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Sula nebouxii

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Senescencia en el bobo de patas azules *Sula nebouxii.*

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Resumen

La senescencia es el deterioro de las funciones que resulta en menor probabilidad de supervivencia y éxito reproductivo a edades avanzadas. En poblaciones silvestres se conoce poco sobre cuáles componentes del ciclo reproductivo y la fisiología son afectados por la senescencia. En este trabajo estudiamos la senescencia reproductiva en el bobo de patas azules, un ave marina de larga vida. En primer lugar, analizamos datos longitudinales de tres cohortes para determinar cómo el éxito reproductivo de hembras que sobrevivieron al menos 14 años cambiaba a lo largo de su vida. En segundo lugar, evaluamos la contribución de los efectos pre- y post-natales hacia la disminución en el éxito reproductivo de las hembras viejas mediante un experimento de intercambio de puestas entre hembras de distintas edades. Posteriormente, evaluamos si los carotenoides, que los bobos de patas azules obtienen únicamente a través de la dieta, son un factor limitante en la reproducción de los machos viejos. Para ello realizamos un experimento de suplemento de carotenoides a machos de diferentes edades. Finalmente, estudiamos las posibles repercusiones de la senescencia sobre la conducta de cortejo en parejas de edad conocida. Evaluamos si los individuos viejos modifican su estrategia de cortejo y si las parejas de animales viejos disminuyen el cortejo intra-pareja y realizan más conductas extra-pareja.

Los resultados indican que el éxito reproductivo (numero de crías emplumadas) de las hembras aumenta con la edad hasta los 8 años y posteriormente disminuye, aunque la tasa a la que disminuye difiere entre cohortes. Del experimento de intercambio de puestas encontramos que las hembras viejas pusieron primeros y segundos huevos más pequeños que dieron lugar a crías más pequeñas al día 5. El efecto negativo de la edad de la hembra que puso los huevos continuó en las segundas crias hasta el día 30 con tasas de crecimiento mas bajas. Además, el crecimiento esquelético al día 30 y la respuesta inmune al día 20 de las segundas crías disminuyó con la edad de la hembra que las crió. Sin embargo, el resultado más interesante fue que el éxito reproductivo de las hembras viejas dependió de la edad de la hembra que puso los huevos: hembras viejas que cuidaron huevos de hembras viejas tuvieron un éxito reproductivo bajo en comparación con hembras viejas que cuidaron huevos de hembras maduras.

En el experimento de suplemento de carotenoides encontramos que, independientemente de la edad, los machos que recibieron carotenoides perdieron menos peso que los machos controles, pero no hubo efecto del tratamiento o la edad del macho en nuestros indicadores de estrés oxidativo. Además, encontramos una relación positiva entre la edad y el crecimiento de las crías de machos que recibieron carotenoides pero no para crías del grupo control. Finalmente, durante la etapa de cortejo, la tasa de cortejo que los machos dirigieron hacia la pareja social aumentó con la edad y fue menor hacia hembras mayores a 9 años. Por su parte, las hembras aumentaron con la edad su tasa de cortejo y cópulas con la pareja social además de la presencia en el territorio. La edad no afectó la conducta extra-pareja, pero la presencia de la hembra disminuyó la conducta extra-pareja de su macho.

Los resultados experimentales sugieren que la senescencia afecta negativamente la producción de huevos y pollos en hembras y que los carotenoides limitan la reproducción de machos viejos. Además, los resultados de las observaciones de cortejo sugieren que animales viejos de ambos sexos aumentan su cortejo para compensar la disminución en atractivo, los machos discriminan a las hembras viejas y las hembras viejas modifican su conducta para resguardar a su pareja. En conjunto los resultados sugieren que en los bobos, los animales viejos son capaces de ajustar su conducta reproductiva, durante el cortejo, en función de su edad y la edad de su pareja (solo machos), durante el cuidado post-natal, en función de cambios en la condición corporal, en el caso de machos, o cambios en el valor de la progenie que cuidan, en el caso de las hembras. La capacidad para ajustar positivamente el nivel de cuidado parental en animales viejos sugiere que estos restringen su inversión reproductiva pues si estuvieran limitados en sus capacidades no podrían aumentarlo. En animales viejos, la plasticidad en el cuidado parental puede ser una estrategia adaptativa, sobre todo en especies que

dependen de recursos impredecibles.

Palabras clave: senescencia reproductiva, cuidado parental, carotenoides, estrés oxidativo, cortejo, aves marinas.

Capítulo 1

Introducción

La senescencia es la pérdida de las funciones a edades avanzadas que generalmente resulta en la disminución de la probabilidad de supervivencia y éxito reproductivo (Ricklefs 2008). Es un fenómeno de amplia distribución filogenética pues se ha observado en organismos tan diversos como las bacterias, levaduras, gusanos, moscas, aves y mamíferos (Rose 1991, Barker y Walmsley 1999, Kirkwood y Austad 2000, Ackerman et al. 2003, Stewart et al. 2005, Nyström 2007). A partir de estudios en modelos clásicos de laboratorio (e.g. rata, mosca, gusano, cultivos celulares) se ha generado información sobre mecanismos genéticos y fisiológicos involucrados en el deterioro somático (Halliwell y Gutteridge 2007) y germinal (Risch et al. 1987, te Velde y Pearson 2002), así como cambios que ocurren en la fisiología de los animales senescentes (Hollyday 1995). Se ha reportado un declive al avanzar la edad en la capacidad de aprendizaje, memoria, función de los sentidos, locomoción, función reproductiva y función inmune en aves, mamíferos e invertebrados (Martin y Grotewiell 2006). Dichos estudios requieren muchas veces de manipulaciones genéticas, obtención de muestras de tejido o sacrificio de animales, por lo que las especies de laboratorio han resultado ideales. En contraste, el nivel de conocimiento que tenemos acerca de la relación entre la senescencia y las interacciones ecológicas de los organismos o de la simple existencia de la senescencia en poblaciones silvestres es mucho menor pues hasta hace relativamente poco tiempo se pensaba que la mortalidad extrínseca causada por depredación, parasitismo o daño mecánico, eliminaba a la mayoría de los individuos de la población antes de alcanzar la edad en que ocurre la senescencia (Weissman 1892, Medawar 1952, Williams 1957). Sin embargo, la acumulación de información en poblaciones con seguimiento a largo plazo de individuos marcados ha permitido analizar los cambios en los patrones de reproducción y supervivencia que ocurren a lo largo de la vida. Los resultados de estos estudios han mostrado, por un lado, evidencia de senescencia reproductiva, y por otro lado variación individual en la

edad a qué dicha disminución ocurre (Beneth y Owens 2002, McElligot et al. 2002, Yoccoz et al. 2002, Velando et al. 2006a, Jones et al. 2008, Bowhuis et al. 2009). Para el caso particular de la reproducción, en algunas especies se ha observado que el éxito reproductivo aumenta con la edad hasta un máximo y luego disminuye (Schwartz et al. 2003, Velando et al. 2006a, Sharp y Clutton-Brock 2010) y en otras solamente se ha reportado disminución en el desempeño reproductivo al aumentar la edad (Benneth y Owens 2002). En poblaciones silvestres existen dos revisiones recientes muy importantes al respecto de la senescencia reproductiva. En el primero de estos estudios se encontró evidencia de senescencia reproductiva en 13 de 16 especies de aves de zonas templadas (Benneth y Owens 2002), mientras que en el estudio mas reciente se encontró senescencia reproductiva en 10 de 13 especies de mamíferos y aves con diferencias importantes en la longevidad y número de descendientes producidos por cada evento reproductivo (Jones et al. 2008). Posiblemente en las especies donde no se detectó senescencia reproductiva los estudios no han alcanzado a cubrir la edad a la cual ocurre este fenómeno. A partir de los resultados de estos estudios se ha reconocido a la senescencia reproductiva como un fenómeno fundamental y ampliamente distribuido en poblaciones silvestres de vertebrados.

Al ser un proceso que disminuye el desempeño de los organismos de edades más avanzadas, la senescencia podría afectar los compromisos entre distintos componentes de historia de vida como son la reproducción y mantenimiento así como las estrategias reproductivas de los organismos a lo largo de la vida (McNamara et al. 2009). La ecología evolutiva explica la senescencia cómo resultado de disyuntivas de historia de vida entre los recursos que se asignan a la reproducción y al mantenimiento somático (Stearns 1992). La hipótesis del soma desechable propone que, cuando hay recursos que se utilizan tanto para funciones reproductivas como de mantenimiento, los recursos asignados a la reproducción no estarán disponibles para funciones de mantenimiento y esto resultará en la aparición de defectos en los tejidos corporales, tales como mutaciones en las células somáticas, proteínas glicosiladas o daño a las membranas celulares (Kirkwood y Rose 1991). Estos defectos, al irse

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acumulando a lo largo de la vida darán lugar a la aparición del fenotipo senescente (Kirkwood y Rose 1991). La inversión en la reproducción impacta negativamente en la inmunocompetencia y capacidad antioxidante de los organismos (Deerenberg et al. 1997, Hansen et al. 2005, Wiersma et al. 2006) y la inversión reproductiva a edades tempranas afecta negativamente la longevidad (Reid et al. 2003, Descamps et al. 2006) y el éxito en la reproducción a edades avanzadas (Nussey et al. 2006). En teoría, en el fenotipo senescente la capacidad para asignar recursos entre la reproducción y el mantenimiento se verá más comprometida que en los organismos más jóvenes pues, al estar más deteriorados, los organismos viejos necesitarán más recursos para el mantenimiento somático y esto limitará su capacidad reproductiva (McNamara et al. 2009). Sin embargo, en poblaciones silvestres se conoce muy poco sobre qué componentes del ciclo reproductivo son afectados negativamente por la senescencia, cuáles son los factores que están relacionados con la variación individual en las tasas de senescencia o cómo cambia la fisiología con la edad (Saino et al. 2003, Catry et al. 2006, Bouwhuis et al. 2009, Lecomte et al. 2010). En esta tesis nos propusimos estudiar la senescencia reproductiva en una población silvestre del bobo de patas azules (*Sula nebouxii*), investigando qué componentes del ciclo reproductivo se ven afectados por la edad. En particular estudiamos el efecto de la senescencia en el cortejo de machos y hembras, en efectos maternos en el huevo, y en la capacidad de crianza de machos y hembras. También estudiamos los posibles mecanismos fisiológicos asociados a un desempeño reproductivo pobre a edades avanzadas y evaluamos si existe variación en las estrategias reproductivas de los organismos viejos, dependiendo de la edad de su pareja y de cambios en el valor reproductivo de la progenie y condición corporal individual.

Efecto de la senescencia sobre los patrones de cortejo

Estudios teóricos han propuesto que los animales deberían preferir aparearse con individuos viejos porque sus genes han probado habilidad para sobrevivir y reproducirse muchas veces (Kokko y

Lindström 1996) o bien porque los animales que sobreviven a edades avanzadas tienen menores

expectativas reproductivas a futuro y por tanto invierten más en sus señales sexuales, haciéndolas más

confiables (Proulx et al. 2002). Sin embargo, estos modelos no consideraron el deterioro somático que

ocurre a edades avanzadas y que podría afectar negativamente la expresión de señales sexuales. Por

ejemplo, si la capacidad antioxidante dismnuye con la edad (Halliwell y Gutteridge 2007, ver más

abajo) entonces la capacidad para mantener ornamentos dependientes de antioxidantes carotenoides

podría disminuir a edades avanzadas. Los estudios teóricos sobre preferencias de apareamiento en

relación a la edad que consideran además a la senescencia proponen que los animales deberían evitar

aparearse con individuos viejos cuando la calidad individual disminuye con la edad (Beck et al. 2002,

Beck y Promislow 2007). Por lo tanto, la senescencia podría ser una fuerza selectiva importante que

afecte los patrones de cortejo y estrategias reproductivas de los animales.

Durante el cortejo los animales utilizan características sexuales secundarias y despliegues

conductuales para elegir y evaluar a las parejas potenciales. Tales despliegues podrían haber

evolucionado para señalizar la calidad individual (atractivo sexual, inmunocompetencia, buenos genes

para la viabilidad, capacidad física y de cuidado parental) y consecuentemente los animales podrían

ajustar su inversión reproductiva de acuerdo al atractivo de la pareja (Sheldon 2000, Schneider y

Lesmono 2009). Debido a que la calidad individual, el desempeño reproductivo (Haussman et al.

2005, Catry 2006, Palacios et al. 2007, Jones et al. 2008, Torres y Velando 2007, Lecomte et al. 2010),

y la calidad en la línea germinal (Velando et al. 2010a) disminuyen con la edad, las parejas de animales

viejos podrían experimentar un bajo éxito reproductivo. Por lo tanto, se esperaría que evolucionen

mecanismos para detectar y discriminar negativamente a los animales viejos durante la elección de

pareja (Beck et al. 2002, Beck y Promislow 2007) o que la selección favorezca estrategias alternativas

que amortigüen la baja en el éxito reproductivo en las parejas de animales viejos. Por ejemplo, los

animales viejos podrían recibir menores tasas de cortejo y conseguir menos cópulas extra-pareja,

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mientras que las parejas de animales viejos podrían mostrar mayores tasas de cortejo y cópulas extra-pareja que podrían resultar en éxito reproductivo fuera del nido o en cambio de pareja.

Además de la edad de la pareja, la edad propia podría afectar la intensidad y la calidad de los ornamentos y las conductas de cortejo (Kokko 1997). El despliegue de conductas de cortejo puede verse afectado negativamente por la pérdida de la condición con la edad, por lo que se esperaría una disminución en el desempeño durante el cortejo (i.e., duración o frecuencia de conductas de cortejo) en organismos senescentes. Sin embargo, en especies que despliegan conductas y ornamentos durante el cortejo para los animales viejos el costo de los despliegues conductuales podría ser menor que el costo de mantener ornamentos sexuales atractivos. Los ornamentos dependen en muchas especies de pigmentos carotenoides que influyen en la capacidad antioxidante del individuo (Hill y McGraw 2006), sin embargo, la capacidad antioxidante se ha visto que disminuye con la edad (Beckman y Ames 1998, Alonso-Alvarez et al. 2006, Devevey et al. 2010). Consecuentemente para un animal viejo el costo de asignar antioxidantes a las señales sexuales, en términos de deterioro somático, sería mayor que para los animales más jóvenes y debería producir ornamentos menos coloridos (Torres y Velando 2007). Por lo tanto, sería factible que en los animales viejos aumente la intensidad de las conductas de cortejo para compensar una baja en el atractivo de los ornamentos. De tal manera que tanto aumento como disminución en las conductas de cortejo en los animales viejos es posible.

A pesar de que los teóricos se han interesado por investigar la variación de las preferencias sexuales con la edad (Kokko y Lindström 1996, Beck et al. 2002, Proulx et al. 2002, Beck y Promislow 2007), existe poca evidencia empírica del efecto de la senescencia sobre las características sexuales, los despliegues conductuales, las preferencias de pareja y las estrategias extra-pareja que podrían adoptar las parejas de los individuos viejos (Ritchie et al. 1995, Hoikkala et al. 2007, Velando et al. 2010b). En las moscas de la fruta *Drosophila montana* y los grillos de campo *Ephippiger ephippiger* se ha registrado una disminución en la frecuencia y longitud de las sílabas del canto de cortejo al aumentar la

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edad (Ritchie et al. 1995, Hoikkala et al. 2007), y además, las hembras del grillo discriminan negativamente a machos con cantos de mala calidad (Ritchie et al. 1995). En el bobo de patas azules se ha observado una disminución con la edad en la intensidad del color de las patas, un rasgo dependiente de carotenoides y sujeto a selección sexual (Torres y Velando 2007, Velando et al. 2010b). Las hembras de los bobos apareadas con machos de color de patas opaco, cortejan y copulan menos, además de poner huevos más pequeños que las hembras apareadas con machos con patas más brillantes (Torres y Velando 2003, Velando et al. 2006b). Estos estudios sugieren que las hembras podrían mediante señales sexuales reconocer y discriminar negativamente a los machos viejos.

Finalmente, las estrategias reproductivas podrían variar para cada organismo dependiendo de su edad pero también de la edad de su pareja. A pesar de que se ha reportado apareamiento concordante por la edad (*i.e.* viejos con viejos, Black y Owen 1995), no en todas las poblaciones se presenta este fenómeno y el éxito del evento reproductivo para cada individuo podría verse afectado por su edad y la de su pareja. Por lo tanto, es de esperarse que los organismos maximicen su éxito reproductivo ajustando su inversión en ornamentos sexuales y despliegues de cortejo en función de su edad y de la edad de su pareja. Sin embargo, no se tiene información sobre el efecto de la edad del individuo y de su pareja en lo patrones de cortejo y estrategias de apareamiento en especies de larga vida. En este estudio evaluamos si los individuos viejos modifican su estrategia de cortejo y si las parejas de animales viejos disminuyen el cortejo intra-pareja y realizan más conductas extra-pareja.

Efecto de la senescencia sobre la calidad de los huevos y el cuidado parental
El pobre desempeño reproductivo a edades avanzadas puede deberse a una disminución en la provisión de recursos a los huevos/placenta (efectos prenatales), a un menor cuidado de los críos después del nacimiento (efectos postnatales) o a ambos procesos (Sydeman y Emslie 1992, Saino et al. 2002). El tamaño y supervivencia de la progenie puede verse afectado por la transmisión diferencial por parte de

las hembras viejas de nutrientes y otros elementos claves para el desarrollo de los embriones (e.g. hormonas, antioxidantes, etc. Mosseau y Fox 1998, Blount et al. 2000), o por la menor calidad del genoma de la línea germinal de machos y hembras viejos (Tarin et al. 2000). Si bien en algunas especies se ha reportado que hembras viejas ponen huevos más pequeños (Davis 1975, Sydeman y Emslie 1992), poco se sabe del efecto de la calidad del huevo dependiente de la edad de la madre sobre el desempeño de la progenie en especies silvestres (Bogdanova et al. 2006). Además, la supervivencia de la progenie de hembras viejas puede verse afectada negativamente por una disminución en la capacidad de incubar los huevos, forrajear y alimentar a las crías (Catry et al. 2006, Saino et al. 2002).

En aves se ha observado que el tamaño del huevo influye positivamente en el éxito de eclosión y desarrollo temprano de los críos, sin embargo, típicamente los efectos negativos de un huevo pequeño se pierden pronto después de la eclosión (revisado por Williams 1994, Christians 2002), lo que implica una atenuación del efecto de la calidad del huevo durante la crianza. Sin embargo, el deterioro somático de las madres a edades avanzadas puede llevar a una covariación positiva entre los efectos prenatales y postnatales (Lock et al. 2007), dando lugar a una disminución tanto en la calidad de los huevos que ponen como en la calidad del cuidado parental que proveen, lo que resultaría en la producción de progenie de mala calidad con pocas probabilidades de sobrevivir. En especies con cuidado parental prolongado esta covariación podría no ocurrir si en estos animales han evolucionado mecanismos para ajustar su nivel de cuidado prenatal o postnatal en función de las condiciones ambientales o cambios en la condición corporal (Räsänen et al. 2003, Lock et al. 2007). Ajustes en el nivel de cuidado postnatal podrían amortiguar o incluso revertir los efectos de un decline en la calidad de los huevos en hembras viejas (Lock et al. 2007). En este trabajo separamos la contribución relativa de los efectos de la calidad del huevo y de la capacidad de crianza hacia la disminución en el desempeño reproductivo observado a edades avanzadas. Para ello realizamos un experimento de intercambio de puestas entre hembras de diferente edad y evaluamos si el crecimiento y la

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supervivencia de la progenie está relacionado con la edad de la madre que puso el huevo (efectos prenatales) o con la edad de la madre que cuidó a los pollos (efectos postnatales).

Senescencia reproductiva y carotenoides

Los estudios teóricos que explican los patrones de desempeño reproductivo a lo largo de la vida proponen que la disminución en el éxito reproductivo a edades avanzadas puede ser el resultado de limitaciones o de restricciones (Cichoń 2001, McNamara et al. 2009). Los organismos senescentes podrían estar limitados en sus capacidades a causa del deterioro fisiológico y esto resultaría en menor éxito reproductivo a pesar de aumentar la inversión reproductiva (Cichon 2001). Por otro lado, la hipótesis de restricción reproductiva a edades avanzadas propone que los organismos viejos, al ser más susceptibles al daño somático, deberían restringir su inversión en la reproducción para disminuir el deterioro al organismo y tener mas oportunidades reproductivas pues el nivel de deterioro determina la mortalidad y expectativa reproductiva futura (McNamara et al. 2009). Si los animales restringen su inversión reproductiva, se esperaría que el suplemento de nutrientes con capacidad para mitigar el deterioro somático tenga un efecto positivo en el desempeño reproductivo de los animales viejos.

Algunos estudios han mostrado que el estrés oxidativo puede ser un mecanismo involucrado en el compromiso entre mantenimiento y reproducción (Salmon et al. 2001, Wang et al. 2001, Alonso-Álvarez et al. 2004, Wiersma et al. 2004), y se ha sugerido que puede ser uno de los principales compromisos fisiológicos entre diferentes componentes de historia de vida (Monaghan et al. 2009). El estrés oxidativo es el desbalance entre el daño oxidativo y la capacidad antioxidante de un organismo; mientras mayor es el desbalance en favor del daño mayor es el nivel de estrés oxidativo (Halliwell y Gutteridge 2007). Niveles bajos de estrés oxidativo son necesarios para el funcionamiento celular cotidiano, sin embargo, niveles altos de estrés son negativos para los organismos dada su relación con

el deterioro somático (Martin y Grotewiel 2006). En la mosca y el diamante mandarín *Taenopygia guttata* se encontró que un esfuerzo reproductivo elevado está relacionado con una menor capacidad antioxidante (Salmon et al. 2001, Wang et al. 2001, Alonso-Alvarez et al. 2004, Wiersma et al. 2004). Estudios en ratas, gerbos y moscas han demostrado que la generación de radicales libres aumenta mientras que la capacidad antioxidante disminuye con la edad (Beckman y Ames 1998). La capacidad antioxidante en plasma disminuye con la edad en el flamingo *Phoenicopterus ruber roseus* (Devevey et al. 2010) y el diamante mandarín (Alonso-Alvarez et al. 2006), mientras que en el patirrojo *Alectoris rufa* tanto el daño oxidativo como la capacidad antioxidante aumentan con la edad (Alonso-Alvarez et al. 2010). Estos resultados sugieren que el esfuerzo reproductivo incrementa el estrés oxidativo y que los individuos más viejos son más susceptibles al estrés oxidativo. Por lo tanto, dado un esfuerzo reproductivo similar, el costo oxidativo generado por la reproducción (Alonso-Alvarez et al. 2004) y el deterioro somático podría aumentar con la edad. En este contexto, la capacidad para adquirir y asimilar antioxidantes a través de la dieta podría ser un factor clave en la capacidad de los organismos viejos para asignar recursos a la reproducción.

