VARIABILIDAD INTRA-POBLACIONAL EN LA UTILIZACIÓN DEL NICHO TRÓFICO: FLEXIBILIDAD FISIOLÓGICA Y ESPECIALIZACIÓN INDIVIDUAL EN *ZONOTRICHIA CAPENSIS*

Universidad de Chile

Tesis

Entregada a La Universidad De Chile

En Cumplimiento Parcial De Los Requisitos

Para Optar Al Grado De

Doctor en Ciencias con Mención en Ecología Y Biología Evolutiva

Facultad De Ciencias

Por

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Octubre, 2009

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FACULTAD DE CIENCIAS

UNIVERSIDAD DE CHILE

INFORME DE APROBACIÓN
TESIS DE DOCTORADO

Se informa a la Escuela de Postgrado de la Facultad de Ciencias que la Tesis de Doctorado presentada por el candidato

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Ha sido aprobada por la comisión de Evaluación de la tesis como requisito para optar al grado de Doctor en Ciencias con mención en Ecología y Biología Evolutiva, en el examen de Defensa de Tesis rendido el día 16 de Octubre del 2009.

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Karin nació en Copiapó, Chile, en mayo del año 1982. Ingresó a la Universidad de Chile en el año 2000 a la carrera de Licenciatura en Ciencias con mención en Biología. Tras realizar una unidad de investigación en el laboratorio de Ecofisiología animal en el año 2001, se vio fascinada por el estudio del funcionamiento animal. Así, en el año 2005, ingresó al programa de Doctorado en Ciencias Biológicas con mención en Ecología y Biología Evolutiva, realizando su tesis en el laboratorio de Ecofisiología animal, bajo la dirección del Dr. Pablo Sabat.

Sus intereses se enfocan en la comprensión de la variación fenotípica en un nivel intra e inter específico. Particularmente su investigación intenta responder cómo las variables ecológicas afectan la diversidad fisiológica. Para ello, ha estudiado rasgos osmorregulatorios, energéticos, digestivos en aves que enfrentan condiciones ambientales disimiles. De esta manera su trabajo conecta el nivel organísmico con la ecología y la búsqueda de patrones evolutivos.

Agradecimientos

A Fernando Novoa por mostrarme el fascinante mundo de cómo funcionan los animales y por vincularme al Laboratorio de Ecofisiología Animal de la Universidad de Chile. A Pablo Sabat, por enseñarme los gajes del oficio, por su paciencia y por instruirme e insertarme en el área de la investigación científica. A todos los integrantes del Laboratorio de Ecofisiología Animal especialmente a Daniela Figueroa, Andrés Sazo, Grisel Cavieres, Sandra Gonzalez, Natalia Ramírez, que además de brindarme su amistad participaron en algún quehacer de esta tesis. A Claudio Veloso por su gran disposición, sus consejos y su calidad humana. A Hugo Torres, María José Pérez, Carolina Simón, por su simpatía y cotidianidad. A Wouter van Dongen por su colaboración en los experimentos conductuales. A los profesores Mauricio Canals, Carlos Martínez del Río, Francisco Bozinovic, Rodrigo Vásquez, Ariovaldo Cruz-Neto, Carlos Navas que en alguna forma han participado cercanamente en mi formación académica. A Angela Valiente, Patricia Luz, Catalina Sabando, Carolina Aguayo, Alejandra Troncoso y Renzo Vargas grandes amigos que han aguantado mi ingratitud y excusas durante los últimos meses de la realización de esta tesis. A Pablo Razeto por sus valiosos aportes durante el doctorado, en esta tesis y por apoyarme siempre. A mi familia, Manuel Maldonado, Juana Pacheco, Carlos Pacheco que además de su cariño, me han brindado ayuda invaluable que forma parte de esta investigación. Finalmente, agradezco a la Comisión Nacional de Investigación Científica y Tecnológica (CONICYT) por el apoyo económico brindado por medio de diversas becas otorgadas durante el curso de este doctorado.

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Dedicada a mis padres

Resumen

Debido a que el rango de distribución de las especies incluye diferentes ambientes con características bióticas y abióticas generalmente contrastantes, la adecuación de los distintos genotipos de una misma especie a menudo difiere entre un hábitat y otro. De esta manera, la variabilidad en las características ecológicas que experimentan las poblaciones podría promover diferencias inter-poblacionales en la expresión y flexibilidad de los rasgos fisiológicos, morfológicos y conductuales, afectando finalmente la organización interna de las poblaciones. Esta tesis tuvo como **objetivo central** estudiar en qué medida las diferencias en temperatura, precipitaciones y heterogeneidad temporal del ambiente (e.g., estacionalidad de la dieta) que enfrentan las poblaciones afecta la expresión y magnitud de la flexibilidad fenotípica de ciertos atributos morfológicos, fisiológicos y conductuales y en qué medida estas diferencias determinan el grado de variabilidad individual en la utilización de recursos tróficos al interior de una población. Para esto, se abordaron diferentes preguntas a través de la evaluación explícita de hipótesis de trabajo, utilizando un modelo ecológico consistente en tres poblaciones de aves de la especie *Zonotrichia capensis* en un gradiente climático latitudinal presente a lo largo de Chile. Para ello, se realizaron experimentos de terreno y de laboratorio, determinando una serie de variables tanto fisiológicas, morfológicas y conductuales como también variables ecológicas, entre las que se encuentran:

1.- La determinación de la marca isotópica de nitrógeno (∂^{15}N) de la sangre y plumas como una variable proxy de la posición relativa en la trama trófica y el consumo de proteínas, de las tres poblaciones estudiadas de *Z. capensis*.

2.- La cuantificación de la variación estacional (i.e., heterogeneidad temporal) del consumo de proteínas para cada una de las poblaciones utilizando como proxy la marca isotópica de nitrógeno (∂^{15}N) de tejidos con distinta tasa de recambio (i.e., sangre y plumas).

3.- La determinación de los ajustes morfológicos, fisiológicos y conductuales asociados al nicho trófico poblacional (la señal isotópica de ∂^{15}N obtenida en objetivo 1) y su heterogeneidad temporal en *Z. capensis* de diferentes localidades, mediante el examen de: a) la conducta exploratoria, b) la tasa de pérdida de agua evaporativa, c) el tamaño de órganos digestivos, d) la actividad de tres enzimas digestivas (las disacaridasas maltasa y sacarasa, y una proteasa, aminopeptidasa-N) y e) la tasa metabólica basal.

4.- La determinación de la correspondencia entre la magnitud de la flexibilidad fisiológica (para los casos anteriores c), d) y e)), medida como la repuesta frente a aclimatación dietaria, y la heterogeneidad temporal de la dieta (referida en 2).

5.- La estimación del nicho trófico individual a través de la determinación de la señal isotópica de $\partial^{15}N$ en diferentes tejidos con distinta tasa de recambio (i.e, plumas, sangre).

6.- La cuantificación de la variabilidad en el grado especialización individual que presentan las distintas poblaciones de *Z. capensis* a través del índice propuesto por Roughgarden (1974), que relaciona el componente entre individuos de una población con la amplitud total del nicho poblacional (*WIC/TNW*).

De los resultados generales de esta tesis (detallados en la introducción) se puede concluir que las diferencias en el gradiente ambiental de temperatura y precipitaciones parecen ser determinantes en las respuestas fisiológicas, morfológicas y conductuales de *Z. capensis*. A su vez, que la heterogeneidad ambiental (estacionalidad de la dieta) influye sobre la flexibilidad de la norma de reacción de características digestivas de los organismos, y con ello, sobre el grado de especialización individual en las distintas poblaciones.

Introducción general

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Tradicionalmente, los esfuerzos orientados a entender y explicar la diversidad fisiológica se han centrado en estudios de carácter comparativo entre especies de diferentes ambientes (Schmidt-Nielsen 1997). No obstante, debido a que los rangos de distribución de las especies abarcan diferentes ambientes con características bióticas y abióticas a menudo contrastantes, la adecuación de los distintos genotipos de una misma especie probablemente difiera entre un hábitat y otro (Spicer & Gaston 1999). Considerando así, que distintas poblaciones están sometidas a diferentes condiciones ambientales, el estudio de las diferencias intra-específicas podría ser importante debido a que puede entregar información relevante respecto a la evolución de los caracteres en los animales (Garland & Adolph 1991). A pesar de la importancia de la variabilidad intra-específica en el proceso evolutivo, son pocos los estudios centrados en comprender la variabilidad fenotípica tanto a un nivel individual (plasticidad fenotípica) (McKechnie 2007) como inter e intra-poblacional (Bozinovic et al. 2007,

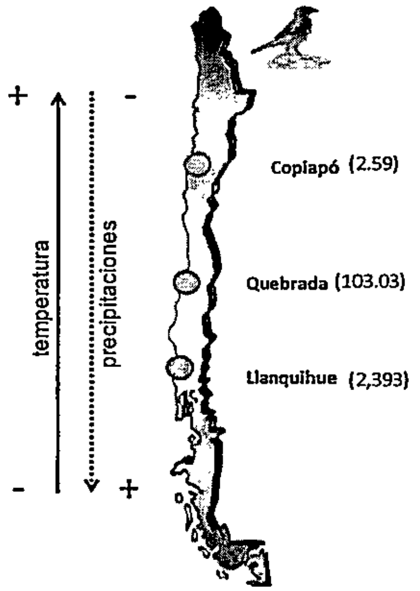

Figura 1.

Figura 1. Poblaciones de *Zonotrichia capensis* a lo largo de un gradiente de precipitaciones y temperatura. Los números contiguos a las localidades indican la productividad primaria del lugar, obtenida a través de las temperaturas y precipitaciones, i.e., Q_{index}. Para mayores detalles de dicho índice véase Capítulo 1.

Quispe 2009, Sabat et al. 2006, 2009). El interés central de esta tesis es comprender cómo las diferencias geográficas de las especies pueden ser reflejadas en variaciones intra-específicas en rasgos fisiológicos, morfológicos y conductuales de los animales en distintos entornos ecológicos, y cómo estas diferencias pueden influenciar la organización interna de las poblaciones. Para ello, se estudió la variabilidad y diferenciación fenotípica a distintos niveles de organización biológica (individual, intra-poblacional e inter-poblacional) en la búsqueda de la comprensión de las causas próximas y últimas que explican y restringen esta variabilidad. Se examinaron los posibles factores ecológicos que pudiesen promover las diferencias fenotípicas y su efecto sobre distintos rasgos de los organismos, centrándose principalmente en algunas características energéticas, digestivas y en la conducta exploratoria de los individuos.

El modelo de estudio utilizado fue la especie *Zonotrichia capensis*, un ave Paseriforme que se encuentra ampliamente distribuida a lo largo de Chile (Araya and Millie 2005). Esto permitió seleccionar tres poblaciones residentes con condiciones climáticas contrastantes y por consiguiente, con entornos ecológicos disímiles. Las zonas de estudio fueron Copiapó (27°18´S, 70°25´W), Quebrada de la Plata (33° 31´ S, 70° 50 ´W) y Llanquihue (41°16´S, 73°00´W), localidades que se encuentran en un gradiente latitudinal de productividad primaria, precipitaciones y temperatura (Figura 1). Por estas características, este modelo biológico es particularmente adecuado para estudiar los efectos ecológicos que afectan la diversidad fenotípica.

La diferenciación entre poblaciones es notoria cuando se examinan algunos rasgos morfológicos y fisiológicos (Figura 2) y conductuales (Capítulo 4, Figura 3). Así, las diferencias en temperatura, precipitaciones y en la oferta alimenticia de las localidades, parecen ser factores relevantes en la diferenciación fenotípica de esas poblaciones.

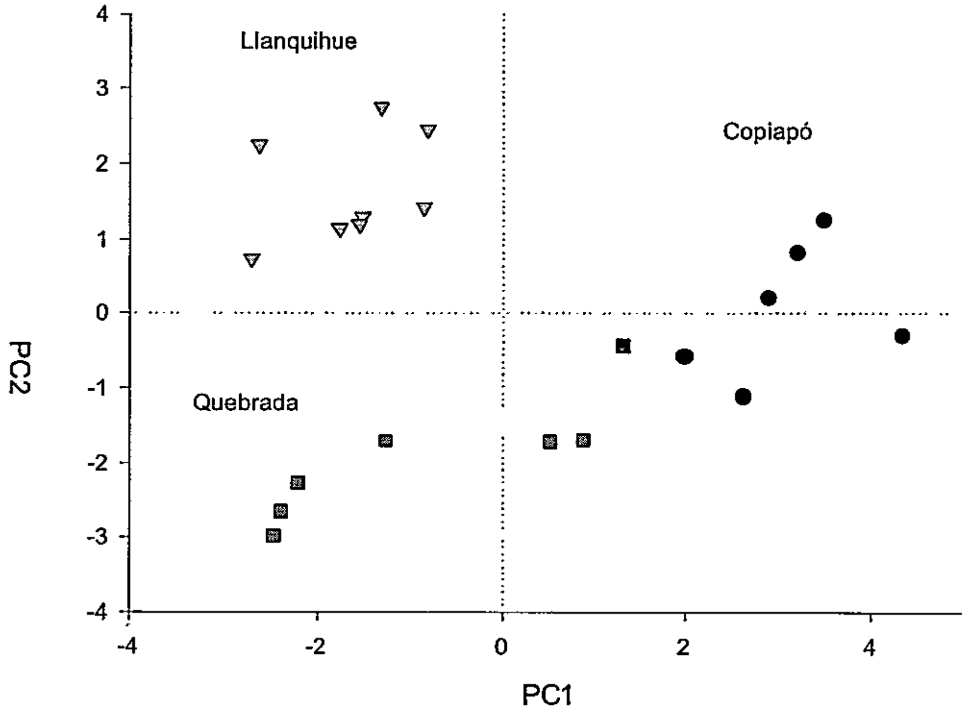

Figura 2. Resultados del análisis de componentes principales (PCA) sobre las variables morfológicas y fisiológicas estudiadas en *Z. capensis* provenientes de tres localidades a lo largo de Chile. El PCA1 resultó estar correlacionado con Q_{index}, el nivel trófico y la glucosa sanguínea al momento de la captura y el PC2 fue correlacionado con la morfología digestiva y la actividad de disacaridasas y aminopeptidasa-N. Ambos componentes representan el 70% de la variabilidad total.

Además de las diferencias inter-poblacionales, un aspecto particularmente novedoso examinado en esta investigación fue la variación fenotípica intra-poblacional asociada a la utilización diferencial del nicho trófico poblacional por parte de los individuos. Los esfuerzos de investigación sobre la variación intra-poblacional en la utilización de recursos fueron drásticamente disminuidos en la década del ochenta, debido a la suposición que la especialización individual era escasa y/o débil, y que por lo tanto ejercería un impacto trivial sobre los procesos ecológicos (Case 1981; Patterson 1983; Schoener 1986). Sin embargo, diversas investigaciones acerca de la variabilidad intra-poblacional han revelado la existencia de subdivisión del nicho poblacional en varios taxa (e.g., Hutchinson 1957; Colwell and Futuyma 1971; Pielou 1972; Skúlason and Smith 1996; Araujo and Gonzaga 2007; Araujo et al. 2009). Recientemente, la variación intra-poblacional ha vuelto a ser considerada relevante, a raíz de estudios en especiación ecológica y radiación adaptativa (Schluter 2000; Bolnick et al. 2003). En este contexto, determinar la proporción de individuos dentro de una población que utilizan una pequeña parte del nicho trófico poblacional total atribuible a características individuales y no debido a diferencias en sexo, morfo discreto o edad (i.e., “individuos especialistas”), es particularmente importante. Sin embargo, los estudios acerca del grado de especialización individual dentro de las poblaciones presentan dos debilidades importantes que esta investigación pretende afrontar. Primero, los estudios que abordan esta problemática se han enfocado en las diferencias entre especies (e.g., Bolnick 2007). Sin embargo, las diferencias poblacionales pueden ser aún más determinantes como explicación del cambio evolutivo, siendo las diferencias entre especies la consecuencia y no la causa de la especiación ecológica o la radiación adaptativa (Schluter 2000).

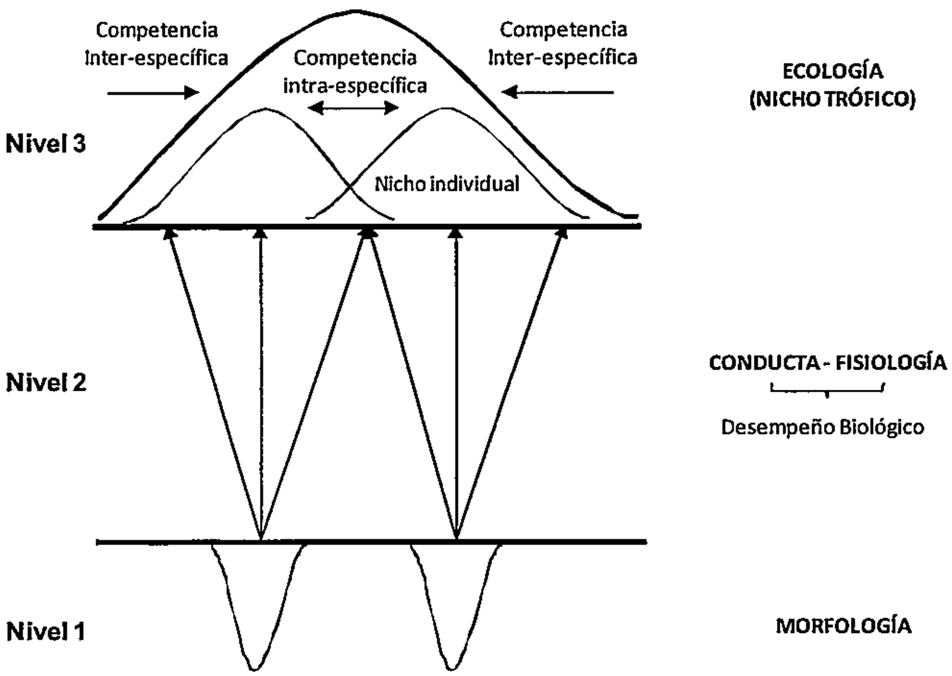

Figura 3. Se describe la relación existente entre morfología (nivel 1) y la ecología del organismo (nivel 3), mediada por variables más plásticas ubicadas en el nivel 2. El nivel 3, representa el nicho poblacional, el cual se muestra subdividido en nichos individuales. Se indican además, las principales interacciones ecológicas que pueden estar involucradas, en el proceso de especialización individual (modificado de Wainwright & Reilly, 1994).

En esta tesis se aborda el estudio cuantitativo de la diferenciación del nicho trófico entre poblaciones, un nivel de estudio no abordado anteriormente y que es fundamental para la comprensión de las implicancias ecológicas y microevolutivas de la especialización individual. La segunda limitante radica en que los estudios de especialización individual han concentrado sus preguntas sólo en el nivel ecológico de los organismos, es decir, en el efecto de la competencia intra e inter-específica sobre el grado de subdivisión del nicho poblacional (Valen 1965; Roughgarden 1974; Bolnick 2001; Svanbäck and Bolnick 2005; Araujo et al. 2008). Sin embargo, es necesario considerar la influencia de otros niveles que podrían ser determinantes en el grado de especialización individual de las poblaciones (véase Figura 3). Así, siguiendo la estructura de la Figura 3, nuestra investigación se diferencia de estudios previos que se han concentrado en un solo nivel de análisis (nivel 3), a través de la incorporación de información morfológica, fisiológica y conductual (niveles 1 y 2) que podría ser determinante en los procesos de diferenciación de los individuos dentro de una población. La inclusión de estos dos niveles puede ser fundamental por cuanto estos rasgos constantemente interactúan a través de la ontogenia del organismo pudiendo generar, en cada una de las etapas, nuevas estructuras producto del desarrollo, aclimatización y flexibilidad fisiológica. Por otra parte, debido a que las diferencias ambientales que experimentan las poblaciones podrían traducirse en diferentes presiones de selección, estas podrían afectar la diversidad de recursos utilizada por los individuos a través de modificaciones del fenotipo y de los compromisos entre rasgos. Esta diversidad fenotípica fue estudiada siguiendo diferentes preguntas y afrontando diversas hipótesis contrastadas con las que previamente se han propuesto en la literatura. Las problemáticas y los resultados obtenidos son presentados en los diferentes capítulos de esta investigación, cada uno de los cuales destaca diversos aspectos empíricos y teóricos asociados a la variabilidad fenotípica de los organismos dentro un mismo modelo ecológico de estudio.

Una de las herramientas clave utilizadas para esta investigación fue la utilización de la caracterización isotópica de distintos tejidos para estimar la posición relativa en la cadena trófica (Capítulo 1), la variabilidad estacional de la dieta en las poblaciones (véase Capítulo 2, 3 y 4) y la amplitud del nicho trófico individual y poblacional (Capítulo 3). El uso de isótopos estables como estimador de la dieta de especies, poblaciones e individuos, se ha incrementado enormemente en los últimos años (Gannes et al. 1998; Bearhop et al. 2004; West et al. 2006; Newsome et al. 2007) siendo esta información incluida como covariable en diversos estudios acerca de caracteres fisiológicos en poblaciones naturales (e.g., Sabat et al. 2006; Sabat et al. 2009) y como herramienta poderosa en el estudio del nicho trófico (para una revisión véase Newsome et al. 2007). Las marcas o señales isotópicas particulares (*isotopic signatures*) de la dieta son reflejadas en las marcas isotópicas del consumidor, lo que permite establecer una escala continua de variación en la utilización de recursos y brinda la posibilidad de estudiar los hábitos alimenticios a diferentes escalas de tiempo. Esto representa una ventaja con respecto a los análisis de dieta tradicionales, que presentan importantes limitaciones (discutido en Capítulo 3). Una manera de obtener un índice certero del tipo de dieta de los organismos es a través del estudio de los isótopos estables de nitrógeno. La composición isotópica de nitrógeno depende principalmente de la posición en la trama trófica de los organismos (Tieszen et al. 1983, Chisholm & Nelson 1982), ocurriendo un enriquecimiento en la proporción de $^{15}N/^{14}N$ entre consumidor y su dieta (Post 2002). Además de la estimación de la posición relativa en la trama trófica, el uso de $\partial^{15}N$ como estimador de la ingesta de proteína ha sido utilizada como covariable en estudios de fisiología de campo en vertebrados, revelando que esta variable es un buen predictor de variables digestivas, metabólicas y osmorregulatorias (Sabat et al. 2006, 2009, Schondube et al. 2001). Así, la determinación de la marca isotópica ($\partial^{15}N$) fue el método utilizado en esta investigación para estimar la dieta de los individuos. El protocolo de determinación de la marca isotópica se explica en detalle en Capítulo 1 y 2.

Por otra parte, la capacidad de asimilar carbohidratos y proteínas como resultado de la aclimatización y aclimatación a la dieta natural y experimental, respectivamente, se determinó mediante la estimación de la actividad enzimática intestinal de tres enzimas digestivas. Estas fueron las disacaridasasas maltasa y sacarasa, como indicadores de la capacidad de *Z. capensis* de asimilar carbohidratos, y la aminopeptidasa-N (proteasa), como indicador de la capacidad de digerir y asimilar proteínas. El protocolo de determinación de la actividad de la maltasa y aminopeptidasa-N fue realizado de acuerdo a lo descrito por Martínez del Río (1990) y Meynard et al. (1999), respectivamente, siendo detallado en los capítulos correspondientes (Capítulo 1 y 2).

A continuación se exponen los resultados generales obtenidos en esta investigación. Mayores especificaciones con respecto a éstos, y el marco conceptual con el cual son discutidos, están explicados en detalle en cada capítulo.

En primer lugar, se estudiaron las diferencias poblacionales de las características digestivas en relación al nivel trófico y las condiciones ambientales que experimentan las aves en las distintas localidades (Capítulo 1). Se encontró que las poblaciones difirieren significativamente en el nivel trófico (Capítulo 1, Figura 3). Además, la morfología de los órganos digestivos fue diferente en las tres localidades estudiadas. La masa de la molleja, la masa y el largo del intestino delgado y la masa del riñón, corregidos por la masa corporal, difirieron significativamente entre localidades (Capítulo 1, Tabla1). Con respecto a las enzimas digestivas, los niveles más altos de actividad de aminopeptidasa-N fueron encontrados en la población que mostró un nivel trófico mayor (Quebrada de la Plata), lo que concordaría con la hipótesis adaptativa de la modulación enzimática (AMH), en el sentido de que mayores niveles de proteína y sus derivados en el tracto intestinal se correlacionarían con una mayor capacidad de digerirlos (véase Karasov 1996). No obstante, los niveles de actividad para las disacaridasas no se correspondieron con lo esperado según el nivel trófico de las poblaciones, i.e., no se encontró una asociación negativa entre el nivel trófico y la capacidad de hidrólisis de azúcares. Esto sugiere que *Z. capensis* no presenta modulación de estas enzimas frente a variaciones en los niveles de carbohidrato en la dieta natural. Este resultado contradice la AMH para las disacaridasas a nivel inter-poblacional, un nivel no estudiado previamente. Este resultado podría relacionarse con la ventana temporal de la ontogenia donde se puede dar esta modulación (discutido en Capítulo 1), no pudiendo descartar que puede existir modulación de las actividades de disacaridasas en etapas tempranas de la ontogenia.

Además de estudiar las características digestivas (Capítulo 1) y metabólicas (Capítulo 4) de las aves en terreno, a través de experimentos de aclimatación se examinó la magnitud de la flexibilidad fenotípica de estos rasgos y su correspondencia con la heterogeneidad temporal de la dieta (Capítulo 2). Encontramos que la tasa metabólica basal (BMR) fue modificada en respuesta a la aclimatación dietaria en aves provenientes de Copiapó (Capítulo 2, Figura 1). Éstas mostraron una disminución del BMR en respuesta a dietas de baja calidad, lo cual es consistente con la hipótesis de hábitos dietarios (McNab 1986; McNab 1988). Con respecto a la flexibilidad de la actividad de las enzimas digestivas, la actividad de la sacarasa (disacaridasa) difirió entre dietas para aves de la localidad de Llanquihue, existiendo modulación específica con respecto al carbohidrato de la dieta (Capítulo 2, Figura 2). Este hecho es consistente con la AMH (Karasov 1996, véase Capítulo 2), la cual no había sido respaldada anteriormente para disacaridasas en estudios de aclimatación dietaria en aves paseriformes adultas. En esta misma línea, la magnitud de la flexibilidad de la actividad de aminopeptidasa-N difirió entre poblaciones, existiendo una clara correspondencia entre la heterogeneidad temporal del consumo de proteínas (medida a través de la variación en el $\partial^{15}N$) y la flexibilidad de esta enzima (Capítulo 2, Figura 4, 6). Esto último apoya la hipótesis evolutiva que predice la existencia de rasgos fenotípicamente más flexibles en animales que habitan ambientes más heterogéneos en comparación con aquellos que habitan ambientes más estables (Pigliucci 1996; Pigliucci 2005). Por otra parte, en aves de Copiapó la tasa de absorción de glucosa también se vio modificada producto de la aclimatación dietaria (Capítulo 2, Figura 5). Las aves aclimatadas a dietas ricas en insectos presentaron una mayor tasa de absorción de glucosa, en comparación a aves aclimatadas a carbohidratos. Esto concuerda con el supuesto que dietas de baja calidad promueven ajustes digestivos que permiten la mantención del desempeño digestivo (Karasov 1996, véase Capítulo 2). Finalmente, debido a que las respuestas metabólicas y digestivas varían en cada población, se concluyó que el BMR y los ajustes digestivos no representan fuerzas antagonistas como ha sido sugerido previamente (Geluso and Hayes 1999).

Sobre la base de la existencia de una correspondencia entre heterogeneidad temporal de la dieta y flexibilidad de rasgos digestivos (Capítulo 2), se propuso que el grado de especialización individual dentro de una población podría diferir entre localidades debido a que ésta sería afectada por la flexibilidad de los rasgos digestivos (Capítulo 3). La cuantificación del grado de especialización individual se realizó de acuerdo al marco cuantitativo para el análisis de la amplitud del nicho propuesto por Roughgarden (1972, 1974). Roughgarden (1974) sugiere que la amplitud del nicho total (*total niche width,* **TNW**), referida a la variedad de recursos que una población explota, está compuesto por la suma de dos componentes: el intra-individual (*within individual component,* **WIC**), el cual refleja el promedio de la amplitud de nicho individual, y el componente entre individuos (*Between Individual Component,* **BIC**) que representa las diferencias entre individuos en la utilización de recursos; de esta manera **TNW** = **WIC + BIC** (ver Figura 4).

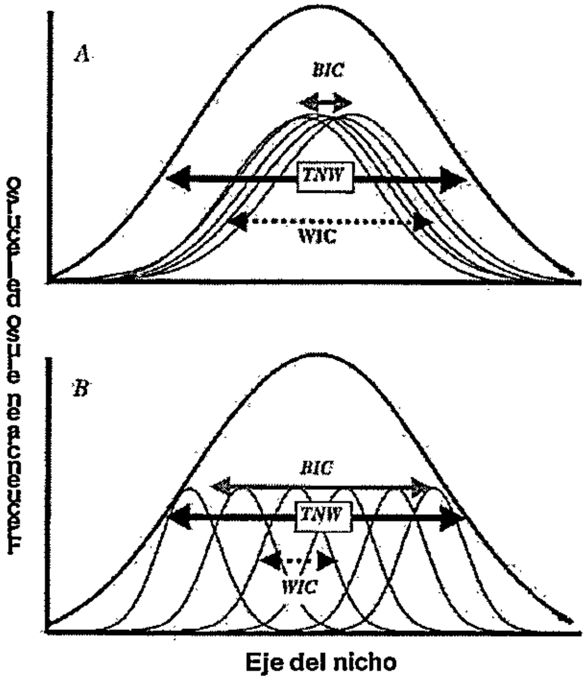

FIGURA 4: Representación de la amplitud total y los componentes de un eje del nicho en una población compuesta por individuos generalistas (A) y una población con variación intra-poblacional en la explotación de recursos, i.e., compuesta por individuos especialistas (B). Modificado de Bolnick et al 2003.

Como mencionamos anteriormente, la diferencia en la amplitud del nicho individual entre especies ha sido relacionada principalmente a fenómenos de competencia intra e inter-específica, pero no a la influencia directa del ambiente. En esta investigación se propuso a la heterogeneidad temporal de los recursos tróficos como un predictor del grado de especialización individual de las poblaciones. La base de la principal hipótesis planteada en esta parte de la investigación, específicamente que la razón WIC/TNW estaría determinada por la flexibilidad digestiva, se apoya en que existirían compromisos funcionales (morfológicos, fisiológicos y conductuales) que determinan que la mayor eficiencia de explotación de un recurso vuelve menos eficiente la explotación de uno alternativo (Ferry-Graham et al. 2002; Svanbäck and Eklöv 2003; Muchhala 2007). En el caso de los compromisos fisiológicos, esto puede ocurrir a un nivel post-ingestivo, ya que el procesamiento de recursos alternativos puede requerir diferentes condiciones de hidrólisis, transporte o mecanismos de detoxificación (véase Capítulo 3). De esta forma, al considerar que la heterogeneidad temporal promueve normas de reacción más flexibles en los rasgos relacionados con ésta (Capítulo 2), se puede deducir que los compromisos adquiridos en la explotación de recursos diferentes también deberían verse modificados, afectando el grado de especialización individual de las poblaciones (para detalles de esta hipótesis véase Capítulo 3). Bajo este panorama, las diferencias en heterogeneidad temporal de la dieta entre las distintas localidades llevan a predecir diferencias en el grado de especialización individual de las poblaciones (Figura 5). La hipótesis planteada en esta investigación acerca del grado de especialización individual de las poblaciones puede ser resumida a través de la Figura 5, donde se representan tres condiciones en las cuales se podrían encontrar las poblaciones examinadas, la influencia de la heterogeneidad temporal de la dieta y la magnitud de la flexibilidad de las características digestivas. De esta manera, se predice que individuos con normas de reacción rígidas deberían verse restringidos en su capacidad de cambiar de dieta, constituyendo por lo tanto una población con un nicho poblacional estrecho y nichos individuales similares entre sí en amplitud y promedio (Figura 5, población a). A su vez, poblaciones en donde los individuos tienen normas de reacción más flexibles, se espera que los costos de aclimatización a los cambios de dietas sean escasos o nulos, lo cual permitiría a los individuos cambiar continuamente de dieta y ocupar diferentes partes dentro del rango del nicho poblacional total. De lo anterior se predice nichos tróficos individuales amplios, constituyéndose así una población de individuos generalistas que utilizan todo el rango del nicho poblacional total (Figura 5, población c). Finalmente, en individuos con normas de reacción de flexibilidad intermedia, los costos de aclimatización a cambios de dieta debieran ser moderados. Según esto, se predice que los individuos tenderían a mantener su nicho trófico individual, evitando los cambios secuenciales de dieta; por lo tanto es de esperar nichos individuales estrechos distribuidos en distintas partes del rango del nicho poblacional total (Figura 5, población b).

