MOLECULAR ANALYSIS OF THE DIET OF *PARASTRELLUS HESPERUS*, THE AMERICAN PARASTRELLE

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MOLECULAR ANALYSIS OF THE DIET OF

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DEDICATION

I dedicate this thesis to my grandfather, Fred M. Teagarden, who instilled in me a love for science, and whose continued faith, support and encouragement has shown me that the possibilities are limitless.

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ABSTRACT

The objective of this study was to use a molecular approach to analyze the diet of *Parastrellus hesperus,* the American parastrelle, and determine if the diet varied across sex and age-classes. I collected guano pellets from a total 147 *P. hesperus* from May - July 2015 over nine nights in Big Bend National Park. A fragment of the cytochrome c oxidase gene was sequenced from the fecal pellets of 79 individuals and the identity of prey items was inferred from DNA reference databases. Using conservative molecular identification criteria I assigned molecular operational taxonomic units to eight orders, 28 families, 36 genera and 27 species of arthropods of which two orders and 20 families contain new prey items for *P. hesperus*. Significant variation in the diet was found between males and females. No dietary differences were observed between the age-classes or across female reproductive condition.

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INTRODUCTION

Approximately 70% of the world's documented 1,200 bat species, including 24 of 27 species that occur in the Chihuahuan Desert region of Texas, are insectivorous (Ammerman et al. 2012). Despite the invaluable ecosystem services and economic benefits to humans, the diet of many of these species is poorly understood. In many cases, studies that do document the feeding ecology of these species have failed to investigate potential shifts in diet due to differences in age, morphology, physiological needs, reproductive condition, and experience. Ontogenetic characteristics such as these may reveal age-structure and reproductive components of niche width (Anthony and Kunz 1977; Adams 1996; Adams 1997; Hamilton and Barclay 1998). For example, newly volant juveniles likely lack the capability of successfully competing with adult bats for similar prey items due to growth and development factors such as maturity of musculature, flight abilities, and skills associated with capturing and handling prey (Rolseth et al. 1994; Adams 1996). Similarly, the physical demands that reproduction places on female mammals may ultimately influence foraging strategies and result in temporal fluctuations in diets across maternity seasons (Anthony and Kunz 1977; Barclay 1989; Valdez and Cryan 2009; Clare et al. 2011). These conditional factors present an opportunity to evaluate potential dietary shifts in insectivorous bats.

Parastrellus hesperus, the American parastrelle, is a common bat in the deserts of the southwestern United States and is abundant in areas of desert scrub habitat near rocky canyon drainages throughout the summer months (Barbour and Davis 1969; Ammerman et al. 2012). In spite of their prominence in these ecosystems, relatively few studies have documented the

diet of the American parastrelle, an assumed dietary generalist. Prior to the 1960's only notes of foraging behavior, and the extent of prey consumption were documented (Bailey 1905; Davis 1960). In 1967 the first conventional identification effort was made to classify insect fragments in the stomachs of 138 individuals in Arizona (Ross 1967). Using a dissecting microscope and key morphological characteristics of insect taxa, Ross (1967) identified 7 orders, 24 families, and 11 genera of insects that ranged between 2 and 10 mm in length. Among the identified prey items were caddis flies (Trichoptera), moths (Lepidoptera), small beetles (*Anthicus* and *Disonycha*), leafhoppers (*Draeculacephala*), flies (*Simulium*), mosquitoes (*Aedes*), ants (*Acromyrmex*, *Neivamyrmex*, *Camponotus*, and *Formica*), and wasps (Braconidae). This dietary list was further supported when the stomach contents of a single individual, captured in Big Bend National Park, were analyzed (Easterla 1973). In 1981, the analysis of fecal pellets from 67 parastrelles captured in Dog Canyon, New Mexico, revealed that two individuals had consumed spiders (Araneidae), adding a new class of arthropods to the dietary list (Fries 1981).

Documented dietary shifts.— Dietary analyses of other insectivorous bat species have demonstrated dietary shifts associated with reproduction (Kurta and Kunz 1987; Barclay 1989; Kunz et al. 1995; Clare et al. 2011). Vespertilionids that produce twins, such as *P. hesperus*, have shown high litter mass investments which can amount to 50% of the maternal mass (Kurta and Kunz 1987). Reproductive condition might also have an effect on the foraging behavior of bats as foraging time has been documented to increase by 73% between early lactation and fledgling (Barclay 1989). A stomach content analysis of the dietary energetics of Mexican free-tailed bats (*Tadarida brasiliensis*) revealed increased nightly food intake from mid- to late pregnancy (Kunz et al. 1995). Food intake stabilized during late

pregnancy, before increasing again during early to mid-lactation. Using a molecular approach to analyze diet, Clare et al. (2011) documented temporal variation between early, middle, and late maternity season for *Myotis lucifugus*. This study also documented a more variable diet across lactating adult female *M. lucifugus* than in adult males and attributed this to the potential increased energy requirements of reproduction.