El sistema inmune es un rasgo importante del mantenimiento somático y dado el riesgo constante de ser atacado por patógenos no puede ser comprometido (Lozano y Lank 2003). Se ha reportado que la capacidad inmune disminuye con la edad en aves y mamíferos (Haussman et al. 2005, Lavoie 2006, Martin y Grotewiell 2006, Marzal et al. 2007) y que la reproducción impacta negativamente en la capacidad inmune (Deerenberg et al. 1997, Hansen et al. 2005). Por lo tanto, se esperaría que comparado con animales más jóvenes, la inversión en la reproducción tuviera un impacto mayor en la capacidad inmune de los animales viejos, lo cual podría comprometer severamente la probabilidad de supervivencia y reproducción futura. Además de cumplir una función importante como moduladores del estrés oxidativo, algunos antioxidantes tienen funciones inmunoestimulantes, por lo que se esperaría que la disponibilidad de antioxidantes sea un factor importante en la capacidad de los

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organismos para mitigar los costos en términos de la disminución en la capacidad de respuesta inmune resultante de la reproducción (Blount et al. 2003, Aguilera y Amat 2007).

Los carotenoides son moléculas que tienen efectos positivos en la elaboración de señales sexuales, en el desempeño reproductivo, en la masa corporal, en la capacidad inmune y en la neutralización de radicales libres (Blount et al. 2004, Biard et al. 2005, Alonso-Álvarez et al. 2004, Hôrak et al. 2007, Pike et al. 2007, Safran et al. 2010; pero ver Remeš et al. 2007, Constantini y Møller 2008). En el diamante mandarín, el suplemento de carotenoides modula la relación entre el número de huevos que pone una hembra y su capacidad antioxidante en plasma: hembras sin suplemento de carotenoides que pusieron más huevos mostraron una disminución en las defensas antioxidantes mientras que está relación negativa no se encontró en hembras suplementadas con carotenoides (Bertrand et al. 2006). En el pinzón verde *Carduelis chloris* el suplemento de carotenoides tuvo un efecto positivo en la producción de anticuerpos frente a un reto inmune (Aguilera y Amat 2007). En el pez espinoso *Gasterosteus aculeatus* el suplemento de carotenoides aumentó la capacidad de cuidar los huevos durante la incubación y esto resultó en un mayor éxito de eclosión con respecto al grupo control, además en esta misma especie se ha reportado que el suplemento de carotenoides amortigua la pérdida con la edad de la capacidad de nado durante la reproducción (Pike et al. 2010). Debido al efecto positivo de los carotenoides en la capacidad antioxidante y desempeño físico durante la reproducción se esperaría que el suplemento de carotenoides disminuya el deterioro somático en términos de estrés oxidativo y aumente el desempeño reproductivo de los animales viejos, que son particularmente susceptibles al estrés oxidativo y deterioro físico. Sin embargo, no existe evidencia de cómo afectan los carotenoides la capacidad antioxidante, el daño oxidativo, y el desempeño reproductivo de animales silvestres senescentes. En este trabajo evaluamos la hipótesis de que los carotenoides limitan la inversión reproductiva de los machos viejos. Se evaluó el efecto de suplementar carotenoides en la condición (peso, color de patas, capacidad antioxidante, daño oxidativo)

y desempeño reproductivo (crecimiento, respuesta inmune y supervivencia de las crías) de machos con distintas edades.

El bobo de pata azules S. nebouxii como modelo de estudio de la senescencia reproductiva

El bobo de patas azules es un ave marina de larga vida que anida en islas del Océano Pacífico (Nelson 1978). Tiene un sistema de apareamiento socialmente monógamo y presenta cuidado biparental durante la incubación (41 d) y crianza (~ 4 meses; Nelson 1978, Torres y Drummond 1999). Establece puestas de 1-3 huevos y la puesta modal es de dos (Drummond et al. 1986). Entre los pollos se establece una jerarquía de dominancia-subordinación por el acceso al alimento, en la que usualmente la cría mayor vence a la cría menor mediante el uso de gritos, picotazos y mordeduras sin la intervención de los adultos. En casos extremos de escasez de alimento la cría dominante acapara los recursos y la cría subordinada muere de inanición (Drummond et al. 1986).

Durante el cortejo, machos y hembras caminan juntos, exponen repetidamente sus patas, colocan ramitas sobre las patas de la pareja y exhiben otras características sexuales como vocalizaciones y posiciones ritualizadas antes y durante la puesta (Nelson 1978, Torres y Velando 2003, 2005). Estas conductas son realizadas por machos y hembras dentro de cada pareja (conducta intra-pareja) pero también son desplegadas hacia machos y hembras fuera de la pareja (conducta extra-pareja; Osorio-Bersitain and Drummond 1998, Pérez-Staples y Drummond 2005). El color de las patas es una señal sexualmente seleccionada en esta especie pues machos y hembras ajustan su cortejo en función de la intensidad del color de patas de su pareja: animales con color de patas ópaco (color observado en animales con baja condición nutricional e inmune (Velando et al. 2006b), reciben menores tasas de cortejo intra-pareja en comparación con animales de color de patas brillante (Torres y Velando 2003, 2005). Finalmente, se ha registrado que el color de las patas de los machos disminuye en intensidad con el esfuerzo reproductivo y la edad (Velando et al. 2010b).

Se han encontrado efectos positivos de los carotenoides en la reproducción del bobo. El

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suplemento de carotenoides a machos durante el cortejo tuvo un efecto positivo en el color de las patas (Velando et al. 2006b). Además, hembras suplementadas con carotenoides durante la etapa de puesta mostraron un color de patas más brillantes y pusieron huevos más grandes en comparación con hembras sin suplemento de carotenoides (Morales et al. 2009). Estos resultados sugieren que los carotenoides son limitantes en la reproducción del bobo de patas azules.

Por otra parte, un estudio longitudinal de tres cohortes en los bobos de patas azules mostró que en esta especie hay senescencia reproductiva en los machos: el éxito reproductivo aumenta con la edad hasta un máximo aproximadamente a los diez años y posteriormente declina a edades avanzadas (Velando et al. 2006a). En otro estudio donde se activó experimentalmente el sistema inmune de machos mediante la inoculación intraperitoneal de un lipopolisacárido de la membrana de *Escherichia coli* se observó que los animales mayores a diez años presentaban mayores niveles de daño oxidativo y sufrían una mayor disminución en la intensidad del color de sus patas en comparación con machos menores a diez años que recibieron la vacuna (Torres and Velando 2007). Esta línea de evidencia indica que en el bobo de patas azules los machos de edades avanzadas experimentan senescencia reproductiva y deterioro fisiológico y hace a esta ave un modelo muy interesante para estudiar en más detalle este fenómeno.

El Laboratorio de Conducta Animal del Instituto de Ecología, UNAM mantiene un estudio a largo plazo sobre la colonia reproductiva del bobo en Isla Isabel, Nayarit desde 1982. Cada año se monitorean todos los nidos que se reproducen en el área de trabajo (detalles en Drummond et al. 2003). Se registra la identidad del macho y la hembra, la fecha de puesta, el número de huevos que conforman la puesta, el número de huevos que eclosionan y el número de crías que empluman, además de otros parámetros (Drummond et al. 2003). Los nidos se monitorean cada 3 días hasta que los pollos alcanzan la edad de 30 días y cada 6 días hasta el día 70, edad cercana al emplumado. Los pollos son marcados individualmente con anillos de plástico al eclosionar y con anillos de metal al día 70. El bobo es una

especia altamente fiel a su sitio de anidamiento (Osorio-Beristain y Drummond 1993, Kim et al. 2007),

la dispersión natal es, en promedio, 24.1 m para machos y 28.3 m para hembras (Osorio-Beristain y

Drummond 1993) y la dispersión entre eventos reproductivos es 13.1 m para machos y 15.6 m para

hembras (Kim et al. 2007). Como resultado de este monitorio anual y la alta fidelidad al sitio de

reproducción se puede seguir a individuos marcados y registrar sus historias reproductivas a lo largo de

la vida de aquellos animales que se reproducen dentro del área de trabajo.

Objetivos

El objetivo general de la tesis fue estudiar la senescencia reproductiva en una población silvestre del

bobo de patas azules utilizando animales marcados individualmente e información del estudio a largo

plazo de la colonia de bobos de Isla Isabel, Nayarit. Para determinar si existe senescencia reproductiva

en hembras se estudió el éxito reproductivo a lo largo de la vida en tres cohortes de hembras que

sobrevivieron al menos 13 años. Además, se investigó en detalle si el bajo éxito reproductivo de los

individuos viejos se debe a una disminución en su desempeño durante el cortejo, a una baja asignación

de recursos a los huevos, a una reducida capacidad para criar a la progenie, o a una disminución en

todos estos componentes de la reproducción. Finalmente se evaluó el efecto de suplementar

carotenoides sobre el peso, capacidad antioxidante, daño oxidativo y desempeño reproductivo en

machos de distintas edades. En la primer parte de la tesis se investigó de manera experimental al

respecto de los mecanismos de la senescencia reproductiva (Capítulo 2 y 3) mientras que en la segunda

se investigó de manera descriptiva si la senescencia tiene efecto sobre la conducta de cortejo de los

animales (Capítulo 4).

Capítulo 2

Senescencia en la calidad del huevo y capacidad de crianza.

Senescence of maternal effects: aging influences egg quality and rearing capacities of a long-lived

bird.

En primer lugar se evaluó si hay senescencia reproductiva en hembras utilizando información de la

base de datos de Isla Isabel. Se analizó el desempeño reproductivo a lo largo de la vida de hembras que

sobrevivieron al menos 13 años en la colonia. En segundo lugar, se investigó la importancia relativa

de la inversión en la puesta (efectos pre-natales) y el cuidado de la progenie (efectos post-natales) en el

declive reproductivo a edades avanzadas mediante un experimento de intercambio de puestas entre

hembras de distintas edades. Evaluamos si el crecimiento y la supervivencia de la progenie está

relacionado con la edad de la madre que puso el huevo o con la edad de la madre que cuidó a los

pollos. Este estudio permitió además evaluar si las hembras son capaces de ajustar su esfuerzo

reproductivo en relación a la calidad de la progenie que cuidan. Los resultados de este capítulo fueron

aceptados para su publicación en la revista The American Naturalist en 2009.

Capítulo 3

Efecto de los carotenoides en la condición corporal y desempeño reproductivo de machos de distinta

edad

Positive age-dependent effect of carotenoids on male reproductive performance of a long-lived seabird.

Para investigar si los antioxidantes carotenoides son limitantes en la reproducción de machos viejos se

realizó un experimento de suplemento dietético de carotenoides a machos de diferente edad. Se evaluó

el efecto de suplementar carotenoides en la condición (peso, color de patas, capacidad antioxidante,

daño oxidativo) y desempeño reproductivo (crecimiento, respuesta inmune y supervivencia de las crías)

de machos con distintas edades.. Este experimento permitió evaluar si la capacidad reproductiva de los

machos viejos cambia al disminuir el riesgo de deterioro somático. Los resultados de este capítulo se han formateado para someter a revisión en una revista indizada de alto impacto de ecología funcional.

Capítulo 4

Efecto de la edad en la conducta de cortejo

Love at older ages: courting behaviour in old blue-footed boobies

Para evaluar si la senescencia afecta el desempeño de machos y hembras durante la fase de cortejo se realizó un estudio descriptivo de la conducta reproductiva en parejas de edad conocida. Se registró la frecuencia de los principales despliegues conductuales dirigidos hacia la pareja y a otros individuos así como el tiempo que pasaron los animales en la arena de cortejo. Evaluamos si la edad individual afecta las tasas de cortejo y cópulas intra-pareja así como la presencia en el territorio reproductivo. Además evaluamos la hipótesis de que los animales apareados con individuos viejos disminuyen el cortejo intra-pareja y realizan más conductas extra-pareja. Los resultados de este capítulo serán envíados a revisión en una revista indizada de alto impacto de conducta animal u ornitología.

Cuadro 1. *Descripción del Parque Nacional Isla Isabel (2005).*

Isla Isabel (25" 52' N, 105" 54' W) se encuentra ubicada a 30 km al NO de la costa de Nayarit. Con una

superficie de 82.1 ha fue declarada Parque Nacional en 1980 y actualmente la conservación y manejo

se encuentra a cargo de la Comisión Nacional de Áreas Naturales Protegidas (SEMARNAT). Es una

Isla de origen volcánico que data de hace unos 3.5 millones de años, clasificada como Sistema de

Lomerio en donde si bien la altura máxima registrada es de 85 metros, el 70% de las alturas registradas

se encuentran por debajo de los 40 metros. En general, la isla presenta rocas volcánicas lávicas y

piroclásticas interestratificadas y suelos derivados de ellas.   Presenta un clima tropical subhúmedo con

lluvias en verano. La temperatura máxima es septiembre con 30.3 ºC y la mínima es enero con 22.6ºC.

No existe registro de precipitación. Aproximadamente el 73% de la isla está cubierto por bosque

tropical caducifolio, en donde el roache *Crataeva tapia* y el papelillo *Euphorbia schelchtandlii*

representan el 87% y por pastizal dominado por *Cyperus lingularis*, *Jouvea Pilosa* y *Cenchrus viridis*.

Se han registrado 100 especies de vertebrados, de las cuales 92 son de aves, y 9 de estas aves

establecen colonias de anidación, entre las que se encuentra el bobo de patas azules *S. nebouxii* con una

población estimada de tres mil individuos.

Fuente: Programa de Conservación y Manejo del Parque Nacional Isla Isabel, México. Comisión Nacional de Áreas

Naturales Protegidas, 2005 y Contreras Koob, C. 2010. Isla Isabel, santuario de aves (Textos H. Drummond y R. Torres).

Fig. 1. Parque Nacional Isla Isabel, Nayarit.

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Capítulo 2

Senescencia en la calidad del huevo y capacidad de crianza.

Senescence of maternal effects: aging influences egg quality and rearing capacities of a long-lived bird

En este capítulo se analizaron los cambios en el desempeño reproductivo a lo largo de la vida en tres cohortes de hembras que sobrevivieron al menos 14 años en la colonia. Para ello se utilizó la información de la base de datos del proyecto a largo plazo en la colonia de bobo de patas azules de Isla Isabel. Se encontró que el desempeño reproductivo aumenta con la edad hasta aproximadamente los ocho años de edad y posteriormente hay un declive significativo en el número de emplumados producidos. Interesantemente, la tasa de declive es diferente para las cohortes analizadas. Posteriormente, para separar la contribución relativa de los efectos de la calidad del huevo y la capacidad de crianza hacia la disminución en el desempeño reproductivo de las hembras viejas realizamos un experimento de intercambio de puestas entre hembras de diferentes edades. Este diseño nos permitió evaluar si el crecimiento, respuesta inmune y supervivencia de la progenie está relacionado con la edad de la hembra que puso el huevo (efectos prenatales) o con la edad de la hembra que lo cuidó (efectos postnatales).   Encontramos que las hembras viejas pusieron primeros y segundos huevos más pequeños que dieron lugar a crías más pequeñas al día 5. El efecto negativo de la edad de la hembra que puso los huevos continuó en las segundas crias hasta el día 30 con tasas de crecimiento mas bajas. Además, el crecimiento esquelético al día 30 y la respuesta inmune al día 20 de las segundas crías disminuyó con la edad de la hembra que las crió. Sin embargo, el resultado más interesante fue que el éxito reproductivo de las hembras viejas dependió de la edad de la hembra que puso los huevos: hembras viejas que cuidaron huevos de hembras viejas tuvieron un éxito reproductivo bajo en comparación con hembras viejas que cuidaron huevos de hembras maduras. Los resultados sugieren que en el bobo de patas azules las condiciones experimentadas al principio de la vida, ya sea durante el crecimiento en el nido o en los primeros años de vida, tienen un efecto importante en la tasa de senescencia reproductiva. Además, los resultados del experimento sugieren que la senescencia en hembras afecta negativamente la capacidad para producir huevos y criar a los pollos. Finalmente los resultados sugieren que las hembras viejas son capaces de ajustar su nivel de cuidado post-natal en función de cambios en el valor reproductivo de la progenie que cuidan. Los resultados de este estudio están plasmados en el manuscrito que a continuación se presenta y que fue aceptado para su publicación en la revista The

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Senescence of Maternal Effects: Aging Influences Egg Quality and Rearing Capacities of a Long-Lived Bird

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ABSTRACT: Senescence could depress prenatal and postnatal capacities of mothers to invest in offspring. Longitudinal observations on the blue-footed booby (*Sula nebouxii*) revealed a quadratic effect of female age on fledgling production and cohort differences in rate of reproductive decline. By swapping clutches between females of different ages, we tested whether reproductive senescence is due to decline in egg quality or capacity to care. As laying mothers aged, egg size, ulna length of 5-day-old chicks, and ulna growth of second chicks up to age 30 days declined, and as rearing mothers aged, ulna growth and cellular mediated immune response of second chicks diminished. Oddly, senescent females (>11 years) produced more fledglings when rearing offspring of middle-aged females (8–11 years) than when rearing offspring of senescent or young females. Thus, senescence reduced egg quality and rearing capacities, and reproductive success of senescent mothers depended on prenatal effects associated with the age of the laying mother. Reproductive senescence of boobies may involve constraints on resources allocated to reproduction as well as adaptive adjustment of provision and care according to offspring value, implying that negative effects of senescence on offspring survival can be ameliorated by plasticity in postlaying or postnatal care.

Keywords: senescence, *Sula nebouxii*, maternal effects, age-dependent maternal investment.

Introduction

In iteroparous animals, senescence, the progressive loss of function with advancing age, is now recognized as a life-history trait affecting fitness components in age-structured populations (Kirkwood and Austad 2000). Traditionally, reproductive senescence in wild animals had been considered to be absent or hard to observe because extrinsic mortality eliminates individuals long before they show detectable senescence (Williams 1957, 1992). However, more and more long-term studies of wild species document an increase in reproductive performance until a maximum followed by a decline (in ungulates, McElligot et al. 2002; Yoccoz et al. 2002; Nussey et al. 2006; in birds, Newton and Rothery 1997; Bennett and Owens 2002; Rattiste 2004; Velando et al. 2006; Reed et al. 2008). Here, we characterize reproductive senescence in a natural population of blue-footed boobies.

In species with extended parental care, reproductive senescence can be mediated through two forms of maternal effects: via the provisioning of eggs or the quality of offspring at birth or via the care provided to the young after birth. Phenotypic variation in offspring due to maternal phenotype rather than offspring genotype is known as a maternal effect (Mousseau and Fox 1998; Roff 1998). Mothers may influence offspring size and survival by transmitting different amounts of substances to their embryos and also after birth through variation in postnatal care (Mousseau and Fox 1998). Moreover, the quality and survival of chicks might also be influenced by deteriorating quality of both male and female germ-line DNA at older ages (Tarin et al. 2000; Velando et al. 2008). Female age can influence both prenatal and postnatal maternal effects (Bogdanova et al. 2006). For example, in species with prolonged parental care, females of different ages may adjust their care behavior (Cameron et al. 2000; Clark et al. 2002), and this plasticity can potentially ameliorate or even reverse the effects of the decline in prenatal maternal effects associated with increasing mother age (Lock et al. 2007).

In female birds, effects of age on egg production (size and composition), incubation, and chick rearing can result in differences in reproductive success (Bogdanova et al. 2006). Compared to mature mothers, young mothers lay their eggs later in the breeding season, have smaller clutches, raise smaller chicks, and have overall lower reproductive performance (Clutton-Brock 1988; Perdeck and Cavé 1992; Martin 1995; Daunt et al. 1999; Hegyi et al. 2006; Bogdanova et al. 2007). In contrast, few studies have analyzed factors possibly associated with age-related reproductive decline at older ages (Saino et al. 2002; Catry

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et al. 2006; Heidinger et al. 2006) or investigated whether decline (when present) is due to prenatal or postnatal maternal effects or both (Bouwhuis et al. 2009).

Egg production is costly in time, energy, and nutrients (Monaghan and Nager 1997), and female birds may influence the fitness of their offspring through the amounts of substances they transmit to their eggs (Mousseau and Fox 1998). For instance, yolk carotenoids have beneficial effects during the development of avian embryos by protecting tissues from reactive oxygen metabolites (Blount et al. 2000), and yolk androgens influence chick development by decreasing or promoting pre- and postnatal growth and competitive ability (Sockman and Schwabl 2000; Groothuis et al. 2005). However, although in some species females at older ages lay smaller eggs (Davis 1975; Sydeman and Emslie 1992; but see Robertson et al. 1994), nothing is known about senescence of maternal effects via eggs on chick performance in wild species.

Senescence may decrease not only female capacity for egg production but also capacity to care for eggs and chicks. In birds, egg size is positively related to hatching success and early chick development, but typically, negative effects of small egg size are lost within days of hatching (reviewed in Williams 1994; Christians 2002), implying amelioration during the rearing period. However, coadaptation between prenatal and postnatal maternal effects associated with age (Lock et al. 2007) could drive senescence to involve decline in both egg quality and parental quality (Bogdanova et al. 2006), resulting in progeny of overall lower quality. Reduced performance of senescent females (e.g., lower foraging skills [Catry et al. 2006], immunosenescence [Palacios et al. 2007]) may result in production of low-quality eggs (smaller eggs or eggs with lower nutrient levels), poor incubation capacity, and leaner provisioning of chicks, with these effects combining to prejudice offspring phenotype. Age-dependent covariation between pre- and postnatal maternal effects could disappear if mothers maximize their fitness by facultatively modifying investment in their eggs, in postlaying care, or in postnatal care in response to environmental variation or changes in body condition (Räsänen et al. 2003; Lock et al. 2007). Understanding the mechanisms of reproductive senescence requires assessing the relative contributions of egg effects and rearing effects to age-related decline in reproductive performance. The contributions of egg quality and rearing capacity to reproductive senescence can be teased apart by cross-fostering clutches between different-aged females (Bize et al. 2002; Van de Pol et al. 2006).

The blue-footed booby (*Sula nebouxii*) is an iteroparous, long-lived tropical seabird with a long period of biparental care (up to 6 months; Drummond et al. 2003). The modal clutch is two eggs, and there is facultative brood reduction when food is scarce (Nelson 1978; Drummond et al. 1986).

Egg mass is positively correlated with hatching probability and chick mass at hatching (D'Alba and Torres 2007). After age 10 years, males experience reproductive senescence (Velando et al. 2006) and increased susceptibility to oxidative damage caused by immune system activation (Torres and Velando 2007). In the present study, we first analyze long-term data to determine how reproductive success of individual females surviving at least 14 years changes over the life span and whether this pattern is consistent among cohorts. Second, we cross-foster clutches between different-aged females to test whether reproductive senescence is due to age-related variation in egg quality, parental care, or both.

Methods

Study System

Field data were from a long-term study of the breeding colony of blue-footed boobies on Isla Isabel, in the Mexican Pacific (25°52′N, 105°54′W; described in Drummond et al. 2003; Velando et al. 2006). In every reproductive season (February to July) from 1988 to 2005, all breeding attempts in the study area were monitored until the end of the fledgling period (mean number of nests per year = 969 ± 281; Drummond et al. 2003). Nests were marked and inspected every 3 days, until most chicks in the colony had reached an age of 30 days, and then every 6 days, until all had fledged (reached 70 days) or died. At every nest, observers recorded parental identities, laying date, hatching date, and survival of eggs and chicks. Chicks were individually marked with plastic rings at hatching and with numbered metal rings at fledging. These boobies show lifelong fidelity to their natal neighborhoods (Osorio-Beristain and Drummond 1993; Kim et al. 2007), enabling us to analyze reproductive histories of known-age recruits over their lifetimes.

Reproductive Success over Female Life Span

We described changes in individual female reproductive performance over the lifetime by analyzing longitudinal data from female fledglings banded in 1988, 1989, and 1991 and comparing these three cohorts. We included only female boobies that survived at least 14 years and bred repeatedly in the colony, using data up to 2005. Because apparent changes in reproduction with age can be due to differential survival of individuals with different capabilities, the use of longitudinal data of long-lived females is appropriate to test for within-individual reproductive senescence (McCleery et al. 2008). The 1990 and 1992 cohorts were excluded because in those years chicks were

not banded or failed to fledge (because of an El Niño event), respectively.

The annual reproductive success (number of fledglings) of focal females in each breeding season between 1988 and 2005 was calculated as the z-normal standardized residual of the mean number of fledglings produced by all breeding pairs in the study area in the same season. Age-dependent variation in individual reproductive success was analyzed using a mixed model (PROC MIXED in SAS; SAS Institute 1999), with individual birds nested within cohorts as repeated-measures factors with missing values. We controlled for heterogeneous cohort variances, and we used the Satterthwaite approximation for the denominator degrees of freedom (Littell et al. 1996). Statistical significance of the repeated-measures factors was assessed using changes in the likelihood ratio (-2 log likelihood) of the model with and without the repeated measures. This difference is distributed as χ^2 (Littell et al. 1996). To assess whether the decline in reproductive success at older ages was significant, we also modeled data with a general additive model in R ("gam" procedure; R Development Core Team 2008) to determine age at onset of senescence (i.e., the point of maximum reproductive success) and then adjusted independent mixed models (as above) to data from the beginning of reproduction to age 8 years and from the onset of senescence onward (Jones et al. 2008).

Cross-Fostering Experiment between Females of Different Ages

During January–March 2006, we located recently laid clutches of known-age females by inspecting the study area every 2 days. Banded male partners were of known ages; unbanded male partners were deemed to be 4-year-old recruits (male recruiting ages of 4.7 and 4.3 years were reported by Osorio-Beristain and Drummond [1993] and Drummond et al. [2003], respectively). Clutches laid by females of different ages but with similar laying dates were paired (average laying date difference 3.08 ± 0.94 days) and swapped after completion of both clutches. This design controls for differences in laying dates and reproductive stages among experimental females. We classified females as young (4–7 years; $n = 15$), middle-aged (8–11 years; $n = 20$), or senescent (12–17 years; $n = 11$) and swapped clutches between and within these age classes ($n = 46$ clutches). We included 39 two-egg clutches and seven three-egg clutches. All third eggs were removed within 24 h of laying, so that all pairs incubated two eggs. We measured egg width and length with a caliper to the nearest 0.1 mm to calculate egg volume (Hoyt 1979).

To register survival, nests were visited every 10 days during incubation, daily as hatching approached (incubation period is 41 days; Nelson 1978), and every 6 days after hatching, until chicks reached age 70 days (close to fledging; Drummond et al. 2003). To evaluate growth of chicks, we measured (± 1 mm) the length of the ulna (the bone parallel to the radius in the forearm), by measuring the two end points of this bone, and body mass at ages 5 and 30 days (Pesola spring balance accuracy: day 5, ± 5 g; day 30, ± 20 g); this period includes the maximum growth rate of chicks (Torres and Drummond 1999a). For the analyses, we calculated a daily growth rate of chicks as ulna length or body mass at age 30 days minus ulna length or body mass, respectively, at age 5 days divided by 25 days. The immune response (T lymphocyte response) of chicks was estimated using the phytohemagglutinin skin test (PHA, Sigma-Aldrich; Smits et al. 1999). At age 20 days, we applied a subcutaneous injection in the right wing web of 0.2 mg of PHA in 0.1 mL of a solution of phosphate-buffered saline. Immune response to PHA was estimated as the increase in thickness of the wing web 24 h later. The point of injection was marked with an indelible marker, and wing-web thickness was measured (three times, to yield an average) before injection and 24 h later with a Mitutoyo micrometer (± 0.001 mm). Thickness measures were highly repeatable, both before (intraclass correlation coefficient $r = 0.975$, $P < .001$) and after ($r = 0.984$, $P < .001$) injection.

For statistical analysis, ages of rearing and laying mothers were included as continuous variables to allow us to test for a quadratic effect of age on the response variables. Polynomial regression models (Freund and Wilson 1998) were used to analyze the effect of age on hatching and fledgling success, egg volume, and the growth and immune responses of chicks. The models for the analyses of egg volume, growth, and immune response included data from first and second eggs or chicks in the same model with laying or hatching order as a fixed factor and nest identity as a random factor, using the Satterthwaite approximation for the denominator degrees of freedom (Littell et al. 1996). The initial model for the analysis of egg volume included laying date as a covariate. Initial models of growth and immune responses of chicks included as covariates the age of the male partner, egg volume, hatching date, brood size, and, only for the analyses of growth, chick mass or ulna length at age 5 days. For the analysis of hatching success, the initial model included as covariates clutch volume, laying date, and the age of the male partner. Initial models of reproductive success included clutch volume, female reproductive experience (number of previous reproductive attempts), and the age of the male partner as covariates. Full models included main effects, quadratic effects, and interactions of laying-mother age and rearing-mother age; nonsignificant terms were removed by backward deletion. Reproductive success (number of fledglings produced) and number of eggs that hatched were analyzed

using generalized linear models with a Poisson error distribution and a log link function. Overdispersion was not found in our analyses (Crawley 2007). Additionally, to evaluate whether swapping clutches influenced reproductive success, age-dependent reproductive successes of experimental females that raised eggs laid by similar-age females and unmanipulated females with two-egg clutches and laying dates within the same range as experimental nests were compared using a generalized linear model with a Poisson error distribution and a log link function. The model included the age of the mother (linear term, age, $\chi^2_{1,160} = 3.01$, $P = .08$; quadratic term, age^2, $\chi^2_{1,160} = 3.28$, $P = .07$), the treatment ($\chi^2_{1,160} = 2.67$, $P = .10$), and the interactions (age × manipulation, $\chi^2_{1,160} = 2.77$, $P = .09$; age^2 × manipulation, $\chi^2_{1,160} = 3.09$, $P = .08$). For all other analyses, generalized linear mixed models were used because residuals were normally distributed (Shapiro-Wilk test). We used Cook's distances to assess any possible influential data (Cook value > 1) in all analyses performed, but no influential data were detected. All analyses were carried out in SAS (SAS Institute 1999).

Results

Reproductive Success over Female Life Span

Female reproductive success varied with age. The longitudinal analysis revealed a progressive increase in reproductive success until roughly 8–9 years of age, followed by a progressive decline at older ages (mixed model without interactions: age, $\beta = 0.03$, $F_{1,998} = 34.16$, $P < .001$; age^2, $\beta = -0.01$, $F_{1,998} = 35.86$, $P < .001$; table 1; fig. 1A–1C). Reproductive success over the life span of females from different cohorts differed (table 1; fig. 1A–1C), and differences among individual females were significant (female identity $P < .0001$). The generalized-additive-model analysis showed that the peak of reproductive success in the three cohorts occurred at age 8 years (fig. 2). Independent analyses of females younger than 8 years showed

that reproductive success increased with age and that there were no differences among cohorts (table 1). Reproductive success of females older than 8 years declined with age, but the rate of this decline differed among cohorts: cohort 1988 showed a steeper decrease and a higher peak in reproductive success with age than did cohorts 1989 and 1991 (table 1; fig. 1D).

Cross-Fostering Experiment between Females of Different Ages

Egg Volume and Hatching Success. Mean egg volume of first and second eggs differed ($F_{1,44} = 5.98$, $P = .02$) and declined progressively with female age ($\beta = -412.21$, confidence interval [CI] = -758.40 to -66.01, $F_{1,44} = 5.76$, $P = .02$; fig. 3). No evidence of a quadratic effect of age ($F_{1,44} = 0.54$, $P = .46$) or an interaction between female age and laying order was found (laying-mother age × laying order, $F_{1,44} = 0.01$, $P = .99$; laying-mother age^2 × laying order, $F_{1,43} = 0.13$, $P = .71$). Egg volume was not affected by laying date ($F_{1,43} = 0.36$, $P = .55$), and differences among clutches were significant (random effect $P < .001$). Hatching success was not related to the age of the rearing or the laying mother (linear and quadratic terms of age $P > .40$ in all cases), nor was it affected by clutch volume, laying date, or the age of the male partner ($P > .11$ in all cases).

Chick Body Size and Body Mass. Ulna length of 5-day-old first- and second-hatched chicks was negatively related to laying-mother age ($\beta = -0.20$, CI = -0.39 to -0.01, $F_{1,46} = 4.49$, $P = .03$). Similarly, body mass of 5-day-old first- and second-hatched chicks declined with laying-mother age, but the effect was only marginal ($\beta = -1.58$, CI = -3.29 to 0.11, $F_{1,46} = 3.51$, $P = .06$). Five-day-old chicks from nests with a brood mate were larger ($F_{1,46} = 8.50$, $P < .001$) and heavier ($F_{1,46} = 4.31$, $P = .04$) than singleton chicks, but the interactions between

Table 1: Age-dependent reproductive success of blue-footed booby females from cohorts 1988, 1989, and 1991

	Females age 3–16 years[a]			Females younger than 8 years[b]			Females older than 8 years[c]		
	df	F	P	df	F	P	df	F	P
Age	1, 254	30.33	<.001	1, 316	16.630	<.001	1, 182	16.580	<.001
Age^2	1, 285	30.23	<.001	...	...	...	...	...	...
Cohort	2, 361	1.82	.160	2, 88.5	.160	.850	2, 207	4.840	.008
Age × cohort	2, 346	2.89	.056	2, 296	.900	.400	2, 181	3.740	.020
Age^2 × cohort	2, 327	2.97	.052	...	...	...	...	...	...

Note: Reproductive success (fledglings produced per pair) was estimated as the standardized residuals from annual reproductive success of the colony.

[a] Polynomial mixed model; female identity $P < .0001$; $n = 1,056$ reproductive attempts; see fig. 1A–1C for estimates by cohort.

[b] Linear mixed model using females before the reproductive peak only; age estimate and confidence interval $\beta = 0.16$, confidence interval = 0.08–0.24, $P < .001$; female identity $P < .0001$; $n = 389$ reproductive attempts.

[c] Linear mixed model using females after the reproductive peak only; female identity $P < .0001$; $n = 667$ reproductive attempts; see fig. 1D for estimates by cohort.
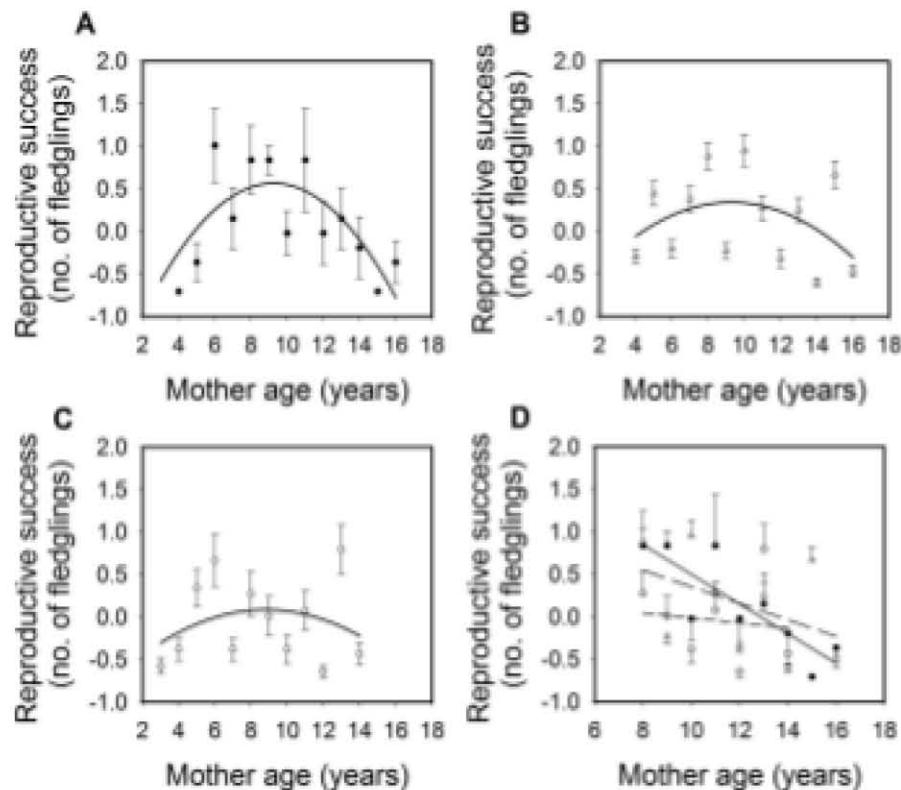
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Figure 1: Lifetime reproductive success up to age 16 years (until 2005) in relation to female age in three cohorts of *Sula nebouxii*. Reproductive success (fledglings produced per pair) was estimated as the standardized residuals from annual reproductive success of the colony (mean ± SE are shown). On the basis of longitudinal data from (*A*) eight females from cohort 1988 (estimate and confidence interval [CI]: age, $\beta = 0.58$, CI = 0.30–0.86, $P < .001$; age², $\beta = -0.03$, CI = -0.04 to -0.01, $P < .001$), (*B*) 63 females from cohort 1989 (age, $\beta = 0.29$, CI = 0.15–0.43, $P < .001$; age², $\beta = -0.01$, CI = -0.02 to -0.009, $P < .001$), and (*C*) 21 females from cohort 1991 (age, $\beta = 0.16$, CI = -0.04 to 0.36, $P = .12$; age², $\beta = -0.008$, CI = -0.02 to 0.003, $P = .16$). *D,* Reproductive success of females older than 8 years in three cohorts: filled circles and solid line represent cohort 1988 ($\beta = -0.18$, CI = -0.28 to -0.08, $P < .001$); triangles and long-dashed line represent cohort 1989 ($\beta = -0.10$, CI = -0.15 to -0.05, $P < .001$); open circles and short-dashed line represent cohort 1991 ($\beta = -0.001$, CI = -0.09 to 0.08, $P = .96$).

brood size and laying-mother age were not significant (ulna length $F_{1,46} = 0.02$, $P = .90$; body mass $F_{1,46} = 0.10$, $P = .75$). Ulna length and body mass of 5-day-old chicks were not related to the age of the rearing mother, hatching order, the age of the male partner, hatching date, or egg size ($P > .28$ in all cases). Differences among nests were significant (random effects: ulna length analysis $P < .001$, body mass analysis $P < .001$).

In the analysis of chick ulna growth up to age 30 days, there were significant interactions between laying-mother age and hatching order and between rearing-mother age and hatching order (table 2). Increased maternal age was associated with slower ulna growth of second chicks (laying-mother age $\beta = -1.00$, CI = -2.51 to -0.50, $P = .01$; rearing-mother age $\beta = -1.80$, CI = -3.36 to -0.25, $P = .02$) but was not related to ulna growth of first chicks (laying-mother age $\beta = 0.72$, CI = -0.57 to 2.02, $P = .26$; rearing-mother age $\beta = -0.006$, CI = -1.39 to 1.38, $P = .99$; table 2; fig. 4*A*, 4*B*). The chick's ulna growth was

not related to egg volume, hatching date, brood size, or age of the male partner ($P > .18$ in all cases).

The chick's increment in body mass until age 30 days was not related to the age of the laying or the rearing mother, hatching order, egg volume, hatching date, brood size, or the age of the male partner ($P > .19$ in all cases). Differences among nests were significant (random effect $P < .001$).

Chick Immune Response. There was a significant interaction between rearing-mother age and hatching order on chick immune response (table 3). The immune response of second chicks decreased as the rearing-mother age increased ($\beta = -0.16$, CI = -0.31 to -0.02, $P = .02$), but no effect was detected for first-hatched chicks ($\beta = 0.01$, CI = -0.10 to 0.13, $P = .81$; fig. 4*C*). Laying-mother age, chick's body mass, egg volume, hatching date, brood size, and age of the male partner were unrelated to the immune
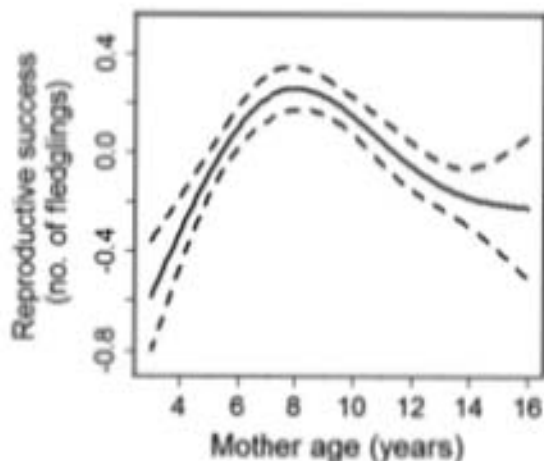
Figure 2: Lifetime reproductive success up to age 16 years in relation to female age in three cohorts of *Sula nebouxii*. Reproductive success (fledglings produced per pair) was estimated as the standardized residuals from annual reproductive success of the colony (mean ± confidence intervals are shown). The analysis is based on pooled longitudinal data from 92 females from cohorts 1988, 1989, and 1991. To determine the peak of maximum reproductive success, a generalized additive model using a spline smoother with 4 df was fitted. Approximate significance of the spline term is $P < .001$. Solid line represents spline smoother.

response of first- and second-hatched chicks ($P > .37$ in all cases).

Reproductive Success. Mother-age effects on reproductive success were complex. The experimental manipulation had no apparent effect on the reproductive success of females rearing eggs laid by females of similar age. For females raising eggs laid by females of similar age (± 1 year; fig. 5A), the model predicted an increase in reproductive success up to a maximum, followed by a decline, similar to the pattern in the long-term descriptive study of reproductive success over female life span. Furthermore, age-dependent reproductive success did not differ between the females that raised eggs laid by similar-age females and unmanipulated females of the colony with two-egg clutches and laying dates similar to those in experimental nests (age^2 × manipulation, $\chi^2_{1, 144} = 3.09$, $P = .08$; see "Methods"). Thus, swapping clutches alone did not alter the reproductive success of females of different ages.

In our experiment, the age of the rearing female affected the number of fledglings produced, but the effect depended on the age of the laying mother: the interactions between the linear term of rearing-mother age and the linear and quadratic terms of laying-mother age were significant (table 4). Reproductive decline with advancing age of the experimental rearing mothers was evident when eggs were laid by old females (fig. 5D) but less evident when

laid by young and middle-aged females (fig. 5B, 5C). Reproductive success was unrelated to clutch volume, female reproductive experience, or age of the male partner ($P > .62$). In addition, when the simplest model that included only the main effects of the age of the laying and rearing mothers (linear and quadratic terms) was tested, no age effects on reproductive success were detected (laying-mother age, $\chi^2_{1, 41} = 0.67$, $P = .41$; laying-mother age^2, $\chi^2_{1, 41} = 0.01$, $P = .94$; rearing-mother age, $\chi^2_{1, 41} = 0.68$, $P = .41$; rearing-mother age^2, $\chi^2_{1, 41} = 0.001$, $P = .95$). Overall, the results suggest that reproductive senescence results from complex interactions between prenatal and postnatal effects.

Discussion

Reproductive Success over Female Life Span

This study documents reproductive senescence in blue-footed booby females similar to the pattern found in two cohorts of male boobies (Velando et al. 2006) and reports experimental evidence of decreased egg quality and rearing capacities with advancing age, as reflected by poor chick growth and reduced immunocompetence of second-hatched chicks. The long-term descriptive data showed an initial increase in female reproductive success, reaching a maximum at roughly 8–9 years, followed by progressive decline in fledgling production with advancing age. Reproductive success varied among individual females, suggesting that age-specific reproductive success could be related to heterogeneity in individual quality or differences in breeding decisions taken early in life (Cam et al. 2002; Reed et al. 2008). Cohorts differed in the rate of decline
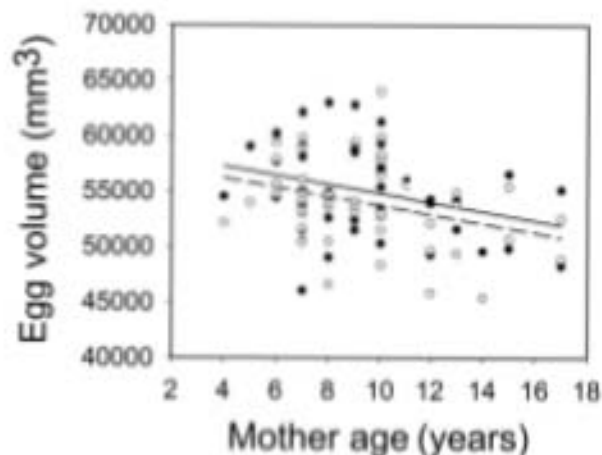

Figure 3: Egg volume in relation to female age ($n = 46$ clutches). Filled circles and solid line represent volume of first eggs, and open circles and dashed line represent volume of second eggs.

