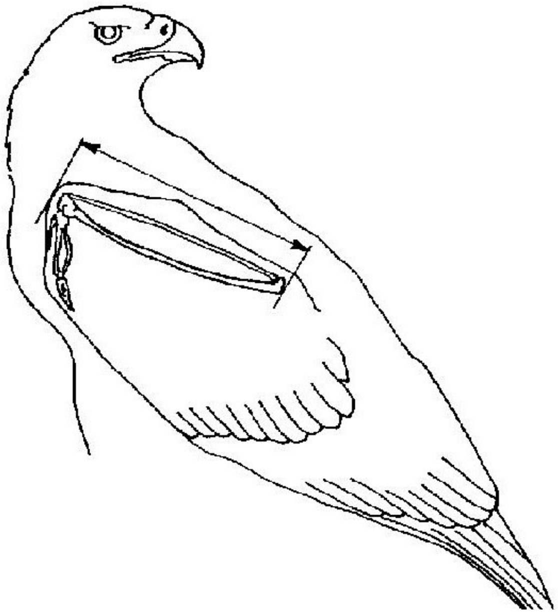
We used a sample of the breeding population of Booted eagles in Doñana National Park. The park is located in south western Spain (37ºN, 6º80' W). It has a Mediterranean climate with an Atlantic Ocean influence. Marshes, Mediterranean scrubland mixed with scattered cork oak (*Quercus suber*) or stone pine (*Pinus pinea*), and costal sand dunes are the main habitats found in the area. A more detailed description of this area is presented in Rogers & Myers (1980).

Six morphometric measurements were taken from wild adult and immature eagles. To obtain measurements, we visited nests when young were 35-45 d old and their skeletons were completely grown but their feathers were still growing. Young leave the nest when they are about 55 d old (Ferrer unpubl. data). A total of 100 young were measured between 1996-98. Adults were trapped using a 2x3 m dho gaza net and an unreleasable captive owl (*Bubo bubo*) lure. Forty-two adults were caught using this method, 12 in 1997 and 30 in 1998. We took measurements of wing, tail, bill with cere, and tarsus lengths using a digital calliper to the nearest 0.1 mm and metal rulers to the nearest 1 mm (Bortolotti 1984). We also measured the forearm length, or the length from the front of the folded wrist to the proximal extremity of the ulna using callipers (Fig. 1) (Ferrer & De Le Court 1992). All the individuals were weighted with 1 kg or 2.5 kg Pesola scales with precisions of 5 g and 10 g, respectively.

We extracted 2 ml of blood from the brachial vein of each eagle and stored part of it (50 µl) in buffer and kept it refrigerated for later analysis. The cellular fraction was used to sex the eagles following Ellegren (1996). We used primers 2945F, cfR, and 3224R to amplify the W-chromosome gene following Ellegren's (1996) recommendations. Using this technique, we identified the sexes of 81 immature (41 females, 40 males) and 41 adult eagles (16 males, 25 females) (Fig. 2). This sample of known-sex individuals was used to derive the discriminant function using morphometric data.

Because young often differ in size from adults (Bortolotti 1984b), we used multivariate analysis of variance (MANOVA) to check for differences in size between males and females and young an adult eagles. Six measurements taken from all age and sex classes were compared using univariate analysis of variance (ANOVA) and nonparametric statistics for those variables when homogeneity of variance was not met. We used the SPSS program (Norusis 1992) to do this analysis. We separated young from adults to examine differences between sexes. First, we checked for sexual differences for each of the six morphological characters using *t*-tests. We derived a discriminant function using DISCRIM procedure of the SAS System program (version 6.12). A jackknife procedure was applied to test the efficacy of the estimated discriminant function (Lachenbruch & Mickey 1968). Each individual in the sample was classified using a discriminant function derived from the total sample, excluding the individual being classified (Chardine & Morris 1989, Amat *et al.* 1993). We chose the function which had the lowest percentage of misclassification based on the molecular determination of gender.

Figure 1. *Measurement of forearm length in Booted eagle*



Results and Discussion

Our analyses of the morphometric data showed that adult Booted eagles differed significantly in size from young eagles and that males were significantly smaller than females (MANOVA: Sex – $F = 72.0$, df = 6,111, $P < 0.001$; age – $F = 181.85$, df = 6,111, $P < 0.001$). Tail, wing, and culmen measurement showed the greatest difference between age groups, with the features of adult individuals larger than those of immatures (Table 1). There were no significant age- or sex-specific differences in bone measurements such as tarsus and forearm lengths but forearm length was significantly smaller in young female eagles than in adults (Table 1). Booted eagles show high sexual dimorphism in size and both adults and young differed significantly in the majority of the variables we studied. Adult females were significantly larger than males for all measurements taken, with forearm and body mass the most dimorphic characters (Table 2). Young females are also larger than males and they have also longer forearms and beaks, but similar-sized wings and tails. Our discriminant function analysis classified 100% of the adult female and male eagles correctly using body mass and forearm as predictor variables. The discriminant function equation for adults was:

$$D = -187.885 + 0.05613\,(\mathrm{MASS}) + 0.95937\,(\mathrm{FOREARM})$$

Young were classified most accuracy using the four variables forearm, tail, bill, and tarsus as predictors in the model. The discriminant function misclassified only one female. The discriminant function for young was:

$$D = -197 + 0.6761\ (\mathrm{FOREARM}) - 0.19286\ (\mathrm{TAIL}) + 2.99438\ (\mathrm{BILL}) + 0.5858\ (\mathrm{TARSUS})$$

Values of $D > 0$ represent females and values of $D < 0$ represent males. By Deleting tail and wing measurements which are highly variable from the model, young eagles were also classified with 84% accuracy using only tarsus and forearm measurements in the discriminant function:

$$D = -33.815 + 0.147\ (\mathrm{FOREARM}) + 0.207\ (\mathrm{TARSUS})$$

The equations we derived for sexing Booted eagles should be useful for future work on the biology of this species. For immature eagles, measurements of wings and tails should be taken carefully if they are used to discriminate gender because the feathers of young birds keep growing after they first take flight. Adults were correctly classified to gender in 100% of cases examined by using the two variables, body mass and forearm. The latter is an easy measurement to take and repeated measurements taken by different observers showed low variances (Ferrer & De Le Court 1992). Gender discrimination for young eagles is valid at 35-45 d of age when nestlings have almost completed their growth.

Figure 2. *Gender identification using PCR test. A multiple amplification with 2945F and cfR specifically amplify a 210 bp fragment of the W chromosome in females and 2945f+ 3224R that amplifies 630 bp fragments in both sexes. Females are indicated by the arrow*

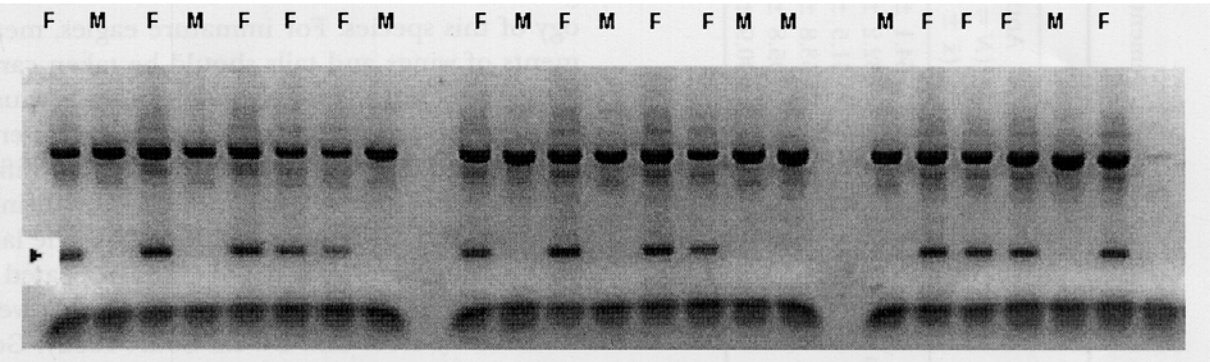


Table 1. *Morphometric measurements in mm of young and adult Booted eagle*

	MALES					FEMALES				
	YOUNG (n = 40) X ± SD	ADULT (n = 16) X ± SD	*F*	Z	*P*	YOUNG (n = 41) X ± SD	ADULT (n = 25) X ± SD	*F*	Z	*P*
Tarsus	64.4 ± 2.51	64.1 ± 2.77	0.33		0.563	69.3 ± 3.30	69.4 ± 3.2	0.168		>0.05
Forearm	131.5 ± 2.64	132.2 ± 4.72	0.65		0.658	140.0 ± 4.85	143.5 ± 3.2	10.26		<0.01
Culmen	28.8 ± 1.29	31.5 ± 1.14	54.1		<0.001	30.9 ± 1.0	34.8 ± 1.3	164.1		<0.001
Wing	244.4 ± 25.9	363.8 ± 7.99		-5.80	<0.001	244.6 ± 28.8	389.2 ± 9.4		-6.77	<0.001
Tail	121.0 ± 18.6	195.8 ± 8.65		-5.80	<0.001	112.9 ± 21.5	208.7 ± 9.2		-6.74	<0.001
Mass	656.3 ± 68.7	690.9 ± 40.9		-1.76	>0.05	828.7 ± 88.3	973.2 ± 76.9	45.59		<0.001

Table 2. *Differences in morphometric measurements between male and female young and adult Booted eagles*

	ADULTS				YOUNGS			
	MALE	FEMALE			MALE	FEMALE		
	(n = 16)	(n = 25)	*t*	*P*	(n = 40)	(n = 41)	*t*	*P*
	X ± SD	X ± SD			X ± SD	X ± SD		
Tarsus	64.1 ± 2.77	69.4 ± 3.23	-5.72	<0.001	64.4 ± 2.51	69.1 ± 3.30	-7.15	<0.001
Forearm	132.2 ± 2.64	143.5 ± 3.20	-12.40	<0.001	131.5 ± 4.72	140.0 ± 4.85	-7.95	<0.001
Culmen	31.5 ± 1.14	34.8 ± 1.32	-8.60	<0.001	28.8 ± 1.29	30.9 ± 1.09	-7.93	<0.001
Wing	355.0 ± 27.8	389.2 ± 9.41	-5.71	<0.001	244.4 ± 25.9	244.6 ± 28.8	-0.03	0.970
Tail	195.6 ± 8.41	208.7 ± 9.24	-4.76	<0.001	121.0 ± 18.6	112.9 ± 21.5	1.78	0.078
Mass	690.3 ± 40.9	973.2 ± 76.9	-13.46	<0.001	656.3 ± 68.7	828.7 ± 88.3	-9.81	<0.001

Acknowledgments

We wish to thank to H. Le Franc and J. Cuesta for their help with the field work. J.M. Arroyo did the lab work and F. Moreno helped with the drawings. We are indebted to L. Garcia for his help assisting, locating, and showing us eagle territories. We also wish to thank M. Bertolotti for reviewing an early draft of this article.

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4. How to test different density-dependent fecundity hypotheses in an increasing or stable population

Referencia: FERRER, M., I. NEWTON & E. CASADO. 2006. How to test different density-dependent fecundity hypotheses in an increasing or stable population. *Journal of Animal Ecology* 75: 111-117.

Resumen

En este capítulo se presenta un estudio de simulación realizado con poblaciones teóricas, tanto estables como en crecimiento, y cuya denso-dependencia en la fecundidad surgiría bien por heterogeneidad de hábitat (HHH) o por ajuste individual (IAH). El objetivo era encontrar diferencias críticas entre las dos hipótesis sobre regulación de poblaciones, que puedan aplicarse a su detección. Para las simulaciones se utilizaron datos extraídos de la población de águila imperial del Parque Nacional de Doñana.

Las poblaciones que se regulan mediante heterogeneidad de hábitat muestran una relación negativa entre su fecundidad media y el coeficiente de variación de la misma. También se detecta una fuerte y negativa relación entre su fecundidad media y la asimetría de su distribución, demostrando que, según la fecundidad desciende las puestas son de menor tamaño y la forma de la distribución se agolpa en la izquierda mientras que la cola se prolonga hacia la derecha. Esto se debe a que cada vez más territorios producen un menor número de crías.

Esta fuerte relación surgió únicamente en las simulaciones correspondientes a poblaciones reguladas mediante heterogeneidad de hábitat, se encontrasen estables o en crecimiento, bajo diferentes proporciones de territorios buenos y malos y con diferentes tamaños de población. Por el contrario, en las simulaciones de poblaciones reguladas mediante ajuste individual no surgió relación entre la fecundidad media de la población y la asimetría de la distribución de la fecundidad.

Las poblaciones reguladas mediante ajuste individual mostraron una relación significativa entre la fecundidad media y su coeficiente de variación, aunque con una pendiente menor que la encontrada en poblaciones reguladas por heterogeneidad de hábitat.

Como conclusión, la asimetría de la fecundidad resultó ser un método crítico que mostró una relación fuertemente significativa con la fecundidad media de una población, solo si en dicha población es la heterogeneidad de hábitat el mecanismo que conduce a la fecundidad denso-dependiente, se encuentre la población estable o en crecimiento. Este método es útil para especies que poseen una distribución discreta del número de crías, con un número pequeño de categorías, lo que incluye a la mayor parte de especies de aves y mamíferos.

Palabras clave: Heterogeneidad de hábitat, ajuste individual, dinámica poblacional, fecundidad denso-dependiente, *Aquila adalberti*.

Abstract

We report on a simulation study of increasing and stable populations working under two different hypotheses of density-dependence of fecundity: the habitat heterogeneity hypothesis (HHH) and the individual adjustment hypothesis (IAH). Our aim was to find critical differences between the two regulatory hypotheses in natural populations.

Populations under HHH showed a strong negative relationship between fecundity and the coefficient of variation of fecundity. We also found a strong negative relationship between fecundity and skewness, demonstrating that, as fecundity decreases, the form of the distribution of brood sizes changes, being more right-skewed due to more territories failing to produce any offspring.

This strong relationship was found only in the simulations of populations under HHH; whether increasing or stable, and under different ratios of good-poor territories and different population sizes. In contrast, no relationship between mean fecundity and skewness was found among simulations under IAH.

Populations under IAH also showed a significant relationship between mean fecundity and the coefficient of variation of fecundity, but with lower slope than in populations under HHH.

In conclusion, skewness was found to be an adequate critical test that showed significant and strong relationships with mean fecundity only in populations under HHH, whether increasing or stable. This test is useful for species with a discrete distribution of offspring with a small number of integer categories, including most bird and mammal species.

Key words: habitat heterogeneity, individual adjustment, population dynamics, density-dependent fecundity, *Aquila adalberti.*

Introduction

The nature and extent of population regulation by density-dependent processes remains a central question in population ecology (Ferrer & Donázar 1996; Rodenhouse, Sherry & Holmes 1997; Newton 1998; Penteriani, Balbontin & Ferrer 2003). Identification of proximate mechanisms by which density can affect demographic parameters is of fundamental importance. Density-dependent effects in population regulation have been well described, especially in fecundity (Newton 1991, 1998; Ferrer & Donázar 1996). However, operating mechanisms are unclear, although two major hypotheses have been proposed (Fretwell & Lucas 1970). The observed density-dependent patterns in mean fecundity could arise either by a higher proportion of individuals occupying poor quality habitats in a heterogeneous environment at high population densities (Andrewartha & Birch 1954; Pulliam & Danielson 1991; Dhondt, Kempenaers & Adriaensen 1992; Ferrer & Donázar 1996; Krüger & Lindström 2001), or by individuals adjusting their behaviour as a response to changing densities within the same habitat, with density affecting all territories and individuals equally (Lack 1954; Both 1998; Fernandez, Azkona & Donázar 1998). The first mechanism is called the habitat heterogeneity hypothesis (HHH) (Dhondt *et al.* 1992; Ferrer & Donázar 1996). According to these authors, as density rises, an increasing proportion of individuals is relegated to lower quality habitats, as a result of which mean population fecundity declines (Andrewartha & Birch 1954; Brown 1969). The second mechanism is named the individual adjustment hypothesis (IAH) or interference competition hypothesis. According to some authors (Lack 1966; Fretwell & Lucas 1970; Dhondt & Schillemans 1983; but see Kempenaers & Dhondt 1992), density-dependent depression of fecundity can arise from an increased frequency of aggression and interference among territorial pairs, resulting in a hostile social environment that leads to a relatively uniform decrease in bird performance. On this hypothesis, as densities increase, all individuals should show reduced fecundity.

As stated by Ferrer & Donázar (1996), these two hypotheses generate the same predictions about mean fecundity in an increasing population: as density rises, mean fecundity declines. Predictions for the expression of fecundity variance, however, are different. For the IAH, no relationship is expected, but for the HHH, fecundity variance must increase with density, because at high densities more poor sites (giving lower fecundity) are occupied. In consequence, Ferrer & Donázar (1996) suggested that a critical test between the two regulatory mechanisms in an increasing population would be a strong negative relationship between mean fecundity and its coefficient of variation. This test was subsequently used by several authors (Krüger & Lindstrom 2001; Penteriani, Gallardo & Roche 2002; Sergio & Newton 2003). Nevertheless, the possibility of spurious correlations between the mean and coefficient of variation in a discrete distribution, with a small number of categories and limited by boundaries, such as brood-size distribution in birds or litter-size in mammals, led us to search for complementary criteria.

Here, we report on an individual-based simulation study of increasing and stable model populations, one pair working as predicted by HHH and the other as predicted by IAH. Our aim is to analyze changes in the parameters of the distribution of both types of populations in an attempt to find critical differences that allow us to discriminate clearly between the two regulatory hypotheses in natural populations, whether these populations are increasing or stable (or declining). Additionally, we use data from a population of the Spanish Imperial eagle (*Aquila adalberti* Brehm 1861) in Doñana National Park (SW Spain) as a case study.

Material and methods

COMPUTER SIMULATION AND MODEL ASSUMPTIONS

Individual-based models envisage a population as an assemblage of individuals. They have been applied to a wide variety of ecological problems (Lomnicki 1978; Huston, DeAngelis & Post 1988, DeAngelis & Gross 1992; Uchmanski & Grimm 1996; Wilson 1998; Fahse, Dean & Wissel 1998; Grimm 1999; Ferrer, Otalora & García-Ruiz 2004), and have a number of advantages over other, more traditional types of modeling techniques. In our case, we developed a stochastic "territory-based" simulation, using turbo Pascal to model the dynamics of the population. Breeding outputs of up to 30 territories were simulated simultaneously. In this way we created a "simulated breeding population" from which "simulated field data" were obtained. Simulation models were adjusted as appropriate to breeding parameters of the Imperial eagle *Aguila adalberti,* using data from a well-known population in Doñana National Park, SW Spain (Ferrer & Calderón 1990; Ferrer & Donázar 1996; Ferrer 2001; Ferrer *et al.* 2004). *A. adalberti* is a large (2500-3500 g), sedentary and territorial bird of prey, with a low reproductive rate and a relatively high age of maturity (adult plumage at age four-five years). Territories, with a mean size of 1,200 ha, are exclusive and vigorously defended throughout the year. Models were used to generate the expected distribution of brood-sizes in theoretical populations of eagles that can only have four integer values of brood size: 0, one, two and three nestlings. This represents a common and realistic scenario for most territorial raptors, owls, and some other species of birds (Newton 1979; Del Hoyo, Elliot & Sargatal 1994), as well as for most mammals. We used a time step of one year. Territories were created and decisions about their production each year were made through the use of random number generators. For each cycle (equivalent to one year), we computed the number of active territories and number of offspring produced in each. We also computed the mean and the variance of fecundity by years and by territories. We made 200 replicates of the same population, and the simulation closest to the mean of all the 200 replicates of each case was selected. This allowed us to work with a brood-size distribution having a discrete, small number of categories, limited by boundaries, as in many large birds.

