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RESUMEN

Los colibríes (Trochilidae) son las aves más especializadas en el consumo de néctar floral. El néctar es una solución de azúcares y agua. Al ser rico en energía, los colibríes cubren prácticamente todos sus requerimientos energéticos y construyen reservas de grasa a partir de este simple alimento. Sin embargo, el néctar contiene cantidades traza de otros nutrientes tales como proteínas, aminoácidos, vitaminas y minerales. Por lo tanto, los colibríes complementan su dieta con pequeños artrópodos para cubrir todas sus necesidades nutricionales. La mayoría de las especies de colibríes (~90%) consumen artrópodos regularmente para cubrir sus requerimientos mínimos de nitrógeno y mantener sus pesos corporales. Para lograr esto, los colibríes dedican entre el 2 y 15% de su tiempo de forrajeo diario a la captura de artrópodos. Sin embargo, los colibríes pueden incrementar sustancialmente su consumo de artrópodos en respuesta a la variación estacional en la disponibilidad del néctar. A pesar de la importancia que los artrópodos pueden tener como un alimento complementario o alternativo para los colibríes, en general, sabemos poco sobre los factores que afectan su consumo en la naturaleza, y del papel que los artrópodos juegan como fuentes de energía en los momentos del año en los que el néctar es poco abundante o no está disponible. Adicionalmente, a la fecha, no sabemos nada sobre las estrategias de regulación de consumo de nutrientes empleadas por los colibríes, y consecuentemente, de la relación entre las estrategias de balance de nutrientes y algunos atributos de sus ensambles.

El estudio de la importancia relativa de los artrópodos para los colibríes no ha sido una tarea fácil, debido principalmente: 1) a la dificultad para realizar observaciones focales de colibríes individuales por periodos largos de tiempo, 2) al pequeño tamaño corporal de

las presas seleccionadas (1-4 mm de longitud), 3) a la dificultad para determinar si las capturas de artrópodos son exitosas, y 4) a la falta de protocolos adecuados para cuantificar la disponibilidad de artrópodos en el ambiente. Los pocos trabajos que se han realizado sobre el uso de artrópodos por colibríes (hasta donde sabemos, únicamente 10 estudios publicados a la fecha) se han enfocado en conocer: 1) la diversidad de las presas de artrópodos ingeridas, 2) el papel de la morfología del pico y las alas de los colibríes sobre las tácticas de forrajeo de artrópodos, 3) el efecto de la disponibilidad del néctar sobre la ingestión de artrópodos, y 4) el papel de los artrópodos como una fuente de energía para los colibríes.

En la presente Tesis, se incluye una revisión sobre el uso de alimentos alternativos por las tres familias de aves nectarívora más especializadas: colibríes, aves sol y comedores de miel. Adicionalmente, se abordan tres temas sobre la importancia de los artrópodos para los colibríes, los cuales han sido poco o nada estudiados: 1) el papel que los artrópodos juegan como un alimento complementario y alternativo para los colibríes que viven en un ecosistema estacional, 2) la regulación del consumo de proteína y azúcar por colibríes, y 3) el papel de las micropiedras (“grit”) en la digestión mecánica de los artrópodos ingeridos por los colibríes. Esta Tesis esta conformada por una introducción general y cuatro capítulos. Los manuscritos de los tres primeros capítulos están en preparación para su publicación, mientras que el cuarto capítulo es un artículo científico que fue publicado recientemente en una revista indexada.

En el primer capítulo, titulado **“Una revisión sobre el uso de alimentos alternativos por aves nectarívoras”** se explora el papel que diferentes factores intrínsecos (morfología del pico y la lengua y tamaño corporal) y extrínsecos (disponibilidad de recursos alimenticos) juegan sobre el consumo de alimentos alternativos al néctar, tales como artrópodos y fruta, por colibríes, aves sol y comedores de miel. En esta revisión, encontramos que la morfología de las lenguas y picos juega un papel central en el consumo de diferentes tipos de alimentos por estos grupos de aves. Adicionalmente, el peso corporal de las aves nectarívoras de las tres familias se correlaciona positivamente con sus tasas metabólicas totales, y con sus requerimientos de nitrógeno, afectando sus necesidades de nutrientes y energía, y definiendo su dieta. Por lo tanto, las especies más grandes presentan dietas más diversas que las especies pequeñas. En estas aves, el consumo de alimentos alternativos al néctar es más importante cuando: 1) estos alimentos son más abundantes o presentan una mayor calidad nutricional; 2) cuando la abundancia del néctar en el ambiente es baja o no está disponible; y/o 3) durante el periodo reproductivo y la crianza, cuando los requerimientos de nutrientes, principalmente de proteínas y energía, son mayores.

En el segundo capítulo, titulado **“Ingestión de artrópodos en un ensamble de colibríes”** (Arthropod ingestion in a high altitude hummingbird ensemble), determinamos el uso de artrópodos por diferentes especies de colibríes (*Colibri thalassinus*, *Lampornis amethystinus*, *Bassilina leucotis*, *Eugenes fulgens*, *Selasphorus platycercus* y *S. rufus*) en un ecosistema estacional del oeste de México, y exploramos el efecto de la variación estacional de la disponibilidad de los recursos alimenticios sobre el consumo de artrópodos. Encontramos que los colibríes ingirieron presas pertenecientes a los ordenes Araneae, Hemiptera, Psocoptera, Hymenoptera y Diptera. También encontramos que la tasa de intentos de capturas de artrópodos de *B. leucotis*, la única especie que observamos forrajeando para artrópodos durante todas las estaciones, fue significativamente mayor durante la estación seca-caliente, cuando el néctar no estuvo disponible y la abundancia de artrópodos fue más alta, que en las estaciones lluviosa y seca-fría, cuando el néctar estuvo disponible y las abundancias de artrópodos fueron menores. Adicionalmente, encontramos una relación positiva y significativa entre la abundancia de artrópodos en el ambiente y la tasa de intentos de capturas de artrópodos de *B. leucotis*. Nuestros resultados indica que, en ausencia de néctar, algunas especies de colibríes son capaces de usar a los artrópodos como un alimento alternativo, manteniendo poblaciones residentes en nuestro sitio de estudio, mientras que las especies que no son capaces de sobrevivir con dietas altas en proteína se ven forzadas a migrar localmente en búsqueda de recursos florales, modificándose la estructura del ensamble de colibríes.

En el tercer capítulo, titulado **“Análisis geométrico nutricional del consumo de proteína por colibríes”** (Life is not always sweet! A nutritional geometry analysis of protein intake by hummingbirds), llevamos a cabo un estudio de geometría nutricional en el laboratorio. La geometría nutricional es una herramienta metodológica que permite estudiar la selección de alimentos y las estrategias de balance del consumo de nutrientes (es decir, carbohidratos, lípidos, proteínas) utilizadas por los animales, determinando las equivalencias de los nutrientes en unidades de energía. De este modo, nuestro diseño experimental nos permitió explorar el papel que la proteína tiene como un alimento complementario o alternativo. Si los colibríes consumen proteína únicamente para cubrir sus requerimientos de nitrógeno, estarían utilizando a la proteína como un alimento complementario, mientras que, si consumen mayores cantidades de este nutriente, podrían estar utilizándolo como un alimento alternativo (fuente de energía). Utilizamos como modelos de estudio a dos especies de colibríes (*Cynanthus latirostris* y *Saucerottia* *beryllina*) que difieren en su comportamiento de forrajeo y patrones de movimiento local. Encontramos que los individuos de ambas especies de colibríes mantuvieron constante el consumo de la relación proteína:sacarosa (durante un periodo de 13 días), en lugar de priorizar el consumo de un único nutriente. Aunque la proporciones proteína:sacarosa seleccionadas por los individuos de *C. latirostris* y *S. beryllina* no fueron significativamente diferentes entre las dos especies, los consumos diarios y acumulados de proteína y de sacarosa por unidad de peso metabólico fueron significativamente mayores en *C. latirostris* que en *S. beryllina*. Nuestros resultados sugieren que la mayor capacidad de los individuos de *C. latirostris* para consumir y metabolizar proteína, les permite utilizar a los artrópodos como una fuente de energía y permanecer como residentes en un sitio cuando los recursos florales son poco abundantes o no están disponibles, mientras que los individuos de *S. beryllina* se ven forzados a migrar localmente a sitios donde hay una mayor disponibilidad de néctar.

Por último, en el cuarto capítulo titulado **“Ingestión de micropiedras por colibríes”** (True grit: Ingestion of small stone particles by hummingbirds in West Mexico), exploramos si la ingestión de micropiedras (“grit” en inglés) está relacionada con la ingestión de artrópodos. El uso de micropiedras por colibríes ha sido poco documentado, y se ha planteado que son ingeridas por estas aves para ayudar a la digestión mecánica de los artrópodos ingeridos, y/o para la obtención de micronutrientes específicos como calcio. En este estudio cuantificamos el número de micropiedras ingeridas por los miembros de un ensamble de colibríes (*Colibri thalassinus*, *Lampornis amethystinus*, *Bassilina leucotis*, *Eugenes fulgens*, *Selasphorus platycercus* y *S. rufus*) en diferentes estaciones de un año. Relacionamos la cantidad de micropiedras encontradas en los estómagos con la biomasa de los artrópodos ingeridos y con su contenido de quitina. Encontramos que las micropiedras fueron ingeridas principalmente por las hembras, con solamente un macho de una especie (*C. thalassinus*) presentando micropiedras en su estómago. Adicionalmente, encontramos que los colibríes únicamente ingirieron micropiedras durante la estación lluviosa y la seca-fría, cuando se reproducen y anidan. En cambio, durante la estación seca-caliente, cuando la disponibilidad y consumo de artrópodos fueron más altas, no encontramos micropiedras en los estómagos de los colibríes. Por último, no encontramos relaciones significativas entre el número de micropiedras ingeridas por las diferentes especies de colibríes, y la biomasa y el contenido de quitina de los artrópodos ingeridos. Estos resultados soportan la hipótesis de que las micropiedras son usadas por los colibríes, específicamente por las hembras, para cubrir requerimientos de micronutrientes asociados a la reproducción. Sin embargo, encontramos que los individuos de ambos sexos de *C. thalassinus*, además de presentar una mayor cantidad de micropiedras en sus estómagos, ingirieron presas de cuerpos más duros, sugiriendo que en esta especie las micropiedras podrían tener el papel adicional de ayudar en la digestión mecánica de los artrópodos ingeridos.

ABSTRACT

Hummingbirds are the most specialized nectar-feeding birds. Nectar is a solution of sugars and water. Being rich in energy, hummingbirds meet practically all their energy requirements and built fat reserves from this simple food. However, nectar contains trace amounts of other nutrients such as proteins, amino acids, vitamins, and minerals; therefore, hummingbirds supplement their diet of nectar with small arthropods to meet their needs for these nutrients. Most hummingbird species (~90%) ingest arthropods regularly to cover their minimum nitrogen requirements and maintain their body masses. To achieve this, hummingbirds spend between 2 and 15% of their daily feeding time capturing arthropods. However, hummingbirds can substantially increase their arthropod consumption in response to seasonal variation in the availability of floral resources. Despite the importance of arthropods as a complementary or alternative food for hummingbirds, in general, we know little about the factors that affect their ingestion in the field, and their role as an energy source, particularly during periods of the year when nectar is scarce or not available. Additionally, to date, we do not know anything about the nutrient intake regulation strategies used by these birds, and consequently, about the relationship between nutritional strategies and some attributes of hummingbird ensembles.

The study of the relative importance of arthropods for hummingbirds in nature has not been easy, mainly due to: 1) the difficulty of making focal observations of hummingbird individuals for long periods, 2) the small body size of the selected prey items, 3) the difficulty to determining if arthropod captures are successful, and 4) to the lack of adequate protocols to measure arthropod availability in the environment. The few studies that have been conducted on the use of arthropods by hummingbirds (as far as we know,

about 10 studies published to date) have focused on understanding: 1) the diversity of ingested arthropod prey, 2) the role that hummingbird wing and bill morphology play on arthropod foraging tactics, 3) the effect of nectar availability on arthropod use by hummingbirds, and 4) the arthropods role as an energy source for hummingbirds.

This Doctoral Thesis includes a review on the use of alternative foods by specialized nectar-feeding birds: hummingbirds, sunbirds, and honeyeaters. In addition, three topics are addressed on the importance of arthropods for hummingbirds, which have received little or no study, and which are not mutually exclusive: 1) the role that arthropods play as a complementary and alternative food for hummingbirds inhabiting a seasonal ecosystem, 2) the regulation of protein and sugar intake by hummingbirds, and 3) the role of small stones (“grit”) in the mechanical digestion of ingested arthropods by hummingbirds. This Thesis consists of a general introduction and four chapters. The chapters one, two, and three, are manuscripts in preparation for publication, while the fourth chapter is a recently published research paper.

In the first chapter, entitled **“A review on the use of alternative foods by nectar-feeding birds”** we explore the role that different intrinsic (bill and tongue morphology and body size) and extrinsic (food availability) factors play in the use of alternative foods, such as arthropods and fruit, by hummingbirds, sunbirds, and honeyeaters. In this review, we found that the morphology of tongues and bills plays an important role in the consumption of different types of foods by these bird groups. Additionally, the body mass of the nectar-feeding birds of the three families is positively correlated with the total metabolic rates, and nitrogen requirements, affecting their nutrient and energy needs, and defining their diet.

Therefore, larger species have more diverse diets than small species. In nectar-feeding birds, the consumption of alternative foods to nectar is more important when: 1) these foods are more abundant or have higher nutritional quality, 2) when the abundance of nectar is low or not available, and 3) during the breeding period when the nutrient requirements, mainly protein, are higher.

In the second chapter, entitled **"Arthropod ingestion in a high altitude hummingbird ensemble"** we determine the use of arthropods by different species of hummingbirds (*Colibri thalassinus*, *Lampornis amethystinus*, *Bassilina leucotis*, *Eugenes fulgens*, *Selasphorus platycercus*, and *S. rufus*) in a seasonal ecosystem of West Mexico, and we explored the effect of seasonal variation in food resources availability on arthropod ingestion. We found that hummingbirds ingested prey items belonging to the orders Araneae, Hemiptera, Psocoptera, Hymenoptera, and Diptera. We also found that the arthropod capture-attempts rate of *B. leucotis*, the only species we observed foraging for arthropods during all three climatic seasons, was significantly higher during the warm-dry season when nectar was not available and the abundance of arthropods was higher than in the rainy and cold-dry seasons when nectar was available and the arthropod abundances were lower. Additionally, we found a positive and significant relationship between the abundance of arthropods in the environment and the arthropod capture-attempts rate of *B. leucotis*. Our results suggest that, in absence of nectar, some hummingbird species can use arthropods as an alternative food, maintaining resident populations at our study site, while species that are not able to survive on rich-protein diets are forced to migrate locally in search of floral resources, modifying the structure of the hummingbird ensemble.

In the third chapter, entitled **"Life is not always sweet! A nutritional geometry analysis of protein intake by hummingbirds"** we perform a geometric analysis of nutrients in the laboratory. The nutritional geometric approach allows for determining the strategies of the balance of the consumption of nutrients (i.e., proteins, carbohydrates, fats) used by animals, determining the equivalences of nutrients in energy units. We performed free-choice feeding trials, offering solutions varying in protein (0.2%-2%) and sucrose (5%-30%) concentrations to compare the nutritional strategies of Broad-billed (*Cynanthus latirostris*) and Berylline (*Saucerottia beryllina*) Hummingbirds. Our experimental design allowed us to explore the role of protein as a complementary or alternative food. If hummingbirds consume protein only to cover their nitrogen requirements, they would be using protein as a complementary food, while if they consume higher amounts of protein, they could be using it as an alternative food (i.e., as an energy source). We found that individuals of both species maintained constant the consumption of the protein:sugar ratio, rather than prioritizing the consumption of a single nutrient. Although the protein:sugar ratios selected were not significantly different between the two hummingbird species, we found that daily and cumulative protein and sugar intakes per unit of metabolic mass were significantly higher in *C. latirostris* than in *S. beryllina*. Our results suggest that the greater capacity of *C. latirostris* individuals to consume and metabolize protein allows them to use arthropods as an energy source and remain resident in a site when floral resources are scarce or unavailable, while *S. beryllina* individuals are forced to migrate locally to other sites where the floral resources are abundant.

Finally, in the fourth chapter entitled **"True grit: Ingestion of small stone particles by hummingbirds in West Mexico"** we explored whether the consumption of

small stones ("grit") is related to the ingestion of arthropods. The use of grit by hummingbirds has been little documented, and it has been suggested that they are consumed by these birds to facilitate the mechanical digestion of ingested arthropods, and/or to obtain specific micronutrients such as calcium for egg-shell production. In this study, we quantified the number of grit particles ingested by members of a hummingbird ensemble (*Colibri thalassinus*, *Lampornis amethystinus, Bassilina leucotis, Eugenes fulgens*, *Selasphorus platycercus*, and *S. rufus*) in different seasons of a year. We related the number of grit particles found in hummingbird stomachs with the biomass of ingested arthropods and their chitin content. We found that grit particles were ingested mainly by female individuals, with only a male of one species (*C. thalassinus*) presenting grit in its stomach. The use of grit by hummingbirds was seasonal, with grit in their stomach being present during the rainy and cold-dray seasons when breeding occurs. In contrast, during the warm-dry season, when both arthropod availability and arthropod ingestion were higher, we did not find grit particles in hummingbird stomachs. Finally, we did not find significant relationships between the number of grit particles in hummingbird stomachs and the biomass of ingested arthropods and arthropod chitin content. These results support the hypothesis that grit particles are used by female hummingbirds to cover their micronutrient requirements associated with breeding. However, we found that individuals of both sexes of *C. thalassinus,* in addition to presenting the greater amount of grit particles in their stomach, ingested the largest amount of hard-bodied prey, suggesting that in this species grit plays an additional role as a grinding agent.

INTRODUCCIÓN GENERAL

Los colibríes son un grupo de aves especializadas en el consumo de néctar floral. El néctar es una solución de azúcares y agua que presenta cantidades traza de aminoácidos, vitaminas y minerales. Al ser rico en energía, los colibríes cubren prácticamente todos sus requerimientos energéticos y construyen reservas de grasa a partir de este simple alimento. Sin embargo, el néctar impone dos desafíos para los colibríes. Primero, estas aves deben de ser capaces de manejar grandes volumen agua conservando electrolitos, y segundo, deben de complementar su dieta con artrópodos para alcanzar un balance nutricional y cubrir todos sus requerimientos nutricionales.

En términos nutricionales, los artrópodos son ricos en proteínas, ya que estas constituyen, en promedio, alrededor del 60% de su peso seco. Los artrópodos también son ricos en ácidos grasos, aunque su contenido varía ampliamente con su estado de desarrollo (5-85% del peso seco). Adicionalmente, las vitaminas y minerales constituyen alrededor del 5% del peso seco de los artrópodos. Los artrópodos también son ricos en energía, ya que 1 g de una dieta de artrópodos suministra ~19.3 kJ de energía metabolizable para las aves que los consumen.

En general, los colibríes ingieren pequeños artrópodos de 1-4 mm de longitud pertenecientes a una variedad de grupos taxonómicos, incluyendo ácaros, arañas, colémbolos, saltamontes, termitas, chinches, psocópteros, escarabajos, avispas, moscas, y pequeñas mariposas, capturándolos directamente de sustratos o en el aire. Para cubrir sus requerimientos de nitrógeno, los colibríes dedican entre el 2 y 15% de su tiempo de forrajeo diario a la captura de artrópodos. Sin embargo, los colibríes pueden incrementar sustancialmente su consumo de artrópodos durante el periodo reproductivo y de anidación y durante los periodos del año en los que el néctar es poco abundante o no esta disponible. Bajo estos escenarios, el tiempo de forrajeo utilizado por los colibríes para la captura de artrópodos puede incrementarse hasta representar entre el 70 y 100% de su tiempo de alimentación total.

A pesar de la importancia nutricional de los artrópodos para los colibríes, sabemos muy poco sobre los factores que afectan su consumo en la naturaleza, y del papel que los artrópodos juegan como una fuente de energía. Adicionalmente, a la fecha, no sabemos nada sobre las estrategias de regulación del consumo simultaneo de nutrientes implementadas por estas aves, y consecuentemente, de la relación entre las estrategias de balance de nutrientes y algunos atributos de sus ensambles. Esta falta de conocimiento ha sido generada, en gran parte, por algunas limitaciones metodológicas que afectan nuestra capacidad para medir el forrajeo de artrópodos por colibríes en la naturaleza, tales como: 1) la dificultad de realizar observaciones focales de colibríes individuales por periodos largos de tiempo, 2) el pequeño tamaño corporal de las presas seleccionadas, 3) la dificultad para determinar si las capturas de artrópodos son exitosas, y 4) la falta de protocolos adecuados para medir la disponibilidad de artrópodos para los colibríes. Por lo tanto, necesitamos utilizar múltiples herramientas metodológicas que nos permitan aumentar nuestro conocimiento sobre importancia de los artrópodos para los colibríes.

En la presente Tesis se abordan cuatro temas sobre la importancia del consumo de artrópodos para los colibríes, los cuales han sido poco o nada estudiados: 1) el uso de alimentos alternativos por aves nectarívoras especializadas, 2) el papel que los artrópodos juegan como un alimento complementario y alternativo para un ensamble de colibríes que vive en un ecosistema estacional, 3) la regulación del consumo de proteína y sacarosa por colibríes, y 4) factores conductuales (uso de grit) que ayudan a la digestión mecánica de los artrópodos ingeridos y a la regulación de nutrientes.

CAPÍTULO 1. UNA REVISIÓN SOBRE EL USO DE ALIMENTOS ALTERNATIVOS POR AVES NECTARÍVORAS

Resumen

El néctar floral es una solución de azúcares y agua que presenta bajas cantidades de aminoácidos, vitaminas y minerales. Los colibríes (Trochilidae), las aves sol (Nectariniidae) y los comedores de miel (Meliphagidae), son las familias de aves que presentan un mayor nivel de especialización morfológica, fisiológica y conductual para el consumo de néctar. Estas aves utilizan el néctar como principal fuente de energía para cubrir sus gastos metabólicos. Sin embargo, debido a que el néctar solo aporta energía, estas aves deben incluir en su dieta alimentos complementarios como artrópodos y/o fruta para obtener nutrientes cruciales como aminoácidos, ácidos grasos, vitaminas y minerales. En condiciones de ausencia o poca disponibilidad de néctar, los artrópodos y la fruta se pueden convertir en alimentos alternativos para estas aves. La morfología de las lenguas y picos juega un papel central en el consumo de diferentes tipos de alimentos por estos grupos de aves. Adicionalmente, el peso corporal de las aves nectarívoras de las tres familias se correlaciona positivamente con sus tasas metabólicas totales, y con sus requerimientos de nitrógeno, afectando sus necesidades de nutrientes y energía, y definiendo su dieta. Por lo tanto, las especies más grandes presentan dietas más diversas que las especies pequeñas. En estas aves, el consumo de alimentos alternativos al néctar es más importante cuando: 1) estos alimentos son más abundantes o presentan una mayor calidad nutricional; 2) cuando la abundancia del néctar en el ambiente es baja o no está disponible; y/o 3) durante el periodo reproductivo y la crianza, cuando los requerimientos de nutrientes, principalmente proteínas y energía, son mayores.

Palabras clave: aves nectarívoras especializadas, consumo de artrópodos y fruta, estacionalidad, néctar floral, requerimientos nutricionales y de energía

El néctar floral como recurso alimenticio

Las aves son capaces de consumir un amplio rango de recursos alimenticios, incluyendo en sus dietas frutas, granos, semillas, néctar floral, invertebrados, e incluso vertebrados, entre otros (ver de Juana, 1992). Entre todos estos tipos de alimentos, químicamente el néctar es el más simple (Martínez del Rio *et al.*, 2001). El néctar es una solución de azúcares en agua (principalmente sacarosa, glucosa y fructosa en proporciones variadas), que presenta cantidades traza de aminoácidos, minerales y vitaminas (Baker, 1977; Baker y Baker, 1983). Dado que el néctar floral es fácil de asimilar y proporciona grandes cantidades de energía, la nectarivoría ha evolucionado por lo menos ocho veces de manera independiente dentro de la Clase Aves (Ford, 1985; Lotz y Schondube, 2006). Sin embargo, la mayor especialización para alimentarse de néctar en aves se encuentra representada en cuatro familias: Trochilidae, Nectariniidae, Meliphagidae y Drepanididae (Ford, 1985; Schuchmann, 1999; Cheke y Mann, 2008; Higgins *et al.*, 2008; Pratt, 2010; Tabla 1).

Las aves nectarívoras asimilan muy bien los azúcares presentes en el néctar (Martínez del Rio, 1990; Martínez del Rio y Karasov, 1990; Schondube y Martínez del Rio, 2003; Lotz y Schondube, 2006). La mayoría de ellas presentan altos niveles de actividad de la enzima sacarasa, que es responsable de la hidrólisis de la sacarosa en sus respectivos monosacáridos glucosa y fructosa, y altas tasas de absorción intestinal de monosacáridos. Esto les permite aprovechar la energía del néctar de forma muy eficiente (Martínez del Rio, 1990; Martínez del Rio y Karasov, 1990; Lotz y Schondube, 2006). Sin embargo, aunque este alimento oferta grandes cantidades de energía y agua, las concentraciones de aminoácidos, ácidos grasos, vitaminas y minerales presentes en el néctar son insuficientes para cubrir las necesidades nutricionales de las aves nectarívoras (Brice y Grau, 1991; Roxburgh y Pinshow, 2000). Por lo tanto, estas aves requieren consumir alimentos complementarios para obtener los nutrientes que faltan en su dieta de néctar, tales como artrópodos, fruta, granos de polen, excreciones de insectos, secreciones de plantas, e incluso pequeños vertebrados (Ford, 1977; Paton, 1980; Southwicke y Southwick, 1980; Craig *et al*., 1981; Collins y Briffa, 1982; Remsen *et al*., 1986; Bergquist, 1987; Stiles, 1995; Daniels *et al*., 2001; Lara *et al*., 2011; Tabla 1).