Table 2: Daily ulna growth (mm/day) of first- and second-hatched chicks until age 30 days

	df	F	P
Rearing-mother age	1, 28.7	2.08	.16
Laying-mother age	1, 30.4	.05	.81
Hatching order	1, 15.8	6.51	.02
Ulna length at age 5 days	1, 21.6	6.44	.01
Rearing-mother age × hatching order	1, 15.5	6.23	.02
Laying-mother age × hatching order	1, 12.6	7.12	.01

Note: Mixed models were used for the analysis, with the identity of the nest included as a random effect ($P = .002$).

in reproductive success after the peak age of reproductive performance. Blue-footed boobies are exposed to strong annual variation in food availability (Torres and Drummond 1999*b*), and cohorts differ in chick growth (body mass), recruitment probability, and age at first nesting (Drummond et al. 1986, 2003). Thus, conditions early in development and during the years before recruitment may affect female reproduction over the long term (Lindström 1999; Metcalfe and Monaghan 2001; Beckerman et al. 2003).

Cross-Fostering Experiment between Females of Different Ages

The study showed senescence of prenatal maternal investment. First, mean egg volume declined with the age of the laying mother. In the blue-footed booby, egg mass positively affects hatching success and chick body mass 24 h later (D'Alba and Torres 2007). In our study, egg volume did not affect hatching success or chick ulna length and body mass of chicks 5 days after hatching. We measured egg size in a sample of 87 known-age females in 2007 and found a similar negative relationship between egg size and female age ($r = -0.26$, $P = .01$), suggesting that the decline of egg size with female age is consistent among years. However, even though egg size did not affect chick growth, immune response, or survival, the age of the laying mother was negatively related to ulna length and body mass of 5-day-old chicks and to the growth of second-hatched chicks, suggesting that egg quality declines as mother's age increases and can potentially influence the chick's development.

The negative effect of laying-mother age on egg quality may be due to deterioration of egg composition or embryonic DNA that is independent of egg size (Giron and Casas 2003; Velando et al. 2008). Maternal age affects egg quality in populations in which female condition varies with age (Giron and Casas 2003; Bogdanova et al. 2006), and senescence may reduce the availability of key resources for reproduction, prejudicing egg production and self-maintenance (Kirkwood and Rose 1991). For instance,
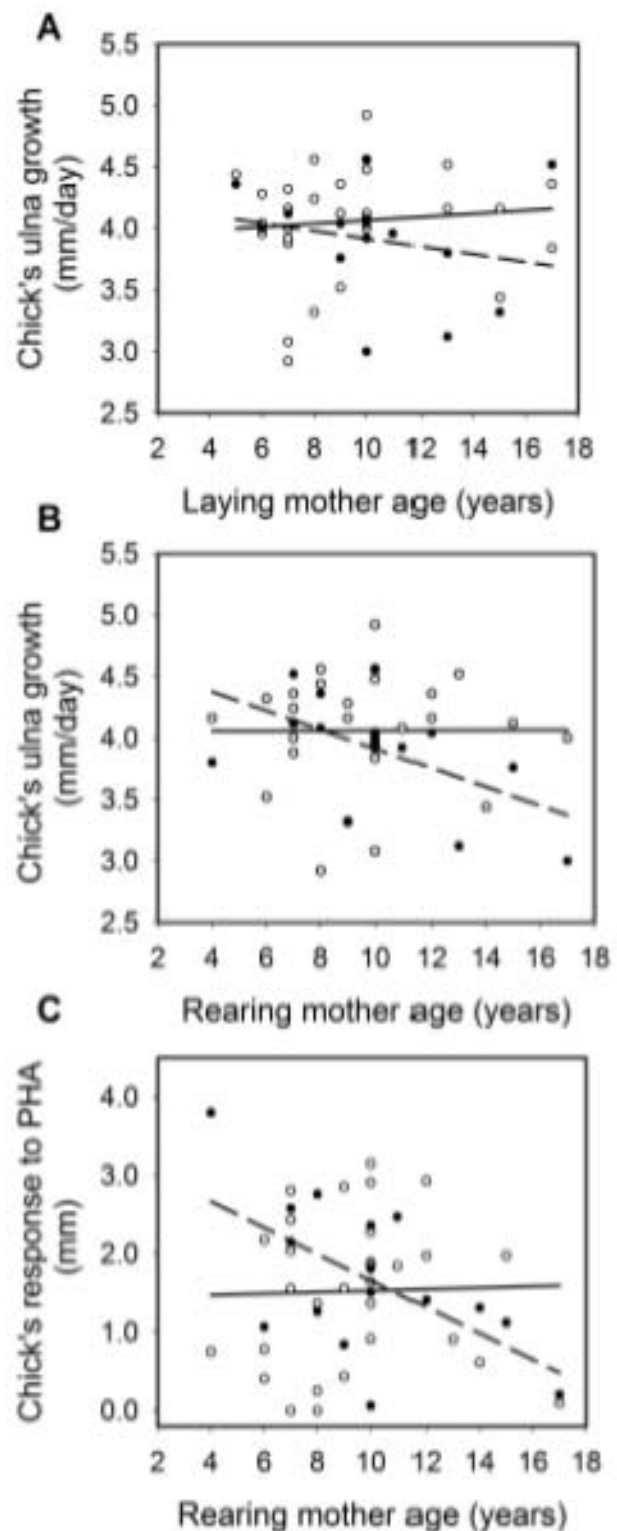

Figure 4: Chick's growth and immune response. *A, B,* Daily ulna growth (mm/day) up to age 30 days in relation to (*A*) laying-mother age and (*B*) rearing-mother age. *C,* Response to phytohemagglutinin (PHA) of 20-day-old chicks in relation to rearing-mother age. Open circles and solid lines represent first chicks; filled circles and dashed lines represent second chicks.

Table 3: Immune response to phytohemagglutinin from 20-day-old first- and second-hatched chicks

	df	F	P
Rearing-mother age	1, 43	2.82	.10
Laying-mother age	1, 43	.87	.35
Hatching order	1, 43	4.35	.04
Rearing-mother age × hatching order	1, 43	3.86	.05

Note: Mixed models were used for the analysis, with the identity of the nest included as a random effect ($P < .001$).

limited availability of antioxidants in senescent females (Beckman and Ames 1998) might constrain the amount of antioxidants furnished to eggs, with adverse effects on offspring development (von Schantz et al. 1999; Blount et al. 2000; Török et al. 2007). In blue-footed booby males, experimental activation of the immune system induced greater lipid peroxidation in senescent birds than in younger birds, suggesting a decline with age in the capacity to deal with oxidative stress (Torres and Velando 2007). Thus, female capacity to produce eggs may decline with age because physiological functions underlying trade-offs between investment in reproduction and self-maintenance are constrained by senescence.

Reduction in female rearing capacities with advancing age was indicated by poor skeletal growth and diminished cellular immune responses of second-hatched chicks. Blue-footed booby chicks compete aggressively for parentally provided food, and usually the first chick outcompetes the second one (Drummond et al. 1986). If the amount of food brought to the nest by the female decreases with age, second chicks will likely bear the brunt. Immune response to phytohemagglutinin (PHA) at age 20 days was lower for second-hatched chicks when reared by a senescent mother but unaffected by laying-mother age, implying that development of nestling immune response is prejudiced by the diminished care provided by senescent mothers. Thus, senescence may involve not only age-related decline in physiological functions affecting investment in eggs but also diminished ability to forage (Catry et al. 2006; but see Ricklefs 2008) and allocate resources to chicks.

Overall, fledgling production was dependent on the combined effects of rearing-mother age and laying-mother age, indicating that age effects are complex. Consistent with our longitudinal observations, females raising offspring from eggs laid by similar-aged females showed an increase in reproductive success up to a maximum, fol-
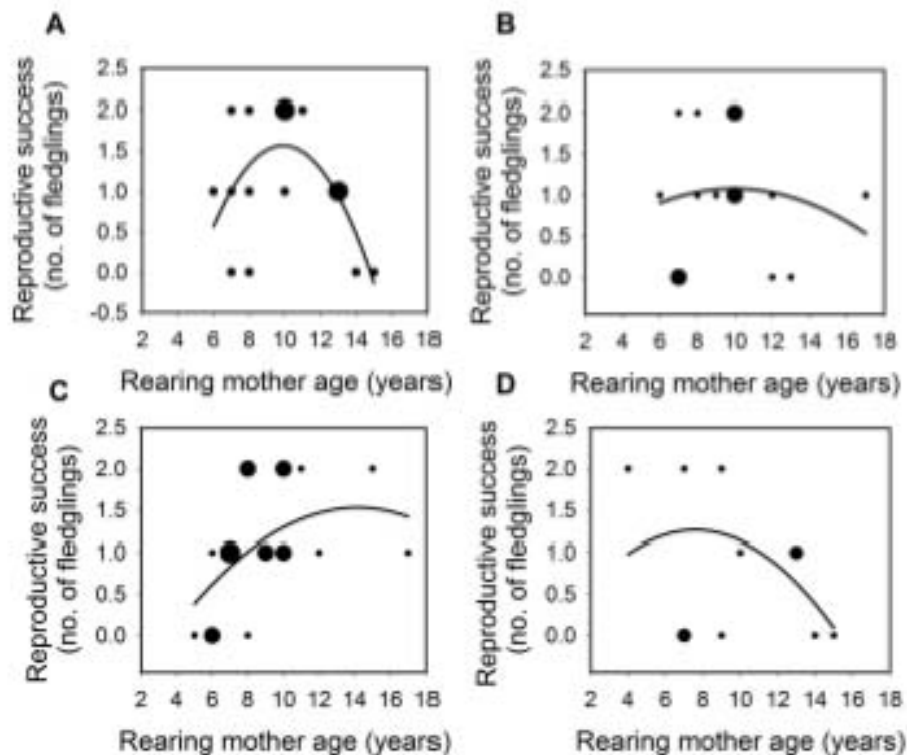
Figure 5: Reproductive success (number of fledglings produced) according to the age of the rearing mothers raising offspring from eggs laid by (A) females of similar age (± 1 year), (B) females of ages 4–7 years (n = 15), (C) females of ages 8–11 years (n = 20), and (D) females of ages >11 years (n = 11). To simplify visualization, conditional plots from the predicted values of the interaction rearing-mother age × laying-mother age² are presented. Dot size is related to number of points overlapping.

Table 4: Effects of rearing-female and laying-female ages on production of fledglings by females of the experiment (n = 46 nests)

| | Estimate | Confidence interval | | χ^2 | P |
		Lower	Upper		
Rearing-mother age	−.74	−2.12	.62	1.17	.28
Rearing-mother age[2]	−.01	−.04	.01	1.50	.22
Laying-mother age	−2.23	−4.56	.09	3.77	.05
Laying-mother age[2]	.11	.01	.22	4.92	.02
Rearing-mother age × laying-mother age	.25	−.01	.51	4.53	.03
Rearing-mother age × laying-mother age[2]	−.01	−.02	−.01	5.66	.01

Note: Model residual deviance is 27.9 with 39 df (ratio is 0.71).

lowed by a decline. These results suggest that the experimental manipulation did not distort the natural outcome of pre- and postnatal effects on reproductive success at late ages. Senescence of the ability to care for offspring was evident when old mothers raised eggs laid by old mothers but not when they raised eggs laid by middle-aged mothers (8–11 years old). Old mothers (>11 years) rearing offspring of middle-aged mothers achieved 100% hatching success and 75% fledging success, compared to 62% hatching success and 40% fledging success when the offspring came from old mothers. Egg quality of senescent mothers may be constrained by their limited ability to acquire resources and allocate them to reproduction and subject to deterioration of ova DNA (as discussed above), and these limitations may prejudice offspring development.

Additionally, in fluctuating environments senescent mothers may be selected to plastically adjust parental care in relation to environmental conditions and the reproductive value of their offspring (Uller 2008). Low reproductive performance of senescent mothers rearing offspring of senescent laying mothers suggests that senescent females facultatively reduce their investment in postnatal care in order to secure their own survival and invest preferentially in future offspring of higher quality (Phillipi and Seger 1989). Likewise, senescent mothers could increase current reproductive investment whenever their own survival prospects decrease, under favorable environmental conditions, or when the reproductive value of the current clutch is high. Enhanced investment is especially expected in old individuals because their prospects for reproduction in the future are meager (Clutton-Brock 1984; Velando et al. 2006). In our experiment, greater reproductive success of senescent foster mothers rearing offspring of middle-aged laying mothers may have occurred because they responded to the perceived high quality of eggs and chicks by upgrading their care of them. We do not know the mechanism females could use to assess egg quality, but nestling quality could be estimated from cues such as begging behavior or chick growth rate, which can increase

when yolk androgens or carotenoids are high (Groothuis et al. 2005; Helfenstein et al. 2008). Thus, our data suggest that positive adjustment of postlaying and postnatal care occurs when senescent mothers are faced with a high current reproductive value and highlight the importance of egg quality in subsequent levels of female provisioning and care.

We investigated reproductive senescence by analyzing longitudinal data on reproductive performance over the female's lifetime and by performing an experiment to tease apart the effects of senescence on egg quality and rearing capacities. Longitudinal studies are needed to test individual reproductive senescence because in cross-sectional studies, apparent changes in reproduction with age can be due to differential mortality of individuals that invest heavily in reproduction (Reid et al. 2003; Nussey et al. 2008). However, experiments are needed to assess causality between variables and generally have to be conducted in cross-sectional samples because long-term experiments on long-lived species in the wild are difficult to perform. Thus, although in principle results of the cross-fostering experiment might have been influenced by females' individual life histories, our study highlights the insights into the life-history constraints and phenotypic plasticity underlying patterns of aging that can be gained through cross-sectional experiments.

In conclusion, senescent blue-footed booby females produced chicks of lower quality and raised fewer fledglings. The egg-swapping experiment suggests that the negative effect of female senescence on egg quality is important early in offspring development but that senescent mothers can facultatively upgrade postlaying and postnatal care in response to high offspring quality, thereby increasing offspring survival chances. Decline in reproductive performance with age may be related to constraints on resource allocation to reproduction at older ages and ameliorated by an adaptive strategy whereby level of provision and care is calibrated to offspring value. Plasticity in postlaying and postnatal care by senescent females could be particularly

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adaptive in long-lived animal species that depend on unpredictable resources.

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Senescent blue-footed booby females adjust their postlaying and postnatal care according to offspring quality. Photograph by Claudio Contreras Koob.

Capítulo 3.

Efecto de los carotenoides en la condición corporal y desempeño reproductivo de machos de distinta edad

Positive age-dependent effect of carotenoids on male reproductive performance of a long-lived seabird

Positive age-dependent effect of carotenoids on male reproductive performance of a long-lived seabird

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Abstract

Understanding the mechanisms mediating age-dependent reproductive effort in wild animals is a major challenge for evolutionary ecology. A recent theoretical model proposed that somatic damage affects reproductive investment and predicts that animals benefit by restraining reproduction late in life to increase time for reproducing. Under this scenario, the acquisition of molecules able to deter somatic damage could potentially improve reproductive performance of old individuals. Carotenoids are antioxidants and immuno-enhancers that animals can only obtain through diet. We tested whether carotenoid supplementation during incubation improves condition and reproductive performance, particularly of older males in the blue-footed booby. Independent of age, carotenoid supplemented males lost less body mass than non-supplemented birds. We found a positive effect of male age on first chick body mass and second chick ulna growth only for supplemented males. However antioxidant capacity and peroxidative damage were not affected by treatment. The positive effect of carotenoids on body mass and reproduction of old males could be independent of changes in male oxidative status. Alternatively, old males could increase their reproductive performance without increasing oxidative stress when a surplus of carotenoids is available. Our data suggest that dietary carotenoids can potentially ameliorate the decline in breeding performance at old ages and that old animals adjust their levels of parental care in response to changes in body condition and risk of somatic deterioration.

Introduction

Evolutionary ecology explains the occurrence of senescence as a result of life-history trade-offs

between reproduction and maintenance (Stearns 1992). For instance, reproduction impacts negatively

on immunocompetence and antioxidant capacity (Deerenberg et al. 1997, Hansen et al. 2005, Wiersma

et al. 2004, Alonso-Alvarez et al. 2004), whereas a high reproductive effort early in life impacts

negatively on individual longevity (Reid et al. 2003, Descamps et al. 2006) and accelerates the rate of

reproductive senescence (Nussey et al. 2006). However, in wild animals few is known about the

physiological mechanisms mediating the rates of reproductive decline at older ages. A recent

theoretical model has proposed that decline in reproductive success in older animals could result from a

late-age restraint strategy to maximize lifetime reproductive success, with individuals investing less in

reproduction at older ages to reduce somatic damage (McNamara et al. 2009). Under this scenario,

supplementation of molecules that reduce damage, such as dietary antioxidants (Halliwell and

Gutteridge 2007), might improve reproductive performance, particularly of older animals. Here, we

assessed the effect of carotenoid supplementation on condition (body mass, foot color and oxidative

status) and reproductive performance (chicks' growth rate, immune response and survival) of different

aged blue-footed booby males.

Susceptibility to oxidative stress has been proposed as a cost of reproduction and as a possible

mediator among life-history trade-offs (Alonso-Alvarez et al. 2004, Monaghan et al. 2009). Oxidative

stress is defined as the unbalance between pro-oxidants production and antioxidant defenses, the

greater the unbalance in favor of the former the greater the oxidative stress levels (Halliwell and

Gutteridge 2007). Low levels of oxidative stress are required during normal physiological functioning

and high levels of oxidative stress are negative for organisms because of their association with somatic

damage and disorders (Martin and Grotewiel 2006). Experimental increase of reproductive effort has

been related to decreased resistance to oxidative stress, assessed as organismal mortality in response to a free radical attack in fruitflies (*Drosophila melanogaster;* Salmon et al. 2001, Wang et al. 2001), and as the reduced resistance of red blood cells to a free-radical attack or lower activity of superoxide dismutase enzyme in zebra finches (*Taenopygia guttata;* Alonso-Alvarez et al. 2004, Wiersma et al. 2004). Older animals are expected to become more susceptible to the oxidative costs of reproduction because as animals age their capacity to deal with oxidative stress declines (Beckman and Ames 1998). Accordingly, resistance to oxidative stress is lower for old compared to middle-aged birds in greater flamingo, *Phoenicopterus ruber roseus* (Devevey et al. 2010), whereas in red-legged partridge *Alectoris rufa* both antioxidant capacity and oxidative damage increase with age (Alonso-Alvarez et al. 2010). Thus, if reproduction imposes a greater oxidative damage to older breeders, they would either require a greater amount of antioxidant resources to mitigate these costs, thus reducing the amount of antioxidants resources available for reproduction, or they could reduce reproductive effort to decrease somatic damage (McNamara et al. 2009). Therefore, at least one of the proximal physiological mediators of the decline in reproductive performance in old animals might be the increased oxidative costs of reproduction that older animals suffer. However, we know little about the relationship between age and the oxidative cost of reproduction in wild animals (Nussey et al. 2009, Lecomte et al. 2010).

Carotenoids are antioxidants that can increase maintenance capacities and buffer the oxidative costs of reproduction (Bertrand et al. 2006, Aguilera and Amat 2007). Several studies have assessed the relationship between antioxidants and reproduction (Wiersma et al. 2004, Pérez et al. 2008, review in Catoni et al. 2008). Particularly, carotenoids, which animals cannot synthesize and must obtain from the diet, have positive effects on free radical scavenging and immunocompetence (Alonso-Álvarez et al. 2004, Aguilera and Amat 2007, Hŏrak et al. 2007; but see Constantini and Møller 2008), and in some cases, influence the quality of colorful sexual signals and reproductive performance (Blount et al. 2004, Biard et al. 2005, Pike et al. 2007, Safran et al. 2010). In zebra finches, carotenoid

supplementation modulated the relationship between the number of eggs laid and the resistance to oxidative stress: non-carotenoid supplemented pairs that laid more eggs exhibited a decrease in antioxidant defenses, whereas this negative correlation vanished for carotenoid-supplemented individuals (Bertrand et al. 2006). In greenfinches *Carduelis chloris,* males that received a carotenoid supplementation showed higher titers of antibodies after an immune challenge than control males (Aguilera and Amat 2007). Moreover, carotenoid supplementation in sticklebacks (*Gasteorsteus aculeatus*) prevented the decline in swimming performance of older breeding males (Pike et al. 2010). The decline with age of antioxidant capacity (Beckman and Ames 1998) and of the intensity of carotenoid-dependent sexual ornaments (Torres and Velando 2007) suggest that carotenoids might be limited resources for old animals, either because they have reduced foraging skills (Catry et al. 2006), because absorption capacities decline with age, or because an increased amount of them are necessary for maintenance functions. Therefore, the acquisition of dietary antioxidants such as carotenoids might be a key factor underlying the trade-off between reproduction and maintenance, particularly for older animals.

The blue-footed booby (*Sula nebouxii*) is an iteroparous long-lived tropical seabird with a long period of biparental care: both parents incubate the modal clutch of two eggs for 41-45 d and feed the chicks for up to four months (Nelson 1978, Torres and Drummond 1999, Drummond et al. 2003). There is facultative brood reduction when food is scarce, frequently resulting in the dead of the youngest chick in the brood during the first 15 days of chick rearing (Nelson 1978, Drummond et al. 1986). In the blue-footed booby, there is evidence of reproductive senescence: longitudinal data from males and females surviving up to age 13 years have shown that reproductive success increases with age until 9-10 years and then decline afterwards (Velando et al. 2006a, Beamonte-Barrientos et al. 2010). Moreover, foot color, a sexually selected carotenoid-dependent trait (Torres and Velando 2003, 2005, Velando et al. 2006b) declines in brightness with age (Torres and Velando 2007, Velando et al.