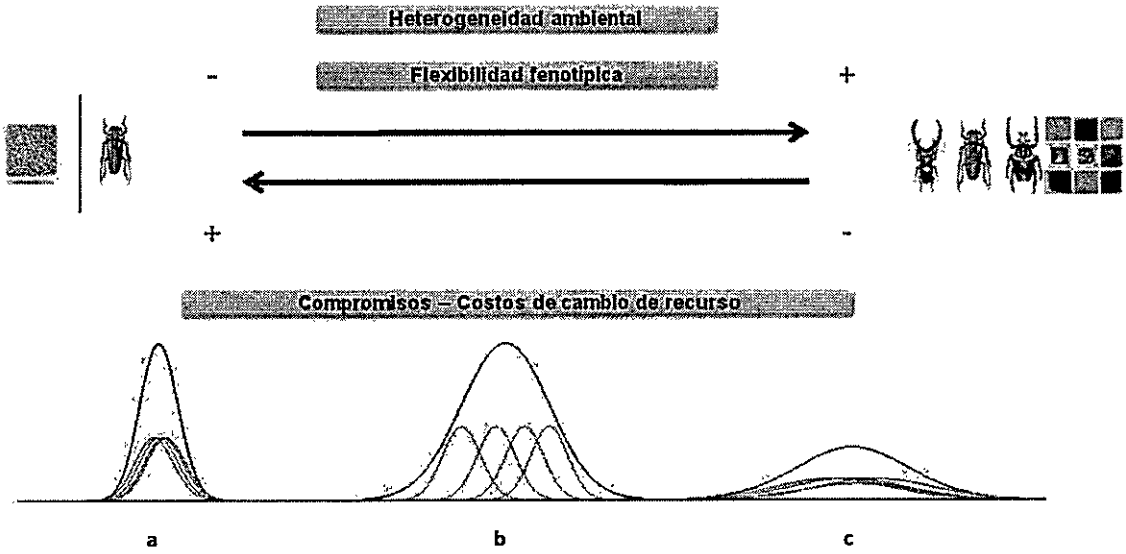

Figura 5. Diagrama representando la relación entre el grado de heterogeneidad temporal, flexibilidad fenotípica y los compromisos y costos del uso de recursos. Las letras representan distintas poblaciones con grados diferentes de especialización individual.

Los resultados encontrados se correspondieron claramente con lo esperado para cada localidad de acuerdo a los criterios planteados anteriormente basados en la heterogeneidad ambiental y flexibilidad fenotípica (Capítulo 3, Figura 2). Así, el grado de especialización individual difirió entre localidades siendo la población de Llanquihue la que presentó un menor WIC/TNW, es decir, fue la población que presentó el mayor grado de especialización individual (Capítulo 3, Figura 2). Este resultado es coherente con la alta correlación encontrada entre ∂^{15}N de las plumas primaria 1 y primaria 9, las cuales representan variaciones en la dieta de corto plazo (1 mes), siendo una evidencia de la mantención de la dieta de los individuos en el tiempo (véase Capítulo 3, Figura 3). Además, la mantención de la especialización individual supone la existencia de compromisos funcionales en la explotación de recursos alternativos. En esta investigación, se manifestaría este supuesto si los individuos especialistas se encuentran aclimatizados a la composición química de la dieta, es decir, si presentan ajustes en la morfología y fisiología digestiva en relación a su dieta natural. En esta línea, se encontró una relación positiva entre la proteína dietaria consumida (medida a través ∂^{15}N de la sangre) y la actividad de aminopeptidasa-N para aves provenientes de la localidad de Llanquihue (Capítulo 3, Figura 4). Esto concuerda con la visión plantada anteriormente, donde la especialización individual dentro de la población se vería beneficiada por la aclimatización dietaria, siendo a su vez una causa de la mantención de la especialización de los individuos al interior de la población (discutido en Capítulo 3).

Por último, se analizó la variabilidad fenotípica de la conducta, tanto a nivel inter-poblacional como intra-poblacional y se relacionó con la fisiología de los animales (Capítulo 4). La variación conductual se estudió en términos de caracteres de la personalidad de los individuos. La personalidad animal es definida como diferencias conductuales correlacionadas que persisten a lo largo de un amplio rango de contextos (Bell 2007, Sih et al. 2004). De esta manera, animales con diferentes personalidades pueden diferir consistentemente en sus respuestas a un mismo estímulo ambiental. Este tipo de variabilidad también es considerado como un ejemplo de variación continua dentro de una población (Goldsmith et al. 1987). Un importante componente de la personalidad animal es la conducta exploratoria, la cual a su vez ha sido relacionada a las características del hábitat y a la especialización dietaria (Greenberg and Mettke-Hofmann 2001, Mettke-Hofmann et al. 2002, Tebbich et al. 2009). En este estudio, la conducta exploratoria fue relacionada al BMR, a la tasa de pérdida de agua evaporativa una vez alcanzado un estado estable (TEWL) y a dos nuevos indicadores propuestos del efecto de la conducta exploratoria sobre la tasa metabólica y la pérdida de agua evaporativa (que llamamos MR_{30} y $TEWL_{30}$) que representan el promedio de la tasa metabólica y la pérdida de agua evaporativa durante los segundos treinta minutos del registro experimental, respectivamente (véase Capítulo 4, Figura 1).

Las poblaciones analizadas difirieron en TEWL y $TEWL_{30}$ entre poblaciones, siendo ambas tasas menores para la localidad de Copiapó (Capítulo 4, Figura 2; Tabla 2). También se encontraron diferencias en la asociación entre rasgos conductuales y rasgos fisiológicos entre las poblaciones. $TEWL_{30}$ en aves de la zona árida de Copiapó se correlacionó negativamente con la conducta exploratoria (Capítulo 4, Figura 5). Esto significa que aves con mayores tasas de pérdida de agua evaporativa frente a situaciones novedosas, presentaron a su vez, menores niveles de exploración en los experimentos conductuales, y viceversa. Esto apoya el modelo de asignación de agua (WAM) que se propuso en esta parte de la investigación, el cual considera al agua como un recurso limitante, existiendo entonces un compromiso entre la conducta exploratoria y la pérdida de agua (véase Capítulo 4). Por otra parte, las aves de la localidad de Quebrada de la Plata presentaron una asociación positiva entre la conducta exploratoria y BMR, donde animales con mayor BMR mostraron ser más exploradores en los experimentos conductuales (Capítulo 4, Figura 4). Esto concuerda con lo esperado según el modelo de desempeño propuesto por Careau et al. (2008) que plantean una relación positiva entre BMR y los niveles de actividad de los organismos (véase Capítulo 4). Finalmente, para la localidad de Llanquihue la conducta exploratoria no estuvo asociada con ningún rasgo fisiológico medido en este estudio. Nosotros adjudicamos este resultado a la baja repetibilidad de la conducta exploratoria y, por lo tanto, a la alta variación debida a cambios en las condiciones ambientales. Este fenómeno ha sido reportado en un estudio previo en esta localidad (véase Anexo 1).

Por otro lado, los niveles de exploración difirieron entre poblaciones, siendo mayor para la localidad de Llanquihue (Capítulo 4, Figura 3). Se ha propuesto que altos niveles de exploración estarían relacionados a una mayor plasticidad ecológica de las especies (e.g., nichos tróficos poblacionales más amplios) y a su vez, que bajos niveles de exploración implicarían mayores niveles de especialización dietaria (i.e. nichos tróficos poblaciones más estrechos) (véase Tebbich et al. 2009). En este estudio no se verificaron las relaciones predichas, siendo más bien la heterogeneidad temporal en los recursos tróficos el factor que pareció ser más influyente en la conducta exploratoria (discutido en Capítulo 4). Este resultado es consistente con lo encontrado en las otras secciones de la investigación realizada en este modelo ecológico (Capítulos 1, 2 y 3). En efecto, las diferencias en heterogeneidad temporal a lo largo del gradiente latitudinal, parece ser un factor ecológico relevante en la explicación de la variación fenotípica en los distintos niveles estudiados. A lo largo de esta investigación el resultado de ello estuvo reflejado en un complejo y amplio espectro de tipos de fenómenos y patrones de variación fenotípica tanto en rasgos fisiológicos como morfológicos y conductuales (Capítulos 1, 2, 3 y 4). Finalmente, los estudios realizados revelaron además que la variabilidad fenotípica no sólo está influenciada por las condiciones ecológicas, sino también por la compleja forma en que los distintos rasgos interactúan entre sí. De este modo, se comprobó que una mirada integrativa y multifactorial puede ser particularmente útil para la comprensión de los fenómenos detrás de la organización de las poblaciones y de la diversidad fenotípica a un nivel intra-específico.

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Chapter 1

Dietary habits and digestive modulation in three populations of *Zonotrichia capensis*

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Introduction

The range of food types that individuals may consume could be constrained by their digestive structures determining in part their trophic niche (Karasov 1996; Sabat et al. 1998). In birds, the relationship between digestive features (i.e., morphology and gut biochemistry) and dietary patterns has been widely studied (Karasov 1990; Martínez del Rio 1990; Afik et al. 1995a; Battley et al. 2005). For instance, it has been reported that passerine birds consuming mainly insects have shorter intestines than those consuming fruit, seeds and mixed diets (Ricklefs 1996). In turn, the birds' intestine biochemistry, i.e., the level of specific enzymes that deal with the chemical breakdown of food components, seems to be related to feeding habits (Karasov 1996; Karasov et al. 1997). For example, data on sucrase activity have shown the highest values for hummingbirds, once these data are compared to the activities of nine passerine species utilized for the analysis. This result would be a consequence of unusual sucrose-rich nectars of hummingbird diets (Martínez del Rio 1990).

Ecological factors, such as climate, may lastly determine the food diversity and its availability (Rosenzweig 1968; Zaks et al. 2007). If the food resource for a single species varies, e.g., because geographical differences and/or seasonal climate, individuals could maintain their energy and nutrient demands by means of digestive adjustments according to the amount and biochemical composition of food that animals experienced (McWilliams et al. 2001). It has been proposed that vertebrates have the ability of modifying the levels of digestive enzymes according to their dietary chemistry (the adaptive modulation hypothesis, AMH). The AMH has been mainly suggested for omnivore species which face changes in the biochemical composition of resources (Biviano et al. 1993; Karasov et al. 1997). Nevertheless, in birds, the specific modulation of carbohydrases, i.e., the up regulation of enzyme levels when they consume high carbohydrate diets, has been reported only in adults of the order Galliformes, but not for passerines birds (Caviedes-Vidal et al. 1994; Karasov et al. 1997; Sabat et al. 1998; Caviedes-Vidal et al. 2000). Nevertheless, differences within a single species may be determined by irreversible modifications due to environmental changes which occur in early stages of development (Tracy et al. 2001; Piersma et al. 2003; Sabat et al. 2008). Recently, Brzek (2009) found that specific maltase activity increased significantly during development in nestlings of House sparrows (*Passer domesticus*) fed with starch diet, compared to birds fed with diet lacking starch. Moreover, this difference in the magnitude of maltase activity was at least twice as high at the time of fledging (Brzek et al. 2009). Since the house sparrows have not shown a positive correlation between dietary carbohydrate and maltase in adulthood (Caviedes-Vidal et al. 2000) suggesting the presence of developmental plasticity for this biochemical trait. In other words, they suggested that phenotypic flexibility of enzyme expression may be lost later in life. Taking into account the foregoing, we expected that dietary habits experienced by a single species during their life, modulate the enzymatic activities among populations, including the carbohydrases activities. In this work, we evaluated whether or not a passerine species satisfies AMH with respect to natural diets birds experience during life time (i.e., field dietary acclimatization) as well as the putative differences in digestive morphology (i.e., intestine mass and length). To accomplish this, we investigated those digestive features in three populations of *Zonotrichia capensis* along a latitudinal gradient in Chile with marked differences in primary productivity. Besides, in order to evaluate the correspondence between the digestive characteristics and the population feeding habits, we employed the nitrogen stable isotope ratios as indicators of the relative food web position (Post 2002) and as an indirect index of the protein consumption of birds (Schondube et al. 2001, Sabat et al. 2009). We measured the activity of three intestinal membrane-bound digestive enzymes: sucrase and maltase, two disaccharidases that we used as indicator of carbohydrates ingestion. Those enzymes hydrolyze sucrose and maltose, yielding glucose and fructose for their further absorption. We included aminopeptidase-N activity as a measure of protein digestion (Caviedes-Vidal et al. 2000), since it cleavages NH_2 terminal amino acid residues to produce the final dipeptides and amino acids for absorption (Maroux et al. 1973; Sjostrom et al. 1978).

Methods

Animals and study sites

We measured the digestives features of Rufous-collared sparrows captured from three study areas during fall of 2008. *Zonotrichia capensis* (Paseriformes: Emberiziidae) is broadly distributed between southeast Mexico and Cabo de Hornos in southern Chile (Goodall et al. 1951). Its distribution in Chile is almost ubiquitous and includes both, deserts and rain forests (Araya et al. 2005). This omnivorous species is described by feeding on mixed diets comprised of seeds and insects, besides it has been reported populations that differ in their trophic niche (Lopez-Calleja 1995; Novoa et al. 1996; Sabat et al. 1998, Sabat et al 2009). Accordingly, *Z. capensis* is a well-suited model to study population differences in digestive features. The three study areas differ greatly with respect to their climatic conditions; Copiapó (27°18´S, 70°25´W), the northernmost site is surrounded by the Atacama Desert and it is characterized by a rigorous aridity, with mean temperatures of 15.2°C and an total annual precipitation of 12 mm. Quebrada de la Plata (33° 31´ S, 70° 50 ´W) has a Mediterranean climate with seasons quite variable in temperature and rainfall (i.e., hot and dry summers, and cold and rainy winters). The annual average temperature is about 14.4° C and the total annual precipitation of 312.5 mm (http://www.meteochile.cl; Di Castri et al. 1976). Finally, Llanquihue (41°16´S, 73°00´W), the most southern region, exhibits plentiful rainfall all year round and lower temperatures compared with the other localities. The mean annual temperature is about 9.4° C and the total annual precipitation of 3112 mm (Centro de Informacion de Recursos Naturales, Ciren).

In order to represent the magnitude of food availability among populations, we estimated the primary productivity (Tieleman et al. 2003) via the aridity index (Q) as:

$$Q = \frac{pp}{(T_{\max} + T_{\min})(T_{\max} - T_{\min})} \cdot 1{,}000$$

Where pp is the average annual precipitation (mm) and $T_{\max}$ (°C) and $T_{\min}$ (°C) are the mean maximum and minimum temperature for the hottest and coldest month, respectively (Tieleman et al. 2003).

The annual mean of this index is low in hot, dry deserts, and high in cool, wet areas. Our calculations demonstrated a north-south increase in primary productivity from Copiapó ($Q = 15.8$), through to Santiago ($Q = 360$) and Llanquihue ($Q = 9{,}185$). We collected historical data for both precipitation and temperatures from national institutes of information (Dirección meteorológica de Chile, Centro de información de recursos naturales), from literature (Di Castri et al. 1976), and from the web (http://www.meteochile.cl).

Procedures at field sites and samples preparation

Immediately after capture, birds were weighed (to the nearest 0.05 g) and a blood sample from the brachial vein was collected into heparinized microhematocrit tubes (~ 100 µl). The samples were stored to a posterior isotopic analysis. Then, sparrows were sacrificed by decapitation for later dissection at the laboratory.

Laboratory measurements

Birds were dissected abdominally, the large and small intestine (SI) were extracted, weighed and measured their length. SI contents were carefully removed and washed with a 0.9% NaCl solution, and immediately frozen in liquid nitrogen for further enzymatic determinations. Then, we removed the heart, gizzard, liver and kidneys, which were immediately weighed in an electronic balance (± 0.001 g).

We determined the activity of three intestinal membrane bound disaccharidases, maltase (E.C. 3.2.1.20), sucrase (E.C. 3.2.1.48) and one oligopeptidase, aminopeptidase-N (E.C. 3.4.11.2) by colorimetric method in whole intestinal tissue homogenates. The advantages of considering the activities in whole tissue are discussed by Martinez del Rio et al. (1995) and Caviedes et al. (2000). Briefly, tissues were thawed and homogenized for 30 s using an ULTRA TURRAX T25 homogenizer at maximum setting in 20 volumes of 0.9 % NaCl solution. Disaccharidase activity was determined according to the method described by Martinez del Rio 1995. In brief, tissue homogenates (100 µL) were incubated at 40°C with 100 mL of 56 mmol L^{-1} sugar solutions in 0.1 M Maleate/NaOH buffer, pH 6.5. After a incubation period, 3 min to maltase and 5 min to sucrase assay, the reactions were stopped by adding 3 mL of a stop/develop Glucose-Trinder (Sigma Diagnostics, Saint Louis, Missouri). This solution was consisting of 500mL in 250 mL 0.1 mol L^{-1} TRIS/HCl, pH 7 plus 250 mL of 0.5 NaH_2PO_4, pH 7. After 18 min, the absorbance was measured at 505 nm with a UV-VIS spectrophotometer (Shimadzu, UV mini-1240) at 20°C. Aminopeptidase-N assays were done using L-alanine-*p*-nitroanilide as a substrate. We started the reaction adding 1 ml of assay solution (2.04 mmol l^{-1} L-alanine-*p*-nitroanilide in 0.2 mol μl^{-1} NaH_2PO_4/Na_2HPO_4, pH 7) to the tissue homogenate (100µl). The reaction was incubated during 10 min at 40°C and then arrested using 3 mL of ice-cold acetic acid (2 mol L^{-1}); the absorbance was measured at 384 nm. On the basis of absorbance, standardized intestinal enzymatic activities were calculated. The protein content of SI tissue was measured using bovine serum albumin as standard. Absorbance was read at 595 nm (Bradford 1976).

Isotopes analysis

We obtained nitrogen stable isotope ratios ($\partial^{15}N$) of the whole blood in order to rely on an indicator of sparrows' diet. The isotopic signature of blood corresponds nearly to resources assimilated during the 14 days before taking the samples (Hobson et al. 1992). Before isotopic measurements, blood samples were dried until constant mass at 70 °C. Then, samples were loaded into tin capsules, after grounded into a fine powder. Stable nitrogen isotopic compositions of samples were determined by a conventional method using elemental analyzer/isotope ratio mass spectrometry (EA/IRMS, Finnigan Delta V Advantage interfaced in continuous flow with FlashEA 1112 HT, Thermo Finnigan, Bremen, Germany). Accuracy of the measurements was monitored by analysis of international standards (IAEA-N1 and N2). Precision was better than ±0.1‰. Nitrogen isotope ratios were expressed using standard delta notation ($\partial^{15}\mathrm{N}$) in parts per thousand (‰) as:

$$\partial^{15}\mathrm{N} = \left[(R_{sample} / R_{standard}) - 1\right] \cdot 1{,}000$$

where R_{Sample} and R_{standard} are the molar ratios of $^{15}\mathrm{N}/^{14}\mathrm{N}$ of the sample and reference, respectively.

To compare the dietary habits of birds we calculated the trophic level (TL) as follows:

$$\mathrm{TL} = \left(\frac{\partial^{15}\mathrm{N}_{\mathrm{blood}} - \partial^{15}\mathrm{N}_{\mathrm{plants}}}{3.4} \right) + 1$$

Where $\partial^{15}\mathrm{N}_{\mathrm{blood}}$ represent the isotopic signature ($\partial^{15}\mathrm{N}$) of bird blood sample and $\partial^{15}\mathrm{N}_{\mathrm{plants}}$ the isotopic signatures ($\partial^{15}\mathrm{N}$) of primary producers i.e., the average of $\partial^{15}\mathrm{N}$ from plants obtained in the locality in which the bird was captured. We used the value 3.4 as the enrichment of $\partial^{15}\mathrm{N}$ per TL (~3.4‰) (Post 2002).

Data Analysis

Prior to all statistical analyses, data were tested for normality and homogeneity of variance using Kolmogorov–Smirnov and Levene tests, respectively. To explore the association between variables we performed linear regressions analysis (LRA). In order to evaluate whether the relationship between variables can be explained by a linear or a power function, we tested if the allometry coefficient *b* of the model: $y = aM_b^{\,b}$ were significantly different from 1, with t-test (α=0.05). Because body mass among populations almost differed significantly (P= 0.056), when dependent variables were correlated with M_b we evaluated differences among populations by analysis of covariance (ANCOVA) using M_b as covariate. Because means of SI protein content was not statistically different among localities, data on enzyme activities was analyzed as total hydrolytic activity (μmol min^{-1}). When we found significant differences among groups (at P<0.005) we used *a posteriori* Fisher test for multiple comparisons. Data are presented as mean ± SD. Statistical analyses were performed using STATISTICA 6.0.

Results

Body and organ mass

Body mass did not differ among populations (ANOVA; $F_{2,26}$=3.22, P=0.06; Table 1). Because SI mass was correlated with body mass (LRA; r^2= 0.27, P=0.004), to determine the effect of locality on this variable we performed one-way analysis of covariance (ANCOVA). This analysis revealed significantly higher SI mass

(ANCOVA; $F_{2,25}$=4.91, P=0.02; Table 1) for birds from Quebrada compared with the other populations (Fisher test, P<0.05). Also, the length of SI differs among populations (ANOVA; $F_{2,26}$=5.0442, P=0.01; Table 1). The SI length of sparrows from Llanquihue was approximately 15% higher than SI length of birds from Quebrada and Copiapó (Fisher test, P<0.05). Also, we found significant differences by locality for gizzard mass (ANCOVA; $F_{2,25}$= 5.81, P= 0.008; Table 1); birds from Copiapó showed the smallest values (Fisher test, P<0.05). Kidney mass was significantly higher in birds from Quebrada in compared with birds from Copiapó and Llanquihue (ANCOVA; $F_{2,25}$= 4.47, P= 0.02; Table 1). We found no differences for liver and heart masses among localities (ANCOVA P>0.05; Table1).

Digestive enzymes

Total protein from SI did not vary among localities when we used intestine mass (ANCOVA; $F_{2,25}$=2.77, P= 0.08) and body mass as covariates (ANCOVA; $F_{2,25}$=2.89, P= 0.07). The analysis of the total enzymatic activities showed a strong positive relationship among them when all data were pooled (LRA: r^2 = 0.56; r^2 = 0.84; r^2 = 0.57, respectively; P < 0.001; Figure 1). Among populations, the ANCOVA analysis of total enzyme activities (μmol min^{-1}) revealed that locality has a significant effect on digestive enzymes. In the case of disaccharidases, we found that intestinal sucrase activity was significantly lower in birds from Copiapó (ANCOVA; $F_{2,25}$=7.58, P=0.003, Figure 2). Unlike sucrase, maltase activity did not differ among populations (ANCOVA; $F_{2,25}$=2.22, P=0.13, Figure 2). The hydrolysis rates of aminopeptidase-N showed significant differences among populations (ANCOVA; $F_{2, 25}$=9.69, P<0.001). The *a posteriori* analysis of multiple comparison, revealed that sparrows inhabiting in Quebrada had the highest activities for this enzyme, followed by Llanquihue and Copiapó birds, which once again evidenced a reduced enzymatic activity (Figure 2).

Blood isotopic composition and trophic level

Trophic level differs among all three localities (ANOVA: $F_{2, 48}$=13.82, P<0.001), being higher in birds from Quebrada, follows by Copiapó and Llanquihue (Figure 3). There was a negative relationship between protein ingestion and Q_{index} (LRA: Q_{index}= 2,95 – 0.0004 TL, r^2=0.4; P<0.001). Also, there we found a significant and negative association between SI length and TL, when all data were pooled (LRA: SI length=.19.71-1.99 TL, r^2= 0.3; P<0.001, Figure 4). We did not found a significant relationship among TL and digestives enzymes (LRA, P> 0.05).

Table 1. Morphological data of *Z. capensis* from different localities. Different letters denote significant differences ($P<0.05$) among localities after *a posteriori* Fisher test. The number of animals in each locality is in parenthesis. Data are reported as mean ± SD.

	Copiapó (10)	Quebrada (10)	Llanquihue (9)
Body mass (g)	15.98 ± 2.5^a	18.1 ± 1.69^a	17.38 ± 1.21^a
Gizzard (g)	**0.66 ± 0.08^a**	0.85 ± 0.11^b	0.82 ± 0.10^b
SI (g)	0.35 ± 0.09^a	0.52 ± 0.11^b	**0.42 ± 0.06^a**
SI length (cm)	13.5 ± 1.7^a	13.6 ± 1.5^a	**15.5 ± 1.3^b**
Liver (g)	0.46 ± 0.16^a	0.62 ± 0.16^a	0.48 ± 0.09^a
Heart (g)	0.19 ± 0.06^a	0.24 ± 0.04^a	0.25 ± 0.04^a
Kidney (g)	0.18 ± 0.03^a	**0.22 ± 0.03^b**	0.19 ± 0.02^a

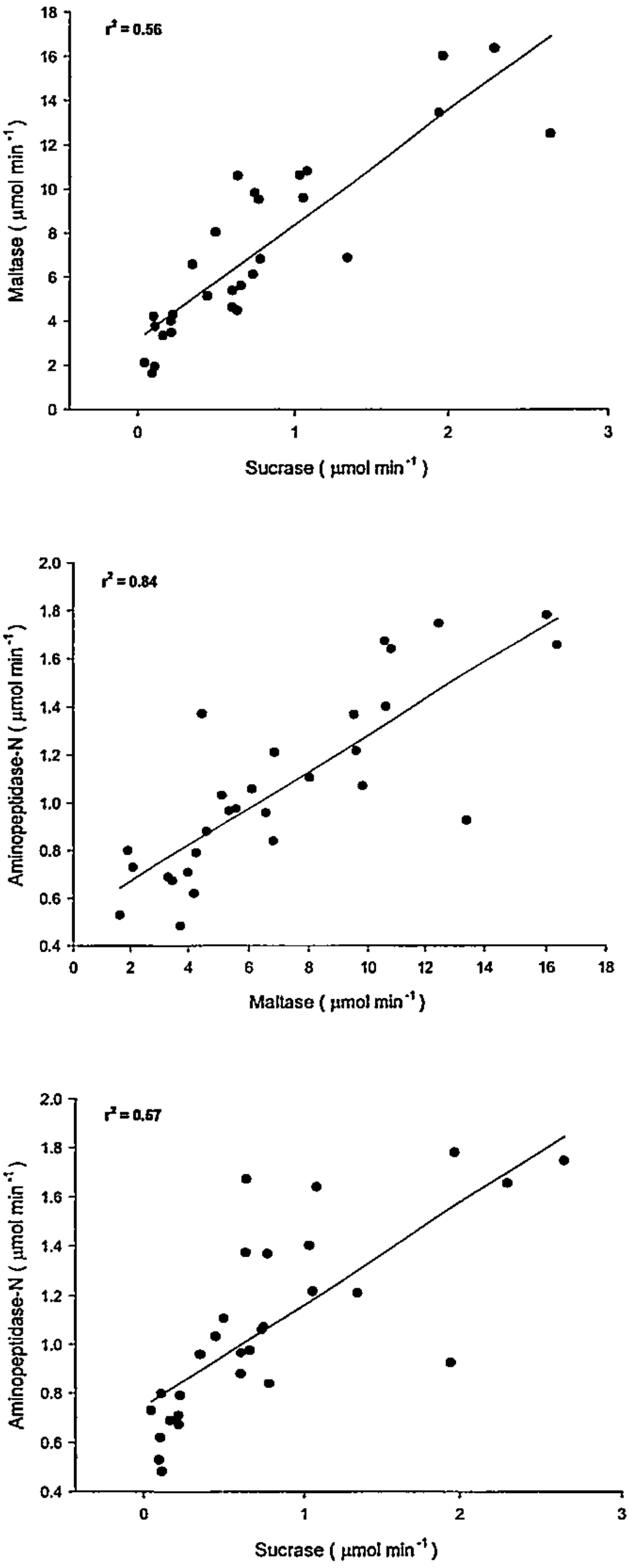

Figure 1. Relationship between intestinal enzymatic activities in field-caught birds. Note that all the LRA shown are statistically significant ($P < 0.05$).

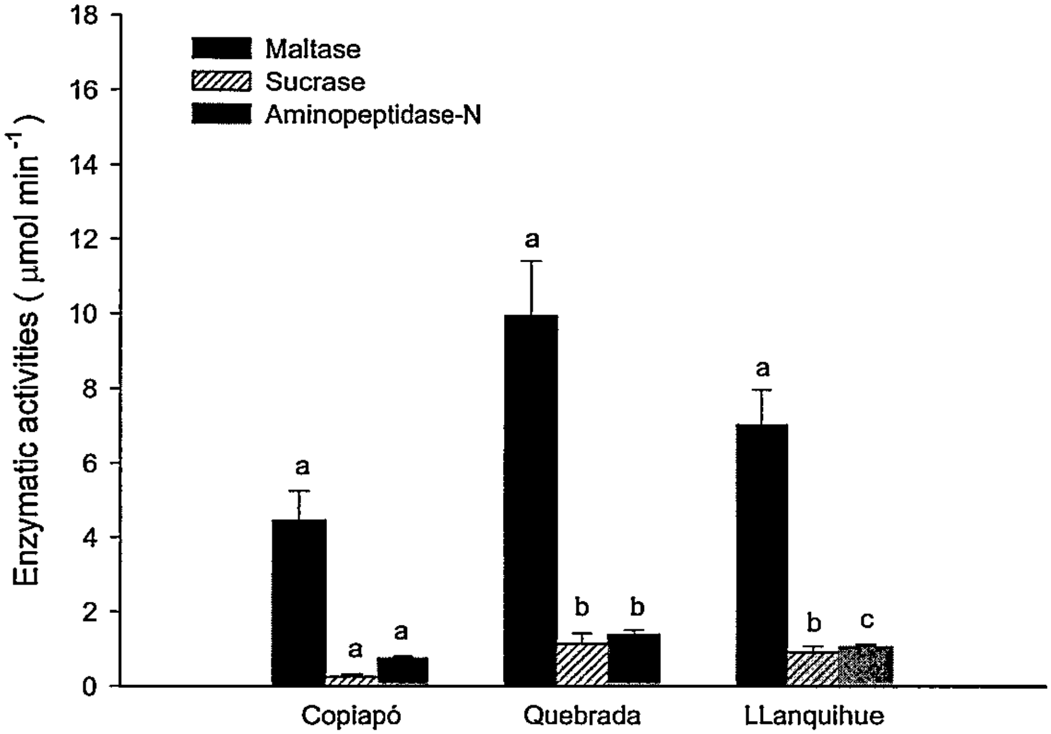

Figure 2. Maltase, sucrase and aminopeptidase-N activities in *Zonotrichia capensis* from three localities along Chile. Common letter reflect means that are not significantly different (P > 0.05) among localities after *a posteriori* Fisher test. Data were expressed as mean ± SE.