SIMULATIONS UNDER HABITAT HETEROGENEITY HYPOTHESIS

Two different situations were simulated, involving increasing and stable populations. The increasing populations were allowed to increase from a starting number of 10 pairs until they reached the ceiling number of 30 pairs, and simulations were run for 30 breeding seasons (equal to years). During this period of population growth, we included a decrease in fecundity according to a density-dependent fecundity function that reduced fecundity linearly from starting values of 1.59 to final values of 0.7 young per pair (44% of the initial mean fecundity, Ferrer & Donázar 1996). As we assumed that density-dependent fecundity arose by the HHH, we maintained the same fecundity values from the beginning (low density situation) until the end (saturated situation) in the first-occupied territories. In consequence, the decrease in mean fecundity was due only to the occupation of new territories, where birds had lower fecundity. Two different ratios between good-poor territories were used: 10 high quality territories from a total of 30, and 10 poor from a total of 30. To account for stochasticity in fecundity values, we selected for each territory each year one value at random from a normal distribution with mean fecundity according density and territory quality (high quality territories: 1.59 nestlings; low quality: 0.47 nestlings; in the real population fecundity was normally distributed, see Ferrer & Donázar 1996), and variance according to observed variance in the natural population, that is 0.901 (Ferrer & Donázar 1996; Ferrer et al. 2004). The integer value closest to the random number was selected as brood size for the territory. During population increase, low quality territories were occupied only when no high quality territory was unoccupied.

We also simulated stable populations that stayed at the ceiling level of 30 pairs over 30 years with a mean fecundity value for the whole population of 0.7 young per pair per year. Again, two different ratios of good-poor territories were used (10/20 and 20/10). For each territory each year, one value at random from a normal distribution was selected. In the high quality territories, mean fecundity was 1.59 and in the low quality territories 0.47, with variance again set at 0.901. Simulations during a short time, 10 years, were also conducted to analyze the effect of sample size (numbers of years), as well as small populations with only 15 breeding pairs (five good and 10 poor territories).

SIMULATION UNDER INDIVIDUAL ADJUSTMENT HYPOTHESIS

Here we again simulated two different situations. The increasing population was allowed to rise from 10 to 30 territories during 30 years, using the same density-dependent fecundity function as above. Because we assumed that density-dependent regulation of fecundity was by IAH, we used the same mean fecundity for all the territories (mean according density and variance of 0.901). So as density increased, fecundity decreased with the same probability in all the territories. To account for stochasticity in fecundity values, we selected for each territory each year one value at random from a normal distribution with mean fecundity according density (low density 1.59; high density 0.7, the same value for all the territories), and variance according to observed variance in the natural population, (that is 0.901, see above). The integer value closest to the random number was selected as the brood size for the territory. During the period of population increase, new territories were occupied at random.

We also simulated 30 years of a stable population that stayed at the ceiling level of 30 pairs, with a mean fecundity value for the whole population of 0.7. Again, we selected for each territory each year one value at random from a normal distribution, but in this occasion, mean fecundity was 0.7 (mean value for eagle populations under saturation) for all the territories, and variance was again 0.901. Ten-year simulations with 30 and 15 pairs were also conducted to analyze the possible effect of sample size.

REAL POPULATION DATA

A. adalberti is the most endangered bird of prey in Europe and one of the rarest raptors in the world (Collar & Andrew 1988). Its total population is estimated to be about 150 pairs (Ferrer 2001). In Doñana National Park, its numbers increased from six to 15-16 breeding pairs, and then remained stable from 1976 until 1992 (long-term monitoring data (1959-1991) from the Doñana archives). From this pattern, it may be inferred that the population was close to the environmental carrying capacity during the last years. Coincident with the population increase, mean fecundity decreased and then stabilized with population size. The entire National Park area was surveyed at the beginning of each breeding season (January-February, during the courtship and nest site selection period; Ferrer 2001) to determine if pairs were present on territories. The sedentary behavior of birds and their tendency to call repeatedly greatly facilitated their detection. We believe that all breeding and non-breeding pairs were detected every year.

STATISTICAL ANALYSES

Statistical analyses were conducted using STATISTICA package. All the variables were normally distributed. We tested for trends with linear analysis using the F-ratio statistic to find whether the slope of the data was significantly different from 0. Variances of the linear models were tested for homogeneity using Cochran's C statistic. Mixed ANOVAs with random factors were employed. Statistical significance was set at $p<0.05$.

Results

POPULATIONS UNDER HHH

The coefficient of variation of fecundity in an increasing population with a good-poor territory ratio of 10/20, showed a significant increase from 45% to 130% as the population grew from 10 to 30 pairs (Figure 1). Mean fecundity, coefficient of variation of fecundity, skewness and kurtosis showed highly significant relationships with population density (four-way ANOVA; *fecundity,* F=139.31, df=4, 26, P<0.001; *CV,* F=54.43, df=4, 26, P<0.001; *skewness,* F=43.40, df=4, 26 P<0.001; *kurtosis,* F=3.18, df=4, 26, P=0.03). As population density increased, coefficient of variation, skewness and kurtosis increased, while fecundity decreased. Mean fecundity was inversely related to skewness of fecundity (Figure 2). In addition, the coefficient of variation showed significant variation according to mean fecundity (Table 1).

Using a ratio of 20 good to 10 poor territories, again mean fecundity, coefficient of variation, skewness and kurtosis showed highly significant relationships with population density (four-way ANOVA; *fecundity,* F=14.16, df=4, 26, P<0.001; *CV,* F=7.80, df=4, 26, P<0.001; *skewness,* F=4.72, df=4, 26 P<0.001; *kurtosis,* F=0.65, df=4, 26, P=0.652). As the model population grew, coefficient of variation of fecundity and skewness increased, and fecundity decreased. Coefficient of variation of fecundity was inversely related to mean fecundity, as was skewness (Table 1).

Simulations of stable populations under HHH with 10 good territories out of 30 over a period of 30 years showed that the coefficient of variation was inversely related to mean fecundity, as was skewness (Table 1). Reducing the simulation time to 10 years to analyze the possible effect of length of study, we obtained the same results (Table 1), as well as on a good-poor territory ratio of 20:10 (Table 1). Ten-year simulations with a 20:10 good:poor territory ratio showed that the coefficient of variation and skewness were significantly related to mean fecundity (Table 1).

Simulations conducted with populations of only 15 pairs (five good and 10 poor territories) over 30 years showed significant relationships between mean fecundity and coefficient of variation and skewness (Table 1). Reducing the simulation period to ten years again revealed a significant relationship between mean fecundity and skewness, but not with coefficient of variation (Table 1).

POPULATIONS UNDER IAH

The coefficient of variation of fecundity in an increasing population under IAH increased from 45% to 80% as the population grew from 10 to 30 pairs (Figure 1). Only mean fecundity and its coefficient of variation showed highly significant relationships with population size, but not skewness or kurtosis (four-way ANOVA; *fecundity,* F=365.56, df=4, 26, P<0.001; *CV,* F=17.26, df=4, 26, P<0.001; *skewness,* F=1.03, df=4, 26 P=0.409; *kurtosis,* F=0.717, df=4, 26, P=0.588). The coefficient of variation was inversely related to mean fecundity (Table 1). In contrast, skewness was not related to mean fecundity (Figure 2).

Simulations of stable populations under IAH over 30 years showed that the coefficient of variation was inversely related to mean fecundity, while skewness showed no significant relationship (Table 1). Ten-year simulations showed a significant negative relationship between mean fecundity and coefficient of variation, but not with skewness (Table 1).

Simulations with a population of only 15 pairs over 30 years also showed a significant negative relationship between mean fecundity and coefficient of variation, but not with skewness (Table

1). Ten-year simulations with 15 pairs showed that mean fecundity was not related to coefficient of variation, or skewness (Table 1).

REAL POPULATION

The proportion of high versus low quality territories in the real population was 6/10 (Ferrer and Donázar 1996). Mean fecundity was negatively related to coefficient of variation of fecundity (r=-0.890, df=23, P<0.001; Ferrer & Donázar 1996), and to skewness (r=-0.860, df=23, P<0.001). In addition, kurtosis showed significant variation according to mean fecundity (r=-0.656, df=23, P<0.001).

Table 1. *Results of linear regressions with mean fecundity as the independent variable and Coefficient of Variation (CV), Skewness and Kurtosis as dependent variables. Simulations were made under different hypotheses, ratios of good/poor quality territories, population trends (increasing or stable populations) and during different number of years (see text). ** P<0.001 and* P<0.5*

					CV			Skewness			Kurtosis		
Hypothesis	Pairs	Fecund.	Years	good/poor	r	df	P	r	df	P	r	df	P
HHH-increas.	10-30	1.6-0.7	30	10/20	-0.954	28	**	-0.935	28	**	-0.412	28	*
HHH-increas.	10-30	1.6-0.7	30	20/10	-0.682	28	**	-0.770	28	**	0.124	28	ns
IAH-increas.	10-30	1.6-0.7	30	-----	-0.715	28	**	-0.258	28	ns	-0.193	28	ns
HHH-stable	30	0.7	30	10/20	-0.715	28	**	-0.926	28	**	-0.383	28	*
HHH-stable	30	0.7	10	10/20	-0.939	8	**	-0.877	8	**	-0.635	8	*
HHH-stable	30	0.7	30	20/10	-0.898	28	**	-0.786	28	**	0.466	28	*
HHH-stable	30	0.7	10	20/10	-0.945	8	**	-0.735	8	**	0.551	8	*
HHH-stable	15	0.7	30	5/10	-0.545	28	**	-0.652	28	**	-0.110	28	ns
HHH-stable	15	0.7	10	5/10	-0.492	8	ns	-0.879	8	**	-0.162	8	ns
IAH-stable	30	0.7	30	-----	-0.474	28	*	0.301	28	ns	0.579	28	**
IAH-stable	30	0.7	10	-----	-0.672	8	*	0.124	8	ns	0.445	8	ns
IAH-stable	15	0.7	30	-----	-0.674	28	**	-0.183	28	ns	0.325	28	ns
IAH-stable	15	0.7	10	-----	0.317	8	ns	0.103	8	ns	0.621	8	*

Discussion

Simulation models enable us to analyze differences in the distribution of parameters according to the mechanisms used to generate density dependent depression of fecundity. Increasing populations under HHH showed a strong negative relationship between fecundity and coefficient of variation of fecundity: as population size increased, mean fecundity decreased and coefficient of variation increased. We found a strong negative relationship between fecundity and skewness, demonstrating that as fecundity decreased, the form of the distribution of brood sizes changed, being more right-skewed. This strong relationship was found in the simulations of populations under HHH; whether increasing or stable, and under different ratios of good-poor territories and different population sizes. Also a significant relationship between mean fecundity and coefficient of variation was found in stable populations under HHH.

On the other hand, significant negative relationships between fecundity and coefficient of variation of fecundity in increasing and stable populations were found under IAH. As fecundity decreased, coefficient of variation increased significantly, but with a lower slope than in populations under HHH. In contrast, no relationship between mean fecundity and skewness was found among simulations under IAH, whether in increasing or stable populations, or in populations of different sizes.

Figure 1. *Trend of mean and coefficient of variation of fecundity in increasing simulated populations according Habitat Heterogeneity and Individual Adjustment Hypotheses*

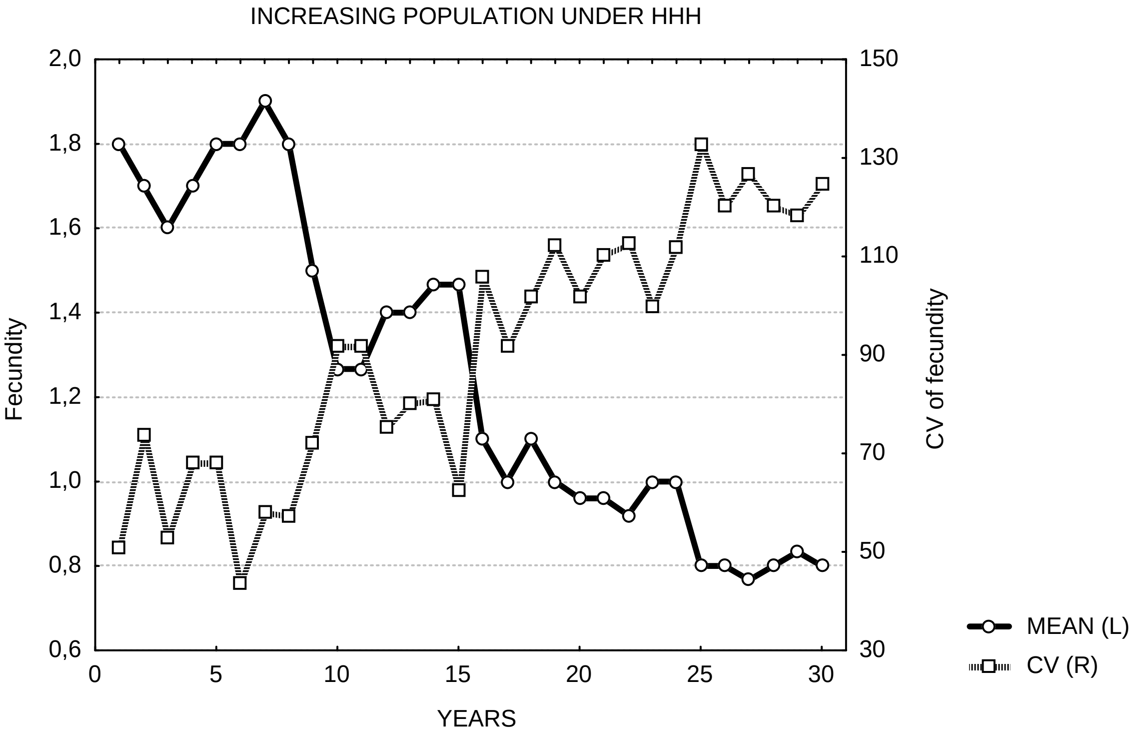
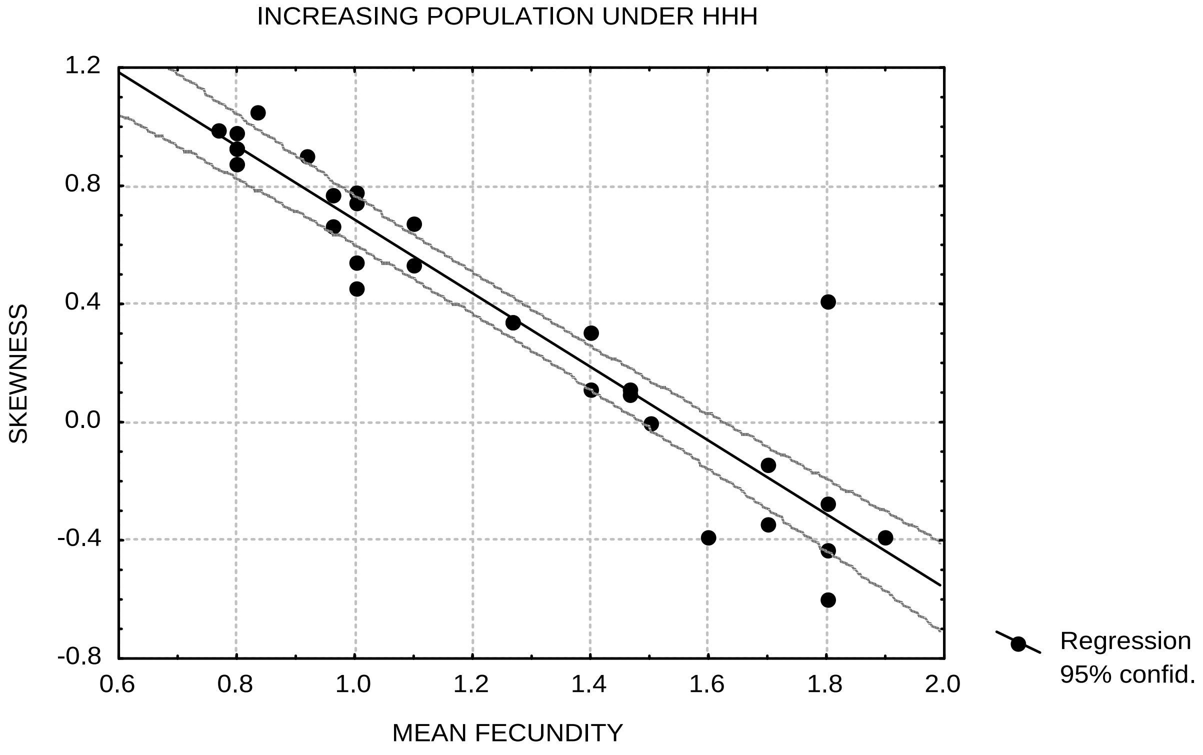
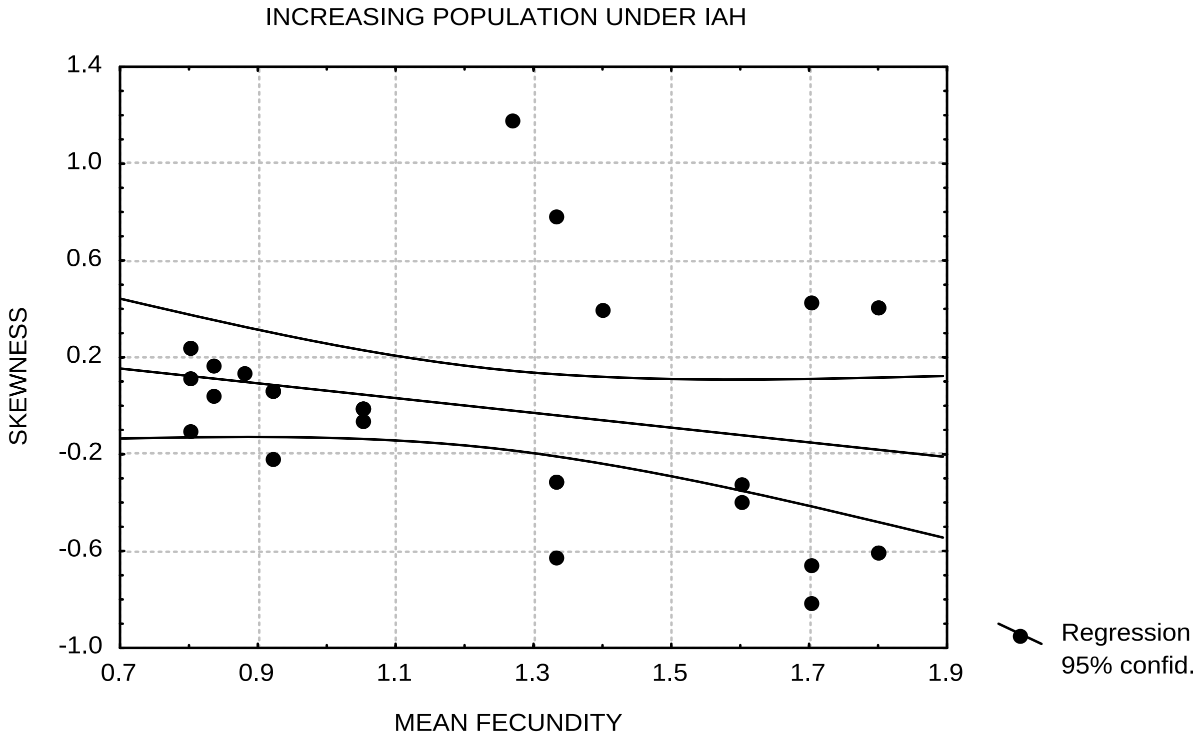


Figure 2. *Linear regressions between skewness and mean fecundity in increasing simulated populations according Habitat Heterogeneity and Individual Adjustment Hypotheses*





Using data from a real population of eagles known to be under density dependent regulation of fecundity through habitat heterogeneity (Ferrer & Donázar 1996), a strong negative relationship between mean fecundity and skewness was found.