2010). After mounting an immune response, males older than ten years lowered foot color brightness and produced higher lipid peroxidative damage than younger males (Torres and Velando 2007). Carotenoid supplementation to courting males had a positive effect in the cellular immune response (Velando et al. 2006b), whereas females that received a surplus of carotenoids during egg laying, increased foot color intensity and egg size (Morales et al. 2009), and enhanced egg coloration (Morales et al. 2010). Thus, in the blue-footed booby carotenoids influence positively some reproductive traits and data suggests that carotenoids are limited for older birds. Here, we evaluated if carotenoid supplementation during incubation improves condition and reproductive performance, particularly of older males. We predicted that old males that received a supplement of carotenoids (1) will have lower lipid peroxidative damage, higher antioxidant capacity and will be heavier, and (2) will raise offspring with greater growth rates, immune response and survival chances than non-supplemented old males.

Material and Methods

Study site

The study was conducted from January to June 2007 in the breeding colony of blue-footed boobies on Isla Isabel, in the Mexican Pacific (25° 52' N, 105° 54' W). All males included in the experiment were previously banded in the course of a long-term study (see Drummond et al. 2003). Since 1988, in every reproductive season (February to July) all breeding attempts in the study area are monitored until the end of the fledging period (Drummond et al. 2003). Chicks are individually marked with plastic rings within 3 days after hatching and with numbered metal rings at fledging, enabling us to know the exact age of individuals that recruit to the breeding population. Only males older than 6 years with at least one reproductive attempt were included in the experiment (male recruitment age is 4.3 ± 0.09 years; Drummond et al. 2003), to avoid greater variation in parental performance due to inexperience. Although most female partners of focal males were banded and of known age, 8% of female partners

were unbanded and were deemed to be 3-year old recruits (mean female recruiting age 3.8 ± 0.08 years; Drummond et al. 2003).

Experimental procedure

Recently laid clutches of known age males (range 7 - 18 years) were located by inspecting the study area every two days and half of the nests of each age were randomly assigned to either the experimental (n = 22) or control group (n = 23). At days 34, 41 and 48 after the laying of the first egg, males were supplemented with an oral dose given by means of a syringe of either 1 mL of drinking water in the control group, or a solution of 0.955 mg of carotenoids in 1 mL of drinking water in the experimental group. Carotenoid supplemented males received a mixture of 0.455 mg of Xamacol (16 g/kg of lutein and 1 g/kg of zeaxanthin) and 0.500 mg of Canthacol (100 g/kg of canthaxanthin; CaroTech, Carotenoid Technologies S.A., IQF Group, Tarragona, Spain). Supplementation with the mixture of these three carotenoids resembled natural conditions; blue-footed booby diet is mainly based on anchovies, mackerels, and sardines (Nelson 1978), which provide birds with lutein, zeaxanthin, and canthaxanthin among other carotenoids (Shahidi et al. 1998). Additionally, these are the three most common carotenoids in plasma of blue-footed booby (Morales et al. 2009).

Before the first carotenoid supplementation at day 34 of incubation, males were weighed (Pesola Spring balance ± 20 g) and their foot color was measured by taking the average of three measures on the foot web using a spectrophotometer (Minolta CM-2600d). Reflectance spectrum was determined from 360 to 740 nm wavelength at 10 nm intervals. Because the highest visual sensitivity of blue-footed boobies occur between 460 and 620 nm (Reed 1987), we analyzed the chroma of foot color in this range (hereafter green chroma) estimated as the sum of reflectances between 460 and 620 nm divided by the total spectrum reflectance (Torres and Velando 2007). Additionally, males were sampled for blood (1 mL) from the brachial vein to assess plasma antioxidant capacity and lipid

peroxidation levels. Blood samples were kept on ice for a maximum of 3 h until centrifugation at

10000 rpm for 10 minutes. The plasma was separated and preserved immediately on liquid nitrogen

until laboratory analysis. Thirteen days (13.23 ± 0.12 days) after first capture, males were sampled for

blood and weighed, and their foot color was measured again. Birds were captured and manipulated at

night to reduce perturbation and handling time per bird was less than 5 min. Two out of 45 nests from

our experiment were abandoned between the first and the third capture (both from the carotenoid

supplemented group).

At ages 1 and 15 days chick's ulna length (± 1 mm) and body mass (± 2g for measures at day 1

and ± 10g at day 15) were measured. For the analyses of chick growth, we calculated a daily growth

rate as the difference of ulna length or body mass at the ages of 1 and 15 days divided by 15 days. The

immune response (T lymphocytes response) of chicks was estimated using the phytohemagluttinin skin

test (PHA, Sigma-Aldrich; Smits et al. 1999). At age 15 days we applied a subcutaneous injection in

the right wing-web of 0.2 mg of PHA in 0.1 mL of a solution of phosphate buffered saline. Immune

response to PHA was estimated as the increase in thickness of the wing-web 24 h later. The point of

injection was marked with an indelible marker and wing-web thickness was measured (three times, to

yield an average) before injection and 24 hours later with a Mitutoyo micrometer (± 0.001 mm).

Thickness measures were highly repeatable, both before (Intraclass correlation coefficient ± Standard

Error: $r = 0.990 ± 0.002$, $P < 0.01$) and after ($r = 0.989 ± 0.002$, $P < 0.01$) injection. To determine the

sex of chicks, at age 10 days we obtained a blood sample (~80 ul) from the brachial vein. Blood was

stored in lysis buffer until laboratory analyses and the sex of chicks was determined by DNA test

according to Griffiths et al. (1998).

All experimental procedures comply with the current laws of Mexico (General Law for

Environmental Protection and Ecological Equilibrium).

Antioxidant capacity

Antioxidant capacity was measured using a colorimetric reaction method described by Erel (2004); this technique has been previously used in blue-footed boobies (Morales et al. 2009). Briefly, a molecule ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) is decolorized by sample antioxidants according to their concentration and antioxidant capacity. The change in color after 5 min is measured as the change in absorbance at 655nm. Results were calculated as Trolox equivalent antioxidant capacity (TEAC) estimated from a standard curve and expressed as mmoles TEAC/mg protein.

Lipid peroxidation

Oxidative damage of lipids was estimated as lipid peroxide concentration by the thiobarbituric acid reactive substances (TBARS) technique. Lipid peroxidation (hereafter peroxidative damage), a major indicator of oxidative damage, was detected by the formation of reactive malondialdehyde products (MDA) measured by spetrophotometry. Sample proteins were precipitated with 10% tri-chlore-acetic-acid before incubation with TBA. Samples were then incubated at 92º C for 45 min in a solution containing a mixture of TBA (0.375%) and acetic acid (2%). After reaction, samples were put on ice for 10 min and then color was measured with a spectrophotometer at 532 nm of absorbance. Results were calculated as MDA equivalents estimated from a standard curve and expressed as pmoles MDA/mg protein.

Total protein levels

We calculated total protein levels to express antioxidant capacity and peroxidative damage in terms of protein quantity. The levels of plasma protein were measured using the Bradford protein assay calibrated with a standard curve of bovine albumin (Thermo 23225). We determined the amount of protein (mg) contained in plasma volume used for TEAC (5 ul) and MDA (50 ul) assays. Similarly, we

calculated the amount of TEAC and MDA moles in plasma volume used for each assay. Afterwards, we divided the amount of moles by protein amount. This procedure was conducted to ensure that the oxidative status calculations were not affected by including samples with different levels of protein, which might strongly bias our estimations of oxidative status. Although it is not a common use in evolutionary ecology studies, expressing concentrations of antioxidant capacity and peroxidative damage by protein content is a common and recommended use in biomedical sciences.

Statistical analyses

To evaluate whether the initial antioxidant capacity and peroxidative damage of incubating males varies with age and incubation effort we used general linear models. The models included as explanatory variables the linear and quadratic terms of male age and clutch volume, as a possible indicator of incubation effort. Initial peroxidative damage was log transformed to use models with normal error distribution.

 To evaluate the effect of carotenoid supplementation on male condition we analyzed the change of male body mass, foot color, antioxidant capacity and peroxidative damage (value at day 34 minus value at day 48 after laying of first egg) using general linear models. Initial models included treatment and male age and the interaction treatment*male age as factors, and laying date, brood size at day 48 after laying, and initial measures of the response variable as covariates. Only for the analyses of the change in peroxidative damage and antioxidant capacity, models included the change in antioxidant capacity and peroxidative damage, respectively, as covariates, since these two variables might be negatively correlated. The quadratic term of male age ($P > 0.10$) or the interaction treatment*quadratic male age ($P > 0.28$) were not significant, therefore the quadratic term of male age was not include in the final analyses.

 We analyzed chick growth, chick immune response and chick survival using mixed models with

the Satterthwaite approximation for the denominator degrees of freedom (Littel et al. 1996). Models

included data from first and second eggs/chicks in the same model with laying/hatching order and

treatment as fixed factors and nest identity as a random factor (Littel et al. 1996). Initial models

included age of focal male, age of female partner, laying date, and brood size. All second- and third-

order interactions between hatching order, treatment and male age were included. Preliminary analyses

showed no significant effect of the quadratic term of male age ($P > 0.14$) or the interaction quadratic

age*treatment ($P > 0.88$), thus the quadratic terms of male age were not included in final models of

offspring growth, immune response and survival. To analyze offspring survival, we used generalized

linear models with binomial error distribution, for all other analyses we used linear models because

residuals were normally distributed. Non-significant terms were removed by backward deletion

procedures. We used Cook's Distances to assess any possible influential data but none was detected.

All analyses were carried out in R (R core Development team 2008) and SAS (SAS Institute 1999).

Results are expressed as Mean ± Standard Error.

Results

At day 34 of incubation (before carotenoid supplementation), antioxidant capacity of males was

unrelated to male's age (linear term, $F_{1,43} = 0.02$, $P = 0.88$; quadratic term, $F_{1,43} = 0.34$, $P = 0.55$) or

clutch volume ($F_{1,42} = 0.76$, $P = 0.38$). Peroxidative damage varied with the age of the male (linear

term, $F_{1,43} = 0.02$, $P = 0.96$; quadratic term, $F_{1,43} = 4.43$, $P = 0.04$; Figure 1), but was unrelated to clutch

volume ($F_{1,42} = 0.14$, $P = 0.70$). To assess whether the quadratic effect of male age on peroxidative

damage was due to increase or decrease with age in this parameter, we analyzed the significance of the

slopes before and after the age at the peak of damage (12 years). The apparent increase with age in

peroxidative damage was not significantly different from zero ($\beta = 0.13 \pm 0.07$, t = 1.74, $P = 0.09$; n =

30), in contrast after age 12 years the decline with age of peroxidative damage was significantly different from zero (β = -0.21 ± 0.09, t = -2.34, P = 0.03; n = 15). Thus, the quadratic relationship we found was due to a decline in peroxidative damage levels at older ages.

Experiment of Carotenoid supplementation

Male Condition

Prior to manipulation, carotenoid supplemented and control males did not differ in their age (Carotenoid supplemented: 11.00 ± 0.66 *versus* Control: 10.76 ± 0.65 years; $F_{1,43}$= 0.12, P = 0.73), clutch volume (114,104 ± 17,846 *versus* 117,956 ± 25,361 mm³; $F_{1,43}$ = 0.34, P = 0.55), body mass (1362.73 ± 21.59 *versus* 1361.43 ± 25.28 gr; $F_{1,43}$= 0.06, P = 0.80), foot green chroma (0.48 ± 0.006 *versus* 0.49 ± 0.006 % of reflectance; $F_{1,43}$= 0.15, P = 0.69), antioxidant capacity (64.61 ± 10.82 *versus* 65.28 ± 9.07 nmoles TEAC/mg protein; $F_{1,43}$ = 0.01, P = 0.96) or peroxidative damage (7.9 ± 1.5 *versus* 8.8 ± 1.1 pmoles MDA/mg protein; $F_{1,43}$ = 0.23, P = 0.62).

Males were on average lighter during chick rearing than during the incubation period (body mass change: -29.30 ± 17.99 gr), and control males lost more mass than experimental males (Table 1; Figure 2). The change of body mass was related to initial body mass but unrelated to male age, laying date, brood size, or the interaction male age*treatment (Table 1).

Supplementation of carotenoids did not influence the change in green chroma of male feet (Experimental: -0.009 ± 0.006, Control: -0.010 ± 0.006 change of % of reflectance). We found no effect of male age, the interaction male age*treatment or brood size on the change of foot green chroma (Table 1). Instead, laying date affected the change of foot green chroma: males laying earlier lost more color reflectance than males laying later (β = 0.003 ± 0.006).

Treatment did not affect the change of antioxidant capacity (Experimental: 15.82 ± 11.83,

Control: 0.52 ± 12.63 nmoles TEAC/mg of protein), nor the change of peroxidative damage (Experimental: 9.0 ± 2.1, Control: -10.0 ± 1.2 pmoles MDA/mg of protein). Male age, brood size and the interaction male age*treatment did not affect the change of antioxidant capacity and peroxidative damage (Table 1). Laying date was positively related to the change of peroxidative damage ($\beta = 0.03 \pm 0.01$), but not with the change of antioxidant capacity (Table 1).

Chick Body Size and Mass

The interaction between hatching order, treatment and male age affected the daily body mass increase of chicks (Table 2). Body mass growth of first chicks was not related to male age in the experimental ($\beta = 0.51 \pm 0.35$, t = 1.44, $P = 0.16$; n = 19) or control groups ($\beta = 0.43 \pm 0.29$, t = 1.47, $P = 0.15$; n = 19). In contrast, body mass growth of second chicks was positively related to male age in the experimental group ($\beta = 1.37 \pm 0.51$, t = 2.66, $P = 0.03$; n = 8), but not in the control group ($\beta = -0.07 \pm 0.25$, t = -0.31, $P = 0.77$; n = 6; Figure 3). Chick body mass growth was negatively related to laying date ($\beta = -0.37 \pm 0.08$, t = -4.65, $P < 0.01$). Chicks raised in nests with broodmates had greater body mass growth than singleton chicks (Table 2). Female age ($F_{1,31.5} = 0.27$, $P = 0.60$) and chick's sex ($F_{1,40.3} = 0.66$, $P = 0.42$) were unrelated to the chick's body mass growth.

In the analyses of chick ulna growth, there was a significant interaction between hatching order, treatment and male age (Table 3). During the first 15 days of life, ulna growth of first chicks was related to male age in the experimental group ($\beta = 0.06 \pm 0.02$, t = 2.34, $P = 0.03$; n = 19; Figure 4), but not in the control group ($\beta = 0.02 \pm 0.02$, t = 0.76, $P = 0.45$; n = 19), whereas ulna growth of second chicks was unrelated to male age in both groups (experimental, $\beta = 0.09 \pm 0.04$, t = 1.96, $P = 0.09$, n = 8, and control, $\beta = -0.02 \pm 0.04$, t = -0.60, $P = 0.58$, n = 6). Laying date had a negative effect on chick's ulna growth ($\beta = -0.03 \pm 0.009$, t = -3.30, $P = 0.002$; Table 3). Female age ($F_{1,32.1} = 0.32$, $P = 0.57$), chick's sex ($F_{1,17.1} = 2.26$, $P = 0.15$) and brood size ($F_{1,33.2} = 2.61$, $P = 0.11$) did not affect chick's

ulna growth.

Chick Immune Response

There was no effect of treatment on the immune response of chicks ($F_{1,32.9} = 0.01$, $P = 0.99$, all second and third order interactions that included treatment, $P > 0.19$). Male age had a marginal positive relationship with the first chick immune response ($\beta = 0.05 \pm 0.02$, t = 1.75, $P = 0.08$) but was unrelated to the second chick response to PHA ($\beta = -0.05 \pm 0.04$, t = -1.14, $P = 0.26$; male age*hatching order, $F_{1,15.9} = 5.0$, $P = 0.040$). Male nestlings showed a greater immune response (1.79 ± 0.13 mm) than female nestlings (1.43 ± 0.10 mm; $F_{1,42.7} = 9.0$, $P = 0.04$). Brood size ($F_{1,32.6} = 1.98$, $P = 0.16$), laying date ($F_{1,29.3} = 0.75$, $P = 0.39$), and female age ($F_{1,30.4} = 0.53$, $P = 0.47$) were not related to the immune response of chicks.

Offspring Survival

Hatching success, chick survival until ages 15 and 70 days were unrelated to treatment ($P > 0.64$), male age ($P > 0.55$), hatching/laying order ($P > 0.10$), or female age ($P > 0.49$). Second and third order interactions involving male age, treatment and laying/hatching order were not related to the probability of offspring hatching and survival ($P > 0.51$ and $P > 0.78$, respectively).

Discussion

In this study we report age-dependent positive effects of carotenoid supplementation on reproductive performance of male blue-footed boobies. Contrary to expectations, carotenoid supplementation and male age were unrelated to changes of foot color, plasma antioxidant capacity or peroxidative damage. Nonetheless, independently of their age, carotenoid supplemented males lost less body mass than

control males. Moreover, the expected negative effects of male senescence on chick growth (but not on chick immune response) were at least temporarily ameliorated in the carotenoid supplemented group: there was a positive effect of male age on the growth of first chick body mass and second chick ulna length in the carotenoid supplemented group but not in the control group. The positive effects of carotenoids on male body condition and offspring growth could result if carotenoid supplementation had a positive impact on the male's phenotypic quality that elicited greater investment by the female partner (Sheldon 2000). Indeed, supplementation of carotenoids in male blue-footed boobies during courtship influences foot color, a sexually selected trait (Torres and Velando 2003, Velando et al. 2006b), and females are able to modify investment in the eggs according to male foot color during egg laying (Velando et al. 2006b, Dentressangle et al. 2008, Torres and Velando 2010). However, in the present experiment no effect of carotenoid supplementation on male's foot color or changes on female body mass ($F_{1,43} = 0.22$, $P = 0.63$) were detected. In the blue-footed booby, male foot color brightness declines along the breeding season with the lowest values occurring during chick rearing (Torres and Velando 2010), thus it is possible that in our experiment males allocated the extra carotenoids to offspring instead to signaling. Hence, it is unlikely that maternal effects are responsible for the positive effect of carotenoid supplementation on male condition and offspring growth.

Carotenoid supply could positively influence male body mass and paternal effort through effects of carotenoids on physiological process that enhanced male condition and/or increased male foraging performance. In our experiment, control males lost 4.0 % of their body mass whereas carotenoid supplemented males lost only 0.2 %. Positive effects of carotenoid supplementation on body mass have been reported in pheasants (*Phasianus colchicus*) during courtship, and in zebra finches during egg laying (Bertrand et al. 2006, Smith et al. 2007), but not in great tits during egg laying, incubation or chick rearing (Constantini et al. 2007, Remeš et al. 2007). Furthermore, in sticklebacks, carotenoid supplementation increased parental care: carotenoid supplemented males fanned their eggs at a higher

rate and had higher hatching success than non-supplemented males (Pike et al. 2007), and the decline

with age in physical performance of breeding males was ameliorated with carotenoid supplementation

(Pike et al. 2010). It has been shown that carotenoid supplementation enhances flight (Blount and

Matheson 2006) and visual performance (Kvansakul et al. 2006), which might result in shorter and

possible energetically cheaper and more effective foraging trips. In addition, carotenoids might

influence some physiological traits that improved male condition: enhancing the activity of other

antioxidants or increasing immunocompetence, traits that decline in activity at older ages (Krinsky

1998, Aguilera and Amat 2007, Hõrak et al. 2007, Vleck et al. 2007; but see Constantini and Møller

2008). Hence, although we do not know the mechanisms, in the blue-footed booby a carotenoid supply

during incubation and early chick rearing resulted on males being able to maintain body mass and, in

the case of older males, produce offspring with greater growth rates.

Despite a positive effect of carotenoid supplementation on chick growth of older males, extra

carotenoids on the diet of males had no effect on the chick's survival or immune response.

Independently of the treatment, first chicks' immune response (but not second chicks) increased with

adult male age, but the effect was marginal. In the blue-footed booby, incubation last for roughly 41-45

days and feed the chicks for up to 120 days (Nelson 1978, Torres and Drummond 1999). In our

experiment, males received three doses of carotenoids during a short period of time (13.23 ± 0.12

days), and late in the incubation period. It is possible that the time and duration of the treatment was

insufficient to influence the cellular immune response and survival of old male's progeny but enough to

distort the natural age-associated variation in reproductive success (Velando et al. 2006, Beamonte-

Barrientos et al. 2010).

Carotenoid supplementation was unrelated to the change of antioxidant capacity or peroxidative

damage, and contrary to our expectation, we found no effect of age on the change in antioxidant

capacity or peroxidative damage after carotenoid supplementation. In birds, the rate of carotenoid

uptake varies between species but can be high, increasing plasma carotenoid concentration rapidly in response to dietary supplementation: eight days in greenfinches (Aguilera and Amat 2007), and one week in kestrels *Falco tinnunculus* (Constantini et al. 2007). In contrast, effects of carotenoids on plasma oxidative status are not detected so rapid. Plasma antioxidant capacity was unaffected four days after a single supplementation of carotenoids in blue-footed boobies (Morales et al. 2009), but increased four weeks after daily supplementation in gulls (Blount et al. 2002), whereas serum levels of oxidative damage decreased after two weeks of daily carotenoid supplementation in kestrels (Constantini et al. 2007). Hence, it is possible that in the blue-footed booby three weekly dosages were insufficient to produce positive effects on males' plasma antioxidant capacity and peroxidative damage . Interestingly, males that received a supply of carotenoids maintained their body mass, and the offspring of older males showed greater growth rates, without detrimental effects on the males' oxidative status. Blue-footed booby females that were supplemented with carotenoids during egg laying produced larger eggs and improved their foot color, but no effect on the change of antioxidant capacity was detected four days after manipulation (Morales et al. 2009). Thus, it is possible that blue-footed boobies, and particularly old males, maintain low levels of oxidative stress, as evidenced by the low initial peroxidative damage, and allocated extra carotenoids to maintenance functions related to body mass storage and to paternal effort. However, our experiment provides no evidence of carotenoids improving the oxidative status of males.

When extrinsic mortality is low, as for seabirds, animals should vary reproductive investment in relation to somatic damage because longevity is determined by somatic deterioration (McNamara et al. 2009). According to the hypothesis of reproductive restraint late in life, investment in reproduction is adjusted by the risk of somatic damage and older individuals are expected to decrease their reproductive investment due to an increased risk of somatic deterioration (McNamara et al. 2009). Reproduction impacts negatively on two key components of maintenance: antioxidant capacity

(Alonso-Alvarez et al. 2004) and immunocompetence (Hansen et al. 2005), and carotenoids have positive effects on immunocompetence (Aguilera and Amat 2007) and probably oxidative status (Blount et al. 2002, Alonso-Alvarez et al. 2004, Hŏrak et al. 2007). Since older animals are more deteriorated, any increase in resources for maintenance would have a greater impact for them compared to younger, and presumably, less deteriorated animals. Thus, it would be expected that old males with increased body condition allocated more resources to reproduction than younger males. The results of the experiment suggest that older animals restraint their reproductive investment, but can facultatively adjust their investment to offspring based on changes in their body condition, a strategy particularly adaptive when resources are unpredictable (Lock et al. 2007).

In conclusion, in blue-footed boobies carotenoid supplementation had positive effects on body mass and positive effects of age on offspring growth were observed in males that received an extra surplus of carotenoids. Male parameters of oxidative status were not affected by carotenoids, thus the physiological mechanisms mediating this positive effect of carotenoids needs further investigation. By ameliorating the decline in breeding performance at old ages differential carotenoid acquisition among individuals could have an important effect on senescence rates of wild long-lived animals.

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n = 43 males	Change of body mass		Change of feet color		Change of TEAC		Change of MDA	
Effect	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Treatment	4.25	0.04	0.01	0.90	1.16	0.21	1.03	0.31
Male Age	2.24	0.14	1.16	0.28	1.19	0.28	0.24	0.62
Laying Date	0.14	0.7	6.34	0.01	0.98	0.32	8.51	0.005
Brood Size d5	2.58	0.11	0.07	0.78	0.17	0.68	0.6	0.44
Change of TEAC[1]							0.12	0.72
Change of MDA[2]					0.29	0.59		
Treatment * Male Age	0.27	0.6	1.45	0.23	0.26	0.61	0.02	0.87
Initial value	42.49	<0.001	28.58	<0.001	42.9	<0.001	21.13	<0.001

[1] TEAC refer to Trolox equivalents of antioxidant capacity (nmoles TEAC/mg protein)
[2] MDA refer to Malondyaldehyde (pmoles MDA/mg protein)

Table 1. Effect of carotenoid supplementation on body mass, green chroma, antioxidant capacity and peroxidative damage of adult male blue-footed boobies. The change is defined as the initial minus the last measurement. Change of TEAC and MDA were only included as covariates in models analyzing the change of MDA and TEAC, respectively, since it is possible that these effect covary.

n = 49 chicks	df	F	P
Hatching order	1,20.8	1.29	0.26
Treatment	1,35.7	10.03	0.002
Male age	1,36.7	4.76	0.04
Laying date	1,30.0	22.6	<0.001
Brood size	1,33.2	6.76	0.01
Treatment * Male age	1,36.2	8.97	0.004
Hatching order * Male age	1,21.5	0.04	0.85
Hatching order * Treatment	1,21.2	4.35	0.04
Hatching order * Treatment * Male age	1,21.6	5.29	0.03

Table 2. Daily body mass growth (grams/day) of first- and second- hatched chicks until age 15 days. Note: Mixed models were used for the analysis, with the identity of the nest included as a random effect (P = 0.02).

n = 49 chicks	df	F	P
Hatching order	1,10.2	0.06	0.81
Treatment	1,37.6	9.49	0.003
Male age	1,38.2	2.65	0.11
Laying date	1,33.5	10.85	0.002
Treatment * Male age	1,38	8.83	0.005
Hatching order * Male age	1,10.2	0.04	0.85
Hatching order * Treatment	1,10.2	4.95	0.04
Hatching order * Treatment * Male age	1,10.2	6.62	0.02

Table 3. Daily ulna growth (mm/day) of first- and second- hatched chicks until age 15 days. Note: Mixed models were used for the analysis, with the identity of the nest included as a random effect ($P < 0.001$).

Fig. 1

Fig 2.

Fig 3.

Male age (years)
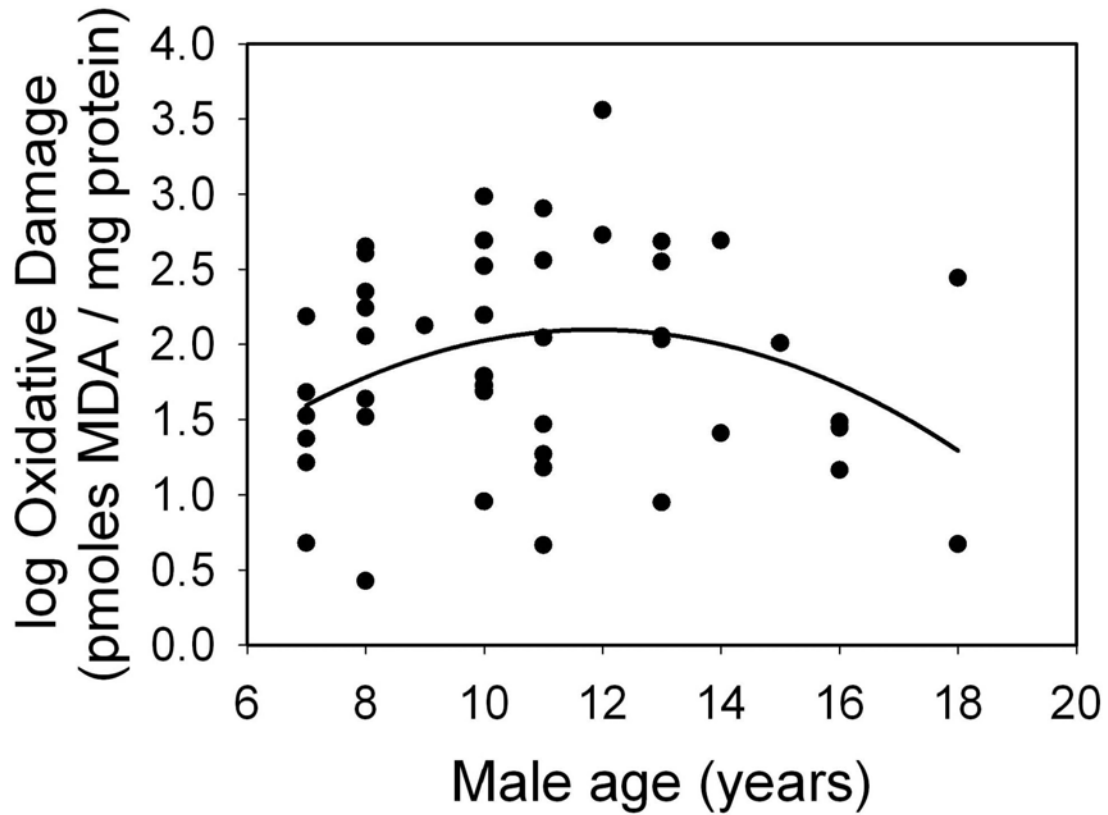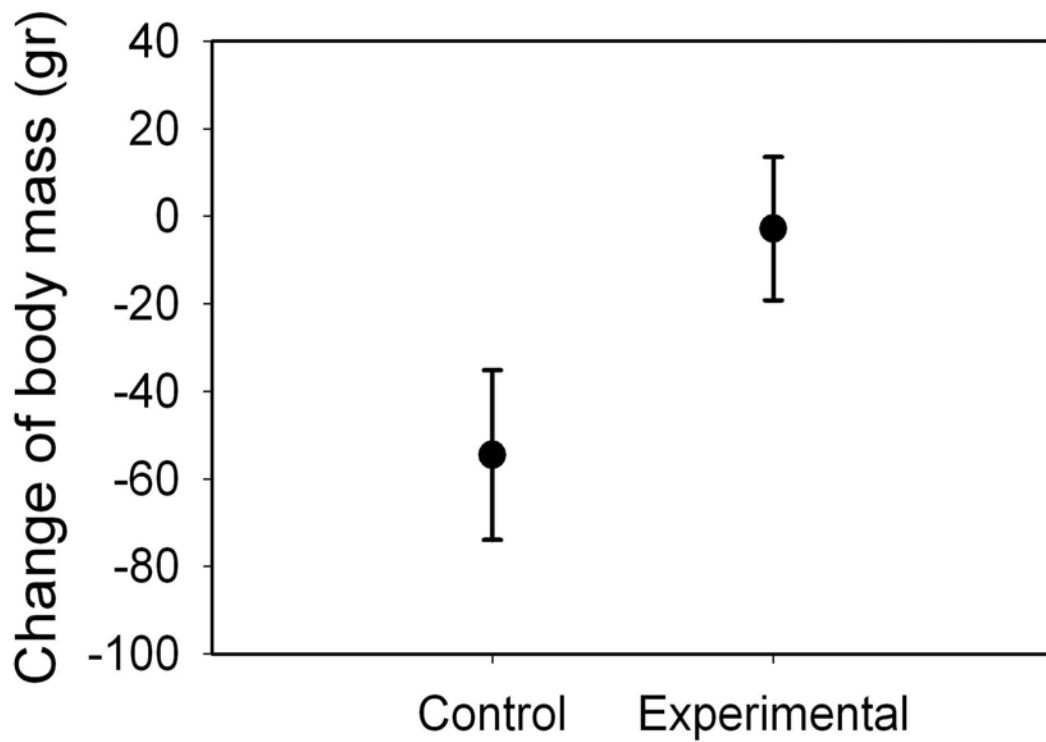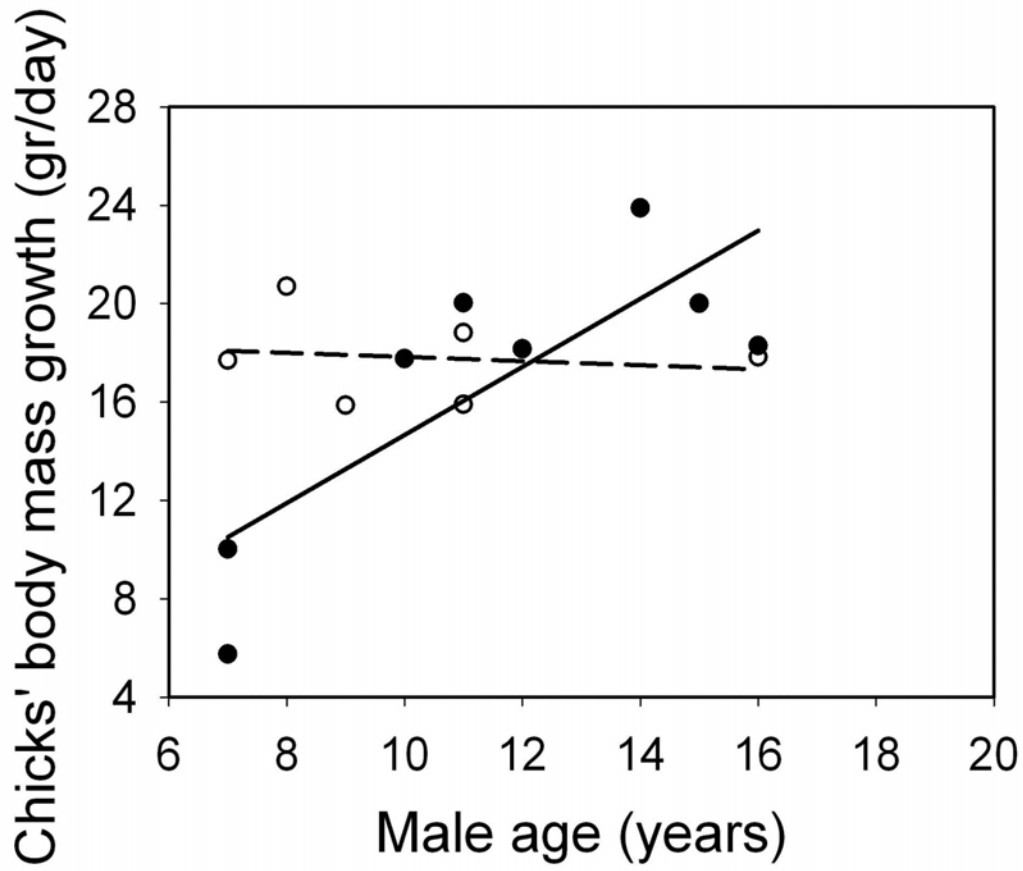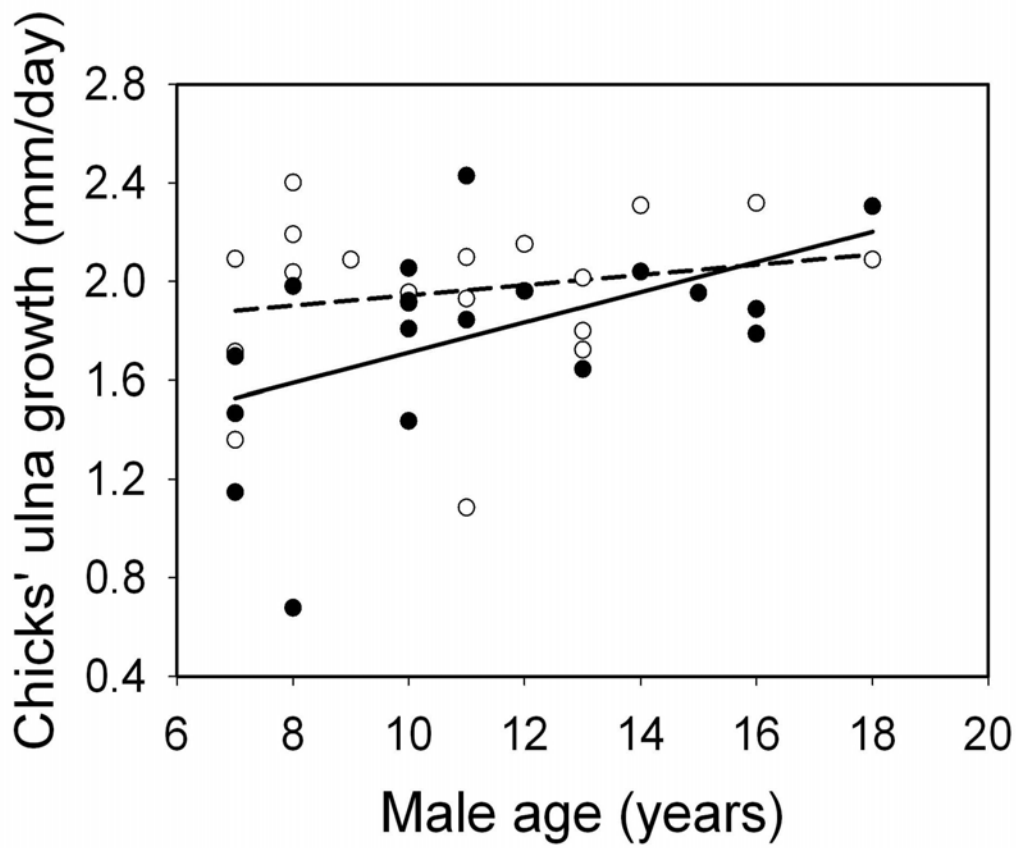
Fig 4.

Capítulo 4

Efecto de la edad en la conducta de cortejo

Love at older ages: courting behaviour in old blue-footed boobies

Love at older ages: courting behavior in old blue-footed boobies

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Keywords: aging, senescence, courtship, *Sula nebouxii*

Manuscript

Abstract

Aging has been highlighted as an important selective force shaping the mating preferences and breeding strategies of animals. Animals should discriminate against old mates when individual quality deteriorate with age and this should affect mate choice and courtship behavior. Here we registered courtship behavior of known-age pairs in blue-footed boobies, a socially monogamous long-lived seabird. Firstly, we assessed if rates of courtship and copulation and presence at the breeding territory are affected by age and mate age. Secondly we tested if animals paired to old individuals are more prone to perform extra-pair behavior. Male and female rates of intra-pair courtship increased with age. Males displayed lower rates of courtship when paired to females older than nine years, but female intra-pair courtship was not affected by mate age. Females increased copulation rates and presence at the breeding territory with age whereas extra-pair courtship rates were not affected by age or mate age. Finally, reproductive success was positively associated to male age. Courtship behavior of old males could signal parental capacities or females increased their parental care when paired to males with high courtship rates. Previous studies showed old females have low reproductive performance, thus, low courtship rates toward old females might represent discrimination against poorly attractive mates. Females may compensate their poor attractiveness and guard their mates by increasing courtship, copulation and presence at the territory. Our results showed differences between sexes in courting behavior in relation to own and mate age and highlights the role of aging on shaping the breeding strategies of animals during courtship.

Introduction

Aging has been highlighted as an important selective force shaping the mating preferences and breeding strategies of animals (Hansen and Price 1995, Brooks and Kemp 2001). Recent studies on wild populations have clearly shown that animals experience a decline in reproductive performance and survival probabilities at old ages (Nussey et al. 2006, McCleery et al. 2008, Reed et al. 2008, Jones et al. 2008, Sharp and Clutton-Brock 2010). Consequently, animals should discriminate against old mates because the reproductive value and quality of an individual as a mate decreases at advanced ages (Beck et al. 2002, Beck and Promislow 2007). In particular, for monogamous long-lived species it is expected that age have a profound effect on the rates of courtship behavior and promiscuity of both the old animals and their mates but the effect of age on courtship behavior has rarely been addressed (Hoikkala et al. 2007). Here, we observed intra- and extra-pair courtship behavior of known-age pairs in a long-lived socially monogamous seabird and provide evidence on how male and female ages influence the rates of courtship behavior.

The role of age on breeding strategies has long been recognized (Manning 1985, Hansen and Price 1995). Some theoretics have proposed that animals should prefer to mate with old individuals because they possess genes that proved ability to live long and reproduce many times (Kokko and Lindström 1996) or because their sexual signals are more reliable (Proulx et al. 2002). Evidence supporting this view derives from studies on short-lived species or comparisons between juveniles and older animals. For instance, in the yellow hammer *Emberiza citrinella* and Bullock's orioles *Icterus galbula* females preferred males older than two years as extra-pair sires (Sundberg and Nixon 1996, Richardson and Burke 1999). Moreover, males older than two years have more complex vocal displays in the willow warbler *Phylloscopus trochilus* whereas in the blue tit *Parus caerulues* adult bird plumage is more chromatic and brighter compared to yearling birds (Gil et al. 2000, Delhey and Kempenaers 2006). However, recent models have challenged the preference for older mates by

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suggesting that animals should discriminate negatively to old mates when condition or quality deteriorates with age, as occur during senescence (Beck et al. 2002, Beck and Promislow 2007). In short lived species it has been shown that old hide beetle *Dermestes maculatus* has lower fertilization and competitive capacities than middle-age and females prefer middle-age mates and discriminate against old individuals in field cricket *Ephippiger ephippiger* and lekking sandfly *Lutzomya longipalpis* (Ritchie et al. 1995, Jones et al. 2000). In long-lived species, compared to younger mates, old males show duller sexual ornaments and lower quality sperm in blue-footed boobies *Sula nebouxii* (Velando et al. 2010, Velando et al. *in press*) but there is no information on whether females discriminate against old mates. Finally, in long-lived monogamous species it is necessary to assess whether the rates of courtship behavior and promiscuity vary for each individual depending on the own and their mate age.

During courtship animals show stereotyped behaviors and ornamental displays to inform about their individual quality and to gather cues on the quality of potential mates (Schneider and Lesmono 2009). Despite being more experienced breeders, older animals are expected to have a reduced physical condition, which may negatively affect their foraging capacities (Catry et al. 2006, Lecomte et al. 2010), immune response (Palacios et al. 2007), investment in sexual ornaments (Torres and Velando 2007) as well as courtship behavior. In species that show sexual ornaments and behavioral displays during courtship, colorful carotenoid-dependent ornaments might be more costly to maintain in old organisms because their antioxidant capacity is low (Alonso-Alvarez et al. 2006, Devevey et al. 2010). Consequently old individuals could partially compensate a decline in sexual attractiveness by increasing their rates of courtship behavior. Therefore, an increase or a decrease in the rates of courtship behavior at increasing age is possible.

An important side-effect of reproductive senescence is that individuals paired to senescent mates will have lower reproductive success (Alatalo et al. 1986, Price and Hansen 1998, Jones et al.

2000). The low reproductive value and the deteriorated physiological functioning should favor the evolution of mechanisms to detect and discriminate against old mates, or to follow alternative breeding strategies when paired to an old mate (Beck and Promislow 2007). For instance, older animals should receive less extra-pair courtship and gain less extra-pair copulations whereas animals paired with old mates are expected to look for more extra-pair interactions. If compared to young birds, germ line of old individuals has lower quality (either spermatozoa or ova) and lower chances of fertilization (Jones and Elgar 2004, Radwan et al. 2005, Velando et al. *in press*) then animals paired to old mates may benefit of engaging in extra-pair copulations to ensure fertilization and increase offspring viability.

In this study we assessed the effect of aging on courtship behavior in the blue-footed booby. The blue-footed booby (*Sula nebouxii*) is a long-lived seabird, socially monogamous and with a long period of biparental care (up to six months; Nelson 1978). Boobies have an active courtship period (up to 40 days) prior to egg laying where they show stereotyped behaviors directed toward the mate and defend small ground patches (Osorio-Beristain and Drummond 1998; Stamps et al. 2002). Mates increase the time spent together progressively until they are present roughly 6 hours on the 5 days before laying start. Similarly they start copulations on average 29 days before laying date and peak to 1.71 ± 0.64 copulations *per* day during the 5 days before egg laying (Osorio-Beristain and Drummond 1998). Male and female perform extra-pair courtship and copulations (Osorio-Beristain and Drummond 1998, Pérez-Staples and Drummond 2005) and there is evidence that extra-pair behavior decrease in the presence of their social mate (Osorio-Beristain and Drummond 1998, Pérez-Staples and Drummond 2005). Foot color is a condition-dependent sexually selected trait whose brightness intensity decreases with age (Velando et al. 2006a, Torres and Velando 2007) and both male and female decrease the intra-pair courting rates when their mate foot color is experimentally dulled (Torres and Velando 2003, 2005). In boobies, reproductive success increase with age until a maximum and then decline at older ages, with onset of reproductive senescence occurring in males after age 10 years

(Velando et al. 2006b) and in females after age 9 years (Beamonte-Barrientos et al. 2010). Egg size, chick growth rate and chick immune response is negatively related to female age (Beamonte-Barrientos et al. 2010). However, nothing is known about the age-dependent courtship patterns in blue-footed boobies. Here, we first evaluated the effect of individual and mate age on the rates of intra-pair courtship and copulation and on the presence at the breeding territory. Secondly, we tested the hypothesis that individuals are more prone to perform extra-pair behavior with increasing mate age. We predicted that extra-pair courtship and copulation rates will increase with mate age.