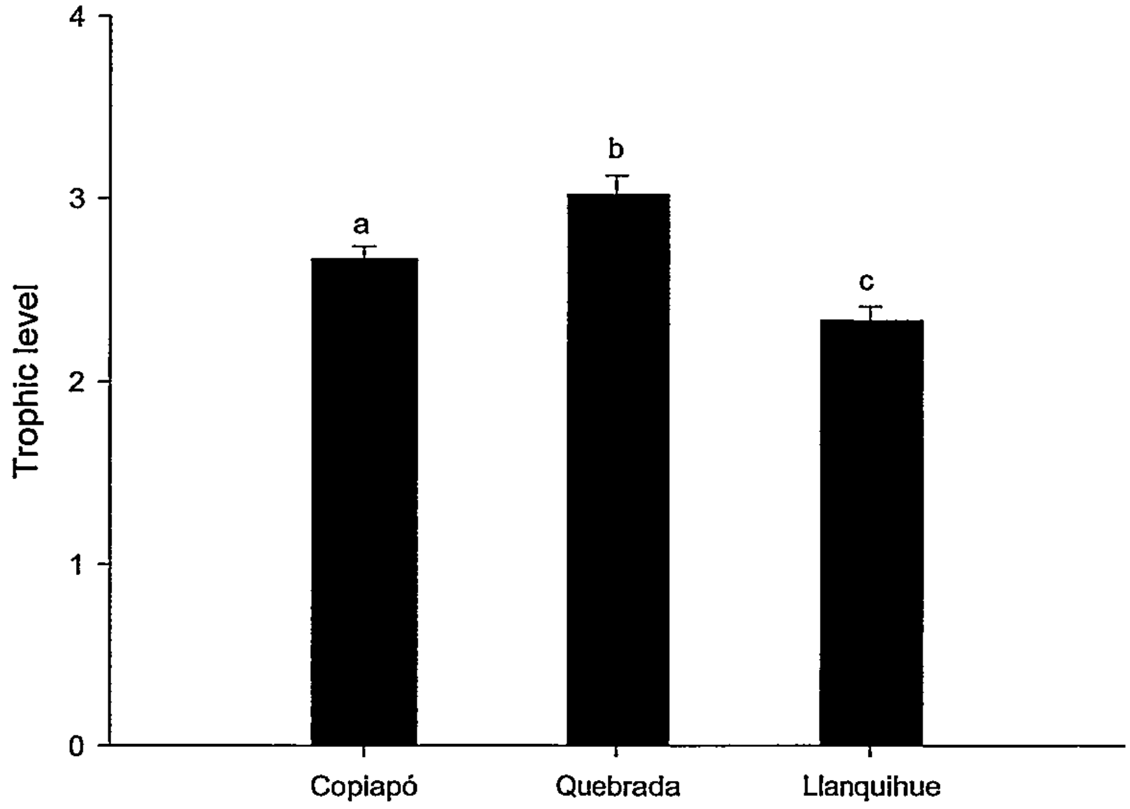

Figure 3. Trophic level (from isotopic analysis $\partial^{15}N$) in three populations of *Zonotrichia capensis*. Different letters denote means that are significantly different (P > 0.05) among localities after *a posteriori* Fisher test. Data were expressed as mean ± SE.

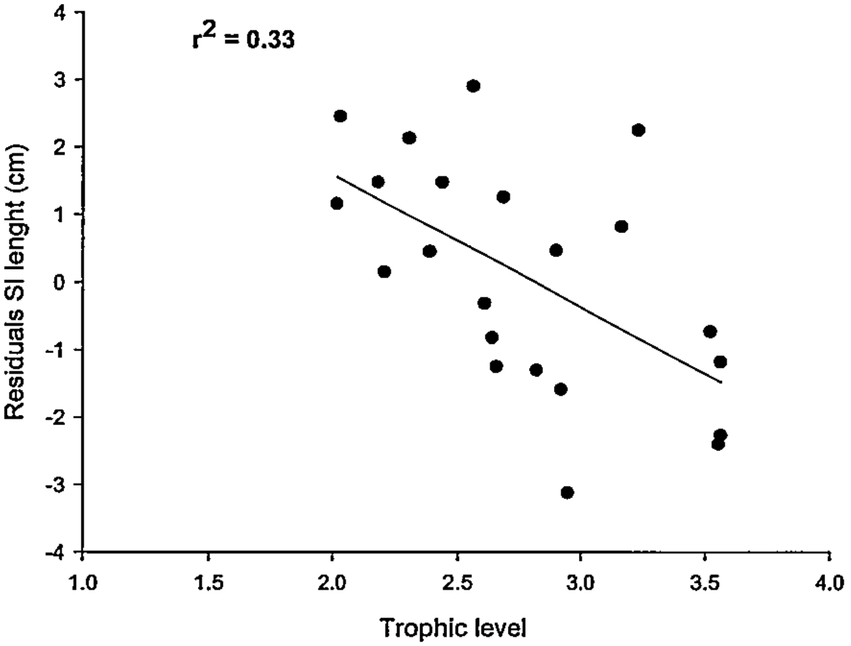

Figure 4: Linear regression among residuals of SI length (against body mass) and trophic level determined from isotopic analysis ($\partial^{15}N$). This LRA included data from all populations. Note that LRA showed be statically significant (P= 0.001).

Discussion

Geographically separated populations may experience markedly different ecological conditions, which may influence the natural diets and digestive features of individuals. Here, the three examined bird populations differed consistently in their abiotic conditions and in their trophic niche. Considering all populations, the range of trophic level was between 1.8 and 3.6, with birds feeding in average on the second level. *Z. capensis* has been reported as a seed and insect consumer (López-Calleja 1995). This observation along with the TL values suggests that birds from Quebrada incorporate a higher proportion of insects in their diets compared to the remainder populations. In turn, birds from Llanquihue, which had the lowest TL average, probably consume a higher amount of seeds than Quebrada and Copiapó birds. Based on the observed dietary differences, in the following paragraphs we discuss how dietary habits may influence both the intestine biochemistry, focused on AMH as well as the gut morphology.

Digestive morphology

In birds, it has been reported that conditions favoring the increase of food intake, such as low temperatures and lower dietary quality, should lead to a higher intestinal mass and length (Savory et al. 1976; Karasov et al. 1990; Brugger 1991; Starck 1996; Geluso et al. 1999; Battley et al. 2005; McWilliams et al. 2005). On the other hand, among species, insectivorous diets which commonly are composed by low carbohydrate and high protein content, have been associated with shorter and smaller intestines than granivorous or mixed diets (Ricklefs 1996). Similar results have been found in seasonal acclimatization to insect diets compared to more fibrous or high plant material diets (Pulliainen and Tunkkari 1983, Al-Dabbagh et al. 1987). However, experimental acclimations to diets that differ in protein content have not shown conclusive results (e.g., Afik et al. 1995, Novoa et al. 1996, Sabat et al. 1998, Caviedes-Vidal et al. 2000). In this study, we found geographical significant variation in SI mass. In birds from Quebrada, SI mass comprises in average the 2.87% of body mass being significantly higher than the 2.19% and 2.41% found in sparrows from Copiapó and Llanquihue, respectively (Table 1). Besides, we found that, in average, birds from Llanquihue had longer SI than the remainder populations. In this vein, it has been reported in an experimental study in *Z. capensis* from the same populations, that dietary acclimation to insect and seed diets modified SI mass (being heavier in birds acclimated to insect diet) without any changes in the SI length (Chapter 2). Here, because *Z. capensis* from Quebrada showed to be the most insectivorous population and also had higher SI mass, the results are agree with those found by insect diet acclimation. In turn, birds from Llanquihue are challenged with colder temperatures throughout the year, which results in greater thermoregulatory costs that those achieved by an increment of energy intake (Denbow 2000; Cavieres & Sabat 2008). Hilton et al. (2000) demonstrated that organ size variation among species is an important component of variation between geographically separated Icelandic seabird populations, even though that study considered a narrow species range. The authors suggested that high daily energy expenditures, probably associated to a broad foraging range and high thermoregulatory costs (i.e. lower ambient temperatures) result in greater intestine mass. Based on these assertions, we suggest that longer SI may be an adjustment to an increase in food consumption. Examinations on freshly captured sparrows, indicate that birds from Llanquihue could effectively have higher rates of food intake. First, birds from Llanquihue had a significant elevated plasma glucose concentration immediately after captured compared with Quebrada and Copiapó (Maldonado K., unpublished data). In addition, they had visibly higher subcutaneous fat reserves in their interclavicular depression and abdomen than the other two populations (Maldonado K., personal observations). Furthermore, we found a significantly and negative relationship between TL and SI length (Figure 5). In average, sparrows from Llanquihue consume in lower TL than birds from Quebrada and Copiapó. Thus, it seems that differences in the SI length also could be the result of adjustments to the proportion of insects and seeds they consume, that is, a decrease of insect consumption and an increase of seed ingestion could result in longer intestines.

In a number of studies in bird species, it has been reported that fresh gizzard mass (corrected by body mass) is correlated positively with dry-matter intake (reviewed by Karasov 1996). The rationale for this relationship is that muscle hyperplasia can occur from increased contractions associated with a higher food intake (Moss 1989; Moore 1998). In this study, the lowest gizzard mass was observed in birds from Copiapó, which seems to be consistent with the lower food availability in this arid zone and the expectable reduced food consumption due to lower thermoregulatory cost of this population (Cavieres et al. 2008).

The geographical variation found in organ sizes appears to be associated to the different ecological conditions that birds experienced. Ecological factors previously described, such as temperature and food composition, appear to jointly shape the SI characteristics. In this sense, the relationship between interspecific and intraspecific responses to dietary habits may appear unclear because there is no unique factor controlling them. The implications of dietary habits and energetic demands on digestive features clearly need more investigation, since digestive variations among natural populations is a possible widespread phenomenon and an important component of local adaptation of geographically separated populations.

Digestive enzymes

In passerine birds, studies have failed to found a complete support for the AMH in acclimation experiments; those results may be summarized in two types of effects on the expression of intestinal enzymes (Afik et al. 1995b; Martinez Del Rio et al. 1995; Sabat et al. 1998; Caviedes-Vidal et al. 2000). First, the specific modulation, defined as the up-regulation of a particular enzyme activity (e.g., sucrase) as a result of the consumption of specific substrates (e.g., sucrose), i.e.

the AMH. Secondly, the non-specific modulation in which changes in diet quality leads to correlated changes in all digestive enzymes (Caviedes-Vidal et al. 2000). Both effects have been reported for *Z. capensis* (Sabat et al. 1998). These authors found a specific modulation of aminopeptidase-N when animals were fed with a carbohydrate-free (high protein) diet coupled with a non-specific increase in maltase and sucrase activity, which contradicts the prediction of the AMH hypothesis. As it was expected, we found that birds from Quebrada, which are in a higher trophic level position, also exhibited higher levels of aminopeptidase-N activity compared with the other populations. Nevertheless, our results did not conform exactly to the expected predictions, despite Llanquihue, which exhibited the smallest proportion of protein consumption in their natural diet, showed higher values of aminopeptidase-N than sparrows from Copiapó. On the other hand, disaccharidases activities did not show to be modulated in relation to the TL exhibited by the bird populations. In spite *Z. capensis* from Llanquihue were feeding on a lower TL (i.e., they incorporated higher proportion of seeds), the amount of disaccharidases was similar with respect to birds from Quebrada. These results suggest a non-specific modulation in which differences in the amount of assimilated dietary protein leads to correlated changes in all digestive enzymes. That is, the aminopeptidase-N activity of freshly caught birds as well as in diet acclimation experiments (Sabat et al. 1998), seems to be modulated by the amounts of protein consumption (as judged by $\partial^{15}N_{blood}$), increasing also the levels of disaccharidases activities.

Besides, the three enzymes were strongly correlated, so it seems reasonable that the Quebrada population showed higher levels in all digestive enzymes. Birds from Copiapó exhibited the lowest values to sucrase and aminopeptidase-N activities and similar values for maltase activity compared to Quebrada and Llanquihue. We suggest that the condition of low primary productivity and low energy requirements that should have sparrows from Copiapó lead in a depressing effect on the activities of digestives enzymes. However, it has been observed that the primary digestive adjustment to long-term (i.e., weeks) and low energy food acclimation is through modifications in the gut mass rather than in the absorption rate of tissue-specific enzymes or nutrients (McWilliams et al. 2005). According to this observation, the possibility that low energy requirements reduced the overall digestive enzymes merits further study.

Our measurements seem to support the hypothesis that flexible digestive morphology is more responsive to dietary switches than disaccharidases in passerine birds. Nevertheless, Brzek's (2009) findings about specific change in maltase in nestling house sparrows are reasons to doubt the idea that passerines do not modulate their digestive enzymes in respect to dietary carbohydrates. These facts suggest that sparrows could modulate the activity of maltase (or sucrase) only in a short-term time window in early ontogenetic stages where their diet is controlled by parental care. Our suggestion is based on the assumption that nestling birds are provided by their parents early in the ontogeny with rich insect diets (Klasing 1998), which may buffer the diet differences among populations. This could explain the lack of differences in the disaccharidases in the adulthood state. Nevertheless, further studies are needed to confirm these findings and to clarify the flexibility of disaccharidases enzymes in passerine birds. In summary, geographic variation in ecologically relevant traits, like the physiology and morphology of gastrointestinal organs, may be the rule rather than the exception in single species that occupy dissimilar geographic distributions. It may occur because many ecologic factors appear to influence the digestive features. The understanding of these geographical variation patterns could be useful to gain insight about the ultimate and proximate causes of microevolutionary change in physiological and morphological gastrointestinal features, but also to highlight the influence of these variations on the trophic niche breath of natural populations, which could have profound consequences in community ecological patterns.

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Chapter 2

The influence of foods habits on the flexibility of energetic and digestive traits in three populations of an omnivorous species of passerine bird

Chapter 2. The influence of foods habits on the flexibility of energetic and digestive traits in three populations of an omnivorous species of passerine bird

Introduction

Generalist species must be able to find, digest and metabolize a potentially wide range of food types which often exhibits very different biochemical compositions (Diehl 2003). The rate at which animals acquire and process energy is thought to be linked to the rate at which animals process and spend energy (Cruz-Neto et al. 2004; Bozinovic et al. 2007). Accordingly, several studies have been conducted to validate the so-called food habits hypothesis (FHH), which states that basal metabolic rate (BMR), i.e., the minimum energy necessary to maintain the homeostasis in endotherm animals, is correlated with the food habits of species (McNab 1986; McNab 1988). The specific prediction of this hypothesis is that certain diet attributes (quality, availability and predictability) are correlated with mass specific BMR (see Cruz-Neto et al. 2004). For instance, it has been reported that herbivorous birds consuming food with high fiber content exhibit lower metabolic rates than expected based on body mass, as a result of both poor quality and low energy contribution of their diets (Daan et al. 1990; Grajal et al. 1991; Hinds et al. 1993). However, despite FHH has been tested both at an inter and intraspecific level (Veloso and Bozinovic 1993; Geluso et al. 1999; Veloso and Bozinovic 2000a,b; Cruz-Neto et al. 2004; Bozinovic et al. 2007;

Bozinovic et al. 2009), the existent literature fails in gathering conclusive results. In this sense, Cruz-neto and Bozinovic (2004) concluded that at the proximate level, the lack of BMR response to low quality diets observed in several studies appears to be influenced by the ability of animals in triggering a combination of compensatory ingestive and digestive mechanisms known as integrated-processing responses (*sensu* Batzli et al. 1994).

The physiology of the digestive tract, which is tightly linked to the process of energy acquisition, represents a functional link between energy intake and the utilizable energy (Naya et al. 2008a; Naya et al. 2008b). In birds, laboratory studies have shown that the digestive performance could be maintained by modifications in gut size, nutrient transport activity or hydrolysis rate of tissue-specific digestives enzymes, when birds are fed with chemically different diets (Karasov 1996; Karasov et al. 1997; Sabat et al 1998; McWilliams et al. 2005). In vertebrates, generally the digestive enzymes such as intestinal aminopeptidase-N, sucrase and maltase, can be regulated by the concentration of specific substrates in the diet (Biviano et al. 1993; Karasov 1996). This hypothesis (the adaptive modulation hypothesis, AMH) stems from the assertion that high constitutive levels of enzymes entail a valuable metabolic cost, for synthesis and maintenance, which is unnecessary when animals deal with diets with low levels of the specific substrates (Diamond 1991). However, the AMH has not been supported universally. Indeed, several experimental studies conducted in different taxa have reported a "hard-wired" or rigid response for the levels of disaccharidases in the small intestine (e.g., Sabat et al. 1998; Sabat et al. 1999;

Caviedes-Vidal et al. 2000; Sabat & Bozinovic 2002; Naya et al. 2005; Sabat et al. 2005). Moreover, to the best of our knowledge, there are no studies in birds that have investigated the validity of the AMH at the intraspecific level.

Physiological flexibility in energetic and digestive traits is often hypothesized as a critical component of the adaptive repertoire of animals to cope with changing environmental conditions (Pigliucci 1996; Piersma et al. 2003; Pigliucci 2005; Cavieres et al. 2008). Nevertheless, how these responses take place together appears to be a no trivial question. In this sense, Geluso and Hayes (1999), suggest that there is a trade-off between digestive and metabolic flexibility which could be explained by the opposed forces that they represent. The authors argued that there is a contradictory response when animals are forced to consume low quality diets. In order to maintain the energy assimilation, animals might increase their food intake, and hence organs of the gastrointestinal tract such as stomach, small intestine, colon, and cecum get larger. This fact results in a higher energetic cost of maintenance and it is in the opposite direction of the expected decrease in BMR to reduce the overall energy expenditure (Geluso et al. 1999). So, if occurs that digestive and metabolic responses present unavoidable trade-offs, it is expectable that the number of possible flexible responses would be restricted among populations within and between species.

Intraspecific differences in the physiological responses of geographically separated populations may provide insights into how energetic and digestive traits evolved together and to what extent the ability to modify the physiology depends on the ecological features of species. An increasing number of studies reveal considerable phenotypic flexibility in avian BMR and digestive features; however, these adjustments have typically been examined as a result of seasonal acclimatization, i.e., the physiological adjustments associated with long-distance migration, or short-term thermal acclimation in artificial environments (McKechnie 2008). In fact, very few integrative studies have been conducted in order to evaluate the interplay between digestive and metabolic processes in response to dietary changes among populations. The intraspecific variation observed in avian physiological traits reflects an important source of phenotypic variation (Karasov 1996; McKechnie 2008). Hence, we investigate whether changes both in BMR and digestive features in response to changes in diet quality represent antagonistic forces and if these changes depend of birds' dietary habits. In order to do that, we evaluated if digestive features (such as intestine mass, activity of digestive enzymes and rate of glucose absorption) and BMR of sparrows (*Zonotrichia capensis*) from three populations along an aridity gradient in Chile, respond in a dissimilar way to the dietary acclimation. In addition, we examined the magnitude of these flexible responses in order to test the hypothesis which predicts more flexible traits in environments with higher temporal heterogeneity. In this ecological model populations differ noticeably in their biotic and abiotic conditions, so we expected that primary productivity and birds' diets characteristics (i.e., quality and temporality) to be different among localities, influencing the birds' physiological traits. To validate this last assumption we relied in an index of primary productivity (Q) (Tieleman et al. 2003) and on nitrogen stable isotope ratios, as indicators of food availability and as an indirect index of protein consumption, respectively (Schondube et al. 2001; Newsome et al. 2007, Sabat et al 2009).

Methods

In Chile, *Zonotrichia capensis* (Paseriformes: Emberiziidae) is a ubiquitous species, inhabiting in deserts and rain forests (Araya et al. 2005). This species is described as opportunistic, feeding on mixed diets comprised of seeds and insects (López-Calleja 1995,Sabat et al 1998). Furthermore, this species include populations that exhibited seasonal changes in dietary pattern (Novoa et al. 1996; Sabat et al. 1998). Rufous collared sparrows were captured in three localities separated by more than 1,800 kilometers. Copiapó (27°18´S, 70°25´W), is characterized by a light rainfall in some winters, with mean temperatures of 15.7°C and an total annual precipitation of 12 mm (di Castri and Hajek 1976, http://www.meteochile.cl). Quebrada de la Plata (33° 31´ S, 70° 50 ´W) henceforth Quebrada, have a warm temperate climate with rainy winters follow by a long dry period with an annual average temperature of 13.8° C and a total annual precipitation of 312.5 mm (di Castri and Hajek 1976, http://www.meteochile.cl/). Llanquihue (41°16´S, 73°00´W), the most southern studied region, has a wet temperate climate with rainfall present throughout almost all the year and with lower temperatures than the other sites. The mean annual temperature is about 10.1° C and the total annual precipitation of 3112 mm (Centro de Informacion de Recursos Naturales, Ciren). In order to represent the magnitude of food availability among populations, we calculated the primary productivity (Tieleman et al. 2003) via the aridity index (Q):

$$Q = \frac{pp}{(T_{\max} + T_{\min})(T_{\max} - T_{\min})} \cdot 1{,}000$$

Where pp is the average annual precipitation (mm) and $T_{\max}$ (°C) and $T_{\min}$ (°C) are the mean maximum and minimum temperature for the hottest and coldest month, respectively (Tieleman et al. 2003). The annual mean of this index is low in hot, dry deserts, and high in cool, wet areas. Our calculations demonstrated a north-south increase in primary productivity from Copiapó to Llanquihue (Table 1).The historical data for both precipitation and temperatures was obtained from national institutes of information (Dirección meteorológica de Chile and Centro de información de recursos naturales), from literature (Di Castri et al. 1976) and from web sites (http://www.worldclimate.com; http://www.atmosfera.cl).

Field methods

Non reproductive adult birds were captured with mist nets during the austral fall in 2008. We collected 16 individuals in Copiapó; 14 in Quebrada and 16 individuals in Llanquihue. Immediately after capture birds were weighted (to the nearest 0.05 g). A flight feather (primary 1) from the left wing and a blood sample from the brachial vein, which was collected into heparinized microhematocrit tubes (~ 100 µl) were obtained. Both samples were stored for a posterior isotopic analysis.

Laboratory methods

After completing field procedures, sparrows were transported to the laboratory in Santiago, Chile (33° 27'S, 70° 42' W). They were held in individual plastic-mesh cages (35 x 35 x 35 cm) and maintained at constant temperature room 22 ± 2 °C, and photoperiod 12L:12D. For a habituation period of one day sparrows were feeding with larvae of mealworms (*Tenebrio molitor*), seeds and water *ad libitum*. After that, BMR was determined in order to obtain a pre-acclimation baseline (for procedures see below basal metabolic rate). Once BMR measurements were accomplished, sparrows were weighed and randomly divided into two dietary treatments. One group was fed with an insect-based diet (ID; *Tenebrio molitor*) and the second with a seed-based diet (SD; canaryseed) with water (with vitamins added) provided *ad libitum*. We chose these diets, in order to resemble possible natural food types of the species diets (Afik et al. 1995; López-Calleja 1995). Thus, ID, represent high protein and low carbohydrate diet and SD, represent a high-carbohydrate and low protein diet (Table 2). Diets were provided *ad libitum* for 7 weeks, and then measurements of oxygen consumption (BMR) were performed.

Basal metabolic rate

The rates of oxygen consumption were carry out at 30°C, temperature which is within the thermoneutral zone for this species (Sabat et al. 2006), in post-absorptive resting birds during the inactive phase. The oxygen consumption was recorded in a computarized open-flow respirometry system (Sable Systems, Henderson, Nevada). First, birds were weighed using an electronic balance (± 0.05 g), placed in a dark metabolic chamber (1 L) perched on a wire-mesh grid that allowed excreta to fall into a tray containing mineral oil (to trap the water). After that, the chamber was positioned inside a controlled temperature cabinet (Ta = 30 ± 0.5°C). The open-flow respirometry system was calibrated with a known mix of oxygen (20%) and nitrogen (80%) that was certified by chromatography (INDURA, Chile). The measurement and calibration protocols we followed were after Williams et al. (2000). Because CO_2 was scrubbed before entering the O2 analyzer, oxygen consumption was calculated as (Withers 1977): $VO_2 = \left[FR \cdot 60(F_iO_2 - F_eO_2)\right]/(1 - F_iO_2)$, where *FR* is the flow rate in mL/min after STP correction, and F_i and F_e are the fractional concentrations of O_2 entering and leaving the metabolic chamber, respectively. We used a respiratory quotient (RQ) of 0.71, assuming that fasting sparrows rely mainly on stored lipids (King et al. 1961; Walsberg et al. 1995). At the end of each measurement cloacal body temperature (T_b) was recorded by Cole Palmer copper constantan thermocouple attached to a Digisense thermometer (Model 92800–15). Output from oxygen analyzers (%) was digitized using a Universal Interface II (Sable Systems) and recorded on a personal computer using data acquisition software

(EXPEDATA, Sable Systems). Our sampling interval was 5 s. Birds remained in the chamber for at least 4 hrs (Maldonado et al. 2009). Posterior to BMR measurements, birds were euthanized by CO_2 exposure

Digestive Enzymes

After birds were scarified by CO_2 exposure, they were dissected abdominally. We removed the small and large intestine, which were weighed inmediately in an electronic balance (± 0.0001 g). Small intestine (SI) contents were carefully removed and washed with a 0.9% NaCl solution, and immediately stored frozen (Martinez Del Rio et al. 1995; Sabat et al. 1998). After that, we extracted the heart, gizzard, liver and kidneys, which were inmediately weighed in an electronic balance (±0.0001 g).

For enzyme analysis, the small intestine tissue samples were thawed out and homogenized (30 s in an ULTRA TURRAX T25 homogenizer at maximum setting) in 20 volumes of 0.9 % NaCl solution. Disaccharidase activities were determined according to the method of Dahlqvist (1964), as modified by Martínez del Río (1990). Briefly, 100 µL of tissue homogenates were incubated at 40°C with 100 µL of 56 mmol L^{-1} of substrate (maltose or sucrose) in 0.1 M Maleate/NaOH buffer, pH 6.5. After incubation of 3 or 5 minutes for maltase and sucrase respectively, the reactions were ended by 3 ml of stop/develop Glucose-Trinder (Valtek, Chile). This solution was consisting of 500mL in 250 mL 0.1 mol L^{-1} TRIS/HCl, pH 7 plus 250 mL of 0.5 NaH_2PO_4, pH 7. After an incubation period of 18 min at 20°C, the absorbance was measured at 505 nm with a UV-

VIS spectrophotometer (Shimadzu UV mini-1240). Aminopeptidase-N assays were done using L-alanine-*p*-nitroanilide as a substrate. The assay beginning adding 1 mL of substrate solution (2.04 mmol L^{-1} of L-alanine-*p*-nitroanilide in 0.2 mol μl^{-1} NaH_2PO_4/Na_2HPO_4, pH 7) to 100µl tissue homogenate. The reaction was incubated during 10 min at 40°C and then arrested using 3 mL of ice-cold acetic acid (2 mol L^{-1}). The absorbance was measured at 384 nm. On the basis of absorbance, standardized intestinal enzymatic activities were calculated. The protein content of SI tissue was measured using bovine serum albumin as standard. Absorbance was read at 595 nm (Bradford 1976).

Glucose absorption rates

In order to estimate the rates of glucose absorption we studied the postprandial glucose levels during one hour after the administration of sugar solutions following (Malcarney et al. 1994). First, we obtained an initial blood sample (≥ 1.5 µL) puncturing a toe as a baseline (time 0) in fasted birds. Then animals were fed with either sucrose (0.1 mL administered in 0.87 M solutions) or a 1:1 mixture of glucose and fructose (0.1mL administered in 1.66 M solutions) with the aid of a micropipette (Gatica et al. 2006). After 10 minutes, we measured the glucose concentration of whole blood and so forth each 10 min during 1 hour. Because we were concerned that taking blood samples too frequently could stress the birds and added that previous essays reveled us that glucose peak is in general at the first 20 minutes, we collected samples at time 0', 10', 20', 30', 60'. In addition, to discard the effect of stress in the increase of blood glucose, we performed experiments providing water (0.1 mL) rather sucrose or glucose-fructose solutions.

In all cases, blood drop obtained was applied to the electrode (strip) of a glucose meter (Medisense Optium Xceed). The glucose of blood reacts with the chemicals on the electrode, producing a small electrical current that is measured by the glucose sensor of the device. The data obtained were expressed in mg/dL. The measurements were repeated twice for each sugar solution. Once the glucose curves were obtained, we register the time in which the peak of glucose concentration (T_{max}) was reached. We considered this measure as indicator of the rate of glucose absorption. All the described protocol has little modifications with respect to others previously utilized.

Isotope analysis

In order to investigated whether birds effectively switch diet seasonally, we relied on isotopic measurements ($\partial^{15}N$) of feathers and blood. Because, *Z. capensis* molt from late September to October, the isotopic composition of feathers corresponds to the resources assimilated for the few weeks during which the feather grew in the spring. The isotopic composition of blood corresponds to resources assimilated during the fall, due to diet nitrogen signatures incorporated in this tissue correspond to the diet consumed nearly 14 days before taking the samples (Hobson et al. 1992). Hence, the isotopic composition of these two tissues provides information about that animals were consuming and assimilated during those two contrasting seasons.

Before isotopic measurements, feathers samples were cleaned from external contaminants with a 2:1 methanol: chloroform solvent. All samples were dried until constant mass at 70 °C. After grounded into a fine powder, samples were loaded into tin capsules. Stable nitrogen isotopic compositions of feather and blood were determined by a conventional method using elemental analyzer/isotope ratio mass spectrometry (EA/IRMS, Finnigan Delta V Advantage interfaced in continuous flow with FlashEA 1112 HT, Thermo Finnigan, Bremen, Germany). Accuracy of the measurements was monitored by analysis of international standards (IAEA-N1 and N2). Precision was better than ±0.1‰. Nitrogen isotope ratios were expressed using standard delta notation (∂^{15}N) in parts per thousand (‰) as:

$$\partial^{15}\mathrm{N} = \left[(R_{\mathrm{sample}} / R_{\mathrm{standard}}) - 1\right] \cdot 1{,}000$$

where R_{Sample} and R_{standard} are the molar ratios of ^{15}N/^{14}N of the sample and reference, respectively.

Data Analysis

Prior to all statistical analyses, data were tested for normality and homogeneity of variance using Kolmogorov–Smirnov and Levene tests, respectively. To explore the association between variables we performed linear regressions analysis (LR).

Because the protein content in the homogenate was not statistically different among diets, data on enzyme activities was analyzed as total hydrolytic activity (µmol min^{-1}). Because, body mass (M_b) was different between diets

($P<0.05$), the variables correlated (organ masses, BMR and digestive enzymes) were corrected for M_b. Avian metabolic rate is a power function of mass (Lasiewski and Dawson, 1967), consequently we used $\log_{10}$ transformed mass and BMR to linearize these variables prior to comparative analysis. For the remainder variables, we tested if the allometry coefficient *b* of the model: $y = aM_b^{\,b}$ were significantly different from 1; t-test ($\alpha=0.05$). When *b* was significantly different from 1 we used $\log_{10}$ transformed data to perform the analysis. In the case that variables were correlated with M_b; we utilized the residuals of variables to analyze them removing the M_b effect. Because we included two factors: locality of origin and diet to evaluate the effect of the interaction between them, we performed a factorial ANOVA or factorial ANCOVA (with M_b as covariate when necessary). When the effect of interaction between locality and diet was no significant the F and P values were not reported.