Differences in diet and foraging habits have also been observed between adult and newly independent juvenile species of vespertilionids (Anthony and Kunz 1977; Rolseth et al. 1994; Adams 1996; Adams 1997; Hamilton and Barclay 1998). Ecological segregations between age groups have been shown in *M. lucifugus* with shifts in adult foraging areas occurring once juveniles have become volant (Adams 1997). These young *M. lucifugus* predominantly forage in low cluttered microhabitats, but quickly display differences in habitat use and diet as wing size increases (Adams 1996). Also, the diet of juvenile *Lasiurus cinereus* was found to encompass a broader range of prey items with a lower mean hardness than was that of the adults (Hamilton and Barclay 1998). These differences in dietary features have been explained by a lack of capture experience (Rolseth et al. 1994), poor handling skills of larger insects (Rolseth et al. 1994; Hamilton and Barclay 1998), and a reduced ability to discriminate between prey species (Anthony and Kunz 1977; Hamilton and Barclay 1998). Each of these explanations presents a situation which could prevent juveniles from maintaining a level of prey selection similar to adults (Anthony and Kunz 1977; Rolseth et al. 1994).

Need for and advantages of molecular diet analysis.— Morphological analysis and identification of prey fragments in the stomachs or fecal material of target species has traditionally been the method of determining species diet (Kunz and Whittaker 1983).

Unfortunately, this method is biased if key features used for identification are damaged by chewing, digestion, or decomposition (Kunz and Whitaker 1983). Thus, results of this technique may be skewed toward hard-bodied insects whose fragments frequently survive mastication, while leaving easily degraded remains of soft-bodied species undocumented. Additionally, the difficulty of identifying prey fragments to lower taxonomic levels is a major limitation of traditional fecal analysis (Kunz and Whitaker 1983; Whitaker et al. 2009).

In recent decades, new advances in technology have allowed for dietary studies to be conducted using molecular techniques. As a result, large databases of taxonomically validated reference sequences, such as those in the Barcode of Life Data System (BOLD, Ratnasingham and Herbert 2007) and Genbank (http://www.ncbi.nlm.nih.gov), are available to quantify biodiversity using genetic data (Clare et al. 2014). These databases provide a powerful means to determine the identity of DNA sequences from fecal samples once they are matched to database references. However, the application of this technique to understanding chiropteran diets is fairly recent with the first full molecular analysis of bat diet occurring in 2009 (Clare et al. 2009). With this molecular approach, DNA sequences from prey fragments that survive digestion and are recovered from feces can allow for species-specific verifications of food items eaten (Clare et al. 2011). The use of the polymerase chain reaction (PCR) technique to obtain cytochrome c oxidase subunit I gene (COI) sequences from prey items, followed by DNA-sequence analysis to identify prey species, should help bridge the gaps in species-level identification of insect remains. This technique could limit bias between frequently preserved hard-bodied species and easily degraded soft-bodied species, thus providing a more complete dietary analysis.

Because of the paucity of data on their diet and their relative abundance, *P. hesperus* was identified as an ideal target species for expanding and refining a list of known prey items while investigating potential ontogenetic and reproductive shifts in diet. Therefore, the objective of this study was to document the diet of *P. hesperus* using molecular analysis methods and to evaluate potential dietary differences due to ontogenetic and reproductive shifts. I hypothesized that 1) the diet items of female *P. hesperus* would vary significantly with reproductive status (pregnant, lactating, post-lactating, and non-reproductive) and 2) that prey consumption of newly volant juvenile *P. hesperus* would vary significantly from that of adults feeding in the same general area during the same time of year. Further, I hypothesized that a molecular approach to diet analysis would expand the known prey items of this species.

METHODS

Study site.— Based on the natural history of *P. hesperus* and the previous collection history of parastrelles in the area, Big Bend National Park was the chosen study site. Located in Brewster County along the United States/Mexico border, Big Bend National Park occupies the Chihuahuan Desert ecoregion of Texas and is located between 29°41' – 28°58' N and102°50' – 103°46' W. The 324,219-hectare park is characterized by various habitats but is primarily dominated by desert badlands occasionally interrupted with riparian corridors and steep walled canyons. Within the eastern half of the park two specific locations were established as survey sites, Ernst Tinaja (29 15'22" N, -103 00'42"W) and Carlota Tinaja (29 16'45"N, -103 2' 8"W). At an elevation of approximately 2300 ft., both sites exhibit deep canyon walls comprised of thin-bedded limestone, and shale with vegetation on surrounding slopes comprised of creosote bush (*Larrea divaricata*), lechugilla (*Agave lechugilla*), ocotillo (*Fouquieria splendens*), blind pricklypear (*Opuntia rufida*), and dog cholla (*O. grahamii*) (Wauer and Fleming 2002).