Methods

Field data was obtained in 2008 from the breeding colony of blue-footed boobies on Isla Isabel, in the Mexican Pacific (25° 52' N, 105° 54' W). All individuals included in the study were previously banded as part of a long-term study (details in Drummond et al. 2003). Since 1988, in every reproductive season (February to July) all breeding attempts in the study area are monitored until the end of the fledgling period (Drummond et al. 2003). Chicks are individually marked with plastic rings within 3 days after hatching and with numbered metal rings at fledging (age 70 days) enabling us to know the exact age of all individuals observed.

We observed courtship behavior within the boobies colony using canvas blinds in areas with active courting pairs and single adults. All marked pairs within 10 m of the blind were observed, provided they did not have an egg, were actively courting, and could be easily observed from the blind. Courting pairs were observed a single day from 7:00 to 11:00 and from 15:00 to 18:00 hrs, the periods of greatest courtship activities in this colony. Birds were considered a pair if both defended the same site (patch of ground), were observed courting together in two scans 7 days apart and established a clutch afterwards. We observed a total of 33 pairs where both male and female ages were known (age

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range for both males and females was 2-19 years old). Six pairs had previous experience reproducing together and 27 were reproducing together for the first time, however in preliminary analyses a pair's previous experience together did not affect courtship behavior (data not shown) and was not included in the final statistical models. Observed pairs did not show assortative mating by age ($r = 0.06$, $P = 0.70$) and the number of days between the observation session and the laying date was 13.0 ± 2.14 days. The mean laying date for pairs observed was January 29th, a date close to the mean laying date of the colony: January 16th (colony range of laying dates for the breeding season 2008 was: December 4th, 2007 to June 24th, 2008). The courtship displays recorded were sky-pointing (when the bird's neck was lengthened with the bill pointing skywards, accompanied by elevation of the tail and lateral spreading of the wings), parading (an exaggerated foot raising, flaunting the webs upwards and outwards) and twiglifting (when a bird picked up a fragment of any type of material and with an upward movement of the head in a smooth high arc deposited it near the feet of the courting bird; Nelson 1978). Copulations were recorded whenever the two birds' cloacae came into contact or, when these were out of sight, the female rotated and elevated her tail, and the male simultaneously rotated and lowered his tail then stepped down to the ground (Osorio-Beristain and Drummond 1998). We recorded all courtship displays and copulations and whether they were performed within the pair or with another bird (extra-pair mate). We registered the presence at the breeding territory for each bird of the focal pairs every five minutes (we did not register presence of extra-pair mates). Finally, to assess if the age-dependent courtship rates were related to pair's reproductive success we recorded the number of chicks that survived until age 70 days.

To ensure inter-observer reliability, each observer registered all behaviors alongside another observer for 120 min. During the first 30 min the two observers recorded behavior jointly, discussing their criteria; during the next 90 min they recorded independently, and then compared their records. The exercise was then repeated until there was a 90% agreement on the frequency of each behavioral

category.

We estimated the rate of intra-pair courtship and copulations as the sum of all within pair behaviors (sky-pointing, parading and nest material presentation) performed by each bird divided by the time spent with the mate at the breeding territory. To calculate the rates of extra-pair courtship and copulation rates we summed all behaviors directed to an individual different than the mate divided by the total time the bird was present at the breeding territory. For statistical analyses we included the age of the focal bird as continuous variable and the age of the mate as categorical variable (males: younger and older than 10 years according to Velando et al. 2006; females: younger and older than 9 years according to Beamonte-Barrientos et al. 2010). Results did not differ if we include mate age as continuous variable (data not shown). To analyze intra and extra-pair courtships rates, presence at the breeding territory and the rate of copulations we used general linear models separated for each sex. Male extra-pair and female intra-pair courtships displays were square root transformed to normalize the error distribution of models. To analyze the number of fledglings produced we used generalized linear models with Poisson distribution and included male and female age (linear and quadratic) and the rates of courtship as explanatory variables. Initial models analyzing courtship and copulation rates and presence at the breeding territory included the linear and quadratic terms of focal male or female age, mate categorical age, mate courtship rates, number of days between the observation and the laying date and the interaction between focal individual age (linear and quadratic) and mate age. In extra-pair behavior models only, we included the time that the mate spent at the breeding territory as explanatory variable because in boobies mate presence reduces extra-pair behavior (Perez-Staples and Drummond 2005). Non-significant terms were removed using backward deletion procedures. We used Cook's Distances to assess any influential data (Cook Value > 1) in all analyses performed, but no influential data was detected. All analyses were carried out on R 2.8.0 (R core development team). Results are expressed as Mean ± S.E.

Results

Presence at the breeding territory

Pairs were present together at the breeding territory for 257 ± 16.36 min (range: 86 to 436 min).

Females were present at the territory for 330.76 ± 17.15 min (range: 86 to 480 min) and males were

present for 363.78 ± 11.70 min (range: 211 to 475 min). The amount of time that pairs spent together at

the breeding territory was unrelated to male ($F_{1,30} = 0.05$, $P = 0.80$) or female age ($F_{1,30} = 2.56$, $P =$

0.11) but increased with closeness to the laying date ($\beta = 0.006$, $F_{1,31} = 6.84$, $P = 0.01$). Female total

presence at the breeding territory increased with age ($\beta = 0.01$, $F_{1,30} = 11.37$, $P = 0.002$), and with

closeness to the laying date ($\beta = 0.005$, $F_{1,30} = 4.12$, $P = 0.05$), but was unrelated to mate age ($F_{1,29} =$

0.01, $P = 0.90$). Male total presence at the breeding territory was unrelated to age ($F_{1,31} = 0.14$, $P =$

0.70), mate age ($F_{1,31} = 0.30$, $P = 0.58$) nor the number of days to the laying date ($F_{1,31} = 0.07$, $P =$

0.78). Quadratic terms of male and female age, the interaction between male/female age and mate age,

and courtship rates of mates did not affect the presence of individual males ($P > 0.32$) or females ($P >$

0.08) at the breeding territory nor the amount of time that pair members spent together ($P > 0.09$).

Intra-pair courtship and copulations

The intra-pair courtship rate of males increased with age ($\beta = 0.02$, $F_{1,30} = 7.56$, $P = 0.01$) and was

lower for females older than 9 years ($F_{1,30} = 4.61$, $P = 0.03$; Figure 1). The intra-pair courtship rate of

females increased with age ($\beta = 0.01$, $F_{1,31} = 6.58$, $P = 0.01$; Figure 2) and was unaffected by mate age

($F_{1,30} = 0.008$, $P = 0.92$). The intra-pair rate of copulations was positively related to female age ($\beta =$

0.19, $F_{1,31} = 7.16$, $P = 0.01$; Figure 2) and unrelated to male age ($F_{1,30} = 0.001$, $P = 0.99$). Quadratic

terms of male and female age, the interaction between male/female age and mate age, courtship rates of

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mates and the number of days to the laying date were not related to the intra-pair courtship rates of males ($P > 0.17$) and females ($P > 0.12$) nor to the intra-pair copulation rate ($P > 0.09$).

Extra-pair courtship and copulations

Extra-pair courtship rates were very low for females compared to males. Only two females displayed extra-pair courtship behaviors: one seven years old female and one two-years old female paired to males of fourteen and two-years old, respectively. In contrast, twenty-three males (70%) displayed extra-pair courtship behavior. The extra-pair courtship rate of males was unrelated to age ($F_{1,29} = 0.03$, $P = 0.85$) or mate age ($F_{1,30} = 0.10$, $P = 0.75$) and was negatively affected by female presence at the breeding territory ($\beta = -0.41$, $F_{1,31} = 15.34$, $P < 0.001$). Intra-pair courtship rate of females, number of days to the laying date, quadratic male age or the interaction between male and female age did not affect the extra-pair courtship rate of males ($P > 0.13$). We did not register any female extra-pair copulation and only two males engaged in extra-pair copulations (males of eight and nine years paired to females of nine and fourteen years old respectively).

Reproductive success

The rates of male ($z_{1,29} = 0.09$, $P = 0.73$) and female courtship behavior ($z_{1,30} = -0.65$, $P = 0.51$) were not related to the number of fledglings produced. Male age was positively associated with the number of fledglings produced (linear term: $\beta = 0.09$, $z_{1,31} = -2.40$, $P = 0.01$) and female reproductive success was not affected by age (linear term: $z_{1,28} = -0.12$, $P = 0.92$). Laying date, quadratic terms of male and female age and the interaction between linear and quadratic terms of male and female age were not related to the number of fledglings produced ($P > 0.11$).

Discussion

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This study documents the effect of age on the patterns of courtship behavior in a socially monogamous and long-lived seabird. Intra-pair courtship rates increased with age in both sexes but males courted less intensely when paired to females older than nine years. Additionally, the intra-pair copulation rate increased with female age. We found no effect of male or female age on rates of extra-pair behavior. This is the first evidence of age-dependent courtship behavior using old individuals of a long-lived seabird and suggests the occurrence of mating strategies followed by individuals depending on the own and mate age.

Different to other stages of boobies' reproduction (egg laying and chick rearing) that show decreased performance with increasing age (Beamonte-Barrientos et al. 2010), the rates of intra-pair courtship behavior increased with age in males and females. Decrease in courtship intensity with age after the age at peak of reproductive success was expected as a result of decreased physical condition, a common feature in old animals, that result in decreased foraging capacities and immune response at old ages (Catry et al. 2006, Palacios et al. 2007, Lecomte et al. 2010). Previous research conducted on flies *Drosophila montana* showed that courtship capacity declined with male age (Hoikkala et al. 2007) suggesting that senescence can affect negatively the courtship performance of old males. In contrast, rates of intra-pair courtship behavior in blue-footed boobies increased with age and did not decline at advanced ages. It might be possible that courtship performance increase with age because old birds are less attractive and need to display for longer to elicit a response from its mate. The courting displays of boobies include stereotyped behavior directed toward the mate and conspicuous displays of turquoise-colored foot webs (Nelson 1978, Torres and Velando 2003, 2005). Foot color is a condition-dependent sexually selected trait whose brightness intensity decline with age (Torres and Velando 2007, Velando et al. 2010). Reduced foot color can prejudice individual success, decreasing copulation probabilities as well as the chances of receive intra- and extra-pair courtship (Torres and Velando 2003, 2005) and

mate investment in parental care (Velando et al. 2006a, Dentressangle et al. 2008). In species that display sexual ornaments and courtship behaviors the investment in each display could be affected by age-specific physiological constraints. Many colorful ornaments depend on antioxidant pigments for expression (Hill and McGraw 2006, Pérez et al. 2008) and studies have found that antioxidant capacity decrease with age (Beckman and Ames 1998, Alonso-Alvarez et al. 2006, Devevey et al. 2010). Consequently, the capacity to maintain bright sexual ornaments is expected to decline with age (Velando et al. 2010). In contrast, in different species the assessment of the energetic expenditure during courtship displays has shown that, even when net metabolic rate increases during courtship, the increase is low compared to the daily energy expenditure (Benneth and Houck 1983, Dearborn et al. 2005). If for older animals courtship displays are cheaper to maintain than colorful traits then they could maintain high rates of courtship behavior even when foot color is low. Courtship displays prior to egg laying might be used by animals to evaluate mate quality and adjust their reproductive investment accordingly (Schneider an Lesmono 2009). Thus, older animals might partially compensate a decline in sexual attractiveness by increasing the rates of courtship behavior.

Although we found no relationship between the intra-pair courtship rate and reproductive success, we found a positive relationship of male age with both the intra-pair courtship rate and the number of fledglings produced. These results suggest that for old males in our study, the rate of courtship behavior was an honest indicator of their capacity to raise chicks until independence. Alternatively, the higher reproductive success of older males might be due to increased reproductive effort of females mated to males with high courtship rates (Sheldon 2000).

Male intra-pair courtship rate was lower when paired to females older than nine years compared to younger females but female intra-pair courtship rates was unrelated to male age. Theoretical models suggest that animals should discriminate against old mates when condition deteriorates with age (Beck et al. 2002, Beck and Powell 2007), thus reduced courtship rates toward older females might represent

discrimination against old and less attractive females. Senescent female boobies produce low quality eggs, raise chicks with low immune response and growth rates (Beamonte-Barrientos et al. 2010) which suggest that pairing to old females could be more costly to males than pairing to younger female boobies. Thus, males paired to old females could be less motivated to court its mate and consequently display low rates of intra-pair courtship behavior. In contrast, in our study old males produced more fledglings than younger males, thus female reproductive success could be penalized if they reduce courtship rates toward old males. Interestingly, the low courtship rates of males towards old females was independent of male age which means that old males courted less to old females than to younger females. Differences in the rates of courtship behavior in relation to the own and mate age between males and females suggests the occurrence of mating strategies that responded to age-related differences in reproductive performance between sexes.

The rate of copulation and presence at the breeding territory increased with female age but not with male age. In boobies copulation is under female control and forced copulations have never been reported (Osorio-Beristain and Drummond 1998). Then, old and poorly attractive females might increase their presence, courtship and copulation rates as a strategy to maximize their reproductive success. Female increase with age in presence at the breeding territory can reflect a mate-guarding strategy since in our study male extra-pair courtship rate decreased with female presence at the breeding territory and a previous study on boobies found male-extra pair copulations to decrease with female presence (Osorio-Beristain and Drummond 1998, Perez-Staples and Drummond 2005). Old females could increase their copulation rate as a mate guarding strategy, to assure their ova fertilization or to increase mate parental care (Hunter et al. 1993). By increasing the number of copulations, old female boobies might decrease the male's opportunities to copulate with other females and reduce the chances of male mate switching. Male discrimination against old females is expected because of the low reproductive value and attractiveness of those mates (Beck and Promislow 2007). Additionally,

females should copulate repeatedly to obtain young sperm from males since ova fertilization by old sperm reduces hatching success and offspring quality (Siva-Jothy 2000, Wagner et al. 2004). If the structures responsible for maintaining sperm quality after copulation in females deteriorate at old ages (den Boer et al. 2009), females might be selected to increase their copulation rate in order to decrease the amount of time that ova fertilizing sperm is stored within the genital structures. Additionally, the increased rates of copulation might be used by females to increase paternity confidence and obtain good paternal care to its offspring by their mates. Thus, female increase in rates of copulation with age can reflect a physiological constraint in sperm management, mate-guarding behavior, or an adaptive strategy whereby senescent female boobies increase the parental care provided by their mates.

Extra-pair courtship and copulation rates were not affected by age. A previous study in blue-footed boobies showed high levels of extra-pair courtship and copulations and found higher extra-pair behavior in males compared to females (Pérez-Staples and Drummond 2005). We registered very low levels of extra-pair copulations but found clear differences in the occurrence of extra-pair courtship between sexes with males being more promiscuous than females. Differences in observation time between studies and the fact that boobies show low levels of extra-pair copulation rates (0.5 extra-pair copulations per day; Osorio-Beristain and Drummond 1998) could prevent us to register extra-pair copulations. Thus, in our study there is no evidence that animals paired to old mates seek more extra-pair interactions.

In conclusion, blue-footed booby males and females increased their courtship behavior displays with age and only males decreased their courting behavior toward old mates. Increased courtship rates could reflect an adaptive strategy whereby individuals compensate low foot color attractiveness, particularly important in old dulled foot color animals. Increase in presence and copulation rates of old females suggest the occurrence of mate-guarding behavior or a strategy to increase mate paternity certainty and male parental care. Our results showed differences between sexes in courting behavior in

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relation to own and mate age and highlights the role of aging on shaping the breeding strategies of

animals during courtship. Because reproductive value changes with age, the occurrence of mating

strategies in relation to own and mate age is expected to occur in iteroparous animals.

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Figure 1. Intra- pair courtship rates of males in relation to male and female age. Courtship rate was calculated as the total number of all within-pair displays of sky-pointing, parading and twiglifting (see methods) performed by focal males over the total time a pair was at the arena. Male courtship rate increased with male age and decreased with age of female but the interaction between male and female age was not significant (see results). Filled circles and solid line represent female partners younger than 9 years; open circles and dashed line represent female partners older than 9 years. Rates are expressed as number of behaviors per minute

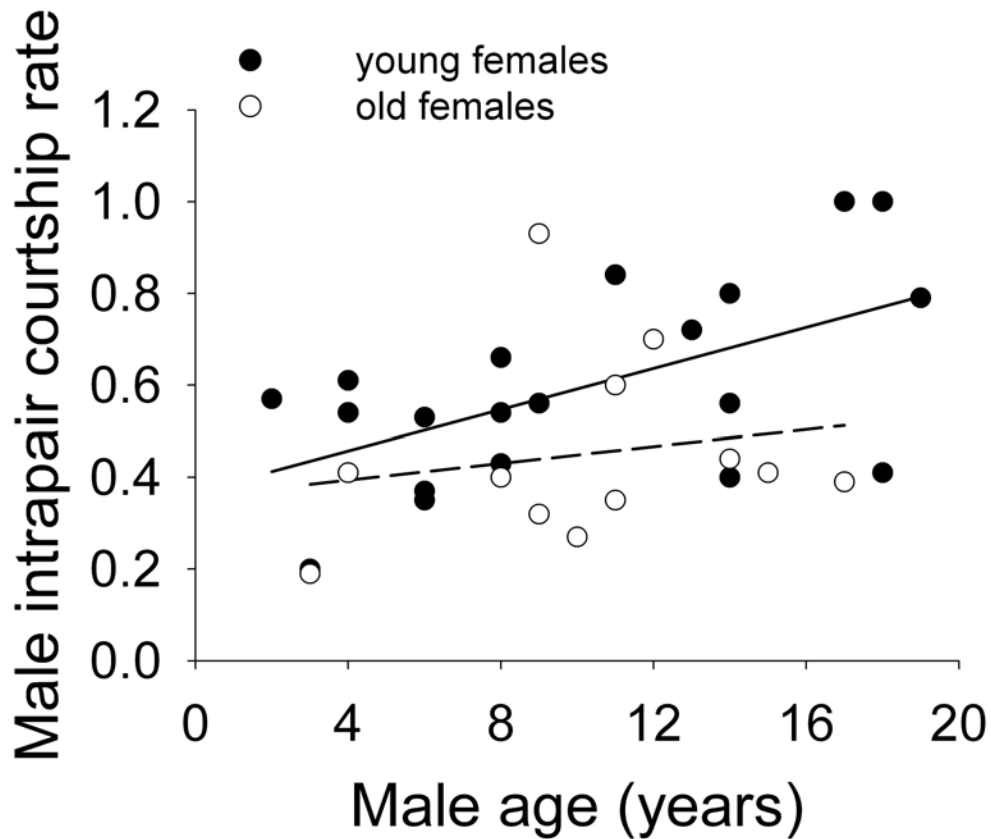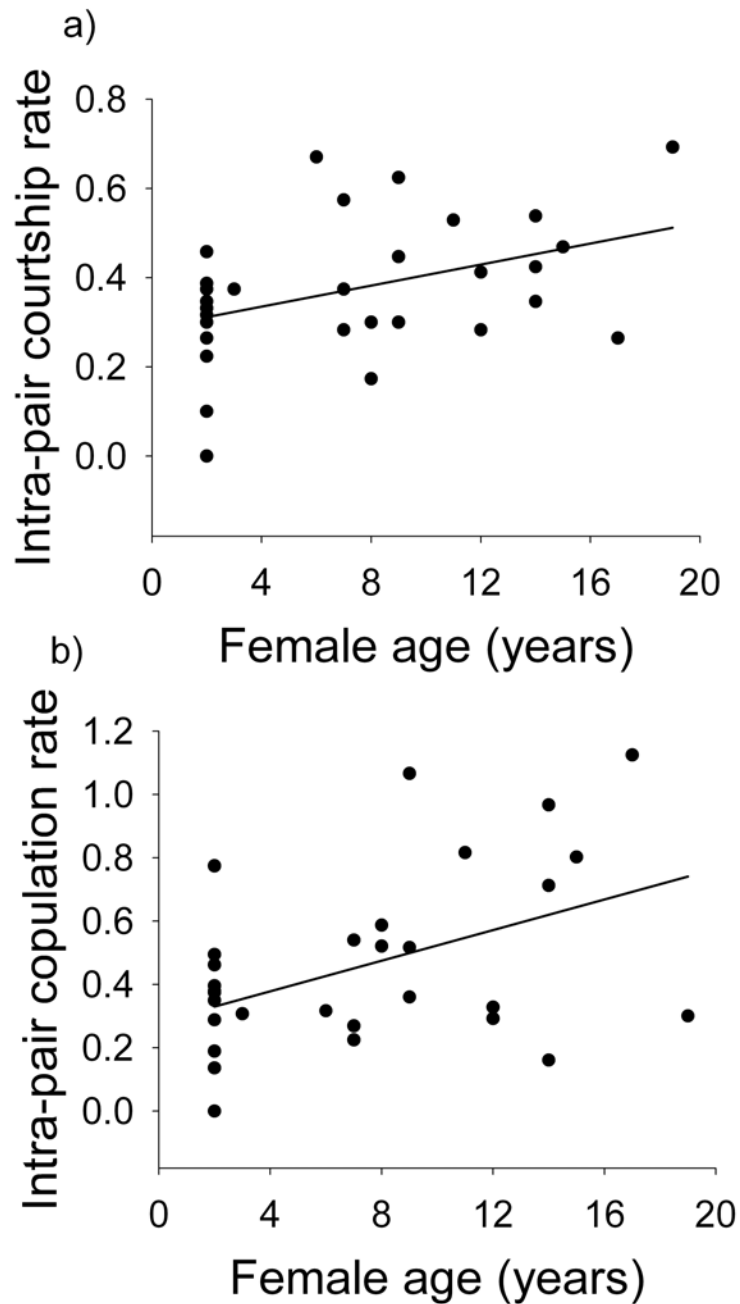Figure 2. Behavior in relation to female age. a) Intra-pair courtship rate of females in relation to female age. Courtship rate was calculated as the total number of all within-pair displays of sky-pointing, parading and twiglifting (see methods) performed by focal females over the total time a pair was at the arena (expressed as number of behaviors per minute). b) Intra-pair copulation rate in relation to female age. Copulation rate was estimated as the total number of copulations over the time a focal pair was at the arena (expressed as number of copulations per hour).