In consequence, the suggestion of Ferrer & Donázar (1996) that a critical test between the two regulatory mechanisms in an increasing population would be a strong negative relationship between mean fecundity and its coefficient of variation under HHH but not under IAH, must be taken with caution. In fact, this expected relationship is true and strong in populations under HHH, but could also emerge as statistically significant in populations under IAH. The reason is that, in a discrete integer distribution, with a low number of brood-size categories and limited by fixed boundaries, spurious correlations between the mean value and the coefficient of variation can be found. As brood-size distributions are limited by fixed boundaries (for example it is impossible to have a negative fecundity), as mean fecundity decreases, variance of fecundity would increase. So the differences in the relationship between mean fecundity and coefficient of variation among both hypotheses is only a matter of degree.

On the other hand, skewness was found to be an adequate critical test that showed significant and strong relationships with mean fecundity only in populations under HHH, whether increasing or stable. Skewness is at best as an indicator of changes in the form of distributions fitting well with the original idea of different parts of the population responding in different ways to density changes, whether long term or year-to-year. An important point is that results seem to show that the skewness test is very robust even with a short time series. This test could therefore be used even with relatively short-term studies.

Of course, additional criteria could distinguish between the two hypotheses; for example: (1) high quality sites have the same fecundity at high or low population densities (Newton & Marquiss 1976; Newton 1991; Ferrer & Donázar 1996; Rodenhouse *et al.* 1997), (2) high quality sites must be the first and most frequently occupied sites (Ferrer & Donázar 1996; Sergio & Newton 2003), or (3) high quality sites must be less variable among years than low quality sites (Ferrer & Donázar 1996). Another complementary criterion is that low quality sites may be more frequently occupied by non-adult individuals in species with deferred sexual maturity (Ferrer & Bisson 2003; Ferrer & Penteriani 2003). But use of all these criteria requires a good database with all occupied sites, territories and occupants monitored year after year.

Such studies are not common, and more often we have access only to long series of fecundity data with no clear geographical reference points. For these types of data, the present test of coefficient of variation and skewness seems ideal to find the mechanism of density dependence over the prevailing density range.

With the skewness test, we are able to determine whether the response of a population to long-term changes in density or to inter-annual fluctuations in density is heterogeneous or not. Territory quality is not necessarily the only source of heterogeneity, however, for breeding success is a function of both territory quality and individual quality. If the best territories were occupied by the best performing animals, rather than by a random selection of animals, this would add to the level of heterogeneity resulting from territory quality alone (Newton 1991; Ferrer & Donázar 1996; Rodenhouse *et al.* 1997). Whatever its cause, the existence of consistent heterogeneity is always important from a theoretical point of view, as well as for informing conservation programs.

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5. Density-dependence hypotheses and the distribution of fecundity

Referencia: FERRER, M., I. NEWTON & E. CASADO. 2008. Density-dependence hypotheses and the distribution of fecundity. *Journal of Animal Ecology* 77: 341-345.

Resumen

Beja & Palma (2008) intentan ofrecer un análisis crítico de la efectividad y limitaciones de un método publicado previamente (Ferrer *et al.* 2006) para discriminar entre la hipótesis de heterogeneidad de hábitat (HHH) y la de ajuste individual (IAH), utilizando datos reales de una población de Águila perdicera (*Hieraaetus fasciatus*).

Concluyen que una correlación fuerte y significativa entre la media de la fecundidad y su CV o asimetría de la distribución son esperables bajo una asunción plausible sobre la distribución del tamaño de puesta, y que por lo tanto no se puede distinguir entre ambas hipótesis utilizando estos métodos.

Ellos utilizaron la misma distribución quasi-Poisson del tamaño de puestas (típica para una población saturada bajo la hipótesis de Heterogeneidad de Hábitat) para ambas familias de simulaciones. De modo que no es sorprendente que ambos grupos les den resultados similares. Ellos argumentaron que esta aproximación era "empírica", libre de asunciones teóricas. Pero el objetivo de testar ambas hipótesis, es precisamente encontrar diferencias entre las distribuciones teóricas del tamaño de puestas que se predicen bajo ambas hipótesis.

Resumiendo, con la misma fecundidad media en situación de alta densidad, ambas hipótesis deben tener diferentes distribuciones del tamaño de puesta. De modo que usar una única distribución sesgada hacia la derecha, típica de una población real saturada (probablemente bajo la hipótesis de Heterogeneidad de hábitat) para intentar distinguir entre ambas hipótesis mediante el re-muestreo consecutivo sobre la misma distribución según hicieron Beja & Palma, es claramente inapropiado.

Palabras clave: Distribución del tamaño de puesta, fecundidad denso-dependiente, heterogeneidad de hábitat, ajuste individual, simulación orientada a objetos.

Abstract

Beja & Palma (2008) attempt to provide a critical analysis of the effectiveness and limitations of a previously published method (Ferrer, Newton & Casado 2006) to discriminate between Habitat Heterogeneity Hypothesis (HHH) and the Individual Adjustment Hypothesis (IAH) using real data from a Bonelli's eagle *Hieraaetus fasciatus* population.

They conclude that significant and strong correlations between mean and CV or skewness are expected under a biologically plausible assumption about brood size distribution, and that the two hypotheses cannot therefore be distinguished.

A major concern we have with their paper centres on this biologically plausible brood-size distribution. They used the same quasi-Poisson distribution of brood sizes (typical for a saturate population under HHH) for both families of simulations. So, is not surprising that both groups gave similar results.

They argued that this approach was 'empirical', free of theoretical assumptions. But in testing between hypotheses, what we are looking for is precisely the differences among theoretical brood-size distributions predicted under the two hypotheses.

Summarizing, with the same mean fecundity at high densities, both hypotheses *must have* different brood-size distributions. So the use of a single right-skewed distribution, typical of a real saturated population (most likely under HHH) in attempts to distinguish between the two hypotheses by re-sampling several times on the same right-skewed distribution, as done by Beja & Palma (2008), is clearly inappropriate.

Keywords: Density-dependent fecundity; brood size distribution; habitat heterogeneity; individual adjustment; object-oriented simulation.

Introduction

The nature and extent of population regulation by density-dependent processes remains of central concern in population ecology (Ferrer & Donázar 1996, Rodenhouse, Sherry & Holmes 1997, Newton 1998, Penteriani, Balbontín & Ferrer 2003, Sergio & Newton 2003). Identification of proximate mechanisms by which density can affect demographic parameters, as well as the way they operate, is therefore of fundamental interest.

Density-dependent effects in bird population regulation have been well described, especially in fecundity (Newton 1991, 1998, Ferrer & Donázar 1996, Rodenhouse *et al.* 1997). Two major hypotheses have been proposed, in which the observed density-dependent patterns in fecundity could arise either by (1) a higher proportion of individuals occupying poor quality areas at high than at low population densities (Andrewartha & Birch 1954**,** Pulliam & Danielson 1991, Dhondt, Kempenaers & Adriaensen 1992, Ferrer & Donázar 1996, Krüger & Lindström 2001); or by (2) individuals adjusting their behaviour as a response to changing densities within the same area (Lack 1954, Both 1998, Fernandez, Azkona & Donazar 1998). The first mechanism is called the habitat heterogeneity hypothesis (HHH) or site-dependence hypothesis (Ferrer & Donázar 1996, Rodenhouse *et al.* 1997), and the second the individual adjustment hypothesis (IAH) or interference competition hypothesis.

Distinguishing between these two potential regulatory mechanisms is important not only to the theoretical population biologist, but also to the conservation biologist working on endangered species, because it informs management needs (Ferrer & Donazar 1996, Sergio & Newton 2003, Ferrer, Newton & Casado 2006, Carrete *et al.* 2006). Under HHH, populations close to saturation level are perceived as source-sink systems, with pairs in the best parts of the habitat (source areas) producing more young than necessary to replace themselves, and pairs in other parts (sink areas) producing insufficient young, so that their numbers are maintained only by over-production in source areas (Ferrer & Donazar 1996). Consequently, partial habitat destruction could affect population viability in different ways, depending on which parts of the habitat were affected (Newton 1991, 1998, Ferrer & Donázar 1996). Under IAH, the population is perceived as living in homogeneous habitat, destruction of any part of which should have the same effect on mean reproductive rate as destruction of any other part. For these conservation reasons, several attempts have been made at distinguishing between these two main mechanisms which could produce density-dependence in fecundity (see Ferrer & Donazar 1996, Sergio & Newton 2003, Ferrer *et al.* 2006). The methods depend critically on changes in the distribution of brood sizes (including zeros) at different population densities.

TWO HYPOTHESES, TWO DISTRIBUTIONS

In their paper, Beja & Palma (2008) attempt to provide a critical analysis of the effectiveness and limitations of a previously published method to discriminate between the Habitat Heterogeneity Hypothesis (HHH) and the Individual Adjustment Hypothesis (IAH). To this end, they use real data from a Bonelli's eagle *Hieraaetus fasciatus* population to parameterize a simulation model which is used to test the skewness and CV trajectories of brood-size distributions according population size. They conclude that, under both hypotheses (HHH and IAH), significant and strong correlations between mean and CV or skewness are expected under a biologically plausible assumption about brood size distribution, and that the two hypotheses cannot therefore be distinguished.

A major concern we have with their paper centres on this biologically plausible brood-size distribution. They used the same quasi-Poisson distribution of brood sizes for both families of simulations (based on HHH and IAH respectively), because this was the 'real' brood-size distribution in the natural population of eagles. But this distribution could itself result from a density-dependent process, most likely based on HHH (as far as they are able in their study to distinguish between good and bad territories). In any case, they used the same brood size distribution in both groups of simulations, so is not surprising that both groups gave similar results.

Both density-dependent hypotheses generate the same prediction for the distribution of brood-sizes in a population at low density, but differ about the final distribution of brood-sizes in populations close to saturation level. The idea of different distributions under the two hypotheses was implicit in their formulation. Under AIH, depression in fecundity arises from some factor that acts more or less uniformly across the population, such as an increasingly hostile social environment resulting from increasing agonistic encounters and interference that has a similar impact on all individuals. As density rises, practically all individuals could show reduced fecundity, and variance in brood-sizes among individuals would not alter (Lack 1966, Fretwell & Lucas 1970, Dhondt & Schillemans 1983, Ferrer & Donazar 1996). In contrast,

under HHH, the depression of mean fecundity results from habitat heterogeneity, and as density rises, an increasing proportion of individuals are forced to occupy poorer habitat, where individual reproductive rates are lower.

During a period of population increase, therefore, mean population brood-size decreases while variance in brood size increases (Andrewartha & Birch 1954, Brown 1969, Fretwell & Lucas 1970, Dhondt *et al.* 1992, Ferrer & Donazar 1996). It is true that nothing about the real form of the distribution was said, but it is clear that, with the same mean fecundity, the two hypotheses must have different brood-size distributions. As density rises, the HHH predicts an increasingly left biased distribution of brood sizes, with some territories producing consistently large broods, against an increasing number producing smaller broods. Conversely, the IAH predicts a closer-to-normal distribution of brood-sizes at all densities, centred on a decreasing mean value as density rises. It is this distribution of brood-sizes, or its change during population growth, that is crucial to testing between the two hypotheses.

The bootstrapping procedure used by Beja & Palma (2008) is a general technique for estimating sampling distributions by treating the observed data as if it were the entire (and unique) statistical population under study. On each replication, a random sample of size *N* is selected, with replacement, from the available data. Under HHH, Beja & Palma divided their territories, on the basis of brood sizes, into good and bad, sampling from good and poor territories separately and after pooling them. Under IAH, they sampled only from the total data base (of both good and poor territories) and presented the results altogether. (Note that some of the outliers are the same in the two "different" families of simulations.) They argued that this approach was 'empirical', free of theoretical assumptions. But in testing between hypotheses, what we are looking for is precisely the differences among theoretical brood-size distributions predicted under the two hypotheses.

IS THE LEFT-BIASED BROOD SIZE DISTRIBUTION UNIVERSAL AMONG LONG-LIVE BIRDS?

Beja & Palma (2008) claimed that long-lived species with low fecundity often show left-biased brood-size distributions (close-to-Poisson) regardless of situation. They concluded that the use of a close-to-normal distribution in Ferrer *et al.* (2006) was an unrealistic approach, likely to generate spurious correlations. Nevertheless, a quasi-normal distribution of brood-sizes is the most common situation in raptor populations at low densities (see Ferrer & Donázar 1996).

Interestingly, using their own data on brood-sizes in good territories (the only territories occupied at low density) in their Bonelli's eagle's population, the distribution of brood-sizes was close-to-normal, showing no left-bias (Figure 1a). In contrast, their data from poor territories (those occupied only in a high density situation) showed a strong left-biased distribution (Figure 1b). This is as expected under HHH.

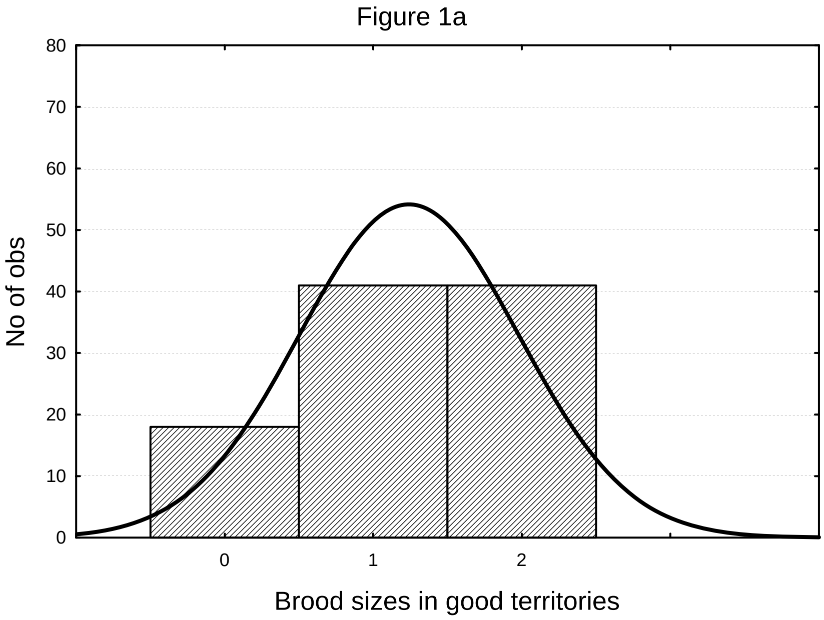
Nevertheless the number of brood-size categories will clearly influence the power of the test, and the likelihood of obtaining a significant test result. The Spanish Imperial eagles *Aquila adalberti* discussed in our original paper had four brood size categories (0-3), while their Bonelli's eagles had three (0-2). In species that lay only one egg (brood sizes 0 or 1), it is extremely unlikely that the two hypotheses could be distinguished using this kind of approach. But as the number of brood size categories increases, it should become increasingly easy to detect differences between the two expected distributions.

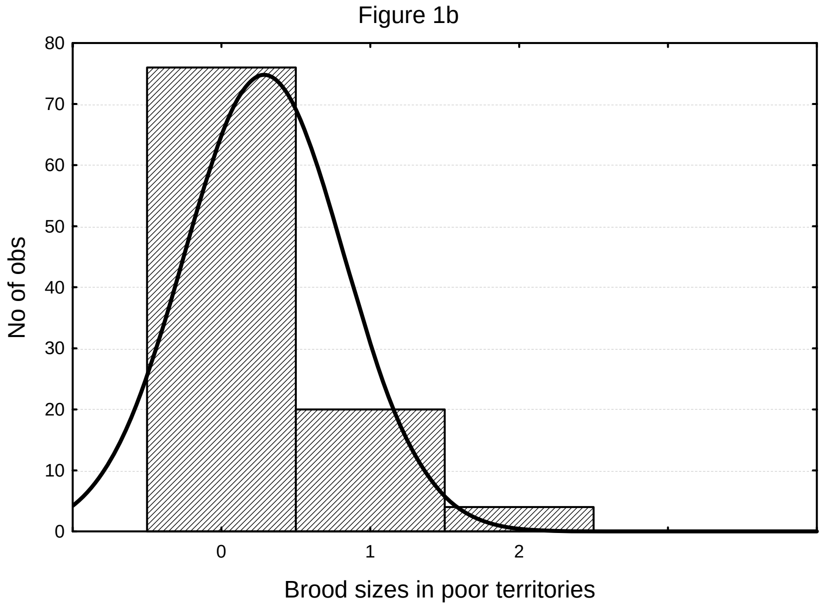
SIMULATION PROBLEMS

Beja & Palma stated that the use of the normal distribution is unwarranted because it causes simulation problems, such as predicted values below zero. It is true that using a normal distribution, when mean fecundity values decrease, some of the randomly selected values would be less than zero, but this problem can be corrected easily during the simulation, first by raising negative values to zero, which gives a mean value slightly higher than the stipulated mean. This higher mean must then be reduced accordingly for the next simulation. These correction procedures were employed in Ferrer *et al.* (2006).

Owing to scant procedural details, we find it difficult to follow the logic of the simulation in Beja & Palma (2008), but nevertheless it is easy to see that simulations of increasing populations were done starting with a mean fecundity of 1.23 (corresponding to mean value for high quality sites) and ending with 0.28 (corresponding with the mean value for low quality sites, see table 1 of Beja & Palma 2008). By this procedure, they effectively forced the whole final population into in low quality sites (or greatly affected by interference) with a mean fecundity far below the observed, which seems to be around 0.75 (see Figure 1 in Beja & Palma 2008). They thereby constrained the results of the simulation into an unrealistic and extremely left-biased distribution. With this extremely low mean value of 0.28, the population would probably become extinct without continuing immigration.

Figure 1a) *Using data from Beja & Palma (2008), at low density, brood-sizes of Bonelli's eagles showed a non left-biased distribution.* **Fig. 1b)** *Poisson (Left-biased) distribution of brood sizes in poor territories of Bonelli's eagles (those occupied only in a high density situation, data from Beja & Palma 2008)*





SPURIOUS CORRELATIONS

Beja & Palma stated that the regression relationship between mean fecundity and its coefficient of variation should not be used in further studies owing to potential spurious correlation in the general form of X *vs* Y/X, in which X and Y are non-independent variables. We agree that caution should be exercised, as stressed in our previous paper, but disagree that only spurious correlations are expected (a point also made by Brett (2004) cited by the authors in support of their view). According to Chayes (1949), the expected spurious correlation of the form X *vs* Y/X is:

$$r = - CV_x \ / \ (CV_y^2 + CV_x^2)^{1/2}$$

Using data from our previous paper, the expected coefficient of determination due to spurious correlation was $r^2 = 0.54$ for an increasing population under IAH and $r^2 = 0.52$ for an increasing population under HHH. The values obtained in the previous analyses were $r^2 = 0.511$ for increasing population under IAH and $r^2 = 0.92$ for increasing populations under HHH. This implies that the relationship found between mean fecundity and its CV under IAH could have been solely the result of a spurious correlation, as stated by Ferrer *et al.* (2006). But the relationship found in an increasing population under HHH between mean fecundity and its CV is clearly stronger than expected solely due to spurious correlation. To adequately analyze this problem when using the relationship between mean fecundity and CV to look for density dependence, we have to use the above approach or conduct simulations using, for example, the Monte Carlo procedure to generate the null distribution against which we can test the significance of the correlation coefficients. These problems complicate the use of CV and mean fecundity as a clear test to discriminate between the two main hypotheses, as stated in our previous paper. On the other hand, this kind of problem does not arise in the relationship between mean brood size and skewness. For this reason among others, we suggest the use of this test (Ferrer *et al.* 2006).

Beja & Palma stated that, by and large, caution should be exercised when interpreting results of previous studies relying on the mean fecundity-CV relationship to draw inferences about population processes (they cited Ferrer & Donázar 1996, Blanco *et al.* 1998, Krüger & Lindström 2001, Sergio & Newton 2003, Penteriani *et al.* 2003, Carrete *et al.* 2006). However, as is clearly evident in the papers they cited, all the authors used several different and complementary criteria, including the CV test, in drawing conclusions about which mechanism was the most relevant in their study populations. So the concerns expressed by Beja & Palma do not seem well supported in practice.

Beja & Palma further stated that our previous suggestion of the use of the skewness test as the simpler way to differentiate between density-dependent fecundity hypotheses was largely inadequate. We believe that they are too dismissive of the test we propose. We did not claim that this test was the only and definitive way to assess the kind of regulation operating. We said that, if a significant test result is obtained, this provides strong support for the overwhelming operation of HHH. If the result is statistically insignificant, this does not wholly exclude the operation of HHH, but indicates that its role is small compared to other mechanisms of density dependence. As stated in Ferrer *et al.* 2006, additional criteria are recommended, namely (1) high quality yield similar brood sizes at high or low population densities under HHH, (2) high quality sites are the first and most frequently occupied sites, (3) high quality sites are less variable in productivity among years than low quality sites, or (4) low quality sites may be more frequently occupied by non-adult individuals in species with deferred sexual maturity. Use of all these criteria requires a good data base. When only long-term data on brood size are available, the use of the skewness test seems ideal to check for the operation of one of these regulatory mechanisms.

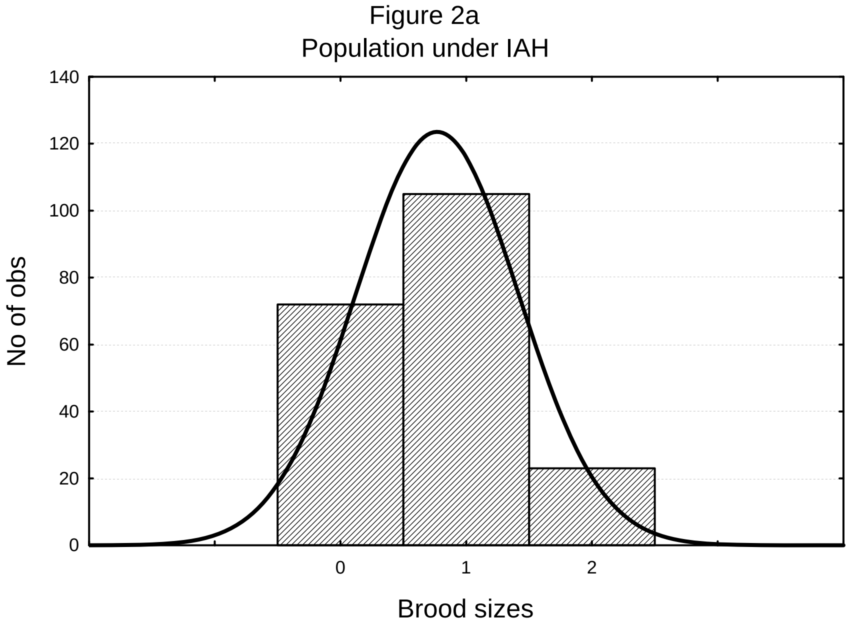
HABITAT AND INDIVIDUAL QUALITY

Beja & Palma also stated that Carrete *et al.* (2006) reported immature birds breeding more frequently in certain territories than expected by chance, and concluded that differences in fecundity among territories could arise simply from variability in occupant age rather than in territory quality. While the age of an eagle it is without doubt an eagle characteristic, differences in the probability of occupancy of territories by immature birds seems to be a characteristic of the territories themselves, being a component of territory quality. Hence, the differences in brood-sizes they detect are in fact partly a consequence of the differences in territory quality, specifically in the turn-over rates. This is true irrespective of the possible cause generating these differences (mortality, emigration rate, etc). As stated by Ferrer & Bisson (2003), heterogeneity in individual performance within populations need require only two assumptions: (1) breeding outputs differ among territories, and/or (2) occupant turnover rates by either mortality or emigration differ among territories.

Conclusion

Summarizing, the HHH predicts an increasingly left-biased distribution of brood-sizes as density rises, with only some territories producing consistently large broods, and an increasing number producing few or no chicks. Conversely, the IAH predicts a more close-to-normal distribution of brood-sizes at all densities with only minor differences among territories. Consequently, with the same mean fecundity at high densities, both hypotheses *must have* different brood-size distributions (Figure 2). So the use of a single left-biased distribution, typical of a real saturated population (most likely under HHH) in attempts to distinguish between the two hypotheses by re-sampling several times on the same left-biased distribution, as done by Beja & Palma (2008), is clearly inappropriate.

Figure 2a). *Non left-biased brood sizes distribution of Bonelli's eagles in a saturated population with a mean fecundity of 0.75 (i.e. under IAH).* **Figure 2b)**. *Brood sizes distribution in a saturated population under HHH with a mean fecundity of 0.75 showing a quasi-Poisson distribution (using data from Beja & Palma 2008)*



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6. Density-dependent fecundity by habitat heterogeneity in the Booted eagle

Resumen

En el presente capítulo se analiza la relación entre fecundidad y densidad de la población en crecimiento de águila calzada del Parque Nacional de Doñana. El objetivo era testar las diferentes predicciones generadas por las dos principales hipótesis que intentan explicar qué mecanismo conduce a la fecundidad denso-dependiente. Dichas hipótesis son la de Heterogeneidad de Hábitat (HHH) y la del Ajuste Individual (IAH). Para ello se empleó una serie de 18 años de datos. Bajo la hipótesis de Heterogeneidad de Hábitat se esperaría encontrar correlación entre características de hábitat de los territorios y su fecundidad media, así como una relación significativa entre dicha fecundidad y su sesgo en la distribución (skewness). Los resultados obtenidos apoyan la HHH en la situación de alta densidad en la que actualmente se encuentra la población del águila calzada del Parque Nacional de Doñana, ya que la fecundidad en los mejores territorios (aquellos ocupados desde hace más tiempo y más frecuentemente) fue mayor que la encontrada en los territorios de peor calidad; además la fecundidad de los mejores territorios no se vio afectada por el tamaño de la población en el periodo de alta densidad. No obstante, el tamaño poblacional sí parecía afectar, aunque no significativamente, a la fecundidad de los territorios de alta calidad durante el periodo de baja densidad. Esto sugiere que el Ajuste Individual pudo actuar cuando únicamente se ocupaban territorios de alta calidad. Estos territorios, los más antiguos, fueron utilizados con mayor frecuencia que los territorios de reciente ocupación y sus pollos nacieron antes. Se ha encontrado una relación inversa y significativa entre la fecundidad media y su sesgo en la distribución, lo que también apoya la HHH.

Se encontraron diferencias significativas en características del hábitat que afectan a la densidad de presas entre los territorios más antiguos y los de reciente ocupación.

Palabras clave: Características del hábitat, parámetros reproductores, heterogeneidad de hábitat, ajuste individual.

Abstract

In the present chapter the relationship between density and fecundity of the expanding population of Booted eagles (*Hieraaetus pennatus*) in Doñana National Park was analyzed to test the predictions of the two main hypotheses that attempt to explain how density-dependence fecundity is generated, that is, the Habitat Heterogeneity Hypothesis (HHH) and the Individual Adjustment Hypothesis (IAH). An 18-year data series was used. Under HHH, differences in habitat variables among territories that correlate with mean fecundity values could be expected as well as significant relationships between mean fecundity and Skewness. The results support the HHH hypothesis in the present situation of high density, since fecundity in the best territories (older and more frequently occupied) was higher than in low quality territories and it was not affected by population density in high density period. Nevertheless, fecundity of high quality territories was affected (although not significantly) by population density in low density period, suggesting that IAH could acted when only high quality territories were occupied. Older territories were used more frequently and chicks hatched earlier than in new territories. We found a significant negative relationship between mean fecundity and its skewness, a finding that also supports the HHH hypothesis.

Older territories showed significant differences in habitat features, affecting prey density, comparing with new ones.

Keywords: Habitat features, reproductive parameters, habitat heterogeneity hypothesis, individual adjustment hypothesis.

Introduction

Identification of proximate mechanisms by which density can affect demographic parameters is of fundamental importance in ecology.

The effect of density-dependent fecundity on population regulation has been well described (Newton 1991, 1998; Ferrer & Donázar 1996, Rodenhouse *et al.* 1997) and two major hypotheses, not necessarily mutually exclusive (Rodenhouse *et al.* 2003), have been proposed to explain how density-dependent fecundity arises. Individuals will be less fecund in an increasing population (Lack 1966), either because of increasing intraspecific interference affecting all pairs –the Individual Adjustment Hypothesis- (Lack 1954, 1966, Fretwell & Lucas 1970, Haller 1996, Both 1998, Fernandez *et al.* 1998), or because an increasing number of breeding pairs nest in territories with lower fecundity prospects –the Habitat Heterogeneity Hypothesis (Andrewartha & Birch 1954, Pulliam & Danielson 1991, Dhondt *et al.* 1992, Ferrer & Donázar 1996, Rodenhouse *et al.* 1997, Krüger & Lindström 2001, Horne & Fielding 2002).

Assuming that the habitat quality remains constant within patches over time, both hypotheses result in a decrease in mean fecundity related to an increase in population density, although predictions for other reproductive parameters differ from one to another in territorial animals (Ferrer & Donázar 1996, Ferrer *et al.* 2006). Under the habitat heterogeneity hypothesis 1) Fecundity does not decrease in high-quality territories as population densities increase; 2) Territories that are occupied more often or earlier during population growth have higher fecundity; 3) The fecundity distribution is more rightwards skewed when mean fecundity is lower (i.e there are more low-values of fecundity when mean fecundity is lower).

Conversely, the individual adjustment hypothesis in territorial animals predicts that 1) Individuals in all different patches are affected by density roughly to the same extent; 2) The occupation frequency of available territories does not differ from a random distribution pattern. 3) No correlation is expected between mean fecundity and the skewness of fecundity distribution.

Articles discussing the regulation mechanisms operating on both short-lived (Goodburn 1991, Dhondt *et al.* 1992, Both 1998) and long-lived bird species (Ferrer & Donázar 1996, Haller 1996, Krüger & Lindström 2001, Penteriani *et al.* 2002, Penteriani *et al.* 2003) are frequent and show, for instance, contradictory results within each group (Ferrer & Donázar 1996 vs. Haller 1996, Dhondt *et al.* 1992 vs. Both 1998). Differences may be due to differences in habitat suitability (it must be constant within patches during the study period), in population status (HHH is easier to detect in a saturated population -Rodenhouse *et al.* 1997) or in differences in the tests employed (Ferrer *et al.* 2006).

The objective of the present chapter was to contribute to the debate concerning IAH and HHH by using new tests (Ferrer *et al.* 2006) in an expanding population of a raptor. To this end we analyzed fecundity in relation to density in the Booted eagle (*Hieraaetus pennatus*, Gmelin 1788) population in Doñana National Park (SW Spain) over a 18-year period, which allows to analyze population dynamics across a range of population densities. Furthermore, the habitat structure of the study area was studied by checking major habitat features which would affect territorial suitability.

Methods

MECHANISMS OF POPULATION REGULATION

Although the first breeding record for the Booted eagle in Doñana National Park dates from 1976, more comprehensive surveys were carried out between 1983 and 2000. Consequently, only fecundity data from the last 18 years were analyzed. From 1983 to 2000 the breeding population size (number of pairs occupying territories at the beginning of the breeding cycle), brood size at hatch, and number of fledglings (chicks that reached at least 30 days of age, when they are ringed) were monitored. Breeding population size and fecundity parameters between 1983 and 1996 were taken from the Doñana field diaries (Doñana Biological Station Archives). The density of the population was measured as the number of territorial pairs in the area, since the size of the study area remained constant throughout the study period. Every spring, all previously known territories were visited to record occupancy and the rest of suitable but previously unoccupied areas were also checked for new pairs. All the nests detected were visited at least twice by the same person, at the beginning and end of the breeding season, to band the nestlings.

From 1996 to 2000 our own team monitored changes in the Booted eagle population. Nest sites were easily detected due to the conspicuous behavior of the Booted eagle, and the nests themselves were easily distinguishable from those of other raptors because Booted eagles cover their nests with green leaves. Nest sites were visited every 2-3 days to check for signs of occupation. Once we suspected incubation had started, we waited for 40 days (incubation period) before climbing up to the nests for the first time to mark the first chicks. If the chicks had not hatched, we returned at a later date. The laying date of the first egg was recorded in nests where we had previously installed a camera linked to a time-lapse video cassette recorder (Mitsubishi HS-7424EDC).

Laying and hatching dates (both converted to the number of days from January 1 of the year in question) were estimated from the timing of nest visits and examinations of nest contents; we only used dates that we were sure had an error lower than seven days. The next time the nests were climbed was to mark chicks. In all, the nests where eggs and/or chicks were marked were climbed four times; the others were only climbed three times.

The location of all known nests was recorded using a Garmin III Plus GPS. Territories were identified by mapping the UTM coordinates of nests: territories held potentially more than one nest site, because alternative nests may be used in the same or different years. Since the average inter-nest distance was 1.06 km (Suárez-Seoane *et al.* 2000), nest coordinates were overlaid with a 1-km grid and all nests within the same 1 km grid square were considered as belonging to the same territory.

The skewness of the fecundity distribution was used to discriminate between HHH and IAH (Ferrer *et al.* 2006, Ferrer *et al.* 2007). To analyze the annual relationship we used the fecundity of all the pairs within a year, whereas we used all attempts over the years within a territory when we analyzed the territorial effect. Territories were divided into two groups according to the date of first occupation, being older territories those occupied for the first time in the period 1983-1991, and new territories those first occupied in the period 1992-2000. This factor paired to the occupation frequency calculated as percentage, were used as measures of territorial quality (Ferrer & Donázar 1996, Sergio & Newton 2003).

The regulating mechanisms of the Booted eagle population in Doñana National Park were explored using STATISTICA 6 except when other program is specified. Statistical significance was set at Alpha= 0.05.

Breeding population size and annual fecundity, both all over the population and distinguishing between territory qualities, were related by regression models. We selected the best model based on the Akaike index.

Nest-site dispersion was analyzed per year with the GMASD statistic (Brown 1975) determined as the ratio between the geometric and arithmetic means of the squared averages of the minimum distances to the nearest neighboring nests (Newton *et al.* 1977). G-statistic values ranged from 0 to 1 and values higher than 0.65 indicate regularity in nest spacing.

HABITAT FEATURES

Habitat was characterized by 19 non-correlated (assessed by Pearson-test, see Table 1) environmental variables, according to previous studies (Suárez-Seoane *et al.* 2000). Variables were measured using Geographic Information Systems (GIS) and digital cartography from different sources: (1) Land cover map at 1:50,000 (SINAMBA, Regional Government of Andalusia, 1990, 1995); 2) Digital maps of linear features at 1:200,000 (National Geographic Institute); and (3) Unpublished maps made from aerial photographs 1:50,000. All data were overlapped on 50m resolution grids. As habitat features were practically unchanged from the 1980s until current days, the variables could be measured using the same cartography for the whole study period. Data were transformed into quantitative variables calculated within windows of 20 x 20 pixels (equivalent to 1 km^2, the territory size; see Suárez-Seoane *et al.* 2000): (1) As the average of the distances measured from the center of every 50m-pixel to the nearest habitat features, which provides information about habitat structure; (2) As the percentage of each feature within the territories. Analyses were performed using the commands “distance”, “filter” and “contract” in IDRISI 32.2 (Eastman 2000).

In order to identify the environmental variables explaining the territories occupation frequency, (1) an univariate analysis (t test with Bonferroni corrections) and (2) a multivariate linear regression were run. Model was fitted using a backwards stepwise procedure with a 5%

significance level for the variable to remain in the equation on each step. The occupation frequency in the pixels at 1km resolution occupied by Booted eagle was the dependent variable, and the variables included in the Table 1 were the environmental predictors. Predictive performance was assessed through 10-fold cross-validation (Verbyla & Litvaitis 1989) and both fit and predictive performances were measured through R-squared. SPSS 13 was used.

Results

MECHANISMS OF POPULATION REGULATION

Booted eagle breeding population of Doñana National Park has been increasing during all the study period (Figure 1), from one pair in 1976 to 62 pairs in 1999. At least 372 young fledged, 199 nests failed and 87 nests had an unknown fecundity because the nestlings were ringed before 30 days old, or age was unknown. UTM locations were obtained for more than 500 nests within 79 territories.

From this point, only data from the period 1983-2000 inclusive were used. Seventy-seven territories were occupied in this 18 year period, and the population increased from 13 breeding pairs in 1983 to 62 in 1999 (in 2000, last year of study, 60 breeding pairs were recorded). G-statistic values were lower than 0.60 for all years indicating an irregular distribution of the nests. Nevertheless, values increased from 1983 to 2000 and were significantly correlated with the percentage of occupation ($r = 0.778$, $n = 18$, $P < 0.001$), suggesting an increasingly regular distribution.

The mean population fecundity was 1.010 (SD = 0.277). A negative relationship between population fecundity and population size was detected ($r = -0.711$, df = 16, $P = 0.0009$). The relationship occurred both in old ($r = -0.657$, df = 16, $P = 0.003$) and new territories ($r = -0.751$, df = 7, $P = 0.020$). The effect of population density on old territories' mean fecundity was strong although not significant through the low density period ($r = -0.600$, df = 7, $P = 0.088$), and there was not significant relationship between them in high density period ($r = -0.566$, df = 7, $P = 0.112$). Mean fecundity in low density period was 1.111 (SE= 0.072, n=135) and 0.828 in high density period (SE= 0.039, n=378). The skewness of the distribution was more rightwards as fecundity decreased (Figure 2).

Figure 1. *Annual changes in the size of the Booted eagle population (continuous line) in Doñana National Park and its fecundity (dotted line) between 1976-2000*

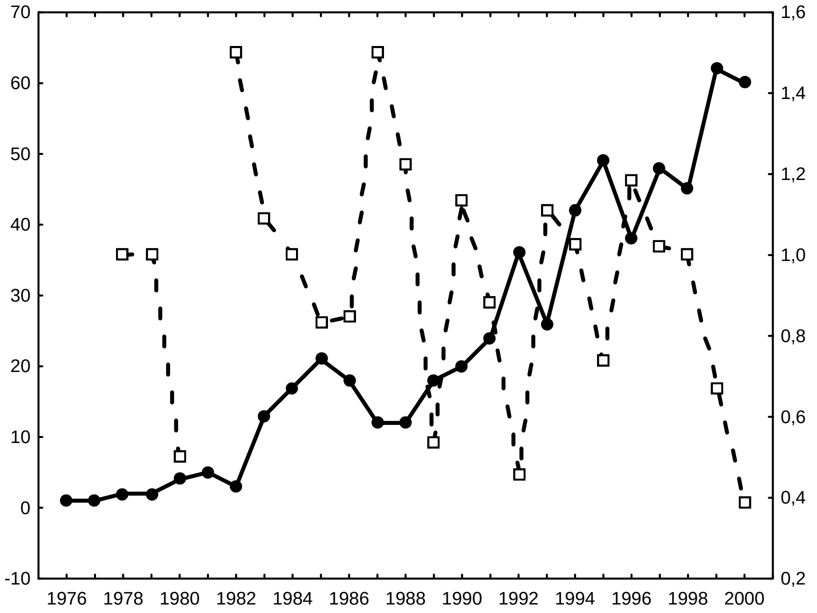


Figure 2. *Relationship between mean fecundity and its skewness (r = -0.676, df = 16, P = 0.002)*

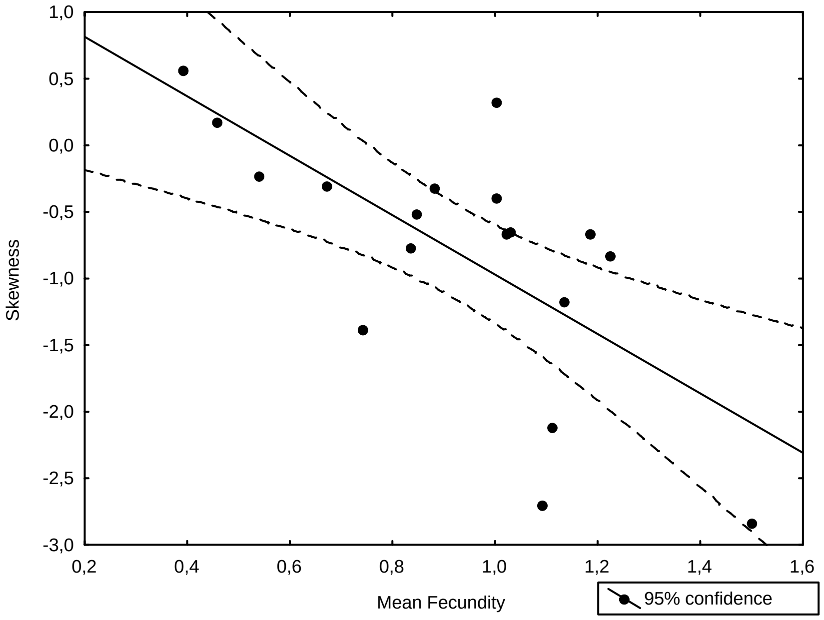
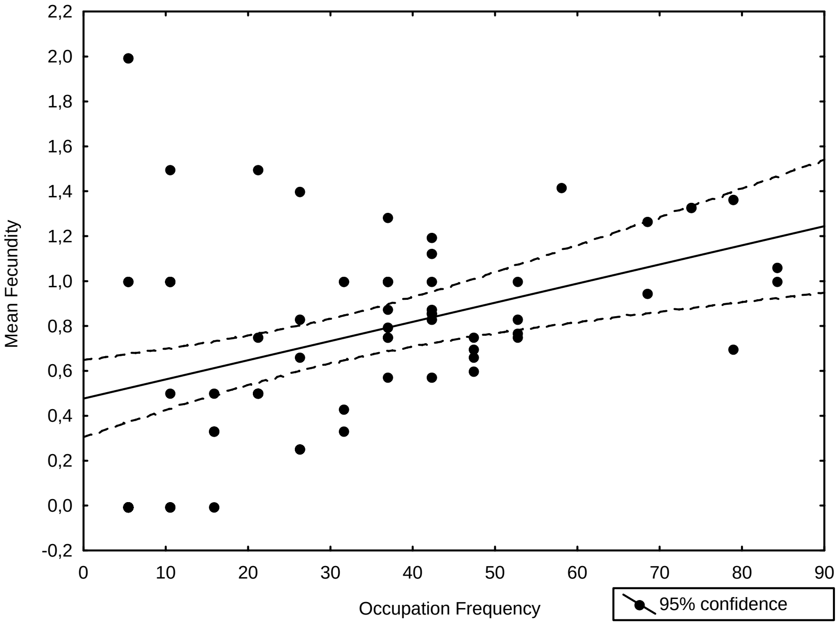


Figure 3. *Relationship between territorial mean fecundity and occupation frequency of the territory*



The mean fecundity of territories was significantly related to their occupation frequency (r = 0.423, df = 76, P = 0.0001), being higher in more frequently occupied territories (Figure 3). More frequently occupied territories showed earlier hatching dates than less frequently occupied ones (r = -0.242, df = 50, P = 0.084). A significant relationship between occupation frequency and the year of first occupation was found (r = -0.525, df = 76, P < 0.001): older territories were more frequently occupied during the study period.

HABITAT FEATURES

More frequently occupied territories were located significantly closer to towns, marshland and "pilgrim trail" (the main trail crossing the Doñana National Park, which is intensively used during "Rocio Virgin" celebration- one million people crossing it through one week) and contained more cork oaks than the less frequently occupied, as the univariate analysis evidenced (Table 2). The backwards stepwise regression analysis showed that occupation frequency was significantly related to only two variables: distance to the marshland and distance to pine forests. Territories more frequently occupied were located nearer to the pines and the marshland than the less frequently occupied (Table 3). This model had an R-square value of 0.151. When it was 10 cross-validated, this value decreased little, to 0.091, which support its predictive power.

Discussion

The Booted eagle population in Doñana National Park has been increasing since 1976 and was still increasing at the end of our study period. This corresponds to a more general trend observed in Western Europe (Carlon 1996). Only fairly approximate estimates of population sizes in other countries are available, although most are apparently stable (del Hoyo *et al.* 1994); likewise trends are unknown in most of Spain since regular censuses have never been carried out. A population decrease has been recorded in one province (Madrid), whereas populations are increasing in other regions such as Catalonia, Extremadura, and Murcia (Bosch 2003, Martí & Del Moral 2003). The increase of the Booted eagle population in Doñana National Park seems to be above average compared to that of other known areas.

The increase in number of breeding pairs was accompanied by a decrease in fecundity, which reveals the density-dependent nature of fecundity. At the same time, the nests were irregularly distributed but became more regularly distributed with time.

The results obtained in the present chapter suggest that in the Doñana's Booted eagle population, the habitat heterogeneity is the regulating mechanism that reduces the rate of population increase when the population density is high, although interference among individuals could work when low density; it means, both mechanism would regulate the fecundity of the studied Booted eagle population although at different stages. Occupation frequency was used as a measure of territorial quality in this study: the most frequently used territories (i.e., the best territories) showed higher fecundity rates, a more high-values of fecundity (leftwards skewed), and were generally occupied earlier in the study period. This demonstrates that there are different territory qualities in the study area. Furthermore, the older territories (also considered those of high quality), opposite new ones, did not show to be affected by population increase in high density period which supports the habitat heterogeneity hypothesis.

Nevertheless, we consider the tendency of fecundity in older territories to be negatively affected by the increase in population size in low density period. This supports the idea of when all the breeding pairs are nesting in a homogeneous patch of high quality territories and population is growing, the interference among individuals reduce the fecundity rate as been demonstrated by Rodenhouse *et al.* (2003). In fact, this interference in an increasing population would force to some individuals to move into poorer territories and then, the habitat heterogeneity would start to be the main regulating mechanism of fecundity. Consequently, the trend towards yearly decreases in fecundity can be explained by an increasing number of new pairs nesting in low quality territories, as has been shown for the Spanish Imperial Eagle (Ferrer & Donázar 1996). These findings are consistent with the notion that the Booted eagle population of Doñana is actually regulated through source-sink dynamics generated by density-dependence in fecundity through habitat heterogeneity (Newton 1991, Ferrer & Donázar 1996). Under this model, in which a relatively small number of territories may sustain a much larger population, conservation plans must take into account the fact that disturbances have different consequences on population viability depending on the quality of the territories affected. The same impact could be negligible in sink territories or determinant in source ones (Ferrer & Donázar 1996, Sergio & Newton 2003).

Since the Booted eagle is a migratory bird, conditions during the non-breeding season also will contribute to fluctuations in the breeding population size, but the processes operating during the breeding period are sufficient for regulating the population of a migratory species (Rodenhouse *et al.* 2003).

Table 1. *Variables used to characterize Booted eagle habitats, including human disturbances and prey availability, within the 1km square territories. Multicolinearity among the predictors was avoided by previously calculating pair wise Pearson correlations (other variables showing a coefficient over than 0.8 were eliminated from the subsequent analyses)*

CODE	VARIABLE
Shape	Shape of scrubland patches. Calculated as the ratio area/ perimeter (both measured in meters)
Droad	Average of the distances measured from the center of every 50m-pixels to the nearest road or track within the 1km^2-territory
Dtwon	Average of the distances measured within the 1km^2-territory to the nearest urban area or isolated building
Dmarsh	Average of the distances measured within the territory to the marshland
Pmarsh	Percentage of marshland within the territory
Dpil	Average of the distances measured within the territory to the “pilgrim trail”
Driver	Average of the distances measured within the territory to the nearest river
Plake	Percentage of lagoons within the territory
Dlake	Average of the distances measured within the territory to the nearest lagoon
Dirri	Average of the distances to the nearest irrigated crop (including rice fields)
Ddry	Average of the distances measured within the territory to the nearest dry crop
Dsand	Average of the distances to the nearest sand dunes
Dscrub	Average of the distances to the nearest scrubland
Poak	Percentage of cork oak trees within a window of 1km^2 around each site
Doak	Average of the distances to the nearest cork oak woodland
Ppine	Percentage of pine forest within the territory
Dpine	Average of the distances to the nearest pine forest
Pypine	Percentage of young pine forest within a window of 1km^2 around each site
Peuca	Percentage of eucalyptus forest within the territory

Table 2. *Significance of the univariate Student t-test, with Bonferroni correction (for difference between means with equal or different variances, according to each case) for testing the relationship between territory occupancy and environmental features*

CODE	t	df	P	CODE	t	df	P
Shape	0.878	1	0.352	Ddry	0.905	1	0.344
Droad	2.291	1	0.134	Dsand	1.927	1	0.169
Dtown	**5.728**	**1**	**0.019**	Dshrub	0.181	1	0.672
Dmarsh	**5.648**	**1**	**0.020**	**Poak**	**4.833**	**1**	**0.031**
Pmarsh	1.021	1	0.316	Doak	3.131	1	0.081
Dpil	**5.686**	**1**	**0.020**	Ppine	0.397	1	0.531
Driver	2.893	1	0.093	Dpine	0.626	1	0.431
Plake	0.785	1	0.378	Pypine	1.746	1	0.190
Dlake	2.714	1	0.104	Peuca	0.933	1	0.337
Dirri	0.119	1	0.731				

Table 3. *Linear multiple regression summary for occupation frequency as dependent variable and environmental variables as predictors*

N=77	Non-standardized coefficients		Standardized coefficients		
	B	SE	Beta	t	P
INTERCEPT	8.978	1.053		8.528	0.000
Dmarsh	-0.001	0.000	-0.453	-3.526	0.001
Dpine	-0.002	0.001	-0.341	-2.656	0.010

Factors others than territorial quality, such as the individual quality of breeders, the fact that higher quality individuals occupy best territories, and the age of breeders, may all affect demographic parameters (Ferrer & Bisson 2003, Penteriani *et. al.* 2003, Carrete *et al.* 2006). It is difficult to clearly separate individual effect from that of habitat quality on breeding performance (Newton 1991, Ferrer & Donázar 1996, Rodenhouse *et al.* 1997, Krüger & Lindstrom 2001, Ferrer & Bisson 2003, Ferrer *et al.* 2006). In our specific case, the duration of the study period was longer than the life spans of most Booted eagle individuals (mean lifespan of a Booted eagle 7.95 years, SD = 3.623, ringing center of Doñana Biological Reserve Archives) and so HHH undoubtedly helps explain the trends we observed in fecundity. Based on a 7-year study, Martínez *et al.* (2006) showed that a given territory with successful previous reproductive events had a higher probability of being occupied by a Booted eagle, a finding that is consistent with our results (more productive territories are more frequently used). Nevertheless, the authors leaps to conclude that individual quality could be the main factor affecting population fecundity, without showing individualized data of breeding birds (e.g, age, ring number, territory occupied every year), which turns that conclusion into a speculation. Since other raptor studies have demonstrated that individuals move into better territories when they are able -e.g. loss/emigration of one of the partners- (Ferrer & Bisson 2003, Penteriani & Ferrer 2004), it would be interesting to conduct more comprehensive studies in Booted eagles concerning this idea.

Newly settling pairs tend to establish themselves in areas where prey is less abundant, specially the wild rabbit (Moreno & Villafuerte 1995, Lombardi *et al.* 2003) such as further from towns (in the shoreline of the main marshland) and marshland, and contained less cork oak savannas. This tend suggests that habitat with higher abundance of prey were pre-emptively occupied, again supporting HHH. Recently occupied territories were also further from "pilgrim trail" which could indicate avoidance of human disturbance. Pilgrim trail is far from food resources such as lagoons, scrubland or ecotone scrubland-marshland. It is surrounded by grassland cover where wild rabbit shows high abundance but also tends to show a more nocturnal activity due to the high pressure predation by raptors (Lombardi *et al.* 2003), being consequently less available.

The importance of the habitat variables is relative since the variation in occupation explained by habitat variables is low in the multiple regression. The model indicates that territories more frequently used were closer to marshland and to pine forest; the first variable is close related to prey abundance, especially wild rabbit, whereas the distance to pine forest (the nearest distance is zero) could be related with nest site availability. Consequently, wild rabbit density and nest site availability could explain the low productivity observed in newly settling pairs compared to those established earlier.

Acknowledgements

We are in debt to ICONA and RBD fieldworks, which have monitored Booted eagle fecundity in Doñana National Park for 20 years, especially to Luis Garcia, who furthermore spent much of his time showing us every old nest occupied by the species, and to Javier Balbontin who carried out the monitoring for 3 years. We are grateful to A. Dekker who helped me in field work for one year, during his ethologie research project (Zoological Laboratory, University of Groningen). Patrick Osborne assisted with habitat features analyses, which were carried out by Susana Suarez-Seone. The maps from SINAMBA, used in habitat analyses, were provided by J. Bustamante, scientific researcher from Biological Station of Doñana.

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7. Brood reduction in a population regulated by habitat heterogeneity

Resumen

La reducción en el número de crías nacidas (reducción de nidada) es un mecanismo que interviene en la regulación de las poblaciones animales y que puede generarse por fecundidad denso-dependiente. La reducción de nidada en un sistema de cría territorial, puede surgir bien como respuesta a un alto nivel de interferencia entre individuos de diferentes territorios en una situación de alta densidad poblacional, o bien como resultado de la heterogeneidad de hábitat. En el presente capítulo se ha estudiado la ocurrencia y frecuencia de reducción de nidada en la población de águila calzada del Parque Nacional de Doñana, con el fin de conocer tanto los factores que le influyen como sus efectos posteriores.

Los resultados del presente capítulo indican que la reducción de nidada en la población de águila calzada fue consecuencia de la estructura heterogénea del hábitat, con unos territorios con mayor probabilidad de sufrirla que otros. Los territorios más antiguos fueron ocupados también más frecuentemente, tuvieron fechas de eclosión más tempranas y sufrieron menos mortalidad en nido que en los territorios ocupados más recientemente.

Durante los años de escasez de alimento, los territorios ocupados menos frecuentemente sufrieron una mayor tasa de reducción de nidada.

La condición física de los padres no influyó en la ocurrencia de reducción de nidada.

En este capítulo se discuten además el efecto de la reducción de nidada sobre la calidad de los pollos y sobre la dinámica de la población.

Palabras clave: águila calzada, denso-dependencia, disponibilidad de alimento, estado nutricional.

Abstract

Brood reduction is a mechanism for regulating populations that can be generated by density-dependence in fecundity. Brood reduction may arise in a territorial breeding system as either a response to a high level of territorial interference in a situation of high density or as a result of habitat heterogeneity. We have studied the occurrence and frequency of brood reduction in the population of Booted eagle in Doñana National Park in order to appreciate its further effects and the factors that influence its occurrence and frequency. The present study indicates that brood reduction in the studied Booted eagle population was consequence of the heterogeneous structure of the habitat, with some territories having higher probability of brood reduction than others. Those nests with higher brood reduction frequency were those less frequently used and with latter hatching dates. Those years of food shortage, the less frequently occupied territories suffered higher rates of brood reduction. Parental nutritional condition did not affect brood reduction. The effect of brood reduction on nestling's quality and population dynamic is also discussed.

Keywords: Booted eagle, dense-dependence, food shortage, nutritional condition.

Introduction

Species produce higher rates of progeny than the number that can reach adult age. It varies from several millions of eggs in some fish species to only one offspring in some bird or mammal species. Overproduction is also noticed in raptor species, that suffer from brood reduction (Edwards & Collopy 1983, Simmons 1988) in spite of high costs spent in nestling rearing. At the time of egg laying when future food levels cannot be predicted, parents may lay an optimistic clutch size (Lack 1947, Mock & Forbes 1995). Variations in brood reduction frequency within a population would influence in some extent on population fecundity.

Changes in fecundity lead to density dependent processes in natural populations (Sinclair 1989). Ferrer & Donázar (1996) compared two different hypotheses explaining how density-dependent fecundity arises. Individuals would have a reduced fecundity in an increasing population (Lack 1966, Fernandez *et al.* 1998) either because of interferences among them – Individual Adjustment Hypothesis- (Fretwell & Lucas 1970, Haller 1996, Both 1998), or because of an increasing number of breeding pairs nesting on territories with lower fecundity perspectives -Habitat Heterogeneity Hypothesis- (Andrewartha & Birch 1954, Dhondt *et al.* 1992, Rodenhouse *et al.* 1997, Krüger & Lindström 2001, Horne & Fielding 2002).

The aim of this study was to investigate the density-dependence of brood reduction and the mechanism through brood reduction affects population growth. For that, we have analysed the brood reduction frequency in relation to density in the Booted eagle (*Hieraaetus pennatus*) population of Doñana National Park. The density of the chosen population has increased during an 18 years period (Chapter 6). The long-term study on Booted Eagle allowed investigating the effect of territory quality, food resource and birds nutritional condition on brood reduction.

Population fecundity is partly a function of brood reduction and if the latter is a density-dependent process then an increase in population density should lead to an increase in the frequency of brood reduction. If the density-dependence fecundity of a population is the result of habitat heterogeneity, then we would expect to find a higher frequency of brood reduction in a subset of territories (defined as low quality territories) rather than in all of them. Otherwise, if intraspecific interference is the primary influence, then brood reduction frequency would be found equally in all nests and/or territories.

Methods

BREEDING PARAMETERS

From 1983 to 2000 the breeding population size (number of pairs occupying territories at the beginning of the breeding cycle), brood size at hatch, and number of fledglings (chicks that reached at least 30 days of age, when they are ringed) were monitored. Breeding population size and fecundity parameters between 1983 and 1996 were taken from the Doñana field diaries (Doñana Biological Station Archives). The density of the population was measured as the number of breeding pairs in the area, since the size of the study area remained constant throughout the study period. Every spring, all previously known territories were visited to record occupancy and the rest of suitable but previously unoccupied areas were also checked for new pairs. All the nests detected were visited at least twice by the same person, at the beginning and end of the breeding season, to band the nestlings.

From 1996 to 2000 our own team monitored changes in the Booted eagle population. Nest sites were easily detected due to the conspicuous behavior of the Booted eagle, and the nests themselves were easily distinguishable from those of other raptors because Booted eagles cover their nests with green leaves. Nest sites were visited every 2-3 days to check for signs of occupation. Once we suspected incubation had started, we waited for 40 days (incubation period) before climbing up to the nests for the first time to mark the first chicks. If the chicks had not hatched, we returned at a later date. The laying date of the first egg was recorded in nests where we had previously installed a camera linked to a time-lapse video cassette recorder (Mitsubishi HS-7424EDC).

Laying and hatching dates (both converted to the number of days from January 1 of the year in question) were estimated from the timing of nest visits and examinations of nest contents; we only used dates with an error lower than seven days. The next time the nests were climbed was to mark chicks. In all, the nests where eggs and/or chicks were marked were climbed four times; the others were only climbed three times.

The location of all known nests was recorded using a Garmin III Plus GPS. Territories were identified by mapping the UTM coordinates of nests: territories held potentially more than one nest site, because alternative nests may be used in the same or different years. Since the average inter-nest distance was 1.06 km (Suárez-Seoane *et al.* 2000), nest coordinates were overlaid with a 1-km grid and all nests within the same 1 km grid square were considered as belonging to the same territory.