In the case of glucose absorption we also used a Factorial ANOVA model, but we used diet treatment and solution (glucose or glucose + fructose) as factors, testing for significant differences separately by each locality. To evaluate putative differences in the isotopic signatures between feather and blood samples we performed a t-test for dependent samples for each locality. We considered significant differences at $P<0.05$. All statistical analyses were performed using STATISTICA 6.0.

Results

Body and organ masses

Diet acclimation significantly affected M_b of sparrows (Factorial ANOVA; $F_{1, 40}=20.71$, $P<0.001$). When analyzed separately (Fisher test) sparrows from Copiapó and Llanquihue acclimated to the ID tend to have higher M_b than those acclimated to SD. Birds from Quebrada showed similar means between groups (Table 3). However, we did not found significant effect of locality on body masses (Factorial ANOVA $F_{2, 40}= 0.37$, $P=0.69$). In respect to organ masses, there was a significant effect of interaction between locality and diet on gizzards masses (Factorial ANCOVA; $F_{2, 39}=5.47$, $P=0.008$); birds from Llanquihue showed different masses between acclimation diets (Table 3). The SI masses were affected by both locality (Factorial ANCOVA; $F_{2, 39}=6.45$, $P=0.004$) and diet (Factorial ANCOVA; $F_{1, 39}=6.4$, $P=0.02$; Table 3), but we did not find effect of interaction between diet and locality. Birds from Quebrada had smaller intestines than Llanquihue and Copiapó (Table 3). Besides birds acclimated to ID tended to have heavier small intestines than those acclimated to SD, but only birds from Quebrada showed statistically significant differences between diets (Table 3). Also, we found a strong effect of locality on SI length (ANCOVA; $F_{2, 39}=11.83$, $P< 0.001$); birds from Copiapó had shorter SI. Neither diet nor the interaction between locality and diet had an effect on SI length (Factorial ANCOVA; $F_{1, 39}=0.71$, $P=0.053$; Table 1). For all populations kidney mass were similar between diets (Factorial ANCOVA; $F_{1,39}$=3.46, P=0.07; Table 3), but a significant effect of locality was found (Factorial ANCOVA; $F_{2,39}$=8.06, P=0.001). Birds from Copiapó have smaller kidneys in comparison with the remainder localities (Table 3). Finally no significant effects of diet, locality or the interaction were found for liver, heart and large intestine masses (Table 3).

Basal metabolic rate

BMR was positively correlated with body mass (double log LR r^2=0.14; P=0.006). The analysis of the residuals obtained from this correlation, showed that there was an effect of the interaction between the locality and diet on BMR (Factorial ANOVA: $F_{2,40}$=3.93, P=0.03), but neither diet nor locality separately affected BMR significantly (Factorial ANOVA; diet: $F_{1,40}$=1.21, P=0.28; locality: $F_{2,40}$=0.39, P=0.68; Figure 1). The *a posteriori* analysis revealed that only, birds from Copiapó changed their BMR in response to diet; SD acclimated group showed a 15% higher BMR than ID group (Figure 1).

Digestive enzymes

We found a positive and significant relationship between total activities of the three enzyme activities and body mass when all data were pooled (double log LR; M: r^2=0.53, P<0.001; S: r^2=0.56, P<0.001; A: r^2=0.48, P<0.001; Figure 2). The analysis of the residuals obtained from these LRs indicated that maltase activity was not affected by dietary treatment (Factorial ANOVA; $F_{1,40}$=0.02; P=0.9; Figure 2). However a significant effect of locality on total maltase activity was found (Factorial ANOVA; $F_{2,40}$=4.14, P=0.02). Birds from Copiapó had higher total maltase activity than birds from Llanquihue. Moreover, there was a clear association between maltase and sucrase activities in each locality (Figure 3). This was an expectable result; maltase activity is the integration of the maltase-glucoamylase and sucrase-isomaltase effects (Biviano et al. 1993). Thus, an increase in sucrase-isomaltase expression leads to an increase in maltase activity (Sabat 1998). For this reason, we tested for effect of diet on maltase glucoamylase, using factorial ANCOVA with total sucrase activity as covariable. The results showed that maltase glucoamylase activity was not influence neither by both locality (Factorial ANCOVA; $F_{2,39}$=2.36, P=1.08) nor by dietary treatment (Factorial ANCOVA; $F_{1,39}$=2.37, P=0.13).

Besides, we found a significant effect on sucrase activity by both, locality (Factorial ANOVA; $F_{2,40}$=4.66, P=0.015) and by the interaction between locality and diet (Factorial ANOVA; $F_{2,40}$=5.17; P=0.01). The posteriori test revealed that sparrows from Copiapó had lower total activities of sucrase than birds from Llanquihue and Quebrada. Furthermore, sparrows from Llanquihue showed higher sucrase activity in birds acclimated to the SD (Figure 2). Aminopeptidase-N activity was affected by diet (Factorial ANOVA; $F_{1,40}$=16.72, P<0.001). Birds acclimated to ID showed higher values of aminopeptidase-N than SD acclimated birds (Figure 4). We found no effect of locality on Aminopeptidase-N (Factorial ANOVA; $F_{2,40}$=2.06, P=0.14), but a significant effect of the interaction between locality and diet was found (Factorial ANOVA; $F_{2,40}$=11.53, P<0.001). Birds from

Quebrada and Llanquihue increased the aminopeptidase-N activity in response to ID acclimation but it did not occur for birds from Copiapó (Figure 4).

Glucose Absorption

We found differences in the postprandial response of birds among localities. For birds from Copiapó we found an effect of diet on T_{max} (Factorial ANOVA; $F_{1,28}$=4.43, P=0.04), but there was not an effect of the sugar solution (Factorial ANOVA; $F_{1,28}$=0.81, P=0.37). Copiapó birds consuming ID showed a decrease in T_{max}, suggesting higher rates of glucose absorption (Figure 5). In the others two populations we did not found neither effect of diet (Factorial ANOVA; Quebrada: $F_{1,24}$=0.48, P=0.5; Llanquihue: $F_{1,28}$=2.03 P=0.17) nor of the sugar solution on T_{max} (Factorial ANOVA; Quebrada: $F_{1,24}$=0.85 P=0.36, Llanquihue: $F_{1,28}$=0.15 P=0.7; Figure 5).

Isotopes analyses

We found significant differences in isotopic signatures $\partial^{15}N$ between seasons to Quebrada (t_{19}= -2.60, P= 0.01), but not for Copiapó (t_{14}= 1.06, P= 0.3) and Llanquihue (t_{14}= -0.96, P= 0.35). Sparrows from Quebrada showed an increase in about 22% the values of $\partial^{15}N$ during the fall with respect to spring. The coefficient of variation between the $\partial^{15}N$ in spring and fall was in average 0.05 for Copiapó, 0.22 for Quebrada and 0.13 for Llanquihue. The coefficient of variation differs among populations (ANOVA; $F_{2,47}$ = 7; P= 0.02), being higher to Quebrada than the remainder two populations (Figure 6).

Table 1. Climatic data and diet variation in three populations of Rufous-collared sparrows. The mean annual temperature (calculated as average of the monthly temperatures) and total annual precipitations (calculated as the sum of monthly precipitations) are reported. Primary productivity was calculated as Q_{index} following Tieleman et al. 2003. Coefficient of variation (CV) of $\partial^{15}N$ from feathers and blood, which represent diet from spring and fall seasons, was calculated for each locality.

Localities	T° annual mean	PP annual mean	Q_{index} mean	$\partial^{15}N$ (c.v)
Copiapó	15.2	12	15.8	0.05
Quebrada	14.4	312.5	360	0.22
Llanquihue	9.4	3112	9.185	0.13

Table 2. Proximate values to experimental diets obtained from published data (Jones et al. 1972; Abdel-Aal et al. 1997; Barker et al. 1998) on composition of diet constituents. Data is expressed as the percentage of dry matter.

	Canary seed	**Mealworms**
Proteins (%)	18.7	48.75
Crude fat (%)	8.7	31.1
Carbohydrate (%)	62.7	1.35
Fiber (%)	5.19	14.5*
Ash (%)	2.1	4.3
Energy content (Kcal)	212	274

* Correspond to neutral detergent fiber (NDF), a measure of dietary fiber that includes chitin (Barker et al. 1998).

Table 3. Body mass and internal organs mass of *Z. capensis* from different localities acclimated to one of two contrasting diets (SD and ID). Different letters denote significant differences ($P<0.05$) between diets for each locality. The number of animals of each treatment is in parenthesis. Data are reported as mean ± SD.

	Copiapó		Quebrada		Llanquihue	
	SD (8)	ID (8)	SD (7)	ID (7)	SD (8)	ID (8)
Body mass (g)	**14.02 ± 1.34**^{a}	**18.37 ± 1.42**^{b}	15.09 ± 1.89^a	16.03 ± 3.09^a	**14.08 ± 1.04**^{a}	17.77 ± 3.57^b
Gizzard (g)	0.61 ± 0.16^a	0.57 ± 0.05^a	0.54 ± 0.10^a	0.51 ± 0.14^a	**0.44 ± 0.04**^{a}	**0.62 ± 0.18**^{b}
SI (g)	0.38 ± 0.06^a	0.50 ± 0.08^a	**0.29 ± 0.09**^{a}	**0.44 ± 0.03**^{b}	0.40 ± 0.07^a	0.53 ± 0.14^a
Kidney (g)	**0.17 ± 0.02**^{a}	**0.2 ± 0.03**^{b}	0.22 ± 0.06^a	0.22 ± 0.06^a	**0.19 ± 0.02**^{a}	**0.25 ± 0.03**^{b}
Liver (g)	0.56 ± 0.14^a	0.65 ± 0.04^a	0.6 ± 0.28^a	0.514 ± 0.12^a	0.5 ± 0.13^a	0.72 ± 0.29^a
Heart (g)	0.16 ± 0.03^a	0.22 ± 0.02^a	0.2 ± 0.03^a	0.24 ± 0.09^a	0.18 ± 0.04^a	0.24 ± 0.10^a
SI length (cm)	12.99 ± 0.79^a	11.95 ± 0.77^a	13.14 ± 1.31^a	13.87 ± 2.22^a	14.09 ± 1.15^a	15.16 ± 1.57^a

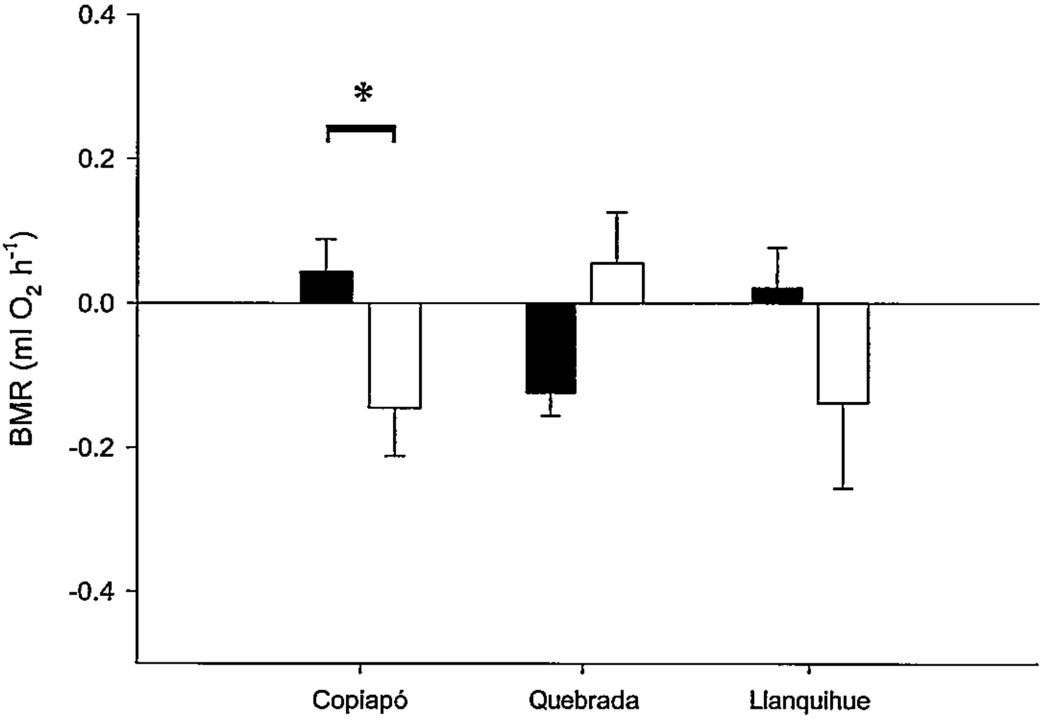

Figure 1. Residuals of BMR (mean ± SE) in sparrows after acclimation to SD and ID. Asterisk denotes statistically significant difference between diets after Fisher test (critical P= 0.05).

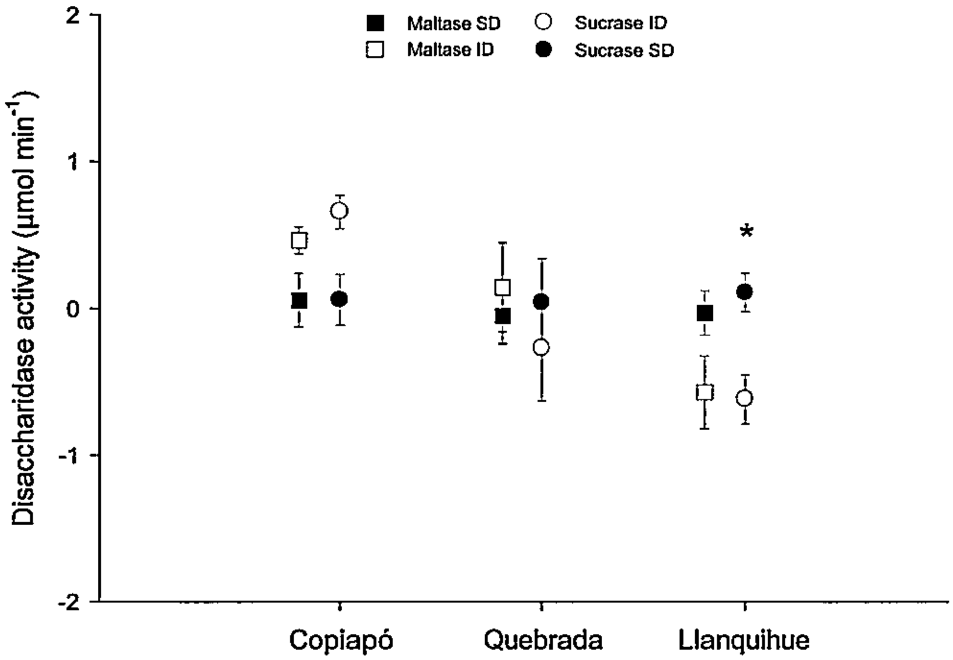

Figure 2. Residuals of total disaccharidases activities (UI) against body mass of *Zonotrichia capensis* fed on SD (black symbols) and ID (white symbols). Asterisk denotes significantly different means ($P > 0.05$) between diet acclimation after Fisher test. Data were plotted as mean ± SE.

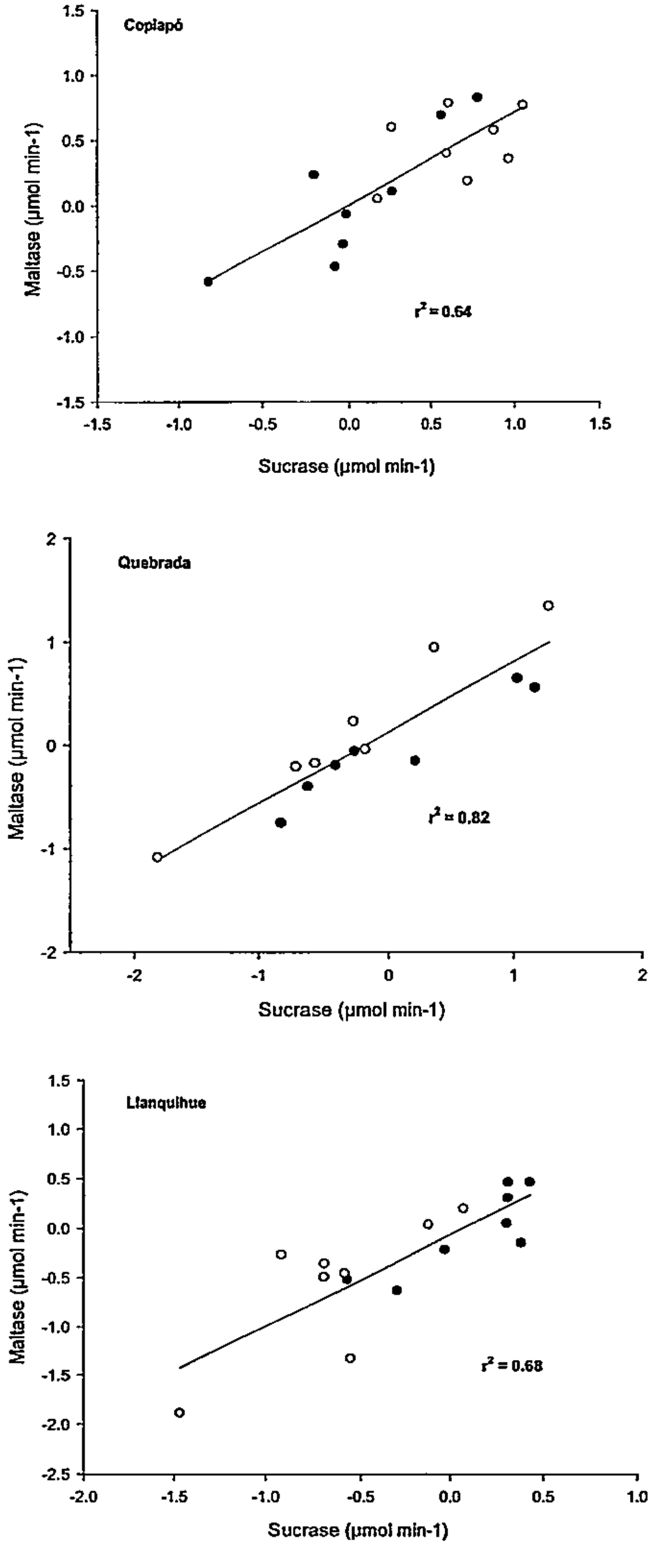

Figure 3. Relationship between intestinal sucrase and maltase activities in sparrows after correcting by body mass in birds fed on SD (closed circles) and ID (open circles) for each locality.

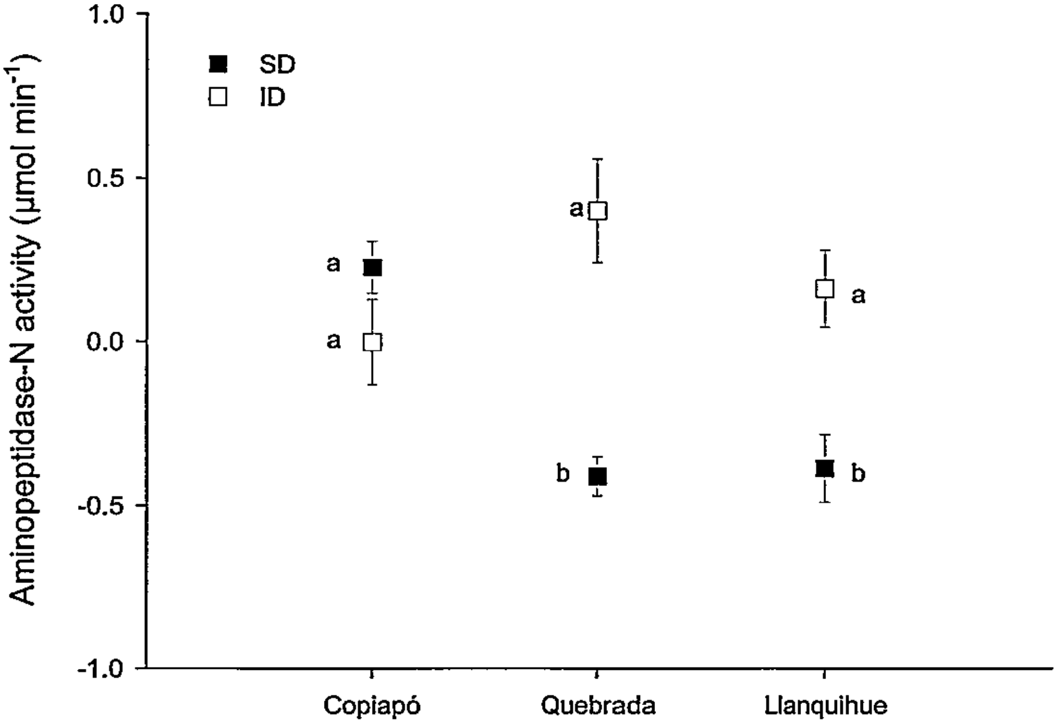

Figure 4. Residuals against body mass of aminopeptidase-N activities (UI) of small intestinal tissue in *Zonotrichia capensis* fed on SD (black symbols) and ID (white symbols). Common letter reflect means that are not significantly different (P > 0.05) between diet and for each locality after Fisher test. Data were plotted as mean ± SE.

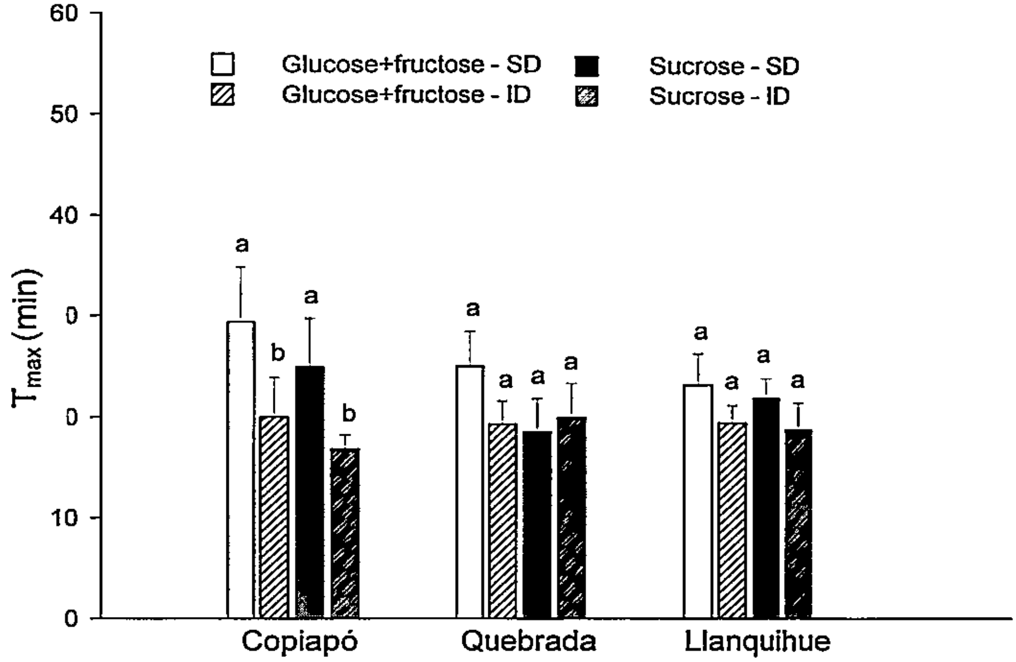

Figure 5. Time to reach the maximal value of plasma glucose (T_{max}) represented as mean ± SE, in *Z. capensis* after 7 weeks of acclimation to SD and ID. The measure was recorded after an ingestion of glucose+fructose (white bars) or sucrose (grey bars) solution. Common letters indicate no statistically significant difference between diets for each drinking solution after Fisher test (critical P= 0.05).

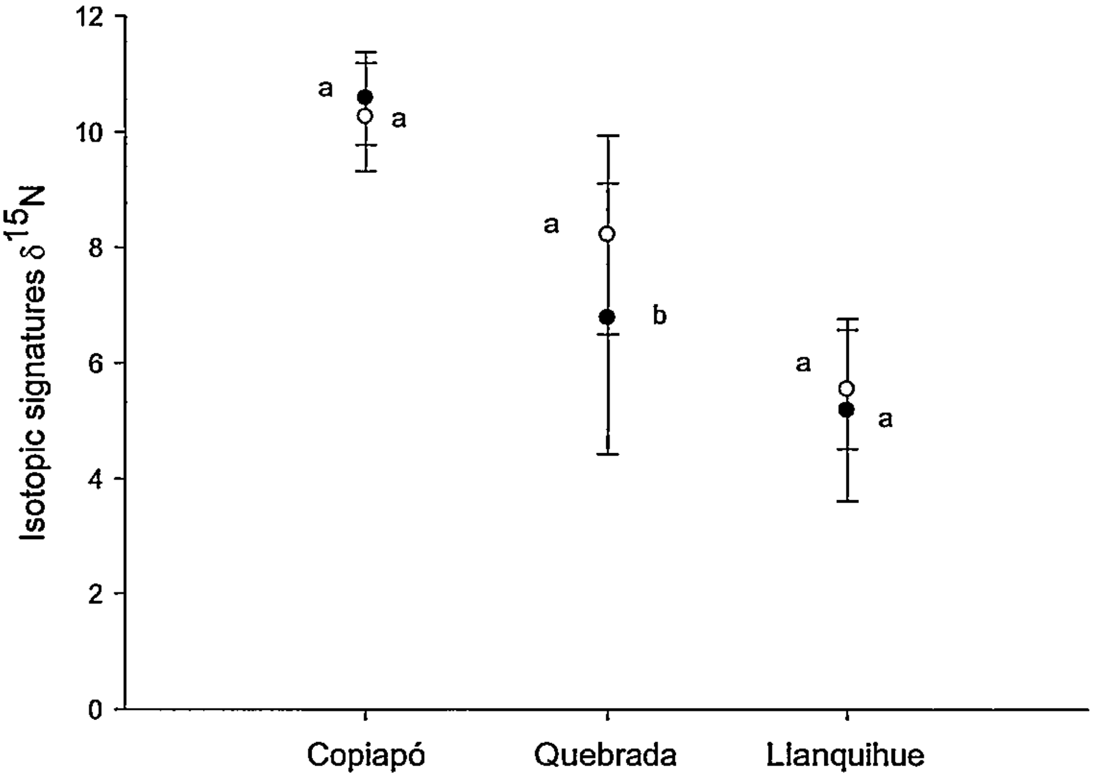

Figure 6. Seasonal diet variation represented by the isotopic signature from feathers (black symbols) and blood (white symbols) in *Zonotrichia capensis.* Common letter reflect means that are not significantly different ($P > 0.05$) between tissues. Data were plotted as mean ± SD.

Discussion

In this study we evaluated the putative tradeoff between the energetic and digestive features in a bird model. Also, we examined how dissimilar ecological characteristics affect the magnitude and shape of acclimation response to dietary chemistry. At the end of the acclimation period, differences in diet composition caused several morphological and physiological adjustments, including changes in gut size, BMR, digestive enzymes and in the rates of glucose uptake. Moreover, we found a strong effect of locality on almost all the variables we measured. In the following paragraphs we discuss the characteristics of these modifications.

Digestive morphology

Zonotrichia capensis maintained or even gained body weight after the dietary acclimation (Table 3). This fact suggested that birds could maintain a positive energy and nutritional balance, in spite of the significant differences in food composition (Table 2). The small intestine mass increased in average 51.7% in *Z. capensis* from Quebrada population when they were fed with the ID, although the length of SI remained unchanged for all populations. An increase in the size of digestive tract may be beneficial because it permits the maintenance of digestive and absorptive efficiency (Karasov et al. 1997; Battley et al. 2005). In fact, several experimental studies have reported that animals increase their SI mass or length in response to low-quality diets (e.g., high fiber content) and also in response to increased energy needs such as cold acclimations (Savory et al. 1976; Gross et al. 1985; Brugger 1991; Dykstra et al. 1992; Savory 1992; Karasov 1996; Geluso et al. 1999). Those modifications have been also reported for field-caught sparrows that experience significant changes in diet composition. Novoa et al. (1996) reported seasonal changes in both dietary habits and the digestive morphology in Z. *capensis* from central Chile. In winter, sparrows exhibit an increase in the amount of insects in their diets, which is coupled with an increase in the mass of digesta and in gut size (mass and length, Novoa et al. 1996). The authors hypothesized that this could be the result of the higher energy demands that animals experience in winter seasons. Besides, they suggested that diet switch from seeds to insects, under constant energy demand, would promote a decrease in gut size. Our results revealed that insect-based diet did not lead to a decrease in gut size; moreover, we found that ID promotes higher SI mass. Although, our experimental diets had similar energy content, the SD diet had near twice the dietary fiber than insect-based diet (Jones et al. 1972, Abdel-Aal et al. 1997, Barker et al. 1998). We suggest that despite higher fiber in ID, it was not enough to produce differences in intake rates (or digesta volume) to result in longer guts (e.g., Starck and Rahmaan, Savory 1992). For instance, Starck and Rahmaan (2003) reported an increase of 24% in SI length in Japanese quails acclimated during 4 weeks to high fiber diet (40% of fiber) than these acclimated to low fiber diet (3% of fiber). Contrarily, little differences in the amount of fiber between acclimation diets (and similar to this study) yield in small or any change in the SI length (see Brugger 1991, Afik et al. 1995, Geluso and Hayes 1998).

As well as the SI mass, the size of organs such as the liver, kidney, gizzard, pancreas, and heart have been correlated with dietary chemistry (Driedzic 1993; Karasov 1996; Sabat et al. 2004; McWilliams et al. 2005). Accordingly, we found significant effects of diet treatment on gizzard mass in birds from Llanquihue. In addition, birds from Copiapó and Llanquihue had heavier kidneys when acclimated to ID compared to SD. Our results agree with laboratory studies that have reported an increase of kidney mass in *Z. capensis* as a result of the ingestion of diet with high protein content (Sabat et al. 2004; Aldea et al. 2007). Both results are consistent with the idea that an increase of protein consumption and the need to eliminate nitrogen waste might increase the blood flow to the kidneys, which in turn cause the hypertrophy of medullary tissue (Goldstein et al. 2001). In summary, birds' organ masses did not respond in a uniform manner to SD and ID acclimation showing a large effect of locatity of origin in *Z. capensis.* Thus, among populations, the maintenance of a positive nutritional balance when birds face diets of contrasting biochemical composition does not seem to be explained by a single response.

Basal metabolic rate

The food habit hypothesis predicted that animals facing nutritional bottlenecks or low-quality foods should decrease BMR in order to compensate the lower energy intake (McNab 1986; McNab 1988; Cruz-Neto et al. 2004). BMR reduction, on the one hand results in a lower daily energy requirements and also may enhance digestive efficiency by maintaining food for a longer period in the digestive tract, hence extracting more nutrients from each unit of food ingested (Veloso et al. 1993). In this study, diet acclimation had an effect on BMR, but this effect was only significant for birds from Copiapó. The BMR recorded after the acclimating period (observed BMR) was compared with BMR values expected for the allometric equation described from Tieleman and Williams (2000) obtained from several birds species from mesic and arid environments. After 7 weeks of acclimation, birds fed with the ID diet decreased its BMR compared with birds fed with the SD; the percentage of mass expected for BMR was 102.45% and 96.91% for SD and ID, respectively. Moreover, BMR of SD acclimated birds did not show any difference between the pre and post acclimation period. Lower BMR values found in ID acclimated birds supports the food habits hypothesis. As it was already stated, the FHH predicts a decrease in the energy expenditure when the energetic return from food is diminished. In this sense, digestibility of seeds by passerines is about 75%, and in *Z. capensis* the value is nearly 80% (Novoa et al., 1996). On the other hand, insect digestibility in birds ranges from 50 to 80% (Karasov, 1990; Weiser et al., 1997). In addition, the exoskeleton of insects has variable amounts of chitin, whose digestibility in passerines is ca. 10% (Weiser et al., 1997). This fact might determine large differences in prey digestibility (See Novoa 1996). Hence, it is possible that subtle differences in digestibility between seeds and larvae, but not in the net energy (Table 2), may explain the observed differences in BMR. Besides, ID had lower fiber and carbohydrate content than the SD diet, which could make it harder and take more time to digest and absorb (Martinez del Rio et al. 1990). Nevertheless, only birds from Copiapó exhibited BMR changes after dietary acclimation, which is a perplexing result. Recently, Cavieres and Sabat (2008) reported a positive relationship between environmental heterogeneity (seasonality) and BMR flexibility in *Z. capensis.* Birds acclimated to contrasting temperatures differed in the magnitude of the plastic response. They found that sparrows inhabiting in Copiapó had an inflexible reaction norm of BMR, opposed to birds from Quebrada, which showed higher BMR changes. Here, we found the contrary trend for dietary acclimation, which would be expectable, in analog way, if birds experienced population differences in the availability of different food types in a temporary basis. However, we did not find any evidence of seasonal changes in the dietary protein consumption that would make this response expectable. In fact, the variation coefficient in ^{15}N was minor from the rest of bird populations. Copiapó is characterized by its higher aridity and remarkably low primary productivity (Table 1). Therefore, it is likely that *Z. capensis* from this locality confronts a shortage in the availability of resources all year round. Moreover, the rainfall is very low (12mm) and in some winters there are no precipitations. Thus, it is expected that low food availability in these years will result in a stressful condition that favors the BMR reduction. We suggest that the decrease in BMR is an ability of birds to face life in habitats that exhibits nutritional bottlenecks. Since, BMR is often correlated with daily energy expenditure (Daan et al. 1990), lower BMR also means lower energetic requirements (i.e., food) to survive.

Digestive enzymes and glucose absorption

It is well known that birds can maintain the nutrient extraction efficiency by adjustments in the rates of both hydrolysis and nutrients uptakes (reviewed in Karasov 1996; Karasov et al. 1997). To date, the adaptive modulation hypothesis has not been supported by the results obtained in passerine bird (Afik et al. 1995; Caviedes-Vidal et al. 2000; Sabat et al. 2008). Here we found that *Z. capensis* from Llanquihue increased the intestinal sucrase activity after acclimating to SD. It has been proposed that passerine birds are unable to show specific disaccharidases modulation, because birds rely less on active transport for absorption of glucose and more on passive absorption of glucose (Karasov et al. 1994; McWilliams et al. 2001). Nevertheless, Brzek (2009) recently found that specific maltase activity increased significantly during development in nestlings of House sparrows (*Passer domesticus*) fed with starch diet, compared to birds fed with diet lacking starch. Moreover, this difference in the magnitude of maltase activity was at least twice as high at time of fledging (Brzek et al. 2009). Due to house sparrows have not shown a positive correlation between dietary carbohydrate and maltase in adulthood (Caviedes-Vidal et al. 2000), the authors suggested the possibility of developmental plasticity for this biochemical trait. In other words, they suggested that phenotypic flexibility of enzyme expression may be lost later in life. To our knowledge, this is the first documentation of modulation of disaccharidases on high carbohydrate diet in adulthood passerine birds. A previous study in *Z. capensis* revealed that birds from Llanquihue consume a higher proportion of seeds (had lower trophic level) than the other two populations (*Chapter* 1). We suggest that these differences of feeding habits may be correlated with the ability of those birds to modulate the activity of sucrase. Nevertheless, since we obtained only the variability of protein ingestion through $\partial^{15}N$, we do not have information about the carbohydrate changes they may experience.

Aminopeptidase-N increased in response to ID in birds from Quebrada and Llanquihue, but not from Copiapó. These results agree with the variability of nitrogen incorporation of the birds' diet (Figure 6). The change in the activity of aminopeptidase-N in *Z. capensis* from Quebrada was greater in magnitude than the response in birds dwelling in Llanquihue. The coefficient of variation of $\partial^{15}N$ among populations was greater for Quebrada population and despite this coefficient was statistically similar between sparrows from Llanquihue and Copiapó, the hypothesis that they differ was in the limit of significance ($P= 0.08$). Schlichting and Pigliucci (1998) proposed that phenotypic plasticity should be restricted to specialist individuals and that it should have an adaptive value in organisms that experience temporary variations in their biotic and physical environment. The observed pattern in this study supports this prediction; we found that the magnitude aminopeptidase-N flexibility is related to the temporal variation in the protein consumption, as revealed by isotopic analysis.

In this study, it is shown that *Z. capensis* fulfill with FHH and AMH, but the way in which energetic and digestive adjustments are accomplished differ among populations. The significant effect of climate on the phenotypic characteristics, lead us to believe that the evolutionary forces that modulate the BMR and the digestive characteristics are not necessarily antagonistic. Birds from Copiapó exhibited lower BMR after acclimating to a rich insect diet, and ID acclimation in these birds result in an increase in the rate of glucose uptake. In addition, *Z. capensis* from Quebrada showed a great increase in the SI length with any change in BMR between diets. These facts suggest that changes in digestive features not necessary result in an increase of energetic demands. On the contrary, these adjustments seem to operate together in order to confront a decrease in diet quality. On the other hand, the BMR flexibility appears to be very sensitive to the type of environmental cues. Birds from Copiapó modify their BMR in response to diet changes, whereas it remains unchanged when birds are acclimated to contrasting temperatures (and vice versa to Quebrada sparrows) (Cavieres & Sabat 2008). In addition, the flexibility of aminopeptidase-N appears to be related to the magnitude of the changes in protein consumption experienced by birds under natural conditions. Together, these results highlighted the importance of considering the circumstances in which phenotypic plasticity evolves (see de Jong 1995; Pigliucci 1996; Ernande et al. 2004; Pigliucci 2005) and the specific environmental cues that induce their expression. Finally, by means of understanding the correlation between environmental signals and the associated phenotypic response, may serve to explain general ideas about the evolution of adaptive plasticity in present and future environments.

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Chapter 3

Individual specialization within populations is related to environmental heterogeneity in a species of passerine bird (*Zonotrichia capensis*)

Chapter 3. Individual specialization within populations is related to environmental heterogeneity in a species of passerine bird (*Zonotrichia capensis*)

Introduction

Individual variation within populations plays an essential role in the evolution through natural selection (Grant et al. 1976; Endler 1986; Mousseau 2000). However, only in the last decades its magnitude, causes and effects have again taken up attention following the renewed interest in adaptative radiation and ecological speciation (Schluter 2000; Bolnick et al. 2003). There are several studies that have been focused in the intrapopulation variation in the use of trophic niche (Roughgarden 1974; Chabot 1986; Durell 2000; Araujo et al. 2008; Araujo et al. 2009). In general, the differential use of resources among individuals may result from differences in age classes, sex, morphs and individual specialization (Bolnick et al. 2003). Roughgarden (1972) provided a quantitative framework to determine the level of intrapopulation niche variation. He considered the total niche width (TNW) of a population equal to the sum of two components: the *within-phenotype (individual) component* (WIC), which reflects the variability of resources utilized by the average individual, and the *between phenotype (individual) component* (BIC), which reflects differences between individuals in their resource utilization (Roughgarden 1972; Roughgarden 1974; Bolnick et al. 2002). Accordingly, many apparently generalist species may consist of phenotypes that use a small subset of the population's niche, where TNW is made up mostly of the BIC. In spite of important dietary differences among individuals showed to be due to sex and age class differences, recently it has been shown that individual specialization also may contribute significantly to niche differentiation (reviewed by Bolnick et al. 2003). Individuals within a population using a subset of the whole population trophic niche attributable to individual features and not to sex, age or morph differences are considered as "specialist individuals" (Bolnick et al. 2003). This individual source of variation developed inside the populations, generally are attributed to adaptive genetic differences (Grant et al. 1976; Wilson 1998; Bell 2007). However, there isscarce evidence about the existence of adaptive genetic variation inside the populations and how it can influence the phenotypic variation in ecological traits (Jaenike and Holt 1991; Leibold et al. 1994; Tessier and Leibold 1997). The mechanism of maintenance of individual specialization seems to be related to the existence of constraints on an individual's ability to efficiently exploit a wide variety of resources. Such constraints, generally arise from functional trade-offs (morphological, physiological or behavioral) in which consumers efficiently exploiting one kind of resource are less efficient when consuming an alternative resource (Ferry-Graham et al. 2002; Bolnick et al. 2003; Svanbäck and Eklöv 2003; Muchhala 2007). In the case of physiological trade-offs, this can occur after prey is ingested, because alternative prey may require different digestive conditions, enzymes, or detoxification mechanisms (Afik and Karasov 1995; Bolnick et al. 2003). For instance, it has been observed that changes in birds' diet involve immediately costs in terms of a reduced digestive performance and subsequently in gut structure and functional changes, although after an acclimation time animals may be able to feed normally (Hilton et al. 2000). Consequently, it appears that the increase in fitness resulting from acclimation to a particular environment is only possible at the cost of reducing fitness in other environments (Gomulkiewicz and Kirkpatrick 1992; Via et al. 1995). These costs could be maximized when the norm of reaction of a particular trait is inflexible (Van Tienderen 1997; Sabat and Bozinovic 2000). Hence, in ecological terms, the ability to change dietary habits may depend on the flexibility of traits involved in these switches.

Although functional trade-offs have necessarily an effect on the individual niche breadth that cannot be neglected, it has not been considered that traits underlying these trade-offs are subject of evolution, that differ among environment and hence influence the evolution of niche breath. In evolutionary terms, it is expectable that species or populations that inhabit heterogeneous environments should exhibit a more flexible reaction norm (Schlichting and Pigliucci 1995; Kingsolver and Huey 1998; Pigliucci 2005; Cavieres and Sabat 2008). Commonly studies referring to individual specialization consider the environmental heterogeneity as a prior assumption, which provide individuals different alternatives to yield the niche subdivision (Van Valen 1965; Roughgarden 1974; Grant et al. 1976; Ackermann et al. 2004). Nevertheless, the role of heterogeneity as a selective pressure that promote more flexible reaction norms has not been considered in studies of intrapopulation variation. Instead, these studies are mainly focused on the effect of intraspecific and interspecific competition on the levels of niche subdivision (Valen 1965; Bolnick 2001; Svanbäck and Bolnick 2005; Svanbäck and Bolnick 2007; Araujo et al. 2008). The aim of this study is to analyze how environmental heterogeneity could directly influence the degree of individual specialization within population, focusing at the organism level.

We evaluated quantitatively the correspondence between the environmental temporal heterogeneity and the level of individual specialization in the utilization of the trophic resource within populations. We calculated the level of individual specialization using Roughgarden's (1979) measure of individual specialization, i.e., the WIC/TNW ratio. To do that we studied three populations of a passerine bird, *Zonotrichia capensis*, along a latitudinal gradient in Chile that differ noticeably in their biotic and abiotic conditions and also in the strength of its temporal heterogeneity (i.e., seasonality). To evaluate the trophic niche among individuals, we used the nitrogen stable isotope ratios as indicators of the food web position (Post 2002) and as an indirect index of the protein consumption (Schondube et al. 2001). In addition, we used data of digestives features (enzymatic activity and the mass of small intestine) in the field and after acclimation to contrasting diets (see Chapter 2) to evaluate: 1) the correspondence between diet ($\partial^{15}N$) and digestive features. Such correlations in freshly caught birds would indicate the presence of digestive trade-offs in specialist individuals; and 2) the correspondence among environmental heterogeneity and phenotypic flexibility. We hypothesized that heterogeneity should be related with levels of flexibility in the digestive features and hence related with the level of individual specialization within populations.

Methods

Animals and study site

Rufous-collared sparrows are distributed between southeast Mexico and Cabo de Hornos in the southern Chile (Goodall et al. 1951). In Chile, its distribution is almost ubiquitous and includes both deserts and rain forests (Araya and Millie 2005). Although we have no information about the mobility of the studied populations, the race we studied here, *Z. capensis chilensis*, has been reported as non migratory (Chapman 1940). The only recognized migratory race is *Z. c. australis*, which is distributed southern than our range of study. This omnivorous species is described by feeding on mixed diets comprised of seeds and insects (López-Calleja 1995; Novoa et al. 1996; Sabat et al. 1998).

The three study areas differ greatly with respect to their climatic conditions; Copiapó (27°18´S, 70°25´W), the northernmost site is within the Atacama Desert and it is characterized by a rigorous aridity, with mean temperatures of 15.2° C and an total annual precipitation of 12 mm. Quebrada de la Plata (33° 31´ S, 70° 50 ´W) has a Mediterranean climate with seasons quite variables in temperature and rainfalls (i.e., hot and dry summers, and cold and rainy winters). The annual average temperature is about 14.4° C and the total annual precipitation is 312.5 mm (www.meteochile.cl ; Di Castri and Hajek 1976). Llanquihue (41°16´S, 73°00´W), the most southern studied region, is characterized by a wet temperate climate with a mean annual temperature about 9.4° C and a total annual precipitation of 3112 mm (Centro de Informacion de Recursos Naturalales, Ciren). The primary productivity, calculated via the annual aridity index (*Q*) (Tieleman et al. 2003) demonstrated a north-south increase in primary productivity from Copiapó to Llanquihue (Table 1).

Individual and population niche breath

Several studies have shown that individual diet inferred from stable isotope data are consistent with those observed from gut content, evidencing that isotopic variation reflects long term foraging strategies (Gu et al. 1997; Sabat and Martinez del Rio 2002; Sabat et al. 2006). In this sense, the use of isotopic signatures has a temporal advantage often unattainable by a single snapshot analysis of gut contents (Araujo et al. 2007). Nevertheless, most of studies employed the isotopic variance measured from a set of single samples of individuals as an indirect measure of the level of diet specialization within a population (Gu et al. 1997; Darimont and Reimchen 2002; Araujo and Gonzaga 2007). Here, we used different animal tissues because, they are synthesized and replaced at different rates they reflect the diet of animals at the time of tissue synthesis and hence represent the temporal variability of the individual' diet in a better way (Hobson and Clark 1992b; Hobson and Clark 1992a; Bearhop et al. 2002; Cherel et al. 2005; Newsome et al. 2007). In this way, we reconstructed the individual niche breadth in a more reliable way, obtaining individual temporal consistency in resource use, which is necessary to infer individual specialization.

Immediately after capture, we obtained two flight feathers (*P1* and *P9*) from the left wing. Because feathers (after growth) are an inert structure, the isotopic signature indicates the diet at time of molt (Bearhop et al. 2004). Since its growth is sequential (Campbell and Lack 1985) we removed *P1* and *P9 to* be ensured that their development (and the signatures) occurs at different times. It has been reported that renewal of the primary feathers requires about 60 days in several subequatorial populations of rufous-collared sparrows (Davis 1971; King 1972), so we used the isotopic signature of feathers as an indicator of short term diet variation. Also, we obtained a blood sample from the brachial vein, which was collected into heparinized microhematocrit tubes (~ 100 µl). The blood isotopic composition of blood corresponds to resources assimilated nearly 14 days before taking the samples (Hobson and Clark 1992a). Because, the molt occurs from September to October (spring) and birds were captured during the fall (2008), the isotopic composition of feathers and blood correspond to the diet assimilated during contrasting seasons. Therefore, comparisons between feathers and blood corresponds to long-term variation in the individual' diet. All samples were stored and dried (70ºC) for posterior isotopic analysis. Our data was supplemented with data from *Chapter* 2 obtained for the same individuals.

Before isotopic measurements, feathers samples were cleaned from external contaminants with a 2:1 methanol: chloroform solvent. All samples were dried until constant mass at 70 °C. After grounded into a fine powder, samples were loaded into tin capsules. Stable nitrogen isotopic compositions of feathers and blood were determined by a conventional method using elemental analyzer/isotope ratio mass spectrometry (EA/IRMS, Finnigan Delta V Advantage interfaced in continuous flow with FlashEA 1112 HT, Thermo Finnigan, Bremen, Germany). Accuracy of the measurements was monitored by analysis of international standards (IAEA-N1 and N2). Precision was better than ±0.1‰. Nitrogen isotope ratios were expressed using standard delta notation ($\partial^{15}\mathrm{N}$) in parts per thousand (‰) as:

$$\partial^{15}\mathrm{N} = \left[(R_{\mathrm{sample}} / R_{\mathrm{standard}}) - 1 \right] \cdot 1{,}000$$

where R_{Sample} and R_{standard} are the molar ratios of $^{15}\mathrm{N}/^{14}\mathrm{N}$ of the sample and reference, respectively.

Digestive features

It is assumed that an individual maintains specialized in return of individual performance benefits (Ferry-Graham et al. 2002). The increase in capacity to absorb nutrients may be related with digestive adjustments, such as the amount of digestive enzymes and gut size (Karasov and Hume 1997). In passerine birds it has been reported that aminopeptidase-N activity and the small intestine is modulated in response to the dietary protein (Sabat et al. 1998 and *Chapter* 2). Based on this, we evaluated the correspondence among natural diets ($\partial^{15}\mathrm{N}$) and these digestive features in freshly caught birds in order to test whether birds exhibit phenotypic adjustments with respect to individual' diets that justify the individual specialization. We obtained data previously reported in *Chapter* 1 for aminopeptidase-N activity and small intestine mass. Sabat (2000) used the ratio of aminopeptidase-N and maltase as a predictor of the different proportions of protein and carbohydrates in diet of some bird species. Because, it has been reported a strong correlation between aminopeptidase-N and maltase and sucrase (Sabat et al. 1998, *Chapter* 1 and 2), we also calculated this ratio and performed a regression analysis against the nitrogen isotopic signature (∂^{15}N) of tissues which is an indicator of the amount of dietary protein assimilated by Z. capensis (Sabat et al 2009).

Data analysis

Individual specialization

To estimate the individual specialization in trophic niche, we used Roughgarden's (1974) measure of individual specialization for continuous data, the ratio WIC/TNW. This measure exclude the requirement of the knowledge of frequency distribution of resources in the environment, a process that is difficult and may be riddled with full of assumptions (Bolnick et al. 2002). The WIC/TNW index of individual specialization uses the population's total diet to define resource availability, so individual diets are compared with those from the population instead with the environment. The total variance of resources corresponds to the total niche width of the population (TNW) and can be divided into the two aforementioned components. Thus, TNW = WIC +BIC. The higher the values of WIC relative to TNW, the less variable individuals are, and vice versa. Therefore, WIC/ TNW varies from 0 (maximum individual specialization) to 1 (no individual specialization). For details of index calculation, readers are referred to Bolnick et al. (2002) and Roughgarden (1974).

To determine WIC, BIC, TNW and WIC/TNW we used IndSpec1, a program developed by Bolnick et al. (2002) to calculate these measurements. After obtaining the index, a non-parametric Monte Carlo procedure was performed to test the null hypothesis that any observed diet variation arose from individuals sampling stochastically from a shared distribution. Each individual was randomly reassigned a new diet via multinomial sampling from the observed population resource distribution, and WIC/TNW was recalculated for the resulting population diet variation. IndSpec1 generated 10,000 such populations, and the null hypothesis was rejected if the observed value of WIC/TNW was less than 5% of the null WIC/TNW values. In order to evaluate significant differences in the level of individual specialization among localities, we calculated the individual variance with respect to the population variance, i.e., the quotient between $\partial^{15}N$ variance obtained from feathers and blood, and TNW. Then, these values were log 10 transformed to satisfy the assumption of normality to perform an analysis of variance (ANOVA) and *a posteriori* Fisher test. To test the effect of the locality on TNW we performed a Snedecor test (F test). In order to avoid multiplicity errors all P values were adjusted for multiple comparisons according to Bonferroni; P value of less than 0.02 was considered statistically significant.

To explore the association between variables we performed linear regressions analysis (LRA). Before that, we tested if the allometry coefficient *b* of the model: $y = aM_b^{\,b}$ were significantly different from 1; t-test (α=0.05). When *b* was significantly different from 1 we used $\log_{10}$ transformed data to perform the analysis. For variables correlated with M_b; we utilized their residuals against M_b to take account the Mb effect.

Results

We found a significant effect of the origin locality of birds on niche breath. Birds from Quebrada had a broader niche breadth than the remainder two populations (F-test, P< 0.001; Table 2, Figure 1). TNW for birds from Quebrada was about 4.17 and 2.79 times higher than Copiapó and Llanquihue TNW, respectively (Table 2, Figure 1). Sparrow population from Llanquihue exhibited the higher level of individual specialization (ANOVA, $F_{2,47}$= 4.01, P= 0.02), being birds from Copiapó and Quebrada population 94% and 86% less specialist than those from the Llanquihue population (Table 1, Figure 2). The level of individual specialization between Copiapó and Quebrada did not differ statistically (Table 1). The null hypothesis that any observed diet variation arose from individuals sampling stochastically from a shared distribution was rejected for birds from Quebrada, where 3.62% of the bootstrapped populations had a WIC/TNW less than the observed WIC/TNW and Llanquihue (0.02% had a WIC/TNW less than the observed). However, the null hypothesis could not be rejected for birds from Copiapó (6.18% had a WIC/TNW less than the observed).

We found for all populations a positive and significant relationship between the *P1 and P9* ∂^{15}N (Figure 3) ($r^2 = 0.49$, $P = 0.001$; $r^2 = 0.22$, $P = 0.03$; $r^2 = 0.89$, $P < 0.001$) for Copiapó, Quebrada and Llanquihue, respectively (Figure 3). However, there a non significant relationship between the average of ∂^{15}N of both feathers and blood were found ($r^2 = 0.05$, $P = 0.68$; $r^2 = 0.02$, $P = 0.07$; $r^2 = 0.62$, $P = 0.14$) for Copiapó, Quebrada and Llanquihue, respectively.

The association between ratio A/M and nitrogen isotopic signature (∂^{15}N) was different among populations. Only birds from Llanquihue showed a positive and significant association between A/M and ∂^{15}N from feathers (LRA; $r^2 = 0.62$; $P = 0.01$); however a non significant correlation was found with the blood ∂^{15}N (Figure 4). The small intestine mass was not correlated neither with ∂^{15}N from feathers (LRA; Copiapó, P=0.93; Quebrada, P= 0.65, Llanquihue, P=0.67) nor with ∂^{15}N from blood (LRA; Copiapó, P=0.97; Quebrada, P= 0.91, Llanquihue, P= 0.068).

Table 1. Climatic data for the three populations of rufous-collared sparrows. The mean annual temperature (calculated as average of the monthly temperatures) and total annual precipitations (calculated as the sum of monthly precipitations) are reported. Primary productivity was estimated via Q_{index} (Tieleman et al. 2003). Q_{index} tends to be low in hot, dry months and in more mesic sites. The seasonal coefficient of variation (c.v) of $\partial^{15}N$ was calculated by the birds' isotopic signatures from feathers and blood (which represent diets from different seasons).

	T° annual (mean)	PP annual (mean)	Q_{index} (mean)	$\partial^{15}N$ (c.v)
Copiapó	15.2	12	15.8	0.05
Quebrada	14.4	312.5	360	0.22
Llanquihue	9.4	3112	9.185	0.13

Table 2. Measures of the components of total niche width (TNW) and the index of the level of individual specialization within population (WIC/TNW) for *Zonotrichia capensis* from three different localities. Asterisks denote a statistically different population after *a posteriori* Fisher test (critical P>0.005).

	WIC	BIC	TNW	WIC/TNW
Copiapó	0.618	0.538	1.156	0.535
Quebrada	2.48	2.351	**4.831***	0.513
Llanquihue	0.477	1.251	1.728	0.276*

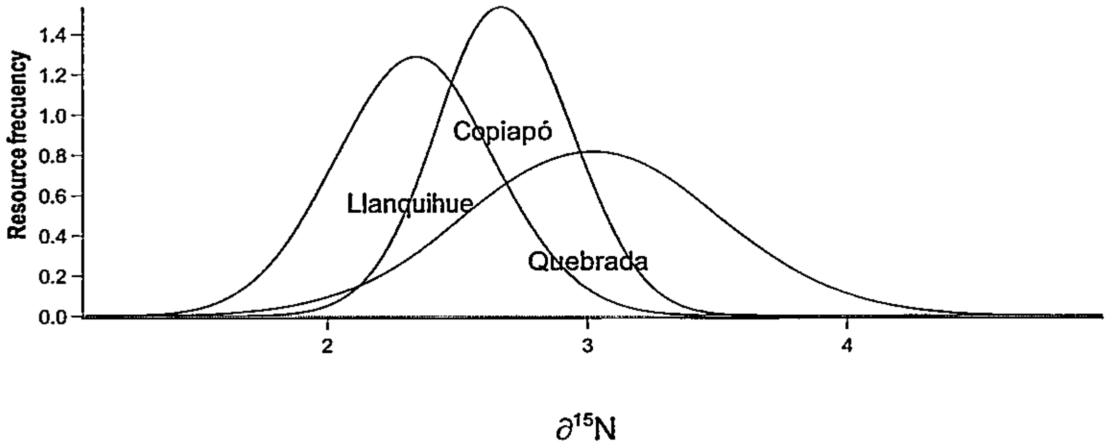


Figure 1. The curves correspond to normal function N (μ_t, σ_t), where μ_t, σ_t are the mean and standard deviations of $\partial^{15}N$ of all individuals of the population. Therefore each curve represents the total niche width of a population (TNW). In order to convert $\partial^{15}N$ in values of trophic level, $\partial^{15}N$ were corrected by the isotopic signatures of primary producers i.e., the average of $\partial^{15}N$ from plants obtained in the locality in which the bird was captured and divided for 3.4, the enrichment of $\partial^{15}N$ per trophic level (~3.4‰). For more details see *Chapter* 1.

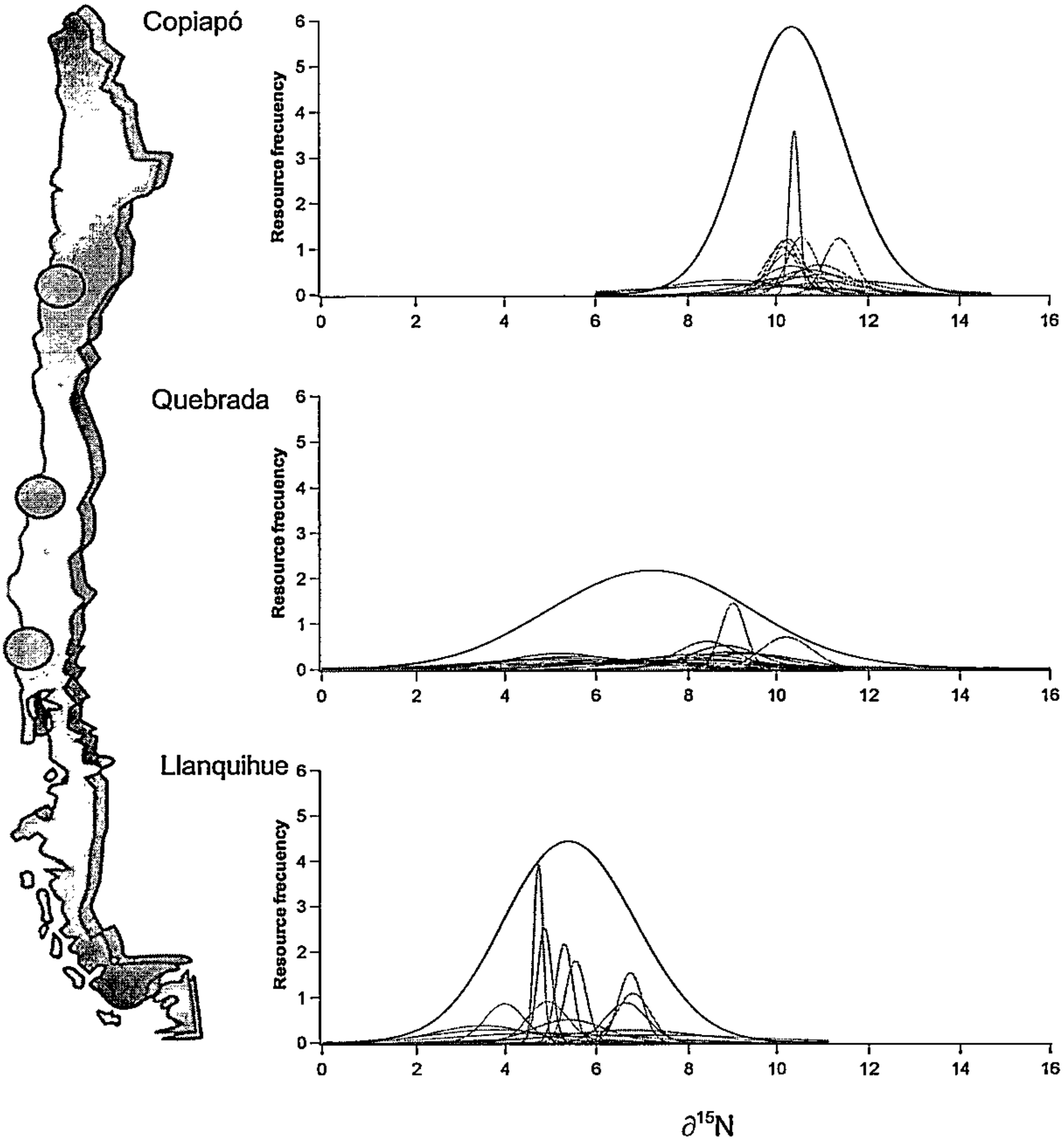


Figure 2. Brighter curves correspond to normal functions N (μ_i, σ_i) with unitary area, where μ_i and σ_i are the mean and standard deviations of ∂^{15}N from different tissues from a same bird, namely their individual trophic niche. Darker curves correspond to normal function N (μ_t, σ_t), where μ_t, σ_t are the mean and standard deviations of ∂^{15}N of all individuals of the population, namely TNW. For visuals convenience curves where equally scaled for the three populations, with an area equals to 16.

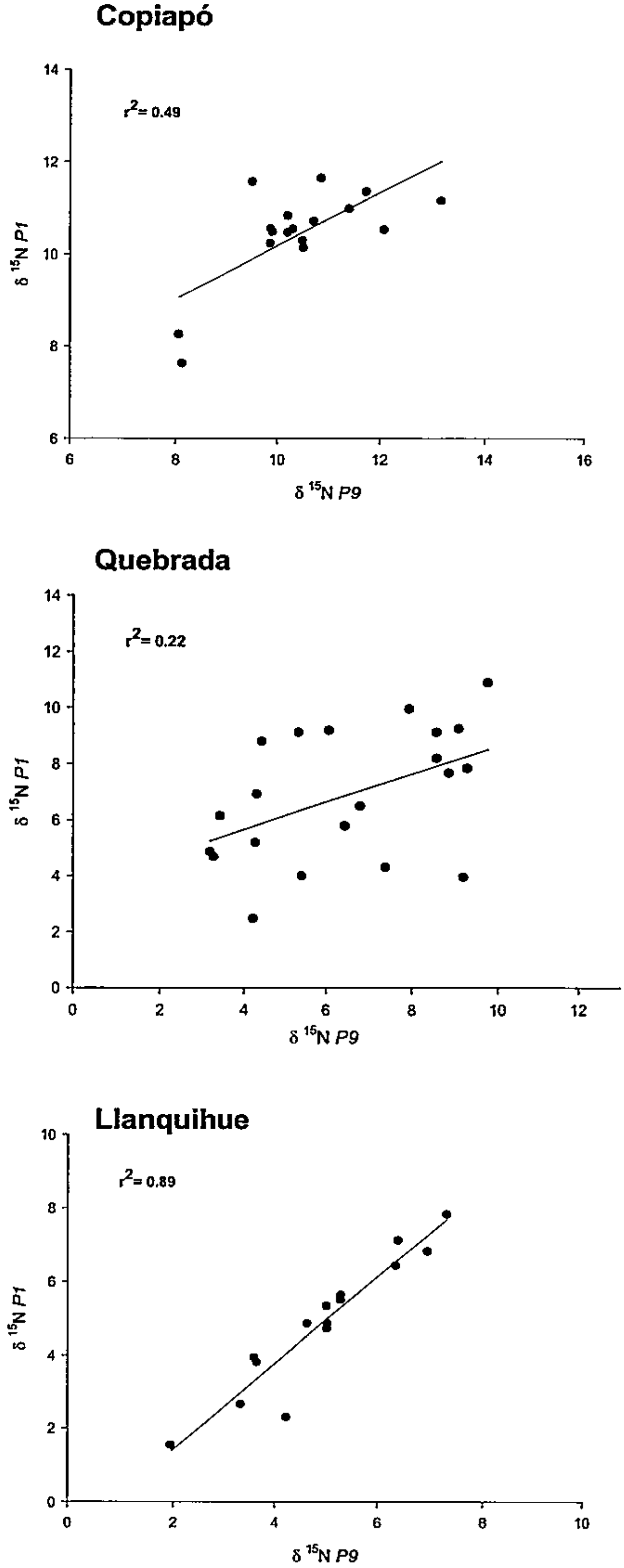
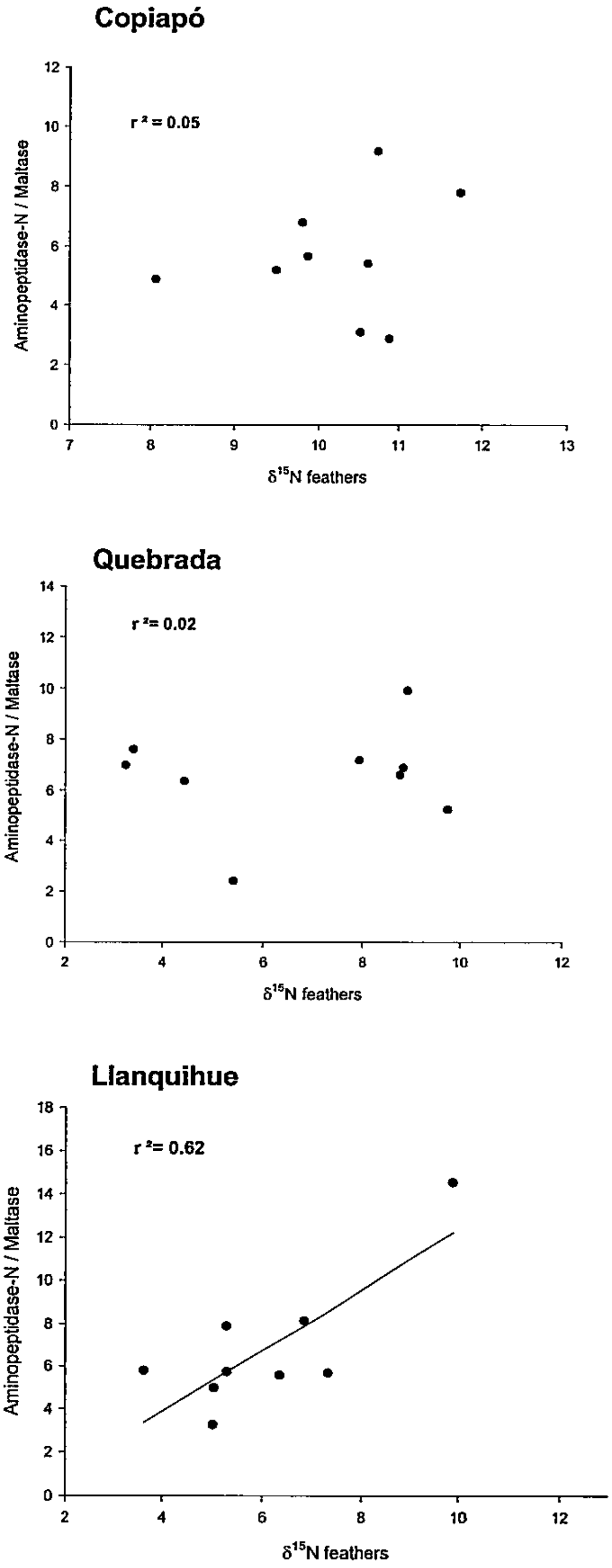


Figure 3. Association between the isotopic signature ($\partial^{15}N$) of two primary feathers differing in the time of synthesis. The *P1* is commonly synthesized approximately one month after the *P9*. Note that all the relationships shown are statistically significant ($P < 0.05$).


Copiapó

Quebrada

Llanquihue

Figure 4. Relationship between the ratio of the activity of aminopeptidase-N and maltase and the nitrogen isotopic signature ($\partial^{15}N$ average from *P1* and *P2*). Note that only for birds from Llanquihue, LRA was statistically significant ($P < 0.05$).

Discussion

An important objective in the study of individual differentiation has been the understanding how species evolve to a particular niche width. Van Valen (1965) through the niche-variation hypothesis (NVH) predicted that under conditions of relaxed interspecific competition niche expansion is achieved by increased between-individual variation in resource use as a result of individual adaptation to different micro-environments. Hence, it is expected that ecological generalists should exhibit stronger individual specialization; however this relationship has been repeatedly rejected by empiricists (Bolnick et al. 2003). Recently, Bolnick et al. (2007) in spite of the long standing of NVH, described the first general pattern in several animal taxa in which more generalist populations showed higher degrees of among-individual diet variation. Nevertheless, Araujo et al. (2009) reexamined this pattern, adding four new frogs species from the same region, and could not reject the null hypothesis, i.e., this relationship is caused by stochastic sampling effects when there is limited information on individuals' diets. Accordingly, we believe that intraspecific differences in the level of individual specialization in geographically separated populations of a single species can provide insight into the micro-evolutionary processes that entails the expansion of niche breath. Here, we discussed the possibility of the existence of a general pattern, which dissent with respect to the Bolnick et al. (2007) and NVH proposition, which is based on how organismal traits has evolved with respect to the environmental conditions that they experienced.

Total niche width

Schlichting and Pigliucci (1998) proposed that phenotypic flexibility have an adaptive value in organisms that experience temporary variations in their biotic and physical environment. Accordingly, physiological flexibility in digestive traits is often hypothesized as a critical component of the adaptive repertoire of animals to cope with changing environmental conditions (Pigliucci 1996; Piersma and Drent 2003; Pigliucci 2005; Cavieres and Sabat 2008). Our previously reported observations (*Chapter 2*) revealed a correspondence between temporal heterogeneity (seasonality) and the magnitude of the aminopeptidase-N flexibility. *Zonotrichia capensis* from Copiapó, the more climatically stable locality and in which birds had a smaller coefficient of variation in $\partial^{15}N$ between seasons (Table 1), exhibited an inflexible reaction norm after acclimating to contrasting diets. Instead, birds from Quebrada the most heterogeneous region, showed a more flexible reaction norm than the remainder two populations. Besides, *Z. capensis* from Llanquihue had an intermediate level of flexibility in the aminopeptidase-N activity, which corresponds with their intermediate levels of climatic heterogeneity and seasonal diet variation (c.v. of $\partial^{15}N$ Table 1). In this vein, it has been reported that in some cases phenotypic flexibility may influence population niche width, and a positive correlation between dietary and digestive flexibility should occur (Karasov 1996; McWilliams et al. 2005; Sabat and Bozinovic 2008). Our data seems to support this idea (Figure 1). In fact, TNW of birds from Quebrada was significantly higher and at least twice the TNW of Z.

capensis from Copiapó and Llanquihue. Moreover, the narrow TNW showed in birds from Copiapó under natural conditions seems to reflect the observed lack of flexibility in the acclimation experiments (*Chapter* 2).

Individual specialization and digestive features

Based on the aforementioned assumption of trade-offs acquisition we suggest that the level of individual specialization within population, namely WIC/TNW, should be related with the magnitude of phenotypic flexibility of the trait involved in that specialization. In this case, this hypothesis is based in the notion that the individual cost incurred by a dietary change, i.e., occupy a different part of TNW, is lesser pronounced in more flexible animals. Indeed, birds from Quebrada exhibited a flexible reaction norm of aminopeptidase-N. This ability to change reversibly the protein digestion capacity may allow birds to switch for example between prey of animal and vegetal origin without a significant decrease in its digestive performance. On the contrary, birds having an inflexible reaction norm in their digestive capacities should incur in a significant decrease in digestive performance when forced to change diets. Thus, it is likely that more flexible birds can occupy the whole TNW in an opportunistic manner (Figure 2). In contrast, more inflexible birds from Copiapó, which cannot modulate the activity of aminopeptidase-N, could be physiologically constrained in maintaining its digestive performance when switch between high protein and high carbohydrate diets. In this vein, it is also expectable that those birds share a similar average in the TNW, i.e., similar individual niche width placed in a similar part of the restricted TNW (Figure 2). Birds from Llanquihue showed an intermediate level of flexibility in the activity of aminopeptidase-N. Following our rationale, it likely causes that birds may change their diet with intermediate costs in their digestive performance. Therefore, sparrows from Llanquihue would maintain their individual diet average, setting in a part of the TNW rather fall into sequential costs of diet changes (Figure 2). The regression analysis also reveals this pattern (Figure 3). Compared with those from Llanquihue and Copiapó, sparrows from Quebrada showed the highest short-term variation in their individuals' diet expressed by the lower correlation coefficient between *P1* and *P9.* Birds from Llanquihue showed the higher correlation coefficient, suggesting that individuals maintain their average trophic niche, at least in a short-term. *Zonotrichia capensis* from Copiapó showed a lower correlation coefficient with respect to birds from Llanquihue and higher than Quebrada (Figure 3). As was already stated, we expected that these birds maintained their individual trophic niches in a restricted TNW. We found that in a short term, most birds are feeding in a similar average with a scarce variance among them. We believe that this low variability, besides explaining the little changes in the individuals and among individual diets, also may explain the reduction of the regression coefficient (Figure 3).

The results of WIC/TNW among populations support our hypothesis proposed, which suggest that the level of individual specialization within population should be related with the magnitude of phenotypic flexibility. This is described by a no uniform pattern. The WIC/TNW ratio in the more heterogeneous locality reveals a population composed by generalist individuals feeding in all parts of TNW (Figure 2). This result coupled with the value of TNW allows us to infer that Quebrada birds represent a generalist population composed by generalist individuals. On the other hand, *Z. capensis* from Copiapó, also showed higher level of individual specialization than birds from Llanquihue (and similar to Quebrada), suggesting that individuals also consuming in the whole TNW, but in a smaller TNW (Figure 1). Hence, this sparrow population is composed by generalist individuals, but consuming in a restricted TNW, i.e., in a specialist population (Figure 2). Finally, birds from Llanquihue showed a smaller WIC/TNW, which represent a population constituted by more specialist individuals distributed along the whole niche width. Thus, that population can be considered as a generalist one, but composed by specialist individuals, in which the BIC contribute in a 72.4% of the whole niche in comparison with the only 27.6% that contribute the WIC.

The individual specialization assumes the existence of functional trade-offs. Here we related the benefit of individual specialization with functional and morphological adjustments in the digestive tract in birds under natural conditions. We expected whether birds are specialized on a part of TNW the activity of aminopeptidase-N and SI mass was correlated with the protein intake, in order to increment their digestive efficiency. We found a positive relationship between dietary protein (∂^{15}N feathers) and aminopeptidase-N for birds from Llanquihue that also showed the highest level of individual specialization.

Unfortunately, there are few studies having examined the individual specialization within populations via the isotopic analysis (e.g., Fry et al. 1978; Gu et al. 1997; Araujo et al. 2007; Layman et al. 2007; Araujo et al. 2009). In a seminal study, Fry et. al. 1978 examined the feeding relationships among Grasshopper species finding that in three generalist species, individuals exhibited substantial differences in their $\partial^{13}C$ values. The authors suggested that this variability indicates that individuals are feeding on different proportions of C_3 and C_4 plants, and that these species are generalist species in which individuals differ in their resource use. Although this appears support the NVH, high levels of variability do not necessarily means individual specialization. Here we showed that WIC/TNW was similar among Quebrada and Copiapó populations, although they showed high and low variability in the $\partial^{15}N$ values, respectively (Figure 3). In this sense, and despite that isotopes in the studies of trophic niche have their criticisms (Matthews and Mazumder 2004), we highlight the advantage of obtaining different isotopic signatures (i.e., from different tissues) from the same individual. Moreover, our findings contrast with the current view that individual from more generalized populations also should exhibit a more individual niche variation. However, this view is based and probable applicable for interspecific level studies, which no necessarily reflect that could happen in an intraspecific level, which is ultimately the target of the microevolutionary processes shaping the functional traits of species. Evidently, the uses of both inter and intrapopulation approaches are necessary and complementary to elucidate the underappreciated phenomenon of individual specialization within the populations.

In conclusion, we have showed that digestive features may act as a functional trade-offs that might explain an important part the diet variation among individuals. Nevertheless, despite it was showed that physiological adjustments may be modified by acclimation (i.e., phenotypic flexibility), we cannot rule out the possibility that the individual variation within population may due to genetics differences. The extent to which ecological variation may be represented by genetic diversity requires further investigation. Finally, evaluating the causes of individual specialization within population by excluding the ecological contexts that may influence in the evolutionary pathways of populations is likely to be ineffective and more effort should be developed to investigate the mechanism behind the evolution of niche breath.

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Chapter 4

Population differences in water, energy economy and the exploratory behavior in Rufous-collared Sparrow (*Zonotrichia capensis*)

Chapter 4. Population differences in water, energy economy and the exploratory behavior in Rufous-collared Sparrow (*Zonotrichia capensis*)

Introduction

Animal personality or "coping styles" is defined as behavioral correlated differences which persist across a wide range of contexts (Sih et al. 2004; Bell 2007). Thus, animals with different personalities might consistently differ in their behavioral response to the same environmental stimuli (Boissy and Bouissou 1995; Clarke and Boinski 1995; Dingemanse and Réale 2005). These types of behavioral differences are considered an example of continuous variation among species, populations and individuals (Goldsmith et al. 1987; Wilson et al. 1994; Wilson 1998; Gosling 2001; Careau et al. 2009). Recent studies have suggested that personality traits are a target of natural selection, because individual behavior affects both survival and reproductive success (Reale et al. 2000; Dingemanse et al. 2003; Dingemanse et al. 2004; Dingemanse and Réale 2005; Sinn et al. 2006; Bell 2007; Smith and Blumstein 2008). In several taxa, exploratory behavior has shown to be an important component of animal personality, relating the organism and their surrounding environment through the information acquisition (Dingemanse et al. 2002). Moreover, given that explorative animals collect information such as profitable feeding sites, food availability and distribution territory qualities, this behavior has been linked to the characteristics of the habitats and with levels of diet specialization (Greenberg and Mettke-Hofmann 2001; Mettke-Hofmann et al. 2002; Careau et al. 2009). For instance, Tebbich et al. (2009) found that exploratory behavior of Darwin's finches increased with diet diversity, corroborating the hypothesis that suggests a functional link between generalist dietary habits and higher levels of exploration (e.g., Greenberg 1983; Greenberg and Mettke-Hofmann 2001; Mettke-Hofmann et al. 2002).

On the other hand, empirical and theoretical studies on animal personality have shown that superficial and thorough exploration strategies are associated with proactive and reactive coping styles, respectively (Sih et al. 2004b; Wolf et al. 2007). Because different coping styles involve behaviors that differ in energetic demands, they have been associated with the energy budget of individuals (Røskaft 1986; Bryant and Newton 1994; Metcalfe et al. 1995; Yamamoto et al. 1998). In this vein, basal metabolic rate (BMR), i.e., the minimum energy necessary to maintain the homeostasis in endotherms organisms (Kleiber 1961), has been related to individual coping styles patterns (see Careau et al. 2008 and references therein). In order to explain the relationship between BMR and animal personalities, two hypothetical contrasting models have been proposed. The performance model (PM) considers that BMR determines the total energy available to an individual, so higher BMR allows expending more energy in different activities (behaviors). In turn, the allocation model (AM) predicts that animals might allocate a fixed amount of energy between competing processes, such as, BMR or activity, and then a negative association between BMR and activities is expected (Levins 1968; Speakman 1997; Careau et al. 2008). This relationship between the management of energy budgets and the expression of energy demanding behaviors has been investigated in several studies, providing support for both models (Careau et al. 2008).

Passerine birds are characterized by diurnal habits, high mass-specific metabolisms and high body temperatures, which result in proportionately high rates of water flux (Williams 1996). Given these features, water budgets maintenance also may comprise an important challenge for these birds. Thus, total evaporative water loss (TEWL), i.e., the sum of cutaneous water loss and respiratory water loss, is also considered an important physiological trait (Sabat and Bozinovic 1996; Williams 1999; Tieleman and Williams 2002). TEWL is involved in water balance, thermoregulation and survival, being the main avenue of water loss in birds (Williams 1996; Wolf and Walsberg 1996). Because TEWL is related positively with the rates of metabolism and with activity levels, we suggest that this physiological feature can also influence the behavioral responses of animals. As an analogous to the allocation model, we postulated a water allocation model (WAM) which predicts that animals must allocate a fixed amount of water between evaporative cooling and activity. Thus, we expect a negative association between TEWL and activity among individuals.

There are several studies that have been reported variations in BMR and TEWL between species and populations as a consequence of diet, altitude, latitude, temperature and aridity differences (Dawson and Bennett 1973; Williams 1999; Williams and Tieleman 2000; Bozinovic et al. 2009). It is well known that foraging and thermoregulation behavior are based on a combination of physiological variables, including body temperature, hydration state and level of energy reserves. Nevertheless, studies on the relationship between behavior and physiological traits are scarce and often consider behavioral flexibility as unlimited, immediate, and reversible (Sih et al. 2004). Recently, studies in animal behavior have reported a very limited behavioral plasticity, which has awaken the interest in empirical studies that attempt to understand the link between animal personalities and physiological variables (Lahti et al. 2002; Finstad et al. 2007; Careau et al. 2009). The study of the geographical variation of personalities and physiological traits can give an important insight into how these traits are influenced by different environmental factors. Although the existence of considerable geographic variation among populations for both physiological and behavioral traits is recognized, the understanding of how animal personalities and physiological features have evolved together is far from being solved. The relationship between physiological and behavioral traits could be a consequence of correlational selection, genetic correlation, or by a shared regulation pathway (e.g., hormonal regulation). Thus, if traits are no genetically correlated and do not share a regulatory pathway, some population differences could be found in the relationship among those traits (Foster 1999).

In this study, we investigated the relationship between exploratory behavior, ecological features (diet specialization) and two physiological traits: BMR and TEWL, in an omnivorous passerine bird (*Zonotrichia capensis*). In order to do that, we chose three sparrows populations which differ in their degree of diet specialization (*Chapter* 2) along a gradient of precipitation and temperature in Chile. We predicted a correspondence between higher levels of exploration and diet specialization, and an increase in the exploratory behavior associated to higher metabolic rates, for all populations (the expected according to the PM). Besides, we predicted a negative association between exploratory behavior and TEWL (following the WAM). Furthermore, the activity in an unfamiliar environment is recognized as an interesting measure of exploratory behavior (Verbeek et al. 1994). Because we determined BMR and TEWL in a metabolic chamber, which could be considered as a novel environment for animals (Careau et al. 2008), we estimated the average oxygen consumption and total evaporative water loss during the second 30 min of experimental trials. Thus, we introduced this measure as a proxy of the individual level effect of exploratory behavior on BMR and TEWL in an unfamiliar environment.

Methods

Rufous-collared sparrows were collected from three Chilean localities – Copiapó, Quebrada de la Plata and Llanquihue. Copiapó (27°18′S, 70°25′W) is surrounding by the Atacama Desert and it is characterized by a rigorous aridity, with mean temperatures of 15.2°C and an total annual precipitation of 12 mm (di

Castri and Hajek 1976, http://www.meteochile.cl). Quebrada de la Plata (33° 31′ S, 70° 50 ′W) has a typical Mediterranean climate, characterized by hot dry summers and cold and rainy winters. The annual mean temperatures and total precipitations are 14.4 °C and 312.5 mm respectively (di Castri and Hajek 1976, http://www.meteochile.cl/). Llanquihue (41°16′S, 73°00′W) the most southern region, experiences both high rainfall and low temperatures with a mean annual temperature of 9.4° C and total annual precipitation of 3,112 mm (Centro de Informacion de Recursos Naturalales, Ciren). In order to represent the magnitude of food availability among populations, we calculated the primary productivity (Tieleman 2003, Bozinovic 2007) via the aridity index (Q):

$$Q = \frac{pp}{(T_{\max} + T_{\min})(T_{\max} - T_{\min})} \cdot 1{,}000$$

Where pp is the average annual precipitation (mm) and $T_{\max}$ (°C) and $T_{\min}$ (°C) are the mean maximum and minimum temperature for the hottest and coldest month, respectively (Tieleman et al. 2003a).

The annual mean of this index is low in hot, dry deserts, and high in cool, wet areas. Our calculations demonstrated a north-south increase in primary productivity from Copiapó to Llanquihue (Table 1). We collect historical data for both precipitation and temperatures from national institutes of information (Dirección meteorologica de Chile and Centro de información de recursos naturales) from literature (di Castri and Hajek 1976) and from http://www.worldclimate.com. Also, we used reported data about total trophic niche width and the coefficient of variation (between seasons) of diets for each population. These data were estimated using the isotopic signatures obtained from different tissues ($\partial^{15}N$ from feathers and blood), which have different turnover rates (for details see *Chapter* 1 y 3). These measures were used in order to assess the level of diet specialization among populations (Table 1).

After capture, birds were transported at the laboratory and housed together in individual plastic-mesh cages (35 x 35 x 35 cm) at a constant temperature room and photoperiod of 22 ± 2°C and 2L: 12D, respectively. They had ad lib access to mealworms, bird seeds and water.

BMR and TEWL

Following an habituation day, rates of oxygen consumption (BMR) and total evaporative water loss were measured at 30°C, which is well within the thermoneutral zone for this species (Sabat et al., 2006a). Measurements of BMR and TEWL were made in post-absorptive, resting birds during the inactive phase by recording oxygen consumption in a computarized open-flow respirometry system and by flow-through water vapor analyzer (Sable Systems, Henderson, Nevada). First, birds were weighed using an electronic balance (± 0.1 g), placed in a dark metabolic chamber (1 L) perched on a wire-mesh grid that allowed excreta to fall into a tray containing mineral oil, thus trapping the water from this source and then situated inside a controlled temperature cabinet (Ta = 30 ± 0.5°C). The open-flow respirometry system was calibrated with a known mix of oxygen (20%) and nitrogen (80%) that was certified by chromatography (INDURA, Chile). The measurement and calibration protocols we followed were after Williams and Tieleman (2000). Because CO_2 was scrubbed before entering the O_2 analyzer, oxygen consumption was calculated as (Withers 1977, p. 122): $VO_2 = \left[FR \cdot 60(F_iO_2 - F_eO_2)\right] / (1 - F_iO_2)$, where FR is the flow rate in mL/min after STP correction, and F_i and F_e are the fractional concentrations of O_2 entering and leaving the metabolic chamber, respectively. We calculated absolute humidity (kg/m^3) of air entering and leaving the chamber as $\rho = P / (T \cdot R_w)$, where P is water vapor pressure of the air in Pascal, T is the drew-point temperature in Kelvin and R_w is the gas constant for water vapor (461.5 J/kg K, Lide 2001). The P_{in} was determining using the average value of the vapor pressure of the air entering the empty chamber (i.e., the baseline period of 15 min) before and after each experiment with a dew-point hygrometer (RH- 200, Sable System, Henderson, Nevada). TEWL (mg/mL) was calculated as $TEWL = (V_e \cdot \rho_{out} - V_i \cdot \rho_{in})$; ρ_{out} and ρ_{in} are the absolute humidity in kg/m3 of the inlet air and the outlet air, respectively, V_i is the flow rate of the air entering the chamber as given by the mass flow controller (500 mL min^{-1}), and V_e is the flow of exiting air. V_e was calculated following Williams and Tieleman (2000): $Ve = V_{in} = \left[VO_2(1 - RQ)\right] + VH_2O$; V_{in} and VO_2 (mLmin^{-1}) are known. We used a respiratory quotient (RQ) of 0.71, assuming that fasting sparrows rely mainly on stored lipids (King and Farner 1961; Walsberg and Wolf 1995). At the end of each measurement cloacal body temperature (T_b) was recorded by Cole-Palmer copper-constantan thermocouple attached to a Digisense thermometer (Model 92800–15). Output from the H_2O (kPa) and oxygen analyzers (%) was digitized using a Universal Interface II (Sable Systems) and recorded on a personal computer using data acquisition software (EXPEDATA, Sable Systems). Our sampling interval was 5 s. Birds remained in the chamber for at least 4 hrs (Maldonado et al. 2008). We averaged water vapor pressure and O2 concentration of the excurrent air stream over a 20 min period after steady-state was reached (following Tieleman et al. 2002).

As we already stated, metabolic chambers can be considered as a novel environment, where individual differences in exploratory responses may influence the recordings (Careau et al., 2008). So, we propose two novel measurements MR_{30} and $TEWL_{30}$, as a way of including information about animal's personality in physiological measurements. MR_{30} corresponds to oxygen consumption average during the second 30 min of the trial in a BMR recording (Figure 1) and $TEWL_{30}$ as the average of water loss during the second 30 min of the trial in a TEWL recording (Figure 1). We take the second 30 minutes, in order to diminish the stress response to handling when animals were located inside the metabolic chambers.

Exploratory behavior

According to van Dongen et al. (unpublished results, Annex 1), exploratory behavior in these rufous-collared sparrow populations is a complex, multi-component behavior. In this vein it was proposed exploration diversity as a reliable indicator of true exploratory tendency. Thus, individuals visiting a greater diversity of areas rather than only exploring a subset of the available area, would benefit in localities where the location of available food is unknown. Following this rationale we used this measure in order to estimate the exploratory behavior of individuals. After physiological measurements were done, exploration behavior was quantified by means of novel environment experiments in a large field-portable cage (270 cm length x 150 cm width x 150 cm height) constructed of PVC poles and semi-transparent black shading cloth. Five wooden perches (80 cm in length, 2 cm in diameter) were hung throughout the cage at varying heights (between 50-110 cm above ground). One perch was placed diagonally in the opposite corner at each far end of the cage and the remaining three spaced at regular intervals (70 cm apart) along the long axis of the cage. The cage was placed in a fixed position within the laboratory. Before each trial, the subject was placed in a small holding cage (30 cm length x 25 cm width x 39 cm height) in a corner of the experimental cage and covered with a cloth during a 5-minute habituation period. Each experimental trial lasted ten minutes. At the beginning of the experimental period, the cloth was removed and the door of the holding cage was opened. An observer, hidden from view but with full sight of the cage, dictated all the subjects' movements onto a digital voice recorder including: 1) number and destination of flights and 2) number and destination of hops. Flight and hop destination included both the perches and the walls of the cage. We also distinguished between cases where the birds landed on the front and back ends of the two side-walls. This resulted in 11 areas of the cage where the birds regularly perched (five wooden perches and six wall regions). At the termination of each trial, sparrows were recaptured within the cage using a butterfly net and returned to the housing. Exploration diversity was quantified via the Shannon's diversity index, via $H = -\sum p_i \ln p_i$, where p represents the total number of times perch i was visited, expressed as a proportion of the total number of perch visits throughout the trial. Individuals were assumed to be more thorough explorers when they visited a higher diversity of perches during the trial.

Data analysis

To evaluate differences among populations, the samples were analyzed by a bootstrap (10,000 resamplings) ANOVA using the BioEstat 5.0 software, assuming $P < 0.05$ as the level of significance. To estimate the relationship among variables we used linear regression analysis (LRA). As body mass differed significantly among populations ($p<0.001$) (see Table 2), for variables that were correlated with M_b, we analyzed them by the residuals obtained in the LR against M_b. Since, we preferred to evaluate the association between exploratory behavior and physiological variables in each population, we performed multiple forward stepwise regression analysis using exploratory behavior as dependent variables and BMR, TEWL, MR_{30}, $TEWL_{30}$ as independent variables. Because, independent variables were correlated with M_B, this analysis was performed using the residuals of the variables against M_B. For LRA and multiple forward stepwise regression analysis we assumed $P < 0.05$ as the level of significance. All analyses were performed using STATISTICA 6.0. Data are presented as a mean ±1 SD.

Results

Body mass was different among populations (bootstrap ANOVA; $P<0.001$), being significantly higher in Llanquihue, the most southern site (Table 2). BMR values were positively correlated with body mass (LRA, $r^2= 0.38$; $P<0.001$). Residual from this regression were not significantly different among sites (bootstrap ANOVA; $P=0.15$; Table 2). Contrarily, TEWL differ among populations (bootstrap ANOVA; $P<0.001$), even though body mass and BMR effects was removed (bootstrap ANOVA; $P= 0.02$). In this case, the lowest value of TEWL was observed in sparrows from the arid zone (Table 2). MR_{30} was similar among localities (bootstrap ANOVA; $P= 0.11$; Figure 2). Besides, $TEWL_{30}$ differ significantly among populations (bootstrap ANOVA; $P= 0.01$), being the lowest values those observed in sparrows from the arid locality of Copiapó (Figure 2). Body temperature were similar among populations (bootstrap ANOVA; $P=0.177$; Table 2).

We found a significant effect of locality on exploration diversity (bootstrap ANOVA; P=0.041), being higher in birds from Llanquihue than the other two populations (Figure 3). In addition, exploration diversity in birds from Llanquihue was not associated to any physiological parameter. However, in sparrows from Copiapó and Quebrada, exploration diversity was correlated with $TEWL_{30}$ ($P< 0.03$; Figure 4) and with BMR ($P<0.04$; Figure 5), respectively. We did not found any significant correlation between mass and exploratory diversity ($P>0.05$).

Table 1. Climatic data in three populations of Rufous-collared sparrows. The mean annual temperature (calculated as average of the monthly temperatures) and total annual precipitations (calculated as the sum of monthly precipitations) are reported. Primary productivity was estimated via Q_{index} (Tieleman et al. 2003). Q tends to be low in hot, dry months and in more mesic sites. Total niche width (TNW) was obtained from reported data (see *Chapter* 3). The seasonal coefficient of variation (c.v) of $\partial^{15}N$ was calculated by the birds' isotopic signatures from feathers and blood (which represent diets from different seasons) (c.v $\partial^{15}N$ was obtained from *Chapter* 2).

Localities	T° annual mean	PP annual mean	Q_{index} mean	TNW	$\partial^{15}N$ (c.v)
Copiapó	15.2	12	15.8	1.156	0.05
Quebrada	14.4	312.5	360	4.827	0.22
Llanquihue	9.4	3112	9.185	2.031	0.13

Table 2. Body mass and physiological features in *Zonotrichia capensis* from different localities. Different letters denote significant differences ($P<0.05$) among localities. The number of animals is in parenthesis. Data are reported as mean ± SD.

	Copiapó (19)	Quebrada (19)	Llanquihue (24)
Body Mass (g)	17.95 ± 1.03^a	17.72 ± 1.31^a	19.65 ± 2.04^b
TEWL (mgh^{-1})	**67.19 ± 16.13^a**	73.12 ± 25.61^b	103.97 ± 38.45^b
BMR (mlO_2h^{-1})	53.78 ± 5.37^a	49.45 ± 7.9^a	56.59 ± 11.48^a
Body temperature (ºC)	39.6 ± 1.2^a	39.9 ± 1.10^a	39.3 ± 0.9^a

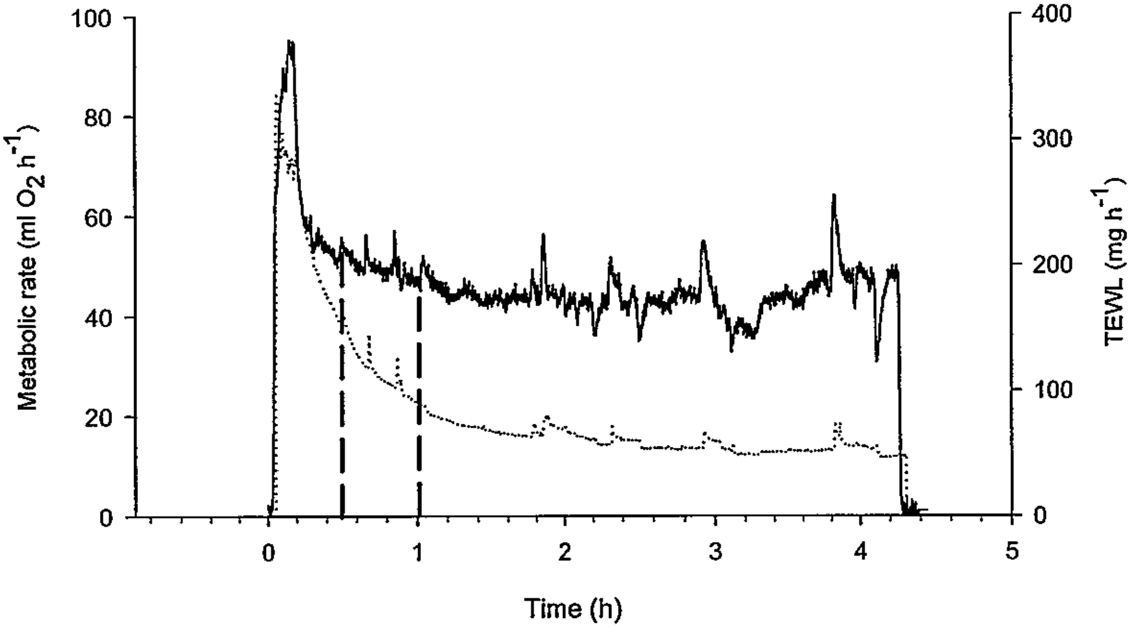


Figure 1. Representative oxygen consumption (solid line) and evaporative water loss (dotted line) records in an individual of *Zonotrichia capensis* during the resting phase. Note that dashed lines represent the range which average represents MR_{30} and $TEWL_{30}$ measurements.

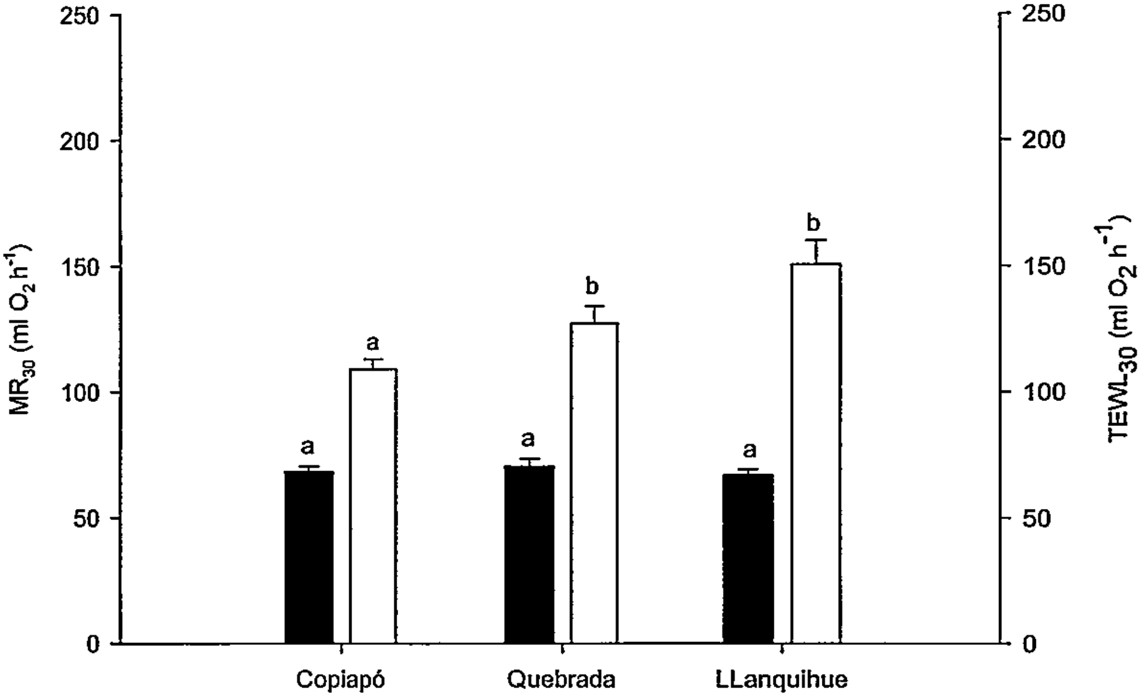


Figure 2. The average oxygen consumption (MR_{30}, black bars) and total water loss ($TEWL_{30}$, white bars) during the second 30 min of experimental trials. Data is represented as mean ± SE.

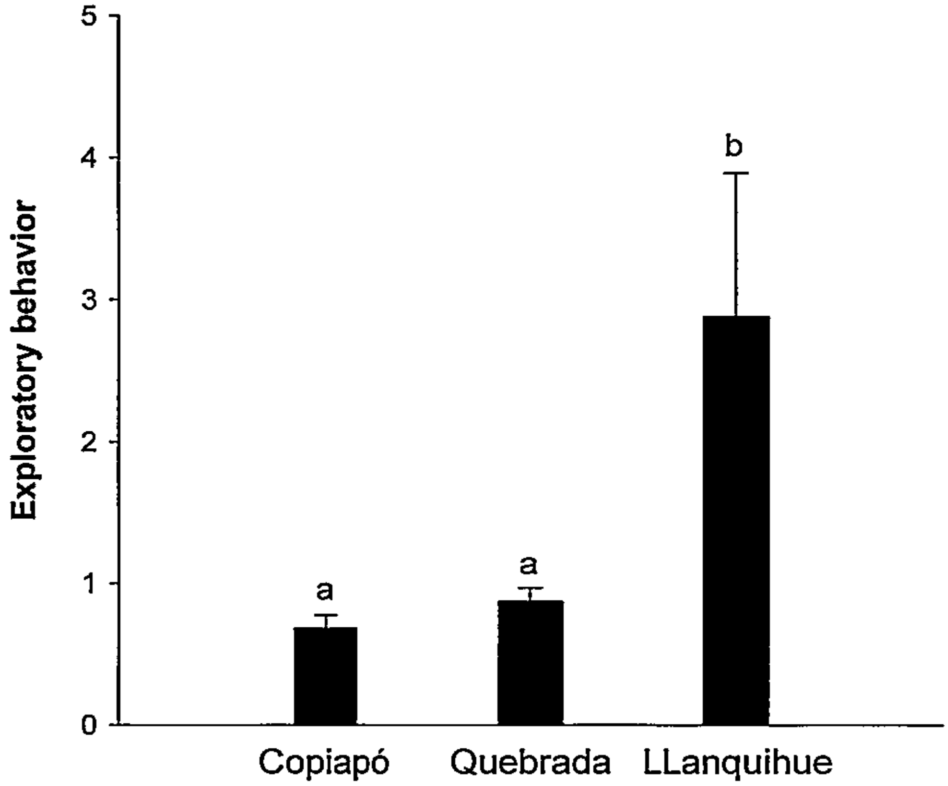


Figure 3. Exploratory behavior in *Zonotrichia capensis* from three localities of Chile. Data is represented as mean ± SD.

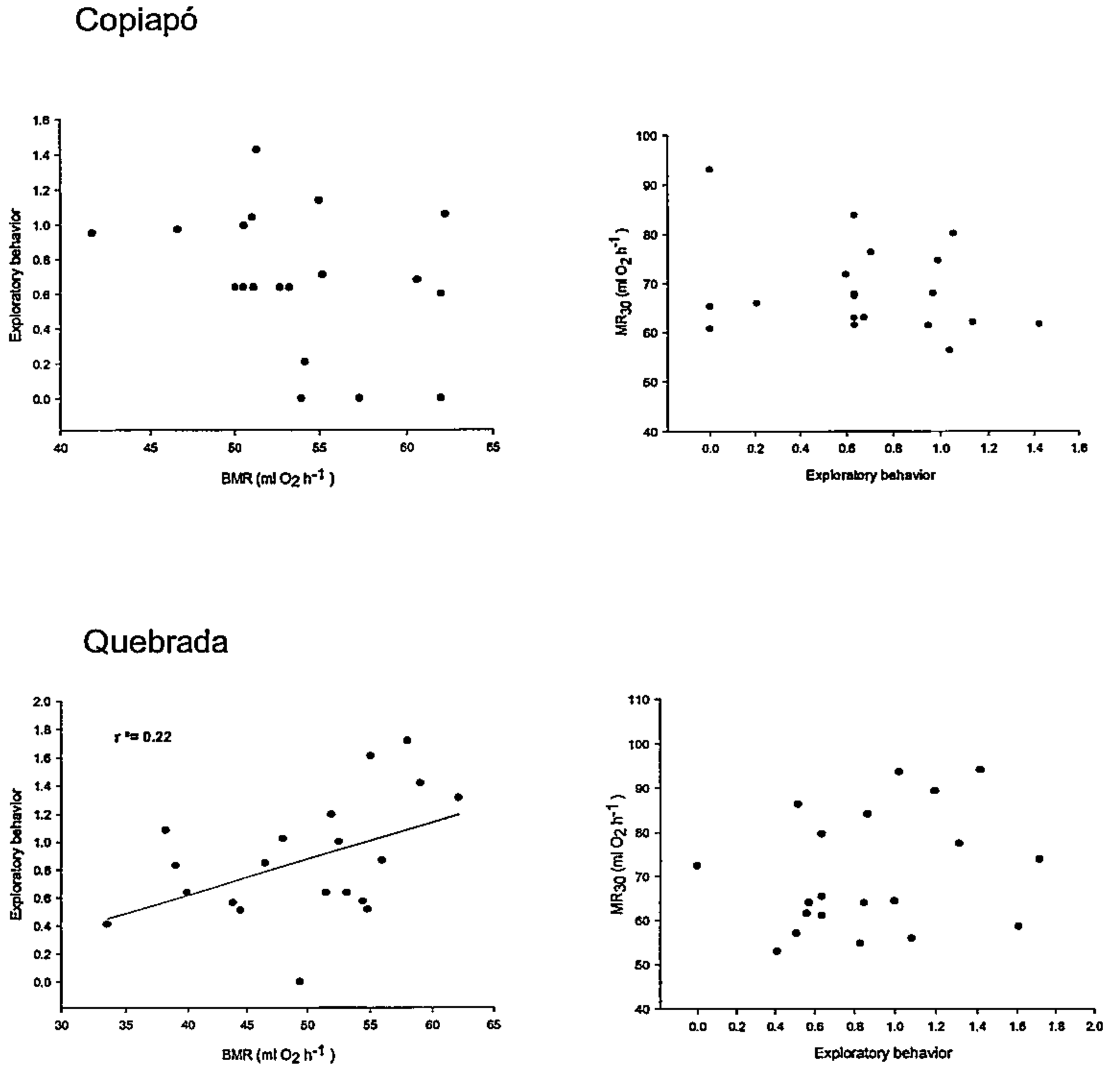
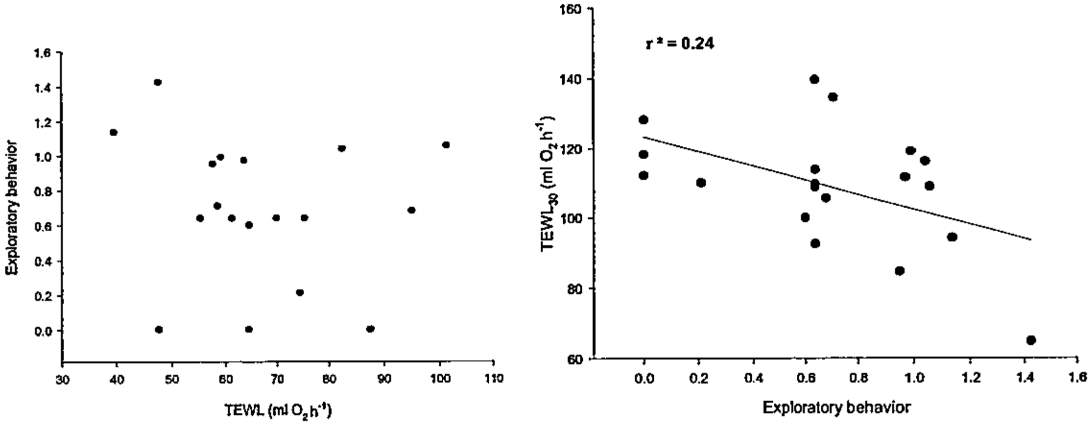


Figure 4. Relationship between basal metabolic rate (BMR) and the average of oxygen consumption (MR_{30}) during the second 30 min of experimental trials and the exploratory behavior in birds from Copiapó and Quebrada. Regression line is drawn to significantly relationships (P<0.05).

Copiapó



Quebrada

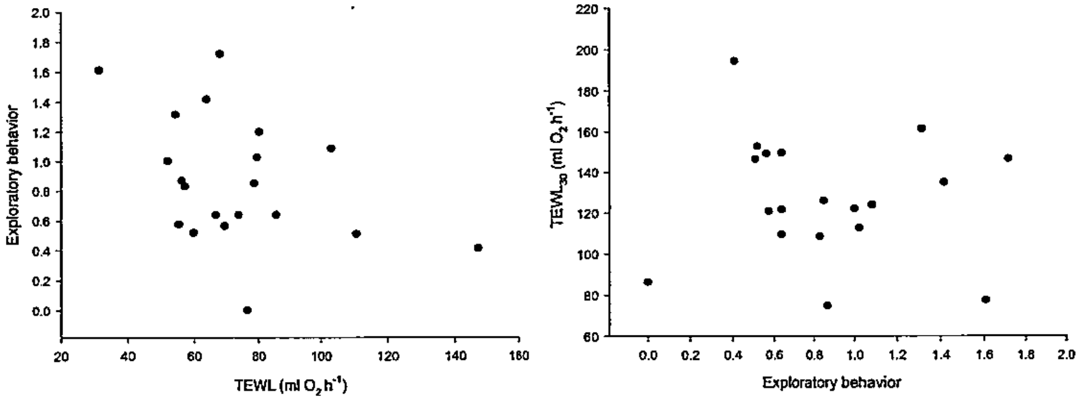


Figure 5. Relationship between Total evaporative water loss (TEWL) and the average of total water loss (TEWL$_{30}$) during the second 30 min of experimental trials and exploratory behavior in birds from Copiapó and Quebrada. Regression line is drawn when relationships was significant ($P<0.05$).

Discussion

In the current study, we tested the hypothesis of a significant association among exploratory behavior, dietary specialization and physiological features. The analysis revealed strong differences in both personality and the physiological traits among populations and within populations. First, we will discuss the variation among populations and then the significance of the intrapopulation variability in behavioral and physiological traits.

Behavioral and physiological variability among populations

Ecological differences among populations have been shown to modulate some animal personality traits (Reale et al. 2007). For instance, it has been reported that island bird populations generally show lower neophobia and higher exploration levels than mainland bird populations (Greenberg and Mettke-Hofmann 2001). Greenberg (1990a, 1999) suggested that low neophobia is related to a greater ecological plasticity of a species, measured as the diversity of habitats used. The author suggested that island conditions that birds experienced in comparison with mainland species i.e., broader trophic niches along with a lesser risk of predation, would reduce the costs of exploration favoring the use of alternative resources. In our study, birds from Quebrada had the highest variability in their diets along with a broader niche breath (Table 1), so we expected these birds to exhibit higher exploration levels. Nevertheless, the exploratory behavior was greater in birds from Llanquihue, which were characterized by intermediate levels of diet variation (Table1). Mettke-Hoffmann et al. (2002), emphasized that in order to understand the function of exploration, one must know the value of information and their pay-offs. The value of information varies according to the environmental conditions that organisms confront, being the highest in moderately variable environments as compared to stable or extremely variable ones (Mettke-Hofmann et al. 2002). The rationale of this hypothesis is that in completely stable environment exploratory behavior is not profitable, because there are no environmental changes, which have get information about. Contrarily, in extremely variable environments, the too rapidly loss of previous information acquired, provoked that useful information could not be properly achieved by individuals. Thus, only in a moderately variable environment exploration behavior may lead to a high increase in knowledge (Winkler & Leisler 1999). Llanquihue is characterized by its high productivity and low seasonality, Quebrada by its seasonal climate and intermediate productivity, and Copiapó is recognized as a stable arid zone with a remarkable low primary productivity (Cavieres and Sabat 2008). Apparently, in *Z. capensis* a higher niche breath did not promote an increase in the levels of exploration. However environmental predictability could be an important factor influencing the levels of exploratory behavior in sparrow populations. According to the aforementioned hypothesis we found that birds from Llanquihue population, with a moderately variable environment, showed the highest levels of exploration.

A recent interspecific study in rodents (Careau et al. 2009) seems to offer an alternative hypothesis. Careau et al. (2009) found that the two desert rodents (*Meriones tristrami* and *Rhabdomis pumilio)* were characterized by high exploration indices, and the hamster, *Mesocricetus auratus*, which lives in open steppes and agricultural lands, with a low exploration score. They argued that an important selection pressure may be food productivity and predictability, where unproductive and unpredictable environments, such as arid zones, should favor higher exploration scores since it increase the likelihood of finding resources pulses (Lovegrove 2000). Despite this assertion appears to be completely rationale (but in opposite sense to the information theory), we think that in our case highly explorative individuals in arid zones are not benefited, especially when we consider the maintenance of hydric balance. Contrarily to mammals, avian kidneys are known to have poor concentrating ability (Sturkie 1976, Dantzler & Braun 1980, Goldstein & Braun 1989, Sabat et al. 2009). Thus, bird distribution could be constrained because the maintenance of osmotic homeostasis in environments where fresh water is scarce or unavailable (Johnston & Bildstein 1990, Sabat 2000, Sabat et al 2006). We suggest that differences among taxa may explain the difference with the results found by Careau et al. 2009.

Additionally, we found that TEWL differed among populations, being the lowest values for birds from Copiapó, the most arid region. This result was not surprising, due to Cavieres and Sabat (2008) reported a positive association between TEWL and latitude in *Z. capensis*. Minor TEWL has been proposed as a physiological adjustment to deal with the high temperatures and low relative humidity of arid environments, which results in a selective pressure that favors individuals with lower water permeability through the skin (Muñoz- Garcia and Williams 2005). Also, we expected that birds with higher levels of exploration had a higher metabolism (BMR and MR_{30}), but we did not find any differences neither in BMR nor in MR_{30} among birds populations. *Z. capensis* from Llanquihue was the most exploratory population, but it did not have the highest levels of metabolism in comparison to the others. Elevated levels of BMR have been associated to a proactive coping style, which is characterized by including behaviors with high energetic costs. In the same vein, we expected that more explorative populations were to show higher MR_{30}, because of their exploratory activities inside the chamber, but it was not the case. Nevertheless, evidence for the association between exploratory behavior and BMR becomes from interspecific studies (Careau 2009), which does not discard the existence of other factors that could be affecting intraspecific processes. In fact, we found a significant and positive intrapopulation correlation between these variables for birds from Quebrada, suggesting that different ecological factors could be affecting this relationship.

Behavioral and physiological variability within populations

Following Wilson (1998) it is expected to find that natural selection will favors traits that are domain-specific, or appropriate for a particular context. However some traits may be constrained by each other, being in this case the relationship between exploratory behavior and BMR and TEWL a general characteristic of species (Bell 2005). We found that relationships among these traits differed among localities. Birds from Quebrada showed a significant association between exploratory behavior and BMR; birds with higher BMR also showed higher exploration levels (i.e., according to the PM). Exploration is part of coping styles of proactive individuals which manage the stress actively by means of manipulating the situation that causes it by avoidance or aggression. In this sense, sparrows having higher BMR are more likely to engage in these energetically costly behaviors (Careau et al. 2008). Thus, animals with higher BMR are probably better set to produce the energy necessary to be spent in exploration.

The relationship between total evaporative water loss and exploratory behavior showed differences between populations. Birds from Copiapó exhibited a significant relationship between $TEWL_{30}$ and exploratory behavior. Although our experimental setup probably could prevent birds to exhibits a large arrange of explorative behaviors while metabolic measurements were performed, animals may differ in their degree of exploration that could be reflected in the physiological measurements (Careau et al. 2008). We found, that individual exploratory behavior explained the 24% of $TEWL_{30}$ variation. Because TEWL variability (measured in the steady-state) was not significantly explained by exploratory behavior, we suggest that the individual levels of exploration may be related to the physiological response when animals confronting to novel situations, rather than to physiological differences in TEWL per se (e.g., skin permeability, nasal turbinate). Although the proximal factors behind this response are difficult to unravel and unfeasible through this work, we argue that due to the importance of water budgets in this desert population, evolutionarily, it has been favored a combination of traits in order to reduce the water expenditure. The importance of hydric balance maintenance in birds from Copiapó is supported by the lowest TEWL values in comparison with mesic sites (see also Cavieres and Sabat 2008). Thus, it is likely that more explorative individuals showing lower rates of water loss when being faced to novel situations are favored in this population. This assumption, considered that water is a limited resource for *Z. capensis* from the arid region and that there is a trade-off between exploration and water loss, following our proposed model WAM.

Birds from Llanquihue did not show any correlation between exploratory behavior and physiological variables. We suggest that this lack of association was due to the high variances in the exploratory trait (Figure 3). van Dongen et al. (unpublished results, *Appendix 1*) analyzed the repeatability of the exploratory behavior in these three populations and reported the lowest values for birds from Llanquihue, opposed to the significant consistency found in the remainder two populations. This low repeatability suggests that exploration is very sensitive to environmental influences (Falconer and Mackay 1996) that could explain the lack of correlation with the physiological variables. It is probably that correlations between exploration and physiological variables in the other populations are due to selection pressures favoring these traits combination. Nevertheless, the low repeatability observed in Llanquihue could reflect low selection response along with the lack of correlation among these traits.

Our results suggest that exploratory behavior has an important contribution in the balance of water budgets in the arid region. Moreover, the fact that BMR within a population could explain some variability in the exploratory levels, suggest that individual physiology may constraint the expression of energetically expensive behaviors in individuals with lower BMR. Thus, differences in traits combinations found within populations with both a heritable basis (*Apéndice 1*) and different fitness payoffs between distinct circumstances, would lead to the stable coexistence of different kind of phenotypes within populations (Dall et al. 2004). Nevertheless, although formerly we adjudicated the variation within population as a result of selective pressures that favors specific combination of traits; there are several proximate and ultimate factors which could maintain different responses within a population. Variable responses may exist between individuals that differ in experience, i.e., due to phenotypic plasticity (Semlitsch et al. 1990, Houston and McNamara 1992). For instance, behavior towards novel situations can be strongly affected by experiences during early stages of ontogeny which could be maintained in adulthood stages (Winkler & Leisler 1999). It is clear that in order to fully understand the inheritance of personality and physiological traits further studies examining the correlations between phenotypic and genetic basis are needed.

The differences among populations found in this study suggest that personality and physiology have evolved together in order to adjust the phenotype to specific environmental pressures. It is to be expected that future studies assess the evolutionary consequences of the relationship between animal personality and physiology.

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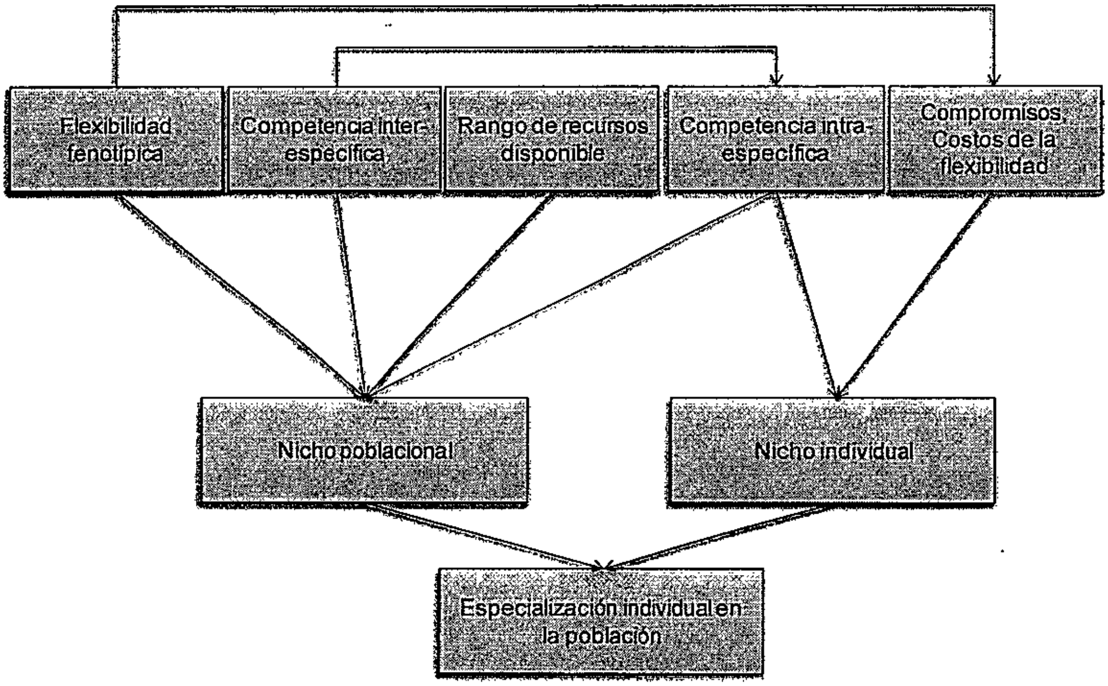
Conclusiones generales

Conclusiones generales

Esta investigación se enfocó en el efecto de la variabilidad climática, a una escala geográfica y estacional, sobre la biología de una especie de ave en distintos niveles de organización. Los resultados demuestran claramente que las diferentes condiciones ambientales que experimentan las poblaciones de *Z. capensis* producto de la estacionalidad climática y otras diferencias geográficas, afectan significativamente la variabilidad fenotípica de los organismos estudiados. Las poblaciones de *Z. capensis* presentaron diferencias a nivel inter-poblacional, intra-poblacional y en la magnitud de la flexibilidad fenotípica, tanto en rasgos fisiológicos como morfológicos y conductuales.

A su vez, se elaboraron dos aproximaciones particularmente novedosas. Por primera vez se estudió el efecto directo de la heterogeneidad ambiental sobre el grado de especialización individual en poblaciones naturales (Capítulo 3). Además, se investigaron las causas que dan origen a la variabilidad intra-poblacional a un nivel organísmico, ampliando de esta manera el rango de factores abordados en estudios previos, donde las preguntas han estado principalmente enfocadas en un nivel ecológico. El núcleo central de esta investigación fue considerar a la heterogeneidad ambiental como una presión de selección que podría dirigir la evolución fenotípica. Enmarcados en lo anterior, concluimos que el nivel organísmico parece ser clave en la determinación de la variabilidad intra-poblacional y que la heterogeneidad ambiental puede influir en especialización dietaria individual en las distintas poblaciones (Capítulo 3). El siguiente esquema propuesto resume cuáles son las variables que inciden y pueden explicar en parte la variación en la amplitud del nicho trófico individual dentro de las poblaciones naturales.



A lo largo de este estudio hemos alcanzado un número de conclusiones generales. Primero, al comparar nuestros resultados con estudios previos acerca de la flexibilidad fenotípica en rasgos energéticos, se evidenció que las respuestas parecen ser específicas a las señales ambientales (Capítulo 2). En esta línea, la correspondencia entre la magnitud de la flexibilidad de la tasa metabólica basal (BMR) y heterogeneidad temporal, parece estar dada principalmente por la estacionalidad en la temperatura ambiental y no por cambios en la composición de la dieta (véase Capítulo 2 and Cavieres & Sabat, 2008). Por otra parte, poblaciones cuyos individuos parecen no responder con principalmente por la estacionalidad en la temperatura ambiental y no por cambios en la composición de la dieta (véase Capítulo 2 and Cavieres & Sabat, 2008). Por otra parte, poblaciones cuyos individuos parecen no responder con modificaciones metabólicas (BMR) frente a una aclimatación térmica parecen ser flexibles frente a cambios en la composición química de la dieta (Capítulo 2). Esto destaca la importancia de considerar bajo qué circunstancias la flexibilidad fenotípica evoluciona. Segundo, el BMR en *Z. capensis* parece ser un rasgo menos flexible de lo esperado frente a cambios dietarios, mientras que la modulación de los rasgos digestivos, parece responder más fácilmente frente a dichas variaciones (Capítulo 2). Tercero, la flexibilidad fenotípica juega un rol tanto a nivel individual como en la organización interna de las poblaciones. En esta línea, nuestros resultados sugieren que la especialización individual implica la adquisición de beneficios otorgados por la aclimatización dietaria y que ésta, a su vez, restringe la ocupación de otras partes del nicho poblacional total (Capítulo 3). Cuarto, debido a que la importancia de los presupuestos energéticos y de agua es distinta para cada población, se observan diversos ajustes fenotípicos entre las distintas poblaciones. Más aún, cuando las restricciones en los presupuestos de agua y energía de los animales son altas, puede haber más de un rasgo involucrado en la economía requerida con respecto a tal restricción. Nuestros resultados mostraron que la población más restringida hídricamente presenta en promedio menores tasas de pérdida de agua evaporativa (TEWL). Además, al interior de esta población, los animales que presentaron mayor $TEWL_{30}$ (ver Capítulo 4) fueron los que mostraron menores niveles de exploración en los experimentos conductuales. Esto sugiere que bajo condiciones de estrés hídrico no sólo la fisiología se ajusta a las necesidades de mantención del balance de agua, sino que también la la conducta exploratoria juega un rol activo frente a este desafío ambiental (Capítulo 4).

Debido a que no se encontró apoyo para la hipótesis adaptativa de modulación dietaria de las disacaridasas entre poblaciones en animales adultos, se propone que las aves podrían modular la actividad de estas enzimas sólo en una corta ventana temporal en los estados tempranos de la ontogenia, etapa en que la dieta es controlada mediante el cuidado parental (véase Capítulo 1). Según esto, queda por resolver si las diferencias ambientales experimentadas entre poblaciones efectivamente son amortiguadas por el cuidado parental durante las primeras etapas de la ontogenia, causando la ausencia del patrón de modulación esperado. De esta manera se necesitan esfuerzos de investigación adicionales para evaluar la hipótesis de modulación dietaria en etapas tempranas del desarrollo y también investigar en qué medida existe una modulación enzimática a nivel poblacional como resultado del cuidado parental. En este sentido, estudios comparativos que involucren aves nidífugas y nidícolas podrían entregar una información valiosa para entender las causas de la variación fisiológica digestiva entre poblaciones naturales.

En esta tesis se demostró que la heterogeneidad tiene un efecto sobre la magnitud de la flexibilidad fenotípica de las poblaciones de *Z. capensis*. Sin embargo, nosotros sugerimos que la diferenciación de los individuos al interior de una población podría estar determinada principalmente por la expresión de esta flexibilidad (i.e., aclimatización) y no necesariamente por diferencias genéticas (véase Capítulo 2 y 3). Esto se apoya en que los rasgos involucrados en la especialización individual mostraron ser flexibles frente a aclimatación experimental, sugiriendo que su expresión tiene un importante componente ambiental. Así, estudios complementarios de medición de la heredabilidad de los rasgos digestivos asociados a la especialización individual en poblaciones que presentan este tipo de variación intra-poblacional, serían especialmente informativos.

Finalmente, como proyecciones futuras de esta investigación, se pretenden evaluar y modelar matemáticamente los costos asociados con las diferencias en la magnitud de la flexibilidad fenotípica. Así, mediante una aproximación del tipo costo-beneficio, se podría estimar de manera explícita los efectos de la heterogeneidad ambiental, los niveles de competencia intra e inter-específica y la flexibilidad fenotípica sobre el grado de especialización individual de las poblaciones naturales.

Apéndice 1

Geographic variation in the repeatability of a personality trait

Anexo 1. Geographic variation in the repeatability of a personality trait

Introduction

Animals often differ consistently amongst individuals in certain behaviours. When these differences persist across multiple behaviours and across different contexts, they are labelled animal personalities, behavioural syndromes or coping strategies (Sih et al. 2004a; Sih et al. 2004b; Bell 2007). These personalities are typically population-specific (Bell 2007) and consist of correlations between seemingly unrelated behaviours such as aggression, risk-taking behaviour and exploratory behaviour. It is now known that population-specific correlations between behaviours can be adaptive and represent different strategies to cope with the local environmental pressures (Sih et al. 2003; Dingemanse et al. 2004; Bell 2005; Bell and Sih 2007; Dochtermann and Jenkins 2007). Although personalities have traditionally been quantified in single populations, increasing research is targeting interpopulational differences in personalities with the realization that the selective pressures acting on certain behavioural combinations may differ amongst populations depending on local environmental constraints. For example, Dingemanse et al. (2007) recently showed that, across 12 populations of three-spined stickleback (*Gasterosteus aculeatus*), high predation pressures can select for the genetic coupling of certain behaviours, resulting in interpopulational differences in personalities based on the occurrence of predators.

An important step in understanding the strength and evolutionary consequences of natural selection acting upon personalities is to document behavioural variability amongst individuals and consistency within individuals. Repeatability is a measure commonly used for such purposes, reflecting the amount of interindividual variation in a trait relative to the total phenotypic variation (i.e. the sum of interindividual and intraindividual variation; Lessells and Boag (1987). The repeatability of a trait will thus be high if individuals behave consistently (low intraindividual variability) or large variation between individuals exists (high interindividual variability). Moreover repeatability can also predict the upper limit for the heritability of a trait, since it includes both genetic and environmental sources of variation whereas heritability includes only interindividual genetic differences (Lessells and Boag 1987; Dohm 2002). However, inter- and intraindividual variability of a trait can arise both due to labile environmental factors and fixed genetic differences between individuals (Falconer and Mackay 1996). If genetic and environmental differences exist between populations, geographic variation in the repeatability of traits may therefore also occur. Understanding these population-level differences in repeatability may well provide important insights on the selective pressures acting upon personality traits and the causes of personality differences between populations. To date, geographic variation in repeatability measures has yet to be documented for a personality trait.

Exploratory behaviour is one behavioural trait that commonly forms parts of animal personalities. This behaviour describes the process of collecting information about the immediate surroundings as individuals move through the environment and has been shown to have strong fitness consequences across diverse taxa (Dingemanse et al. 2003; Dingemanse et al. 2007; Avni and Eilam 2008; Pruitt et al. 2008). Avian exploratory behaviour, which is often coupled with aggressive and risk-taking behaviour in personalities (Groothuis and Carere 2005), has been linked to over-winter survival rates, extra-pair mating patterns and dispersal patterns (Dingemanse et al. 2003; Dingemanse et al. 2004; Van Oers et al. 2008). Previously Dingemanse et al. (2002) documented repeatability in exploratory behaviour in two great tit (*Parus major*) populations and found this trait to be both highly repeatable and heritable. Repeatability estimates, however, did not vary between populations.

Here we quantify geographic variation in repeatability in exploratory behaviour in a bird species, to understand the mechanisms which may control interpopulational differences in animal personalities. Under laboratory conditions, we quantified the exploratory behaviour, across multiple trials, of rufous-collared sparrows (*Zonotrichia capensis*) that originated from three populations across a strong climatic gradient (ranging from arid to mesic environments). This sparrow is a common species found throughout the entire central and south Americas, in a vast range of habitats (Ridgley and Tudor 1989). We also explored which factors can influence our estimates of exploratory behaviour, such as location of origin (i.e. geographic variation), time spent in captivity and familiarity with the experimental apparatus. We expected that birds originating from the arid environments display more thorough exploratory tendencies due to the lower availability of food resources in these habitats. Finally, we measured exploration with both captive and wild-caught birds to document whether the experimental protocol can influence estimates of exploratory behaviour in birds.

Methods

Study sites

We conducted novel environment experiments on rufous-collared sparrows from three Chilean populations – Copiapó, Santiago and Llanquihue. Copiapó (27°18'S, 70°25'W) is situated in an arid environment with little rainfall and high temperatures (mean maximum temperature: 15.2 °C, annual precipitation: 12 mm). Santiago (33°31'S, 70°50'W) is also located in an arid environment but experiences higher rainfall and lower temperatures (mean maximum temperature: 14.4 °C, annual precipitation: 312.5 mm). In sharp contrast, Llanquihue (41°16'S, 73°00'W) experiences both high rainfall and low temperatures (mean maximum temperature: 9.4 °C, annual precipitation: 3112 mm). To further understand environmental differences between the three populations we estimated primary productivity via the Martone aridity index (DMi) calculated via $DM = \frac{\mathrm{P}}{\mathrm{T}} + 10$, where P and T represent monthly precipitation (mm) and average monthly temperature (^{o}C), respectively (Cavieres and Sabat 2008). The annual mean and variance of this index is low in hot, dry deserts, and high in cool, wet areas. In addition, it has previously been shown that this index is strongly related to energy expenditure in rufous-collared sparrows (Cavieres and Sabat 2008). Our calculations demonstrated a north-south increase in primary productivity from Copiapó (DMi – $\overline{\mathrm{X}}$ = 10.10, σ^2=0.02), through to Santiago (DMi – $\overline{\mathrm{X}}$ = 11.44, σ^2=2.75) and Llanquihue (DMi – $\overline{\mathrm{X}}$ = 28.42, σ^2=119.54; climatic data obtained from www.worldclimate.com).

Laboratory based study

Individuals were captured passively with mist nets from the three field sites between April and May 2008 and housed under laboratory conditions at the Facultad de Ciencias, Universidad de Chile in Santiago, Chile. The sparrows were housed together (up to 4 individuals per cage) in plastic mesh cages (35 X 35 X 35 cm) measuring and provided with seed and water *ad libitum*. Temperature and photoperiod were maintained at 22 ± 2°C °C and 12L:12D, respectively. Individuals were allowed to settle in the laboratory for at least three days before the exploration assays (mean time before first trial: Copiapó – 3.0 ± 0.0 days; Santiago – 10.6 ± 0.9 days; Llanquihue – 23.5 ± 1.8 days). Although the three populations differed significantly in mean time elapsed between capture and time of the first trial (Kruskal-Wallis: Z=43.95, df=2, p<0.001), our analyses show that time since capture does not affect exploration behaviour (see Results). The exploration behaviour of each individual was quantified twice (mean time elapsed between first and second trial: Copiapó – 25.0 ± 0.0 days; Santiago – 10.7 ± 0.5 days; Llanquihue – 13.4 ± 1.0 days). Samples sizes for each population were N=16 (Copiapó), N=22 (Llanquihue) and N=19 (Santiago).

Exploration behaviour was quantified via novel environment experiments in a large field-portable cage (270 cm length x 150 cm width x 150 cm height) constructed of PVC poles and semi-transparent black shading cloth. Five wooden perches (80 cm in length, 2 cm in diameter) were hung throughout the cage at varying heights (between 50-110 cm above ground). One perch was placed diagonally in the opposite corner at each far end of the cage and the remaining three spaced at regular intervals (70 cm apart) along the long axis of the cage. The cage was placed in a fixed position within the laboratory.

Before each trial, the subject was placed in a small holding cage (30 cm length x 25 cm width x 39 cm height) in a corner of the experimental cage and covered with a cloth during a 5-minute acclimatization period. Each experimental trial lasted ten minutes. At the commencement of the experimental period, the cloth was removed and the door of the holding cage was opened. An observer, hidden from view but with full sight of the cage, dictated all the subjects' movements onto a digital voice recorder including: 1) number and destination of flights and 2) number and destination of hops. Flight and hop destination included both the perches and the walls of the cage. We also distinguished between cases where the birds landed on the front and back ends of the two side-walls. This resulted in 11 areas of the cage where the birds regularly perched (five wooden perches and six wall regions). At the termination of each trial, sparrows were recaptured within the cage using a butterfly net and returned to the housing.

To quantify variation in the exploratory behavior during the trial, we calculated three variables: 1) proportion of hops during the trial relative to the total number of hops and flights 2) exploration diversity and 3) exploration speed. Exploration diversity was quantified via the Shannon's diversity index, via $H=-\sum \rho_i \ln \rho_i$, where ρ represents the total number of times perch *i* was visited, expressed as a proportion of the total number of perch visits throughout the trial. Finally, exploration speed was calculated by summing the distances travelled between perches (when an individual hopped along the same perch or on the same wall, travel distance was estimated at 10 cm). Dividing the total distance covered during the trial by the duration of the trial period provided exploration speed (i.e. m/min). Individuals were assumed to be more thorough explorers when they hopped more frequently, explored more slowly and visited a higher diversity of perches during the trial.

Field-based study

In addition to quantifying exploration in the laboratory-based study, we were also interested in the effect of housing birds in captivity for an extended period on their exploratory behaviour. We therefore also quantified exploration of sparrows from Santiago at the source population immediately after capture to allow a comparison with exploratory behaviour of the same population that had been held in captivity. Field-based experiments occurred in August 2007. Individuals used for the field-based study were not used for the laboratory-based study to avoid familiarity with the cage confounding our results. We passively captured 12 individuals in mist nets and fitted each with a unique combination of coloured leg rings. Upon capture, each individual was introduced into the novel environment cage for a 10 minute period as previously described. All birds were released at the site of capture at the termination of the trial. The cage was placed in a fixed position within the study site.

Statistical analysis

All data were tested for normal distributions and transformed where necessary. Non-parametric tests used where transformations did not improve normality. In order to investigate the factors which influence inter-individual variation in exploratory behaviour we used general linear mixed models. Each measure of exploratory behaviour was included separately as a dependant variable. Time since capture (in days), trial number (first or second trial – a measure of familiarity with the experimental cage) and location of origin were included as fixed factors. As we included each individual twice in the analysis (i.e. for both the first and second trial) we also included individual identity as a random factor to avoid pseudoreplication biasing our results. Models had either normal errors with identity link (exploration diversity) or Poisson errors with logarithm link (proportion of hops, exploration speed). A range of models were created, consisting of different combinations of the fixed factors as main effects and as interactions. We then used Akaike's Information Criteria (AIC; (Akaike 1974) to select the most parsimonious statistical model. AIC is calculated as the model deviance plus twice the number of estimable parameters of the model (Burnham and Anderson 1998). The model resulting in the lowest AIC was considered the most parsimonious model and competing models with differences in AIC values of more than two were considered significantly different. However, when the AIC values for two competing models differed by less than two, we chose the model with the least number of parameters as the best fitting model (Quinn and Keough 2002).

Repeatability of exploratory behavior was estimated following (Lessells and Boag 1987) where repeatability is given by $r = \frac{S^2{}_A}{S^2{}_W + S^2{}_A}$ ($S^2{}_A$ is the among-groups variance component and $S^2{}_W$ is the within-group variance component). However, low repeatability in a given behaviour can either result from high *intraindividual* variability between trials or low *interindividual* variability. Therefore, to understand which sources of variation drive differences in repeatability between populations, we also calculated the intraindividual and interindividual coefficients of variation for each behaviour, where $CV = \frac{\text{Standard deviation}}{\text{Mean}}$.

Intraindividual CVs were obtained by calculating the CV for each individual (via the mean and standard deviation of the two trials) and taking the average for each behaviour and population. Interindividual CVs were calculated by averaging the values of each individual and behaviour and subsequently calculating the mean and standard deviation within each population. To compare differences in intraindividual CVs within behaviours, but across populations, we conducted general linear models, incorporating population as a fixed effect. Differences in interindividual CVs between populations were calculated following (Feltz and Miller 1996). GLMMs were conducted using Genstat 11.0 and all other analyses in SPSS 15.0 and Systat 12.0. Data are presented as means ± SE.

Results

1. Characteristics of exploration behaviour

During the novel environment experiments, the rufous collared sparrows displayed large variability in their responses upon release into the novel environment cage. Individuals typically flew and hopped throughout the cage, moving between both the wooden perches and mesh walls (mean number of movements to perches = 18.7±2.3 movements; mean number of movements to walls = 47.1±5.4 movements). On average, the sparrows utilized 4.2±0.2 of the 11 available surfaces to perch on (N = 114 trials, range = 1-11 perches utilized) and, although they typically only remained stationary on each perch for a few seconds, some individuals stayed stationary on a single perch for the majority of the trial (mean time spent stationary on a perch = 6.1±0.2 SE seconds, N=8 530, range = 1 - 548 seconds). There was no difference in the number of times each individual hopped or flew during the trials (mean number of flights throughout trial: 33.1 ± 4.4 SE flights; hops: 34.4 ± SE 4.5 hops; Wilcoxon signed rank test: Z=-0.516, N=139, P=0.606).

The correlation matrices between the three measures of exploratory behaviour showed some variability between populations (Table 1). Firstly, in no populations was exploration diversity related to proportion of hops. However, intercorrelations between exploration speed with proportion of hops and exploration diversity were similar for sparrows from Santiago and Llanquihue, but always differed for sparrows originating from Copiapó.

2. Sources of variation in exploratory behaviour

The factors influencing variation in exploratory behaviour depended on what measure of exploration was used. Firstly, the most parsimonious model describing variation in proportion of hops only included location of origin (Table 2; AIC of the model = 59.2 vs. 61.2 for second most parsimonious model [Trial number]). Individuals from Llanquihue hopped the most often, while those from Santiago hopped the least. Variation in the exploration diversity was significantly influenced by location of origin, trial number and the interaction between the two terms (Table 2; AIC of the model = -41.3 vs. -31.3 for second most parsimonious model [Trial number]). The effects of each factor indicate that the sparrows visited a higher diversity of perches during the second trial. However, the interaction between trial number and location of origin indicated that the effect of trial number on exploration diversity was highest for Llanquihue and lowest for Santiago. Similarly, the most parsimonious model for exploration speed included trial number, origin and the interaction between the two terms (Table 2; AIC of the model = 142.9 vs. 148.1 for second most parsimonious model [Time since capture]). Individuals from Llanquihue explored more slowly than those from Copiapó and Santiago. In addition, all individuals explored faster during the second trial, although the effect of trial number was greatest for the birds from Copiapó.

A comparison of exploration behaviour of individuals from Santiago quantified under different conditions revealed that the behaviour of individuals measured under field conditions did not differ significantly from those measured in the laboratory (proportion of hops: field – 0.21 ± 0.06, laboratory – 0.35 ± 0.06: $F_{1,31}$=2.415, P=0.130; exploration diversity: field – 0.99 ± 0.14, laboratory – 1.02 ± 0.11: $F_{1,31}$=0.019, P=0.890; exploration speed: field – 4.8 ± 1.1 m/min, laboratory – 3.8 ± 1.0 m/min: $F_{1,31}$=0.432, P=0.516).

3. Repeatability in exploratory behaviour

The repeatability of exploratory behaviour varied between the three populations (Table 3). Proportion of hops was moderately repeatable between trials for individuals from Copiapó and Santiago, but not Llanquihue. Similarly, exploration diversity was highly repeatable between trials for Copiapó and Santiago but not

Llanquihue. Exploration speed was highly repeatable for all three populations. Inter-populational variation in the repeatability of proportion of hops seemed not to be related to differences in intra-individual variation (Z=0.249, df=2, P=0.883). Therefore, the consistency of the behaviour between trials was similar for individuals from all populations. In contrast, the interindividual variation in proportion of hops was substantially lower in Llanquihue (where no repeatability was detected). However this difference was not statistically different between the three populations (Z=3.017, df=2, p<0.25) although the difference between Santiago and Llanquihue approached significance (Z=2.921, df=1, p<0.10). In sharp contrast, differences in repeatability in exploration diversity appeared to be related to higher intraindividual variation rather than lower interindividual variation in the behaviour by birds from Llanquihue. Interindividual variation in exploration diversity was similar between populations (Z=1.745, df=2,p<0.50), while differences in intraindividual variation between populations approached significance (Z=5.320, df=2, P=0.070) with individuals from Llanquihue being less consistent in exploration diversity between trials than those from Copiapó (Z=-1.953, df=1, P=0.051) and Santiago (Z=-1.936, df=1, P=0.053). There were no interpopulational differences in neither interindividual nor intraindividual coefficients of variation for exploration speed (interindividual variation: Z=0.349, df=2, p<0.900; intraindividual variation: Z=1.951, df=2, P=0.377).

Table 1. Correlation matrix for the three measures used to describe exploratory behaviour of three populations of rufous collared sparrows during novel environment experiments. For all tests N=16 for Copiapó, N=19 for Santiago and N=22 for Llanquihue. Probability of finding three statistically significant tests (with $p \leq 0.017$) out of 9 due to chance alone (calculated via a Bernoulli process; (Moran 2003): P=0.00037.

	Population	Proportion of hops		Exploration Diversity	
		R	P	r	P
Exploration Diversity	Copiapó	-0.084	0.757		
	Santiago	-0.409	0.082		
	Llanquihue	0.270	0.225		
Exploration speed	Copiapó	-0.586	0.017	0.228	0.396
	Santiago	-0.339	0.156	0.596	0.007
	Llanquihue	-0.009	0.970	0.597	0.003

Table 2. Sources of interindividual variation in three measures of exploratory behaviour of rufous collared sparrows during novel environment experiments. Results are from GLMMs including individual identity as a random factor and location of origin, time since capture, trial number and mass at capture as fixed factors. The most parsimonious model explaining variation in each measure was selected via the Akaike's Information Criterion (see main text for explanation).

	Factor	Effect	Wald	P
Proportion of hops [a]	Constant	-0.848±0.149		
	Location (Copiapó/Santiago/Llanquihue)	0.000/-0.191/0.300	6.88	0.039
Exploration diversity [b]	Constant	0.916±0.102		
	Location (Copiapó/Santiago/Llanquihue)	0.000/-0.015/0.154	2.04	0.367
	Trial number	0.495±0.117	35.44	<0.001
	Location*Trial number (Copiapó/Santiago/Llanquihue)	0.000/-0.632/0.246	34.86	<0.001
Exploration speed [c]	Constant	1.308±0.242		
	Location (Copiapó/Santiago/Llanquihue)	0.000/-0.373/-0.664	6.16	0.054
	Trial number	1.305±0.186	30.22	<0.001
	Location*Trial number (Copiapó/Santiago/Llanquihue)	0.000/-1.411/-0.559	29.62	<0.001

a. Random effect = 0.157±0.082, b. Random effect = 0.118±0.035, c. Random effect =0.788±0.199

Table 3. Differences between populations in repeatability (r) of exploratory behaviour by rufous collared sparrows during two novel environment experiment trials. Intraindividual and interindividual coefficients of variation in the measures allow the comparison of repeatability between behaviours. See main text for further explanations of how repeatability and coefficients of variation were calculated.

<table>
<tr><td rowspan="2">Population</td><td colspan="6">Proportion of hops</td><td colspan="6">Exploration diversity</td><td colspan="6">Exploration speed</td></tr>
<tr><td colspan="2">r</td><td colspan="2">F</td><td colspan="2">P</td><td colspan="2">r</td><td colspan="2">F</td><td colspan="2">P</td><td colspan="2">r</td><td colspan="2">F</td><td colspan="2">P</td></tr>
<tr><td>Copiapó</td><td colspan="2">0.549</td><td colspan="2">3.458</td><td colspan="2">0.084</td><td colspan="2">0.932</td><td colspan="2">28.731</td><td colspan="2"><0.001</td><td colspan="2">0.909</td><td colspan="2">21.029</td><td colspan="2"><0.001</td></tr>
<tr><td>Santiago</td><td colspan="2">0.604</td><td colspan="2">4.048</td><td colspan="2">0.061</td><td colspan="2">0.906</td><td colspan="2">20.327</td><td colspan="2"><0.001</td><td colspan="2">0.840</td><td colspan="2">11.536</td><td colspan="2">0.003</td></tr>
<tr><td>Llanquihue</td><td colspan="2">-0.202</td><td colspan="2">0.664</td><td colspan="2">0.423</td><td colspan="2">-0.170</td><td colspan="2">0.709</td><td colspan="2">0.410</td><td colspan="2">0.829</td><td colspan="2">10.687</td><td colspan="2">0.004</td></tr>
<tr><td></td><td colspan="3">Intraindividual CV</td><td colspan="3">Interindividual CV</td><td colspan="3">Intraindividual CV</td><td colspan="3">Interindividual CV</td><td colspan="3">Intraindividual CV</td><td colspan="3">Interindividual CV</td></tr>
<tr><td>Copiapó</td><td colspan="3">75.6</td><td colspan="3">70.2</td><td colspan="3">35.4</td><td colspan="3">43.1</td><td colspan="3">71.2</td><td colspan="3">132.1</td></tr>
<tr><td>Santiago</td><td colspan="3">69.7</td><td colspan="3">72.8</td><td colspan="3">48.5</td><td colspan="3">53.1</td><td colspan="3">53.4</td><td colspan="3">113.1</td></tr>
<tr><td>Llanquihue</td><td colspan="3">65.5</td><td colspan="3">44.1</td><td colspan="3">65.8</td><td colspan="3">35.6</td><td colspan="3">64.0</td><td colspan="3">98.9</td></tr>
</table>

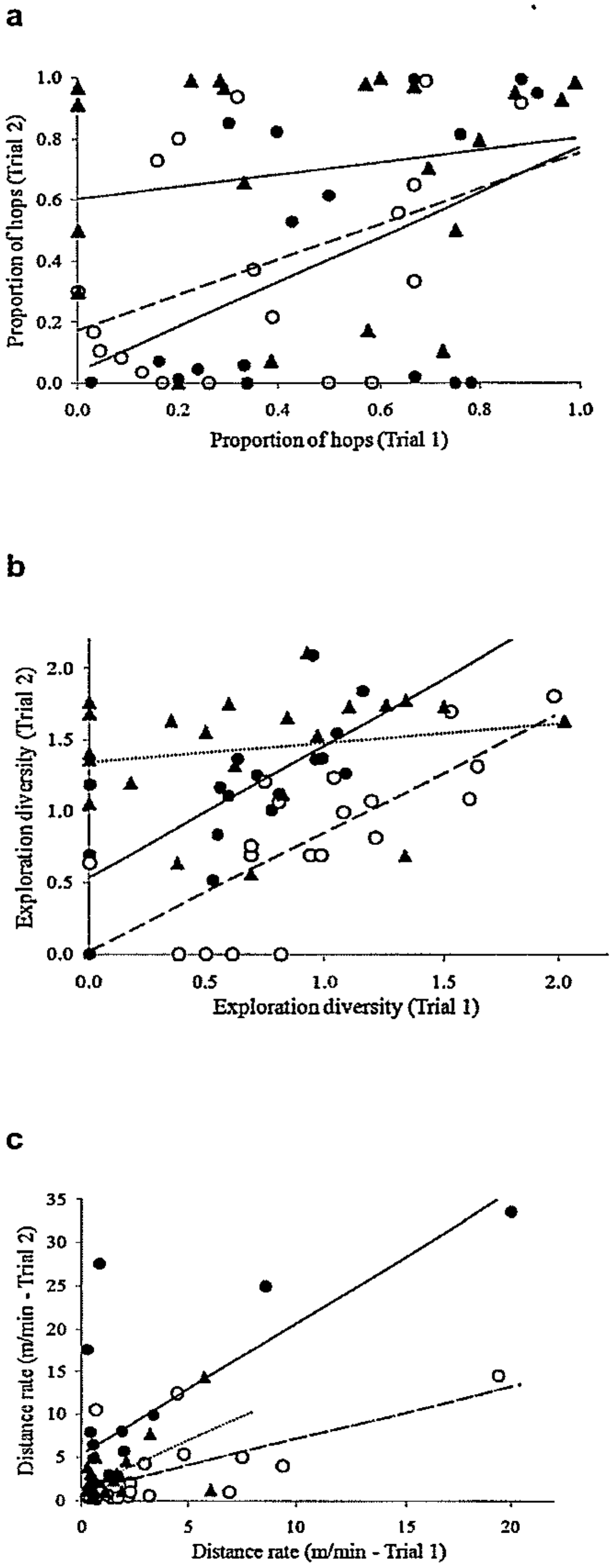


Figure 1. Interpopulational differences in the repeatability of a) proportion of hops, b) exploration diversity and c) exploration speed of rufous collared sparrows between trials of the novel environment experiments. Individuals from Copiapó are represented by filled circles and solid line, Santiago by open circles and dashed lined and Llanquihue by filled triangles and dotted line.

Discussion

We have shown here that exploratory behaviour in rufous-collared sparrows is a complex, multi-component behaviour. Firstly, we have identified a novel measure to estimate exploration, exploration diversity, which has been thus far ignored by other researchers. Exploration diversity would intuitively be a reliable indicator of true exploratory tendency, as an individual in an unknown environment (or indeed a known environment where the location of available food sources are unknown) would benefit from exploring a greater diversity of areas, rather than only exploring a subset of the available area. Secondly, we show that several factors can explain inter-individual variation in exploratory behaviour. During second trials, individuals explored a greater diversity of perches and explored more slowly, suggesting that familiarity with the experimental apparatus can influence estimates of exploratory behaviour. However, the conditions under which exploration was quantified (i.e. under field or laboratory conditions), nor time spent in captivity, affected our estimates of exploration, justifying the use of temporarily captive birds to estimate exploration under field conditions. Location of origin also influenced exploratory tendencies, supporting the prediction that interpopulational differences in local environment pressures (e.g. food availability) may promote variation in exploration. Finally, our three measures of exploration tended to be highly repeatable between trials (with estimates of up to 0.93) suggesting that within individual variation in exploration is much lower than between individual variation in exploration. However, marked variation existed in the repeatability estimates, both between measures and populations, highlighting the complexity of this behaviour.

Dingemanse et al. (2002) have previously demonstrated moderately high repeatability estimates for exploration in great tits (*Parus major*) with estimates ranging from 0.27 to 0.66. However, this is the first study to demonstrate that repeatability in exploration can vary both between populations and between different measures of exploratory behaviour. Since repeatability is calculated as the relative contribution of within and amongst-individual variation, it is important to document the sources of variation in the traits of interest. Proportion of hops was only moderately repeatable in the two most northern populations, but not in Llanquihue. The low repeatability in this measure appeared to be due to low variability between individuals in this population. In contrast, the low repeatability of exploration diversity in Llanquihue appeared to be due to high intraindividual variability in the measure (i.e. low consistency between trials). Although the causes of this geographic variation in repeatability are as yet unknown, it provides some interesting insights into the selective pressures acting upon exploration between populations. As repeatability in a behaviour generally sets an upper limit to heritability of the trait (Lessells and Boag 1987; Dohm 2002), our data suggest that the heritability of exploration is much higher in Copiapó and Santiago. This could be related to the lower primary productivity in these populations (as estimated via the Martone aridity index) and hence stronger selective pressures for consistency and heritability in the ability to find scarce resources. Interestingly, contrary to our prediction, individuals in Llanquihue appeared to be more exploratory than the northern populations. One possibility is that exploration is genetically coupled with another behaviour that also varies between the populations. For example, exploratory behaviour is known to be frequently linked with variation in aggressive behaviour (Groothuis and Carere 2005). Amongst *Zonotrichia* species, aggression is known to vary markedly along latitudinal gradients (Wingfield et al. 2007). Exploration may therefore be evolutionarily constrained and not vary predictably with food resources if the selective pressures on variation in aggression is greater. It is also, however, possible that interpopulational differences in exploration are non-adaptive and only arise due to stochastic genetic processes (Armbruster and Schwaegerle 1996).

An alternate interpretation to our data is that, what we have categorised as exploratory behaviour, may in fact represent varying levels of fear and flight response (Renner 1990; Hughes 1997). This is, in part, supported by our finding that individuals changed their behaviour during the second trial, when fear levels may have diminished with increased familiarity with the experimental setup. Despite possible confounding effects of fear, movement throughout the cage is still likely to be a reliable estimate of exploration since it is likely to result in information acquisition by the birds regardless of the individual's state of stress (Russell 1983). Moreover, we found that keeping the birds in captivity does not appear to affect exploratory behaviour when compared to birds that had been recently captured. Finally, increasing studies using similar techniques have demonstrated that exploration measured under laboratory conditions is strongly related with individual fitness in wild bird (Dingemanse et al. 2003; Dingemanse et al. 2004; Van Oers et al. 2008).

Our findings have important implications for our current understanding of the selective pressures acting upon animal personalities. As personalities typically consist of a suite of interrelated behaviours, it is expected that the characteristics of population-specific personalities will be directly dependant on the repeatability, and hence heritability, of each personality trait. Our finding that repeatability of exploration, a common personality trait, can vary between populations, suggests that the selective pressures acting upon this trait and its association with any other behaviours can also differ between populations. The repeatability of one personality trait in a particular population could therefore directly influence the personality characteristics of that population. These findings also have important implications for studies that assume no intraspecies or inter-population variation in exploratory behaviour repeatability and highlight the importance of reporting repeatability estimates in studies of behavioural ecology.

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GLOSARIO

Entre paréntesis se indica la sigla utilizada en el texto. Corresponde a la abreviación del término en inglés.

Amplitud total del nicho poblacional (TNW): Suma del componente inter-individual (BIC) e intra-individual (WIC) del nicho poblacional. Representa la variabilidad total de recursos consumidos por una población.

Componente inter-individual del nicho poblacional (BIC): Varianza del promedio de cada individuo en la utilización de recursos. Representa las diferencias entre individuos en la utilización de recursos.

Componente intra-individual del nicho poblacional (WIC): Promedio de la varianza de cada individuo en la utilización de recursos. Representa la variabilidad de recursos utilizada por el individuo promedio.

Flexibilidad fenotípica: Variación reversible en el fenotipo de un individuo iniciada por cambios en el ambiente externo.

Grado de especialización individual (WIC/TNW): Cociente entre el componente intra-individual (WIC) del nicho poblacional y la amplitud del nicho poblacional (TNW). Representa el nivel de subdivisión del nicho poblacional debido a las diferencias entre individuos en la utilización de recursos.

Individuo especialista: Individuo cuyo nicho es sustancialmente más estrecho que su nicho poblacional por razones no atribuibles a su sexo, edad o grupo morfológico discreto.

Pérdida total de agua evaporativa (TEWL): Suma de la pérdida de agua respiratoria y cutánea de un organismo.

Personalidad animal: Conjunto de conductas correlacionadas de un individuo que persisten a través de un amplio rango de contextos.

Tasa metabólica basal (BMR): Tasa metabólica medida en un adulto no reproductivo, en la zona de termoneutralidad y en estado post-absortivo, durante la fase inactiva de su ciclo circadiano, con una temperatura corporal en un nivel normotérmico. Representa la energía mínima necesaria para mantener la homeostasis en animales endotermos.