Tabla 1. Características principales de las cuatro familias más importantes de aves nectarívoras especializadas. Datos tomados de Schuchmann (1999) para Trochilidade, de Cheke y Mann (2008) para Nectariniidae, de Higgins *et al*. (2008) para Meliphagidae, y de Pratt (2010) para Drepanididae.

Familia	Número de especies	Peso corporal (g)	Distribución	Dieta
Trochilidae	328	1.75-20.75	América	Néctar floral, artrópodos, excreciones de insectos y savia
Nectariniidae	132	4.12-42.55	África, sur de Asia, y Malasia	Néctar floral, artrópodos, excreciones de insectos, savia, fruta y polen
Meliphagidae	175	7.3-254.75	Oceanía	Néctar floral, artrópodos, fruta, polen y pequeños vertebrados
Drepanididae	23	10.4-37.85	Archipiélago de Hawái	Néctar floral, artrópodos, fruta y semillas

Esta sección de la tesis se concentra en las tres familias de aves nectarívoras que tienen un mayor número de especies: Trochilidae (colibríes), Nectariniidae (aves sol) y Meliphagidae (comedores de miel; Tabla 1). No presento información de la familia Drepanididae debido a que existe muy poca literatura sobre su uso de diferentes tipos de alimentos (ver Pratt, 2010). Aquí describo como la morfología de sus picos y lenguas, y como varios de sus rasgos fisiológicos, afectan su consumo de diferentes tipos de alimentos para completar su dieta, además describo patrones espaciales y temporales en el uso de alimentos alternativos en cada uno de estos grupos de aves nectarívoras. Aunque en su mayoría los miembros de estas tres familias son considerados como nectarívoros, al tener que complementar su dieta con artrópodos y/o fruta, estas aves también pueden ser consideradas como insectívoras, frugívoras u omnívoras. Por lo tanto, para evitar problemas de clasificación, definí a estos grupos de aves dentro de un gremio alimenticio (nectarivoría, insectivoría o frugivoría) con base en el principal recurso alimenticio que utilizan según lo reportado por Schuchmann (1999), Cheke y Mann (2008), y Higgins *et al.* (2008). Adicionalmente, a lo largo de esta introducción utilizo las siguientes definiciones para distinguir diferentes tipos de alimentos: 1) ***Alimento básico***, es aquel que es consumido diariamente en grandes cantidades, y que proporciona la mayoría de los nutrientes principales y energía de la dieta; 2) ***Alimento complementario***, también es consumido diariamente pero suministra un conjunto de nutrientes que no están disponibles en cantidades suficientes en los alimentos básicos; y 3) ***Alimento alternativo***, que es aquel que se consume en ausencia del alimento básico tradicional.

Efecto de la morfología del pico y la lengua sobre el consumo de alimentos alternativos

El pico y la lengua de las aves son cruciales para la colecta, manipulación y consumo de diferentes tipos de alimentos (Paton y Collins, 1989; Erdogan y Iwasaki, 2013; Johnston, 2014). De este modo, el pico y la lengua de las aves nectarívoras permiten la extracción eficiente del néctar, y su morfología juega un papel central en el consumo de este alimento (Paton y Collins, 1989; Erdogan y Iwasaki, 2013; Johnston, 2014). En general, las aves nectarívoras especializadas tienen picos largos que pueden ser rectos o curvos, y lenguas extensibles, con puntas bifurcadas o filamentosa, y provistas con papilas gustativas que les permiten distinguir entre alimentos que difieren en su composición y concentración de azucares, aminoácidos y/o ácidos grasos (Paton y Collins, 1989; Lotz y Schondube, 2006; Erdogan y Iwasaki, 2013; Johnston, 2014).

Para ingerir el néctar, las aves insertan su pico en las flores y extienden su lengua hasta el nectario, donde el néctar es colectado principalmente por capilaridad (Schlamowitz *et al*., 1976; King y McLelland, 1984; Paton y Collins, 1989). La morfología del pico y la lengua difiere en algunos aspectos entre las familias de aves nectarívoras especializadas, limitando el tipo de flores de las que pueden extraer néctar. Los picos de los colibríes son más estrechos y rectos que los picos de las aves sol y los comedores de miel, los cuales son más anchos y curveados; mientras que las lenguas de los comedores de miel son más anchas y con punta en forma filamentosa, y las de los colibríes y las aves sol presentan puntas bifurcadas (Paton y Collins, 1989; Erdogan y Iwasaki, 2013; Johnston, 2014; Tabla 2). A pesar de estas diferencias, los colibríes, las aves sol y los comedores de miel presentan tasas de extracción de néctar muy similares, siendo igualmente eficientes en sus tasas de consumo de néctar (Collins *et al*., 1980; Ewald y Williams, 1982; Tamm y Gass, 1986; Tabla 2). Aunque estas variaciones en la morfología del pico y la lengua entre grupos de aves nectarívoras no son importantes para generar diferencias en el uso de néctar, pueden afectar de forma significativa el uso de otras fuentes de alimento como artrópodos y fruta (King y McLelland, 1984; Johnston, 2014).

Tabla 2. Morfología del pico y la lengua, y tasas de extracción de néctar de las tres familias de aves nectarívoras especializadas. Datos tomados de Paton y Collins (1989).

Familia	Morfología del pico			Forma de la lengua	Tasa de extracción de néctar (µl s^{-1})
	Longitud (mm)	Grosor (mm)	Forma		
Trochilidae	7-42	1.0-4.3	Recto y curvo	con punta bífida	10-72
Nectariniidae	12-39	3.2-7.4	Curvo	con punta bífida	71
Meliphagidae	11-47	3.3-8.1	Curvo	con punta filamentosa	20-74

Los principales rasgos morfológicos del pico que afectan el consumo de néctar y otros tipos de alimentos son su longitud, su grosor y su grado de curvatura (Feinsinger *et al*., 1985; Paton y Collins, 1989; Temeles, 1996). La longitud promedio del pico es muy similar entre las distintas familias de aves nectarívoras. Sin embargo, la variación en la longitud del pico es alta entre especies dentro de cada grupo (Paton y Ford, 1977; Wooller, 1984; Paton y Collins, 1989; Tabla 2). En colibríes, aves sol y comedores de miel, la longitud de sus picos esta correlaciona positivamente con la longitud de las corolas de las especies de plantas que utilizan (Ford y Paton, 1977; Feinsinger *et al*., 1985), afectando sus tasas de extracción del néctar. En general, las aves con picos largos son más eficientes para extraer el néctar que las aves con picos cortos, incrementando su tasa de consumo de néctar (Collins, 2008). Sin embargo, su capacidad para colectar y consumir otros tipos de alimentos se ve afectada con la especialización para extraer néctar (Collins y Briffa, 1982; Montgomerie, 1984; Feinsinger *et al*., 1985). Cuanto más largos y delgados sean los picos, menor será el área para capturar y manipular alimentos sólidos como artrópodos y frutos (Ford, 1979; Richardson y Wooller, 1986). En el grupo de los comedores de miel, la variación en la longitud de los picos es mayor que en los colibríes y en las aves sol (Wooller, 1984; Paton y Collins, 1989; Tabla 2), con especies con picos largos que tienden a ser más nectarívoras, y especies con picos cortos que tienden a ser más insectívoras o frugívoras (Wooller, 1984; Paton y Collins, 1989; Higgins *et al*., 2008).

El ancho del pico de las aves nectarívoras juega un papel importante en el consumo de alimentos, afectando el tamaño máximo de otros tipos de alimentos que puedan llegar a ser ingeridos. Los picos de los colibríes son muy estrechos, mientras que los picos de las aves sol y los comedores de miel son más anchos (Paton y Ford, 1977; Wooller, 1984; Paton y Collins, 1989; Tabla 2). Esto parece estar relacionado con la capacidad de estas aves para ingerir artrópodos. De este modo los artrópodos consumidos por colibríes no sobrepasan los 4 mm de longitud (pequeñas arañas e insectos; Stiles, 1995; Powers *et al*., 2010) mientras que las aves sol y los comedores de miel pueden ingerir artrópodos de un mayor rango de tamaños (desde ácaros hasta mantis religiosas y mariposas), e incluso en el caso de especies grandes de comedores de miel, pequeños vertebrados (Higgins *et al*., 2008).

Aunque la morfología del pico de los colibríes limita el tamaño máximo de las presas de artrópodos que pueden capturar y consumir (≤ 4 mm), estas aves presentan en sus picos una adaptación especializada para el consumo de insectos que consiste en un movimiento de flexión mandibular en el que la mitad distal de la mandíbula se puede mover hacia abajo, incrementando la apertura del pico (Yanega y Rubega, 2004; Smith *et al.*, 2011). Aunque esta capacidad para cambiar la forma del pico no incrementa la capacidad de los colibríes para ingerir presas de mayor tamaño, los hace muy eficientes para la captura de insectos pequeños en vuelo (Yanega y Rubega, 2004; Smith *et al.*, 2011).

Los picos más anchos de las aves sol y de los comedores de miel, además de facilitar el consumo de artrópodos, también les permite consumir frutos que pueden representar una importante fuente de energía y nutrientes para estas aves, sobre todo cuando el néctar no es muy abundante (Craig *et al.*, 1981; Bergquist, 1987). Al igual que los colibríes, las aves sol y los comedores de miel asimilan muy bien la sacarosa del néctar (eficiencias de asimilación de 99-100% en Trochilidae, y de 97-100% en Nectariniidae y Meliphagidae), y suelen no tener preferencias por los tipos de azúcares presentes en sus alimentos (Martínez del Rio, 1990; Martínez del Rio y Karasov, 1990; Schondube y Martínez del Rio, 2003; Lotz y Schondube, 2006). De este modo, consumen tanto néctar como frutos ricos en hexosas de forma indiferente (Martínez del Rio, 1990; Schondube y Martínez del Rio, 2003). La pulpa de los frutos consumidos por las aves sol y los comedores de miel es rica en glucosa y fructosa (la sacarosa solo constituye el 8% de los azúcares), y además contiene altas concentraciones de proteínas y grasas (Snow, 1981; Martínez del Rio *et al.*, 1995; Baker *et al.*, 1998). Ochenta y nueve especies de comedores de miel y 26 especies de aves sol (50.8% y 19.7% de las especies dentro de cada grupo, respectivamente) complementan su dieta de néctar con frutos (Cheke y Mann, 2008; Higgins *et al*., 2008), y dentro de la familia Meliphagidae, 13 especies son predominantemente frugívoras (7.4% de las especies; Higgins *et al*., 2008).

Por último, la morfología de la lengua de las aves nectarívoras especializadas también juega un papel central en el consumo de otros tipos de alimentos. Los colibríes y las aves sol tienen lenguas que se dividen distalmente en dos ramas, son cóncavas transversalmente, y presentan ranuras que se extienden hacia sus extremos distales. En cambio, los comedores de miel tienen lenguas que se dividen distalmente en dos, cuatro u ocho partes, y cada una de las partes distales termina en una estructura en forma de cepillo compuesta de numerosos filamentos (Schlamowitz *et al*., 1976; Paton y Collins, 1989; Erdogan y Iwasaki, 2013; Johnston, 2014; Tabla 2). Las lenguas de los comedores de miel pueden cubrir mayores áreas de superficie que las lenguas de los colibríes y las aves sol, permitiéndoles consumir de forma eficiente, no solo el néctar floral, sino también otras fuentes de carbohidratos, en particular excreciones de insectos (Paton, 1980; Erdogan y Iwasaki, 2013; Johnston, 2014). Las ninfas de los áfidos (Aphididae), cocoideos (Coccoidea) y psílidos (Psyllidae) consumen el floema de los tallos u hojas de las plantas, excretando el exceso del alimento consumido (nombrado en inglés como “honeydew”) el cual es rico en azúcares (Triplehorn y Johnson, 2005). Los comedores de miel lamen estas excreciones sobre la superficie de las hojas y cortezas (Paton, 1980). Veintiún especies de comedores de miel (12% de las especies de la familia Meliphagiade) complementan su dieta con estas excreciones azucaradas producidas por insectos (Paton, 1980; Higgins *et al*., 2008).

Efecto del peso corporal sobre el consumo de néctar y otros alimentos

En adición a las limitantes que la morfología del pico y la lengua imponen para el consumo de artrópodos, frutos y otros tipos de alimentos por aves nectarívoras, los requerimientos metabólicos y nutricionales de las aves determinan que otros tipos de alimentos deberán consumir además del néctar (Ricklefs, 1974; Hainsworth, 1977; Montgomerie y Redsell, 1980; Stiles, 1995; Daniels *et al.*, 2001; Powers *et al.*, 2010). Las aves nectarívoras metabolizan los azucares del néctar y utilizan la energía resultante para cubrir los gastos metabólicos generados por la regulación de su temperatura corporal y sus patrones de actividad (Ford y Patton, 1976; Brown *et al.*, 1978; Paton, 1982). Al mismo tiempo requieren de proteínas, aminoácidos, ácidos grasos, vitaminas, y minerales adicionales para cubrir sus necesidades nutricionales (Ricklefs, 1974; van Tets y Nicolson, 2000).

Las aves nectarívoras y frugívoras tienen menores requerimientos mínimos de nitrógeno (RMN= 152.8 mg N kg$^{-0.69}$ día^{-1}) que aves con dietas omnívoras (RMN= 575.4 mg N kg$^{-0.76}$ día^{-1}; López-Calleja *et al.*, 2003; McWhorter *et al.*, 2003; Tsahar *et al.*, 2006). Aunque las aves que se alimentan de néctar floral presentan requerimientos de nitrógeno bajos (Tabla 3), la cantidad de proteína en el néctar es muy baja para cubrir sus necesidades nutricionales, obligándolas a ingerir otras fuentes de nutrientes. Por ejemplo, el néctar proporciona aproximadamente solo el 14% del nitrógeno requerido por el Colibrí *Calypte costae* (Brice y Grau, 1991). Adicionalmente no hay reportes de que aves nectarívoras puedan cubrir sus otras necesidades nutricionales utilizando únicamente este dulce alimento (Brice, 1992; López-Calleja *et al.*, 2003).

Los colibríes tienen un menor peso corporal promedio ($\bar{x}$ ± D.E.= 5.25 ± 2.26 g, rango= 1.75-20.75 g) que las aves sol y los comedores de miel ($\bar{x}$ ± D.E.= 10.50 ± 5.41 g, rango= 4.12-42.55 g; $\bar{x}$ ± D.E.= 35.82 ± 39.85 g, rango= 7.30-254.75 g, respectivamente; Fig. 1), y esto puede parcialmente explicar su mayor dependencia por el néctar floral. Los colibríes suelen obtener toda la energía que necesitan del néctar floral, y cubren sus necesidades nutricionales principalmente a partir del consumo de artrópodos, ya que son incapaces de digerir eficientemente los granos de polen (Brice *et al*., 1989), y la morfología de sus picos/lenguas les impide consumir otros tipos de alimentos como fruta (Paton y Collins, 1989; Erdogan y Iwasaki, 2013; Johnston, 2014). Las aves sol y los comedores de miel, al ser más grandes, requieren consumir una mayor variedad de tipos de alimentos para cubrir sus mayores requerimientos totales de otros nutrientes y energía. Como resultado de esto, los grupos de aves que tienen mayores tamaños corporales, como las aves sol y los comedores de miel, tienden a ser menos nectarívoras que aquellas aves que tienen un menor tamaño corporal como los colibríes.

Tabla 3. Requerimientos mínimos de nitrógeno (RMN, mg N kg^{-1} $día^{-1}$) de diferentes especies de aves nectarívoras.

Familia	Especie	Peso corporal promedio (g)	RMN (mg N kg^{-1} $día^{-1}$)	Referencia
Trochilidae	*Selasphorus platycercus*	3.4	62.6	McWhorter 1997
	Calypte costae	3.5	77.1	Brice y Grau 1991
	Archilochus alexandri	2.7	85.5	McWhorter *et al.* 2003
	Lampornis clemenciae	7.9	122.4	McWhorter *et al.* 2003
	Eugenes fulgens	7.5	158	McWhorter *et al.* 2003
Nectariniidae	*Nectarinia ósea*	6.9	165	Roxburgh y Pinshow 2000
	Nectarinia chalybea	8	253.2	Van Tets y Nicolson 2000

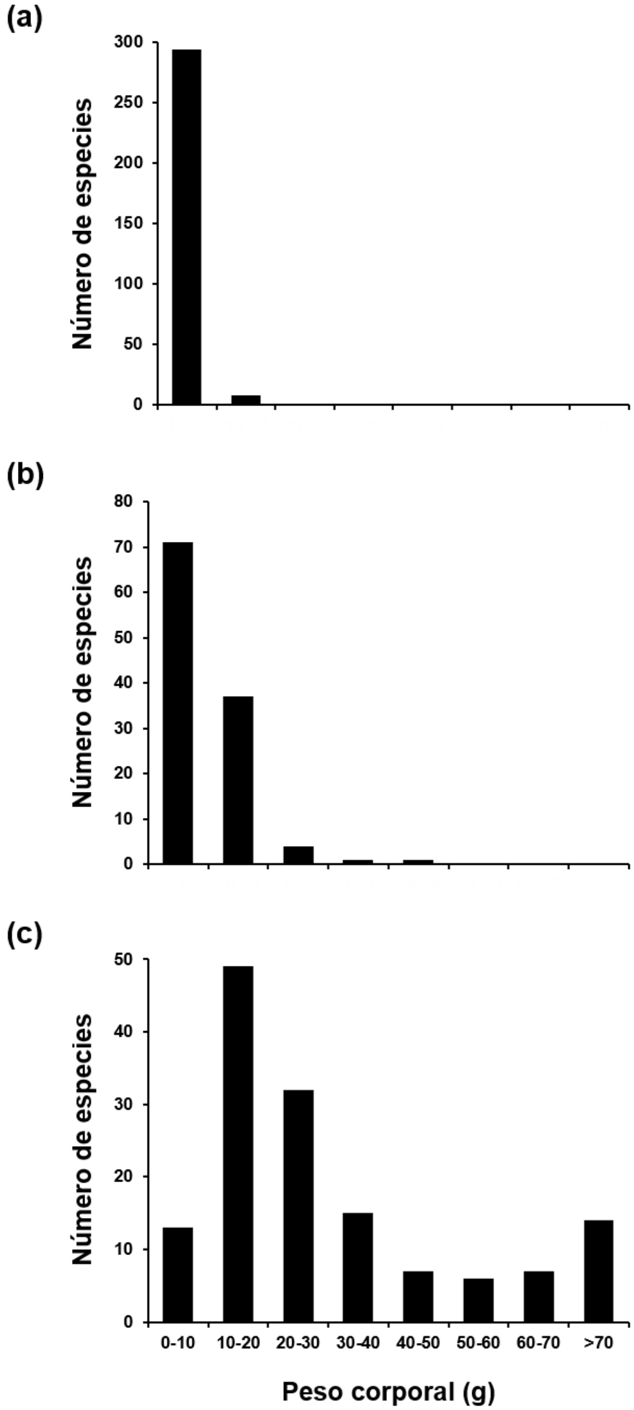

Figura 1. Distribución del peso corporal en (a) Trochilidae, (b) Nectariniidae y (c) Meliphagidae. Datos tomados de Schuchmann (1999) para Trochilidade, de Cheke y Mann (2008) para Nectariniidae, y de Higgins *et al.* (2008) para Meliphagidae.

Variaciones espacio-temporales en el consumo de néctar y otros alimentos

En adición a los factores intrínsecos que llevan a las aves nectarívoras especializadas a consumir otras fuentes de nutrientes y energía, factores extrínsecos, como la disponibilidad de recursos alimenticios, son cruciales para definir la diversidad y variación que estos animales presentan en su dieta (Paton, 1980; Craig *et al.*, 1981; Remsen *et al.*, 1986; Stiles, 1995; Daniels *et al.*, 2001; Tsahar *et al.*, 2006). Por lo tanto, el consumo de otros tipos de alimento como excreciones de insectos, savia, fruta, o artrópodos, puede estar afectado por la abundancia de estos recursos en el tiempo y espacio, y la interacción entre esta disponibilidad y las demandas nutricionales impuestas por momentos clave en los ciclos de vida de las aves (Paton, 1980; Craig *et al.*, 1981; Remsen *et al.* 1986; Stiles, 1995; Daniels *et al.*, 2001; Tsahar *et al.*, 2006).

En adición al néctar, las aves nectarívoras pueden obtener energía de las excreciones ricas en azúcares de algunos insectos ("honeydew"). Esto se ha observado tanto en colibríes en el nuevo mundo, como en otras aves en Asia y Australia (Paton, 1980; Gamper y Koptur, 2010; Lara *et al.*, 2011; Teixeira y Azevedo, 2013). En el caso de los colibríes, se ha reportado que se alimentan de secreciones de insectos del orden Hemiptera (Familia Margarodidae, genero *Stigmacoccus*; Hodgson *et al.*, 2007; Gamper y Koptur, 2010; Lara *et al.*, 2011). Estos insectos tienen un filamento anal largo donde se acumula la excreción azucarada, y los colibríes la colectan directamente de este filamento cuando la abundancia de los insectos que lo producen es alta (Gamper y Koptur, 2010; Lara *et al.*, 2011). El consumo de este recurso ha sido reportado en cuatro especies de colibríes en Norteamérica (*Atthis heloisa*, *Colibri thalassinus*, *Eugenes fulgens*, y *Basilinna leucotis*; Gamper y Koptur, 2010; Lara *et al.*, 2011) y en cuatro especies en Sudamérica (*Amazilia lactea*, *Chlorostilbon lucidus*, *Leucochloris albicollis*, y *Thalurania glaucopis*; Teixeira y Azevedo, 2013). En el viejo mundo, algunas especies de comedores de miel también complementan su dieta de néctar con excreciones de insectos (Paton, 1980; Higgins *et al*., 2008). De este modo, 21 especies de comedores de miel (12% de las especies dentro de la familia; Higgins *et al*., 2008) consumen las excreciones de las ninfas de áfidos (Aphididae), cocoideos (Coccoidea), y psílidos (Psyllidae; Basden, 1970; Triplehorn y Johnson, 2005).

El consumo de otras secreciones de plantas distintas del néctar, como la savia, es relativamente raro en aves nectarívoras. Sin embargo, *Archilochus colubris*, una especie de Colibrí de Norteamérica, consume la savia exudada de los orificios hechos por el pájaro carpintero *Sphyrapicus varius* en los troncos y ramas de los alisos (*Alnus*) y sauces (*Salix*; Southwicke y Southwick, 1980). Adicionalmente, se ha reportado que dos especies de colibríes, *Selasphorus platycercus* y *S. rufus*, consumen las secreciones de las lesiones de las ramas de *Quercus gambelii* (Kevan *et al*., 1983). Estas lesiones, las cuales son producidas por bacterias, secretan una solución rica en sacarosa, hexosas y aminoácidos. Este recurso rico en nutrientes y energía es usado por los colibríes de forma efímera, ya que se evapora o se cristaliza rápidamente al exponerse al sol, y solo está disponible en ciertos momentos del año (Kevan *et al*., 1983). En el viejo mundo, 15 especies de comedores de miel consumen secreciones de plantas (conocidas como "manna" en inglés), lo que representa el 8.6% de las especies de la familia (Higgins *et al*., 2008). El manna es un material cristalizado rico en azúcares que es secretado por diversas especies de eucaliptos (Basden, 1970). Los comedores de miel lamen con sus lenguas filamentosas la superficie de las cortezas y el follaje de estas plantas para ingerirlo, y su consumo es más importante cuando: 1) el néctar es poco abundante; 2) en los momentos en que la abundancia del manna es alta; y/o 3) durante el periodo reproductivo de estas aves (Paton, 1980). Cuando este recurso azucarado es abundante, los comedores de miel consumen en promedio 9 gotas/min de manna, lo que equivale a una ganancia de energía de 45 cal/min (Paton, 1980).

Una fuente de energía adicional para algunas aves nectarívoras son los frutos carnosos de una gran diversidad de plantas (por ejemplo, Caricaceae, Musaceae, Rubiaceae; Cheke y Mann, 2008; Higgins *et al.*, 2008). Aunque se asume que el consumo de frutos carnosos no ocurre en colibríes debido a las limitaciones impuestas por la morfología de sus picos, se ha observado que estas aves pueden consumir los jugos secretados por frutos maduros cuando la cascara de los mismos está rota (Schondube, com. pers.). A diferencia de los colibríes, algunas especies de comedores de miel utilizan los frutos carnosos como alimento complementario, alternativo, o incluso básico. Mientras que en este grupo de aves algunas especies tienden a ser más insectívoras, otras tienden a ser más frugívoras (48 y 13 especies, que representan el 27.4% y 7.4% de las especies de la familia Meliphagidae, respectivamente; Higgins *et al*., 2008). El uso de néctar y fruta como fuente de energía y otros nutrientes por los comedores de miel suele variar estacionalmente, dependiendo de la fenología y diversidad de las plantas de los sitios donde habitan (Ford, 1977; Craig *et al.*, 1981; Collins y Briffa, 1982; Bergquist, 1987). De este modo, los comedores de miel en Nueva Zelanda tienden a ser más frugívoros, mientras que los de Australia tienden a ser más nectarívoros. Se ha propuesto que este patrón es resultado de diferencias en la diversidad de especies de plantas entre las dos regiones (Craig *et al.*, 1981). La flora de Nueva Zelanda presenta una mayor riqueza de especies de plantas productoras de frutos comestibles que la australiana (por ejemplo, plantas de los géneros *Podocarpus* y

Coprosma; Allan, 1961). Contrario a esto, en Australia el número de familias de plantas con flores que usan los comedores de miel es mayor que en Nueva Zelanda (Ford *et al*., 1979).

Debido a que las excreciones de insectos, las secreciones de plantas, y los frutos carnosos son consumidos principalmente como fuentes de energía, funcionan como alimentos alternos al néctar, y pueden convertirse en alimentos básicos estacionalmente en ausencia de este. Hay que considerar que, para las especies grandes de comedores de miel, los frutos carnosos pueden ser considerados como el alimento básico, siendo el néctar un alimento complementario (Higgins *et al*., 2008). Los frutos carnosos, a diferencia de los otros recursos ricos en azúcares consumidos por las aves nectarívoras, pueden contener una alta diversidad de nutrientes (Snow, 1981; Martínez del Rio *et al*., 1995; Baker *et al*., 1998), por lo que estas aves pueden cubrir sus requerimientos nutricionales por completo y no se ven obligadas a consumir alimentos complementarios, o lo hacen solo de manera ocasional. Los colibríes y las aves sol, al verse limitados en su uso de frutos, suelen utilizar a los artrópodos como su principal alimento complementario para obtener los nutrientes y micronutrientes ausentes en el néctar (Hainsworth, 1977; Remsen *et al*., 1986; Stiles, 1995; Daniels *et al*., 2001).

Uso de artrópodos como alimento alternativo o complementario por aves nectarívoras

A pesar de que el néctar floral es la principal fuente de energía de los colibríes, se ha documentado que el 89% de las especies dentro de esta familia consumen regularmente artrópodos (Schuchmann, 1999). El 11% restante de las especies de colibríes también deben de consumirlos, sin embargo, no hay datos de forrajeo de artrópodos para ellas

(Schuchmann, 1999). Este consumo, en general, se refiere a un uso de los artrópodos como alimento complementario, con la función de cubrir sus requerimientos de aminoácidos, ácidos grasos, vitaminas y minerales (Hainsworth, 1977; Remsen *et al*., 1986; Stiles, 1995). En términos nutricionales, los artrópodos son ricos en proteínas, ya que estas constituyen, en promedio, alrededor del 60% de su peso seco. Los artrópodos también son ricos en ácidos grasos, aunque su contenido varía ampliamente con su estado de desarrollo (5-85% del peso seco). Adicionalmente, las vitaminas y minerales constituyen alrededor del 5% del peso seco de los artrópodos (Bell, 1990; Karasov, 1990; Rothman *et al*., 2014). Finalmente, los artrópodos también son ricos en energía, ya que 1 g de una dieta de artrópodos suministra ~19.3 kJ de energía metabolizable para las aves que los consumen (Karasov, 1990). Esto causa que el consumo de artrópodos sea más importante en los momentos del año donde la muda y la reproducción aumentan las demandas de nutrientes de los colibríes (Hainsworth, 1977; Montgomerie y Redsell, 1980; Chavez-Ramirez y Down, 1992; Stiles, 1995; Murphy, 1996). Sin embargo, algunas especies de colibríes también pueden consumir artrópodos para cubrir sus demandas energéticas cuando el néctar es escaso o no está disponible, convirtiéndolos en un alimento alternativo al néctar floral (Hainsworth, 1977; Kuban y Neill 1980, Montgomerie y Redsell, 1980; Chavez-Ramirez y Down, 1992; Powers *et al*., 2010).

En general, los colibríes ingieren pequeños artrópodos (de uno a cuatro mm de longitud) pertenecientes a los órdenes Acari, Araneae, Collembola, Orthoptera, Isoptera, Hemiptera, Psocoptera, Coleoptera, Hymenoptera, Diptera y Lepidoptera (Remsen *et al*., 1986; Poulin *et al*., 1994; Stiles, 1995; Powers *et al*., 2010; Moran *et al*., 2019). Los miembros de las dos sub familias de colibríes muestran diferencias en los grupos de artrópodos que seleccionan. Los colibríes ermitaños (Phaethornithinae) ingieren principalmente arañas y otros artrópodos de cuerpos blandos, capturándolos directamente del follaje, flores, cortezas de árboles, o del suelo (“gleaning”). En cambio, los colibríes no ermitaños (Trochilinae) tienden a ingerir insectos voladores de cuerpos relativamente duros como moscas y avispas, cazándolos durante el vuelo (“hawking”; Stiles, 1995). Esta variación en el comportamiento de forrajeo entre diferentes especies de colibríes es resultado de variaciones en la morfología del pico y las alas que afectan sus decisiones de alimentación (Stiles, 1995).

Debido a que los colibríes deben de mantener un consumo constante de proteínas para cubrir sus requerimientos mínimos de nitrógeno y mantener su masa corporal, estas aves deben de complementar su dieta de néctar con artrópodos (Brice y Grau, 1991; Brice, 1992; López-Calleja *et al*., 2003; McWhorter *et al*., 2003). Para cubrir sus requerimientos nutricionales, los colibríes dedican entre 2 y 15% de su tiempo de alimentación diario a la captura de artrópodos (Gass y Montgomerie, 1981). Sin embargo, los colibríes pueden incrementar sustancialmente su consumo de artrópodos en respuesta a la variación estacional en la disponibilidad del néctar (DesGranges, 1979; Kuban y Neill, 1980; Montgomerie y Redsell, 1980; Chavez-Ramirez y Down, 1992). Incluso se ha reportado que, en ausencia de néctar floral, o cuando este es escaso, algunas especies de colibríes pueden sobrevivir consumiendo exclusivamente artrópodos (Hainsworth, 1977; DesGranges, 1979; Montgomerie y Redsell, 1980). Bajo estas condiciones ecológicas, el tiempo de forrajeo utilizado por los colibríes para la captura de artrópodos puede incrementarse hasta representar entre 70 y 100% de su tiempo de alimentación total (Gass y Montgomerie, 1981). Por lo tanto, los colibríes pueden usar a los artrópodos como un recurso alternativo al néctar floral para cubrir sus requerimientos nutricionales y energéticos (Hainsworth, 1977; Montgomerie y Redsell, 1980; Powers *et al.*, 2010).

De forma similar a los colibríes, la gran mayoría de las especies de aves sol complementan su dieta de néctar con artrópodos. De las 132 especies de aves sol conocidas, 113 de ellas consumen artrópodos como alimento complementario (85.6% de las especies), mientras que las 19 especies restantes los utilizan como alimento básico, considerándose como insectívoras (14.4% de las especies; Cheke y Mann, 2008). La dieta de artrópodos de estas aves incluye una alta diversidad de grupos taxonómicos con diferentes tamaños y estados de desarrollo: Acari, Araneae (huevos y adultos), Ephemeroptera, Orthoptera, Mantodea, Isoptera, Hemiptera, Coleoptera, Neuroptera, Hymenoptera, Diptera, y Lepidoptera (larvas, ninfas y adultos; Daniels *et al.*, 2001; Cheke y Mann, 2008). En estas aves, el consumo de artrópodos se ve afectado por los requerimientos metabólicos y nutricionales asociados a su tamaño corporal, y se incrementa durante el periodo reproductivo y en los momentos en los que el néctar floral es poco abundante o la abundancia de artrópodos es alta (Daniels *et al.*, 2001).

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CAPÍTULO 2. INGESTIÓN DE ARTRÓPODOS EN UN ENSAMBLE DE COLIBRÍES

Arthropod ingestion in a high altitude hummingbird ensemble

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Running title: Arthropod ingestion by hummingbirds

Abstract

Arthropods are the principal source of nitrogen, fatty acids, and micronutrients for hummingbirds. Despite their nutritional importance, we know little about the factors that regulate arthropod ingestion by hummingbirds and their relative role as an energy source for this group of birds. Here, we aimed to determine the arthropod use by the members of a hummingbird ensemble in a Mexican temperate forest during an annual cycle. We compared the arthropod capture-attempt rates among different humming species (Mexican Violetear *Colibri thalassinus*, White-eared Hummingbird *Basilinna leucotis*, Rivoli's Hummingbird *Eugenes fulgens*, Broad-tailed Hummingbird *Selasphorus platycercus*, and Rufous Hummingbird *S. rufus*). Also, we explored the role that seasonal variation of food availability played on the arthropod capture-attempt rate of White-eared Hummingbird, the only species that we observed foraging for arthropods during all three climatic seasons. Our results showed that hummingbirds ingested arthropods belonging to different orders: Araneae, Hemiptera, Psocoptera, Hymenoptera, and Diptera. Arthropod capture-attempt rate was significantly higher in Broad-tailed Hummingbird that in Rivoli's Hummingbird. White-eared Hummingbird arthropod capture-attempt rate was positively and significantly related to arthropod availability, being higher during the warm-dry season when floral nectar was not available, and arthropod availability was high. Some hummingbird species were able to maintain resident populations at our study site by feeding only on arthropods when no floral nectar was available. We concluded that arthropod consumption could play a crucial role for hummingbirds as a source of nutrients and energy, mainly when nectar availability is low or non-existent. Our findings suggest that a decrease in arthropod abundances due to anthropogenic activities and global change could affect hummingbirds' survival at local scales.

Keywords: arthropods as an energy source, arthropod capture-attempt rate, hummingbird nutritional requirements, nectar and arthropod availability, seasonality.

1. INTRODUCTION

Although the nutritional importance of arthropods as a source of nitrogen for hummingbirds is beyond doubt (Brice & Grau 1991, López-Calleja *et al*. 2003, McWhorter *et al*. 2003), we know little about the factors that affect their ingestion in the field and their importance as an energy source. Some authors indicate that hummingbirds ingest arthropods only to satisfy their nitrogen requirements and obtain essential fatty acids and micronutrients that are not present in floral nectar (Brice 1992, López-Calleja *et al*. 2003, McWhorter *et al*. 2003). However, there is evidence that hummingbirds can ingest large amounts of arthropods and use them as an energy source when nectar is not abundant in the environment (Hainsworth 1977, Kuban & Neill 1980, Montgomerie & Redsell 1980, Gass & Montgomerie 1981, Remsen *et al*. 1986, Chavez-Ramirez & Dowd 1992, Powers *et al.* 2010).

Classic studies on arthropod ingestion by hummingbirds (see Remsen *et al*. 1986, Stiles 1995) have revealed a marked difference in arthropods' use by different hummingbird species. This variability in the ingestion of arthropods among hummingbird species is result of the interaction between intrinsic and extrinsic factors affecting the feeding decisions of hummingbirds. Hummingbird intrinsic factors include the wing and bill morphology that limits the hummingbirds' capacity to capture arthropods, minimum nitrogen requirements, and changes in the nutritional needs through their annual cycles (e.g., increases in their protein and mineral requirements during egg production or molting;

Hainsworth 1977, Brice & Grau 1991, Chavez-Ramirez & Dowd 1992, Stiles 1995, Murphy 1996). Extrinsic factors include the variation in the availability of floral resources (Young 1971, Kuban & Neill 1980, Remsen *et al.* 1986, Chavez-Ramirez & Dowd 1992).

Even though the role of some intrinsic factors has been studied in the past (e.g., Stiles 1995, López-Calleja *et al*. 2003, McWhorter *et al.* 2003, Yanega & Rubega 2004), our knowledge of the role that extrinsic factors play in arthropod ingestion by hummingbirds is insufficient (but see Remsen *et al*. 1986, Stiles 1995, Powers *et al.* 2010). This lack of knowledge is generated by methodological restrictions that limit our capacity to measure arthropod foraging by hummingbirds in the field. These limitations include the small body size of arthropods ingested by hummingbirds, the arduousness to determine if arthropod capture-attempts were successful, and the difficulty of performing focal observations of individual hummingbirds for long periods (Stiles 1995, Powers *et al*. 2010). Our objective was to describe the arthropods' use by members of a hummingbird ensemble in a seasonal ecosystem of West Mexico during an annual cycle. Specific objectives were: (1) to determine the diversity and quantity of arthropod ingested, and the arthropod capture-attempt rates by hummingbirds, (2) the arthropod foraging tactics used by hummingbirds, (3) if there are differences in arthropod use among hummingbird species, (4) if arthropod use varies seasonally, and (5) the role that some extrinsic factors such as nectar and arthropod availability played on arthropod ingestion.

Because arthropod ingestion by hummingbirds increases in response to the absence of nectar (Young 1971, Kuban & Neill 1980, Montgomerie & Redsell 1980, Remsen *et al*. 1986, Chavez-Ramirez & Dowd 1992), and also because it increases in other groups of nectar-feeding birds when arthropod abundance is high (Paton 1982, Collins & Briffa 1982, Bergquist 1987, Recher & Davis 1997, Damon 2000, Daniels *et al.* 2001, Timewell & Mac Nally 2004), we predicted that arthropod ingestion by hummingbirds should increase when the availability of nectar in the environment is low and the availability of arthropods is high. In addition, because some studies suggest that at the ensemble level the degree of behavioral dominance of different hummingbird species affects their arthropod foraging rates (Young 1971, Powers *et al*. 2010), we predict that subordinate species will attempt to capture prey more frequently that aggressive species to reduce interspecific competition for nectar and meet all their nutritional and energy requirements.

2. METHODS

2.1. Study site

We conducted fieldwork at the Nevado de Colima National Park (NCNP) in Jalisco, Mexico. The Nevado de Colima is an inactive high-altitude volcano (4,260 m a.s.l.) located in the Trans-Mexican Volcanic Belt (19° N, 103° W; INEGI 2011). The region presents three climatic seasons: 1) rainy season (June to October), 2) cold-dry season (November to February), and 3) warm-dry season (March to May). During the rainy season, temperatures varied from 3.4 to 17.9°C, and heavy rainstorms were present every day (monthly average precipitation was 360.5 mm). The cold-dry season presented temperatures ranging from -0.7 to 20.1°C, and monthly average precipitation of 20.3 mm. Finally, the warm-dry season presented temperatures that fluctuated from 3.9 to 23.8°C and monthly average precipitation of 66.1 mm (CONANP 2006, 2017).

Our study site is located on the northeast slope of the mountain at 3,194 m a.s.l. and has an area of 2 ha. It includes a combination of subalpine scrubland (dominated by plants of the genus *Salvia*, *Ribes,* and *Festuca*), scattered patches of alders (*Alnus*) on exposed ridges, and pine and fir forests (*Pinus* and *Abies*) in ravines. The hummingbird ensemble in the Nevado de Colima is composed of 15 species. Eight species are year-round residents, while the other seven species are long-distance winter migrants (Calder & Contreras-Martínez 1993, Schondube 2012, Maya-García 2014). The most important flowering plants that hummingbirds used at our study site include: *Salvia elegans*, *S. gesneriflora* (Lamiaceae), *Ribes ciliatum* (Saxifragaceae), *Senecio angulifolius* (Asteraceae), and *Penstemon roseus* (Plantaginaceae; Schondube 2012).

2.2. Fieldwork

We sampled during the warm-dry (May 2016), rainy (September 2016), and cold-dry seasons (February 2017). Each sampling-period lasted 15 days. We selected this sampling scheme because weather conditions and floral resources availability vary widely among seasons. During the warm-dry season, no plants were blooming; however, during the rainy and cold-dry seasons, several plant species were in bloom (mainly from the genera *Salvia*, *Ribes*, and *Penstemon*). The composition and structure of hummingbird ensembles also varied among seasons. During the warm-dry and rainy seasons, the hummingbird ensemble was composed of only resident species. In contrast, long-distance migratory species were also present in the cold-dry season (DesGranges 1979, Calder & Contreras-Martínez 1993, Schondube 2012, Maya-García 2014). During each sampling period, we captured hummingbirds using 10 mist-nets (12-m long, 24-mm mesh) for three consecutive days. Mist-nets were opened at sunrise and closed after six hours, and net rounds were conducted every five minutes. We located the mist-nets within a 2 ha area, and their location remained constant during the study. We identified all birds captured and determined their age and sex using plumage characteristics and bill striations (Williamson 2001, Howell 2003, Russell *et al*. 2019). The abundance of all hummingbird species was standardized as the number of individuals captured during 100 net-hours.

2.3. Stomach content analysis

We obtained the stomachs from individuals that were collected as part of an independent stable isotope study conducted at our study site (*n*= 6 in May 2016, *n*= 8 in September 2016, and *n*= 18 in February 2017). Samples were collected with permission from the Secretaría de Medio Ambiente y Recursos Naturales, Mexico (SGPA/DGGFS/712/2767/14). All collected birds were humanely euthanized by carefully placing their heads inside a small vial that contained a cotton ball soaked in isoflurane, following the guidelines to the use of wild birds in research (Fair *et al*. 2010). Stomachs were deposited in plastic vials with saline solution (0.8% NaCl) and frozen in liquid nitrogen for later analysis. Stomachs were thawed and dissected in the laboratory, and their contents were removed. We weighted all arthropod remains using an analytical balance (Ohaus Adventurer, capacity/readability of 110 g × 0.001 g), and separated them into identifiable (partially fragmented) and unidentifiable (very fragmented) using a stereoscopic microscope (AmScope, 7-45x binocular stereo zoom microscope). We identified the recognizable arthropods to the taxonomic level of order following Triplehorn & Johnson (2005).

2.4. Arthropod and nectar foraging rates

During each sampling period, we measured two feeding behaviors of hummingbirds, arthropod capture-attempts, and flower visits for nectar, using the *ad libitum* sampling method (Altmann 1974, Martin & Bateson 2007). *Ad libitum* sampling, which involves opportunistic observations without restrictions in the measurement time in which different behaviors displayed by one or several individuals are recorded, has previously been used to quantify the arthropod foraging behavior of hummingbirds (Stiles 1995, Powers *et al.* 2003). We identified observed individuals to species level and recorded the number of feeding events (i.e., the number of times the hummingbird attempted to capture arthropods, or it introduced its bill into a floral corolla). We recorded all arthropod capture-attempts that occurred when the prey was captured from substrates such as tree foliage, shrubs, herbs, tree bark, or ground ("gleaning"), and/or when the prey was captured in the air ("hawking"; Stiles, 1995). In both foraging tactics, the capture attempts may be made during continuous hovering flight or when the bird left from a perch, made one or more attempts, and then returned to the perch (Stiles, 1995). Additionally, we recorded the arthropod captured-attempts that occurred while birds sitting on a perch, and no flight is involved ("perch- foraging"; Powers *et al.* 2003). We measured the frequency of foraging for arthropods and nectar by dividing the number of feeding events by the observation time (events/s; Altmann 1974, Martin & Bateson 2007). We also measured the intensity of arthropod foraging using frequencies. We consider that a hummingbird individual foraged for arthropods intensely when attempting to capture ≥ one prey item per second. Foraging observations were conducted simultaneously by three observers located in different areas of our site. Observers were trained to follow the same protocol, strolling through an area of our study site until detecting a bird and describing its foraging behavior until it flew away.

Observations were conducted with binoculars from 08:00 to 17:00 hr during two consecutive days in each season (54 hr/season).

2.5. Availability of floral nectar

During each sampling period we measured the availability of floral nectar using the standing crop method (Kearns & Inouye 1993). This technique measures nectar availability at a single point in time allowing for comparisons (Possingham 1989, Kearns & Inouye 1993). We randomly selected 10 individuals of each flowering plant species and 10 flowers per plant (i.e., 100 flowers for each plant species). We extracted nectar from flowers exposed to floral visitors from 08:00 to 10:00 hr using capillary tubes (Drummond Microcaps, 20 µl, 64 mm). We measured nectar volume and concentration to evaluate variation in energy standing crop during the period of hummingbird activity. Nectar concentration was measured using a manual refractometer (Leica DC50), and converted to energy units following Kearns & Inouye (1993). Additionally, to assess the representativeness of nectar data from our study site, we measured energy availability using the same methods in two areas located 2-4 km away from our study site.

We determine the density of flowering plants visited by hummingbirds using the point-centered quarter method (Cottam & Curtis 1956, Pollard 1971, Mitchell 2007). We randomly selected 10 points within our study site using a grid on top of a map and a random number generator to select locations. The area surrounding each sampling point was divided into four quadrants using North-South and East-West directions as axes. At each sampling point, we measured the distance between the central point and the nearest target plant species in each of the four quadrants. We estimated plant density (plants/ha) with the equations proposed by Pollard (1971), while we determined the number of flowers (flowers/plant) by counting the flowers on one plant of each species in each one of the 10 points randomly selected to conduct the point-centered quarter method.

We used energy availability (kJ/ha) as a measure of nectar resources. We calculated this for each plant species used by hummingbirds following Kearns & Inouye (1993). Briefly, we multiplied the average value of nectar volume (µl/flower) by the average number of flowers per plant (flowers/plant), and the resulting number by plant density (plants/ha). We converted this value to energy by taking into account the mean sugar concentration of the nectar (1 mol of sucrose solution contains 56.41 J/ml; Kearns & Inouye 1993). Finally, we added the values obtained for each plant species to obtain the total energy present at our site during each season (kJ/ha).

2.6. Arthropod availability

We estimated the availability of arthropods by using beating method and Malaise traps. Implementing a combination of these sampling methods allows a more extensive and diverse sampling (Basset *et al*. 1997, Nageleisen & Bouget 2019). While these capture methods do not provide an exact measurement of total arthropod abundance, they allowed us to obtain a measurement of the relative availability of arthropods present at a given place and time to compare seasons (Basset *et al*. 1997, Wade *et al*. 2006, Nageleisen & Bouget 2019). We used the beating method to target arthropods that cannot fly, that do not move away quickly when disturbed, or that are associated with tree and shrub foliage (i.e., psocids, bugs or spiders; Basset *et al*. 1997, Nageleisen & Bouget 2019). We randomly positioned seven linear transects (30 m long x 1 m wide each) within our study site. The branches of trees and bushes located within each linear transect were hit three times with a stick while a beating tray of 0.7 m × 0.7 m was placed below the section of the plant. Arthropods deposited in the tray were collected using an insect pooter (Bioquip). Arthropods were capture during a single day per season from 08:00 to 11:00 hr by the same person to minimize sampling bias.

We used Malaise traps for capturing small strong-flying insects (i.e., wasps and flies; Ausden & Drake 1996, Basset *et al*. 1997, Nageleisen & Bouget 2019). We randomly selected two locations within our study site to place Malaise traps (Bioquip, 110 cm × 110 cm × 110 cm). We placed one Malaise trap at ground level, while we suspended the other in a tree 2 m from the ground, leaving them on the same location for seven days (336 hr/season). We preserved all captured arthropods from both capture methods in 70% ethanol. All collected arthropods were identified to the taxonomic level of order and counted to determine their abundances. Subsequently, arthropod samples were dried at room temperature for 3 hr and weighed using an analytical balance. To determine relative arthropod availability for hummingbirds we only counted and weighed organisms within the size range, and taxonomic groups present inside hummingbird stomachs (see results section below). We estimated the relative availability of these arthropods (g of dry weight/season) collected by the beating method and the Malaise traps separately.

2.7. Data analysis

We used different analyses to test our main hypotheses on the arthropod use by hummingbirds. First, we compared the arthropod capture-attempt rates among hummingbird species using a Kruskal-Wallis test (Zar 1999). We used a non-parametric test because our data did not present a normal distribution. Because we only observed one hummingbird species foraging for arthropods during the warm-dry season, and due to the small sample sizes of the rainy season, this analysis was implemented to test differences among hummingbird species within the cold-dry season. Second, we used a generalized linear model (GLM) to determine if arthropod ingestion varied seasonally. In this model, we used the season (warm-dry, rainy, and cold-dry seasons) as the explanatory variable and the arthropod capture-attempt rate as the response variable. Third, we also used generalized linear models to assess the role that the availability of food resources played in arthropod ingestion by hummingbirds. We generated three GLMs with the same response variable (arthropod capture-attempts rate), but different explanatory variables: 1) the total energy available as nectar (kJ/ha), and 2) the availability of arthropods measured as grams of dry weight beating/season, and 3) as grams of dry weight Malaise/season. In all four GLMs, we used exponential distributions with reciprocal link functions, and we only included data from White-eared Hummingbird because this was the only species we observed attempting to capture arthropods during the three climatic seasons. We conducted all analyses in JMP version 9.0 (SAS Institute Inc.).

3. RESULTS

3.1. Hummingbird ensembles

Hummingbird ensemble composition and abundance varied among seasons. The hummingbird ensemble present during the warm-dry season was composed of three species: Amethyst-throated Mountaingem (*Lampornis amethystinus*), White-eared Hummingbird (*Basilinna leucotis*), and Rivoli’s Hummingbird (*Eugenes fulgens*). During this season, White-eared Hummingbird was the dominant species in terms of its relative abundance (13.3 hummingbirds per 100 net-hours), while Amethyst-throated Mountaingem and Rivoli’s Hummingbird species were very low (0.5 hummingbirds/100 net-hours in both). During the rainy season, the hummingbird ensemble was composed of four species: Mexican Violetear (*Colibri thalassinus*), Amethyst-throated Mountaingem, White-eared Hummingbird, and Rivoli’s Hummingbird. In this season, Mexican Violetear was the species more abundant, followed by Rivoli’s Hummingbird (2.2 and 1.1 hummingbirds/100 net-hours, respectively), while Amethyst-throated Mountaingem and White-eared Hummingbird were the less abundant species (0.5 hummingbirds/100 net-hours in both). Finally, the hummingbird ensemble present during the cold-dry season was composed of five species: Mexican Violetear, White-eared Hummingbird, Rivoli’s Hummingbird, Broad-tailed Hummingbird (*Selasphorus platycercus*), and Rufous Hummingbird (*S. rufus*). During this season, again, Mexican Violetear was the species more abundant (7.7 hummingbirds/100 net-hours), followed by Broad-tailed and White-eared Hummingbird species (6.6 and 5.5 hummingbirds/100 net-hours, respectively), while Rivoli’s and Rufous Hummingbird species were the less abundant (1.6 hummingbirds/100 net-hours in both).

3.2. Arthropod ingestion by hummingbirds

During the warm-dry season, all hummingbird stomachs contained arthropod remains, and their biomass varied from 0.001 g dry weight for an adult female Amethyst-throated Mountaingem individual to 0.0038 g dry weight for an adult female Rivoli’s Hummingbird individual (Table 1). During the rainy season, all stomachs again contained arthropod remains, and their biomass varied from 0.0001 g dry weight for an adult female Rivoli’s Hummingbird to 0.0012 g dry weight for two adult Mexican Violetear individuals (Table 1). Finally, during the cold-dry season, except for an adult male Rivoli’s hummingbird individual whose stomach that did not contain arthropod remains, the other 17 stomachs contained arthropods, and their biomass varied from 0.0001 g dry weight for an adult male White-eared Hummingbird individual to 0.0025 g dry weight for an adult male Rivoli's hummingbird individual (Table 1).

Our examination of identifiable arthropods indicated that hummingbirds ingested prey items belonging to the orders Araneae (spiders), Hemiptera (bugs), Psocoptera (psocids), Hymenoptera (wasps), and Diptera (flies; Fig. 1). During the warm-dry season, Diptera and Psocoptera were the most common arthropod orders ingested by White-eared Hummingbird individuals (37.6 ± 27.7% and 36.2 ± 33.0% of total arthropods present in their stomachs, respectively), while Diptera was the main order ingested by a Rivoli's Hummingbird individual (57.1%), and Psocoptera was the only order ingested by an Amethyst-throated Mountaingem individual (100%; Fig. 1). It should be noted that only during the warm-dry season did we find spiders inside the stomachs of White-eared Hummingbird individuals. During the rainy season, Diptera was the main arthropod order ingested by all sampled hummingbird species (Mexican Violetear: 40.3 ± 13.5%, Amethyst-throated Mountaingem: 66.6%, and White-eared Hummingbird: 100%; Fig. 1). Finally, in the cold-dry season, Diptera was also the main arthropod order ingested by Rivoli's (46.4 ± 35.3%), Broad-tailed (36.7 ± 42.5%), and Rufous hummingbird species (31.5 ± 28.0%). In contrast, Hymenoptera was the most common order ingested by Mexican Violetear Hummingbird individuals (45.5 ± 5.0%), and Psocoptera was the predominant order ingested by White-eared Hummingbird individuals (62.0 ± 45.8%; Fig. 1).

3.3. Hummingbird feeding behavior

3.3.1. Foraging rates

During the warm-dry season, only one species, the White-eared Hummingbird, was observed foraging for arthropods, while no individuals visited flowers. The proportion of White-eared hummingbirds that attempted to capture arthropods was relatively high (80 individuals represented 61.5% of the total observed), with a lower proportion of individuals performing other activities that did not involve feeding (50 individuals represented 38.5%; Fig. 2). During this season, White-eared Hummingbird foraged for arthropods at a rate of 0.48 ± 0.30 arthropod capture-attempts per second (mean ± sd, n= 80; Table 2, Fig. 3a). Four White-eared hummingbirds (one female and three males that represented 5% of the total individuals observed) foraged for arthropods intensely (i.e., they attempted to capture one or more arthropods per second).

During the rainy season, Mexican Violetear, White-eared Hummingbird, and Rivoli's Hummingbird species, and a few Broad-tailed Hummingbird individuals present, were observed making both attempts to capture arthropods and visiting flowers for nectar (Figs. 2 and 3a, b). In White-eared Hummingbird, the proportion of individuals attempting to capture prey was higher than the proportions of individuals visiting flowers (44.4% vs. 22.2 %), while in both Rivoli's and Broad-tailed hummingbird species, the proportion of individuals observed visiting flowers was higher than individuals foraging for arthropods (Rivoli's: 30.3% vs. 15.1 %; Broad-tailed: 75% vs. 25%; Fig. 2). In Mexican Violetear, the proportions of individuals observed performing both feeding behaviors were the same (8% in both; Fig. 2). Regardless of the proportions of individuals observed foraging for arthropods and nectar during this season, all hummingbird species had higher floral visit rates than arthropod capture-attempt rates (Table 2, Fig. 3a, b).

During the cold-dry season, all hummingbird species were observed both making attempts to capture arthropods and visiting flowers for nectar (Figs. 2 and 3a, b). The proportion of observed individuals attempting to capture arthropods was lower than the proportion of individuals visiting flowers in all hummingbird species (Fig. 2), and except for Rivoli's Hummingbird, all hummingbird species had higher floral visit rates than arthropod capture-attempt rates (Table 2, Fig. 3a, b). During this season, only one male Riboli's and one male Rufous individual foraged for arthropods intensely (4.3% of the total individuals observed).

We found a significant difference in arthropod capture-attempt rates among hummingbird species during the cold-dry season (Kruskal-Wallis test: $\chi^2_4 = 12.67$, $P = 0.01$). The Tukey-Kramer HSD test revealed that arthropod capture-attempts rate was significantly higher in Rivoli's Hummingbird than Broad-tailed Hummingbird (0.73 ± 0.40 vs 0.08 ± 0.11 arthropod capture-attempts/s, $P = 0.009$; Table 2, Fig. 3a). Arthropod capture-attempt rates did not differ among the other species present ($P > 0.05$ for each comparison between pairs of species). Finally, we found that the arthropod capture-attempt rates of the White-eared Hummingbird, the only species we observed foraging for arthropods during all three climatic seasons (Table 2, Figs. 2 and 3a), varied seasonally. White-eared Hummingbird arthropod capture-attempts rate was significantly higher during the warm-dry season than during the rainy and cold-dry seasons (Table 3).

3.3.2. Arthropod foraging tactics

During the warm-dry season, all hummingbirds attempted to glean arthropods directly from substrates (100% of individuals observed, including White-eared hummingbirds and unidentified individuals; Fig. 4). During the rainy season, the hummingbird individuals observed attempted to capture arthropods both from the substrates and on perch, with most using gleaning (92.9%) while a few used perch-foraging (7.1%; Fig. 4). Finally, during the cold-dry season, the observed hummingbird individuals attempted to capture arthropods from the substrates, on perch, and in the air, with gleaning being the most used foraging tactic (77.1%), followed by foraging perch (14.6%), and hawking (8.3%; Fig. 4).

Taking into account only the attempts to glean arthropods on substrates (the foraging tactic most used by hummingbirds in the three sampling seasons; Fig. 4), during the warm-dry season, most of the individuals observed gleaning arthropods from the tree foliage (48.75%) and shrubs (37.5%), while a smaller proportion of individuals gleaning from herbs (8.75%), tree barks (2.5%), and ground (2.5%; Fig. 5). Similarly, during the rainy season, most of the individuals observed gleaning arthropods from the tree foliage (61.5%), followed by herbs (23.1%) and shrubs (15.4%; Fig. 5). In contrast, during the cold-dry season, most of the individuals observed gleaning arthropods from shrubs (70.3%) with a smaller proportion of individuals gleaning from tree foliage (27%) and a few gleaning from tree barks (2.7%; Fig. 5).

3.4. Food availability

No plants were blooming during the warm-dry season at our study site and surrounding areas. During the rainy season, *Penstemon roseus* was the only plant blooming, presenting a total nectar availability of 4.71 ml/ha (0.09 kJ/ha; Table 4). In the cold-dry season, *Ribes ciliatum* and *Salvia elegans* were in bloom, generating a nectar availability of 1609.92 ml/ha (53.38 kJ/ha; Table 4). We found that the availability of nectar resources in our study area did not differ from adjacent sites (Wilcoxon test: χ^2_1= 0.04, P= 0.83).

Arthropods found at our study site using our two sampling methods included members of the orders Acari, Araneae, Collembola, Hemiptera, Psocoptera, Thysanoptera, Coleoptera, Hymenoptera, Diptera, and Lepidoptera. However, as our stomach analysis showed, hummingbirds only ingested prey items belonging to the orders Araneae, Hemiptera, Psocoptera, Hymenoptera, and Diptera (see Fig. 1). During the warm-dry season, the availability of these arthropod orders collected using the beating method was 0.246 g dry weight/season; while in the rainy and cold-dry seasons, their availability was 0.051 and 0.049 g dry weight/season, respectively (Table 4).

3.5. Factors affecting arthropod ingestion by hummingbirds

We found that White-eared Hummingbird arthropod capture-attempt rates were positively and significantly affected by arthropod availability measured as both g dry weight/beating and g dry weight/Malaise (Table 5). However, the arthropod capture-attempt rates of this hummingbird species were not significantly affected by the energy available from nectar (Table 5).

4. DISCUSSION

4.1. Seasonal changes in hummingbird ensembles

Seasonal changes in hummingbird species composition and abundances occurred paired with significant fluctuations in floral nectar and arthropod availability at our study site. This was caused by both altitudinal movements of hummingbirds following floral phenology (DesGranges 1979, Calder & Contreras-Martínez 1993), and the arrival of latitudinal migrant hummingbirds during the fall and winter months (DesGranges 1979, Calder & Contreras-Martínez 1993, Schondube *et al*. 2004, Maya-García 2014). Additionally, our data indicate that arthropod availability was crucial to determine the presence of some hummingbird species at our study site in those moments of the year when floral nectar was absent or presented low abundance.

Only three species were present during the warm-dry season when flowers were absent and arthropod availability was at its highest point. The White-eared Hummingbird was the most abundant of them. This species is a dietary generalist (DesGranges 1979), and its arthropod capture-attempt rate during this season was one of the highest we found throughout our sampling year (only lower that the arthropod capture-attempt rate of Rivoli's Hummingbird during the cold-dry season; Table 2, Fig. 3a). Additionally, the average amount of arthropod ingested by White-eared Hummingbird during this season was the highest found for any species throughout our sampling year (Table 1). Our results indicate that White-eared Hummingbird individuals survived the absence of nectar during this season by increasing its arthropod ingestion, suggesting that this species can persist by feeding solely on arthropods, as has been suggested for other hummingbird species in the past (DesGranges 1979, Kuban & Neill 1980, Montgomerie & Redsell 1980, Powers *et al*. 2010).

4.2. Effect of availability of food resources on arthropod ingestion

The differences in nectar and arthropod availability among the warm-dry, rainy, and cold dry seasons allowed us to describe two different arthropod-use strategies by hummingbirds. The first strategy involves using arthropods as the primary source of nutrients and as an energy source. In contrast, in the second strategy, hummingbirds use arthropods only as a source of protein, fatty acids, and micronutrients, obtaining energy from floral nectar. Our study indicates that the high abundance of arthropods and the absence of nectar at our study site during the warm-dry season forced hummingbirds to use arthropods as their primary source of energy. This is surprising because some hummingbird species are unable to survive on high protein diets due to the physiological costs of handling the products of protein metabolism (López-Calleja & Bozinovic 2003, López-Calleja *et al*. 2003, Zanotto & Bicudo 2005). Our study indicates that hummingbird species with the physiological capacity to survive only on arthropods should be able to maintain year-round populations at our study site, becoming the dominant species in the ensemble in the absence of nectar resources. While hummingbird species unable to handle protein-rich diets will require local altitudinal movements to find nectar, modifying the composition of the hummingbird ensemble (DesGranges 1979, Calder & Contreras-Martínez 1993).

The arthropod capture-attempts rate of White-eared Hummingbird (the only species that was observed foraging for arthropods during all three climatic seasons) was significantly higher during the warm-dry season than during the rainy and cold-dry seasons

(Fig 3a, Tables 2 and 3). Additionally, the stomachs of this species from the warm-dry season contained on average three times more arthropods than the stomachs collected during the other two seasons (Table 1). Karasov (1990) estimates that the metabolizable energy content of dry arthropods for birds is 19.3 kJ/g. If White-eared Hummingbirds are capturing arthropods of fruit-fly size, and present a 30% capture success rate, they could cover their daily energy expenditure in less than 9 hr by feeding only on arthropods (Field metabolic rate of White-eared Hummingbird= 26.71 kJ/day; Schondube *et al.* 2012). This indicates that the high arthropod foraging rate of the White-eared Hummingbird could allow this species to satisfy all of its nutritional and energetic requirements in the absence of nectar by feeding exclusively on arthropods.

White-eared Hummingbird arthropod capture-attempt rates were positively and significantly affected by the availability of arthropods in the environment (Table 5). This pattern of arthropod use has been reported in other specialized nectar-feeding birds such as honeyeaters (Meliphagidae) and sunbirds (Nectariniidae; Paton 1982; Collins & Briffa 1982; Bergquist 1985, 1987; Recher & Davis 1997; Damon 2000; Daniels *et al*. 2001; Timewell & Mac Nally 2004), but as far as we know, not in hummingbirds. This result indicates, once again, that when nectar is available and abundant, hummingbirds, like sunbirds and honeyeaters, use arthropods as a complementary food, obtaining protein, fatty acids, and micronutrients; however, when nectar availability is low or absent, and arthropod abundance is high, arthropods play a role as alternative food and hummingbirds compensate by ingesting more arthropods to increase their energy intake and meet all nutritional requirement. In addition, during the warm-dry season, the proportion of individuals attempting to capture arthropods intensely (≥1 arthropod capture-attempt/s) was higher than during rainy and cold-dry seasons. Our findings clearly are related to the high prey availability and the high capacity of White-eared hummingbirds to consume protein.

4.3. Arthropod foraging tactics

Gleaning was the arthropod foraging tactic most used by hummingbirds during all three climatic seasons (Fig. 4). This arthropod foraging pattern has been previously reported in other hummingbird ensembles (Chavez-Ramirez & Dowd 1992, Chavez-Ramirez & Tan 1993, Stiles 1995), and it has been suggested that this foraging tactic is energetically less expensive than capturing insects in the air (Stiles 1995). Therefore, at our high-altitude study site, hummingbirds may use gleaning more frequently than other foraging tactics to save energy, mainly during months of the year when floral nectar is not available and/or night temperatures can drop below 0ºC.

During the warm-dry and rainy seasons, hummingbirds attempted to glean arthropods mainly from the tree foliage. In contrast, during the cold-dry season, most of them attempted to glean arthropods from the shrub layer (Fig. 5). This difference in the foraging stratum could be related to seasonal variation in the abundance of different arthropod orders and their patterns of activity through the vegetation. Specifically, during the warm-dry and rainy seasons, bugs (Hemiptera) and psocids (Psocoptera) were the most abundant insect groups in the foliage of trees and shrubs (estimated data using the beating method), while during the cold-dry season, the flies (Diptera) were the most abundant aerial insects (estimated data using Malaise traps). Bugs and psocids live on the bark and foliage of trees and shrubs, and in the litter (Triplehorn & Johnson 2005). The flies have a high diversity in terms of feeding habits and activity patterns, but some of them concentrate their activity around the flowers (Triplehorn & Johnson 2005). Therefore, the seasonal variation in the proportion of prey foraging substrate used by hummingbirds may be affected by these differences in arthropod abundance and activity patterns.

4.4. Differences in arthropod ingestion among hummingbird species

During the cold-dry season, when we observed five hummingbird species foraging for arthropods, the arthropod capture-attempts rate was significantly higher in Rivoli's Hummingbird than in Broad-tailed Hummingbird (Figs. 2 and 3a, Table 2). We suggest that this difference in arthropod use between these two species is related to differences in their foraging behavior. At our study site, Rivoli's Hummingbird is a large, very aggressive species (7-8 g) that acts as a trap-liner, while Broad-tailed Hummingbird is a small long-distance winter migrant species (3-4 g) that aggressively defends territories to obtain floral nectar (DesGranges 1979, Calder & Contreras-Martínez 1993, Schondube 2012, Maya-García 2014). Therefore, during the cold-dry season, when floral resources are abundant, Rivoli's Hummingbird individuals could increase their arthropod ingestion relative to nectar as a strategy to reduce interspecific competition for floral resources and obtaining additional energy from arthropods, while Broad-tailed hummingbirds ingest arthropods at a very low rate only to satisfy their nitrogen requirements while using nectar as their main source of energy and building fat stores in preparation to their migration that occurs at the end of the season (Fig. 3a, b).

4.5. Effects of the population declines of arthropods on hummingbirds in a changing world

Recently, many studies have reported drastic declines in the diversity and abundance of specific arthropod taxa (e.g., bees, ants, beetles, butterflies, and moths) all around the globe (see Hallmann *et al*. 2017, Tsutsui *et al*. 2018, Sánchez-Bayo & Wyckhuys 2019). The main factors of insect species declines include 1) natural-habitat loss, 2) intensive agriculture and urbanization, 3) pollution by pesticides and fertilizers, and 4) climate change (Hallmann *et al.* 2017, Sánchez-Bayo & Wyckhuys 2019 and references therein). Sánchez-Bayo & Wyckhuys (2019) suggest that insect population declines could lead to the extinction of up to 40% of the insect species on the planet in the following decades. While a reduction in arthropod diversity and abundance will have adverse effects on all hummingbird species by limiting their protein intake (Crick 2004, Both & Visser 2005), our results indicate that this reduction can also limit the energy intake of species that can survive on arthropods when nectar is not available. The role of arthropods for the survival of hummingbirds could be even more critical if we consider that climate change can reduce the production and quality of the nectar that hummingbirds use as their primary energy source in several regions of America (Soja *et al*. 2007, Rehfeldt *et al*. 2012). Under this scenario, arthropods could become an essential element for the survival of hummingbirds living in a human-altered planet.

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Table 1. Biomass of ingested arthropods (g dry weight) by hummingbirds each season. We show the mean and standard deviation values for the species with two or more stomachs analyzed.

Season	Individual	Species	Age	Sex	Biomass of arthropods ingested (g dry weight)	Mean biomass of arthropods ingested ± sd
Warm-dry	1	Amethyst-throated Mountaingem	Adult	Female	0.001	
	2	White-eared Hummingbird	Adult	Male	0.0011	0.0019 ± 0.0007 (*n*= 4)
	3	White-eared Hummingbird	Adult	Male	0.0027	
	4	White-eared Hummingbird	Adult	Male	0.0024	
	5	White-eared Hummingbird	Adult	Male	0.0014	
	6	Rivoli’s Hummingbird	Adult	Female	0.0038	
Rainy	7	Mexican Violetear	Adult	Female	0.0002	0.0007 ± 0.0005 (*n*= 4)
	8	Mexican Violetear	Adult	Unknow	0.0012	
	9	Mexican Violetear	Adult	Unknow	0.0012	
	10	Mexican Violetear	Juvenile	Unknow	0.0003	
	11	Amethyst-throated Mountaingem	Adult	Female	0.0006	
	12	White-eared Hummingbird	Juvenile	Female	0.0002	
	13	Rivoli’s Hummingbird	Adult	Male	0.0002	0.00015 ± 7.07e^{-05} (*n*= 2)
	14	Rivoli’s Hummingbird	Adult	Female	0.0001	
Cold-dry	15	Mexican Violetear	Adult	Male	0.0007	0.0004 ± 0.0001 (*n*= 4)
	16	Mexican Violetear	Adult	Unknow	0.0003	
	17	Mexican Violetear	Juvenile	Male	0.0003	
	18	Mexican Violetear	Juvenile	Unknow	0.0004	
	19	White-eared Hummingbird	Adult	Male	0.0002	0.00037 ± 0.00035 (*n*= 4)
	20	White-eared Hummingbird	Adult	Male	0.0001	
	21	White-eared Hummingbird	Juvenile	Male	0.0009	
	22	White-eared Hummingbird	Juvenile	Male	0.0003	
	23	Rivoli’s Hummingbird	Adult	Male	0.0025	0.001 ± 0.0013 (*n*= 3)
	24	Rivoli’s Hummingbird	Adult	Male	0	
	25	Rivoli’s Hummingbird	Juvenile	Male	0.0005	
	26	Broad-tailed Hummingbird	Adult	Female	0.0004	0.00075 ± 0.00037 (*n*= 4)
	27	Broad-tailed Hummingbird	Adult	Female	0.0009	
	28	Broad-tailed Hummingbird	Adult	Female	0.0012	
	29	Broad-tailed Hummingbird	Juvenile	Male	0.0005	
	30	Rufous Hummingbird	Juvenile	Male	0.0003	0.00036 ± 5.77e^{-05} (*n*= 3)
	31	Rufous Hummingbird	Juvenile	Female	0.0004	
	32	Rufous Hummingbird	Juvenile	Female	0.0004	

Table 2. Hummingbird arthropod capture-attempt and flower visit rates (events/s; mean ± sd) per species per season. For each hummingbird species, we pooled data on female, male, and unidentified sex individuals, and "*n*" represents the number of independent observations. The "Unknown" category includes individuals that it was not possible to identify.

Season	Species	Arthropod capture-attempts/s	Flower visits/s
Warm-dry	White-eared Hummingbird	0.48 ± 0.30 (*n*= 80)	
	Unknown	0.30 ± 0.19 (*n*= 6)	
Rainy	Mexican Violetear	0.27 ± 0.00 (*n*= 2)	0.32 ± 0.40 (*n*= 2)
	White-eared Hummingbird	0.16 ± 0.06 (*n*= 4)	0.38 ± 0.13 (*n*= 2)
	Rivoli's Hummingbird	0.26 ± 0.24 (*n*= 5)	0.43 ± 0.28 (*n*= 10)
	Amethyst-throated Mountaingem		0.34 (*n*= 1)
	Broad-tailed Hummingbird	0.04 (*n*= 1)	0.31 ± 0.10 (*n*= 3)
	Unknown	0.45 ± 0.40 (*n*= 4)	0.81 (*n*= 1)
Cold-dry	Mexican Violetear	0.16 ± 0.24 (*n*= 5)	0.25 ± 0.18 (*n*= 16)
	White-eared Hummingbird	0.25 ± 0.24 (*n*= 7)	0.45 ± 0.33 (*n*= 28)
	Rivoli's Hummingbird	0.73 ± 0.40 (*n*= 5)	0.55 ± 0.36 (*n*= 8)
	Broad-tailed Hummingbird	0.08 ± 0.11 (*n*= 10)	0.40 ± 0.31 (*n*= 41)
	Rufous Hummingbird	0.34 ± 0.54 (*n*= 7)	0.41 ± 0.25 (*n*= 22)
	Ruby-throated Hummingbird		0.33 (*n*= 1)
	Unknown	0.30 ± 0.31 (*n*= 12)	0.33 ± 0.32 (*n*= 19)

Table 3. Results of the GLM evaluating the effect of the season on the arthropod capture-attempt rates of White-eared Hummingbird.

Explanatory variable	Estimate	Standard error	χ^2_1	*P*-value
Intercept	4.0405	1.1392	12.578	0.0004
Rainy season	2.1132	2.1104	1.4362	0.2308
Warm-dry season	-1.9615	1.1471	5.217	0.0224

Table 4. Nectar and arthropod availability at our study site per season. Arthropod availability only includes arthropod orders ingested by hummingbirds at our study site.

Season	Nectar availability		Arthropod availability	
	Abundance (ml/ha)	Energy (kJ/ha)	g dry weight/beating	g dry weight/Malaise
Warm-dry	no nectar	no nectar	0.2464	0.0263
Rainy	4.71	0.09	0.0517	0.0015
Cold-dry	1609.92	53.38	0.0495	0.0124

Table 5. Results of the GLMs evaluating the effect of food availability on arthropod capture-attempt rates of White-eared Hummingbird.

Model	Explanatory variable	Estimate	Standard error	χ^2_1	*P*-value
GLM 1	Intercept	2.1464	0.2342	83.9755	<0.0001
	Nectar energy (kJ/ha)	0.0327	0.0279	1.9433	0.1633
GLM 2	Intercept	5.1018	1.7001	30.7795	<0.0001
	g dry weight of arthropods/beating	-12.2675	6.9967	4.7114	0.03
GLM 3	Intercept	5.911	2.107	31.5378	<0.0001
	g dry weight of arthropods/Malaise	-145.7884	81.068	5.1908	0.0227

Figure legends

Figure 1. Relative importance of different arthropod orders in the diet of hummingbirds at our study site. Values represent the mean of the percentage of prey items of each arthropod order ingested by hummingbird species. We excluded from our data birds whose stomachs were empty or contained unidentifiable arthropods.

Figure 2. Percentage of individuals observed each hummingbird species attempting to capture arthropods, visiting flowers, or performing other behaviors such as perching or hovering, during the warm-dry, rainy and cold-dry seasons. For each hummingbird species, we pooled data on female, male, and unidentified sex individuals. We excluded those species with less than four observations. The values at the top of the bars represent the number of observed individuals. MeVi= Mexican Violetear, WhHu= White-eared Hummingbird, RiHu= Rivoli's Hummingbird, BrHu= Broad-tailed Hummingbird, RuHu= Rufous Hummingbird, and Un= Unknown.

Figure 3. The number of arthropod capture-attempts per second (a) and the number of floral visits per second (b) for each hummingbird species during the warm-dry, rainy and cold-dry seasons. We show the mean and standard deviation values, except for *n*= 1. The values at the top of the bars represent the number of observed individuals. MeVi= Mexican Violetear, WhHu= White-eared Hummingbird, RiHu= Rivoli's Hummingbird, AmMo= Amethyst-throated Mountaingem, BrHu= Broad-tailed Hummingbird, RuHu= Rufous Hummingbird, RubHu= Ruby-throated Hummingbird, and Un= Unknown.

Figure 4. Percentage of hummingbird individuals observed attempting to capture arthropods using gleaning, perch-foraging, and hawking during the warm-dry, rainy, and cold-dry seasons. The values at the top of the bars represent the number of observed individuals. We show data for all hummingbird species together per season.

Figure 5. Percentage of hummingbird individuals observed attempting to glean arthropods on the tree foliage, shrubs, herbs, tree bark, or soil during the warm-dry, rainy, and cold-dry seasons. The values at the top of the bars represent the number of observed individuals. We show data for all hummingbird species together per season.

Figure 1

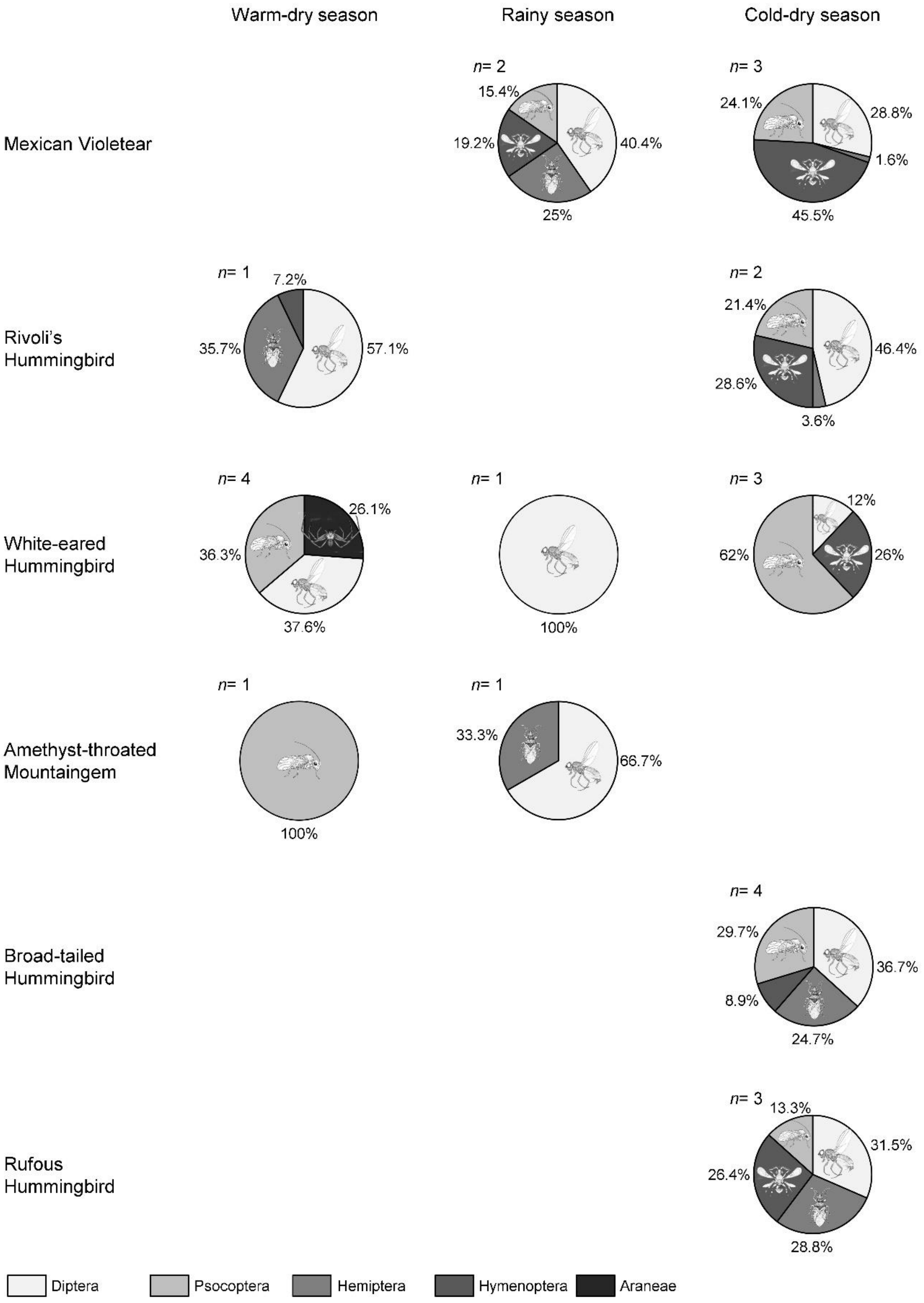

Warm-dry season
Rainy season
Cold-dry season
Mexican Violetear
n= 2
15.4%
19.2%
40.4%
25%
n= 3
24.1%
28.8%
1.6%
45.5%
Rivoli's Hummingbird
n= 1
7.2%
35.7%
57.1%
n= 2
21.4%
46.4%
28.6%
3.6%
White-eared Hummingbird
n= 4
26.1%
36.3%
37.6%
n= 1
100%
n= 3
12%
62%
26%
Amethyst-throated Mountaingem
n= 1
100%
n= 1
33.3%
66.7%
Broad-tailed Hummingbird
n= 4
29.7%
36.7%
8.9%
24.7%
Rufous Hummingbird
n= 3
13.3%
31.5%
26.4%
28.8%
Diptera
Psocoptera
Hemiptera
Hymenoptera
Araneae

Figure 2

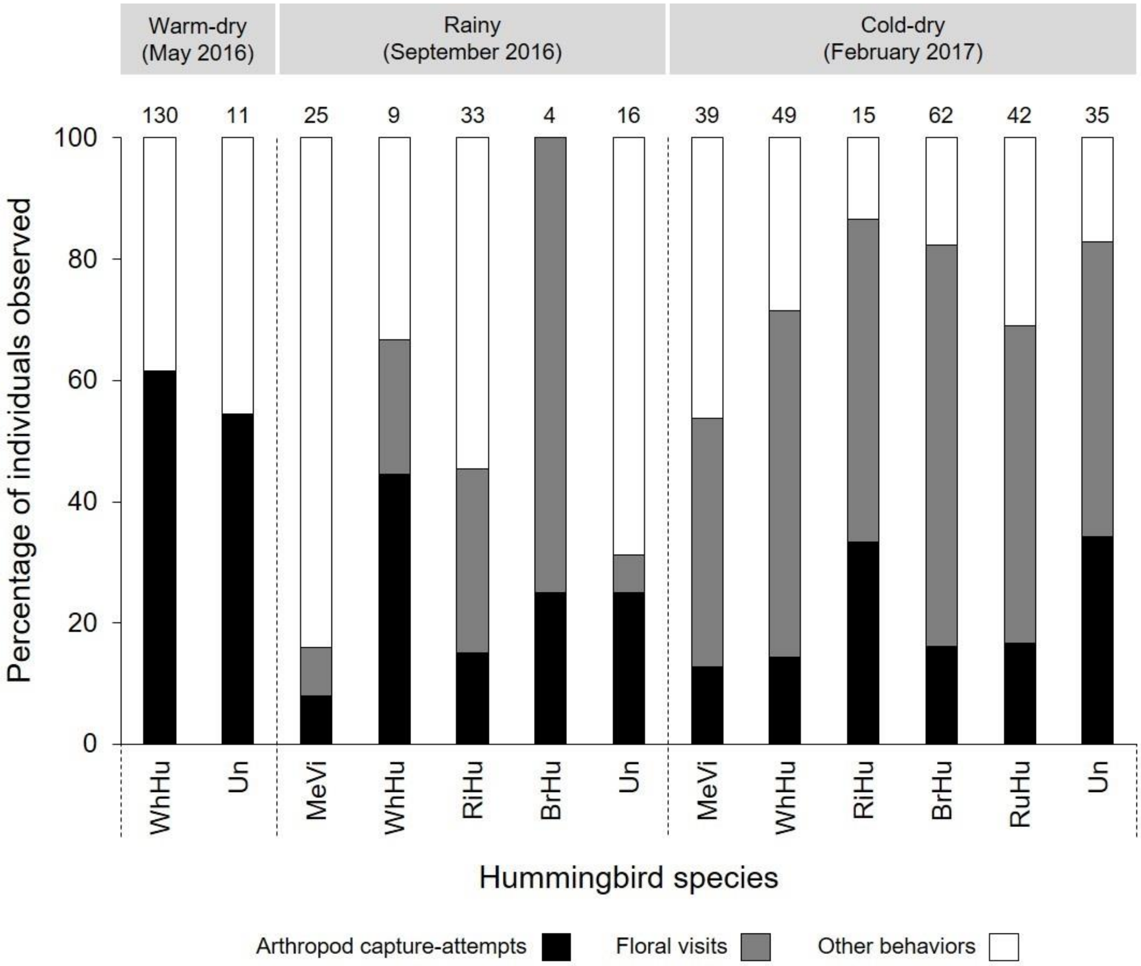

Warm-dry
(May 2016)
Rainy
(September 2016)
Cold-dry
(February 2017)
130
11
25
9
33
4
16
39
49
15
62
42
35
100
80
60
40
20
0
Percentage of individuals observed
WhHu
Un
MeVi
WhHu
RiHu
BrHu
Un
MeVi
WhHu
RiHu
BrHu
RuHu
Un
Hummingbird species
Arthropod capture-attempts
Floral visits
Other behaviors

Figure 3

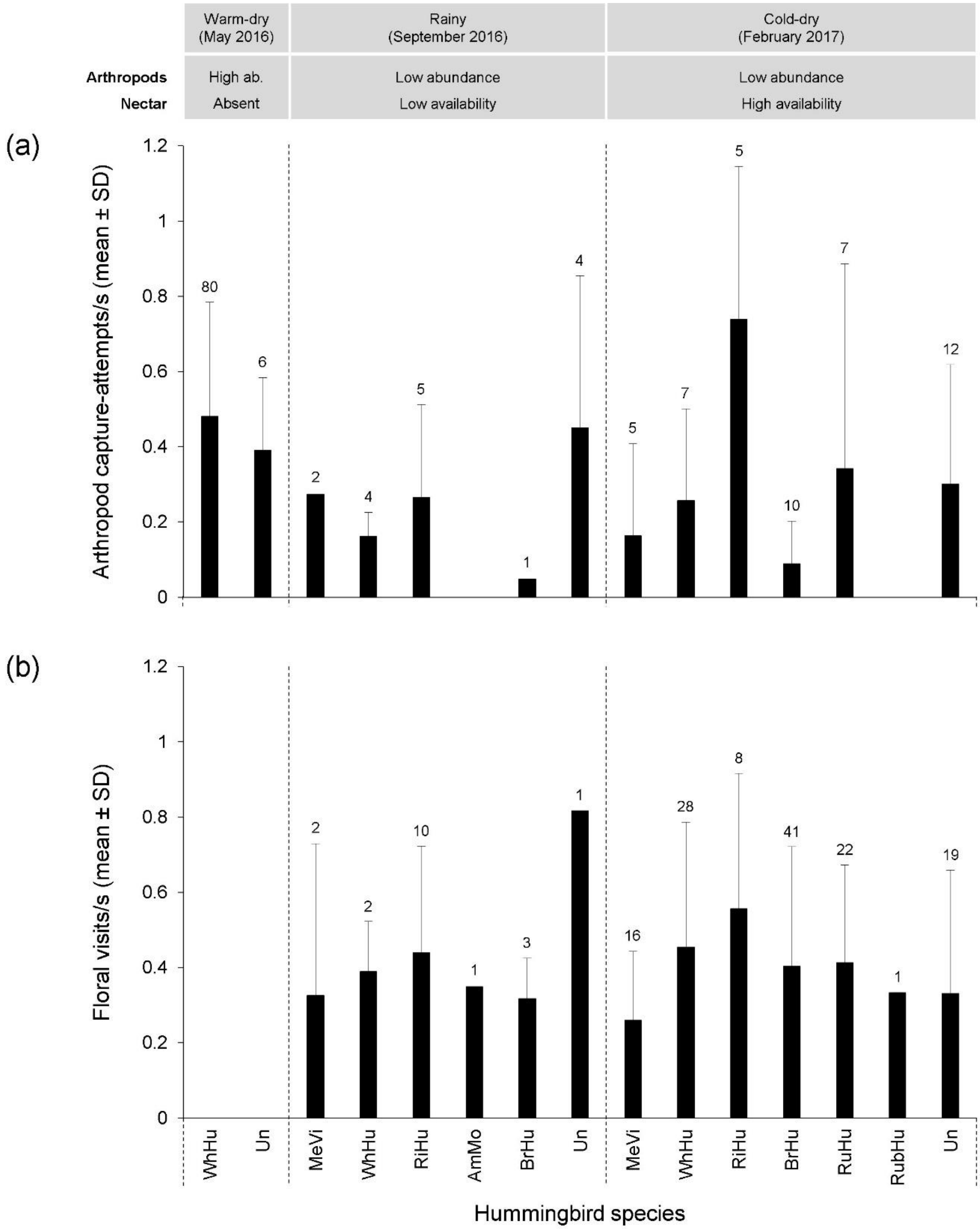

Warm-dry
(May 2016)
Rainy
(September 2016)
Cold-dry
(February 2017)
Arthropods
Nectar
High ab.
Absent
Low abundance
Low availability
Low abundance
High availability
(a)
Arthropod capture-attempts/s (mean ± SD)
1.2
1
0.8
0.6
0.4
0.2
0
80
6
2
4
5
1
4
5
7
5
10
7
12
(b)
Floral visits/s (mean ± SD)
1.2
1
0.8
0.6
0.4
0.2
0
2
2
10
1
3
1
16
28
8
41
22
1
19
WhHu
Un
MeVi
WhHu
RiHu
AmMo
BrHu
Un
MeVi
WhHu
RiHu
BrHu
RuHu
RubHu
Un
Hummingbird species

Figure 4

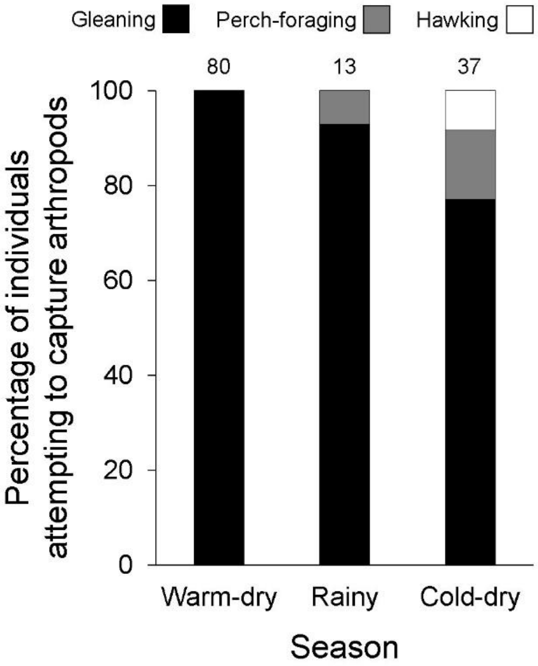
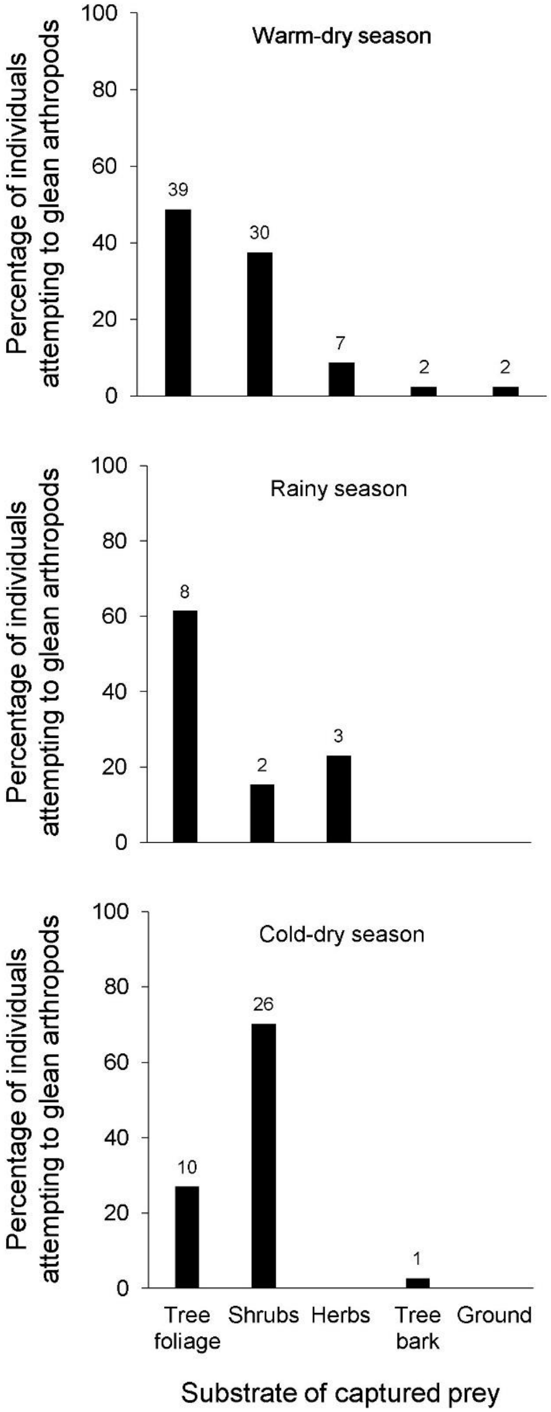

Gleaning
Perch-foraging
Hawking
80
13
37
100
80
60
40
20
0
Percentage of individuals attempting to capture arthropods
Warm-dry
Rainy
Cold-dry
Season

Figure 5


Warm-dry season
Rainy season
Cold-dry season
Percentage of individuals attempting to glean arthropods
100
80
60
40
20
0
39
30
7
2
2
8
2
3
10
26
1
Tree foliage
Shrubs
Herbs
Tree bark
Ground
Substrate of captured prey

CAPÍTULO 3. ANÁLISIS GEOMÉTRICO NUTRICIONAL DEL CONSUMO DE PROTEÍNA POR COLIBRÍES

Life is not always sweet! A nutritional geometry analysis of protein intake by hummingbirds

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Running title: Protein intake by hummingbirds

Abstract

Nectar-feeding birds, while having low protein requirements, must be efficient to acquire and use protein, since it is a crucial nutrient for maintenance, growth, and reproduction and can be a limiting factor under certain ecological circumstances. Even though nectar-feeding birds feed on arthropods, especially during low nectar availability, little is known about their intake and regulation of nutrients other than sugar and water. We applied a nutritional geometry approach to assess the importance of protein and how it is used to balance nutrient requirements by hummingbirds. We performed free-choice feeding trials, offering solutions varying in protein (0.2%-2%) and sucrose (5%-30%) concentrations, to compare the nutritional strategies of Broad-billed (*Cynanthus latirostris*) and Berylline (*Saucerottia beryllina*) Hummingbirds. We found that daily and cumulative protein and sucrose intake per unit of metabolic mass were significantly higher in *C. latirostris* than in *S. beryllina.* In particular, during the first experimental days, the slopes of the accumulated feeding trajectories for the two species almost coincided, showing nearly identical protein:sucrose ratios. However, as the trials proceeded, the slopes diverged, emphasizing a higher protein intake by *C. latirostris*. This difference in the capacity to consume protein seems to affect the ecology of both species. We consider that *C. latirostris* offers an example of how the capacity to ingest more protein permits individuals to remain at a site when floral resources diminish or disappear, while *S. beryllina* individuals were forced to migrate. Our study provides new insights into the role played by nutrient handling capacities in molding the ecology of nectar-feeding birds.

Keywords: macronutrient selection, nectarivory, nitrogen, nutritional ecology, protein as an energy source

1. INTRODUCTION

Hummingbirds and other nectar-feeding animals obtain energy from nectar and complement their nutritional needs by ingesting other sources of food such as arthropods or fruit (Pyke et al. 1980; Remsen et al. 1986; Brice 1992; Stiles 1995; Daniels et al. 2001; Powers et al. 2010). Because nectar is a diluted solution of sugars in water with trace amounts of a few amino acids, vitamins, and minerals, feeding on this sweet diet poses two main problems to animals: 1) they need to be able to handle, and dispose, of enormous quantities of water without losing limited electrolytes (Lotz and Martínez del Río 2004; Nicolson and Fleming 2014); and 2) they need to complement their diet to achieve nutrient balance and cover all their bodily requirements (Brice and Grau 1991; López-Calleja et al. 2003). While nectar-feeding birds such as hummingbirds commonly have low protein requirements (López-Calleja et al. 2003; McWhorter et al. 2003), they can temporarily subsist on a diet of arthropods and can present high protein ingestion under certain environmental conditions (Kuban and Neill 1980; Montgomerie and Redsell 1980). By doing so, they provide an ideal model to address how important protein is for nectar-eating animals, and how they use it to balance their nutritional needs.

Nectar-eating animals must be efficient in acquiring and using nitrogen sources since it is a crucial nutrient for maintenance, growth, and reproduction, and its abundance can be limited under some environmental conditions (White 1993; Sare et al. 2005). However, not much is known about the intake and regulation of nutrients other than sugar and water in nectarivorous vertebrates (Leseigneur et al. 2007). For example, food intake trials conducted with the Green-backed Firecrown (*Sephanoides sephaniodes*) revealed that individuals required approximately 10 mg of nitrogen per day to maintain body mass

(López-Calleja et al. 2003). However, a nitrogen overload (i.e., a saturation in nitrogen absorption capacity) occurred when fed an 11.1% N diet. Similarly, White-breasted Sunbirds (*Cinnyris talatala*) showed no preferences for specific amino acids in artificial nectar and even avoided them when offered at high concentrations (15 mM; Leseigneur et al. 2007).

A valuable tool to understand food choice, nutritional requirements, nutrient regulation and the existence of physiological constraints associated with the use of protein in nectar-eating birds is the nutritional geometry approach (Schaefer et al. 2003; Köhler et al. 2012). Nutritional geometry is a robust framework that takes into account the multidimensional aspects of nutrition and thus analyzes how individuals simultaneously regulate the intake of different macro and micronutrients in response to their changing nutrient needs, metabolic requirements, and availability of nutrients in the environment (Simpson et al. 2004; Behmer 2009). This approach has demonstrated that individuals do not necessarily forage for energy *per se*, being capable of discriminating and selecting different nutrient combinations from complementary foods (Jensen et al. 2012; Machovsky-Capuska et al. 2016; Coogan et al. 2017). Recent studies have stressed that specific nutrient intake ratios (e.g., protein:carbohydrates) are sought out by foraging animals, and are associated with significant variability in their health, lifespan, and reproductive outcome (Solon-Biet et al. 2014; Le Couteur et al. 2016).

Our objective was to determine if hummingbirds can regulate protein intake independently of sugar ingestion. By combining geometric analyses and feeding trials on Broad-billed (*Cynanthus latirostris*) and Berylline (*Saucerottia beryllina*) Hummingbirds we aimed to: 1) identify minimum daily protein intake and nutrient intake targets of individuals of both species under free-choice trials; 2) assess if individuals maximize the intake of any single nutrient, or prioritize (i.e., maintain constant) the intake of a given nutrient or nutrient ratio; 3) evaluate the temporal dimension of such nutrient preferences; and 4) compare the nutritional strategies of two species of hummingbirds that exhibit marked differences in their ecology and foraging patterns.

2. MATERIALS AND METHODS

2.1. Hummingbird capture and maintenance

We captured five adult *Saucerottia beryllina* [Deppe 1830] (sex unknown) and six adult *Cynanthus latirostris* [Swainson 1827] (four males, two females) using traps and mist nets in the gardens of the Morelia Campus of the National Autonomous University of Mexico (UNAM), located in the state of Michoacan, Mexico. All hummingbirds were non-reproductive. We were unable to determine the sex of *S. beryllina* individuals due to the lack of evident sexual dimorphism in this species. *Saucerottia beryllina* is a medium-sized (4-5 g) behaviorally dominant species that conducts local migrations, generally found at our study site from April to September (Lara et al. 2012; López-Segoviano et al. 2018a; personal observations). *Cynanthus latirostris* is a slightly smaller species (3-4 g) that acts as behaviorally non-dominant and does not conduct local movements. After capture, we kept birds in individual indoor cages (60 × 60 × 60 cm) in an experimental room maintained at ambient temperature (17-24°C) with artificial full-spectrum light (12 h:12 h, L:D). We trained hummingbirds to feed from glass feeders and offered artificial nectar (Nektar-Plus, Nekton®, Pforzheim, Germany) *ad libitum* during a three-day acclimation period. We recorded individual body mass daily using an electronic scale (Ohaus Scout®

Pro, 200 g x 0.01 g). At the end of the experiment, we released all hummingbirds at their capture site (permit SGPA/DGVS/12889/13).

2.2. Experimental diets and protocol

We determined individual preferences and nutrient intake targets, which we define as the mean nutrient intake across the days of free choice, by using free-choice experiments. During these experiments, hummingbirds were able to choose from two different solutions offered simultaneously. We offered hummingbirds two diets, each composed by two experimental solutions varying in protein (P; Moviment® collagen hydrolysate dietary supplement, GNT-VAL S.R.L., Jalisco, Mexico) and sugar concentrations (sucrose - S; ≥99,5% (GC), SIGMA®). Each diet included a Low Protein – Low Sugar solution and a High Protein – High Sugar solution. Diet 1 comprised Solution A (0.5% P, 15% S) and Solution B (1.5% P, 25% S). Diet 2 was composed by Solution C (0.2% P, 5% S) and Solution D (2% P, 30% S; Fig. 1). The experiment consisted of five consecutive phases, each lasting three days, except for one recovery day with maintenance diet, which was implemented halfway through the experiment to ensure the health of the experimental individuals (Fig. 1): 1) Diet 1 (solutions A and B), 2) Diet 2 (solutions C and D), 3) Maintenance diet (Nektar-Plus only), 4) Diet 1 (solutions A and B), and 5) Diet 2 (solutions C and D; see Fig. 1).

We provided the solutions using calibrated glass feeding tubes inserted through the mesh wall of the cages (two feeders per individual cage). Food was available for 12 hours, from 08:00 to 20:00. After six hours (half of each feeding session), we switched the position of the two feeders to avoid/detect possible effects of the feeder position on food selection. We recorded the amount of solution ingested by each bird when the feeder position was switched and at the end of each experimental day by measuring the remaining volume (ml) of the solutions in the experimental feeders. Additionally, we used control feeders to take into account evaporative losses. We filled these feeders with the experimental solutions and placed them outside the cages. We also controlled for the amount of solution lost by dripping or spilling by placing small cups with liquid paraffin oil below each feeder and weighing them at the beginning and end of each feeding session.

2.3. Data analysis

The experiments were designed and analyzed using a nutritional geometry framework (Simpson and Raubenheimer 2012). Intake data (grams of protein and sucrose consumed per day) are reported both without body mass adjustments, to interpret them within an ecological context, and divided by the metabolic body mass of the individuals (mbm = $g^{0.75}$), to analyze them within a physiological and comparative perspective. Values of body mass for each bird represent the averages of the daily weights of each individual measured before the feeding session started in the morning. We calculated the coefficient of variation (CV) for protein intake, sucrose intake, and their ratio to determine if nutrient intake was consistent across individuals and diets. The CV is a measure of dispersion that is calculated as the standard deviation divided by the mean. Distributions with CV > 1 are considered high-variance, and a CV value close to zero represents a more constrained nutrient intake than a CV value close to one (Johnson et al. 2013).

We used linear mixed-effects models (LMM) to test the effect of the hummingbird species on nutrient intake corrected by metabolic body mass (‘lmerTest’ package in R, version 3.5.0) (Kuznetsova et al. 2017; R Core Team 2018). The identity of experimental individuals was included as a random factor to account for repeated measurements from the same individual, and the species (*S. beryllina*, *C. latirostris*) was the fixed factor. We ran two models, one with protein intake (LMM1) and the other with sucrose intake (LMM2) as response variables. We additionally ran a generalized linear mixed-effect model (GLMM, Bolker et al. 2009) fitted with a negative binomial distribution with the protein:sucrose ratio as the response variable, which was not normally distributed. We used two-sample t tests to compare energy intake between species when consuming the maintenance diet and the experimental diets.

We plotted the accumulated daily nutrient intake during the 13 consecutive experimental days to analyze temporal patterns of nutrient preferences. We compared the slopes of the accumulated sucrose and protein intake trajectories by individual hummingbirds using the 'smatr' package in R (Warton et al. 2012). This analysis uses a Standardized Major Axis approach equivalent to an analysis of covariance. We used adjusted p-values (Sidak correction) for all pairwise comparisons of slopes to control for family-wise error rate (Warton et al. 2012). We set the statistical significance in all analyses at $\alpha = 0.05$.

3. RESULTS

On the days in which hummingbirds were under optimal nutritional conditions (maintenance diet of Nektar-Plus), both species had similar energy intake values corrected by metabolic body mass (11.8 kJ/mbm *S. beryllina* vs. 14.2 kJ/mbm *C. latirostris*; Two-sample t-test, $t = 1.87$, $p = 0.09$). However, when confronted with our experimental diets, *S.*

beryllina ingested lower amounts of protein, sucrose, and overall total energy than *C. latirostris* (7.4 kJ/mbm *S. beryllina* vs. 10.1 kJ/mbm *C. latirostris*; Two-sample t-test, t = 6.92, p < 0.001). As a result of this, during our experimental trials total nutrient intake was higher in *C. latirostris* individuals, which, despite being characterized by a smaller body mass than *S. beryllina*, consumed on average 0.077 ± 0.01 g of protein and 1.43 ± 0.2 g of sucrose daily, vs. *S. beryllina*'s 0.059 ± 0.02 g of protein and 1.33 ± 0.4 g of sucrose. We present mean individual daily nutrient intakes in Table 1. For *S. beryllina*, when compared to protein intake (CV = 0.34) and sucrose intake (CV = 0.31), the protein:sucrose ratio was the variable that fluctuated least across days and among individuals (CV = 0.27). In contrast, *C. latirostris* sucrose intake was maintained more constant (CV = 0.17) than the protein intake (CV = 0.20) and the protein:sucrose ratio (CV = 0.23).

These patterns held when nutrient intake values were corrected by metabolic body mass. Both daily protein (0.031 ± 0.006 g/mbm *vs*. 0.020 ± 0.007 g/mbm) and sucrose intake (0.58 ± 0.1 g/mbm *vs*. 0.44 ± 0.1 g/mbm) were significantly higher in *C. latirostris* than in *S. beryllina* (LMM1: estimate = 0.011, standard error = 0.001, df = 8.24, t-value = 9.22, p < 0.0001; LMM2: estimate = 0.140, standard error = 0.024, df = 8.51, t-value = 5.78, p = 0.0003). However, we did not find statistically significant differences between the two species in the protein:sucrose ratios of their daily meals (1:18 vs. 1:21 mean protein:sucrose ratios for *C. latirostris* and *S. beryllina*, respectively; GLMM: estimate = 0.17, standard error = 0.78, z-value = 0.21, p = 0.8). The nutritional targets for the individuals of both species are shown in Fig. 2. Our results indicate that when fed Diet 2, *C. latirostris* consumed almost exclusively Solution D (2% P, 30% S), which was characterized by the highest concentrations of protein and sucrose.

The cumulative balance of protein and sucrose intake throughout the experimental period was highly consistent for all individuals in both experiments, with the values of the coefficients of determination (R^2) being close to 1 for all individuals of both species (Fig. 3). Slopes of the accumulated sucrose and protein intake curves did not differ among individuals of *C. latirostris* (test for pairwise differences in slope, $p > 0.3$). However, for *S. beryllina*, individual 2 showed a steeper slope (i.e., a higher sucrose:protein ratio), which was significantly different from individual 1 (test for pair-wise differences in slope, TestStat = 11.68, $p = 0.03$) and individual 5 (TestStat = 11.26, $p = 0.04$). Overall, the average slopes of the accumulated intake differed between the two species (Likelihood ratio = 16.57, df = 1, $p < 0.0001$), indicating a higher sucrose:protein ratio in *S. beryllina* than in *C. latirostris* (Fig. 3). During the first 4-5 experimental days, the slopes of the feeding trajectories for the two species almost coincided, but as the feeding trials proceeded, the slopes diverged, emphasizing a higher protein intake by *C. latirostris*.

4. DISCUSSION

Our study provides new insights on hummingbird nutrition, showing differences in nutrient intake by two sympatric species of hummingbirds that belong to the same clade (Emeralds; McGuire et al. 2014), but differ in their foraging behavior, behavioral dominance, and migration patterns. During the 13-day feeding experiment, we found that *Cynanthus latirostris* consumed more protein and sugar per unit of metabolic body mass than *Saucerottia beryllina*. Both species ingested similar daily protein:sucrose ratios. However, these ratios diverged over time. In this section, we first discuss protein ingestion in hummingbirds. Second, we highlight the importance of looking into the balance of certain nutrients in the diet, instead of taking into account just the energy requirements of hummingbirds. Finally, we consider the ecological and behavioral implications of the patterns we found and examine the possible role that protein consumption has in shaping hummingbird community dynamics.

4.1. Protein ingestion by hummingbirds

Nitrogen requirements for nectar-feeding birds are lower than expected, given their body masses (McWhorter et al. 2003). Interestingly, previous studies on protein requirements and protein intake by different species of captive hummingbirds found a significant variation in protein intake among species (Brice and Grau 1991; López-Calleja et al. 2003; McWhorter et al. 2003; see Table 3). Our observations of daily protein intake for *S. beryllina* (0.020 g/mbm) and *C. latirostris* (0.031 g/mbm) surpass three to 13 times the requirements that have been observed or estimated for other hummingbird species. For example, daily protein requirements for body mass maintenance in Costa's hummingbirds (*Calypte costae*), a species that weighs approximately 3.5 g, are only 4.5 mg N/day, which equals to 0.011 g/mbm protein (Brice and Grau 1991).

According to regressions of nitrogen excretion versus nitrogen intake in birds with different diet specializations (e.g., nectarivores, granivores, insectivores), Preest et al. (2003) estimated that for body mass maintenance *C. costae* requires 5.3 mg N (equivalent to 0.0142 g/mbm protein) and Anna's hummingbird (*Calypte anna)* 6.6 mg N (or 0.0141 g/mbm protein). Larger hummingbird species such as the Magnificent hummingbird (*Eugenes fulgens;* body mass: 7.5 g), and the Blue-Throated hummingbird (*Lampornis clemenciae*; body mass: 7.9 g) have lower estimated daily nitrogen requirements: 4.03 mg (0.0056 g/mbm protein) and 3.24 mg (0.0043 g/mbm protein), respectively (McWhorter et al. 2003). In contrast, while the maintenance requirements for *Sephanoides sephaniodes* (6.2 g) were estimated to be as low as 0.0023 g/mbm protein, it was experimentally observed that individuals required at least 9 mg N (0.014 g/mbm protein) per day to maintain their body mass (López-Calleja et al. 2003). Considering the minimal N requirements of hummingbird species of similar body mass, the experimental protein intake of both of our study species should allow them to use between 0.0057 and 0.0204 g/mbm of the ingested protein as an energy source (Table 2).

Independently of their N requirements, daily protein ingestion in different hummingbird species can be quite variable, as shown by the intakes of captive hummingbirds fed diets with different nitrogen levels (0, 1.2, and 4 g/L of casein acid hydrolysate): Black-chinned hummingbirds, *Archilocus alexandri* (0 – 0.032 g/mbm); *L. clemenciae* (0 – 0.020 g/mbm), and *E. fulgens* (0 – 0.034 g/mbm) (McWhorter et al. 2003). In this sense, it is crucial to consider that protein balance is regulated on a time scale longer than just one day. Hummingbird individuals could present different daily protein intakes to achieve nutritional balance depending on nutrient availability and energetic needs (López-Calleja et al. 2003). This hypothesis was confirmed by our results of the accumulated nutrient trajectories, which started to show a divergence in protein intake between our two study species after 4-5 days of the beginning of the feeding trials. Similar to our results, *S. sephaniodes* individuals feeding on a protein-free diet presented a significant difference in body mass when compared to individuals feeding on low protein diets (1.2 and 2.3% protein) after four days (López-Calleja et al. 2003). After ten days on the protein-free diet, this species lost approximately 15% of their body mass (López-Calleja et al. 2003). In a similar experiment, Costa's hummingbirds lost 16% of their body masses (Brice and Grau

1991). Both our results and these two studies indicate that the time scale at which hummingbirds respond to the protein content of their diets, and start balancing N ingestion, is between four and 10 days, presenting significant structural damage in their organs after this time-period (López-Calleja et al. 2003).

4.2. Protein:carbohydrate ratios in hummingbird diet

The benefits of balancing and selecting specific ratios of nutrients are well documented in several organisms (Simpson and Raubenheimer 2012). Since the 1980s, experiments of ‘self-selection’ (otherwise called nutrient mixing or diet balancing) with insects demonstrated that selecting foods that allow reaching a specific nutrient balance has direct effects on performance (e.g., survival, growth, reproduction) (Waldbauer et al. 1984; Waldbauer and Friedman 1991). However, it was the implementation of the Geometric Framework for nutrition that has made possible to identify optimal nutrient ratios (e.g., protein:carbohydrate) that enhance reproductive success, life-history traits, or lifespan in a vast array of species (Raubenheimer and Simpson 1993; Simpson and Raubenheimer 1993, 1995). However, these optimal ratios are not fixed and can vary not only among different taxa but also across sexes, developmental stages, physiological conditions, or even when associated with specific traits (Simpson and Raubenheimer 2012; Sentinella et al. 2013). For example, in field crickets (*Gryllus veletis*) adult weight gain and egg production are maximized when individuals are fed diets with a high protein:carbohydrate ratio (3:1). In contrast, lifespan is maximized on diets with a low protein:carbohydrate ratio (1:8 in females, and 1:3 in males) (Harrison et al. 2014).

Our results indicate that the daily protein:sucrose ratio (1:21) achieved by *S. beryllina* by mixing the two offered experimental solutions was maintained relatively constrained during the feeding trials. Moreover, when analyzing the accumulated intake values, the protein:sucrose ratio in both species was remarkably constant over the 13 experimental days. This particular ratio could be associated with physiological or health benefits linked to reproduction, aging, and immune function (Huang et al. 2013; Ponton et al. 2011, 2013). However, the relevance of the protein:sucrose ratio we found in these nectar-feeding birds is a subject that requires further exploration.

In general, herbivorous and omnivorous animals do not overconsume protein to increase their energy intake due to the physiological complications derived from dealing with the surplus protein (e.g., elimination of nitrogenous waste), or to the high mortality rates that can be associated with a high protein diet (Pirk et al. 2010; Virtanen et al. 2019). For example, in roundworms, flies, and mice, high systemic protein levels increase signaling of the target of rapamycin (TOR) pathway, which was found to cause reductions in lifespan (Simpson and Raubenheimer 2009; Dussutour and Simpson 2012). However, some species prioritize the use of energy from carbohydrates and lipids while over-eating protein (Rothman et al. 2011), and the hummingbird *C. latirostris* might be favored by a higher protein tolerance which could lead to a higher protein consumption during certain seasons of the year. Analyzing the consumption of arthropods as an energy source by three hummingbird species in southeastern Arizona, Powers et al. (2010) found that *Eugenes fulgens* ate a higher amount of arthropods catabolizing more protein/fat than *Lampornis clemenciae* and *Archilocus alexandri*. However, these authors argue that after considering differences in body size among species, different hummingbird species should show similar anatomy and physiological capacities and suggested that the ability to subsist on arthropods should be a common characteristic of all hummingbirds (Powers et al. 2010). Nonetheless, our results indicate that different hummingbird species might have dissimilar capacities to regulate nutrient intake, assimilation, and metabolism, since when exposed to identical diets, individuals of *C. latirostris* and *S. beryllina* presented different protein and sugar intakes (g/mbm).

4.3. The importance of protein ingestion for the ecology of hummingbirds

Our findings indicate that under optimal nutritional conditions (Nektar-Plus maintenance diet), our study species presented similar energetic intakes corrected by metabolic body mass. However, when confronted with the experimental solutions with different nutrient contents, species-specific constraints became evident, with *S. beryllina* reducing their total energy intake by 37% and *C. latirostris* by 29%. These results indicate that protein content in the diet can constrain energy intake in some nectar-feeding animals. The reduction of the energy intake when protein was present in the diet seems to result from both hummingbird species having physiological limitations related to the use of protein as an energy source, with these limitations being more substantial in *S. beryllina*.

By limiting energy intake, dietary protein can negatively affect hummingbird species that live on the verge of a negative energy balance (McWhorter and López-Calleja 2000). Due to energy balance limitations, hummingbirds always select the most concentrated of two sugar solutions in feeding trials (Tamm and Gass 1986; Schondube and Martínez del Río 2003). However, in our experimental study, *S. beryllina* consumed a higher daily amount of the solutions with lower sucrose concentration (A [15%S] and C

[5% S]: 6.4 ± 0.7 ml; mean ± SE) than of those with higher sucrose content (B [25% S] and D [30% S]: 2 ± 0.3 ml; mean ± SE). It must be noted that the solutions with lower sugar concentration also were characterized by a lower protein content (see Methods section), suggesting that the feeding choices of *S. beryllina* during our experiments were not determined by the sugar content of the experimental solutions, but by its inability to handle diets with high protein content. Similar results have been reported for *Sephanoides sephaniodes*, with individuals feeding on high protein diets presenting increases in liver and kidney masses and kidney hypertrophy (López-Calleja and Bozinovic 2003; López-Calleja et al. 2003). While *C. latirostris* also showed a decrease in energy intake when feeding on the experimental solutions compared to the maintenance diet, this species ingested on average the same amount of both solutions when fed Diet 1 (3.6 ml each, for solutions A and B), or preferred to feed on the high protein-high sugar solution when confronted with Diet 2 (4.3 ml [D] vs.0.4 ml [C]), indicating a higher capacity to deal with protein.

How are these results related to the ecology of both species? Our study species co-occur at several sites in Mexico and present different behavioral dominance hierarchies and local migratory patterns (Rodríguez-Flores and Arizmendi 2016; López-Segoviano et al. 2018b; Márquez-Luna et al. 2018). *Saucerottia beryllina* is behaviorally dominant over *C. latirostris*, but is not a year-long resident at our study site in Morelia, Michoacán, since it departs when nectar quality is low (sugar concentration lower than 15% weight/volume; Meneses Ramírez 2010), showing a high sensitivity to the quality of available nectar-resources and local or regional scale movements following the blooming of plant with high-quality nectar (López-Segoviano et al. 2018a). On the contrary *C. latirostris*, despite being subordinate to *S. beryllina*, is a year-long resident at our site. This could be associated with its capacity to ingest higher amounts of both protein and sugar, metabolize more copious amounts of protein, and handle the nitrogenous waste.

The differences in the way both species balance their nutrient intake, particularly their protein intake, could explain their behavioral and ecological dissimilarities. We argue that *C. latirostris*, with its higher daily and accumulated protein intake, represents an example of how some hummingbird species can use protein as an energy source better than others. This capacity to use protein as an energy source could provide an advantage when nectar abundance or quality is reduced, allowing *C. latirostris* to be resident all year round, compared with *S. beryllina* individuals that are forced to migrate under the same environmental conditions. This was particularly evident from the cumulative intake trajectories, which revealed that *C. latirostris,* at the end of our experiment, ate 60% more protein than *S. beryllina*.

An increment in protein intake for a species with limited capacities to metabolize protein could have critical adverse effects on the hummingbirds. In a pilot study conducted with *S. beryllina* and *Amazilia violiceps* we found that these hummingbirds could not consume solutions containing more than 3% (w/v) protein without presenting health issues, such as inability to fly and problems to stay perched. These symptoms were slowly reversed when hummingbirds were offered solutions with lower protein concentrations. We hypothesized that a nitrogen overload might have occurred, provoking a build-up of uric acid (e.g., articular gout or urate deposition), which albeit not reported in hummingbirds, is commonly observed in poultry (Guo et al. 2005; Eldaghayes et al. 2010). A study with the Black Jacobin (*Florisuga fusca*), showed that hummingbirds feeding on a high protein –

low sucrose solution (2.4% P, 12% S) ingested larger volumes of the diet but decreased the frequency between feeding sessions (Zanotto and Bicudo 2005) when compared to hummingbirds fed a 0.8% P – 36% S diet. This might have been caused by a metabolic feedback to avoid a potentially harmful excess of protein intake, even though sucrose intake was also decreased.

Here we have shown that two hummingbird species belonging to the same clade and having relatively similar body masses can present different strategies to balance their diets and that their nutrient selection might reflect slight but essential differences in their metabolism. Brice and Grau (1989), comparing fluctuations in body mass of *Calypte anna* and *C. costae* fed diets containing 3% and 6% protein, suggested that some species of hummingbirds, especially those living in highlands, are more insectivorous than others, and might have higher nitrogen requirements. Under this scenario, species such as *C. latirostris*, which have the possibility of consuming more protein, by feeding more on arthropods without presenting negative physiological consequences, would not need to move or migrate during periods of nectar scarcity.

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Table 1. Weights of the individual hummingbirds at the beginning (day 1) and end (day 13) of the experiments and observed nutrient intakes throughout the feeding trials. Standard deviations (SD) are shown. AM = adult male, AF = adult female.

Variables	*Saucerottia beryllina*					*Cynanthus latirostris*					
	Ind. 1	Ind. 2	Ind. 3	Ind. 4	Ind. 5	Ind.1 (AM)	Ind. 2 (AM)	Ind. 3 (AM)	Ind. 4 (AM)	Ind. 5 (AF)	Ind. 6 (AF)
Initial weight (g) – before meal	4.26	4.32	4.66	4.71	4.57	3.61	3.63	3.56	3.43	3.43	3.33
Final weight (g) – before meal	3.87	4.21	3.62	4.37	4.33	3.18	3.33	3.34	3.13	3.09	2.95
Mean daily protein intake (g) (±SD)	0.056 ±0.020	0.053 ±0.013	0.059 ±0.023	0.068 ±0.029	0.059 ±0.012	0.078 ±0.008	0.077 ±0.020	0.073 ±0.015	0.078 ±0.016	0.087 ±0.014	0.071 ±0.014
Mean daily sucrose intake (g) (±SD)	1.206 ±0.34	1.285 ±0.43	1.375 ±0.34	1.437 ±0.61	1.205 ±0.23	1.369 ±0.25	1.440 ±0.14	1.352 ±0.19	1.437 ±0.28	1.623 ±0.29	1.365 ±0.21
Mean daily protein/sucrose intake (±SD)	0.047 ±0.01	0.043 ±0.01	0.043 ±0.01	0.049 ±0.01	0.050 ±0.01	0.059 ±0.01	0.054 ±0.01	0.055 ±0.01	0.056 ±0.01	0.055 ±0.01	0.054 ±0.01

Table 2. Body mass and protein requirements and intake in different hummingbird species

Species	Body mass (g)	Protein requirements (mg N/$g^{0.75}$)	Max protein intake (mg/$g^{0.75}$)	Average protein intake (mg/$g^{0.75}$)	References
Archilocus alexandri[1]	2.7	3.00	32.31		McWhorter et al. 2003
Cynanthus latirostris[2]	3.3		45.00	31.45 ± 0.7	This study
Selasphorus platycercus[1]	3.4	2.20			McWhorter 1997
Calypte costa[1]	3.5	10.99			Brice and Grau 1991
	3.1	14.18			Preest et al. 2003
Calypte anna[1]	4.2	14.06			Preest et al. 2003
Saucerottia beryllina[2]	4.3		35.00	19.76 ± 0.9	This study
Sephanoides sephaniodes[3]	6.2	14.73	27.93	11.47 ± 0.8	López-Calleja et al. 2003
Florisuga fusca[4]	7.2			2.3 – 11.47	Zanotto and Bicudo 2005
Eugenes fulgens[5]	7.5	5.56	33.70		McWhorter et al. 2003
Lampornis clemenciae[5]	7.9	4.30	20.01		McWhorter et al. 2003

[1] Bees, [2] Emeralds, [3] Coquettes, [4] Topazes, [5] Mountain Gems

Figure Legends

Fig 1. Nutrient space with rails indicating the four solutions (A-D) with different proportions of protein and sucrose (P:S) used in the feeding trials. The experimental design is also indicated, showing the alternation between the diets offered to hummingbirds during the 13-day experiment. NP = Nektar-Plus (maintenance diet). The areas delimited by the rails represent the nutrient space available to the study individuals

Fig 2. Daily protein and sucrose intake (g/mbm) of all *Cynanthus latirostris* and *Saucerottia beryllina* individuals during the 13-day feeding trial. The nutritional rails characterizing the different solutions offered to the hummingbirds are represented by solid (A+B = Diet 1) and dotted (C+D = Diet 2) lines. Total averages (i.e., intake targets) are shown (± standard deviation) for each species

Fig 3. Average accumulated intake (g/mbm) of protein and sucrose by *Cynanthus latirostris* and *Saucerottia beryllina* individuals across the 13 day-feeding trials. Standard deviations, linear regression equations and coefficients of determinations (R^2) are shown. The cumulative balance of protein and sucrose intake was highly consistent for all individuals, as shown by R^2 values close to 1 of the average slopes

Figure 1

Day	1 -3	4 - 6	7	8 - 10	11 - 13
Solutions	**A + B**	**C + D**	**NP**	**A + B**	**C + D**

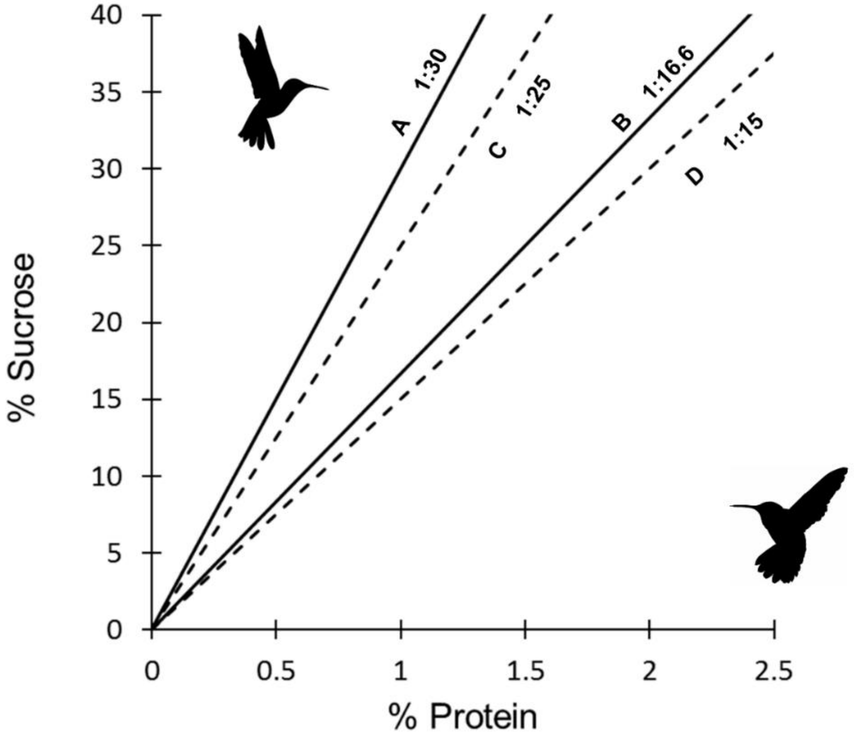


Figure 2

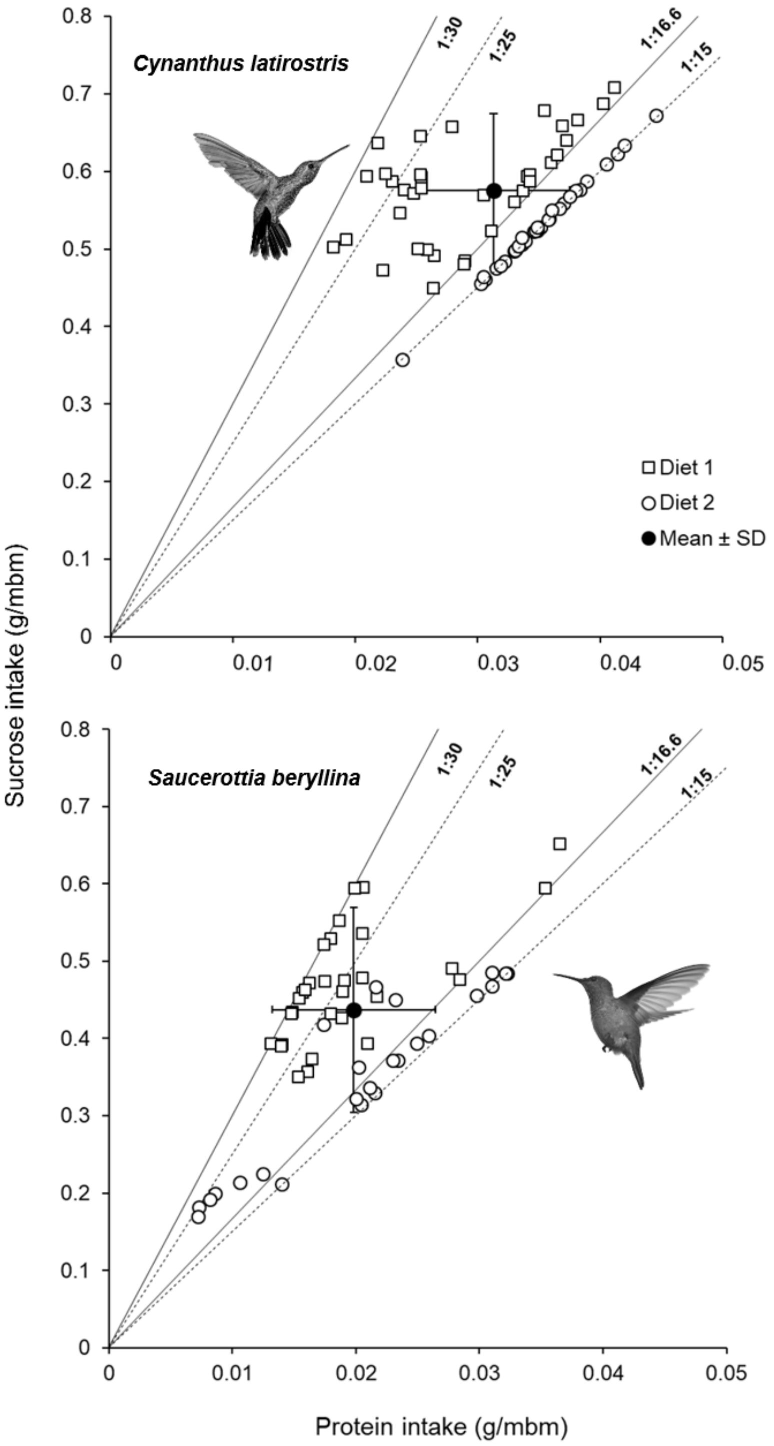
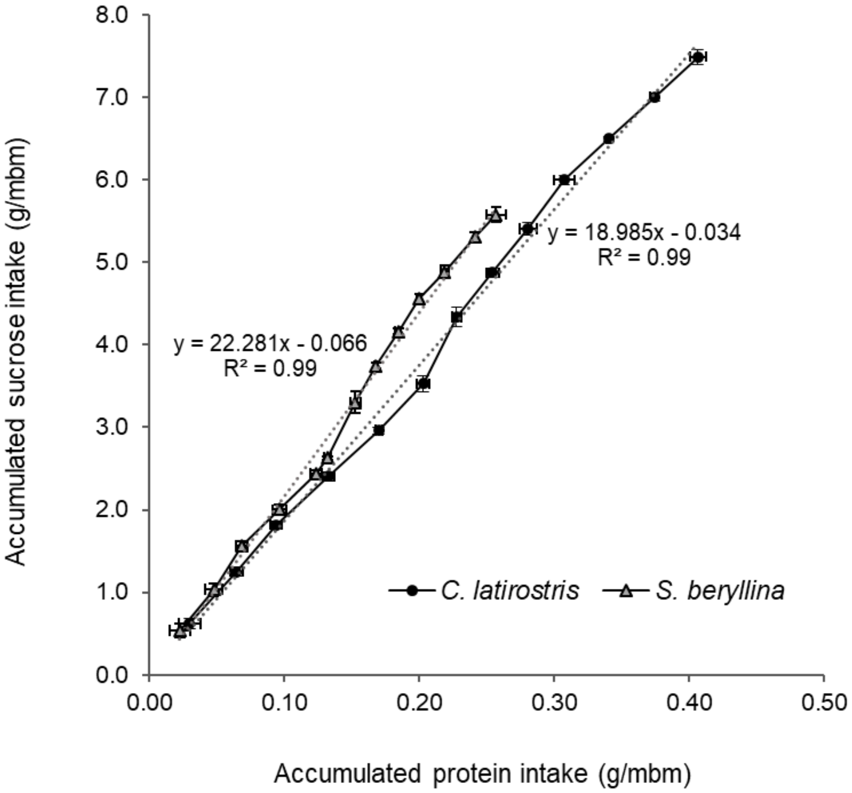

Cynanthus latirostris
Saucerottia beryllina
1:30
1:25
1:16.6
1:15
□ Diet 1
○ Diet 2
● Mean ± SD
Sucrose intake (g/mbm)
Protein intake (g/mbm)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.01
0.02
0.03
0.04
0.05

Figure 3


8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0.00
0.10
0.20
0.30
0.40
0.50
Accumulated sucrose intake (g/mbm)
Accumulated protein intake (g/mbm)
y = 22.281x - 0.066
R² = 0.99
y = 18.985x - 0.034
R² = 0.99
● *C. latirostris*
▲ *S. beryllina*

CAPÍTULO 4. INGESTIÓN DE MICROPIEDRAS POR COLIBRÍES

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RESEARCH **Open Access**

True grit: ingestion of small stone particles by hummingbirds in West Mexico

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Abstract

Background: Grit is used by birds mainly for grinding hard food items but can also serve a nutritional role as a source of minerals. Ingestion of grit by birds has been documented primarily in species that feed on seeds and invertebrates. Although feeding mainly on nectar and small arthropods, hummingbirds also ingest grit, but why they do so is unclear. We quantified the number of grit particles in the stomachs of six species of hummingbirds during an annual cycle in a seasonal ecosystem of West Mexico.

Methods: We compared the number of grit particles in the stomachs of different hummingbird species (Mexican Violetear *Colibri thalassinus*, Amethyst-throated Mountaingem *Lampornis amethystinus*, White-eared Hummingbird *Basilinna leucotis*, Rivoli's Hummingbird *Eugenes fulgens*, Broad-tailed Hummingbird *Selasphorus platycercus*, and Rufous Hummingbird *S. rufus*), and between sex and age categories during the different seasons of a year. To determine if grit was used to grind ingested arthropods, we examined the relationships between the number of grit particles, the biomass of arthropods ingested, and their chitin content.

Results: Although species did not differ in the number of grit particles in their stomachs, we found that grit was mostly ingested by female individuals, with only one male of one species (Mexican Violetear) presenting grit in its stomach. We also found that female hummingbirds had grit in their stomachs during the rainy and the cold-dry season (June–February) but not during the warm-dry season (March–May). Our analyses revealed no relationship between the number of grit particles and the amount of ingested arthropods and arthropod chitin content. However, high grit consumption was related to wasp ingestion on Mexican Violetears.

Conclusions: Our results indicate that grit is used mainly by female hummingbirds. The seasonal variation in the ingestion of grit by female individuals suggests that it can be used to meet mineral requirements related to breeding; however, this topic needs further exploration. Additionally, the use of grit was proportionally higher in juvenile individuals, suggesting it is used for grinding arthropods during a period of fast development.

Keywords: Arthropod chitin content, Arthropod digestion, Breeding season, Mineral nutritional requirements

Background

Grit, defined as small stones or tiny rock fragments, is ingested by many bird species to facilitate the mechanical grinding of ingested hard food items (Ziswiler and Farner 1972; Brown 1976; Barrentine 1980; Bishton 1986; Gionfriddo and Best 1999). However, grit can also serve as a source of minerals such as calcium (Sadler 1961; Harper 1964; Korschgen 1964; Campbell and Leatherland 1983; Adam and des Lauriers 1998). Ingestion of grit by birds has also been found to be influenced by the hardness of

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ingested food (Bird and Smith 1964; Mott et al. 1972; Alonso 1985; Gionfriddo and Best 1996) and by changes in mineral requirements, particularly during egg production and periods of accelerated growth (Harper 1963; Kopischke and Nelson 1966; Taylor 1970; Johnson and Barclay 1996; Reynolds 1997).

Although many different species of birds ingest grit, few research papers have documented the use of grit by hummingbirds. In these reports, different species of hummingbirds ingested materials such as wood ashes, lime dust, sand particles, and small rocks of different geological origins (Haverschmidt 1952; Verbeek 1971; des Lauriers 1994; Adam and des Lauriers 1998; Graves 2007; Estades et al. 2008; Hickman et al. 2012; See Additional file 1: Table S1 for details). Interestingly, most reports of grit ingestion by hummingbirds have been of females during the breeding season (Haverschmidt 1952; Verbeek 1971; des Lauriers 1994; Adam and des Lauriers 1998; Graves 2007; Hickman et al. 2012; Additional file 1: Table S1). As a result, some authors have suggested that female hummingbirds ingest mineral-rich grit to obtain calcium for eggshell production (Verbeek 1971; des Lauriers 1994; Adam and des Lauriers 1998; Graves 2007; Estades et al. 2008; Hickman et al. 2012). However, female hummingbirds also consume more, and higher quality arthropods (e.g. spiders) during the breeding season to provide protein for egg production and to provision nestlings (Hainsworth 1977; Montgomerie and Redsell 1980; Chavez-Ramirez and Down 1992; Stiles 1995; Murphy 1996; Rico-Guevara 2008), so grit could also be used to aid in the mechanical digestion of these arthropods (Ziswiler and Farner 1972; Brown 1976; Barrentine 1980; Bishton 1986; Gionfriddo and Best 1999).

Our objective was to quantify the number of grit particles in the stomachs of several species of hummingbirds and to explore the possible function of grit ingestion by hummingbirds. Specific objectives were to determine: (1) the extent to which hummingbirds ingest grit and how many grit particles are present in their stomachs, (2) if grit ingestion varies seasonally, (3) if there are differences in the use of grit by sex (females vs. males) and age classes (juveniles vs. adults), and (4) if there is a relationship between the number of grit particles in hummingbird stomachs, the quantity of arthropods ingested, and the chitin content of ingested arthropods.

Because the amount of arthropods ingested by hummingbirds changes seasonally with the availability of food resources at our study site, we expected hummingbirds to ingest more grit when they ingested more arthropods. In addition, because the number of grit particles increases in response to food hardness in other species of birds (Bird and Smith 1964; Mott et al. 1972; Alonso 1985; Gionfriddo and Best 1996), we expected that hummingbirds that consumed arthropods with greater chitin content would have more grit particles in their stomachs than those that feed on softer arthropods. Finally, because females ingest more arthropods than males during the breeding season to meet the higher nutritional and energy requirements of producing eggs and parental care (Hainsworth 1977; Montgomerie and Redsell 1980; Chavez-Ramirez and Down 1992; Stiles 1995; Murphy 1996; Rico-Guevara 2008), we expected those female hummingbirds would have more grit particles in their stomachs than adult males.

Methods

Study site

Our study was conducted at the Nevado de Colima National Park (NCNP) in Jalisco, Mexico. Nevado de Colima is an inactive high-altitude volcano (4260 m a.s.l.) located at the western end of the Trans-Mexican Volcanic Belt (19° 33′ 45″–19° 30′ 40″ N, 103° 36′ 30″–103° 37′ 30″ W; INEGI 2011). The climate in the region is highly seasonal (CONANP 2006, 2017). Our study site was located at 3194 m a.s.l. and consisted of subalpine scrublands (dominated by plants of the genus *Salvia*, *Ribes*, and *Festuca*), some scattered alders (*Alnus*) on exposed ridges, and pine and fir forests (*Pinus* and *Abies*) located in ravines (Schondube 2012). The most important flowering plants that hummingbirds feed on include *Salvia elegans*, *S. gesneriflora* (Lamiaceae), *Ribes ciliatum* (Saxifragaceae), *Senecio angulifolius* (Asteraceae), and *Penstemon roseus* (Plantaginaceae) (Schondube 2012).

Fieldwork

We sampled hummingbirds three times over a one-year period. Our sampling corresponded with the three climatic seasons in the region, including (1) a rainy season from June to October, (2) a cold-dry season from November to February, and (3) a warm-dry season from March to May (CONANP 2006, 2017). We selected this sampling scheme because weather conditions and the availability of floral nectar and arthropods varied widely among these three seasons (CONANP 2006, 2017). We sampled during May and September 2016, and February 2017. During each sampling period, we captured hummingbirds using 10 mist-nets (12-m long, 24-mm mesh) for three consecutive days. Mist-nets were opened at sunrise and closed after 6 h. Net rounds were conducted every 5 min. We located the mist-nets within a 2 ha area, and their location remained constant during the study. We identified all captured birds and recorded their age and sex using plumage characteristics and bill striations (Williamson 2001; Howell 2002; Russell et al. 2019). Additionally, for the Mexican Violetear, the only species that did not present a clear sexual dimorphism in plumage at our study site we used wing chord length and bill tip serrations to determine their sex (Rico-Guevara et al. 2019). Data on wing chord differences among sexes for this species was obtained from a 30-year hummingbird banding program located in the same region. We define females as those individuals with a wing chord < 60 mm, and males as those individuals with a wing chord > 63 mm (Contreras-Martínez personal communication).

Stomach content analysis

We obtained the stomachs of hummingbirds collected as part of an independent stable isotope project conducted at our study site ($n=6$ in May 2016, $n=8$ in September 2016, and $n=37$ in February 2017). That study collected blood, liver, pectoral muscle, and bones to extract collagen, and allowed us to use the stomachs. The remaining feathers, skulls, and tongues were deposited at the collection of the Functional Ecology Laboratory of IIES, UNAM. Samples were collected with permission from the Secretaría de Medio Ambiente y Recursos Naturales, Mexico (SGPA/DGGFS/712/2767/14). All collected birds were humanely euthanized by carefully placing their heads inside a small vial that contained a cotton ball soaked in isoflurane, following the guidelines to the use of wild birds in research (Fair et al. 2010), and their stomachs were placed in plastic vials with saline solution (0.8% NaCl) and frozen in liquid nitrogen until processed in the laboratory. The time between hummingbird capture in the nets and stomach freezing was less than 20 min. Because soft arthropods require 3–4 h to be digested completely by hummingbirds (Remsen et al. 1986), this time frame allowed us to quantify stomach arthropod content at the moment of capture. The species sampled were: Mexican Violetear (*Colibri thalassinus*), Amethyst-throated Mountaingem (*Lampornis amethystinus*), White-eared Hummingbird (*Basilinna leucotis*), Rivoli's Hummingbird (*Eugenes fulgens*), Broad-tailed Hummingbird (*Selasphorus platycercus*), and Rufous Hummingbird (*S. rufus*). The number of individuals collected at each season differed due to variation in capture rates among seasons, and due to restrictions on collecting permits (maximum of 10 individuals per species per season).

We analyzed hummingbird stomachs in the lab to determine the number of grit particles they contained. Stomachs were thawed and dissected, and their contents removed. We quantified grit particles by carefully separating them from the arthropod remains in hummingbird stomachs using a stereoscopic microscope (AmScope, 7–45 × binocular stereo zoom microscope). We described the color and shape, and weighed and measured grit particles. To determine their size, we determined the grit area by taking a picture of each grit particle on top of a millimetric grid. Images were analyzed using ImageJ (National Institute of Health, NIH Version v1.32j). Because the role of grit as either a grinding agent or nutritional supplement depends upon its hardness and solubility in the digestive tract (Meinertzhagen 1954; Myrberget et al. 1975; Gionfriddo and Best 1999), we determined grit hardness. We did this by pressing each grit particle twice with fine-point reverse action tweezers. This kind of tweezer allowed us to generate a standard pressure on the grit particle and separate them into two hardness categories: hard (did not break) and soft (did break into smaller pieces).

Physical and chemical characterization of grit particles

We used scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) spectroscopy (Tabletop Microscope Hitachi, Model TM3030Plus) to perform the physical and chemical characterization of the grit particles. Because soft grit broke into tiny particles, we were only able to analyze the hard grit particles. Of these, due to the limitations imposed by the method, we were able to analyze only the largest hard grit particles (diameter of 0.5–1 mm, $n=5$). Analyses were carried out in the Microanalysis Laboratory of the Geophysics Institute, UNAM.

Ingested arthropod biomass and chitin content

We separated all arthropod remains into those that were identifiable (arthropods partially fragmented) and those that were not (very fragmented arthropods) using a stereoscopic microscope. We identified recognizable arthropods to the taxonomic level of order following Triplehorn and Johnson (2005). To determine the biomass of ingested arthropods (g dry mass), we dried both identifiable and unidentifiable arthropod samples at room temperature for 3 h and then weighed them using an analytical balance (Ohaus Adventurer, capacity/readability of 110 g × 0.001 g). Chitin content (percent dry mass) of the different identified arthropod orders was obtained from Rothman et al. (2014). We estimated the mean chitin content of arthropods by averaging the percent chitin content of the different arthropod orders present.

Data analysis

We used different analyses to test our main hypotheses on the use of grit particles by hummingbirds. First, we compared the number of grit particles present in hummingbird stomachs among species using a Kruskal–Wallis test (Zar 1999). We used a non-parametric test because our data did not present a normal distribution. In view of the fact that we did not find grit particles in the stomachs of hummingbirds during the warm-dry season, and due to the small sample size of the rainy season, this analysis was limited to our data for the cold-dry season.

Second, we used a generalized linear model (GLM) to assess whether the number of grit particles (response variable) varied among seasons, and between age and sex classes. In this model, we include season (warm-dry, rainy, and cold-dry seasons), age (adult and juvenile individuals), and sex (female and male individuals) as categorical explanatory variables. We select for this analysis the data of all 25 male and 21 female individuals sampled, excluding data from the 5 individuals whose sexes were unknown. Due to our small sample size, we created a 0–1 binary response variable in which 0 represented the absence of grit and 1 represented the presence of grit, and fitted our model with a Binomial distribution and a logit link function. Additionally, we used the adjusted maximum likelihood estimator for reducing biases of the Binomial logistic regression parameters (following Firth 1993).

Third, because the two response variables of arthropod ingestion (i.e. the biomass of arthropods ingested and their chitin content) presented different distributions, we ran two GLMs with the number of grit particles as the explanatory variable, whereas the response variables differed. For GLM 1, we used the biomass of ingested arthropods (g dry mass) as the response variable and a normal distribution with an identity link function. For GLM 2, we used the chitin content of arthropods ingested by hummingbirds (percent dry mass) as the response variable, and a Poisson distribution with a Log link function. In both models, we only included the data from those individuals whose stomachs presented grit particles ($n=12$ for GLM 1, and $n=8$ for GLM 2; Additional file 1: Table S2).

Finally, we performed some tests to search for differences in the arthropod content and grit particles characteristics between individuals of both sexes. Since we only had a male individual, we use the data of the females to construct a confidence interval to compare it against the male values using a one-sample *t*-test for those variables with normal distributions (number of grit particles and the biomass of ingested arthropods), and a Wilcoxon test for non-normal distributed data (size of grit particles; Sokal and Rohlf 1995). We conducted all analyses using JMP version 9.0 (SAS Institute Inc.). Values are provided as means ± SD.

Results

Characteristics of grit particles ingested by hummingbirds

Grit particles were of two types: crystal-like or opaque and non-crystalline. The average mass of grit particles was 0.057 ± 0.042 mg ($n=50$). The average size of the larger flat side of ingested grit particles was 0.52 ± 0.38 mm^2 ($n=50$). We found that 70% of the grit particles did not break when pressed with the tip of the tweezers (classified as hard, all crystal-like), whereas the other 30% broke after the first or second pressing (classified as soft, mostly opaque, and non-crystalline particles, and a few crystal-like particles).

Examination of hard grit particles using both a stereoscopic and a scanning electron microscope revealed that they were black, angular to rounded, and very porous (i.e. with vesicles; Fig. 1). Additionally, spectroscopy analysis (EDX) revealed that grit particles had high concentrations of oxygen and silicon (40.5 ± 5.8% and 20.5 ± 2.5% of total mass, respectively, mean ± SD, $n=5$), and low levels of minerals such as calcium and sodium (< 10% of total mass; Table 1). Based on their physical characteristics and chemical composition, hard grit particles analyzed were classified as volcanic glasses (Sosa-Ceballos personal communication).

Use of grit particles by hummingbirds

We found that 12 of the 51 hummingbird stomachs analyzed (23.5%) presented grit particles. The majority of collected hummingbird species, including Mexican Violetears, Amethyst-throated Mountaingems, White-eared Hummingbirds, Rivoli's Hummingbirds, and Broad-tailed Hummingbirds, had grit particles in their stomachs (Table 2). Only one species, the Rufous Hummingbird, did not present grit particles. From the birds that presented grit particles, 8 stomachs belonged to female hummingbirds and only one to a male individual. We were unable to determine the sex of the other 3 individuals that presented grit in their stomachs. The only male that presented grit in its stomach had a total of 61 particles, a number that was larger than the average number of grit particles found in the stomachs of female hummingbirds (2.0 ± 0.75, $n=8$). When we compared the mean value of the number of grit particles for females and the value of the only male, we found a significant difference ($t_8=-220.8$, $P<0.0001$). Grit particles found inside the male's stomach had similar color and shape to those present in the females. While grit particles in the male hummingbird tended to present larger areas than those found in the stomachs of females, this difference was not significant ($\chi^2_{1,71}=3.7$, $P=0.053$).

During the warm-dry season, we found no grit particles in hummingbird stomachs (Table 2; Fig. 2). Based on our binomial model, we found that the variation in the use of grit particles in hummingbird stomachs among the three seasons was not significant (Table 3). The percentage of individuals with grit in their stomachs varied among seasons: 50% (four of eight sampled individuals) during the rainy season, 21.6% (eight of 37 sampled) during the cold-dry season, and 0% (0 of 6 sampled) during the warm-dry season (Table 2; Fig. 2). The average number of grit particles found in

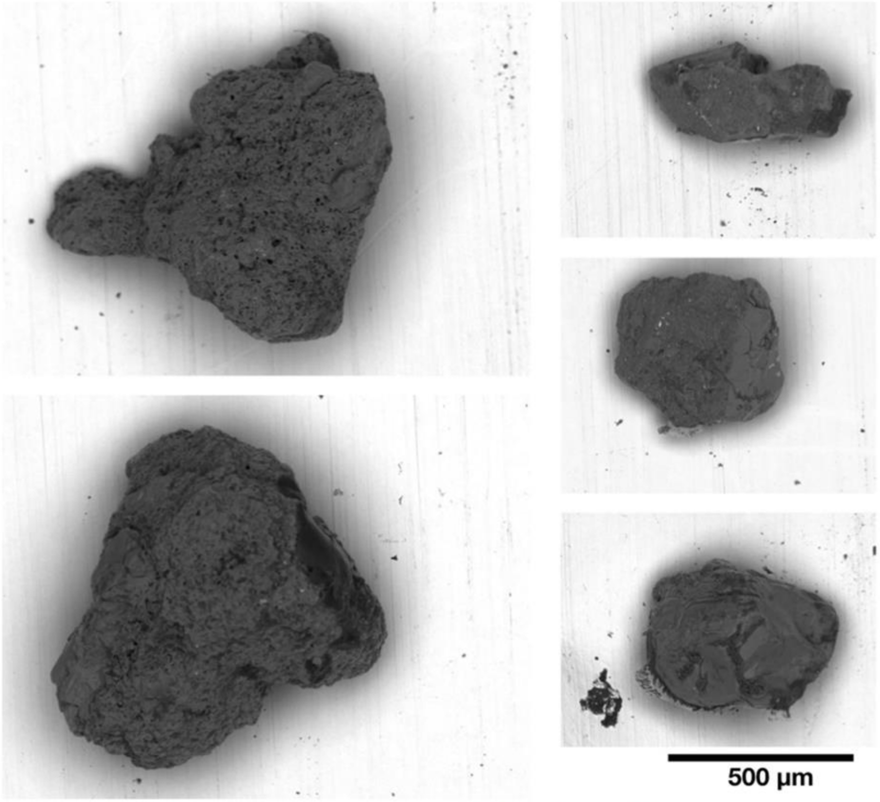

Fig. 1 Morphology of hard grit particles present in the stomachs of Mexican Violetears. All grit particles are presented at the same scale. Pictures were taken at 5 kV using a Hitachi TM3030

Table 1 Chemical composition of hard grit particles found in the stomachs of Mexican Violetears (average grit particle mass = 0.3 ± 0.1 mg, *n* = 5)

Element	Percent of total mass (%)					Mean ± SD
	Grit 1	Grit 2	Grit 3	Grit 4	Grit 5	
O	46	32.8	46.4	38.3	38.8	40.5 ± 5.8
Si	17.8	20.2	23.9	18.6	21.9	20.5 ± 2.5
C	15.3	0	13	0	11.3	7.9 ± 7.4
Al	8.1	5.1	6.5	1.1	6.8	5.5 ± 2.7
Cu	0	10.7	6.9	0	5.01	4.5 ± 4.6
Mg	0	3.4	0	13.1	0	3.3 ± 5.7
Ca	3	7.7	0	0.7	2.7	2.8 ± 3
Na	2.5	0	4.1	2	0	1.7 ± 1.7
Fe	4	0	0	0	0	0.8 ± 1.8
K	0	0	0	0	1	0.2 ± 0.5
Total	96.8	79.9	100	73.8	87.6	87.8 ± 11.3

Elements are presented in order of importance. Elementary analyses were performed using energy dispersive X-ray spectroscopy (EDX)

Table 2 Number of grit particles per stomach (mean ± SD for $n > 1$) of the different members of a hummingbird ensemble in a seasonal ecosystem of West Mexico

Season	Individual	Species	Age	Sex	Grit particles per stomach	Mean number of grit particles per species per season ± SD
Warm-dry	1	Amethyst-throated Mountaingem	Adult	Female	0	
	2	White-eared Hummingbird	Adult	Male	0	
	3	White-eared Hummingbird	Adult	Male	0	
	4	White-eared Hummingbird	Adult	Male	0	
	5	White-eared Hummingbird	Adult	Male	0	
	6	Rivoli's Hummingbird	Adult	Female	0	
Rainy	7	Mexican Violetear	Adult	Female	2	2.5 ± 3.1 ($n = 4$)
	8	Mexican Violetear	Adult	Unknown	7	
	9	Mexican Violetear	Adult	Unknown	0	
	10	Mexican Violetear	Juvenile	Unknown	1	
	11	Amethyst-throated Mountaingem	Adult	Female	1	
	12	White-eared Hummingbird	Juvenile	Female	0	
	13	Rivoli's Hummingbird	Adult	Male	0	
	14	Rivoli's Hummingbird	Adult	Female	0	
Cold-dry	15	Mexican Violetear	Adult	Male	0	11.3 ± 24.4 ($n = 6$)
	16	Mexican Violetear	Adult	Male	61	
	17	Mexican Violetear	Adult	Female	2	
	18	Mexican Violetear	Adult	Unknown	0	
	19	Mexican Violetear	Juvenile	Male	0	
	20	Mexican Violetear	Juvenile	Unknown	5	
	21	Amethyst-throated Mountaingem	Adult	Male	0	
	22	White-eared Hummingbird	Adult	Male	0	0.2 ± 0.6 ($n = 9$)
	23	White-eared Hummingbird	Adult	Male	0	
	24	White-eared Hummingbird	Adult	Male	0	
	25	White-eared Hummingbird	Adult	Male	0	
	26	White-eared Hummingbird	Adult	Male	0	
	27	White-eared Hummingbird	Adult	Male	0	
	28	White-eared Hummingbird	Adult	Female	2	
	29	White-eared Hummingbird	Juvenile	Male	0	
	30	White-eared Hummingbird	Juvenile	Male	0	
	31	Rivoli's Hummingbird	Adult	Male	0	0.1 ± 0.3 ($n = 8$)
	32	Rivoli's Hummingbird	Adult	Male	0	
	33	Rivoli's Hummingbird	Adult	Female	0	
	34	Rivoli's Hummingbird	Adult	Female	0	
	35	Rivoli's Hummingbird	Adult	Female	0	
	36	Rivoli's Hummingbird	Adult	Female	1	
	37	Rivoli's Hummingbird	Adult	Female	0	
	38	Rivoli's Hummingbird	Juvenile	Male	0	
	39	Broad-tailed Hummingbird	Adult	Male	0	0.8 ± 1.3 ($n = 9$)
	40	Broad-tailed Hummingbird	Adult	Male	0	
	41	Broad-tailed Hummingbird	Adult	Female	0	
	42	Broad-tailed Hummingbird	Adult	Female	0	
	43	Broad-tailed Hummingbird	Adult	Female	3	
	44	Broad-tailed Hummingbird	Adult	Female	0	
	45	Broad-tailed Hummingbird	Juvenile	Male	0	
	46	Broad-tailed Hummingbird	Juvenile	Female	3	
	47	Broad-tailed Hummingbird	Juvenile	Female	2	
	48	Rufous Hummingbird	Juvenile	Male	0	
	49	Rufous Hummingbird	Juvenile	Male	0	
	50	Rufous Hummingbird	Juvenile	Female	0	
	51	Rufous Hummingbird	Juvenile	Female	0	

Table 2 (continued)

Mean number of grit particles per species per season is only reported for species in which we had more than one individual of the same species presenting grit in each season

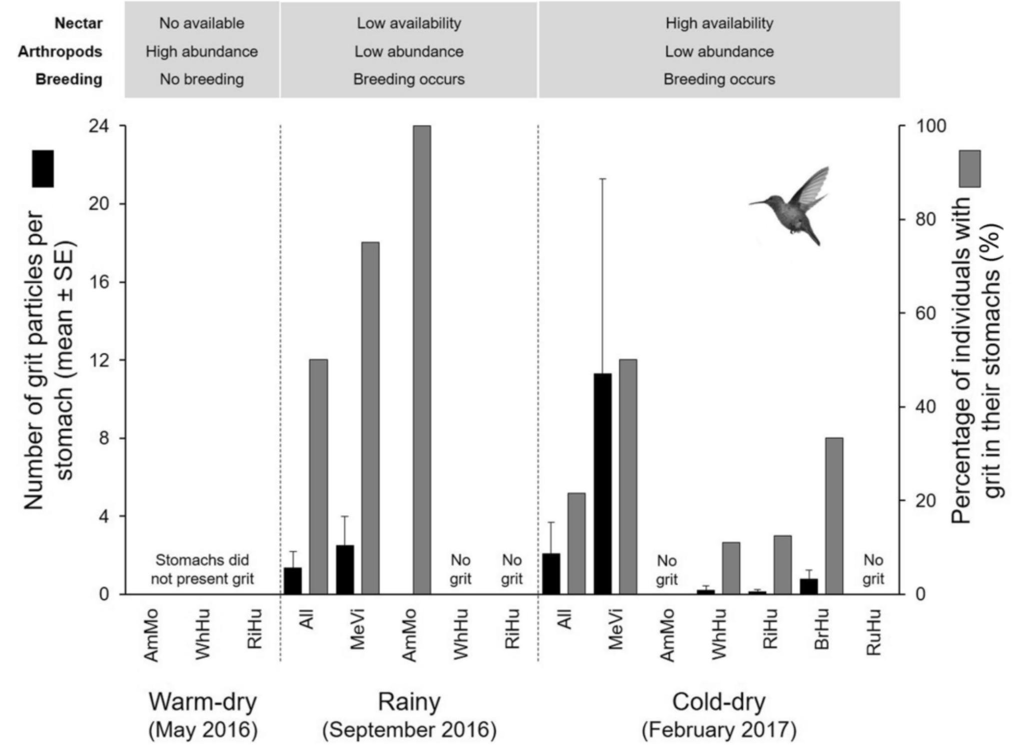

Fig. 2 Seasonal differences in the number of grit particles per stomach (mean ± SE when $n > 1$; black bars) and percentages of individuals with grit in their stomachs (gray bars) for all hummingbird species. During the warm-dry season, no grit particles were present in the stomachs of hummingbirds. *MeVi* Mexican Violetear, *AmMo* Amethyst-throated Mountaingem, *WhHu* White-eared Hummingbird, *RiHu* Rivoli's Hummingbird, *BrHu* Broad-tailed Hummingbird, *RuHu* Rufous Hummingbird, and *All* All species sampled per season

Table 3 Results of the GLM evaluating the effects of season, sex, and age on the presence of grit particles in the stomachs of hummingbirds

Explanatory variable	Estimate	Standard error	χ^2_1	*P*-value
Intercept	1.85	0.78	10.39	0.001
Rainy season	− 0.68	0.87	1.10	0.290
Warm-dry season	1.03	1.19	1.45	0.220
Female hummingbirds	− 1.06	0.46	8.63	0.003
Adult hummingbirds	− 0.14	0.46	1.68	0.190

the stomachs of individuals that presented grit was 2.7 ± 2.8 during the rainy season ($n = 4$; 2 females, and 2 unknown sex individuals) and 9.8 ± 20.6 during the cold-dry season ($n = 8$; 6 females, 1 male, and 1 unknown sex individual; see Table 2).

We found no significant differences in the average number of grit particles per stomach among hummingbird species within the cold-dry season (Kruskal–Wallis test, $\chi^2_{3,32} = 4.9$, $P = 0.2$; Table 2; Fig. 2). However, during this season, Mexican Violetears had the highest average number of grit particles per stomach (11.3 ± 24.4, $n = 6$), and the highest percentage of individuals with grit in their stomachs (50%; Table 2; Fig. 2). Finally, our binomial model showed that the use of grit particles was higher in female hummingbirds when compared to males, while adult and juvenile individuals did not differ (Table 3).

Relationship between the presence of grit particles and arthropod ingestion

The biomass of arthropods ingested by hummingbirds was not related to the number of grit particles in hummingbird stomachs (estimate = 0.00001, SE = 6.99 e^{-6}, $\chi^2_1 = 3.1$, $P = 0.07$). Similarly, the chitin content of arthropods ingested by hummingbirds was not related to the number of grit particles present (estimate = 0.01, SE = 0.05, $\chi^2_1 = 0.04$, $P = 0.8$). The only male that presented grit had higher arthropod biomass in its stomach (0.0014 g dry mass) than the females (0.0006 ± 0.0004 g dry mass/stomach; $t_7 = -4.56$, $P < 0.0013$). Data on the arthropods ingested by hummingbirds is shown on Table 4.

Discussion

All studies that have reported ingestion of grit by hummingbirds have found that only females ingested grit (Haverschmidt 1952; Verbeek 1971; des Lauriers 1994; Adam and des Lauriers 1998; Graves 2007; Hickman et al. 2012). At our study site, we found that both female and male hummingbirds can ingest it. The use of grit particles was more frequent in females (eight of 21 sampled) than males (1 of 25 sampled). However, the only male that presented grit ingested a larger number of grit particles (61 particles) when compared to female individuals (2.0 ± 0.75, $n = 8$). Although the percentage of hummingbirds with grit particles in their stomachs was relatively low (23.5%, including all hummingbirds sampled), our results suggest that the use of grit by hummingbirds is more common than previously thought. However, because of our small sample size, we were unable to determine how common is the use of grit by male hummingbirds and, as a result, the differential use of grit by male and female hummingbirds requires additional study.

The use of grit by hummingbirds at our study site was seasonal, with grit in their stomachs being present only during the rainy and the cold-dry seasons. Because hummingbirds at our study site breed during both the rainy and the cold-dry seasons, these results suggest a relationship between ingestion of grit and hummingbird breeding. Others have also reported that hummingbirds only ingest grit during their breeding season (Haverschmidt 1952; Verbeek 1971; des Lauriers 1994; Adam and des Lauriers 1998; Graves 2007; Hickman et al. 2012).

Table 4 Biomass ingested and chitin content of different arthropod orders eaten by hummingbirds at our study site

Season	Species	Ingested arthropods		
		Biomass (g dry mass)	Orders	Chitin content (% dry mass)
Warm-dry	Amethyst-throated Mountaingem	0.001	Psocoptera	13.3
	White-eared Hummingbird	0.0019 ± 0.0007 ($n = 4$)	Araneae, Psocoptera, Diptera	13.2 ± 0.1 ($n = 3$)
	Rivoli's Hummingbird	0.0038	Hemiptera, Hymenoptera, Diptera	13.6 ± 2.7 ($n = 3$)
Rainy	Mexican Violetear	0.0007 ± 0.0005 ($n = 4$)	Hemiptera, Psocoptera, Hymenoptera, Diptera	13.5 ± 2.2 ($n = 4$)
	Amethyst-throated Mountaingem	0.0006	Hemiptera, Diptera	12.2 ± 1.5 ($n = 2$)
	White-eared Hummingbird	0.0002	Diptera	13.3
	Rivoli's Hummingbird	0.0001 ± 0.00007 ($n = 2$)	Unidentifiable	Unknown
Cold-dry	Mexican Violetear	0.0007 ± 0.0005 ($n = 6$)	Hemiptera, Psocoptera, Hymenoptera, Diptera	13.5 ± 2.2 ($n = 4$)
	Amethyst-throated Mountaingem	0.0006	Psocoptera	13.3
	White-eared Hummingbird	0.0005 ± 0.0003 ($n = 9$)	Psocoptera, Hymenoptera, Diptera	14.4 ± 1.8 ($n = 3$)
	Rivoli's Hummingbird	0.0015 ± 0.0008 ($n = 8$)	Hemiptera, Psocoptera, Hymenoptera, Diptera	13.5 ± 2.2 ($n = 4$)
	Broad-tailed Hummingbird	0.0007 ± 0.0004 ($n = 9$)	Hemiptera, Psocoptera, Hymenoptera, Diptera	13.5 ± 2.2 ($n = 4$)
	Rufous Hummingbird	0.0004 ± 0.0001 ($n = 4$)	Hemiptera, Psocoptera, Hymenoptera, Diptera	13.5 ± 2.2 ($n = 4$)

Mean ± SD values of arthropod chitin content in the stomachs of hummingbirds were averaged when more than one order of arthropods was present. We were unable to identify the orders of arthropod remains ingested by Rivoli's Hummingbirds in the rainy season. In this case, the chitin content of the sample was not determined

During the warm-dry season, no flowers were detected at our capture site (2 ha area) and two additional surrounding areas we used to sample resource abundance located 2–4 km away from it (Maya-García and Schondube, unpublished data). The hummingbirds survived in this season by ingesting more arthropods (0.002 ± 0.001 g dry mass/stomach; mean ± SD; Maya-García and Schondube, unpublished data) than during the rainy and the cold-dry seasons (0.0005 ± 0.0004 and 0.0006 ± 0.0005 g dry mass/stomach, respectively) when several plant species were blooming, and nectar was abundant. This higher content of arthropods, while grit particles were absent from the hummingbird stomachs, suggests that the use of grit is not clearly linked to arthropod ingestion in the majority of the sampled species and that ingestion of grit particles could be associated with obtaining micronutrients as has been suggested previously (Verbeek 1971; des Lauriers 1994; Adam and des Lauriers 1998; Graves 2007; Estades et al. 2008; Hickman et al. 2012).

The proportion of individuals who presented grit in their stomachs was higher in juveniles (28.5%, four of 14 sampled) than in adults (21.6%, 8 of 37 sampled), suggesting that grit ingestion could be more important for juveniles. Grit ingestion by juveniles could possibly serve two complementary roles: (1) due to their high nutritional requirements, juveniles could use grit as a source of minerals to finish their skeleton development after leaving the nest (Harper 1963; Tilgar et al. 2004; Reynolds and Perrins 2010); and (2) because arthropods are an important source of protein for tissue growth, grit particles could also be used by juveniles to facilitate the mechanical digestion of ingested prey.

The absence of significant relationships between the number of grit particles in hummingbird stomachs and the biomass of arthropods ingested by hummingbirds and ingested arthropod chitin content suggests, once again, that the primary role of grit ingestion is not related to arthropod mechanical grinding, and could be associated to obtaining minerals. However, due to the time passed between hummingbirds' capture in the nets and the moment we excised and froze their stomachs (< 20 min), some of the soft-bodied arthropods could have been digested biasing our analysis. As a consequence, our results should be considered with caution. Curiously, most hard grit particles were found in the stomachs of Mexican Violetear hummingbirds, suggesting that the use of grit to obtain minerals may be limited in this species. Nonetheless, we need to consider that soft grit particles break easily and could dissolve in the acidic stomach of hummingbirds, limiting our capacity to find them and quantify their use.

Although we found no differences among hummingbird species in the number of grit particles ingested, Mexican Violetears ingested more grit particles than other species during both the rainy and the cold-dry seasons, accounting for 90.4% of all hard particles present in hummingbird stomachs. Additionally, the only male hummingbird that presented grit particles, a Mexican Violetear captured during the cold-dry season, presented a surprisingly high quantity of 61 grit particles. This individual accounted for 77.2% of all hard grit particles present in Mexican Violetear hummingbird stomachs. These two results seem to be related to the arthropod ingestion patterns of this species. Mexican Violetears ingested more adult wasps (Hymenoptera) during the rainy and the cold-dry seasons than the other hummingbird species (19.2% and 45.5% of total arthropods present in their stomachs in both seasons, respectively; Maya-García and Schondube, unpublished data). This ingestion of hymenopterans is surprising because adult wasps have both low protein and high chitin content compared to other insect groups (Rothman et al. 2014).

Conclusions

Our results show that at the ensemble level, female hummingbirds ingested grit particles during their breeding season. This result suggests that ingested grit could have played a role as a supplement of some micronutrients, such as calcium needed for eggshell production. Although calcium content in hard grit particles was low, we were unable to analyze the elemental content of the soft grit particles and, due to the softness of these particles, we could be underestimating their ingestion. As a result, more information on ingestion, assimilation, and elemental content of soft grit particles is needed. Finally, for both male and female Mexican Violetear hummingbirds, grit particles could have the additional role of facilitating the mechanical digestion of hard-bodied arthropods. Our analyses offer new insights to understand the roles played by grit in several species of hummingbirds, complementing previously published anecdotal information (Additional file 1: Table S1).

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40657-021-00298-x.

Additional file 1: Table S1. Grit ingestion by hummingbirds from the published literature. **Table S2.** Values used in the generalized linear models (GLM).

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Authors' contributions

Conceptualization: OM-G and JES; Methodology: OM-G, JES and MO-F; Data collection: OM-G, JES and MO-F; Formal analysis and investigation: OM-G and JES; Writing – original draft preparation: OM-G and MO-F; Writing – review and editing: JES; Funding acquisition: JES; Resources: JES; Supervision: JES. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and analyzed during the current study are available in the Mendeley Data repository (http://dx.doi.org/10.17632/rzhf2bjw4v.1).

Declarations

Ethics approval and consent to participate

Our methods were approved by the ethics committee of the Institute of Ecosystem Studies and Sustainability, UNAM. Permission to collect samples was provided by SEMARNAT, Mexico (SGPA/DGGFS/712/2767/14).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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DISCUSIÓN GENERAL

La riqueza y diversidad de especies de colibríes a escala local son afectadas principalmente por la estacionalidad climática (variación anual en precipitación y temperatura) y la variación espacio-temporal de la disponibilidad de los recursos florales. Aunque los artrópodos cumplen un papel fundamental para los colibríes como alimento complementario, suministrando nutrientes que no están presentes en el néctar floral, a la fecha, el efecto de la abundancia de los artrópodos sobre la estructura de las comunidades de colibríes ha sido poco estudiado. Nuestro estudio de campo revelo que la disponibilidad de artrópodos en el ambiente puede jugar un papel importante en la determinación de la riqueza y composición de especies de colibríes. Cuando el néctar no está disponible y la abundancia de artrópodos es alta, únicamente las especies que tienen la capacidad de ingerir grandes cantidades de artrópodos pueden sobrevivir y permanecer en un área como residentes, mientras que las especies que no pueden hacerlo, se ven forzadas a migrar localmente a otros sitios en búsqueda de recursos flores, produciéndose cambios en la riqueza y composición de especies. En nuestro sitio de estudio, durante la estación seca-caliente cuando el néctar no estuvo disponible y la abundancia de artrópodos fue más alta, *Bassilina leucotis* fue la especie dominante en términos de su abundancia relativa y la única que observamos forrajeando para artrópodos, presentando altas tasas de intentos de capturas. Bajo este escenario de disponibilidad de recursos alimenticos, los artrópodos podrían jugar un papel importante para ciertas especies de colibríes como alimento alternativo, suministrando tanto nutrientes como energía.

Nuestro estudio de campo también indica que los artrópodos pueden desempeñar un papel importante como alimento alternativo para ciertas especies de colibríes durante los meses del año en los que el néctar es más abundante. Durante la estación seca-fría, cuando la abundancia y contenido de energía del néctar fueron más altos, y la comunidad de colibríes estuvo conformada tanto por las especies residentes como las migratorias de larga distancia, *Eugenes fulgens*, una especie residente que actúa como rutera, presento una alta tasa de forrajeo de artrópodos, la más alta encontrada para cualquier especie a través del año. Este hallazgo sugiere que *E. fulgens* tiene la capacidad de incrementar sustancialmente su consumo de artrópodos como una estrategia para reducir la competencia interespecífica por los recursos florales, obteniendo tanto nutrientes como energía adicional de los artrópodos.

La implementación del enfoque geométrico de nutrientes, nos permitió demostrar experimentalmente que los colibríes, al igual que otras especies de animales terrestres que presentan una variedad de dietas, tienen la capacidad de regular el consumo de varios nutrientes simultáneamente en lugar de priorizar el consumo de un único nutriente o de energía *per se*. Esto significa que los colibríes tienen la capacidad de seleccionar alimentos que difieren en su proporción de nutrientes y de consumirlos en cantidades adecuadas para cubrir sus requerimientos de proteínas y carbohidratos. Específicamente, nuestro diseño experimental nos permitió determinar las estrategias nutricionales empleadas por dos especies de colibríes, *Cynanthus latirostris* y *Saucerottia beryllina*, haciendo frente a combinaciones de pares de alimentos que variaron en sus concentraciones de proteína y sacarosa. El resultado más relevante de nuestro estudio fue un mayor consumo diario y acumulado de proteína en *C. latirostris* que en *S. beryllina*. Esta diferencia en la capacidad para ingerir proteína parece afectar la ecología de ambas especies. En nuestro sitio de estudio, *S. beryllina* es una especie agresiva que realiza migraciones locales, mientras que

C. latirostris es una especie menos dominante conductualmente que permanece como residente durante todo el año. De este modo, nuestro estudio sugiere que la mayor capacidad de *C. latirostris* para consumir y metabolizar proteína le permite permanecer como residente utilizando a los artrópodos como alimento alternativo durante los meses del año en los que los recursos florales no están disponibles, mientras que *S. beryllina* se ve forzada a migrar a otros sitios donde el néctar es abundante.

Nuestros estudios de campo y de laboratorio, demuestran que algunas especies de colibríes tienen la capacidad de consumir y metabolizar altas cantidades de proteína y que, por lo tanto, pueden presentar altas tasas de consumo de artrópodos, lo que les permite sobrevivir y permanecer como residentes en un área durante todo el año. Nuestro estudio de campo también indica que, a nivel de comunidad de colibríes, las micropiedras ("grit") cumplen un papel fundamental para los colibríes como alimento suplementario, suministrando minerales tales como calcio necesario para la formación de la cascara de huevo. Uno de nuestros hallazgos más importes en este estudio, fue la presencia de micropiedras en los estómagos tanto de las hembras como de los machos, ya que previamente, la ingestión de estos materiales había sido reportada únicamente para las hembras de algunas especies de colibríes. Finalmente, aunque no encontramos relaciones significativas entre el número de micropiedras encontradas en los estómagos de los colibríes y la cantidad de artrópodos ingeridos y su contenido de quitina, nuestro estudio indica que para *Colibri thalassinus*, una especie de colibrí que consume altas cantidades de presas de cuerpos duros, las micropiedras cumplen una función adicional como una herramienta que facilita la trituración mecánica de los artrópodos ingeridos. Nuestro estudio es relevante poque proporciona nuevas ideas sobre el papel que las micropiedras tienen para los colibríes como fuentes de micronutrientes y en la digestión mecánica de los artrópodos ingeridos, complementando la poca información anecdótica existente sobre este tema.

CONCLUSIONES

1) Durante el periodo del año cuando el néctar está ausente y la abundancia de artrópodos es más alta (estación seca-caliente), los colibríes aumentan sus tasas de intentos de capturas de artrópodos y la cantidad de artrópodos ingeridos, usándolos como alimento alternativo (fuentes de nutrientes y de energía). En cambio, durante el periodo del año en que el néctar esta disponible y es abundante en el ambiente (estación seca-fría), la mayoría de las especies de colibríes utilizan a los artrópodos como alimento complementario (fuentes de nitrógeno).

2) Los cambios estacionales en estructura del ensamble de colibríes están relacionados a variaciones en la disponibilidad de los recursos alimenticios. Las especies que tienen una mayor capacidad fisiológica de ingerir más artrópodos, de usarlos como fuentes de energía y sobrevivir a partir del consumo exclusivo de este recurso, pueden establecer poblaciones residentes durante todo el año y volverse dominantes en un sitio en ausencia del néctar, mientras que las especies que son incapaces de manejar dietas ricas en proteína deben de realizar migraciones locales en búsqueda de recursos florales.

3) Las diferencias en la forma en que las especies de colibríes balancean su consumo de nutrientes, en particular su consumo de proteína, podrían explicar sus diferencias ecológicas y conductuales. Nuestro estudio de laboratorio sugiere que la mayor capacidad de *Cynanthus latirostris* para consumir proteína por medio de ingerir más artrópodos, y usarla como fuente de energía, podría darle una ventaja durante los periodos de baja disponibilidad de néctar, permitiéndole permanecer como residente

durante todo el año, mientras que *Saucerottia beryllina* se ve forzada a migrar bajo las mismas condiciones ambientales.

4) Debido a que los colibríes ingieren grit únicamente durante el periodo reproductivo (estación lluviosa y seca fría), y debido a la ausencia de relaciones significativas entre el número de partícula de grit presentes en sus estómagos y la cantidad y dureza de los artrópodos ingeridos, nuestro estudio sugiere que, a nivel de ensamble, los colibríes utilizan el grit únicamente para cubrir sus requerimientos de minerales. Sin embargo, para *Colibri thalassinus*, la especie que ingiere la mayor cantidad de grit y las presas de artrópodos más duras, el grit podría tener un papel adicional como herramienta de trituración mecánica de las presas ingeridas.

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