Sampling strategy.— Fecal samples from *P. hesperus* were collected over a three month period (May, June and July) in the summer of 2015 to collectively represent the diet of multiple age classes (adults and juveniles), sexes (male and female), and various reproductive conditions (pregnant, lactating, post-lactating, and non-reproductive). Each month, survey efforts were focused over a period of 2-5 consecutive nights during the new moon phase.

Capture methods.— Bats were collected through the use of monofilament three-tiered mist nets that were deployed at sunset and remained open until approximately 12:00 a.m. (or 3 hours after sunset). This period of deployment corresponded with the peak of activity for *P.*

hesperus (Cockrum and Cross 1964; Barbour and Davis 1969). Two to five mist nets were positioned across small bodies of water and across openings in natural canyon flyways. Mist nets were checked at least every 10 minutes, if not more frequently, for captures as recommended by Kunz et al. (2009).

Upon each capture, the bat was carefully removed from the mist net and handled in accordance with the National Park Service Institutional Animal Care and Use Committee (#IMR_BIBE_Demere_Bat_2015.A2), Angelo State University IACUC (#15-04), and the guidelines of the American Society of Mammalogists (Sikes et al. 2011). The sex and age of captured non-target species were documented and the bat was released. Each captured parastrelle was immediately placed in a clean, ventilated, and individually labeled Dixie cup and held for 5-30 minutes or until defecation was observed. If a fecal sample was not present after 30 minutes, the bat was released. Once defecation had been observed, measurements of mass, forearm length, ear length and hind foot were taken and each individual was examined for any noticeable signs of White Nose Syndrome as specified by the U.S. Fish and Wildlife Service (www.whitenosesyndrome.org) prior to the bat's release. All fecal pellets from an individual were collected and stored as a single fecal sample in a 2-ml cryotube filled with 95% ethanol and labeled with the corresponding specimen number.

Sample categorization.— Fecal samples were grouped into categories based on the sex, reproductive condition at the time of capture, and age of the bat as determined by the ossification of the fourth metacarpal-phalangeal joint (Brunet-Rossinni and Wilkinson 2009). The reproductive condition in females was documented using the following criteria: a) pregnancy was determined by gentle palpation or pronounced swelling of the abdomen, b) females expressing milk or with noticeable swelling of and bare patches around nipples were

categorized as lactating, c) females presenting regrowth of fur around nipples were classified as post-lactation (Krutzsch 1975). Females and males that were not young-of-the-year and that did not exhibit characteristics of established reproductive conditions were classified as non-reproductive.

Analysis of fecal samples.— Pellets from a subset of individuals in each of the established categories were selected for fecal analysis if the mass of the bat was within the standard deviation of weight for their sex, age and reproductive category. DNA from individual fecal samples, ranging between 1-5 fecal pellets, was extracted using a QIAamp DNA Stool Mini Kit (Qiagen Inc., Chatsworth, California) following the manufacturer's instructions with modifications from Zeale et al. (2011). In addition to these modifications, each sample was eluted twice with 50 µl of AE Buffer instead of a single 200 µl elution and the incubation time was increased from 1 to 2 min. Step-up polymerase chain reactions (Research and Testing Laboratory, Lubbock, Texas) were performed in 23 µl reactions for the amplification of a COI fragment using ZBJ-ArtF1c and ZBJ-ArtR2C primers (Zeale et al. 2011). Thermal cycling conditions for annealing steps were 50ºC for 2 minutes, followed by a 0.5ºC increase over 10 cycles reaching 54.5ºC, and finally 30 cycles at 54ºC. PCR products (4 µl) were loaded in 2% agarose gel for electrophoresis and the resulting bands evaluated for amplification success. PCR products with moderate to strong band amplification scores were selected for a second 10-cycle PCR where the remaining 19 µl of the products were incorporated with index labels (unique combinations of 5 forward and 7 reverse tags). A second gel of resulting PCR products was scored and samples with bands of moderate to strong scores were pooled and cleaned for sequencing. Sequencing (Research and Testing Laboratory, Lubbock, Texas) was carried out using an Illumina MiSeq platform.

Upon sequencing completion, all reads were processed through the Research and Testing Laboratory (RTL) data analysis pipeline consisting of two major stages, the denoising and chimera detection stage, and the diversity analysis stage. A stepwise breakdown of the first major stage consists of: 1) removal of short sequences, singleton sequences, and noisy reads; 2) removal of chimeric sequences; 3) base by base correction of remaining sequences to remove noise from each sequence (www.researchandtesting.com/docs/Data_Analysis_Methodology.pdf). At this point each sample sequence was analyzed using the following approach to determine the taxonomic identity. