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**Respuestas fisiológicas de las aves a
perturbaciones antropogénicas de su
hábitat.**

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Carlos Alberto Chávez Zichinelli

TUTOR PRINCIPAL DE TESIS: Dr. Jorge Ernesto Schondube Friedewold

**COMITÉ TUTOR: Dra. Katherine Renton
Dra. Laura Roxana Torres Avilés**

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RESUMEN

El cambio de uso de suelo (agricultura, ganadería urbanización) ha generado efectos negativos en los sistemas naturales. Estas transformaciones de los hábitats generan estímulos estresores en los animales provocándoles cambios a nivel fisiológico y conductual. Los estímulos estresores generan una condición de estrés, que de acuerdo al tiempo que se prolongue puede ser clasificado como agudo y o crónico. El estrés agudo es una respuesta a corto plazo, mientras que el estrés crónico genera efectos negativos a mediano y largo plazo que comprometen otras funciones fisiológicas como la respuesta inmune y la condición física. Las metas de este proyecto fueron 1) Determinar los niveles de estrés de tres especies de aves en hábitats con diferentes niveles de perturbación antropogénica. Las especies de estudio incluyen dos especies adaptables de ambientes urbanos (*Pipilo fuscus* y *Columbina inca*) y una especie explotadora de ambientes urbanos (*Passer domesticus*). 2) Establecer el tipo de estrés (agudo o crónico) generado por las perturbaciones antropogénicas mediante la asociación de la concentración de hormonas de estrés (corticosterona) y la respuesta inmune de las tres especies de aves. 3) Determinar los efectos de la corticosterona en la muda y la coloración estructural del plumaje, utilizando como modelo a *Volatinia jacarina*. Y 4) establecer las implicaciones que tiene la coloración estructural de plumaje como señal honesta en interacciones sociales y sexuales. Este último objetivo se llevo a cabo estudiando a *Passerina caerulea*. Encontramos que existe una relación entre el nivel de estrés de las aves y el grado de perturbación de los sitios, aunque las respuestas de las aves variaron de acuerdo a su historia natural. Para el caso de *Pipilo fuscus* y *Passer domesticus* en sitios con mayor perturbación antropogénica encontramos una relación negativa entre la corticosterona y la inmunoglobulinas, mientras que *Columbina inca* no tuvo una relación entre las dos variables fisiológicas, lo que sugiere que la perturbación antropogénica no está afectando a nivel de estrés crónico a esta especie. Los individuos de *Volatinia jacarina* con menores concentraciones de corticosterona tuvieron una muda reproductiva más avanzada, y una coloración estructural de plumaje más azul, que aquellas aves con mayor estrés. Esto indica que la corticosterona afecta tanto a la muda como a la coloración estructural de plumaje. Finalmente encontramos que estos cambios en la coloración estructural del plumaje asociados a estrés pueden tener efectos en la selección social (machos vs machos) y sexual (hembras vs machos) de las aves. Encontramos que los individuos macho de *Passerina caerulea* que tenían una coloración de plumaje menos intensa preferían percharse con individuos de coloración similar que con individuos de coloración más intensa, que son más dominantes. En el caso de selección sexual no hubo diferencias en la selección por parte de las hembras, por machos de coloración más intensa. Para esta especie la coloración de plumaje estructural parece tener un papel más importante a nivel de dominancia social que en la selección sexual. De acuerdo a nuestros resultados, la corticosterona cuando funge como un elemento regulador ante el estrés crónico genera efectos negativos en los individuos, por lo que altera otras funciones fisiológicas como el sistema inmune y en la condición física determinada en la coloración de plumaje y esta a su vez a nivel ecológico.

CAPÍTULO I

INTRODUCCIÓN

Actualmente entre un 15 y un 25% de la superficie del planeta ha sido alterada por algún tipo de perturbación antropogénica (Vitousek *et al.* 1997). Una proporción considerable del suelo modificado por actividades humanas está cubierto por zonas urbanas, y este tipo de transformación continúa creciendo (USGS 1999). El fenómeno de urbanización está asociado al crecimiento poblacional, económico y a la alta migración de los pobladores de zonas rurales a centros urbanos (Abdullah y Nakagoshi 2006). Se ha calculado que en los próximos 30 años más de la mitad de la población mundial vivirá en las ciudades (Naciones Unidas 2003).

Asociado al deterioro de los sistemas naturales, se encuentran procesos tales como el sobre-explotación forestal y el crecimiento de zonas ganaderas y agrícolas en los alrededores de los centros urbanos (Marzluff *et al.* 2001, Masera 2000). Hasta el momento, se conoce poco acerca de como la urbanización y los procesos de cambio de uso de suelo afectan a la fauna tanto a nivel individual, como a escala del paisaje (Chace y Walsh 2006). Esto se debe principalmente a que la mayoría de los estudios han sido realizadas a escalas pequeñas, estudiando poblaciones y/o en zonas con un grado de conservación (Blair 1996).

La urbanización fragmenta y destruye el hábitat, y es uno de los grandes problemas desde la perspectiva de la ecología de la conservación (Green y Baker 2003). Mientras la urbanización normalmente permite un incremento en la densidad poblacional humana, la diversidad de especies en los sitios urbanos es claramente menor (Maurer 1996). De hecho, la urbanización es considerada como una de las principales causas actuales de extinción de especies (Czech y Krausman 1997). Aunque este proceso antropogénico tiene implicaciones negativas para la mayoría de las especies, existen especies que han logrado adaptarse a estos cambios y coexistir con los humanos (Marzluff *et al.* 2001).

Para la mayoría de las especies, las zonas urbanas generan un hábitat de características hostiles permanentes (Czech y Krausman 1997). Las especies que logran subsistir ante este tipo de ambientes enfrentan nuevos y potenciales eventos negativos, como la presencia permanente de humanos, nuevos depredadores (gatos, perros y

humanos), ruido y contaminación (Clergeau *et al.* 1998). De forma aislada o en conjunto, estos factores de perturbación generan estímulos estresores que modifican o alteran la condición fisiológico en los individuos (Creel 2001, Partecke *et al.* 2006). Cuando es percibido un estímulo estresor por algún órgano sensorial se genera un cambio a nivel fisiológico que altera la homeostasis (Sands y Creel 2004). Este cambio en el equilibrio fisiológico modifica la conducta del individuo y genera un estado de estrés. (McEwen 2000, McEwen y Wingfield 2003). El estrés es una respuesta que implica cambios considerables en una variedad de funciones corporales, tanto a corto como largo plazo, y esta condición puede afectar significativamente el ciclo de vida de los organismos (Wikelski y Cooke 2006). Por lo que si el individuo no consigue equilibrar sus condiciones internas, la consecuencia será un deterioro de su bienestar inmediato, y una reducción posterior de su sobrevivencia y/o adecuación (Jessop 2001).

Respuestas fisiológicas ante eventos estresores

La respuesta de un organismo ante un evento estresor involucra la secreción de hormonas que modifican su condición interna, y generan respuestas conductuales (figura 1). Cuando un estímulo actúa sobre algún sentido del animal, el sistema nervioso aferente lo recibe y lo traslada a las áreas sensitivas del sistema nervioso central. Ante un estímulo estresor el organismo genera una respuesta que va enfocada a disminuir su impacto, utilizando el sistema nervioso autónomo y la actividad neuroendocrina.

El sistema nervioso autónomo en la zona simpática hace que la medula adrenal secrete adrenalina y nor-adrenalina. La actividad neuroendocrina activa la respuesta del eje hipotálamo-hipófisis-corteza adrenal. El hipotálamo secreta una hormona liberadora llamada cortocotropina (CRH). Esta hormona es detectada por la parte anterior de la hipófisis, la que a su vez secreta la hormona adenocorticotropina (ACTH) que induce la secreción de glucocorticoides en la corteza adrenal. Una vez que el organismo del individuo respondió al estímulo se promueve una respuesta de retroalimentación negativa, en la cual los glucocorticoides reducen la secreción de ACTH en la hipófisis y de CRH en el hipotálamo.

Las hormonas esteroides como los glucocorticoides se derivan del colesterol. El colesterol se convierte en pregnenolona, precursor de las hormonas esteroides en todos

los vertebrados. Los principales glucocorticoides son el cortisol y la corticosterona, y su predominio cambia entre grupos taxonómicos. Los anfibios, reptiles y aves utilizan principalmente la corticosterona, mientras que en mamíferos el cortisol es más común. La excepción a este patrón son los pequeños roedores, quienes presentan una dominancia de la corticosterona sobre el cortisol. Se han establecido dos tipos de acciones generadas por los glucocorticoides ante un evento de estrés: 1) acciones modulatorias que alteran al organismo, y 2) acciones preparatorias que preparan al organismo para respuestas futuras. Las acciones modulatorias están enfocadas principalmente a periodos de estrés agudo, mientras que las acciones preparatorias se generan tras periodos prolongado de estrés y ayudan a una aclimatación ante un estrés crónico (Sapolsky *et al.* 2006).

La secreción de los glucocorticoides activa una amplia variedad de funciones fisiológicas, como la regulación de la glucosa sanguínea, el recambio proteico, el metabolismo de las grasas, el equilibrio de sodio, potasio y calcio, la modulación de la respuesta tisular ante lesiones o infecciones y, sobre todo la supervivencia ante cualquier tipo de estrés (Sapolsky *et al.* 2006). Estas hormonas, dentro de procesos homeostáticos normales, controlan el catabolismo de proteínas (transformación de aminoácidos a glucosa) y la lipólisis (degradación de los triglicéridos a glicerol y ácidos grasos), además de estar asociadas con los ciclos circadianos y la vigilia (Buchanan 2000, McEwen 2000, McEwen y Wingfield 2003). Las funciones de los glucocorticoides ante una situación de estrés son organizar una respuesta mediante el desvío de recursos y un incremento de la concentración de glucosa en sangre, esto involucra varios sistemas del organismo que generan una respuesta fisiológica y conductual (Wingfield *et al.* 1998, Sapolsky *et al.* 2006).

La influencia humana sobre los sistemas naturales se incrementa continuamente, por lo cual es crucial entender cuáles son los mecanismos fisiológicos que permiten a los animales sobrevivir y adaptarse a los cambios antropogénicos de su ambiente. El objetivo de este proyecto es determinar cuáles son las respuestas fisiológicas de las aves ante perturbaciones antropogénicas, sobre todo aquellas asociadas a la urbanización. El explorar la relación entre estímulos estresores, respuestas fisiológicas y patrones ecológicos, nos permitirá entender los mecanismos asociados a la supervivencia de especies silvestres ante la perturbación humana. La información obtenida de este

estudio se enmarcará dentro del paradigma de la fisiología de la conservación planteado por Wikelski y Cooke (2006), y permitirá enfrentar problemas de conservación funcional.

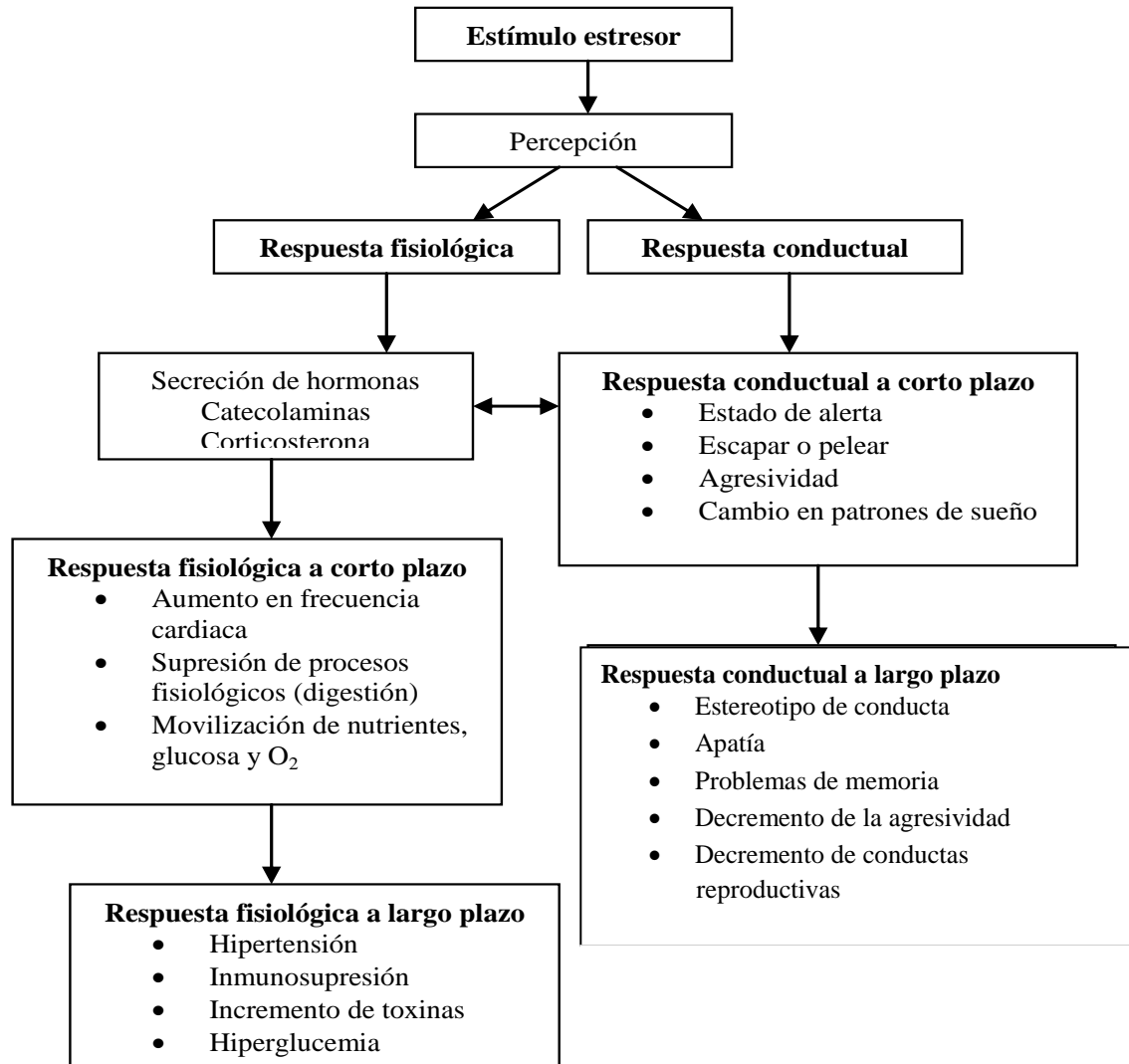


Figura 1. Efectos de un estímulo estresor en animales. El diagrama muestra las consecuencias del estrés en la condición fisiológica y las respuestas conductuales a corto y largo plazo (basado en Buchanan 2000).

Respuestas al estrés agudo y al estrés crónico

El estrés, de acuerdo con tiempo que se prolongue, se clasifica en: 1) estrés agudo y 2) estrés crónico (Buchanan 2000). El estrés agudo es una respuesta fisiológica ante un evento estresor, que no tiene mayores complicaciones fisiológicas ya que es un evento a

corto plazo (Wingfield *et al.* 1995). El estrés crónico es una respuesta que se puede prolongar por semanas, meses, o incluso años (McEwen y Wingfield 2003). Este tipo de estrés genera un impacto negativo en las funciones corporales de los individuos al crear compromisos energéticos entre diferentes sistemas fisiológicos (Touma y Palme 2005).

En periodos de estrés agudo, el enfrentamiento o la huida es la primer respuesta, tanto fisiológica como conductual por parte de los individuos (Buchanan 2000, Walker *et al.* 2005). No obstante, a nivel fisiológico se generan otras respuestas mediante la secreción de hormonas adrenales y su interacción con otras hormonas (Carey 2005). Por ejemplo, las catecolaminas y glucocorticoides promueven memoria del evento estresor, lo que en el futuro permite al individuo identificar posibles factores peligrosos, generando una redirección de la conducta (McEwen y Winfield 2003). Asimismo se realiza la gluconeogénesis que permite sintetizar glucosa a partir de otros elementos no glucídicos (aminoácidos, ácidos grasos etc), además de promover el almacenamiento de glucógeno en el hígado (Sapolyk *et al.* 2006). El sistema inmune también se ve favorecido durante los periodos de estrés agudo, ya que la actividad de las hormonas adrenales facilita el movimiento de células inmunológicas a través del organismo para enfrentar posibles infecciones generando una respuesta inmunológica rápida (Dhabhar y McEwen 1999). Por lo cual los periodos de estrés agudo funcionan como respuestas fisiológicas a corto plazo que le generan beneficios inmediatos a los individuo (Mullner *et al.* 2004).

El estrés crónico, a pesar de funcionar como un mecanismo para lograr la sobrevivencia, se considera un gran adversario de la salud de los individuos (Hadany *et al.* 2006). Una de las principales consecuencias causadas por este tipo de estrés, es el inicio de deficiencias en las respuestas inmunológicas (Buchanan 2000). Múltiples estudios realizados con humanos, y con animales en cautiverio, muestran que el estrés puede generar un estado de inmunosupresión (Ráberg *et al.* 1998). Este estado de inhabilidad inmune se debe principalmente a que se translocan recursos y energía asignados al sistema inmune a otras funciones que son imprescindibles para la sobrevivencia inmediata (Svensson *et al.* 1998, McEwen y Wingfield. 2003, Segerstrom 2007).

La inmunosupresión se define como una disfunción temporal o permanente de la respuesta inmune como resultado de un daño en el sistema inmunológico (Dohms 1991). Esta deficiencia se manifiesta con un incremento en la susceptibilidad a enfermedades debido a que el sistema inmune no reconoce aquellas células patógenas que necesitan ser eliminadas (El-Lethey *et al.* 2003). Numerosos agentes afectan la condición inmune de las aves y mamíferos, incluyendo virus, bacterias, endo y exoparásitos, contaminantes, deficiencias de nutrimentos y estímulos estresores causados por el ambiente (Stevenson *et al.* 2005). El estrés crónico es una de las principales causas que genera inmunosupresión por alteraciones en la fisiología del individuo, principalmente debido a las consecuencias fisiológicas de la secreción de glucocorticoides por tiempos prolongados (Cavigelli 1999).

Los glucocorticoides producidos en concentraciones elevadas y/o por tiempos prolongados, generan efectos adversos en el sistema inmune, ya que disminuyen la síntesis de inmunoglobulinas (Fowles *et al.* 1993). Adicionalmente se ha encontrado que los glucocorticoides inhiben la síntesis de linfocitos, generan una menor actividad en estos, y originan anticuerpos con menor afinidad por su antígeno (Sapolsky *et al.* 2006). También a nivel del sistema inmune celular, los glucocorticoides disminuyen la proliferación de linfocitos T, fibroblastos y los depósitos de fibrina en zonas donde hay sustancias extrañas (Iseki *et al.* 1991). A nivel sanguíneo estas hormonas disminuyen las cantidades de leucocitos e inhiben la síntesis, liberación y eficiencia de las citoquinas y otros mediadores que promueven la respuesta inmune (Owen y Moore 2006, Sapolsky *et al.* 2006).