BROOD REDUCTION

The brood reduction frequency has been expressed as the annual percentage of nests suffering from nestling mortality in which more than one sibling hatched. Predation generally results in total brood lost in raptors (Newton 1979) and so nestling mortality from other causes such as predation or abandonment was excluded from the analyses of brood reduction (Viñuela 1999). As in some years data on hatching rates were not available for some nests, we eliminated those years with less than five records of brood reduction to avoid any possible bias in our estimates of the annual brood reduction frequency, and so analyzed the years 1983-1984, 1988, and 1993-2000 for brood reduction (referred to as the 'selected years'). Yearly mean fecundity was determined as the number of fledglings per breeding pair. From 1997 onwards we marked eggs and/or nestlings in order to try to accurately identify the hatching order.

The mean clutch and brood sizes (at hatch) and fecundity were calculated per territory for the selected years.

Brood reduction frequency between good and poor years was compared. A fecundity that is higher or lower than the average annual fecundity is a commonly used method for the yearly classification of quality (e.g. Penteriani *et al.* 2002), being 'good' years those with a mean fecundity higher than the annual rate, and 'poor' years those with fecundity lower than the annual rate. This method would have led to circular reasoning since fecundity decreased significantly with brood reduction (r = -0.734, n = 10, P = 0.016), then I excluded those nests where brood reduction occurred from our calculations, to suppress their effect on annual fecundity.

BIOCHEMISTRY

During 1996-1998 and 2000, we took blood samples from Booted eagle adults and nestlings to analyze urea and uric acid concentrations in plasma and thus establish an estimate of nutritional condition (Ferrer 1994, and references therein). We extracted nestling blood at the ringing time. Adults were captured during the nestling period using a net and an irrecoverable (alive but unable to fly) Eagle Owl (*Bubo bubo*) as a lure (more details in Casado *et al.* 2002, Chapter 2).

FOOD SUPPLY

The European wild rabbit *Oryctolagus cuniculus* has been identified as the main prey item of the Booted eagle (Veiga 1986). In April 1990, an outbreak of viral hemorrhagic disease in the area of the Doñana National Park seriously affected wild rabbit populations (Villafuerte *et al.* 1994) and caused high mortality rates. Thus, we were also able to investigate the possible effects of the decline in rabbit populations on the fecundity of Booted eagle populations. Owing to the lack of comprehensive censuses of wild rabbit populations during the study period, we used prey remains found in nests during surveys to estimate proportion of wild rabbit in diet and prey diversity. All remains were identified to species level and so we knew year by year the species eaten by Booted eagles and the number of items per species. We estimated the biomass of each prey from published data (for birds: Cramp & Simmons 1980, for hire: Carro 2005, for rabbits Villafuerte 1994) and original data from our study area for reptiles. The contribution of wild rabbits to the diet of Booted eagles was estimated in the following two ways: i) by weight: nº rabbit x 550 (gr)/Σitem x sp weight (gr) (a weight was assigned to every species); ii) by the number of items: nº rabbit x 100/nº items. Prey diversity was considered as the number of species found.

STATISTICAL ANALYSIS

All variables were tested for normality with the Lilliefors test for samples with *a priori* unknown mean and standard deviation. When distribution was non-normal, non-parametric tests were used. STATISTICA 6 was used except when other program was specified. Statistical significance was set at Alpha= 0.05.

One-way ANOVAs were employed when comparing food supply between periods.

Generalized Linear Mixed Models (GLMMs, Littel *et al.* 1996) was used to identify the characteristics influencing the occurrence of brood reduction as the method used by Serrano *et al.* (2001). The population was monitored over a number of years and adults showed a significant attachment to nest sites and so the macro GLIMMIX of SAS permitted to fit nest and year as random terms in the GLMMs. Logistic regressions were developed to predict the probability of occurrence of brood reduction as a function of characteristics representing the nest or territory. The effects of hatching dates and territorial occupancy were evaluated separately. Brood reduction was modeled as a binomial response variable (1 = brood reduction, 0 = no brood reduction) using a logistic link function (Kleinbaum 1994, So 1995, Kuss 2002).

The brood reduction frequency per year was related to the Booted eagle population density and to the proportion of rabbit in diet by multiple regression models. We selected the best model based on the Akaike index.

We performed a Generalized Linear Model to identify the effects of hatching order and the siblinghood environment on the nutritional condition index, while controlling for the nest of birth and year. As in Ferrer (1993), those chicks from the same nest were considered non-independent samples because of their common rearing environment. Therefore, nest was considered as random effect in the GLMM, as well as year. Nutritional condition index was modeled as normal distribution and identity link. The explanatory variables were hatching order and siblinghood environment, of which the latter was divided in three categories: 1) nestlings reared alone, 2) nestlings reared alone after brood reduction, and 3) nestlings reared with a sibling. We used multiple regression models to examine the relationships between the yearly mean of urea residuals and fecundity, population density and brood reduction frequency. Among the possible best subsets we always chose that model with the lowest Akaike index.

Results

Booted eagles laid 1.82 ± 0.44 eggs (*n* = 253) and the mean brood size at hatch was 1.21 ± 0.82 nestlings (*n* = 300). Brood reduction occurred in 33.3% of nests with at least two nestlings (*n* = 129) which means that 44 chicks died whereas 324 fledged. Eight (6.2%) of the nest with at least two nestlings lost whole clutch so were not considered to suffer brood reduction. The most frequent age of nestling death was three days (*n* = 4), although the mean age of death was 5.46 (± 4.96, range 0-19 days). All the nestlings died before being 11 days old, except one that died when 19 days.

Since both measurements of rabbit proportion in diet explained in 'Methods' were strongly correlated (Table 1), we chose for further analysis that based on the number of items because it provided with a longer sample size. Number of breeding pairs was the only variable included in the best model for explaining the yearly frequency of brood reduction (AIC= -12.331, *df*= 1, *P*= 0.0008), being both variables positively correlated.

Years 1997 and 1998 showed higher fecundity than 1999 and 2000. An ANOVA with year quality as a grouping variable showed that, although the laying rate did not differ between good and poor years, the hatching rate was significantly lower in poor years than in good ones (*1997*= 90.63%, *1998*= 84.09%, *1999*= 84.31%, *2000*= 49.12%; $F_{1,9}$ = 7.49, P =0.02) and that brood reduction frequency was significantly higher in poor years ($F_{1,9}$ = 43.770, P <0.001).

Table 1. *Spearman rank order correlation values between the yearly parameters studied for 18 years. Signification level is indicated by **

Variable	Mean fecundity	% occupation	% rabbit (weight)
% occupation	-0.437		
% rabbit (weight)	-0.376	-0.416	
% rabbit (nº item)	-0.121	-0.572**	0.943***

* p<0.05 ** p<0.01 *** p<0.001

The hatching date tended to be earlier in territories with high occupancy (GLIMMIX with Normal distribution and Identity link), although not significantly ($F_{1,20}$ = 3.48, P = 0.076, n = 116). We investigated the impact of hatching dates on demographic parameters averaged over the years for each territory (GLIMMIX with Poisson distribution and Log link,) and on brood reduction: the earlier the hatching date, the higher the clutch size ($F_{1,11}$ = 27.06, P = 0.0003, n = 93), the hatching rate ($F_{1,19}$ = 5.20, P = 0.034, n = 117), and the number of fledglings excluding predated and fallen nests ($F_{1,18}$ = 15.81, P < 0.001, n = 109). Brood reduction occurrence increased with season ($F_{1,4}$ = 11.33, P = 0.028, n = 64), so that the later hatched broods were more likely to suffer nest mortality.

The occurrence of brood reduction was inversely related to the territorial occupation frequency (GLIMMIX, $F_{1,18} = 4.99$, $P = 0.038$). The brood reduction frequency was 18.0 % for those territories occupied more than 70% of the years, while it was 47.9 % for the other territories. This confirms that the brood reduction frequency was significantly lower in the highest quality territories ($\chi^2 = 11.44$, $df = 1$, $P < 0.001$), which corresponds to the relationship previously found between yearly fecundity and the brood reduction frequency.

BIOCHEMISTRY

Urea and uric acid were correlated (Spearman correlation, $r = 0.379$, $n = 102$, $P < 0.001$) and so we decided to work only with data from urea as an index of nutritional condition. Since urea concentrations in nestlings are influenced by sex and age (Casado *et al.* 2002) and may be related to hatching date (Ferrer 2001 and references therein), the residuals obtained from an analysis of covariance (ANCOVA) were used as the index of nutritional condition, with urea as the dependent variable, sex as a factor, and age and hatching date as covariates ($F_{3,92} = 5.29$, $P = 0.002$, $R^2 = 0.15$, $n = 96$ nestlings). Urea residuals were distributed normally (Lilliefors test $P > 0.20$).

The best model explaining the nestlings' nutritional condition was that including "nest" and "hatching order" (AIC= 350.924, df= 33, P= 0.00001): the first hatched chick (mean= -2.131, SE= 1.273, n= 30) had a better nutritional condition than its sibling (mean= 0.866, SE= 2.140, n= 23). Although year was not a good predictor of the nestlings' nutritional condition, showed significant differences (Wald $X^2(3) = 22.005$, $p < 0.0001$), being the nutritional condition worse in good years (1997: urea mean= 0.861, SE= 1.537, n= 19; 1998: urea mean= 1.991, SE= 3.472, n= 14) than in poor ones (1999: urea mean= -3.280, SE= 3.272, n= 5; 2000: urea mean= -4.789, SE= 0.964, n= 15) No effect of siblinghood environment was detected (Wald $X^2(1) = 0.000$, $p = 1.000$).

The best model explaining the yearly nutritional condition of the nestlings was that including brood reduction frequency, population density and fecundity (AIC= 9.492, df= 3, P< 0.0001). Urea residuals decreased with increasing population size and brood reduction frequency, and with decreasing mean fecundity.

Biochemical samples were obtained from 55 adults, although, since many of these birds bred on nests for which we had no information on brood reduction, this reduced our sample size to

30 individuals (15 females and 15 males). The GLIMMIX did not converge (year as random factor) and so we used the Generalized Linear Model Module of Statistica (a bias due to the year effect is possible) with a binomial distribution, the logit link function, and the overdispersion parameter whenever necessary. We used age and urea as estimates of parental quality. Analyses were carried out separately according to sex, since parental roles differ and hence the parental effect on brood reduction may also differ. No significant effect on brood reduction from either of the two sexes was found. The results are presented in Table 2.

Table 2. *Logistic regression parameter estimates, Wald statistics, and P values for the effect of adult nutritional status on the occurrence of brood reduction*

	Variable[a]	Estimate	Df	Wald	*P*
Female	age (7)	0.511	1	1.715	0.190
	urea (8)	0.239	1	0.918	0.338
Male	age (8)	0.126	1	0.063	0.802
	urea (8)	0.006	1	0.013	0.909

[a] Between brackets, adults sample size.

FOOD SUPPLY

The frequency of rabbits in diet decreased with over the years ($r = 0.647$, $n = 18$, $P< 0.005$), although it did not affect population fecundity (Table 1). Nevertheless, the proportion of rabbits in the eagles' diet differed significantly before and after 1990 (outbreak of wild rabbit hemorrhagic disease) by 43.04% and 23.44%, respectively (one way ANOVA $F_{1,16} = 15.247$, $P<0.005$). A negative correlation between diet diversity and the proportion of rabbits in diet ($r = 0.617$, $n = 18$, $P < 0.01$) was also found.

The brood reduction frequency in selected years was negatively related to the percentage of rabbits in diet ($r = -0.710$, $n = 11$, $P < 0.025$).

Discussion

The Booted eagle population in Doñana National Park has grown considerably since the first birds nested in 1976 (Chapter 6) This increase is generally assumed to be a consequence of the species' capacity to adapt to environmental change (Carlon 1996, Suárez-Seoane *et al.* 2000). An evidence of the eclectic nature of the Booted eagle is its flexibility in prey choice: when wild rabbit hemorrhagic disease reduced the availability of the Booted eagle's main prey in Iberian Peninsula, it was able to catch many other prey species as the inverse relationship between the remains of wild rabbits and other prey items found in nests suggests. The reduced availability of wild rabbits only affected those nests in low quality territories, where perhaps, other prey species were also scarce. Years with lower fecundity than the total mean fecundity were characterized by a lower hatching rate. Furthermore, brood reduction was more frequent in less productive years. The relationship between the yearly frequency of brood reduction and the percentage of rabbits in diet suggests that the role of brood reduction in population regulation is stronger when food availability diminishes.

The increase in number of breeding pairs was accompanied by an increase in brood reduction frequency and by a decrease in fecundity, which reveals the density-dependent nature of brood reduction.

For many birds, fecundity decreases as laying dates get later (e.g. Hochachka 1990, Ferrer 1993, Penteriani 1997). In the case of the Booted eagle, both reproductive parameters (Chapter 6) and brood reduction frequencies were linked to hatching dates. This is commonly interpreted as a result of a seasonal decline in food availability during the breeding season, which may increase brood reduction frequency because food shortage would encourage siblicide (review by Drummond 2001, Forbes & Mock 1994 and references therein). Nevertheless, the abundance of wild rabbit in Doñana National Park is at its peak in June and December, and at its lowest in October (Beltrán 1991). Thus, pairs raising chicks later on in the season (mean laying date was April 22 ± 12.75 days, $n = 69$; the earliest laying date was 2nd April and the latest 2nd June; 43.48 % of clutches were laid between 10th and 20th April) would find more favorable conditions. Bigger differences in wild rabbit abundances were found between habitats than between months (Beltrán 1991, Lombardi *et al.* 2003). Other possible explanations could be related to adult physical condition. Those individuals in better condition (probably more skillful birds) would be able to start reproduction soon after arrival and earlier than others. Unfortunately, the Booted eagle is difficult to capture before their eggs hatch (only two individuals from two different breeding pairs were captured) and so we were unable to take blood samples to study their physical condition just after arriving in their breeding grounds. It would be interesting in the future to investigate the relationship between adult condition at the beginning of the breeding season and fecundity.

Biochemical blood analysis indicated that nestling's physical condition varied among territories. Furthermore, the first chicks to hatch were better nourished than their younger siblings. This probably means that either feeding rates are biased towards older nestlings (parental decision) or older siblings have the capacity to dominate their nest-mates during meals through more begging signals and/or aggression (nestling decision). Indeed, parents typically feed first those nestlings that beg at higher intensities and/or who are closer to them (Budden & Wright 2001). In addition, Drummond & Garcia Chavelas (1989) suggested that poor nutritional conditions would increase the aggressiveness of the senior chick. This could lead to the death of younger chick through siblicide. In the best territories, the first chick would be satiated earlier and the second would eat enough to survive; on the other hand, in the worst territories the first chick would go hungry and attack its brood mate to appropriate more food. Nevertheless, the operating mechanism that enhances brood reduction still needs further research.

Differences in nutritional condition between the three kinds of rearing environment (see Methods) were no observed. Thus, the remaining nestlings after brood reduction have no advantage in comparison with situations in which both nestlings survive. Ploger (1997) even found a decrease in food brought to nests with reduced broods. However, Simmons (2003) re-analyzed the experimental data for raptors and found that parents did not reduce food supplies after brood reduction and so in a two-chick nest, the surviving chick should get twice the food than before the elimination of its sibling. The better nutritional condition of nestlings in situation of growing population, when fecundity is decreasing and brood reduction is increasing, could be related to a higher proportion of low quality territories' occupation. This is in accordance with the result obtained when the nutritional condition by nest was analyzed: In years of higher fecundity (1997-1998), more fledglings were reared but in worse nutritional condition because also low quality territories were successful, whereas in poor years (1999-2000) when most of the fledglings flew from high quality territories fewer fledglings were reared but in better nutritional condition. No parental effect was noticed on the occurrence of brood reductions. Nevertheless, we consider that the sample size is too low to draw any conclusions regarding this trend.

The present study indicates that brood reduction occurred mainly in the worst territories. Thus, an increase in brood reduction is probably a consequence of the increasing usage of worse quality territories under higher population densities. In this sense, brood reduction could be considered as a consequence of the regulation of populations by habitat heterogeneity and of food shortage. As the nestlings dying as a result of brood reduction belong to the subset of those born in the worst territories and later on in the year (and thus with fewer possibilities of reaching reproductive age), the real effect of brood reduction on population dynamics may be smaller than the number of nests affected might well suggests.

Acknowledgments

We are in debt to ICONA and the RBD fieldworkers who have monitored Booted eagle fecundity in Doñana National Park for 20 years and, above all, to Luis Garcia, who also spent much of his time showing me every old nest once occupied by the species, and Javier Balbontin, who conducted the monitoring for three years. We are also grateful to A. Dekker for helping me with the field work for a year during his ethological research project (Zoological Laboratory, University of Groningen). G. Högstedt, R. Simmons, M. Stenning, P. Whitfield, and two anonymous referees greatly improved the original manuscript.

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8. Hatching sex ratio is not environmental dependent in the Booted eagle

Resumen

En este capítulo se ha estudiado el sex ratio primario de una población de águila calzada durante cinco años (1996-2000), periodo en el que se encontraba en alta densidad. La población estudiada se regulaba mediante heterogeneidad de hábitat, de modo que comprendía territorios de alta y de baja calidad. Para testar la hipótesis de Ajuste Medioambiental en el sex ratio, según la cual se producen más ejemplares del sexo más barato cuando las condiciones ambientales son adversas, exploramos territorio, año, calidad de territorio (en función de su frecuencia de ocupación) y el aporte de alimento como factores que podrían afectar a la proporción de sexos. También analizamos la edad de los adultos reproductores. La hipótesis de estudio era que si la proporción de sexos en una población heterogénea era dependiente de factores ambientales que condicionan la disponibilidad de alimento, deberíamos encontrar alguno de estos factores como predictores del sex ratio primario; al menos en territorios de baja calidad. Los resultados no apoyaron la hipótesis ya que ninguno de los factores analizados predijo el sex ratio primario y puesto que no se encontraron diferencias en el sex ratio primario entre territorios de alta y baja calidad. La edad de los reproductores no afectó al sex ratio de las nidadas. En la discusión se hipotetiza sobre la importancia de las condiciones que se dan en los cuarteles de invierno sobre la proporción de sexos y sobre el efecto de dicha proporción en la dinámica de la población. El sex ratio primario de la población estudiada no mostró diferencias de 1:1.

Palabras clave: Sex ratio primario, *Hieraaetus pennatus*, calidad del territorio, aporte de alimento, edad de los reproductores.

Abstract

We studied the hatching sex ratio in a Booted eagle population under high density situation, over 5 years period (1996-2000). The studied population was regulated by habitat heterogeneity, comprising both high and low quality territories. We explored territory quality, year and food supply to test the Environment Adjustment Hypothesis for adaptive sex ratio allocation. Age of breeding adults was also analyzed. We predicted that if sex allocation in a heterogeneous population was Environmental Dependent, we should find year and/or territory quality and/or food availability as predictor of the population hatching sex ratio; at least in low quality territories. The results did not support the Environmental Dependent hypothesis since we failed to find any predictor of hatching sex ratio and no significant differences in sex allocation between high and low quality territories were found. We did not find that age of breeders affected sex ratio in the brood. We hypothesized about the importance of the conditions in wintering quarters on sex allocation and about the effect of hatching sex ratio on the population dynamic. Population hatching sex ratio did not show differences from parity in the studied Booted eagle population.

Keywords: Hatching sex ratio, *Hieraaetus pennatus*, territory quality, food supply, parental age.

Introduction

It has been proposed that sex allocation is an adaptive response of animals to a variety of factors (Clutton-Brock *et al.* 1984, Bortolotti 1986). This means that breeding adults would adjust their offspring sex ratio production towards the sex with relatively greater fitness gain (Trivers & Willard 1973), either via current reproductive attempt or via expected future reproduction. The facultative control of primary sex ratio by breeding birds it has been one of the main focus in recent ecology literature (Hardy 1997) with controversial findings, and even it has been concluded that facultative control of offspring sex is not a characteristic biological phenomenon in breeding birds (Ewen *et al.* 2004).

Several hypotheses have tried to explain how adults could obtain larger benefits manipulating their offspring sex ratio. Local Resource Competition Hypothesis (Clark 1978) proposes that individuals produce more offspring of the most dispersive sex to avoid competition for the resources between juveniles and adults, and predicts a higher production of the most dispersive sex in low quality territories. This hypothesis was originally formulated for mammals and it has been supported in cooperative birds where some individuals remain as helpers (Komdeur *et al.* 1997, also see Cockburn & Double 2008), but not in raptors. Early Bird Hypothesis (Zijlstra *et al.* 1992, Smallwood & Smallwood 1998, Krebs *et al.* 2002) predicts that the sex with greater chances of recruitment to the breeding population if born early in the season will tend to be produced earlier in the season, which could explain seasonal trends frequently reported in raptors (Daan *et al.* 1996, Dalhaug *et al.* 1996). Other hypotheses propose that low quality parents would produce more of the cheapest sex (Genetic and Age Dependent sex ratio Hypothesis, Blank & Nolan 1983, Ferrer *et al.* 2009 respectively). The Environmental Sex Determination Hypothesis (Trivers & Willard 1973, Myers 1978) predicts that more offspring of the cheapest sex would be produced under environment stress conditions in sexually size-dimorphic species, because of its lower energetic requirements (Bortolotti 1986, Edwards *et al.* 1988, Wiebe & Bortolotti 1992, Korpimäki *et al.* 2000). But we postulate that in a population regulated by habitat heterogeneity where there are high and low quality territories, a higher proportion of the cheapest sex under environment stress will be specially noticeable in low quality territories, whereas sex ratio in high quality territories will hardly be affected by food shortage.

The main aim of the present study is to determine whether sex allocation was Environmental dependent in a territorial raptor population regulated by habitat heterogeneity. Although environmental predictability was low and then there would not be a strong selection for sex ratio adjustment at hatch (West & Sheldon 2002), the territorial breeding adults it is supposed to guess the quality of the territory where breed. Consequently, if sex ratio is adaptive, it is expected to find some factor related to territory quality or year determining the hatching sex ratio, also named primary sex ratio. Secondary sex ratio would be a consequence of differential mortality during parental care. We propose that in a population regulated by habitat heterogeneity and where sex allocation is Environmental Dependent 1) Territory quality and/or year and/or food supply should rise as the main factor determining hatching sex ratio. 2) Hatching sex ratio in high quality territories would not be affected by variations in food availability 3) Hatching sex ratio in low quality territories would be affected by food availability increasing the production of the cheapest sex under food shortage. Otherwise breeding adults could fail in predicting the environmental conditions during laying clutch, or sex ratio could be controlled by individual characteristics related to individual quality or even sex allocation could be not an adaptive strategy.

We tested the predictions in the Booted eagle population of the Doñana National Park, which has been monitored from 1976 to 2000 and it has been demonstrated that is regulated through habitat heterogeneity, suffering a higher rate of brood reduction those territories occupied less frequently, it means, low quality territories (Chapter 7). The relationships between hatching sex ratio and both territory and population parameters of the Booted eagle population were studied in the period 1996-2000 when population density was high (Casado *et al.* 2008).

Methods

TERRITORY QUALITY

A territory has been designed as a 1x1 km squared and can involve more than one active nest per year (Chapter 6). In that case, the biological parameters of every territory such as brood size and hatching sex ratio were the average from nests belonging to the same territory.

Territory quality was determined through occupation frequency (Ferrer & Donázar 1996, Sergio & Newton 2003, Casado *et al.* 2008), calculated as the number of years with recorded breeding attempts between 1982 and 2000. We used the median value of occupation frequency, 42 % of the studied years, to divide the territories into high quality territories (those territories occupied more frequently) and low quality territories.

To summarize we got for every territory occupied between 1996 and 2000 the occupation frequency, brood size and hatching sex ratio (when any nest of the territory was successful). And for every year we got the population size, sex ratio at hatching, % of rabbit in diet, prey diversity and mean fecundity.

SEX DETERMINATION

We determined the hatching sex ratio by collecting blood samples from the nestlings before brood reduction events, which occurs earlier than 11 days old, usually within the first 3 days of life (Chapter 7). Taking blood samples before brood reduction events was not always possible especially in cold days. Then, we collected blood samples at ringing time from those nestlings with still unknown molecular sex.

Primary or hatching intra-brood sex ratio was calculated as number of hatched males/brood size. When more than one nest was active within a territory, its sex ratio was the average of the nests' sex ratio. Annual hatching sex ratio was the average sex ratio of all the nests occupied that year. Only completely sampled broods were included.

Sexing was done in the molecular laboratory of the Doñana Biological Station. The methodology is detailed in Casado *et al.* (2002).

FOOD SUPPLY

The Booted eagle feeds on a broad range of different-sized prey (Martínez *et al.* 2004, Martínez & Calvo 2005), although the European wild rabbit *Oryctolagus cuniculus* is the main prey item (Veiga 1986). Wild rabbit supply conditioned the brood reduction frequency which occurred in a higher rate those years of low wild rabbit availability (Casado *et al.* 2008). Owing to the lack of comprehensive censuses of wild rabbit populations during the study period, prey remains found on nests during surveys were used to estimate proportion of wild rabbit in diet and prey diversity. All remains were identified to species level. The annual contribution of wild rabbits to the diet of Booted eagles was estimated by the number of items: nº rabbit x 100/nº items. Prey diversity was considered as the number of species found.

POPULATION PARAMETERS

From 1983 to 2000 the breeding population size (number of pairs occupying territories at the beginning of the breeding cycle), brood size at hatch, and number of fledglings (chicks that reached at least 30 days of age, when they are ringed) were monitored. Breeding population size and fecundity parameters between 1983 and 1996 were taken from the Doñana field diaries (Doñana Biological Station Archives). From 1996 to 2000 our own team monitored changes in the Booted eagle population. Every spring, all previously known territories were visited to record occupancy and the rest of suitable but previously unoccupied areas were also checked for new pairs. All the nests detected were visited at least twice by the same person, at the beginning and end of the breeding season, to band the nestlings. Nest sites were easily detected due to the conspicuous behavior of the Booted eagle, and the nests themselves were easily distinguishable from those of other raptors because Booted eagles cover their nests with green leaves.

AGE OF PARENTS

Breeding adults were captured during the nestling period using a net and an alive but irrecoverable Eagle Owl (*Bubo bubo*) as a lure. Then I could know the age of those breeders that had been fitted with metal rings when nestlings. We used from each territory the minimum age recorded, the maximum age recorded, and when we got data from several years, we calculated the mean age.

STATISTICAL ANALYSES

We used the STATISTICA 6 package. Statistical significance was set at Alpha= 0.05.

The effect of territory quality and year on hatching sex ratio in whole population as well as in high and in low quality territories was explored by Generalized Linear Model (GLZ). Sex ratio was the dependent variable with binomial distribution and logit link function. Several predictors were analyzed although not all together since they were related. We introduced territory, year, brood size and territory quality depending on their occupation frequency as categorical predictors. As continuous predictor we used territories' occupation frequency. Age of breeders and food supply were related to territory' and year' sex ratio respectively by Multiple Regression.

We tested deviation of the hatching sex ratio from 1:1 with a 2x2 Fisher test.

Results

It was sexed 189 nestlings from 122 broods between 1996 and 2000 both inclusive, which represent the 98.50 % of the total broods recorded in the studied period.

The 50.00 % of the nestlings in the population during 1996-2000 period were males, which did not differ from 1:1. Neither the total number of sons differed from unity within any of the 5 studied years (Table 1).

Table 1. *Probability of deviation from unity of hatching sex ratio. The 2x2 two tails Fisher test was used to estimate the probability of significant difference*

Year	Hatching sex ratio	N	p
1996	0.452	21	1.000
1997	0.442	26	0.659
1998	0.611	27	0.245
1999	0.563	24	0.651
2000	0.417	24	0.363
All Grps	0.500	122	1.000

Table 2. *Generalized Linear Model of hatching sex ratio built for both whole population and high and low quality territories*

	Degr. Of Freedom	Wald Statistic	p
Population hatching sex ratio			
Intercept	1	0.680	0.410
Year	4	6.035	0.197
Territory quality	1	1.461	0.227
High quality territories sex ratio			
Intercept	1	0.000	0.990
Occupation frequency	1	0.055	0.815
Year	4	3.115	0.539
Low quality territories sex ratio			
Intercept	1	0.141	0.707
Occupation Frequency	1	0.093	0.760
Year	4	5.780	0.216

Forty four territories were considered of low quality since they were occupied less than 43 % of the 1983-2000 period, and 27 territories were considered of high quality. The mean hatching sex ratio in high quality territories was 0.456 and in low quality territories was 0.525. There was not significant difference between mean hatching sex ratio of high and low quality territories (Wilcoxon Matched Pairs Test n=5, Z=1.214, p=0.225) although in 4 of the 5 studied years (1996-2000) hatching sex ratio of high quality territories was more female biased than that of the low quality territories. In Figure 1 the distribution of hatching sex ratio by territory quality is showed. Not territory quality or year were predictors of the population hatching sex ratio (Table 2). Year and occupation frequency neither were predictors of hatching sex ratio in high or low quality territories (Table 2). Brood size did not affect hatching sex ratio in any of the 5 studied years (Table 3).

Food supply did not affect the annual primary sex ratio (Multiple regression with prey diversity and percentage of rabbit in diet, $F_{(2,2)}= 0.180$, $r^2= 0.152$, $p= 0.848$).

Ten females and 7 males breeding adults were captured from 17 territories out of 78. We could not include the age of breeders as factor in a GLZ together with territory and year due to the small and unbalanced sample, but we related it to other factors by regression. Simple regression using occupation frequency as dependent variable and age of adults showed a tendency to the older the individuals the most frequently used were their territories although it was not significant and age of adults did not affect the occupation frequency of every territory (Minimum age Wald Statistic =0.162, p= 0.688; Maximum age Wald Statistic = 0.624, p= 0.430; Mean age Wald Statistic = 0.363, p= 0.547). The age of breeding adults in low quality territories ranged between 4-12 years (mean= 7.11). The minimum age recorded in high quality territories was 3 years and the maximum one was 14 years old (mean= 6.79).

Multiple regression with mean hatching sex ratio of every territory as dependent variable and occupation frequency and age of breeding adults as predictors showed that were not related in high quality territories or low quality territories or analyzing whole population together (Table 4).

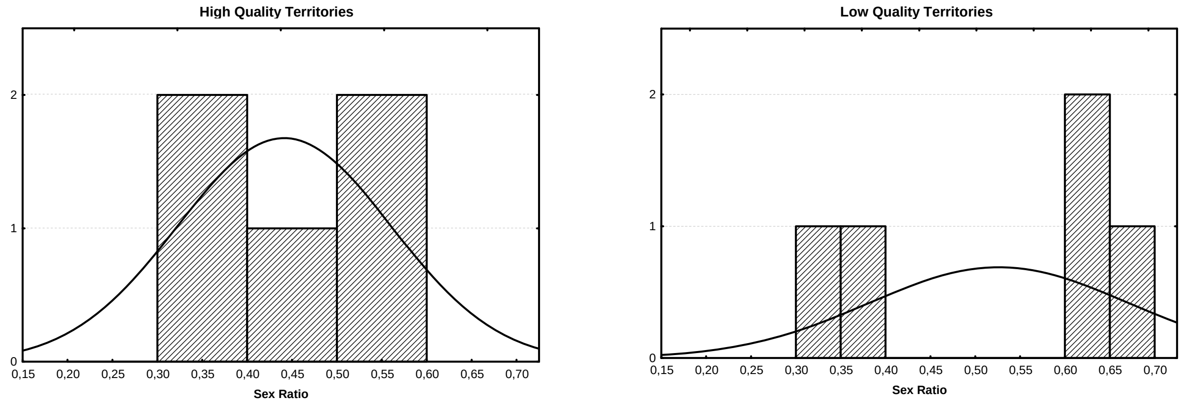
Table 3. *Generalized Linear Model of hatching sex ratio built by brood size every studied year*

Year	Whole population		High Quality territories		Low Quality territories	
	Wald Stat.	P	Wald Stat.	P	Wald Stat.	P
1996	1.177	0.278	0.000	1.000	0.000	0.996
1997	0.116	0.733	0.000	0.996	0.000	0.996
1998			0.000	0.995	0.000	0.995
1999	1.508	0.219	0.000	0.996	0.175	0.676
2000	0.225	0.635	0.000	0.996	0.016	0.898
All Grps	0.274	0.601	0.008	0.930	0.541	0.462

Table 4. *Multiple Regression Models of hatching sex ratio built by Occupation frequency and by age of breeding adults*

Variable	Parameter stimate ±SE	F	P
Dependent variable: **Population** hatching sex ratio	0.266	1.319	0.301
Occupation Frequency			
Minimum age of adults			
Dependent variable: **Population** hatching sex ratio	0.271	1.021	0.387
Occupation Frequency			
Maximum age of adults			
Dependent variable: **Population** hatching sex ratio	0.268	1.170	0.341
Occupation Frequency			
Mean age of adults			
Dependent variable: **High quality** territories hatching sex ratio	0.282	0.556	0.612
Occupation Frequency			
Minimum age of adults			
Dependent variable: **High quality** territories hatching sex ratio	0.277	0.638	0.575
Occupation Frequency			
Maximum age of adults			
Dependent variable: **High quality** territories hatching sex ratio	0.280	0.600	0.593
Occupation Frequency			
Mean age of adults			
Dependent variable: **Low quality** territories hatching sex ratio	0.244	1.614	0.275
Occupation Frequency			
Minimum age of adults			
Dependent variable: **Low quality** territories hatching sex ratio	0.266	0.880	0.462
Occupation Frequency			
Maximum age of adults			
Dependent variable: **Low quality** territories hatching sex ratio	0.257	1.157	0.376
Occupation Frequency			
Mean age of adults			

Figure 1. *Distribution of hatching sex ratio in high and in low quality territories*



Discussion

The results did not support the Environmental Adjustment Hypothesis, since environmental differences among years were not related to variations in hatching sex ratio. Neither territory, which varies in quality, or food availability seemed to affect population primary sex ratio. The lack of significant differences in hatching sex ratio between high and low quality territories is in accordance. Consequently, if sex allocation is adaptive in the Booted eagle, factors determining the sex at hatch must be related to individual quality like in other birds species (Ellegren *et al* 1996) or specifically in raptors (American kestrel -Wiebe & Bortolotti 1992-, Eurasian kestrel -Korpimäki *et al* 2000-). We could also consider that sex allocation is not an adaptive strategy (Radford & Blakey 2000, Ewen *et al* 2004), or even food resources were not scarce enough to affect hatching sex ratio during the study period. Nevertheless, last option has little or not support since wild rabbit population in Doñana National Park, the main prey of the Booted eagle, suffered a strong decline from 1990 (Villafuerte *et al* 1994) and its shortage increased Booted eagle nestling mortality in 1999 and 2000 (Casado *et al* 2008). As far we know it has been not demonstrated a facultative control of the hatching sex ratio by territorial breeding adults related to environmental conditions.

Age Dependent hypothesis predicts that the main factor determining the sex ratio is the age of breeders, independently if the population is regulated or not by habitat heterogeneity. In a population regulated by habitat heterogeneity could be difficult to separate age or territory quality effects, since it has been demonstrated that older individuals choose preemptively the best territories (Ferrer & Bisson 2003, Penteriani *et al* 2003). We failed in finding any relationship between age of breeding adults and territory quality (occupation frequency), although this result should be taken with caution due to the small number of breeding adults with known age. It has been found in some species that the older the breeders are the higher is their fecundity (Sæther 1983, Newton 1989, Desrochers & Magrath 1993, Espie *et al.* 2000, Sedinger *et al.* 2001, Ferrer & Bisson 2003, Penteriani *et al.* 2003), either because they improve skills that are essential to high reproductive performance, or because younger birds abstain from or put less effort into reproduction (Espie *et al.* 2000), or because of hormonal changes related to breeding skills (Angelier *et al.* 2007). Other authors found that birds produced more males or females depending on their age (Forslund & Part 1995, Blank & Nolan 1983, Ferrer *et al* 2009). We did not find any significant relationship between age of breeding adults and hatching sex ratio and brood size (F(1,19)= 0.532, *p*= 0.475). However, due to our small sample size we can not discard the age of breeding adults as a factor affecting hatching sex ratio.

We have found parity in the studied Booted eagle population hatching sex ratio such as predicted by Fisher (1930), and such as it has been found in most of the size-dimorphic raptors studied (Newton 1979, Newton & Marquiss 1979, but see Ferrer *et al* 2009). Due to the strong reverse sexual dimorphism in the Booted eagle a differential cost of rearing sons or daughters was expected; differential mortality during the dependent period of the larger sex occurs because it requires higher food intake (Roskaft & Slagsvold 1985, Slagsvold *et al.* 1986), and consequently it starve more easily if food resources scarce. Hence a significant deviation from unity in the hatching would be also expected; either 1) by parents trying to compensate differential mortality by sex during the period of parental care or 2) by parents trying to compensate the differential cost of rearing nestling deviating the sex ratio towards the cheapest sex under difficult rearing circumstances. Since there was not deviation from unity in hatching sex ratio, some mechanism must be counterbalancing the differential cost of rearing each sex, for example differences in growth dynamics (Newton 1979, Richter 1983). Newton (1978) proposed that males (the smallest sex) have faster growth than females in *Accipiter nisus*, which allow them to leave the nest earlier and to be more competitive than females, and consequently to show higher survival rate. Indeed it has been demonstrated in other bird species the smaller sex has higher rate of growth (Richter 1983, Stamps 1990 and references therein) or matures more rapidly (Fiala & Congdon, 1983). Secondary sex ratio in the Booted eagle between 1996 and 2000 was 0.453 (Casado & Ferrer unpublished data) which indicates a higher males mortality during the nestling period opposite to expected (Trivers & Willard, 1973). However the survival rate after fledging seems to be similar between males and females because in 1996 (first year Booted eagle was sexed by molecular techniques) 15 females and 18 males fledged in Doñana National Park, and one individual of each sex has been recorded as breeder. Moreover, we fitted fledglings with transmitters and we recorded four deaths before migration and they were two males, one female and one unknown sex (Casado unpublished data). What could counterbalance the different survival rate during nestling period is that males leave the nests in better physical condition than females do (Casado *et al.* 2002). Males, we speculate, get higher chance to die when brood reduction happens because of their smaller size.

In short, we have not found any support for the Booted eagle using an adaptive strategy of sex allocation. If there is a facultative control of the hatching sex ratio it would be related to individual quality rather than territory quality. Habitat heterogeneity did not affect sex allocation. Years when more females than males were hatched a higher nestling mortality was recorded. For example 2000 year, that was the poorest one because of the lowest mean fecundity was recorded (Casado *et al* 2008), was poor not only for its lower food availability but also due to a more female biased hatching sex ratio, independently of the brood size. Maybe the migratory character of the Booted eagle affects the sex allocation through variations of conditions in wintering areas, which would affect the physical conditions of adults at the start of the reproductive season rather than age. Conditions in wintering areas should be analyzed to estimated at what extent it affects the sex allocation in the breeding areas. Since hatching sex ratio in the studied Booted eagle population was balanced at population level as well as distinguishing between territory qualities, it would keep the population stable even in case of isolation. A biased mortality during parental care conducting to an unbalance secondary sex ratio would have stronger consequences on the population dynamic than the hatching sex ratio recorded.