Capítulo 5

Discusión y Conclusiones

Discusión y conclusiones generales

Durante mucho tiempo se pensó que la senescencia en vida libre era un fenómeno difícil de observar debido a que la mortalidad extrínseca (*i.e.* depredación, parasitismo, daño mecánico) eliminaba a la mayoría de los organismos de la población antes de alcanzar edades donde se detectara senescencia (Weismman 1889, Medawar 1952, Williams 1957). Al principio de este proyecto el número de publicaciones sobre la senescencia en animales silvestres era muy limitado, sin embargo de 2006 a la fecha se ha incrementado el número de publicaciones donde se analizan los cambios en el desempeño reproductivo y probabilidad de supervivencia en relación a la edad (revisado por Jones et al. 2008). Se ha documentado el efecto de la senescencia sobre las características sexuales secundarias, la capacidad de forrajeo, capacidad inmune, capacidad antioxidante, daño oxidativo, calidad de esperma y producción hormonal en poblaciones silvestres (Catry et al. 2006, Angelier et al. 2007, Palacios et al. 2007, Torres y Velando 2007, Bouwhuis et al. 2009, Nussey et al. 2009, Velando et al. 2010a y b, Lecomte et al. 2010). Inclusive se han publicado estudios teóricos de cómo deberían invertir los animales en la reproducción a edades avanzadas en función del daño somático (McNamara et al. 2009), y cómo las preferencias de apareamiento deberían ser afectadas cuando la calidad individual disminuye con la edad (Beck y Promislow 2007, Velando et al. 2008). Por lo tanto, la acumulación de información en poblaciones de vida libre deja ahora muy claro que la senescencia es un fenómeno fundamental y ampliamente distribuido en poblaciones de aves y mamíferos silvestres con historias de vida tan distintas como las aves paserinas de corta vida y con alta tasa reproductiva, y las ave marinas de larga vida con baja tasa reproductiva. El estudio de la senescencia en poblaciones silvestres y desde una perspectiva evolutiva es un área de investigación muy prometedora que nos permitirá profundizar en nuestro conocimiento de la evolución de las historias de vida, estrategias reproductivas, conducta social y mecanismos de deterioro a edades avanzadas en animales.

El objetivo de esta tesis fue estudiar la senescencia en una población silvestre del bobo de patas azules *S. nebouxii.* En este proyecto, nos propusimos evaluar el efecto de la senescencia sobre componentes del ciclo reproductivo y la fisiología de estas aves marinas de larga vida. En primer lugar, analizamos datos longitudinales de tres cohortes para determinar como el éxito reproductivo de hembras que sobrevivieron al menos 14 años cambiaba a lo largo de su vida. En segundo lugar, evaluamos los efectos de la senescencia en la calidad del huevo y la capacidad de crianza de las hembras. Posteriormente, evaluamos si los carotenoides, que los bobos de patas azules obtienen únicamente a través de la dieta, son un factor limitante en la reproducción de los machos viejos. Finalmente, evaluamos si la conducta de cortejo de machos y hembras varía con la edad y si modifican el cortejo intra y extra-pareja en función de la edad de su pareja.

Senescencia reproductiva y variaciones entre cohortes en la tasa de senescencia

El éxito reproductivo de las hembras que sobrevivieron al menos 14 años aumentó con la edad hasta alcanzar un máximo a los 8 años y posteriormente declinó de forma significativa, un patrón similar al encontrado en machos (Velando et al. 2006a). El patrón de éxito reproductivo en el bobo de patas azules es similar al encontrado en otras especies de aves y mamíferos (Jones et al. 2008). En los estudios de Bennett y Owens (2002) y Jones et al. (2008) se registró senescencia reproductiva en 18 de 23 especies de aves y en 5 de 6 especies de mamíferos; de las cinco especies de aves donde no se detectó, tres pertenecen al grupo de las aves marinas: *Sterna paradisae, Fulmarus glacialoides* y *Larus occidentalis.* Este hecho en sí es muy interesante, pues existen diferencias importantes entre mamíferos y aves en cuanto a su fisiología que podrían repercutir en el deterioro somático acumulado e inicio de la senescencia reproductiva (Constantini 2008). Por ejemplo, si comparamos un ave y un mamífero de similar tamaño, la tasa metabólica es mayor en las aves pero la generación de radicales libres por consumo de oxígeno es menor en aves que en mamíferos (Vleck et al. 2007, Constantini 2008).

Además, las aves tienen menor tasa reproductiva y tiempo de generación que un mamífero de similar tamaño (Jones et al. 2008). El estudio de Jones et al. (2008) mostró que para las especies donde sí se encontró senescencia reproductiva la edad en que ocurre el inicio está relacionada con la velocidad de la historia de vida (edad de maduración, tiempo generacional, número de progenie producida por evento reproductivo). Por lo tanto, la lenta historia de vida de las aves está asociada a la evolución de mecanismos fisiológicos que les permiten alcanzar edades muy avanzadas y esto dificulta la capacidad para detectar senescencia reproductiva en algunas especies de aves marinas.

Interesantemente, la tasa de declive fue diferente entre las tres cohortes analizadas. Desconocemos por qué existen diferencias en la tasa de senescencia entre cohortes, sin embargo, en los bobos de patas azules de Isla Isabel, el éxito reproductivo y la supervivencia se ven afectados por variaciones en la temperatura superficial del mar, un indicador de la productividad marina (Oro et al. 2010, Ancona et al. 2011), por lo tanto es posible que las condiciones experimentadas durante el crecimiento y etapas tempranas de la vida tengan efectos a largo plazo en la calidad de los individuos y por lo tanto en su productividad. Estudios en pinzones zebra *T. guttata* han mostrado que el desempeño reproductivo a lo largo de la vida se ve afectado por las condiciones que los animales experimentan durante el desarrollo temprano (Gorman y Nager 2004, Naguib et al. 2006) por lo que esta posibilidad no puede descartarse.

Senescencia en el huevo y la crianza

En aves, y en particular en bobos de patas azules, se ha observado que el tamaño del huevo está relacionado con su probabilidad de eclosión y tamaño de las crías al eclosionar (Williams 1994, Christians 2002, D'Alba y Torres 2007). Estudios previos en aves silvestres mostraron que el tamaño de los huevos disminuye con la edad (Sydeman y Emslie 1992) y por otro lado, que las crías de hembras viejas son más pequeñas al emplumar, lo que sugiere un efecto negativo de la edad sobre la

calidad de los huevos y la capacidad de crianza (Saino et al. 2002). Sin embargo, ningún estudio en aves había realizado un experimento para separar los efectos pre y post-natales en el éxito reproductivo de hembras de distinta edad. Nuestro experimento de intercambio de puestas entre hembras de diferente edad permitió evaluar si el crecimiento y supervivencia de la progenie está relacionado con efectos de la edad de la madre que influyen en la calidad del huevo (efectos pre-natales) o la calidad de la crianza (efectos post-natales), lo cual permite entender en más detalle que procesos afectan el desempeño reproductivo de las hembras senescentes. Encontramos que las hembras viejas pusieron huevos más pequeños que produjeron crías más pequeñas hasta el día 5, y además dieron lugar a segundos pollos con menor tasa de crecimiento hasta el día 30. Por otro lado, durante la crianza las hembras viejas produjeron segundas crías con menor crecimiento esquelético hasta el día 30 y menor respuesta inmune al día 20. No se detectaron efectos de la edad de la hembra que puso el huevo o la que cuidó de las crías en el tamaño de los pollos al emplumar. Interesantemente, el éxito reproductivo de las hembras viejas fue bajo cuando cuidaron huevos de hembras viejas en comparación con hembras viejas que cuidaron huevos de hembras maduras. Esto último tiene implicaciones importantes para entender la asignación de recursos en la reproducción a edades avanzadas (ver sección *Senescencia reproductiva e historias de vida*). La baja tasa de crecimiento y respuesta inmune de los pollos podría tener efectos negativos en la edad adulta, afectando la edad de reclutamiento o disminuyendo el atractivo sexual, competitividad, y éxito reproductivo a lo largo de la vida (Gustaffson et al. 1995, Gorman y Nager 2004 , Royle et al. 2005). De hecho, estudios recientes en el bobo muestran que las crías de padres viejos tienen una menor probabilidad de reclutar a la población reproductiva (Torres R, Drummond H y Velando A. en prep.), lo que sugiere efectos a largo plazo de un pobre desarrollo temprano bajo el cuidado de un progenitor viejo. Estos resultados muestran que el efecto negativo de la edad sobre la calidad de los huevos y la capacidad de crianza de las hembras viejas repercute en la producción de emplumados y reclutamiento de la progenie.

Sin embargo, estos resultados dejan algunas preguntas por responder. Sería interesante investigar cómo varía la composición de los huevos entre hembras de diferente edad y qué componentes del cuidado post-natal son afectados por la senescencia (frecuencia de alimentación, cantidad de alimento, o duración y efectividad de los viajes de forrajeo). Por otro lado sería también interesante investigar si las diferencias en los efectos pre- y post- natales de hembras de diferentes edades influyen en el sindrome conductual de las crías. En un estudio previo, se encontró que tanto la respuesta inmune como la agresividad de las crías del bobo de patas azules están relacionadas positivamente con la concentración de androstenediona que la hembra deposita en el huevo (Dentressangle 2009). Consecuentemente, la variación dependiente de la edad en hormonas, antioxidantes u otras moléculas importantes puede influir en el desarrollo, competitividad y agresividad de la progenie. En el bobo de patas azules las crías compiten por el alimento y generalmente la primer cría domina a la segunda mediante el uso de gritos, mordidas y picotazos (Drumond et al. 1986). Cuando el crecimiento de la cría dominante es 20-25% debajo de lo esperado la agresión hacia la cría subordinada se incrementa considerablemente y la probabilidad de fratricidio también (Drummond et al. 1986, Drummond y García-Chavelas 1989). Si la provisión de alimentos en nidos de hembras viejas es pobre se esperaría que aumentara la agresión de las crías dominantes hacía sus hermanos. Por otro lado, estudios recientes en albatros han mostrado que los animales más viejos tienen viajes de forrajeo más largos que animales jóvenes pero además cuando regresan a la colonia los animales viejos son menos pesados que los más jóvenes, lo cual sugiere que el decline en el desempeño reproductivo de los albatros viejos está ligado a una pérdida en las capacidades de forrajeo (Catry et al. 2006, Lecomte et al. 2010). No existe evidencia al respecto de cómo varía la conducta de forrajeo en el bobo de patas azules en relación a la edad, sin embargo, sería muy interesante entender a un nivel de detalle más fino el por qué las hembras viejas crían pollos con menores tasas de crecimiento y respuesta inmune.

¿Pueden los suplementos de carotenoides atenuar los efectos de la senescencia?

En el bobo de patas azules el suplemento de carotenoides tuvo un efecto positivo en la condición de los machos y el crecimiento de las crías de los machos viejos. Independientemente de la edad, los machos que recibieron carotenoides durante la última semana de la incubación perdieron menos peso que los machos del grupo control. Además, la tasa de crecimiento de las crías fue mayor en machos viejos que recibieron carotenoides que la de crías de machos viejos del grupo control.

Los carotenoides son substancias que los animales no pueden sintetizar *de novo* y tienen que obtener a través de la dieta. Nosotros evaluamos la hipótesis de que los carotenoides son recursos limitantes para la reproducción de los machos viejos, debido a que los animales viejos tendrían menor capacidad para lidiar con el estrés oxidativo (Beckman y Ames 1998, Alonso-Alvarez et al. 2006, Devevey et al. 2010; Velando et al. 2006a, Torres y Velando 2007). En nuestro estudio no encontramos un efecto del suplemento de carotenoides en la capacidad antioxidante o el daño oxidativo de los machos, y tampoco se detectó un efecto diferencial del tratamiento con la edad de los machos en el estatus oxidativo. Es posible que el efecto positivo de los carotenoides en el peso y reproducción sea independiente de cambios en el estatus oxidativo. Actualmente existe debate al respecto del papel antioxidante de los carotenoides *in vivo* (Alonso-Alvarez et al. 2004, Hõrak et al. 2007, Constantini y Møller 2008). Alternativamente, es posible que los machos viejos pudieron incrementar su esfuerzo reproductivo sin consecuencias en el estatus oxidativo a causa del efecto antioxidante de los carotenoides extra en su dieta. Por lo tanto, aunque su papel como antioxidantes no es claro, los resultados sugieren que un incremento en la disponibilidad de carotenoides influye positivamente en la condición de los machos, independientemente de su edad, y en la reproducción de los machos viejos.

Los resultados de este experimento aportan evidencia al respecto de los posibles mecanismos que subyacen a las variaciones en la tasa de senescencia entre los organismos. En muchos estudios se ha documentado efectos positivos de los carotenoides, independientes de la edad, en las señales

sexuales, sistema inmune, cuidado parental o capacidad antioxidante. (Lozano 1994, Olson 1998, von Schantz et al. 1999, Blount et al. 2004, Biard et al. 2005, Alonso-Álvarez et al. 2004, Hill y McGraw 2006, Velando et al. 2006b, Hŏrak et al. 2007, Pike et al. 2007, Safran et al. 2010, Remeš et al. 2007). La variación en la adquisición de carotenoides podría tener repercusiones importantes en la historia de vida de los animales viejos. Por ejemplo, machos viejos que obtengan más carotenoides durante el cortejo podrían tener ornamentos más atractivos y lograr una mayor inversión de su hembra en la puesta, aumentando de esta forma la probabilidad de eclosión y éxito de emplumado (Velando et al. 2006b, Dentressangle et al. 2008). Esto implica que la fluctuación en la disponibilidad de recursos, y por ende la adquisición de carotenoides, a lo largo de la vida de un individuo tendrá consecuencias importantes en la tasa de la senescencia reproductiva.

¿Los bobos de patas azules modifican el cortejo con la edad?

La edad afectó las conductas de cortejo del bobo de patas azules. Los machos aumentaron su tasa de cortejo intra-pareja con la edad pero cortejaron menos a hembras mayores a nueve años. Algunos estudios teóricos han propuesto que los animales deberían evitar aparearse con animales viejos cuando la calidad individual disminuye con la edad (Beck et al. 2002, Beck y Promislow 2007). Por lo tanto, una menor tasa de cortejo intra-pareja hacia hembras viejas podría ser una forma de discriminación hacia hembras senescentes que ponen huevos de menor calidad y proveen de cuidado materno de menor calidad (Beamonte-Barrientos et al. 2010). Para nuestro estudio se incluyeron solamente parejas establecidas, sin embargo, sería muy interesante determinar si esta discriminación se extiende hasta la formación de las parejas. En la población del bobo de Isla Isabel se ha registrado que los animales más viejos se establecen con mayor frecuencia en etapas tardías de la temporada reproductiva (Peña-Álvarez, 2008) y durante nuestras observaciones encontramos una buena cantidad de animales solitarios, que desplegaban en busca de pareja. No sabemos si los animales viejos se establecen tarde

porque llegan tarde a la colonia o porque están desplegando y no consiguen aparearse. Sin embargo, sería de esperarse que durante la etapa de formación de pareja los machos y las hembras senescentes sean discriminados pues tal conducta tendría ventajas en términos de éxito reproductivo. Tal discriminación podría resultar en que los animales viejos se establezcan tarde en la colonia, cuando hay menos recursos, y además lo hagan con parejas de mala calidad lo cual resultaría en éxito reproductivo bajo, que es lo que ocurre en promedio en la población (Velando et al. 2006a, Beamonte-Barrientos et al. 2010).

Las hembras aumentaron con la edad su tasa de cortejo y cópulas intra-pareja así como su presencia en el territorio reproductivo. Es decir que al pasar mas tiempo en el territorio las hembras podrían estar aumentando con la edad el resguardo de pareja. En el bobo de patas azules, estudios previos en los que no se consideró la edad de los individuos sugieren que las hembras apareadas con machos que despliegan cortejo extra-pareja aumentaron su cortejo intra-pareja hasta 4 veces, además de que la presencia de la hembra disminuyó el despliegue de conducta extra-pareja en los machos (Osorio-Beristain y Drummond 1998, Pérez-Staples y Drummond 2005). Si las hembras viejas son menos atractivas, como sugiere la menor tasa de cortejo que reciben, incrementar el resguardo de pareja podría disminuir la probabilidad de que su macho incurra en actividades extra-pareja, cambie de pareja o le transmita algún parásito adquirido durante las cópulas extra-pareja. Además del resguardo de pareja, nosotros propusimos que el aumento en la tasa de cortejo intra-pareja podría ser seleccionado para compensar una falta de atractivo a edades avanzadas, pues se ha reportado que en machos el color de las patas disminuye en intensidad al aumentar la edad (Velando et al. 2010). Desafortunadamente nosotros no medimos el color de patas de los animales para el estudio de cortejo, por lo que un estudio futuro podría evaluar esta hipótesis al cuantificar tasas de cortejo y medir el color de las patas en animales de edad conocida. Finalmente, es necesario mencionar que muy pocos estudios han abordado el tema de las variaciones en la conducta de cortejo con la edad (Hoikkala et al. 2009), sin embargo

este tipo de estudios es necesario ya que nos permiten poner a prueba las teorías que predicen

discriminación hacia los animales viejos (Brooks y Kemp 2001, Beck y Promislow 2007) después de

que se forman las parejas. Este tipo de estudio nos permitirán evaluar la posible evolución de

conductas discriminatorias y estrategias alternativas de las parejas de animales senescentes.

Senescencia reproductiva e historias de vida

La ecología evolutiva explica la senescencia como resultado de compromisos de historia de vida

(Stearns 1992). En particular, la teoría del soma desechable establece que los recursos utilizados para

el mantenimiento somático compiten con aquellos utilizados para la reproducción, y por lo tanto la

reproducción conlleva un daño que a la larga da lugar al fenotipo senescente (Kirkwood 1991). Los

modelos que analizan la asignación óptima de recursos entre reproducción y mantenimiento a lo largo

de la vida predicen por un lado, que los animales deberían aumentar con la edad su asignación de

recursos a la reproducción y disminuir aquellos asignados al mantenimiento (Cichoń 2001). Bajo este

modelo, la disminución en el éxito reproductivo a edades avanzadas resultaría del hecho que los

individuos viejos están limitados en sus capacidades reproductivas por el deterioro fisiológico

acumulado (Cichoń 2001). Por otro lado, McNamara et al. (2009) proponen que los animales deberían

invertir en la reproducción en función del daño somático resultante y predicen que los animales viejos

reducen su inversión reproductiva porque su soma está deteriorado y obtienen mas daño por esfuerzo

reproductivo que animales jóvenes. Ambos modelos son capaces de predecir las diferentes estrategias

de historia de vida, pero difieren en su explicación de porque se espera una disminución en el éxito

reproductivo a edades avanzadas: limitación (Cichoń 2001) *versus* restricción (McNamara et al. 2009).

Si los animales viejos están limitados en su capacidad reproductiva (Cichoń 2001), se esperaría que

fueran incapaces de aumentar su pobre desempeño reproductivo ya que su inversión está al límite dada

su condición. Por otro lado, si los animales viejos restringen su inversión reproductiva (McNamara et

al. 2009) se esperaría que fueran capaces de aumentarla en caso de ser necesario.

Los resultados del intercambio de puestas entre hembras de diferentes edades (Capítulo 2) mostraron que las hembras viejas tienen un desempeño reproductivo bajo cuando cuidaron progenie de hembras viejas, en comparación con las hembras viejas que cuidaron huevos de hembra maduras (presumiblemente de mayor calidad) que tuvieron un mayor éxito reproductivo. Esto evidencia que las hembras viejas son capaces de ajustar positivamente su nivel de cuidado post-natal. En el experimento de suplemento de carotenoides (Capítulo 3) se observó que los machos viejos del grupo suplementado con carotenoides produjeron crías con mayor tasa de crecimiento que los machos viejos sin carotenoides, lo cual sugiere que también son capaces de aumentar el nivel de cuidado parental. Si los animales estuvieran limitados en sus capacidades reproductivas no podrían aumentar su nivel de cuidado parental. En cambio, estos resultados sugieren que en el bobo de patas azules, la estrategia a edades avanzadas de machos y hembras bajo condiciones naturales es de restringir su inversión reproductiva, pues son capaces de ajustar positivamente su nivel de cuidado parental en función de cambios en el valor de la progenie que cuidan (en hembras) o cambios en su condición corporal (en machos). Dicha estrategia reproductiva debería ser particularmente importante en organismos expuestos a fuertes fluctuaciones en los recursos, como ocurre en la colonia de bobos de patas azules de Isla Isabel (Ancona et al. 2011) y podría además tener implicaciones importantes en la variación individual en las tasas de senescencia.

Conclusiones generales

En conclusión, en el bobo de patas azules la edad tiene un efecto sobre las conductas de cortejo, calidad de los huevos, cuidado postnatal y éxito reproductivo. Los machos viejos aumentan su tasa de cortejo intra-pareja con la edad pero despliegan menos hacia hembras viejas. Las hembras viejas mostraron mayor tasa de cortejo y cópulas intra-pareja, además de estar presentes más tiempo en el territorio

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reproductivo. Esto sugiere discriminación de machos hacia hembras viejas y mayor resguardo de

pareja de las hembras viejas y apoya propuestas teóricas al respecto de evitar aparearse con animales

viejos. La senescencia afecta la calidad del huevo y cuidado de las crías de las hembras por lo que la

disminución en el éxito reproductivo es el resultado de un pobre desempeño a lo largo del ciclo

reproductivo. Las hembras viejas que cuidaron huevos de hembras maduras tuvieron mayor éxito

reproductivo en comparación de hembras viejas que cuidaron huevos de hembras viejas, lo cual sugiere

capacidad para ajustar los niveles de cuidado post-natal en función de cambios en el valor reproductivo

de la progenie. Durante el experimento de suplemento de carotenoides, los machos viejos que

recibieron carotenoides produjeron crías más grandes en comparación con machos viejos sin

suplemento. Por lo tanto en los machos viejos existe capacidad para ajustar el nivel de cuidado

parental en función de cambios en la condición corporal. En el bobo de patas azules la capacidad para

ajustar positivamente el nivel de cuidado parental en animales viejos sugiere que estos restringen su

inversión reproductiva pues si estuvieran limitados en sus capacidades no podrían aumentarlo. La

plasticidad en el cuidado parental en animales viejos de larga vida puede ser una estrategia adaptativa,

sobre todo en especies que dependen de recursos impredecibles.

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