De este modo, el estrés crónico genera desajustes fisiológicos con la finalidad de seguir mantenido la vida del individuo a través de un cambio severo y prolongado en las funciones corporales. Bourgeon y Raclot (2006) administraron corticosterona a través del alimento a una especie de pato (*Somateria mollissima*), encontrando una relación negativa entre los niveles de corticosterona e inmunoglobulinas, indicando un decremento en la inmunidad humoral. Fowles *et al.* (1993) inocularon periódicamente a patos (*Anas platyrhynchos*) con corticosterona a diferentes concentraciones. A partir del sexto día, a una dosis 0.2mg/kg/día, encontraron que las concentraciones de inmunoglobulinas disminuyeron significativamente con respecto a las concentraciones

iniciales. Los datos de ambos estudios sugieren que los individuos de aves que enfrentan un estrés crónico entran en un estado de inmunosupresión.

Estrés crónico, inmunosupresión y sus efectos en la condición corporal y su ecología.

Los primeros efectos de un periodo de estrés crónico y sus consecuencias se ven reflejados directamente en la condición corporal de los organismos (McEwen y Wingfield 2003). La condición corporal puede ser definida operativamente como la variación en peso que es independiente del tamaño corporal. Por lo que las diferencias de peso están asociadas a la presencia de reservas de grasa y desarrollo muscular (Gosler 2004). Estas diferencias en masa corporal, independientes del tamaño, nos indican que individuos, o poblaciones, presentan un mejor condición corporal, además de ayudarnos a entender patrones de dominancia entre individuos, categorías de edad o sexo, y/o la calidad del hábitat utilizado por distintos individuos de una especie (Senar *et al.* 2001). Evidencia reciente sugiere que los caracteres secundarios sexuales del plumaje en algunos casos, y la condición general del plumaje en otros, pueden funcionar como señales de la condición corporal y fisiológica de los individuos (McGraw 2006a). De esta manera los individuos pueden obtener señales directas o indirectas del estado físico de un individuo a través de ver las características de su plumaje (Hill 2006).

Existen dos tipos principales de coloración en el plumaje de las aves: 1) la coloración basada en pigmentos y 2) la coloración estructural (Brush 1978). La coloración pigmentaria se lleva a cabo por la deposición de pigmentos basados en carotenoides y melaninas durante la síntesis de la pluma (Roulin y Dijkstra 2003). Los carotenoides son los pigmentos que generan los colores amarillos, rojos y naranjas, y no son sintetizados por las aves, por lo que tienen que ser incorporados a través de la dieta (McGraw *et al.* 2003). Las implicaciones de la coloración del plumaje basada en carotenoides son consideradas indicadoras potenciales de condición corporal, habilidad para el forrajeo y calidad del territorio del individuo (Hill 2006, McGraw 2006a). De este modo las aves que puedan obtener una dieta rica en carotenoides presentarán plumajes más vistosos que las que tengan dietas con bajos contenidos de este pigmento.

Otra forma de coloración basada en pigmentos es la melanina, existiendo dos tipos: eumelanina y feomelanina. Este pigmento genera colores negros (eumelanina) y marrón y amarillo (feomelanina; McGraw 2006b). Las melaninas son producidas por unos orgánulos llamados melanosomas localizados en la epidermis de las aves (Jawor y Breitwisch 2003). Las implicaciones de esta coloración de plumaje están relacionadas con contextos de interacciones agonísticas y parece tener un componente genético en la determinación de su expresión (McGraw 2006b).

La coloración estructural es la responsable de los colores azules, verde-violeta, blanco, y la coloración iridiscente de las aves (Prum 1999). Esta coloración no depende de pigmentos, sino de la reflexión de la luz en la matriz estructural de la pluma (Prum 1999). La matriz es el conjunto de barbas que forman la pluma, cada barba esta conformada por tres estructuras: 1) un anillo exterior llamado cortex, que contienen el pigmento difuso. 2) la zona colorante hueca, donde tiene lugar la difracción de la luz solar y 3) la medula que esta compuesta por un innumerable número de células principalmente de color negro (Senar 2004). En conjunto estas tres estructuras, el entrelazado de las barbas y la calidad de estas dependen principalmente de que tantos recursos (proteínas) y energía el individuo invirtió para su elaboración (Keyser y Hill 2000, Bortolotti *et al* 2008).

El despliegue de colores que tiene el plumaje representa costos energéticos para su producción y mantenimiento (Moller 1989, Badyaev *et al.* 2001, Pryke 2003, Senar 2004). Cuando un individuo se encuentra en una buena condición corporal, no se originan disyuntivas fisiológicas que comprometan o alteren las señales sexuales secundarias (Buchanan *et al.* 2003). Sin embargo, cuando un organismo presenta altas concentraciones de corticosterona por tiempo prolongado se altera significativamente su condición corporal, y disminuyen significativamente los recursos y energía empleados para el desarrollo de caracteres sexuales secundarios como lo es la coloración del plumaje (Bortolotti *et al.* 2008).

En las aves la condición corporal y fisiológica se refleja directamente en la calidad del plumaje (Chaine y Lyon 2008). Esto se ha visto claramente en el caso de la coloración por pigmentos (Horak *et al.* 2001, Quesada y Senar 2006, Griffith *et al.* 2006). Sin embargo, hasta hace poco tiempo se ha empezado a explorar las implicaciones que tiene la coloración estructural de los individuos en la condición

corporal y a nivel ecológico (Siefferman y Hill 2005). Cuando un individuo tiene una buena condición corporal puede invertir más recursos en la calidad estructural de sus plumas (barbas), que aquellos individuos que tienen una baja condición corporal. Por ejemplo Doucet (2002) encontró que individuos de *Volatinia jacarina* que tenían mayor intensidad en la coloración estructural del plumaje, también tenían mejor condición física medida en tamaño y peso del individuo. Adicionalmente, Siefferman y Hill (2005) encontraron que los individuos machos de *Sialia sialis* que tenían mayor intensidad de reflexión de la luz ultravioletas en sus plumas, eran los que tenían mayor oportunidad de aparearse. Por lo que lo que la coloración estructural es un elemento que puede reflejar la condición física de los individuos, y tener implicaciones a nivel ecológico afectando la capacidad reproductiva de varias especies.

Estructura de la tesis

Los estímulos estresores generados por la perturbación humana asociada al desarrollo urbano, modifican la condición fisiológica de los individuos. Los principales efectos de estos cambios a nivel funcional se manifiesta en un estado de estrés. Se ha encontrado que diferentes grupos de vertebrados como los anfibios (Newcomb *et al.* 2002), reptiles, (Lance 1992), aves (Partecke *et al.* 2006) y mamíferos (Cabezas *et al.* 2007) incrementan significativamente su concentración de glucocorticoides conforme aumentaba la perturbación en sus hábitats. Esta tesis se enfoca a estudiar las respuestas de estrés e inmunológicas de las aves a la perturbación humana, y como estas respuestas pueden ser manifestadas a través de señales honestas de su plumaje.

Para lograr esto, en el Capítulo II ¿Qué tan estresadas están las aves? Relación entre las condiciones de hábitat y respuestas fisiológicas (estrés) de las aves en un paisaje subtropical alterado por actividad humana”, exploramos las relaciones entre tres condiciones de hábitat (zona conservada, zona cultivos y zona urbana) y las respuestas fisiológicas (concentración de corticosterona y concentración de inmunoglobulinas) de dos especies de aves (*Pipilo fuscus* y *Columbina inca*) en un paisaje subtropical de montaña alterado por actividades humanas. Hipotetizamos que las aves que habitan en zonas con mayores niveles de perturbación ocasionada por actividades antropogénicas deben presentar estrés crónico. Predijimos que los individuos que estuvieran sometidos

a mayor estrés crónico causado por perturbaciones antropogénicas, tendrían una relación negativa entre la concentración de corticosterona y la concentración de inmunoglobulinas. Las metas para este capítulo fueron evaluar los niveles de corticosterona (estrés) y la concentración de inmunoglobulinas, y la forma en que estas dos variables se relacionan, en dos especies de aves clasificadas como exploradoras de ambientes urbanos (*Pipilo fuscus* y *Columbina inca*) a través de tres zonas con diferentes niveles de perturbación antropogénica (zona conservada, zona de cultivo y zona urbana).

En el Capítulo III; Respuestas de estrés del gorrión común (*Passer domesticus*) en diferentes usos de suelo urbanos, abordamos los efectos que tienen los diferentes usos de suelo dentro de una ciudad (zona urbana, zona suburbana y zona industrial) en la fisiología (estrés y respuesta inmune) de una especie explotadora de ambientes urbanos. Nuestra hipótesis fue que los individuos que habitan en zonas con mayor actividad humana deben presentar mayores niveles de estrés, y que este estrés debe de ser crónico. Predijimos que los individuos que habitaban en zonas con mayor perturbación por actividad humana, generarían una relación negativa entre la concentración de corticosterona y la concentración de inmunoglobulinas. Nuestra meta fue determinar la concentración de corticosterona y de inmunoglobulinas del gorrión común (*Passer domesticus*) en los principales tipos de usos de suelo urbano de una ciudad Mexicana.

En el Capítulo IV; Efectos del estrés en la muda y la coloración de plumaje durante la reproducción de *Volatinia jacarina* exploramos la relación que existe entre la condición fisiológica (concentración de corticosterona e inmunoglobulinas), la coloración estructural de plumaje y la velocidad de muda de un ave asociada a sitios abiertos con actividad humana. Nuestra hipótesis fue que los individuos que presenten mayores concentraciones de corticosterona como respuesta a estímulos estresores tendrán una baja intensidad en la coloración de plumaje. Predijimos que a concentraciones altas de corticosterona se generaría una alteración en la coloración de plumaje (hue, chroma Brightness). Nuestra meta fue determinar si la condición fisiológica puede estar medida por la coloración estructural de plumaje, y como la calidad del individuo afecta sus patrones de muda.

Finalmente, después de haber determinado la existencia de una relación entre la calidad del individuo, su condición corporal y la coloración estructural de su plumaje,

en el Capítulo V Selección social y sexual basada en la coloración estructural de plumaje en *Passerina caerulea*”. estudiamos experimentalmente los efectos que diferencias en el color del plumaje de *Passerina caerulea* tienen en la selección social y sexual de esta especie con coloración estructural. La hipótesis que planteamos es que los individuos macho de *Passerina caerulea* que tengan una mayor intensidad en la coloración de su plumaje serán individuos más atractivos para las hembras que aquellos individuos machos con coloración de plumaje menos vistosa. Por otro lado con respecto a la selección social los individuos machos preferirán permanecer con individuos machos con una coloración menos vistosa que con los individuos que tengan una coloración de plumaje más intensa. Predijimos que los individuos que manipulamos para mostrar una mayor intensidad en la coloración de plumaje tendrán un mayor éxito en la selección por parte de las hembras que aquellos que manipulamos para atenuar la coloración de plumaje.. Con respecto a la selección social los individuos machos preferirán permanecer con los individuos que aparenten ser menos dominantes (coloración de plumaje opacada) que aquellos con coloración más vistosa). Los objetivos que planteamos fueron 1) Determinar la selección sexual por parte de las hembras de *Passerina caerulea* en machos con la coloración de su plumaje manipulada, y 2) establecer la selección social entre machos de *Passerina caerulea* manipulados para resaltar y en otros atenuar la coloración de plumaje.

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CAPÍTULO II

HOW STRESSED ARE BIRDS? RELATIONSHIPS BETWEEN HABITAT CONDITIONS AND THE STRESS RESPONSES OF BIRDS IN A SUBTROPICAL HUMAN ALTERED LANDSCAPE.

ABSTRACT

Within human altered landscapes, wildlife species confront different levels of disturbance differing in their type, magnitude, intensity, span, and frequency. In this study we measured two physiological traits (corticosterone and immunoglobulin concentrations) and the density of two landbird species (Canyon Towhee–*Melospiza fusca*, Inca Dove–*Columbina inca*) present in three habitat conditions: (1) forest edges; (2) croplands; and (3) urban sites. Our results show that the physiological and density values of both species differ among habitats. Corticosterone concentration showed to be significantly higher in croplands for both species. However, immunoglobulin concentration in urban sites was lowest for *M. fusca* and highest for *C. inca*. In general, our physiological results suggest that *M. fusca* has a greater proportion of physiologically compromised individuals in urban sites, while we did not find any evidence that *C. inca* was physiologically challenged in any of the studied habitat conditions. Although both species are ecologically similar due to their size, feeding resources, and nesting requirements, our results show that a higher proportion of *M. fusca* individuals have physiological limitations in urban areas, while *C. inca* seems to be a habitat generalist, with enough ecophysiological plasticity to allow it to become an urban-exploiter species.

Keywords: Corticosterone, Immunoglobulin, Ecophysiology, Canyon Towhee, Inca Dove, Urban-agricultural matrixes, Forest-edges

INTRODUCTION

Human alteration of ecosystems is immense, representing a threat to biodiversity, and generating complex landscapes (Vitousek et al. 1997, Jetz et al. 2007). Inside human-altered landscapes wildlife species confront different types, magnitudes, intensities, span, and frequency levels of disturbance (Pickett and White 1985, White and Jentsch 2001, Benítez-Díaz and Bellot-Rojas 2007). Regardless of their characteristics, landscape patches under different disturbance regimes always generate stressor stimuli to organisms (Jacobs and Wingfield 2000, Breuner and Hahn 2003, Walker et al. 2005, 2008).

Wildlife species respond to the stressor stimuli of disturbances through physiological mechanisms and behavior, which can shape the dynamics of their populations (Temple and Wiens 1989; Ricklefs and Wikelski, 2002; Romero, 2004; Partecke et al. 2006), and generate evolutionary responses (Badyaev 2005). Physiological responses to stressor agents include acute and chronic stress, both measurable through corticosterone concentration (Romero 2004, Partecke et al. 2006, Ewens 2007). Acute stress, caused by stochastic stressor stimuli, can turn into chronic stress when the stressor agent persists for a long period of time (Buchanan, 2000; Sapolsky et al. 2000, McEwen and Wingfield 2003). Among other effects, chronic stress often alters the immune system, lowering the capability of individuals to respond to pathogens (Fowles et al. 1993, Sapolsky et al. 2000, El-Lethey et al. 2003, McEwen and Wingfield 2003, Morales et al. 2006, Chávez-Zichinelli et al. 2010).

In this study we measured differences in two physiological traits (i.e., corticosterone and immunoglobulin concentrations) and densities of two landbird species (i.e., Canyon Towhee–*Melospiza fusca*, formerly known as *Pipilo fuscus*; Inca Dove–*Columbina inca*) present in three habitat conditions of a human-altered landscape: (1) forest edges; (2) croplands; and (3) urban sites. These habitat conditions include contrastingly different disturbance traits. Although *M. fusca* and *C. inca* are phylogenetically distant (pertaining to Passeriformes and Columbiformes; Hackett et al. 2008), they are ecologically similar due to their size (*M. fusca*: 20-25 cm; *C. inca*: 19-23 cm), main feeding resources (mostly seeds), and microhabitat needs for foraging and nesting (open areas and ground of short vegetation) (Johnson and Haight 1996, Mueller, 2004). We predicted that both bird species would present low corticosterone

concentrations in the condition with less disturbance (i.e., forest edges), increasing its concentration in habitats with higher disturbance (e.g., croplands, urban sites). Conversely, we expected a high range of immunoglobulin concentration values related to low corticosterone concentrations in all habitat conditions, but predicted low immunoglobulin concentrations related to high corticosterone concentrations under high disturbance scenarios, representing physiologically compromised individuals. Finally, based on previous studies relating the physiology of birds to their population numbers (Eeva et al. 1997, Liker et al. 2008, Moya-Laraño et al. 2008), we expected the density of both landbird species to be lowest where higher proportions of physiologically compromised individuals were present.

METHODS

Study area

This study was carried out within the Cuitzeo watershed, located in north-east Michoacán, West Mexico. Our sampling sites include three habitat conditions located at similar elevations (~1950 m asl) with different types and intensities of human disturbance: (1) native forest edges; (2) croplands; and (3) urban sites. Native forest edges (19°39'18''N, 101°06'43''W) comprise the ecotone between pine-oak communities and open areas generated by rocky outcrops and low human disturbance components (i.e., dirt roads, small croplands, scattered suburban settlements). Croplands (19°47'30''N, 101°14'55''W) comprise seasonal agricultural lands (e.g., corn, sorghum) and cattle pastures embedded within a matrix of shrubland patches, which represent an early ecological succession stage resulting from original forest perturbation and/or cropland abandonment. Urban areas include an urban park (19°41'52''N, 101°10'49''W) and a suburban University Campus (19°38'51''N, 101°13'43''W) located in the center and the southwest sections of the city of Morelia, respectively. Morelia is a colonial city established during the sixteenth century, which has undergone rapid and unplanned development in the last decades (López et al. 2001, Vargas Uribe 2008). At present, Morelia covers an area ~100 km² and has a population that exceeds one million inhabitants (Vargas Uribe 2008).

Habitat characterization

To characterize habitat conditions at our survey sites, we measured vegetation structure (tree, shrub, and herb cover and maximum height) and human activities (number of passing cars and pedestrians) in five sites near the areas where we performed our bird captures and sight-recordings. To assess the similarity among our sampling sites, we performed a multivariate cluster analysis (Euclidean distance–single linkage). We assumed that clusters formed after 50% similarity values represent different habitat conditions. Additionally, we evaluated differences among the measured variables in the forest edge, cropland, and urban sites. For this, we performed one-way GLM-ANOVAs between each habitat trait (dependent variable), and each habitat condition (grouping variable), followed by Newman-Keuls post-hoc range tests. Because we carried out several GLM-ANOVAs ($n = 8$), we corrected our p-values following a Bonferroni correction (corrected $\alpha = 0.006$).

Bird captures

We captured *C. inca* and *M. fusca* at the three studied habitat conditions (i.e., forest, croplands, urban) using mist-nets. Both species were captured from 7:00 to 11:00 h during the dry season (January-May) of two years (2008-2009). We captured birds in the dry season of the year to avoid interference with the breeding season, which can alter their physiological condition significantly (Wingfield et al. 1998, Lynn and Porter 2008). We collected fecal and blood samples from individuals of both species, which were frozen for laboratory analyses. Blood samples were obtained from the brachial vein and collected in heparin added capillary tubes. All captured individuals were marked with a numbered metal leg-band and released.

Physiological analyses

Corticosterone concentration (Radioimmunoassay - RIA)

To determine the physiological condition of *C. inca* and *M. fusca* in our sampling sites, we only used fecal samples to determine corticosterone concentration values. We measured corticosterone in feces because they allow the integration of the stress state of the individuals prior to the capture time, and not the stress caused by the capture and

handling of the bird (Millspaugh and Washburn 2004, Touma and Palme 2005, Chávez-Zichinelli et al. 2010).

We defrosted standardized fecal samples collected from *C. inca* and *M. fusca* to 0.2 g samples (wet weight). We ran the samples by duplicate. To extract corticosterone from the fecal samples, we placed each sample in a vial with 1 ml of ethanol (Cavigelli 1999, Sands and Creel 2004), vortexed for 20 min and centrifuged for 20 min (10,000 rpm). In most cases we used a dilution of 1:4. Afterward, we placed 100 μ l of the dilution in RIA tubes and added 100 μ l of anti-corticosterone antibody (Sigma C-8784). Finally, we incorporated 50 μ l of tritiated corticosterone ([1,2,6,7-3H(N)]-Perkin Elmer Boston, Ma. 02118). We incubated our samples for 20 hrs at 4 °C, separating the unbound steroids with carbon. We measured the radioactivity using a scintillation counter (Beckmann Instruments LS-6500). We validated this method for *M. fusca* and *C. inca* by analyzing the recovery of exogenous corticosterone in relation to field samples by adding exogenous corticosterone to one set of samples as control. Two types of results show that the assay was applicable to *M. fusca* and *C. inca* samples: (1) fecal extracts in relation to the corticosterone standard curve showed similar slopes (*M. fusca*: $F = 2.0$, $df = 8$, $P = 0.16$; *C. inca*: $F = 2.1$, $df = 8$, $P = 0.18$); and (2) the successful recovery of exogenous corticosterone.

Immunoglobulin concentration (ELISA test)

To determine the concentration of total immunoglobulins (including IgY, IgM, and IgA) from blood samples, we used an ELISA test, as described by Martínez et al. (2003). Briefly, blood samples were extracted from the capillary tubes, placed in 0.5 ml vials, centrifuged for 10 min, and blood serum was collected. We diluted blood serum samples (1: 4000), placed them in well-plates, and incubated them at 4 °C for 18 hrs. Next, we added a primary antibody (polyclonal rabbit anti-chicken IgG conjugated with peroxidase; Sigma A-9046) and incubated the samples for another two hours at 37 °C. Afterward, we added orthophenyldiamine (OPD) as a substrate. We read the plates for optical densities using an ELISA plate spectrophotometer ($\lambda = 492$). All samples were ran in triplicate.

Bird densities

To evaluate if the densities of *C. inca* and *M. fusca* were related to the studied habitat conditions, we carried out 10 point counts (5 min, unlimited-radius) following Ralph et al. (1996), measuring the distance from each bird individual to the observer using a rangefinder (Bushnell Yardage Pro). Point counts were located at a minimum distance of 250 m from each other to assure survey independence (Ralph et al. 1996, Huff et al. 2000) within, and around, the areas in which birds were captured. All individuals from both species recorded using the surveyed areas were included in our analyses.

Statistical analyses

To compare corticosterone concentrations for both species among the studied forest edge, cropland, and urban sites, we performed Kruskal-Wallis tests because the data did not follow the basic parametric assumptions (i.e., normality, homogeneity of variance). As the data for immunoglobulin concentration were normally-distributed and presented homogeneity of variance, we conducted ANOVAs. To evaluate if corticosterone and immunoglobulin concentrations were related among them in the studied forest edge, cropland, and urban sites, we conducted linear regressions between both physiological variables for each species and the three studied habitat conditions. We analyzed the statistical power (post-hoc) for each regression using G*Power (Faul 2008). As the power of a statistical test increases, the probability of a Type II error decreases, allowing us to evaluate if our results are statistically robust regardless of sample size. We used a minimum power value of 0.80 following Park (2008).

Because individuals with high corticosterone and low immunoglobulin concentrations tend to be physiologically compromised (Buchanan 2000, Sapolsky et al. 2000, Romero 2004), we used two thresholds based on the samples for each species from the forest edge to identify the proportion of physiologically compromised individuals for both species at each habitat condition based on the interaction between the two physiological variables: (1) mean + SD for corticosterone concentration; and (2) mean – SD for immunoglobulin concentration. We used SD values due to the nature of this descriptive tool, which represents the average difference between the data points and their mean (Cumming et al. 2007). We used values from forest edges to establish

the thresholds because this condition represents the least disturbed habitat among the studied ones, and is the studied habitat that represents the closest scenario to what both species use in the absence of human disturbance (Table 1).

To calculate bird densities, we computed individuals/ha (mean \pm 95% confidence intervals) using Distance 6.0 (Thomas et al. 2010). This software calculates the probability of detection of individuals at increasing distances from the observer and estimates the number of bird individuals that exist within a surveyed area (Buckland et al. 2001). We report the Akaike's information criterion (AIC), and key function/series expansion (KF/SE) to describe the coverage of our surveys and the nature of the methods used by the program to calculate bird densities. AIC is a measure of the goodness of fit to a model, which is computed by Distance 6.0 and is recommended for model selection (Buckland et al. 2004, Thomas et al. 2005). KFs are parametric functions to model the detection function (i.e., uniform, half-normal, hazard rate, negative exponential), and SEs are mathematical representations of the functions (i.e., cosine, simple polynomial, hermite polynomial) (Thomas et al. 2002, 2005). To determine if the computed density values for both bird species were statistically different among the studied habitat conditions, we compared their 95% confidence intervals. If confidence intervals did not overlap, we considered the data to be statistically different with an $\alpha < 0.01$ (following Payton et al. 2003).

RESULTS

Habitat characterization and bird captures

The multivariate cluster analysis (Euclidean distance–single linkage), based on the measured variables at each site (Table 1), revealed that: (1) the surveyed sites per habitat condition were more similar among them than in relation to those from other habitat conditions, with lesser similar sites ranging from 61-65% similarity; and (2) the studied urban sites are highly different from the sampling sites in forest edges and croplands (29% similarity), which in turn clustered at 58% similarity (Fig. 1). We captured a total of 119 *M. fusca* and 105 *C. inca* in the studied habitat conditions (Table 1).

Physiological analyses

Corticosterone concentration varied between species and among habitat conditions. *M. fusca* had significantly higher corticosterone concentration values in croplands than in forest edges, however urban sites did not differ from the rest ($H = 8.27$, $df = 118$, $P = 0.01$; Fig. 2; Table 2). *C. inca* had significantly higher corticosterone concentration values in croplands when compared to those from forest edges and urban sites ($H = 9.84$, $df = 102$, $P = 0.007$; Fig. 2; Table 2). Immunoglobulin concentration also varied between species and among habitat conditions. *M. fusca* had significantly higher immunoglobulin concentration values in croplands than in urban sites, with forest edges not differing from the rest ($F = 6.01$, $df = 118$, $P = 0.003$; Fig. 2; Table 2). *C. inca* had significantly higher immunoglobulin concentration values in urban sites when compared to those from forest edges, with values for croplands not differing from the rest ($F = 2.46$, $df = 102$, $P = 0.09$; Fig. 2; Table 2).

Corticosterone and immunoglobulin concentrations only showed a negative significant relationship, considering both the α and statistical power, for *M. fusca* in urban sites. We also found a positive low non-significant trend (statistical power = 0.64) for *M. fusca* in croplands. The remaining regression for *M. fusca* and all regressions for *C. inca* were non-significant and showed no other non-significant trends (Table 3). When we sought for physiologically compromised individuals, based on threshold for both corticosterone and immunoglobulin concentrations, we only found two *C. inca* individuals to fall in the physiologically compromised area of the plot (Fig. 3), one in croplands and one in urban sites (representing 2 and 7% of the total bird captures for this species at each habitat condition, respectively). However, we found a higher number of *M. fusca* individuals to be physiologically compromised in the studied habitat conditions. Although few individuals fell in the physiologically compromised area of the plot in forest edges and croplands (one and two, representing 2 and 3% of the total bird captures for this species at each habitat condition, respectively), we found five *M. fusca* individuals to be physiologically compromised in urban areas, representing 30% of the total bird captures for this species in this habitat condition (Fig 3).

Bird densities

Bird densities recorded for *M. fusca* and *C. inca* varied among habitat conditions. Density values for *M. fusca* were significantly higher in forest edges (4.47 ind/ha; 95% confidence intervals: 3.00–6.67; AIC = 63.79, KF/SE = Uniform/Coisine) and croplands (3.59 ind/ha; 95% confidence intervals: 2.72–4.74; AIC = 74.02, KF/SE = Uniform/Coisine) when compared to urban sites (1.95 ind/ha; 95% confidence intervals: 1.40–2.71; AIC = 175.7, KF/SE = Uniform/Simple polynomial). However, we did not find differences in the density of *C. inca* along the studied habitat conditions: (1) forest edges – 4.02 ind/ha; 95% confidence intervals: 3.09–7.03; AIC = 65.63, KF/SE = Uniform/Coisine; (2) croplands – 3.27 ind/ha; 95% confidence intervals: 2.70–6.00; AIC = 64.93, KF/SE = Uniform/Coisine; urban – 4.66 in /ha; 95% confidence intervals: 3.09–7.03; AIC = 65.63, KF/SE = Uniform/Coisine (Fig. 4, Table 2).

DISCUSSION

Results of this study show that the physiological and density values of both studied landbird species differ among forest edges, croplands, and urban sites in our study region. Corticosterone concentration showed to be significantly higher in croplands for both species. However, immunoglobulin concentrations in urban sites were lowest for *M. fusca* and highest for *C. inca* (Fig. 2). In general, our physiological results suggest that *M. fusca* has a greater proportion of physiologically compromised individuals in urban sites, while we did not find any evidence that *C. inca* is physiologically compromised among the studied forest edges, croplands, and urban sites (Fig. 3). The density of *M. fusca* was significantly lower in urban sites, while the density of *C. inca* did not show statistical differences among the studied habitat conditions, indicating a relationship between bird densities and their physiological condition. In this section, we first focus on the differences found in corticosterone and immunoglobulin concentration values for both species among the studied habitat conditions, and their relationships suggesting physiologically compromised individuals. Second, we contrast the physiological condition of both species with their densities in the studied habitat conditions. Finally, we discuss possible relationships between habitat attributes, including human activity, with our ecophysiological results.

Corticosterone concentration values were highest in croplands for both studied landbird species. As bird captures were carried out during the dry season, and agricultural sites in our study area depend basically on rainfall, crops are not available throughout this time of the year, great part of the land is managed, and few space remains for herbs to grow. Thus, we believe that the scarcity of feeding sites and food resources in croplands rises the corticosterone concentration of the landbird species we studied. Additional costs could be the result of an increase of negative intra- and inter-specific interactions among cropland bird species in this critical time of the year, as recorded by other authors evaluating the effect of antagonistic relationships on stress (Romero et al. 2000, Barbosa et al. 2006, Dickens 2009). On the other hand, urban areas tend to have predictable and abundant food resources throughout the year (Shochat 2004), and the studied forest edge has relatively large areas with unmanaged grasses where we have recorded groups of *M. fusca* and *C. inca* feeding.

Immunoglobulin concentration values differed greatly among species and habitat conditions. For *M. fusca*, higher immunoglobulin concentration values were recorded at forest edges and croplands. Having high immunoglobulin concentration reflects two possible scenarios: (1) strong immunocompetence, allowing individuals to have a successful response to pathogens; and (2) individuals successfully responding to infectious processes (Guillette 1980, Martínez et al. 2003, Perozo et al. 2007). *M. fusca* individuals in forest edges, with low corticosterone and high immunoglobulin concentrations, suggest that they have low stress levels, in a high immunocompetence scenario (Bourgeon et al. 2006). *M. fusca* individuals in croplands, with high corticosterone and immunoglobulin concentrations, suggest that they are under acute stress, probably caused by infectious processes, as corticosterone is an immunoregulator that arrests the production of lymphocytes, which in turn are precursors of immunoglobulins (Hanssen et al. 2004). *M. fusca* individuals in urban sites, with high corticosterone and low immunoglobulin concentrations, suggest that a proportion of their populations are physiologically compromised. Our results show that 30% of the captured *M. fusca* individuals in urban sites have corticosterone and immunoglobulin concentrations values that suggest that they are under a chronic stress scenario (Bourgeon et al. 2006, Bourgeon and Raclot 2006). Our sampling in urban areas was only conducted inside well-vegetated sites (i.e., an urban park, gardens of a University

Campus), and represent the less urbanized condition inside the city. If we consider that in these urban green-oasis three out of 10 *M. fusca* individuals are physiologically compromised, we expect grimmer scenarios for this species more developed urban land-uses.

Contrastingly different to what we found for *M. fusca*, the immune condition of *C. inca* rose significantly from forest edges to urban sites, with intermediate values in croplands. *C. inca* individuals in forest edges, with low corticosterone and immunoglobulin concentrations, suggest that they are in a “physiological standby” scenario, in which the bidirectional interaction between these two components is involved in the maintenance of physiological and immunological homeostasis (Davis 1998, Råberg et al. 2002). *C. inca* individuals in croplands, with high corticosterone and intermediate immunoglobulin concentration, suggest that they are under acute stress (Svensson et al. 1998, Buchanan 2000,). Finally, *C. inca* individuals in urban sites, with low corticosterone and high immunoglobulin concentrations (as *M. fusca* in forest edges), suggest that they have low stress levels, in a high immunocompetence scenario (Barbosa et al. 2006).

M. fusca densities were statistically lower in urban sites when compared to the other habitat conditions. While lower densities could be related to lesser food resources, urban areas tend to favor granivore species (Chace and Walsh 2006), and to have highly predictable food resources in large quantities (Shochat 2004). However, our results show smaller *M. fusca* densities in urban areas, where the largest proportion of physiologically compromised individuals was found. As the physiological condition of birds can alter their population numbers and dynamics (Temple and Wiens 1989, Ricklefs and Wikelski, 2002, Romero 2004, Partecke et al. 2006), our results suggest that the physiological condition of *M. fusca* in urban areas is affecting their densities. Differently to what we found for *M. fusca*, *C. inca* densities did not show statistical differences among the studied habitat conditions. Although we found different physiological scenarios for *C. inca* in the three studied habitat conditions, they were not reflected in their densities. This results could have two possible explanations: (1) that the measured physiological scenarios are not critical enough to modify their densities, making this species ecophysiologicaly capable to withstand human disturbance of different types and intensities; and (2) that meta-population dynamics at different

geographical scales, not evaluated in this study, maintain similar densities in all habitats where the species is present.

Although we cannot directly relate the physiological condition of the studied bird species with the measured habitat traits, we found several associations that could explain our ecophysiological results. Because we did not find a large proportion of physiologically compromised *C. inca* individuals in the studied habitat conditions and their densities were similar, we do not have evidence that the measured habitat traits could determine their ecophysiology. However, our results show significantly lower densities of *M. fusca* in urban sites, where we recorded the largest proportion of physiologically compromised individuals. As shrub cover provides shelter from predators and human disturbance in urban areas (Fernández-Juricic et al. 2001), for which we recorded significantly higher values (i.e., number of passing cars and pedestrians), recording significantly lower shrub cover in urban sites in this study suggests that solitary birds, such as *M. fusca* (Johnson and Haight 1996), could be more exposed to human-based stressor stimuli. Tree cover and height have been identified as factors that affect bird species richness in urban sites (Munyenyembe et al. 1989, MacGregor-Fors 2008). Higher species richness, including several granivore and omnivore species in our study area (MacGregor-Fors et al. in press), could increase the number of antagonistic interactions between species, driving *M. fusca* to a non-optimal physiological state. Additionally to the high-competition scenario, low herb cover in urban sites decreases the microhabitat for foraging of this species (Johnson et al. 1996). Altogether, our ecophysiological results, in addition to previously published studies, suggest that within urban scenarios, those including high tree cover, low shrub and herb cover, and high human activities, could drive the physiological condition of *M. fusca* to a non-optimal state. In fact, our results agree with previous knowledge on the negative association between corticosterone and immunoglobulin concentrations (Bourgeon et al. 2006, Bourgeon and Raclot 2006), where individuals with high corticosterone and low immunoglobulin concentrations tend to be physiologically compromised. However, we do not have any evidence to reference that *C. inca* is physiologically compromised in the studied habitat conditions. Although both species are ecologically similar, our results show that a higher proportion of *M. fusca* individuals have physiological

limitations, while *C. inca* seems to be a habitat generalist, and that the physiology of this species allows it to become a urban-exploiter.

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Table 1. Habitat trait values and human activity (mean \pm SD) among the studied forest edge, cropland, and urban sites. ** = P-values < 0.001. Letters within parenthesis following SD values represent statistical differences from Newman-Keuls post-hoc range test analyses.

Habitat trait	Forest edge	Cropland	Urban	GLM-ANOVA		
Tree cover	27.4 \pm 23.3 (A)	5.1 \pm 3.5 (B)	60.1 \pm 8.9 (C)	$F_{2,12} = 24.86^{**}$		
Tree max. height	8.0 \pm 3.1 (A)	3.6 \pm 2.3 (B)	14.4 \pm 2.5 (C)	$F_{2,12} = 21.47^{**}$		
Shrub cover	21.1 \pm 12.4 (A)	45.6 \pm 8.8 (B)	3.6 \pm 1.3 (C)	$F_{2,12} = 28.59^{**}$		
Shrub max. height	1.9 \pm 0.4 (A)	2.7 \pm 0.8 (A)	2.1 \pm 0.5 (A)	$F_{2,12} = 2.21$		
Herb cover	53.1 \pm 9.7 (A)	82.1 \pm 12.5 (B)	32.0 \pm 16.4 (C)	$F_{2,12} = 18.09^{**}$		
Herb max. height	0.3 \pm 0.2 (A)	0.5 \pm 0.6 (A)	0.2 \pm 0.1 (A)	$F_{2,12} = 1.10$		
Passing pedestrians / min	0.8 \pm 1.8 (A)	2.2 \pm 1.3 (A)	31.0 \pm 14.3 (B)	$F_{2,12} = 20.76^{**}$		
Passing cars / min	4.1 \pm 2.7 (A)	2.6 \pm 3.6 (A)	32.0 \pm 9.1 (B)	$F_{2,12}$	=	40.13**

Table 2. Values of the measure physiological variables (mean \pm SE) and density of *M. fusca* and *C. inca* (mean: 95% confidence intervals) in the studied forest edge, cropland, and urban sites.

Habitat condition	[Corticosterone] (ng/g)	[Immunoglobulin] (optical density)	N	Density -ind / ha (mean: confidence interval)
<i>M. fusca</i>				
Forest edge	34.04 \pm 5.39	0.60 \pm 0.02	43	4.47: 3.00–6.67
Cropland	55.69 \pm 4.60	0.67 \pm 0.02	59	3.59: 2.72–4.74
Urban	44.24 \pm 8.58	0.50 \pm 0.04	17	1.95: 1.40–2.71
<i>C. inca</i>				
Forest edge	27.07 \pm 5.64	0.37 \pm 0.02	37	4.02: 2.70–6.00
Cropland	47.12 \pm 4.76	0.41 \pm 0.02	52	3.27: 2.48–4.32
Urban	21.87 \pm 8.80	0.48 \pm 0.03	15	4.66: 3.09–7.03

Table 3. Relationship between corticosterone and immunoglobulin concentration for *M. fusca* and *C. inca* among the studied forest edge, cropland, and urban sites.

Species	Habitat condition	r^2	df	P	Statistical power
<i>M. fusca</i>	Forest edge	0.04	42	0.19	0.24
	Cropland	0.09	58	0.01	0.64
	Urban	0.49	16	0.001	0.90
<i>C. inca</i>	Forest edge	0.02	38	0.31	0.16
	Cropland	0.02	51	0.29	0.18
	Urban	0.002	14	0.87	0.03

Figure 1. Multivariate cluster analysis (Euclidean distance–single linkage) habitat trait similarity among the studied forest edge, cropland, and urban sites.

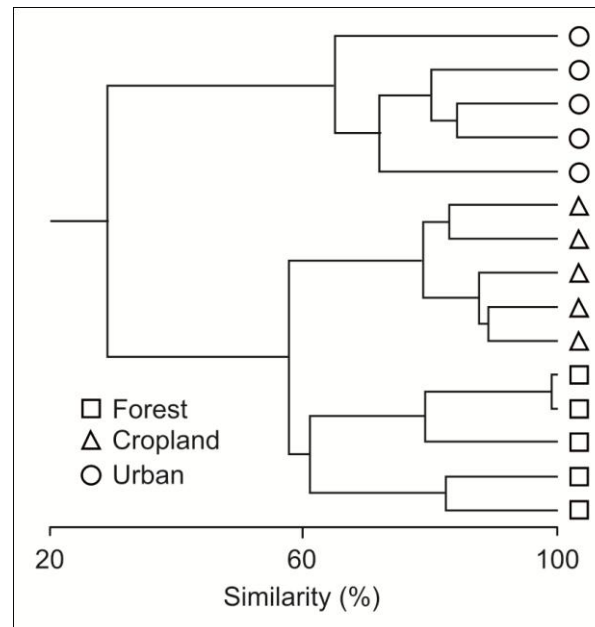


Figure 2. Corticosterone and immunoglobulin concentration values (mean \pm SE) for *M. fusca* and *C. inca* in the studied forest edge, cropland, and urban sites. Letters above error whiskers represent Newman-Keuls post-hoc range test differences ($P < 0.05$). FOR = forest edge, CROP = cropland, URB = urban.

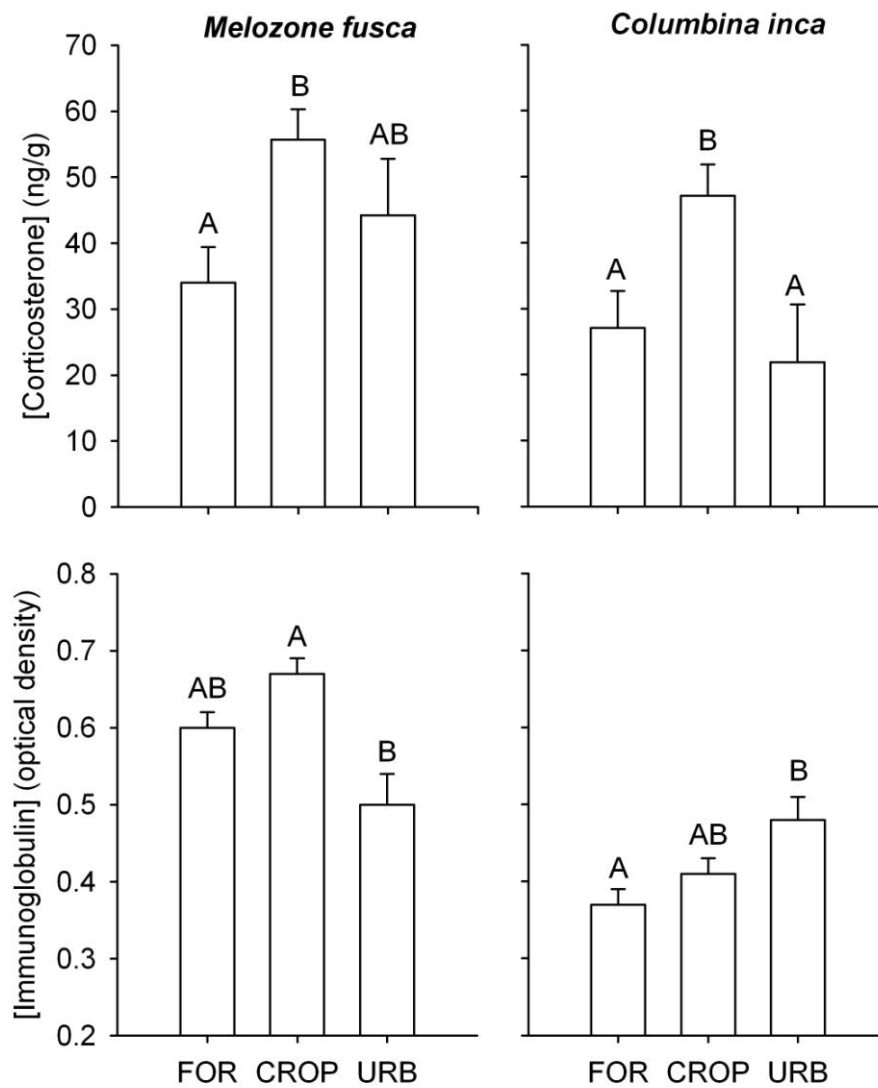


Figure 3. Relationship between corticosterone and immunoglobulin concentration values for *M. fusca* and *C. inca* in the studied forest edge, cropland, and urban sites. Solid gray lines represent mean values for corticosterone and immunoglobulin concentration for each species in the forest treatment, while segmented gray lines depict their positive SD. White circles represent individuals for which no negative ecophysiological assumption can be made in relation to their corticosterone and immunoglobulin concentration value. Black circles represent individuals with corticosterone values that are higher than expected and immunoglobulin concentration values that are lower than expected, and thus could be considered as physiologically compromised.

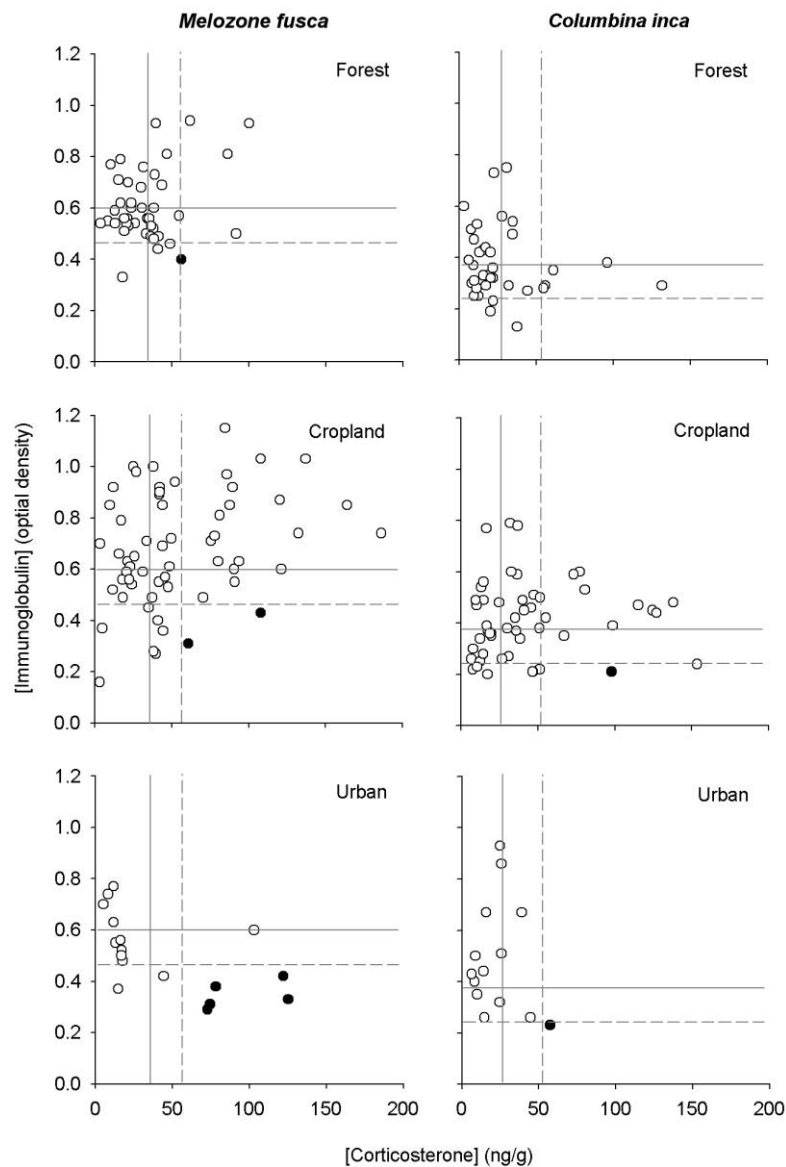
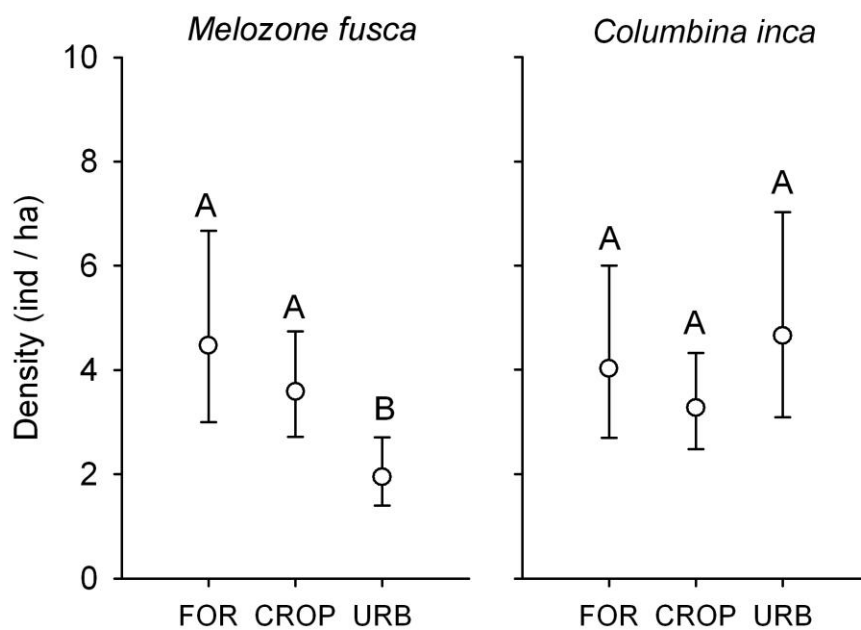


Figure 4. Mean density (\pm 95% confidence intervals) of *M. fusca* and *C. inca* in the studied forest edge, cropland, and urban sites. Letters above error whiskers represent statistical differences based on overlapping 95% confidence intervals (following Payton et al. 2003). FOR = forest edge, CROP = cropland, URB = urban.





Stress responses of the House Sparrow (*Passer domesticus*) to different urban land uses

Carlos A. Chávez-Zichinelli^a, Ian MacGregor-Fors^a, Patricia Talamás Rohana^b,
Ricardo Valdéz^b, Marta C. Romano^b, Jorge E. Schondube^{a,*}

^a Laboratorio de Ecología Funcional, Centro de Investigaciones en Ecosistemas, Universidad Nacional Autónoma de México, Campus Morelia, Antigua Carretera a Pátzcuaro 8701, Colonia ExHacienda de San José de la Huerta, Morelia 58190, Michoacán, Mexico

^b Centro de Investigación y de Estudios Avanzados del I.P.N., Apartado postal 14-740, 07360 México, D.F., Mexico

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ABSTRACT

Urbanization generates human-made habitats with novel resources. Such change is not homogeneous, and includes different urban land uses characterized by specific habitat traits. In this study we explored the ecophysiological response of an urban-exploiter species in three different urban land uses (urban, suburban, industrial). Our results show that corticosterone (obtained through fecal samples) and immunoglobulin (obtained through blood samples) concentrations did not differ significantly among the studied urban land uses. Corticosterone and immunoglobulin concentration showed both high and low values in urban and suburban areas, while industrial areas had low and high levels of corticosterone, and medium-to-low immunoglobulin concentration values. Also, we found a negative relationship between corticosterone and immunoglobulin concentrations in industrial areas. Based on corticosterone and immunoglobulin concentration thresholds established from previous studies, our results suggest that: (1) birds in the three studied urban conditions are both stressed and non-stressed; and (2) the immune system of industrial House Sparrows could not be responding successfully to pathogens. Not finding physiological relationships in urban and suburban areas underline that this species has a large capacity to respond to the different stress and immune challenges found in urban areas. However, our results suggest that the physiological condition of industrial House Sparrows could be compromised by differences in the frequency and intensity of the stressor agents faced by the birds in this land use category.

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1. Introduction

Human activities have dramatically transformed ecosystems around the world, with urbanization being one of the most common agents of land use change (Vitousek et al., 1997; Marzluff et al., 2001; McKinney, 2002). Although urban areas have been described as ecologically poor (Turner et al., 2004), the presence of different urban land uses generates high environmental variability within cities (Blair, 1996; Svoray et al., 2005; Ortega-Álvarez and MacGregor-Fors, 2009). Such urban heterogeneity is closely related to the frequency and intensity of human activities and habitat characteristics that exist within a city, which can generate different stressor stimuli for urban-dwelling wildlife (Blair, 1996; Partecke et al., 2006; Ortega-Álvarez and MacGregor-Fors, 2009).

Urban systems lie open to colonization by the fauna that can reach it and is able to overtake the hazards of a city and to use

its resources (Emlen, 1974). Urban land uses have different habitat traits, several levels of human activity, and diverse types of pollution. Because those species that dwell within urban areas have differential distributions inside them (Blair, 1996; Ortega-Álvarez and MacGregor-Fors, 2009), they may be susceptible to the set of stressor agents present in their land uses. One of the best examples of an urban-exploiter species in North America is the House Sparrow (*Passer domesticus*), which is characterized by its invasive and aggressive behaviors (Kalinowski, 1975; McGillivray, 1980; Gowaty, 1984; Gavett and Wakeley, 1986; Kimball, 1997; MacGregor-Fors et al., 2010). This species is able to exploit the highly predictable food resources generated by human activities in urban areas, becoming one of the most dominating urban-dwelling bird species in most North American towns and cities (Emlen, 1974; Rosenberg et al., 1987; Green and Baker, 2003; Shochat, 2004; MacGregor-Fors et al., 2010).

In this study we used the House Sparrow as a model to determine whether a bird species that has evolved within urban-rural-agricultural matrixes responds physiologically to the different conditions present in three urban land uses of a medium sized neotropical city (i.e., urban, suburban, and industrial). For this, we

* Corresponding author at: 27-3, Santa María de Guido, Morelia, Michoacán, México 58089, Mexico. Tel.: +52(443)222777x42504.
E-mail address: chon@oikos.unam.mx (J.E. Schondube).

measured two physiological traits of House Sparrows, stress (corticosterone concentration) and immune response (immunoglobulin concentration), in the three urban land uses. We also associated the physiological condition of House Sparrows with their abundances. We predicted that the physiological condition and abundance of House Sparrows would be negatively related to sites with low urbanization intensity, assessed using habitat traits, due to its commensalism with humans. Conversely, we expected the physiological condition and abundance of House Sparrows to be positively related with highly developed urban land uses, such as urban and industrial areas where human activities are intense.

2. Materials and methods

2.1. Study area

This study was conducted in the city of Morelia, located in the Cuitzeo watershed (west-central Mexico; 19°42'07"N, 101°11'33"W; ~1925 masl). Morelia is a medium sized city (>100 km²) with an estimated human population of 1,935,524 inhabitants for 2010 (Vargas Uribe, 2008). This city has grown by 400% from 1960 to 1990 with no formal urban planning (López et al., 2001; Vargas Uribe, 2008). Such growth has generated an heterogeneous urban matrix with five distinctive urban land uses: (1) residential areas (urban and suburban); (2) residential-commercial areas; (3) commercial areas; (4) industrial areas; and (5) green areas (e.g., parks, and cemeteries). These urban land uses have different habitat traits (e.g., vegetation and construction cover, socio-economic characteristics) and differ in their human activity levels (e.g., noise, passing cars/min, and passing pedestrians/min).

2.2. Urban land use characterization

We captured House Sparrows in three urban land uses (i.e., urban, suburban, and industrial), each with two replicates within the city of Morelia (Fig. 1). We characterized each of these land uses using a multivariate cluster analysis (Euclidean distance – single linkage) based on 15 environmental and human population variables measured in five sites randomly located within a 1 km² area from each capture site: (1) tree cover; (2) tree species richness; (3) tree abundance; (4) maximum tree height; (5) maximum tree diameter at breast height (DBH); (6) shrub cover; (7) shrub species richness; (8) maximum shrub height; (9) herbaceous plant cover; (10) herbaceous plant species richness; (11) maximum herbaceous plant height; (12) soil cover; (13) built cover; (14) maximum building height; and (15) human population density. Also, we measured the intensity of three human activities among the studied urban conditions: (1) noise, measured as the highest decibel values per min, using a digital sound level meter (BK PRECISION 732A); (2) vehicle traffic, measured as the number of passing cars per min; and (3) pedestrian activity, measured as the number of passing pedestrians per min. We measured noise, vehicle traffic, and pedestrian activity in three sites near each capture replicate. To compare if the measured human activities among the studied urban land uses differed, we performed GLM-ANOVA and post hoc Tukey tests. We did not capture House Sparrows in commercial areas, as these lacked suitable capturing sites; or in parks, because House Sparrows were fed by visitors and did not enter our traps. Hence, the urban conditions used in this study comprise: (1) two urban residential areas; (2) two suburban residential sites; and (3) two industrial areas, one of which was a paper mill, and the other an industrial complex comprised by furniture, food, construction material, shoe, and soap factories (Vargas Uribe, 2008) (Fig. 1).

2.3. Bird captures and surveys

We captured House Sparrows using Yunick traps (Yunick, 1971; Senar, 1988). We baited each capture site previously with a mixture of seeds for two weeks to attract the birds. House Sparrows were captured from 7:00 to 11:00 h for two weeks (August 27–September 7, 2007). We collected fecal and blood samples, which were frozen for laboratory analyses. Blood samples were obtained from the brachial vein and collected in heparin added capillary tubes. Following this, all captured House Sparrows were marked with a numbered metal leg-band and released.

To evaluate if House Sparrow abundances were related to the studied urban land uses, we carried out 10 point counts (25 m radius) for 10 min (Ralph et al., 1996) located at a minimum distance of 250 m from each other to assure survey independence (Ralph et al., 1996; Huff et al., 2000). Point counts were located in the studied urban, suburban, and industrial land uses where the sparrows were captured. All House Sparrows recorded using the surveyed areas were included in our analyses. House Sparrow abundances are displayed as ind/ha, calculated from the average number of individuals observed in each point count within a radius of 25 m (0.19 ha). We used a 25 m radius cut-off limit to calculate the abundance of House Sparrows for several reasons: (1) it was the radial distance at which ~95% of the total House Sparrow were recorded; (2) because most areas of the city of Morelia have buildings with a mean height of 6 m and narrow streets, detectability of urban birds decreases rapidly with distance; and (3) habitat characteristics are also very heterogeneous within the city, so we used a 25 m radius to ensure that the detected birds were using habitats with similar environmental conditions.

2.4. Physiological analyses

Physiological responses to stressor agents include acute and chronic stress. Acute stress, caused by non-constant stochastic stressor stimuli, can turn into chronic stress when the stressor agent persists for a long period of time (Wikelski and Cooke, 2006; Buchanan, 2000; McEwen, 2000; Wingfield and Romero, 2001; Dickens et al., 2010). Chronic stress generates negative effects on the physiology of individuals, such as the presence of hyperglycemia, the loss of muscular mass, and/or immunosuppression (Sapolsky et al., 2000; McEwen and Wingfield, 2003). To determine the physiological condition of House Sparrows in our sampling sites, we used fecal samples to determine corticosterone concentration values. We measured corticosterone in feces because they allow the integration of the stress state of the individuals prior to the capture time, and not the stress caused by the capture and handling of the birds (Millspaugh and Washburn, 2004; Touma and Palme, 2005).

2.4.1. Corticosterone concentration (radioimmunoassay – RIA)

We defrosted standardized fecal samples collected from House Sparrows to 0.5 g samples (wet weight). We ran the samples by duplicate. To extract corticosterone from the fecal samples, we placed each sample in a vial with 1 ml of ethanol (Cavigelli, 1999; Sands and Creel, 2004), vortexed for 20 min, and centrifuged for 20 min (10,000 rpm). In most cases, we used a dilution of 1:4. Afterward, we placed 100 µl of the dilution in RIA tubes and added 100 µl of anti-corticosterone antibody (Sigma C-8784). Finally, we incorporated 50 µl of tritiated corticosterone ([1,2,6,7-3H(N)]-Perkin Elmer Boston, Ma. 02118). We incubated our samples for 20 h at 4 °C, separating the unbound steroids with carbon. We measured the radioactivity using a scintillation counter (Beckmann Instruments LS-6500). We validated this method for House Sparrows by analyzing the recovery of exogenous corticosterone in relation to field samples by adding exogenous corticosterone to

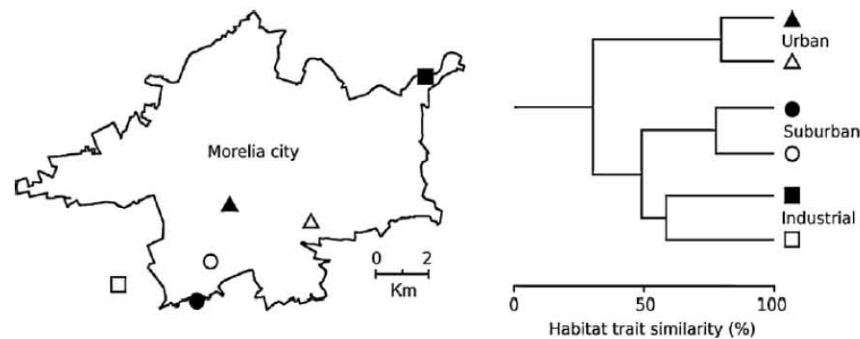


Fig. 1. Location of study sites and differentiation of the surveyed urban land uses. We captured House Sparrow in suburban areas (circles), urban areas (triangles), and industrial areas (squares). Color differences represent the two different replicates for each urban land use. The multivariate cluster analysis clearly revealed that the selected replicates are highly similar for each land use.

one set of samples as control. Two types of results show that the assay was applicable to House Sparrows samples: (1) fecal extracts in relation to the corticosterone standard curve showed similar slopes ($F=0.493$, $df=4$, $P=0.521$) and (2) the successful recovery of exogenous corticosterone. We measured samples in three separate assays, with intra- and interassay coefficients of variation of 5.16% and 3.36%, respectively.

2.4.2. Immunoglobulin concentration (ELISA test)

To determine the concentration of total immunoglobulins (including IgY, IgM, and IgA) from blood samples, we used an ELISA test, as described by Martínez et al. (2003). Briefly, blood samples were extracted from the capillary tubes, placed in 0.5 ml vials, centrifuged for 10 min, and blood serum was collected. We diluted blood serum samples (1:4000), placed them in well plates, and incubated them at 4 °C for 18 h. Next, we added a primary antibody (polyclonal rabbit anti-chicken IgG conjugated with peroxidase; Sigma A-9046) and incubated the samples for another 2 h at 37 °C. Afterward, we added orthophenyldiamine (OPD) as a substrate. We read the plates for optical densities using an ELISA plate spectrophotometer ($\lambda=492$). All samples were analyzed in triplicate.

2.5. Statistical analyses

To avoid bias in corticosterone (fecal samples) and/or immunoglobulin (blood samples) concentration due to possible differences generated by the sex of the captured birds, we performed two-way ANOVA using each physiological variable (corticosterone and immunoglobulin concentration) as dependent variables, and sex (male, female) and urban land use categories (urban, suburban, industrial) as independent grouping variables. To compare corticosterone and immunoglobulin concentrations among the three urban land uses, we performed one-way ANOVA for each physiological variable. To evaluate if different urban land use areas influence the physiological condition of House Sparrows, we computed linear regressions between stress (corticosterone; ng/g – fecal samples) and immunological condition values (immunoglobulin optical density – blood samples) from the three studied urban land uses. We conducted one-way ANOVA to determine whether the abundance of House Sparrows varied with urban land use category. When a significant difference was obtained, we applied Tukey post hoc range tests to determine which land use category differed significantly in their House Sparrow abundances. Furthermore, we evaluated potential relationships between both physiological variables and House Sparrow abundances using linear regressions. All datasets were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Bartlett test), following Sokal and Rohlf (1995).

Finally, we analyzed the statistical power (post hoc) for each regression using G*Power (Faul, 2008). As the power of a statistical test increases, the probability of a Type II error decreases, allowing us to evaluate if our results are statistically robust regardless of our sample size. We used a minimum power value of 0.80 following Park (2008).

3. Results

3.1. Urban land use characterization

The multivariate cluster analysis showed that both replicates for each one of the three studied urban land uses were more similar between them than in relation to the replicates of the other land uses (Fig. 1). Highest similarity was obtained for replicates of urban sites (79%), followed by suburban (77%), and industrial ones (58%). Industrial and suburban areas were clustered at 49% similarity, showing wide differences in relation to the urban cluster (30% similarity; Table 1). Although industrial areas showed a relatively higher habitat trait similarity to suburban areas than urban residential ones, the intensity of human activities differed among the three studied urban conditions. Noise (decibels) was significantly higher in industrial areas than in urban and suburban residential areas (GLM-ANOVA: $F=37.6$, $df=2$, $P<0.001$, Tukey P -values: urban–suburban=0.99, urban–industrial<0.001, suburban–industrial=<0.001). Vehicle traffic (vehicles/min) was also significantly higher in industrial areas than in urban and suburban residential areas (GLM-ANOVA: $F=10.09$, $df=2$, $P<0.001$, Tukey P -values: urban–suburban=0.95, urban–industrial<0.001, suburban–industrial<0.001). The number of passing pedestrians (pedestrian/min) showed a different pattern, with highest values in urban areas, mid values in industrial areas, and low values in suburban areas (GLM-ANOVA: $F=11.15$, $df=2$, $P<0.001$, Tukey P -values: urban–suburban=0.001, urban–industrial=0.11, suburban–industrial=0.02; Table 1).

3.2. Physiological condition

We captured a total of 38 House Sparrows, of which 21 were females, and 17 males. We found no difference in corticosterone or immunoglobulin concentrations between males and females ($F=2.1$, $df=32$, $P=0.13$; $F=0.57$, $df=32$, $P=0.56$; respectively from two-way ANOVA). Thus, the physiological data obtained from males and females were combined for subsequent analyses.

We found no statistical differences between corticosterone concentration nor immunoglobulin optical density, and the three studied urban land uses (ANOVA: $F=0.50$, $df=35$, $P=0.60$;

Table 1
Habitat trait values (mean \pm SE) among the studied urban, suburban, and industrial land uses of the city of Morelia.

Habitat trait	Urban land use		
	Urban	Suburban	Industrial
Tree cover (%)	11 \pm 6.9	18.5 \pm 10.0	11.6 \pm 12.2
Tree species richness	3.3 \pm 2.1	4 \pm 1.7	1.7 \pm 1.5
Tree abundance (ind/0.19 ha)	16.5 \pm 17.4	24.7 \pm 18.8	17.5 \pm 24.8
Maximum tree height (m)	7.6 \pm 4.3	9.8 \pm 3.7	7.3 \pm 5.1
Maximum tree DBH (cm)	25.4 \pm 19.9	57 \pm 42.1	26 \pm 18.5
Shrub cover (%)	3.2 \pm 6.2	2.1 \pm 3.3	6.5 \pm 5.9
Shrub species richness	1.2 \pm 1.6	0.9 \pm 1.2	1.7 \pm 0.8
Maximum shrub height (m)	0.7 \pm 1.1	0.91 \pm 1.9	1.27 \pm 0.8
Herbaceous plant cover (%)	13.9 \pm 20.7	31.6 \pm 25.2	72 \pm 15.5 h
Herbaceous plant species richness	4.1 \pm 4.1	5.1 \pm 2.9	3.5 \pm 3.1
Maximum herbaceous plant height (m)	0.8 \pm 1.2	1.25 \pm 1.2	0.56 \pm 0.6
Soil cover (%)	3.5 \pm 6.7	5.5 \pm 10.7	1 \pm 3.1
Built cover (%)	81.7 \pm 27.4	62.9 \pm 32.6	27 \pm 15.7
Maximum building height (m)	8.3 \pm 2.1	7.25 \pm 1.4	7.4 \pm 3.5
Human population density (ind/km ²)	11329.3 \pm 10766.8	1204.1 \pm 1815.9	213.1 \pm 178.8
Human activity			
Noise (decibels)	57.5 \pm 6.7	58.2 \pm 11.5	77.2 \pm 9.2
Vehicle traffic (vehicles/min)	2.1 \pm 1.7	2.3 \pm 2.8	6.8 \pm 6.9
Pedestrian activity (pedestrians/min)	2.8 \pm 2.5	0.8 \pm 1.5	1.7 \pm 1.6

$F=0.86$, $df=35$, $P=0.42$; respectively). However, when we related immunoglobulin optical density to corticosterone concentration, we found an effect of urban land uses on the physiological condition of House Sparrows (Fig. 2). We found no significant relation between immunoglobulin optical density and corticosterone concentration of birds in urban ($r^2=0.004$, $P=0.86$, statistical power=0.03) and suburban residential areas ($r^2=0.008$, $P=0.81$, statistical power=0.05). However, we found a significant negative relationship between corticosterone and immunoglobulin concentrations in both industrial areas ($r^2=0.499$, $P=0.01$, statistical power=0.86; Fig. 2).

3.3. House Sparrow abundance

The abundance of House Sparrows varied among the three studied urban land uses (ANOVA: $F=4.88$, $df=27$, $P=0.011$; Table 2). The Tukey post hoc test demonstrated that the abundance of House Sparrows in suburban areas was significantly lower than in industrial ($P=0.021$) and urban areas ($P=0.015$), while did not differ between urban and industrial land uses ($P=0.62$; Table 2). Linear regressions showed no significant relationships between the abundance of House Sparrows and corticosterone concentration ($r^2=0.53$, $P=0.09$, statistical power=0.98), or immunoglobulin concentration ($r^2=0.01$, $P=0.81$, statistical power=0.06).

4. Discussion

Animals respond to different degrees of disturbance in their environment through physiological mechanisms and behavior, which in turn could shape the dynamics and size of their populations (Wikelski and Cooke, 2006; Temple and Wiens, 1989; Ricklefs and Wikelski, 2002; Romero, 2004; Partecke et al., 2006). Our results show that the physiological traits measured in House Sparrows (i.e., corticosterone and immunoglobulin concentration) did not vary significantly among the studied urban land uses (i.e., urban, suburban, and industrial). Urban and suburban residential land uses had birds with both low and high levels of corticosterone and immunoglobulin concentrations, while industrial areas had low and high levels of corticosterone and medium-to-low immunoglobulin concentration values. We found that these two physiological variables were negatively related in industrial areas. Also, we found that the abundance of House Sparrows differed among land uses, with higher numbers in urban and industrial

areas, and lower numbers in suburban areas. In this section we first compare our results with those from previous studies. Second, we focus on our results regarding House Sparrow abundances. Third, we briefly discuss the capacity of House Sparrows to respond to the different stress and immune challenges in the studied urban land uses. Finally, we discuss why House Sparrows had a negative relationship between corticosterone and immunoglobulin concentrations in industrial areas.

The range of values recorded in this study for corticosterone concentration (~20–180 ng/g) are within the range of values recorded by other studies that have assessed avian stress using fecal samples, with lowest values being nine times lower than the highest ones. These studies have showed that when a stressor agent is present, corticosterone concentration is 2–17 times higher than in control treatments where stress agents are absent (Buchanan, 2000; Romero, 2004; Thiel et al., 2008; Moreno et al., 2010). For example, Dickcissels (*Spiza americana*) with corticosterone concentrations ~6 ng/g in the absence of stressor agents, increased their values to ~102 ng/g when they were subject to a stressor agent (Kimberly et al., 2003). African Grey Parrots (*Psittacus erithacus*) exhibited low corticosterone concentration values under normal conditions (~75 ng/g), but showed three times higher values when stressed by feather-plucking (~265 ng/g; Owen and Lane, 2006). Spotted Owls (*Strix occidentalis*) also responded to stressor agents, with low corticosterone concentration values in preserved areas (~20–60 ng/g) and almost four times higher concentration values in disturbed areas (~98–110 ng/g; Wasser et al., 1997). Data from these studies suggest a corticosterone concentration baseline in feces for birds living in undisturbed areas that ranges from 6–75 ng/g. Using these values, we consider individuals with corticosterone concentration values <75 ng/g to be non-stressed, while individuals with values >75 ng/g to undergo some kind of stress. In our case, House Sparrows had corticosterone levels below and above this stress threshold in all the three studied urban land uses (Fig. 2). This indicates that birds in the three studied urban conditions confront stressor agents and find refuge from them.

Immunoglobulin concentrations also varied widely in House Sparrow individuals captured in urban, suburban, and industrial areas. Although we did not find any studies evaluating immunoglobulin concentration in House Sparrows, Martínez et al. (2003) reported immunoglobulin optical density values for two passerine species obtained from wild adult individuals: (1) Blue Tits – *Parus caeruleus* (~0.3–0.8); and (2) Pied Flycatchers – *Ficedula*

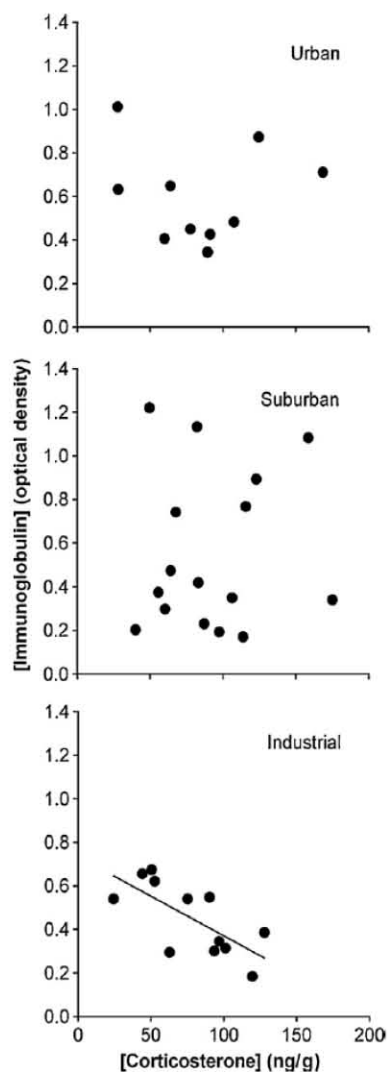


Fig. 2. Relationship between House Sparrow corticosterone and immunoglobulin concentrations at different urban land uses. While both physiological variables were not related at urban and suburban areas, immunoglobulin and corticosterone concentrations were negatively related at industrial areas.

hypoleuca (~0.3–0.9). These values are similar to those recorded for other bird species (i.e., Canyon Towhee – *Pipilo fuscus*, Inca Dove – *Columbina inca*) in our study area (~0.2–0.9 unpublished data) and the data presented in this study (~0.2–0.8). Perozo et al. (2007) showed that immunoglobulin values in Newcastle Disease inoculated chickens rise asymptotically responding to the virus, stabilizing in immunoglobulin concentration (optical density values) ranging from 0.7 to 0.8, while non-inoculated chickens

showed values lower than 0.2. Their results suggest that birds exhibit immunoglobulin concentration values >0.7 are successfully responding to pathogens, and can have values ranging from ~0.2 to 0.7 under other conditions. In our case, House Sparrows had immunoglobulin concentration levels below and above this immune threshold in urban and suburban land uses, and had immunoglobulin concentration levels below 0.7 in industrial areas. Because of the high corticosterone levels of these birds, these values suggest that the immune system of industrial House Sparrows could not be responding successfully to pathogens. However, this hypothesis remains to be tested.

As expected, we found a significantly higher number of House Sparrows in industrial and urban areas when compared to suburban sites (Table 2). Lower House Sparrow abundances in suburban residential areas could be the result of lower urban development and human activity. However, we found no difference in the abundance of House Sparrows between urban and industrial areas, despite the high variation in habitat traits between these two urban land uses. Higher densities of House Sparrows in these two land uses suggest that this species is taking advantage of its ability to withstand human disturbance and use the extra resources present at these sites (as suggested by Shochat, 2004).

Although our results suggest that the immune system of industrial House Sparrows could not be responding successfully to pathogens, we did not find significant differences in stress levels or immune condition of House Sparrows among the three studied urban land uses. This unexpected result suggests that this species has a large capacity to respond to the different stress and immune challenges present in the different land uses of a city and its surrounding areas. This physiological proficiency to defy stressor agents and immune challenges, along with its ability to compete for food and nesting resources with native bird species, could be one of the reasons behind the success of the House Sparrow as an invasive species in human altered environments around the world (Anderson, 2006).

Although we found no differences when comparing House Sparrow corticosterone and immunoglobulin concentrations among urban, suburban, and industrial areas (Table 2), when we related these two physiological variables, we found two different responses. First, bird populations living in urban and suburban areas did not show a relationship between the measured physiological variables. The lack of relationship between these physiological variables suggests that House Sparrows living in these urban land uses present a great array of physiological conditions (e.g., non-stressed, chronic stress, and acute stress; Fowles et al., 1993; Bourgeon et al., 2006; Bourgeon and Raclot, 2006). Both urban and suburban conditions had low to intermediate levels of human activity. These human activity patterns could cause high corticosterone levels in House Sparrow individuals facing stressor agents, but also provide an opportunity for the birds to recuperate from stress (Bhatnagar and Vining, 2003; Romero, 2004; Partecke et al., 2006). Second, House Sparrow corticosterone and immunoglobulin concentrations showed to be negatively related in both industrial areas. Birds with high levels of stress and low immune response tend to be under chronic stress (Fowles et al., 1993; Bourgeon et al., 2006; Bourgeon and Raclot, 2006; Verburg-van and Schreck, 2007). The negative relationship we found between these two variables

Table 2

Values (mean \pm SD) of physiological variables and House Sparrow's abundances recorded at the different urban land use categories.

Land use category (ng/g)	Corticosterone concentration (optical density)	Immunoglobulin concentration birds	Captured (ind/ha)	Bird abundance
Industrial	78.3 \pm 31.9	0.4 \pm 0.1	12	10.6 \pm 3.1
Urban	83.9 \pm 43.1	0.6 \pm 0.2	10	9.1 \pm 2.2
Suburban	92.5 \pm 39.5	0.5 \pm 0.3	16	5.3 \pm 1.9

indicates that industrial House Sparrows do not have an optimal immune condition under high corticosterone concentration levels (Wingfield et al., 1998; Fig. 2). Although industrial areas exhibited high habitat trait similarity in relation to suburban areas, where both physiological variables were not related, the physiological condition of industrial House Sparrows could be caused by differences in the frequency and intensity of the stressor agents faced by the birds in this land use (Table 1). Our results suggest that industrial House Sparrows with low immunoglobulin concentrations and high stress levels could be responding to the continuous presence of human activity, high levels of noise, and vehicle traffic in this urban land use.

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CAPÍTULO IV

EFFECTOS DEL ESTRÉS EN LA MUDA Y LA COLORACIÓN DE PLUMAJE DURANTE LA REPRODUCCIÓN DE *Volatinia jacarina*

RESUMEN

Las características del plumaje son consideradas como señales honestas, principalmente porque la expresión del ornamento depende de la condición física del portador y de otras medidas de calidad individual (genéticas y ecológicas). Nuestra meta en este estudio es establecer si existe una relación entre la condición fisiológica medida como niveles de estrés (corticosterona) y estado inmunológico (inmunoglobulinas), la muda, y la coloración del plumaje estructural de *Volatinia jacarina*. El estudio lo llevamos a cabo en áreas de cultivo del occidente de México. Capturamos a individuos machos de *V. jacarina*, y les tomamos una muestras de plumas para determinar la coloración de plumaje, un muestra de excretas para cuantificar la concentración de corticosterona y una muestra de sangre para medir la concentración de inmunoglobulinas. Encontramos una relación entre los valores de hue del color de las plumas, la corticosterona y lo avanzado de la muda. La aves con a mayores niveles de corticosterona tienen un color mas ultravioleta y una muda menos avanzada, mientras que los individuos con menores niveles de corticosterona eran más azules y tenían una muda corporal más avanzada. Este resultado fue muy marcado en el área de las grandes cobertoras alares, la cual es utilizada como un elemento de comunicación durante los despliegues de los machos. Nuestro estudio indica que la coloración estructural de las plumas pueden reflejar la condición del individuo al momento de la muda, y servirnos como un indicador directo de algunas variables fisiológicas como sus niveles de estrés. La relación entre nivel de estrés y muda muestra que individuos con bajos niveles de estrés mudan antes y más rápido que individuos estresados. Esto les permite iniciar sus cortejos antes, y presumiblemente, aumentar su capacidad reproductiva.

Palabras clave: coloración estructural, corticosterona

INTRODUCCIÓN

El plumaje de las aves cumple entre otras funciones, el servir como un carácter sexual secundario que ayude a las hembras a seleccionar a los machos con los cuales se aparearan (Siefferman y Hill 2005). Se sabe que la condición y/o la coloración del plumaje permite a las hembras evaluar la calidad de los machos (Hill 2002). De este modo, las características del plumaje (color, tamaño de las plumas) son consideradas como señales honestas, principalmente porque la expresión del ornamento depende de la condición física del portador y de otras medidas de calidad individual (genética) y ecológicas (calidad de territorio; Keyser y Hill 2000, Cotton 2004). De este modo, el plumaje puede ser utilizado por distintos individuos para evaluar a sus congéneres y así poder determinar la calidad de un individuo y los posibles tipos de interacción tanto social como sexual (Negro *et al.* 2007).

La coloración del plumaje es una de las características que las aves utilizan para evaluar la calidad de otros miembros de su misma especie (Hill 2002, Siefferman y Hill 2005). Existen principalmente dos tipos de coloración: la coloración pigmentaria y la coloración estructural (Hegyi *et al.* 2007). La coloración pigmentaria ha sido ampliamente estudiada y tiene implicaciones a nivel ecológico debido a que algunos pigmentos se obtienen solo a través de la dieta, y por lo tanto la coloración del plumaje está relacionada con la calidad del territorio, la capacidad para forrajear y la dominancia de los machos (Hill *et al.* 1999). El papel que tiene la coloración estructural como señales honestas en aves es poco conocido. Sin embargo diversos estudios sugieren que producir las coloraciones estructurales es costoso ya que los individuos con mejor condición física despliegan ornamentaciones más vistosas que aquellos con una condición física inferior (Cotton 2004, Siefferman y Hill 2005). La condición física depende en gran medida del contenido y calidad de las proteínas y el estado fisiológica que determina la calidad de este tipo de coloración al momento de la muda (DesRochers *et al.* 2009).

Existen estudios que muestra una relación clara entre la calidad de la coloración estructural y el estado fisiológico del individuo (Badyaev *et al.* 2001). La relación entre el estado fisiológico con el plumaje puede tener un interés teórico y aplicado. Teóricamente nos permite entender cuáles son los mecanismos inmediatos que determinan aspectos

fenotípicos del plumaje, como son las decisiones asociadas con las fechas de muda, el total de plumas que se mudarán, y la calidad final del plumaje (Forsman *et al.* 2008). De forma aplicada, la relación entre la calidad individual y la calidad del plumaje nos permiten utilizar las plumas como un indicador biológico de la salud del individuo a diversos niveles (Doucet 2002, Senar *et al.* 2003).

Nuestra meta en este estudio es establecer la relación existente entre la condición fisiológica medida como niveles de estrés (corticosterona) y estado inmunológico (inmunoglobulinas), la muda, y la coloración del plumaje estructural de *Volatinia jacarina*. Hipotetizamos que individuos con una buena condición corporal deben tener un mayor peso independiente de su tamaño corporal, menor estrés y mejor respuesta inmune. Esto se debe reflejar en mayor cantidad de plumas mudadas y en el color de las plumas. Escogemos como modelo de estudio a *V. jacarina*, debido a que es una especie con coloración estructural, que presenta una muda reproductiva donde los machos cambia el color de sus plumas para atraer a las hembras por medio de despliegues en vuelo donde contrastan manchas blancas contra un fondo negro azulado en las alas (Carvalho *et al.* 2007, Santos *et al.* 2009).

MATERIALES Y MÉTODOS

Área de estudio

Llevamos a cabo nuestro estudio en el poblado de Francisco Villa (19 ° 23' 03''N, 105 ° 03' W) en la costa de Jalisco, México. La zona de estudio es un abierto con campos de cultivo, cercas vivas y remanentes de bosque tropical caducifolio. El paisaje está dominado principalmente por bosque tropical seco (Lott, 1993) y tiene una estacionalidad bien definida, con una marcada estación seca de octubre a junio (Bullock 1986).

El semillero brincador (*Volatinia jacarina*), es una especie granívora que habita en hábitats tropicales abiertos, como pastizales, zonas de cultivo y vegetación secundaria. Esta especie tiene un claro dimorfismo sexual. Las hembras son de color café, con vientre y pecho color crema marcado por líneas medias oscuras. Los machos tienen dos tipos de plumajes: un plumaje no reproductivo de Octubre a Mayo, y un plumaje reproductivo de Mayo a Agosto (Howell y Webb 1995). El plumaje no reproductivo es café con parches de

plumas azul-negro en algunas zonas del cuerpo (cabeza, hombros ala y cola). Durante la temporada reproductiva los machos realizan una mudan completa para producir un plumaje negro/azul brillante (iridiscente), resultado de coloración estructural. Adicionalmente presentan un parche blanco en el ala ubicado entre la muñeca y el hombro (Costa y Macedo 2005).

Capturamos a los individuos de (*V. jacarina*) durante el mes de mayo cuando está presente la muda que genera el plumaje reproductiva. Capturamos con redes de niebla a los machos de esta especie durante las primeras horas de la mañana (07:00 a 10:00). De los individuos capturados tomamos medidas morfológicas (longitud del tarso, ala y cola), peso, número de plumas primarias, secundarias y terciario mudadas, densidad de muda de cuerpo en una escala de (0-4; donde 0 es ausencia de muda, y 4 una muda alta presente en 90% o más del cuerpo del ave), grasa almacenada donde 0 era ausencia completa de grasa y 6 gran cantidad de grasa en fúrcula y costados del aves. Adicionalmente tomamos una muestra de plumas de las cobertoras y del pecho de cada individuo para determinar la coloración de plumaje. Para la estimar la condición fisiológica de los individuos, tomamos una muestras de excretas y de sangre de cada individuo. Las excretas las utilizamos para determinar la concentración de corticosterona (estrés), y cada excreta la colocamos en un microtubo 1.5 ml con un mililitro de etanol absoluto. La sangre fue colectada con un tubo capilar directamente de la vena braquial, y fue utilizada para evaluar el sistema inmunológico. Tanto las muestras de excretas como las desangre fueron congeladas para analizarlas posteriormente en el laboratorio.

Mediciones de coloración del plumaje

Medimos la coloración de plumaje con un espectrofotómetro USB2000 (Ocean Optics, Duiven, The Netherlands, EU) y un PX-2 pulsed xenon light (220-750 nm). Todos los espectros de la muestras se miden con una referencia blanca (WS-1, Diffuse Reflectance Standard) y una referencia oscura. Colocamos de 10 a 15 plumas empalmadas unas entre otras de cada individuo sobre una superficie de terciopelo oscuro (reflectancia 0%). Esto nos permitió imitar la superficie del plumaje de las aves (Quesada y Senar, 2006). Realizamos tres mediciones de las cuales consideramos la media de los espectros con la finalidad de determinar los valores de color. Para cada espectro individual calculamos los

valores de reflectancia en la región ultravioleta del espectro (Andersson *et al.* 1998, Doucet y Montgomerie, 2003). El color se midió como la proporción de la reflexión que ocurre en el pico más alto de la región de UV en relación con el total del espectro (300-700 nm; Andersson *et al.* 1998, Doucet 2002, Doucet y Montgomerie, 2003). La saturación fue medida como la longitud de onda UV donde la reflexión fue máxima (Andersson *et al.* 1998, Doucet y Montgomerie 2003; Keyser y Hill 1999).

Procedimiento de Laboratorio: Corticosterona - Radio Inmuno Ensayo (RIA)

Agitamos los microtubos con las muestras de excretas en etanol en un Vortex por 10 minutos a alta velocidad. Posteriormente, todas las muestras las centrifugamos a 10000 RPM por 20 minutos. De cada muestra extrajimos el sobrenadante o extracto y realizamos a una dilución en buffer RIA diluido en una proporción 1:8. Tomamos 100 µl de la dilución del extracto de excreta de cada individuo y la colocamos en tubos RIA. A cada extracto le adicionamos 100 µl de anticuerpo anti-corticosterona (Sigma C-8784) a una dilución 1:2000 y 50 µl de corticosterona radioactiva ([1,2,6,7- ³H(N)]-Perkin Elmer Boston, Ma. 02118), e incubamos por 20 horas a 4°C. Todas las muestras se realizan por duplicado. Transcurrido el tiempo de incubación, agregamos 500 µl de carbón activado a cada muestra y se dejaron reposar por 10 minutos, para después ser centrifugadas a 10000 RPM a 4 °C por 20 minutos. Posteriormente vertimos las muestras en viales de centelleo, agregando 5 mililitros de líquido de centelleo y midiendo en el contador (LS 6500, Beckman Instruments, Fullerton, CA). Finalmente, las lecturas de las cuentas por minuto fueron analizadas en el programa MS2 corticosterona para calcular la cantidad de corticosterona en picogramos por gramo de excreta.

Concentración de inmunoglobulinas (prueba de ELISA)

Para determinar la concentración de inmunoglobulinas totales (incluidas las IgY, IgM e IgA) en muestras de sangre, utilizamos la técnica ELISA, según lo descrito por Martínez *et al.* (2003). Colocamos las muestras de sangre en microtubos de 0,5 ml. Centrifugamos los microtubos con la sangre durante 10 minutos, y separamos el suero sanguíneo. Diluimos en buffer salino la muestra de suero sanguíneo de cada individuo (1: 4000). Colocamos las

diluciones en placas especiales para técnica de ELISA y las incubamos a 4 ° C durante 18 horas. A continuación, añadimos un anticuerpo primario (polyclonal rabbit anti-chicken IgG conjugated with peroxidase; Sigma A-9046) e incubamos las muestras durante dos horas a 37 ° C. Después, añadimos orthophenyldiamine (OPD) como sustrato. Leímos las placas en un espectrofotómetro ($\lambda = 492$) determinando la densidad óptica de las muestras. Todas las muestras las hicimos por triplicado.

Análisis estadísticos

Con el fin de evaluar la relación entre el estrés fisiológico y la muda se realizó un análisis Regresión de Mínimos Cuadrados Parciales mediante el algoritmo NIPALS. Este método estadístico combina el análisis de componentes principales y modelos múltiples de regresión con el fin de maximizar la varianza explicada entre la variable dependiente y un conjunto de componentes linealmente ortogonales (PC) generados por los predictores originales independientes (Carrascal *et al.* 2009). La ventaja de utilizar estos métodos es que no son sensibles a la multicolinealidad (un problema típico en la regresión múltiple) y que los regresores generan un conjunto de componentes (PC) que maximiza la varianza en relación a la variable dependiente (STATSOFT 6.0). Para validar nuestro modelo, y con el fin de determinar qué componentes lineales fueron significativos, se realizó un análisis de validación cruzada del modelo (similar al método jackknifing; Carrascal *et al.* 2009).

Utilizamos como variable dependiente el número de coberteras primarias mudadas. Se optó por escoger las cobertoras primarias ya que en análisis previos que explicaban la mayor variación en el plumaje de muda entre los individuos que otras zonas y además, se evitaba la colinealidad, y como regresores las concentraciones de corticosterona y de inmunoglobulinas como indicadores de calidad fisiológicas, el tarso y el peso de cómo control para tamaño individual, y por último el número de plumas utilizadas en la medición de la coloración del plumaje, ya que este parámetro influyen en los valores de color y se usó como covariable (Quesada y Senar, 2006). Del mismo modo, para las tres variables de color (lightness, Chroma y Hue), se utilizó un análisis PLSR donde las variables dependientes fueron el lightness, el Chroma y el Hue, y como regresores utilizamos el contenido de corticosterona, la concentración de inmunoglobulinas, la longitud del tarso y

el peso y el número de plumas medidas. Todos los análisis se realizaron con STATISTICA 6.0.

RESULTADOS

Capturamos y procesamos un total de 48 machos adultos. No encontramos ningún componente principal (PC) significativamente asociado a la densidad de la muda. En relación con el color del plumaje, ni la luminosidad ni la saturación del color estuvieron relacionados con alguno de los componentes generados por las variables. Sin embargo, encontramos una relación significativa entre el primer componente y el color del tono del plumaje de los machos de *V. jacarina*. Los regresores que generaron este primer componente explican un 24,2% de la varianza del tono. Este primer componente se generó principalmente por dos variables, el nivel de la muda y el contenido en corticosterona, ya que estas variables explicaban más varianza en el componente que lo esperado por el azar (Tabla 1). Además, estas variables fueron inversamente proporcionales. Por lo tanto, nuestro análisis revela que los individuos que están más estresados tenían una tonalidad más baja y tenía una muda más retrasada que aquellos individuos con una tonalidad mas azul (Figura 1).

DISCUSIÓN

Nuestros resultados muestran que los machos de *V. jacarina* que tenían una mayor concentración de corticosterona, presentaron plumas con coloración más ultravioleta y una muda más retrasada, mientras que los individuos con menor concentración de corticosterona tenían una tonalidad más azul y una muda más avanzada. En esta sección primero discutimos los efectos que puede tener la corticosterona en la coloración de plumaje. Posteriormente mencionamos la relación entre el estrés y el desarrollo de la muda de los machos de *V. jacarina*. Finalmente discutimos los efectos que la coloración de plumaje y la muda tienen a nivel ecológico, y como se relacionan estos aspectos con la calidad individual de las aves.

Niveles de corticosterona y coloración estructural

Es un hecho que las hormonas ejercen efectos sistémicos en muchos aspectos de la fisiología, la morfología, y la conducta de los individuos. (Husack et al., 2009). En las aves la corticosterona ejerce sus efectos en la mayoría de los tejidos, incluida la piel y estructuras epidérmicas como las plumas (Romero 2002, Moore y Jessop 2001). Nosotros encontramos que los individuos macho de *V. jacarina* con mayores concentraciones de corticosterona presentaron plumas con coloración más en el espectro ultravioleta, mientras que aquellos que tenían menos corticosterona presentaban plumas con color más en el rango del azul. Estas diferencias en la coloración de las plumas sugiere que la corticosterona puede estar modificando la coloración de las plumas. Esto puede deberse a que la exposición a altas concentraciones de corticosterona puede alterar la estructura de las plumas.

DesRochers y colaboradores (2009) encontraron en *Sturnus vulgaris* que un aumento en la concentración de corticosterona genera una disminución en la masa total de las plumas. Esta menor masa se debe a un menor número de bárbulas, y una mayor distancia entre bárbulas. Consecuentemente las plumas de aves con altos niveles de corticosterona eran más frágiles y susceptibles a romperse. Roulin y colaboradores (2008) encontraron que conforme se incrementaba la concentración de corticosterona en *Tyto alba* se afectaba la coloración del plumaje debido a que esta hormona inhibe la síntesis de

melanina. La corticosterona afecta la presencia de melanina en las plumas por medio de inhibir la transcripción de la tirosinasa, enzima que cataliza la producción de melanina y otros pigmentos, y de la hormona proopiomelanocortina (POMC) que actúa en forma directa la formación del pigmento melánico de la piel (Heath 2003). De este modo la corticosterona afecta directamente la coloración de plumaje, al modificar la calidad en la estructura de la pluma e inhibir la síntesis de melanina.

Maia y colaboradores (2009) reportaron que en *V. jacarina* la melanina tiene un papel muy importante sobre la coloración estructural. La melanina afecta la coloración por pigmentos y cambia la resistencia de la pluma, por lo que bajas concentraciones de melanina generan plumas menos oscuras y más débiles. Las plumas que lleguen a presentar menores cantidades de melanina pueden tener un menor contraste con el parche blanco de ala, reduciendo la atractividad de los machos durante sus despliegues. Adicionalmente, menos melanina implicaría un mayor desgaste y más susceptibilidad de las plumas a romperse (Bonser 1995). La baja calidad de las plumas puede generar cambios en el color de las plumas por desgaste (McGraw *et al.* 2002). Sin embargo el papel que la relación entre la corticosterona y la melanina tienen sobre la expresión de color en el plumaje de *Volatinia jacarina* requiere aun ser explorada.

Niveles de corticosterona y expresión de la muda.

El plumaje en las aves cumple varias funciones como ayudar a la termorregulación, permitir el vuelo, y servir como una señal visual y en algunos casos auditiva, por lo que es un elemento principal para la selección de pareja (Hill 1990, Siefferman y Hill 2005). Nuestros resultados muestran que los machos de *V. jacarina* que tenían menor concentración de corticosterona, tuvieron una muda del plumaje reproductivo más avanzada que los individuos con mayores concentraciones de corticosterona. La muda del plumaje para las aves es un proceso muy costoso energéticamente (Senar 2004). Por lo tanto un incremento en la concentración de corticosterona puede comprometer el crecimiento de las nuevas plumas al reducir la cantidad de recursos que un ave asigna a su muda. Esto podría limitar la tasa de crecimiento de las nuevas plumas, su calidad y el número de plumas que son mudadas (Rubolini *et al.* 2002).

Romero y colaboradores (2005) encontraron que *Sturnus vulgaris* y *Zonotrichia leucophrys* presentaban una inhibición del crecimiento de sus plumas en respuesta a la presencia de corticosterona, indicando una interferencia de esta hormona sobre el crecimiento de las plumas. Debido a que Romero *et al.* (2005) añadió la corticosterona a la dieta de sus aves experimentales, la inhibición en el crecimiento de sus plumas no estuvo asociada con el costo de producir la hormona, sugiriendo que los cambios fisiológicos que tienen las aves al responder a la presencia de hormonas corticosteroides desvían recursos claves que en ausencia de la hormona serían utilizados en la formación de las nuevas plumas. Nuestros resultados apoyan el trabajo de Romero y colaboradores en el (2005). Los machos de *Volatinia* con menores niveles de corticosterona presentaron mayores intensidades de muda que aquellos con altos niveles de la hormona, lo que sugiere que estos últimos presentan una mayor inhibición en el crecimiento de su plumaje. Adicionalmente, la velocidad de crecimiento y extensión de muda ha sido utilizada como un indicador de la calidad corporal (Romero *et al.* 2005, DesRochers 2009), por lo que nuestros resultados indican que los machos con mayor extensión de muda y menores niveles de corticosterona deben de tener una mejor condición corporal que aquellos con poca muda.

Efectos de la coloración de plumaje y muda a nivel ecológico

La calidad de la ornamentación tiene una gran importancia a nivel ecológico (Hill 1995, Johnsen *et al.* 2003). Diversos ornamentos, en específico la coloración de plumaje (ya sea pigmentaria o estructural), funcionan como elementos de comunicación visual que tiene un papel trascendental en las relaciones intra e interespecíficas (Moller 1989). En *Volatinia jacarina* se ha reportado que la comunicación visual que mantiene los machos con las hembras (cortejo de pareja) se inicia cuando el macho levanta vuelo y posteriormente se deja caer en picada, mostrando los parches de plumas blancas que tiene en ambos lados de las alas (Aguilar *et al.* 2007, Carvalho *et al.* 2007). Esta particular forma de cortejo sugiere que la coloración de plumaje alrededor de las barras blancas de plumas funge como un resaltador de estas barras, señalando la calidad del individuo. Patrones similares de contraste de coloración entre plumas oscuras y plumas de color claro se ha reportado para otras especies de aves como por ejemplo *Parus major* y *Passer domesticus* (Groothuis y Carere 2005, Bokony *et al.* 2006). Por lo tanto podemos suponer que la coloración de

plumaje que mantenga un mayor contraste con las plumas blancas es un indicador de mejor condición física. En nuestros casos, los individuos con menores concentraciones de corticosterona y mayores niveles de muda presentarán un mayor contraste al tener plumas cobertoras nuevas, y presumiblemente de mejor calidad, que los machos que presentan mayores niveles de corticosterona.

Para las aves la muda implica un gran costo energético, sin embargo este proceso es un esfuerzo necesario para cubrir partes cruciales de sus ciclos anuales como la reproducción (Espie *et al.* 1996). La muda tiende a estar muy bien acotada dentro de un periodo de tiempo. Sin embargo, dentro del periodo de muda de una especie, se genera una asincronía entre los individuos (Hall 2000). Esta asincronía varía debido a la cantidad de recursos y las condiciones fisiológicas del individuo (Senar 2004, Romero *et al.* 2005). Nosotros encontramos que los individuos machos de *V. jacarina* que tenían una muda más avanzada eran individuos que presentaban menores concentración de corticosterona, lo que indica que esta hormona altera la velocidad de muda como ha sugerido Romero y colaboradores (2005). Estos autores encontraron que la velocidad de crecimiento de las plumas se reduce con la presencia de corticosterona. A nivel ecológico nuestros resultados sugieren que estén menos estresados terminarán su muda antes, pudiendo tener mejores oportunidades para reproducirse.

Nuestros resultados indican que existe una relación entre la muda activa, la calidad y coloración de las plumas generadas en el proceso, y los niveles de estrés y la calidad individual de las aves. Se ha visto que las plumas registran la calidad del individuo al momento de su crecimiento, y que la escala de tiempo que reflejan puede ser a nivel de días. Esto es claro en las líneas de crecimiento que se marcan en plumas de la cola y las alas tanto en individuos juveniles, como en individuos adultos, y que reflejan la calidad del alimento ingerido en las últimas 24 horas. De este modo nuestro estudio sugiere que la coloración estructural de las plumas pueden reflejar la condición del individuo al momento de la muda, y servirnos como un indicar directo de algunas variables fisiológicas como sus niveles de estrés.

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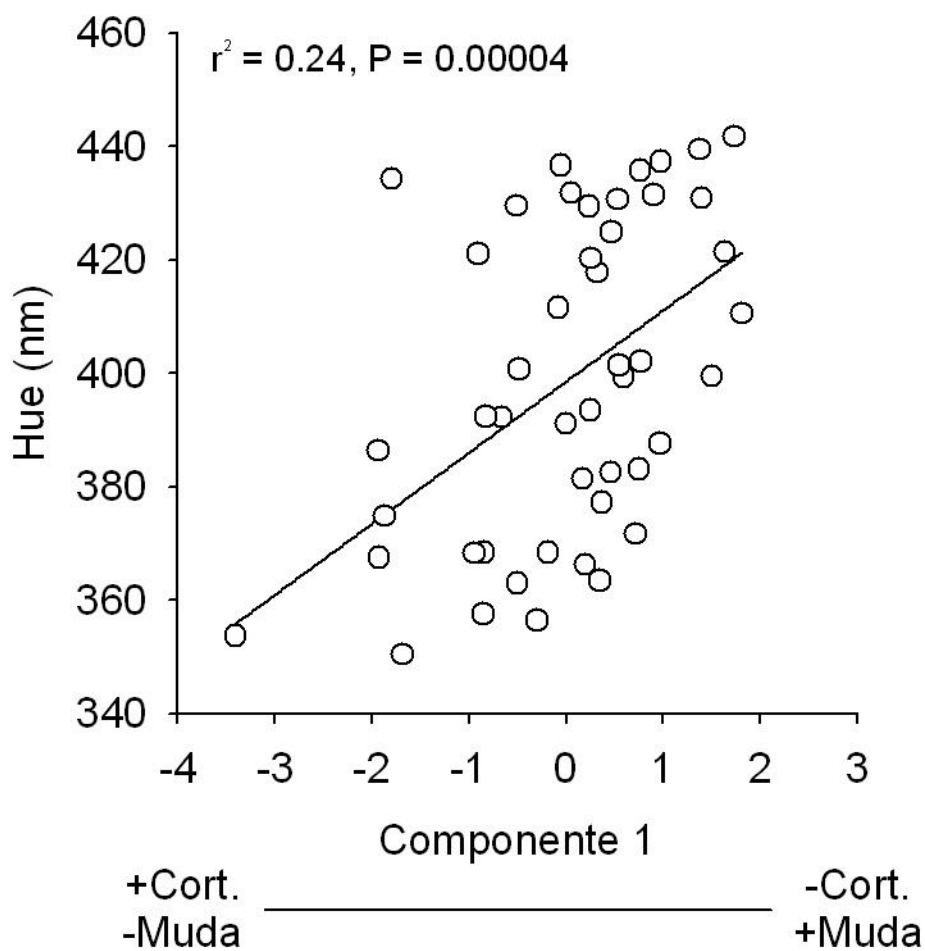
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Tabla 1. Tabla 1. Modelo de mínimos cuadrados parciales (PLS) de la variable (Hue). Se muestra la importancia (peso) de las variables originales que definieron el único Componente significativo del PLS y el porcentaje de varianza explicada en cada componente de los individuos de *Volatinia jacarina*

HUE		
Predictores	Componente 1 (PC1) (Pesos)	Varianza explicada en la componente
<i>Peso</i>	-0.28	8%
<i>Tarso</i>	-0.02	0%
<i>Núm. de cobertoras mudadas</i>	0.55	30%
<i>Inmunoglobulinas</i>	0.27	7%
<i>Corticosterona (na/ml)</i>	-0.64	41%
<i>Núm. de plumas usadas en la medición del color)</i>	0.37	13%
Varianza explicada de la variable dependiente por la PC1	24.2%	

Figura 1. Relación entre el Componente 1 con el tono del plumaje de los individuos (n=47) de *Volatina jacarina*. Los individuos hacia la izquierda son individuos más estresados y con una muda reproductiva más atrasada y generan un color mas UV mientras que los individuos más a la derecha son individuos menos estresados, con una muda más avanzada y generan una coloración más azul. El valor estadístico de la regresión es de: $R^2 = 0.24$; $P < 0.001$



CAPÍTULO V

SOCIAL AND SEXUAL SELECTION OF A STRUCTURAL PLUMAGE COLOR TRAIT: AN EXPERIMENT WITH BLUE GROSBEAK

ABSTRACT

Plumage coloration has been widely studied as a means of understanding how biological and ecological traits have evolved via natural and/or sexual selection. Comparatively, the role of social selection to explain plumage evolution has been less considered, especially in the less well-known case of structural-based plumage color. The aim of this study was to test, through choice trials and plumage manipulations, the role of both sexual and social selection in the evolution of the structural blue plumage coloration in the male Blue Grosbeak (*Passerina caerulea*). In 2007, we captured males and females from two different Blue Grosbeak populations and performed two choice experiments based on the manipulation of plumage color: (1) social - a male has to choose between two males; and (2) sexual - a female has to choose between two males. Males showed a clear preference to forage near less blue birds during their first encounter and on the first day. However, this preference was not evident on subsequent days. Females did not show any preference for any male color treatment. Our data show, for the first time, that plumage color can work as a “first impression” signal, which suggests that plumage coloration in the Blue Grosbeak represents a signal of dominance status to non-familiar individuals, hence providing support to the status-signaling hypothesis. Our results support previous evidence that suggests a lack of direct selection of males by females and that brightly colored males may have evolved through male-male interactions.

Keywords: blue Grosbeak, male-male competition, mate choice, social selection, status-signaling hypothesis, structural plumage color

INTRODUCTION

Plumage coloration is a widely used study model for understanding how traits have evolved via sexual or natural selection (Andersson 1994; Møller, 1994). Both pigmentary and structural-based colors have provided much insight into how these traits are used as honest signals of the qualities of the bearer (*e.g.* parental care, parasites, body condition). They are used in mate choice (Hill and McGraw, 2006a; Hill and McGraw, 2006b) and as a dominance signal in interactive aggressive behavior (Senar, 2006). Comparatively, the role of plumage in sexual selection has been far better studied than in social interaction (Senar 2006) and, consequently, different issues still need to be clarified regarding the role of plumage in social dominance. This is particularly relevant in the case of structural plumage because, while many studies have shown that melanin-based color is mainly related to aggressiveness and status signaling, less evidence has ever been derived from carotenoid- and structural-based color (Senar, 2006).

The status signaling hypothesis (SSH) developed by Rohwer (1975) states that certain plumage patches on birds work as signals indicating social status. The main benefit provided by these signals is that individuals of unequal fighting ability competing for limited resources (females, territories or food) neither risk accidental injury nor waste energy assessing the relative fighting ability of potential opponents (Rohwer, 1975; Rohwer, 1982). Since then, the SSH has been tested in many avian species (see Senar 2006 for a review). Originally, the SSH was posited for species in which individuals interact with numerous competitors or for individuals encountering unfamiliar conspecifics. In fact, this was one of the key-assumptions of the hypothesis (Senar, 2006). Surprisingly, relevant experimental evidence is very scarce (Lemel & Wallin 1993a, Brotons 1998) and has only been generated by experiments that were not devised specifically to test its predictions (Senar, 2006). If status signals work as a first impression, it ought to be possible to design an experiment in which birds that do not know each other do not have to interact physically, and in which the “efficiency” of the status signal is negatively related to the number of interactions, therefore allowing the reliability of the signal to be tested (Senar, 2006).

The aim of this paper was to prove that the blue structural plumage of the Blue Grosbeak (*Passerina caerulea*) works as a sign of social status between birds that do not know each other. We predicted that if the plumage works as a signal of social status for unfamiliar individuals, then this trait must work as a “first impression”. Hence, a male bird that has to choose between feeding close to an apparently dominant or a subordinate flock mate (a “choice test”, Senar and Camerino, 1998; Senar, 2006) would initially avoid the dominant bird, although this avoidance would become diluted as time passes.

Additionally, and given that many status signals have a dual function related both to intra- and intersexual signaling, we also tested the role of the blue plumage of the Blue Grosbeak in sexual selection. Even though a striking trait can evolve through social or sexual selection, both types of pressures may interact, and the sexual selection may work via male-male competition or by direct mate choice by females (Griffith and Pryke, 2006; Senar, 2006). Regarding structural-based color traits, some studies have reported that these traits may potentially be used as indicators of dominance and fighting ability (Alonso-Alvarez et al., 2004; Siefferman and Hill, 2005a) and the UV manipulation of some traits has shown that they have an important role in female mate choice (Bennett et al., 1997; Hunt et al., 1999; Keyser and Hill, 1999; Siefferman and Hill, 2005b). However, very few studies have simultaneously tested within the same species the effects of structural plumage coloration in both male-male competition and female mate choice.

The blue plumage of this species has received much attention in the past (Keyser and Hill, 1999; Keyser and Hill, 2000; Ballentine and Hill, 2003). This trait is sexually dichromatic; adult males are blue with a variable amount of brown, while females are completely brown (Pyle et al., 1997). Previous evidence has demonstrated that there is significant variation in the blue color of the males. The blue plumage in this species reflects in the UV range and it has been shown to be an honest indicator of body condition and to be related to territory size and quality (Keyser and Hill, 1999; Keyser and Hill, 2000).

MATERIAL AND METHODS

General procedures

We captured adult male and female Blue Grosbeaks in March-May 2007 in two localities in the state of Michoacan (Western Mexico). The first locality was the UNAM campus in Morelia, which consists of a suburban habitat with buildings, *Eucalyptus* spp. and many native grasses and shrubs. The second locality was in shrubland near the town of Chiquimitio, 50 km away.

Birds were mist-netted, banded, sexed, measured (tarsus and wing), and weighed. We also took pictures in a standardized position of birds lain on their backs on a 1x1mm referenced surface next to a color reference guide (Figuerola and Senar, 2000). After the pictures were taken, we collected 15-20 breast and rump feathers from each bird. These feathers were used for spectrophotometric color analysis (Quesada and Senar, 2006).

Birds were individually housed in 1m x 0.90m x 1m cages with covered side and back walls so that there was no visual contact between them, although they could hear each other. Birds were fed *ad libitum* with water and a diet of a mixed grain and a vitamin complex. All birds suffered a little stress at the beginning of captivity and usually lost some mass. For this reason, we did not start the experiments until they had regained their original mass (within approximately two weeks).

Plumage coloration analysis

To assess plumage coloration, we layered the plucked breast feathers on a dark velvet surface (reflectance 0%) in an arrangement that replicated the plumage surface of the bird (Quesada and Senar, 2006). We used a USB2000 spectrometer[®] connected to a PC using program SpectraSuite[®] (Ocean Optics, Duiven, The Netherlands, EU) and a DT-MINI-GWS Deuterium Tungsten Halogen light source[®] (220-2000 nm) (Ocean Optics, Duiven, The Netherlands, EU). All sample spectra were measured in reference to a white substance (WS-1, Diffuse Reflectance Standard) with reflectivity of over 98% and a dark spectrum. We took three measurements per sample and then computed the mean.

The UV coloration variables were computed as follows: for each individual spectrum we assessed lightness as the mean reflectance value for the whole spectrum (Andersson et al., 1998; Doucet and Montgomerie, 2003); we calculated chroma as the proportion of reflectance in the UV (300-400) region in relation to the total reflectance of the spectrum (300-700 nm) (Andersson et al., 1998); and we considered hue as the wavelength where the

reflectance was greatest (Andersson et al., 1998; Keyser and Hill, 1999; Doucet and Montgomerie, 2003).

The Blue Grosbeak has brown spots within its mainly blue plumage that may act as a confounding variable and so has to be standardized. To do so, we evaluated the “spottiness” of the birds using digital pictures taken in the field before conducting the choice trials. We drew three straight lines perpendicular to the longitudinal axis of the bird: under the chin, in the middle of the breast, and 1 cm above the cloaca. We assessed the percentage of brown along these lines with respect to the blue and computed the mean of the three lines.

Social and sexual selection trials consisted of releasing an experimental bird into a cage (2.0m x 0.5m x 0.5m; Fig. 1a) in which it had to associate with one of two manipulated birds kept in two smaller independent cages at either end of the larger cage (0.25m x 0.25m x 0.35m; Fig. 1b). We artificially dulled (hereafter, the blackish bird) the plumage of one of these two males and enhanced (hereafter, the bluish bird) the plumage of the other, by completely painting the plumage of the birds with either black or blue markers, following a protocol based on Ballentine & Hill (2003). In this way, we were able to manipulate the single UV peak of a bird in a natural way so that chroma and brightness were changed while hue was unaffected (see Ballentine & Hill, 2003). We used pairs of modified birds, whose the difference of spottiness was less than a 5% between them.

The two cages that housed the manipulated birds consisted of fish tanks placed on an expanded polystyrene base. The fish tanks were turned upside-down and fitted into a groove previously made in the base. Thus, the cages were soundproofed so that the experimental bird was unable to use acoustic cues to select between the manipulated birds. We inserted in the base of the expanded polystyrene a probe connected to a silenced ventilator. All the walls of the fish tanks were covered except for the side facing the experimental cage, where we placed a feeder (Figure 1b). All trials were recorded on a laptop computer with a camera, which was placed in front of the cage of the experimental bird. At the end of the experiment, the recordings were analyzed.

Social selection trials

Trials consisted of the release of a male “experimental bird” into the large cage allowing him the opportunity to feed in front of one or either of the two manipulated birds (blackish

or bluish birds). We also placed two perches in the middle of the experimental cage to allow the experimental bird the possibility of not interacting with either of the manipulated birds (Figure 1a). We located two feeders close to the fish tanks so that, when feeding, the experimental bird was face to face with one of the bird feeders in the cages housing the manipulated birds (Figure 1a). Trials were conducted in a large room with natural light and with external soundproofing.

The whole experiment consisted of a control and then trial experiments. The trial experiment comprised three stages that allowed us to determine whether or not the experimental bird preferred one or either of the manipulated birds, and to evaluate whether or not plumage works as a “first impression” or as a “constant” signal. The first day we evaluated the choice of the experimental bird during its first 5 min of interactions (stage 1); subsequently, we extended the evaluation to two hours (stage 2) and finally observed the bird’s behavior for two further hours on a second day (stage 3).

During the control test, the experimental bird was left for two hours in the cage. During the first hour, we did not provide any food so that it could get used to the cage and starve it. In the second hour, we provided food so as to evaluate its possible preference for one side or the other of the cage. After this, keeping the experimental bird in the cage, we put the manipulated birds into the fish tanks with no physical or acoustic contact with the experimental bird. Then, all birds (experimental and manipulated) spent the night in the cages.

The next day, we carried out the trial experiment with the manipulated birds. We left all birds for two hours without food and then we allowed the experimental bird to have visual contact with the two manipulated birds for two hours. We repeated the same protocol with manipulated birds on a second day.

Sexual selection trials

For sexual selection trials (N=11), we used the same protocol as for social selection (control experiment and two experimental days with manipulated male birds). However, in this case, the experimental bird was a female and, instead of feeders, we used a perch on either side of the cage. In this way, we were able to evaluate the female’s preference for a male rather than her preference for eating close to another bird. Thus, there were four

perches, two in the center of the cage and two in front of the small cages occupied by the manipulated birds.

For both experiments, we used 20 males and 11 females. Each bird performed once in the social selection trials as an experimental bird and twice as a manipulated bird. Because we used the same birds several times, we were able to avoid using the same three birds together in more than one trial and so all trials were always conducted with a pool of birds that were unknown to each other.

Statistical analysis

To evaluate social preference, we carried out a Wilcoxon Rankerized Pairs Tests between the percentages of time spent with the bluish or the blackish bird in the different experimental stages. We first evaluated the “first impression” (hereafter, First impression) as the preference for the blackish or the bluish bird within the first five minutes. Afterwards, we evaluated it for 90 minutes (hereafter, Day 1) and for 90 minutes again in the second day (hereafter, Day 2). We analyzed the sexual preferences of females in the same way. We used STATISTICA 7.0 to perform all statistical analyses. We set the significance level at $\alpha=0.05$.

RESULTS

Social selection trials

We performed 20 male-male trials, but eliminated one event as one of the experimental birds damaged a wing during the experiment. Our control experiment showed that, as expected, male experimental birds showed no preferences for either side of the cage (Table 1).

Experimental birds showed a clear preference for feeding beside the blackish bird during the first impression and during the first day (Figure 2, Table 1). However, although the same tendency held for the second day, this preference was no longer significant (Table 1). Hence, these results reveal that test birds avoided the bluish birds and preferred eating alongside the blackish birds.

Sexual selection

Females did not show any preference for blackish or bluish birds during either their first impression or when we evaluated mate choice on their first and second day (Table 1).

DISCUSSION

Structural colors have received a great deal of attention in recent years. Some studies have demonstrated that UV color manipulations affect conspecific behavior in both sexes and that UV structural-based color may play an important role in signaling individual qualities (Bennett et al., 1997; Andersson and Amundsen, 1997; Hunt et al., 1998; Sheldon et al., 1999; Siitari et al., 2002; Alonso-Alvarez et al., 2004). Although useful for understanding the role of structural color in sexual or social signaling, unfortunately many of the plumage modifications used in these studies consisted of unnatural alterations of plumage color (Ballantine & Hill 2003). Thus, it is difficult to decide whether the effect found is due to a natural response of the receptor as an indicator of a “poor quality” male or is a response to a “bizarre” bird (Ballantine & Hill 2003). For this reason, we sought a more realistic manipulation.

In our study, we followed the same procedure as Ballantine & Hill (2003), but also controlled for confounding variables such as spottiness, male song (birds were kept in sound-proofed cages), and flight displays (manipulated birds were kept in cages that were too small to allow them to display). Our results showed that male Blue Grosbeaks preferentially chose to eat beside less UV-colored male birds, suggesting that Blue Grosbeaks use their plumage to signal dominance status. This result coincides with recent studies that assign a social role to structural plumage color (Alonso-Alvarez et al., 2004; Siefferman and Hill, 2005a). Our results also agree with previous results in the Blue Grosbeak that associate structural plumage with a dominance role. Keyser and Hill (2000) found that more brightly colored birds are in better condition and have better territories. A good territory or good body condition frequently depends on dominance status in males (Beletsky and Orians, 1989; Piper and Wiley, 1990; Smith, 1994; Hake, 1996; Gosler and Carruthers, 1999; Hogstad, 1999; Senar et al., 2000).

Although we controlled for several variables, it could be argued that other variables besides spottiness, song, and display were not controlled for in our experiment – for example, hue, mass or size (tarsus length) – and could have masked our results. However, we found no differences between blackish and bluish birds in sexual or social selection trials (t-test for single means, all $p > 0.20$). Therefore, the variables that we did not actively control for were chosen at random.

Structural plumage color as a “first impression” trait

The most interesting finding was that plumage color worked as a “first impression” signal. Many studies have discussed whether dominance signals work permanently as a faithful trait of fighting abilities or only as a first impression signal (Senar 2006). Experimental evidence of this is practically inexistent or, at best, indirect (Jarvi et al., 1987; Lemel, 1993). In our experiments, we conclusively showed that birds use plumage coloration as a “first impression” trait to evaluate the dominant abilities of other individuals. This fact suggests that the blue plumage is a trait that signals dominant status between unfamiliar individuals in the Blue Grosbeak and hence provides support for one of the main (but rarely tested) assumptions of the status signaling hypothesis. However, it is likely birds probably use even more cues to evaluate the true fighting abilities of a competitor (Bokony et al., 2006).

The role of structural plumage color in mate choice in the Blue Grosbeak

It is well accepted that bright colors in birds are mainly explained by sexual selection. Sexual selection may work by means of direct mate choice or by male-male interaction. Our results support previous evidence in this species that shows there is a lack of direct selection of males by females (Ballentine & Hill 2003). Similarly, other studies have found that socially related traits are likewise not chosen by females of other species that possess structural plumage coloration (Liu et al., 2007) or pigmentary plumage colors (Senar et al., 2005). This suggests that brightly color plumages may have evolved through male-male interactions (Senar 2006). Females may pay more attention to the ability of males to acquire other resources that could be related to plumage coloration or dominance status. For

example, a dominant male may signal his fighting ability to other males in order to acquire a better territory, and then females will use territory quality to choose their mates. Nevertheless, males could use other signs (song, display, etc.) that we did not consider in this study. For instance, we modified brightness and chroma, but did not manipulate any other variable related to plumage color such as hue, which may also be selected by females. More studies are still needed to explore these considerations, since there is evidence to suggest that different features of the same plumage may signal different qualities (Doucet and Montgomerie, 2003; Ferns and Hinsley, 2008; Senar et al., 2008).

Additionally, Senar (2006) has suggested a third way in which selection pressure could explain bright colors in birds. Dominance-related traits such as plumage may evolve through social interaction without any sexual context (“social selection”). For example, in the European Siskin *Carduelis spinus* the black bib in males is a useful signal in winter interactions between males, but is not used by females as a trait for choosing a mate in the breeding season (Senar et al. 1998, Senar 2006). In the Blue Grosbeak, we cannot rule out the potential role of sexual selection in explaining the evolution of plumage color given that previous studies have found that brighter males have better territories (Keyser and Hill, 2000).

In summary, our experiment showed that structural color in the Blue Grosbeak works more as a “first impression” signal of dominance than as a direct sexual trait chosen by females, thereby suggesting that social relationships in some cases may be crucial for the evolution of conspicuous characters that have generally in the past been related to sexual selection.

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Figure 1. Experiment design of the choice test in the social and sexual trials.

Figure 2. Differences in the percentage of time spent with a bluish or blackish manipulated bird in the social selection trials. The choice experiment consisted of three stages: an evaluation of the first impression of the experimental bird during the first 5 min of interaction; an evaluation during the following two hours (Day 1); and an evaluation on a second day in which we measured the bird's behavior for two more hours. * $P < 0.05$; ** $P < 0.01$

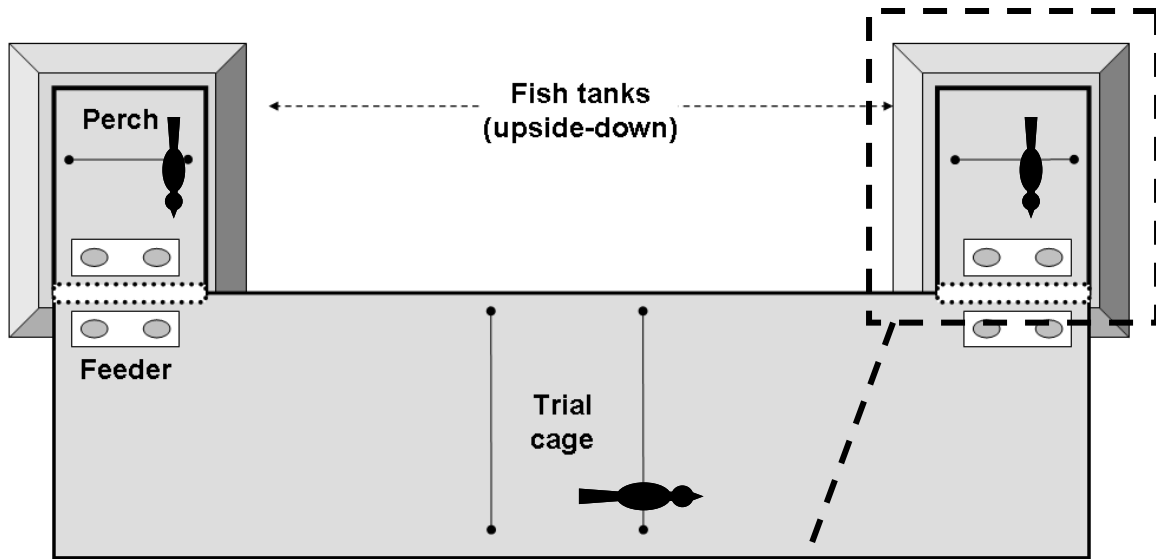
Table 1

	Social selection			Sexual selection		
	W		N	W	p	N
Control	45	NS	16	7	NS	6
5-minutes	24	P< 0.05	15	27,5	NS	10
Day 1	30	P< 0.01	19	21	NS	11
Day2	32	NS	13	29	NS	11

Table 1. Results of the Wilcoxon Rankerized Pairs Tests in the social and sexual selection experiments measured as the percentage of time spent with a bluish or blackish manipulated bird.

Figure 1

a)



b)

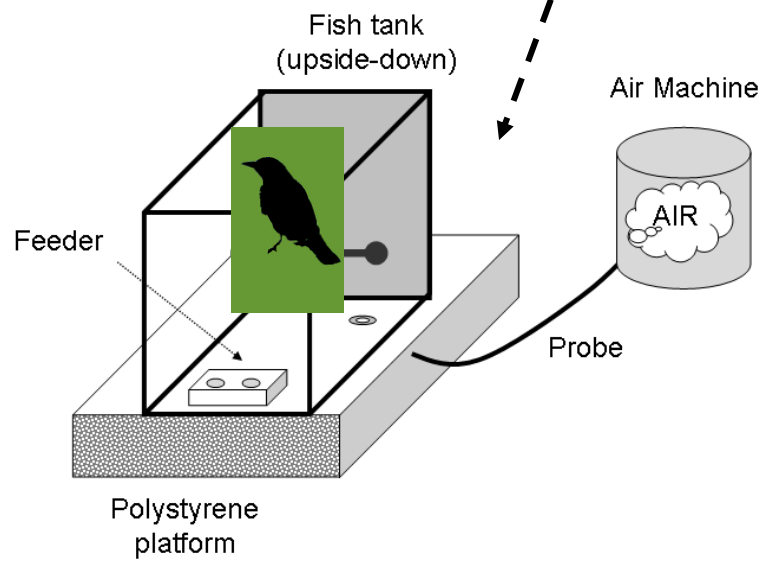
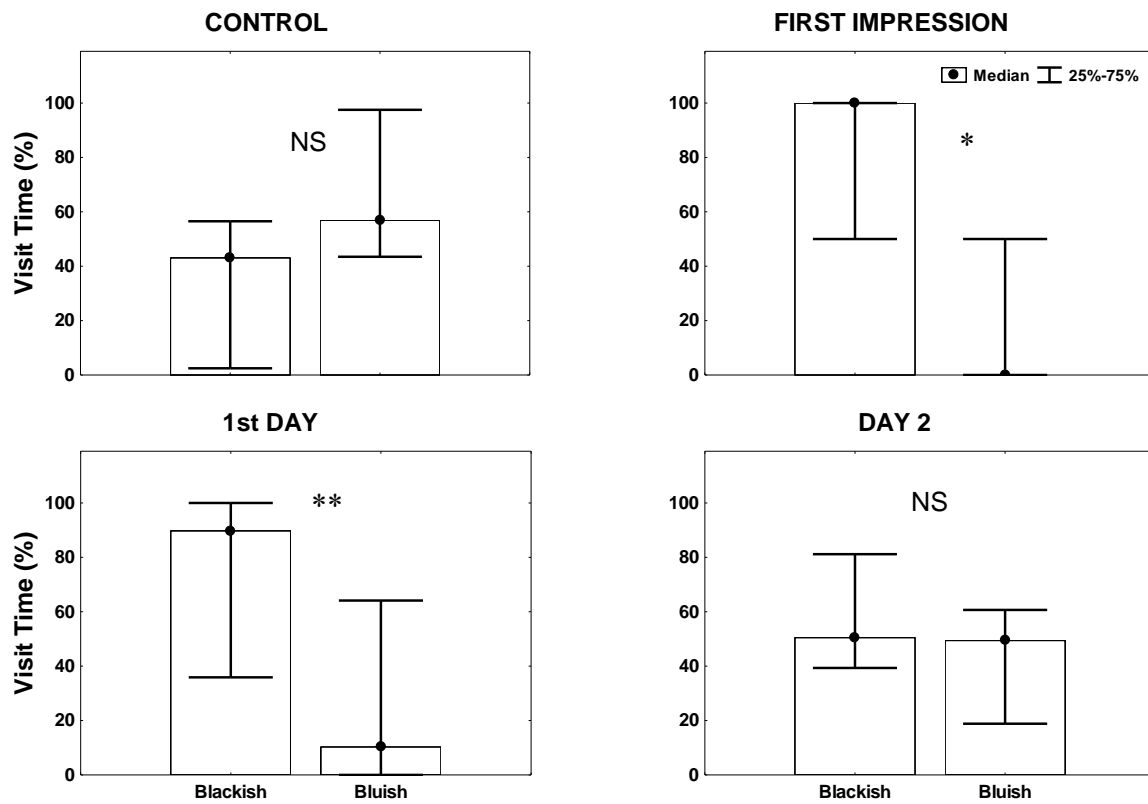


Figure 2



CONCLUSIONES

1.- Nuestros resultados en conjunto con estudios previos sugieren que dentro de las zonas urbanas aun con cierta estructura vegetal para *M. fusca* no genera condiciones apropiada para que los individuos de esta especie alcancen un equilibrio fisiológico, lo que los mantiene en condiciones de estrés crónico. Con respecto a *C. inca*, no tenemos evidencia que indique que esta especie de aves se encuentre comprometida fisiológicamente en las condiciones de los hábitats que estudiados. Aunque ambas especies son ecológicamente similares, nuestros resultados muestran que una mayor proporción de individuos de *M. fusca* tienen limitaciones fisiológicas, mientras que *C. Inca* parece ser un generalista de hábitat, y que la fisiología de esta especie permite convertirse en una urbana-explorador.

2.- Aunque en zonas industriales la estructura vegetal presento semejanzas considerables con las zonas suburbanas Nosotros encontramos un relación negativa en la concentración de corticosterona y la concentración de inmunoglobulinas en individuos del gorrión común (*Passer domesticus*) capturados en zonas industriales. Esta condición de altas concentraciones de corticosterona y bajas concentraciones de inmunoglobulinas podría ser consecuencia de la presencia continua de estímulos estresores que varían en frecuencia en intensidad, en la actividad humana, los niveles ruido y el tráfico constante de vehículos en este uso de suelo dentro de la ciudad.

3.- Con respecto a *Volatinia jacarina* nuestros resultados indican que existe una relación entre la coloración de las plumaje la muda y las concentraciones de corticosterona. De este modo nuestro estudio sugiere que la coloración estructural de las plumas pueden reflejar la condición del individuo al momento de la muda, y servirnos como un indicar directo de algunas variables fisiológicas como sus niveles de estrés.

4.- Con respecto al resultado obtenido de *Passerina caerulea* encontramos que la coloración estructural del plumaje funciona más como una de señal de dominancia, que como una característica de selección sexual.