Acknowledgements

Arjan Deker helped me collecting field data along 2000. We thank to Hugo Le Franc his helpful company in capturing adults.

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9. Síntesis

Analizar la estructura de la población reproductora del águila calzada en el Parque Nacional de Doñana, su fecundidad, frecuencia y ocurrencia de reducción de nidada así como la proporción de sexos, todo ello tanto de toda la población como de cada territorio y año en particular, nos ofrece la posibilidad de saber cómo funciona dicha población y podemos ir recreando su historia. Por extensión podría orientarnos a la hora de interpretar las oscilaciones poblacionales en otras rapaces.

Muy probablemente, el área del Parque Nacional de Doñana era un hábitat adecuado para la reproducción del águila calzada, pero en la que sin embargo no habitaba tal vez por motivos históricos (p.e. persecución directa). Sin embargo, las poblaciones más cercanas iban produciendo individuos que acabarían por colonizar este hábitat disponible y sin competidores. Entorno a 1976 se asentó en el Parque Nacional de Doñana (que, recordemos, se formalizó como tal en 1969 con las consecuentes normas de protección de fauna y flora) la primera pareja reproductora. Los individuos que llegaron primero para reproducirse probablemente serían individuos que no podían criar en su población natal por falta de territorios vacantes, y si los había probablemente eran de baja calidad.

Gracias a determinadas pistas que les indicaban la idoneidad o no de un determinado lugar para instalar su nido, en función tanto de la disponibilidad de sustrato de nidificación como de posibles presas (distancia a la marisma), una a una las nuevas parejas fueron ocupando territorios en el Parque Nacional de Doñana. Inicialmente podrían escoger territorios de alta calidad (con abundante disponibilidad de alimento) puesto que no había competencia intra-específica. Algunos individuos criarían en territorios no favorables bien al fallar en la interpretación de pistas, o bien por ser incapaces de predecir sucesos azarosos como la predación. Estas pocas parejas iniciales se fueron distribuyendo de forma dispersa, alejándose las unas de las otras, ya que las interferencias directas o indirectas entre los individuos reproductores mermarían su éxito reproductor. A medida que la población reproductora del Parque Nacional fue creciendo por incorporación de nuevos individuos procedentes de la propia u otra población, los territorios fueron agregándose distribuyéndose cada vez más regularmente. Llegó un momento en que todos o casi todos los territorios de alta calidad estaban ocupados por parejas que regresaban al mismo año tras año, de modo que los nuevos individuos que se incorporasen tendrían que encontrar un hueco vacante (por mortalidad o emigración) en un territorio ya existente, o bien tuvieron que desplazarse a otro territorio de inferior calidad. Así se llegó, en torno a 1992, a la situación actual de población heterogénea, con territorios buenos y malos que difieren en su éxito reproductivo: En años de alta disponibilidad de alimento todos los territorios, mejores y peores, son capaces de producir un elevado número de crías, mientras que en años de baja disponibilidad de alimento muchos de los territorios de peor calidad fallan en la reproducción o crian un número reducido de pollos. En cambio, los territorios de mejor calidad seguirán siendo capaces de producir un número de crías similar al de otros años. Por este motivo los territorios de mayor calidad muestran menor varianza en la fecundidad y son más estables, mientras que los de baja calidad muestran mayor varianza en la fecundidad.

En esos años malos, la fecundidad media de la población es inferior a la media general, sobretodo porque hay un descenso en la tasa de eclosión especialmente en los territorios de baja calidad (quizá por abandono). Además, algunos de los pollos que nacen en dichos territorios fallecen por inanición: El pollo que nace primero se encuentra en mejor condición física que el segundo, y esto favorece la muerte del segundo en situaciones extremas de escasez de alimento. La reducción del número de pollos de una puesta (reducción de nidada), se produce por lo general antes de la primera semana de vida, y los pollos que mueren son los más jóvenes, y por tanto de menor tamaño y destreza. No obstante, averiguar si es la menor capacidad de los pollos menores para acceder al alimento ofrecido por los padres, o si es la decisión parental sobre su distribución lo que hace que se produzca la reducción de nidada, es un objetivo pendiente. El reducido éxito reproductivo de los territorios de baja calidad desvía la distribución de la fecundidad de la población hacia la izquierda (0 pollos volados). En cambio, en los años de fecundidad media igual o mayor a la general, la forma de la frecuencia de la fecundidad es similar a una curva normal. En estos años poco productivos el sex ratio en el momento de la eclosión es similar al de años mejores, de modo que los padres no parecen ser capaces de predecir cuantos pollos podrán criar y "escoger" qué sexo les conviene criar.

Como norma general los pollos machos se encuentran en buena condición física mientras que los pollos hembra se encuentran generalmente en peor condición, probablemente porque al ser de mayor tamaño requieren mayor gasto energético y mayor demanda. Las hembras adultas también son de mayor tamaño pero se encuentran en mejor condición física que los machos adultos durante la reproducción, probablemente porque debido a su papel, el de aportar alimento a la hembra y a los pollos, sufre mayor desgaste.

Si bien en los inicios de la población del Parque Nacional se le supone una elevada tasa de crecimiento, ésta se ralentizaría, no por un incremento en la reducción de nidada que tiene poca repercusión ya que se produce solo algunos años, en algunos nidos, sino porque se redujo la fecundidad media al ir ocupándose cada vez más territorios de baja calidad. Hasta el año 2001 la productividad media de la población era elevada, y la población continuaba en crecimiento, aunque cerca de la estabilización. Esto sugeriría que algunos pollos nacidos en el Parque Nacional de Doñana tendrían que intentar reproducirse en otro lugar por falta de territorios vacantes en su población natal, continuando un proceso de colonización, y originando una nueva población reproductora. No obstante, si este proceso ya se hubiese iniciado esperaríamos obtener recuperaciones/avistamientos de individuos nacidos en el Parque Nacional en otras áreas en época de cría, lo que de momento no ha sucedido. Aunque puede deberse a una escasa prospección.

En el año 1990 irrumpió en el área de Doñana la enfermedad llamada hemorrágico vírica, que produjo una elevada mortalidad en el conejo diezmando drásticamente su población. A pesar de que esta especie siempre se ha considerado la principal presa del águila calzada, su declive no ocasionó una merma en la fecundidad de la población del Parque Nacional. El descenso en la fecundidad pudo verse contenido por el carácter ecléctico de la especie, que puede cazar sobre un amplio espectro de presas. De hecho, el menor aporte de conejo a los nidos se compensó con el suministro de una mayor variedad de especies presa. El descenso en la disponibilidad de conejo parece que afectó únicamente a los territorios de baja calidad, donde tal vez los individuos reproductores eran poco hábiles en la caza o donde tampoco disponían de otras presas alternativas. Esto haría que se tuviesen que desplazar más lejos en busca del alimento que hembra y pollos demandaban, teniendo que abandonar el cuidado del territorio. Se realizará un próximo estudio relacionando el tamaño del área de campeo con la calidad de los territorios y su fecundidad.

El valor de la presente memoria radica en que aporta conocimiento sobre la dinámica poblacional de esta rapaz poco estudiada, que se puede aplicar al correspondiente manejo encaminado a su conservación. Además, no podemos olvidar el valor que en sí mismas tienen las metodologías desarrolladas, algunas de las cuales se pueden aplicar en el estudio de otras aves.

En una población heterogénea en la que hay territorios con menor varianza en la fecundidad que actúan como fuente y otros con mayor varianza que actúan como sumidero, los impactos negativos tendrán graves consecuencias si perjudican a los territorios fuente, mientras que pueden ser insignificantes si se producen sobre territorios sumidero. Por este motivo es importante conocer la estructura de una población. Si no se tienen datos sobre la productividad anual de cada territorio, los territorios de alta calidad se pueden distinguir por su mayor frecuencia de uso. Además, suelen ser ocupados más tempranamente en la estación reproductora y por tanto sus huevos eclosionan también antes. Este sistema fuente-sumidero acoplado también tiene consecuencias genéticas puesto que los territorios fuente contribuyen desproporcionadamente a la siguiente generación.

10. Conclusiones

1. La asimetría en la fecundidad (skewness) es un método crítico para distinguir entre heterogeneidad de hábitat o ajuste individual como mecanismos que conducen a la fecundidad denso-dependiente. La asimetría muestra una relación fuertemente significativa con la fecundidad media de una población, solo si en dicha población actúa la heterogeneidad de hábitat. Este método es útil para especies que poseen una distribución discreta del número de crías, con un número pequeño de categorías, salvo cuando éstas son dos: 0 - 1.

2. Otro método frecuentemente utilizado para distinguir entre ambas hipótesis sobre el mecanismo regulador de la población, la relación entre la fecundidad media de una población y su coeficiente de variación, únicamente es útil si se conoce qué porcentaje de dicha relación es espuria. Tanto bajo heterogeneidad de hábitat como bajo ajuste individual se da dicha relación significativa, pero la pendiente difiere entre ambas; es decir las diferencias en la relación entre la fecundidad y su coeficiente de variación entre ambas hipótesis es solo cuestión de grado.

3. La población de águila calzada estudiada muestra fecundidad denso-dependiente, que surge por heterogeneidad de hábitat en la actual situación de alta densidad. En esta situación, hay territorios de alta calidad que son ocupados más frecuentemente, desde hace más tiempo, presentan mayor fecundidad y ésta no se ve afectada por la densidad poblacional. No obstante, tal vez el ajuste individual reguló la población en sus inicios, cuando se encontraba en baja densidad y todos los territorios ocupados eran de alta calidad.

4. La reducción de nidada en la población de águila calzada del Parque Nacional de Doñana es denso-dependiente, incrementándose su frecuencia a medida que incrementa la densidad poblacional.

5. La reducción de nidada en la población de águila calzada del Parque Nacional de Doñana es consecuencia de la estructura heterogénea del hábitat, con unos territorios – los de baja calidad- con mayor probabilidad de sufrirla que otros. Como los pollos que fallecen en el nido pertenecen al subconjunto de los nacidos en los peores territorios y

más tarde (por tanto con menores posibilidades de alcanzar la madurez sexual), el efecto real de la reducción de nidada sobre la dinámica de la población puede ser menor de lo que podría sugerir el número de nidos afectados.

6. La condición física de los padres no influyó en la ocurrencia de reducción de nidada.

7. La condición física de los pollos varió con el territorio. El primer pollo nacido se encuentra mejor alimentado que el segundo. Los pollos que sobreviven tras la reducción de nidada no se encuentran en mejor condición física que aquellos que se encuentran en nidos en los que no se da tal fenómeno.

8. Los parámetros sanguíneos indican que los pollos hembra sufrieron mayor estrés alimentario que los pollos machos, y todos los pollos en general mayor estrés que los adultos. Las hembras adultas se encontraron en mejor condición física que los machos, quizá debido a los diferentes papeles que cumplen durante la estación reproductora. Los pollos hembra quizá se encontraron en peor condición física que los machos porque su mayor tamaño, que implica una tasa metabólica y un patrón de crecimiento diferentes, hace que sus requerimientos energéticos sean mayores.

9. En los años en los que se registró mayor número de pollos volados por nido, éstos se encontraban en peor condición física que los pollos volados en años de baja fecundidad. Esto tal vez se debiese a que en los años de alta fecundidad volaron pollos de los territorios de alta y baja calidad, mientras que en los años de baja fecundidad únicamente/mayoritariamente volaron pollos de territorios de alta calidad.

10. El sex ratio de la población de águila calzada del Parque Nacional de Doñana coincide con la unidad mientras que la de los territorios buenos y malos, que no difieren entre sí, se encuentran próximos a la unidad. La heterogeneidad de hábitat no condiciona el sex ratio primario.

11. Los territorios de mejor calidad y por tanto más antiguos se encuentran en áreas de mayor disponibilidad de alimento que aquellos que han tenido que ser ocupados más recientemente.

12. El águila calzada en el Parque Nacional de Doñana no sufrió un duro revés cuando el virus de la hemorragia vírica redujo drásticamente la disponibilidad de conejo, ya que es capaz de capturar muchas otras presas. La reducción en la disponibilidad de conejo solo afecta a aquellos territorios de baja calidad, donde tal vez otras presas son también escasas.

13. El sexo de los adultos se puede determinar con una fiabilidad del 100% gracias con una función discriminante que utiliza el antebrazo y el peso como variables predictoras. El sexo de los pollos se puede determinar con un 98,8% de fiabilidad, con una función discriminante que utiliza como variables predictoras el antebrazo, la cola, el pico y el tarso.

11. Agradecimientos

Desde el inicio de la tesis doctoral hasta su fin han transcurrido muchos años y he conocido a mucha gente. Por esto me supone un gran esfuerzo diferenciar entre aquellas personas que me ayudaron de alguna forma en su desarrollo, que es a quienes va dedicado esto, y esas otras que si bien han influido en mi formación general no tuvieron relación directa con la tesis. Este ejercicio tiene su justificación en que quiero expresar mi sincero agradecimiento a quienes me han ayudado a empezar, continuar y terminar esto que tenéis entre manos.

Siempre he disfrutado de mi estancia en la EBD, pero desde la distancia resultan especialmente entrañables mis primeros años. Los recuerdo como un continuo disfrute, desde el trabajo de campo hasta las copas y cervezas junto con muchos compañeros inolvidables. Con unos coincidí más asiduamente que con otros, pero todos ellos han contribuido en algún momento a hacerme la vida y el trabajo más amenos, muchos dieron buenos consejos y algunos siguen siendo hoy día amigos muy queridos. Tengo multitud de buenos recuerdos con Gema García, Hugo, Paco, David y Cristina, Jordi, Pepe, Esperanza, Ludy, Manu e Isa, Nano, Antonio, Carlos Alonso, Roger, Enrique Luque, Manuela. También pasé buenos momentos con Vincenzo, Marcelo, Hector y Ana, Gema y Jacinto, Fermin, Javichu, Cani, Mariajo.

Vivir en el Palacio de Doñana la mitad del año, me permitió conocer a gente encantadora como Manolito y Fabiola, Rosa y Antonio Otero, Rafa Laffite, Miguel Angel Bravo, Carmen y Antonio Abad, Luis Gutierrez (imprescindible además), Diego, Charina. En general, todo el personal que en esos años trabajó en la Reserva (Juan Calderón, Pilar y Pepe Navas, Cecilia, Manolita y su familia, Chans, Antonio Jimenez) me facilitó llevar a cabo el trabajo de campo. Porque son muchos los problemas que surgen trabajando día tras día en Doñana y muchos los favores que se piden y reciben. Yo recuerdo haber recibido siempre un trato excelente y mucho cariño. Espero que haya sido recíproco.

No recuerdo cuando fue la primera vez, pero desde mi llegada a la EBD he estado consultando en infinidad de ocasiones sobre trampeo de aves, emisores, receptores y otros temas variopintos a Manuel de la Riva, quien siempre ha sido muy amable conmigo y con quien también he compartido cervezas y desayunos. Alvaro y Arjan fueron unos estupendos ayudantes durante el trabajo de campo, que fue muy intenso. José Ayala participó un año en el seguimiento de la reproducción. Vilches también me ayudó en el seguimiento de la población reproductora, puesto que mientras él desarrollaba sus tareas se encontraba en ocasiones con nuevos nidos que yo desconocía. Lo mismo les sucedía a Gema García y a Nano. Hugo me acompañó a menudo durante la captura de adultos y le agradezco tanto su compañía como la ayuda que me prestaba en el montaje de toda la parafernalia. Y hubo excelentes voluntarios como Mara, Ernesto, María y Amaranta, que se esforzaron mucho. A Javier Balbontín debo reconocerle especialmente porque inició el trabajo que yo continué; su experiencia previa me facilitó mucho los comienzos. Pero sin duda quien definitivamente ha posibilitado que dispusiese de un registro completo de coordenadas y de la trayectoria reproductora de cada nido, es Luis García. A Luis debo agradecerle, ya no solo su excelente memoria y los meses que me dedicó mientras interpretábamos sus anotaciones en los diarios de campo y tomábamos las coordenadas de todos los nidos conocidos de águila calzada, sino su disposición, cariño, noches muy divertidas y conversaciones interesantes.

Sin el estímulo ni las ideas de Ernesto y Tenan, probablemente no hubiese conocido la Estación Biológica de Doñana; y sin la experiencia que me dio haber colaborado en GREFA no hubiese tenido posibilidad de acceder a una primera beca. Los años que fui voluntaria de GREFA así como a todos quienes conocí allí son inolvidables. Gracias a Mássimo Pandolfi, profesor y amigo, obtuve una beca Leonardo en 2001. Los numerosos revisores de los artículos realizaron constructivos comentarios. Los diferentes co-autores de los artículos escritos (Susana Suárez-Seoane, Julien Lamelin, Javier Balbontin, Massimo Pandolfi, Ian Newton y Miguel Ferrer) han contribuido grandemente a la elaboración de cada capítulo. Teo Todorov me ha permitido amablemente utilizar una de sus fotos para la portada de esta tesis. Lourdes Encina ha estado siempre disponible para resolverme dudas y asesorarme.

He tenido la suerte de compartir cuarto con Manuela de Lucas desde que llegó, de lo que me alegro muchísimo pues es una maravillosa compañera y amiga.

Nuestros amigos de Madrid y Málaga, así como nuestras familias, nunca han olvidado por qué vinimos Jaito y yo a vivir a Sevilla y siempre han estado insistiendo, preguntando por el fin de la tesis. La compañía de Justo, Chiqui y Lucia, Petu y Ester, y Anita ha sido fundamental para nosotros, que estábamos lejos y solos. Petu hizo de cicerón a nuestra llegada y nos enseñó los lugares clave en Sevilla; gracias a él tuvimos una dulce llegada. Y Victoria nos dejó muy amablemente su casa mientras lo necesitamos.

Saber que me encontraba respaldada por el cariño de mi familia me ha dado fuerza y norte cuando lo he necesitado. Desde que era muy pequeña absorbí de mi hermano Angel la curiosidad por los animales, así que le considero el primer culpable de todo. Mi hermana Gema me ha ayudado de múltiples formas durante todo este tiempo y siempre ha sido un apoyo. No me olvido de Merche. Mis padres han estado conmigo siempre, incondicionalmente, en mis estudios, voluntariados, etc. Incluso me regalaron mi primer coche (ay, cuanto disfruté con él) con el que empecé a trabajar en el campo. Pero sobre todo, impidieron con su persuasión y generosidad que me dedicase a cualquier otra cosa cuando ya estaba decidida a abandonar. Os estaré eternamente agradecida por todo ello, por hacer posible en definitiva que actualmente me pueda dedicar a lo que más me gusta. Esto es una deuda impagable.

La siguiente persona con la que me encontré en este camino y a la que debo agradecer haber hecho la tesis es al director de la misma, Miguel Ferrer, por darme la oportunidad de comenzarla, y porque insistió y me ayudó hasta que la terminé. Mis ideas sobre la dinámica poblacional de las rapaces y su aplicación a la conservación de las mismas son fruto del aprendizaje junto a él. Considero una enorme suerte haberle conocido.

Jaito ha estado conmigo durante todos estos años y espero que muchos más. Vino conmigo desde Madrid y creo que nunca se ha arrepentido. Ha resultado ser el compañero de viaje ideal, y por supuesto irreemplazable. Ya no solo su paciencia y apoyo, sino también sus críticas me han ayudado mucho en todos los aspectos, personales y laborales. Soy muy afortunada porque forme parte de mi vida. Debo agradecerle también su ayuda en la edición de las imágenes que aparecen en esta memoria. El anuncio de la llegada de Vera, mi hija, me impulsó a finalizar este trabajo, sin su llegada no sé cuando me hubiese decidido.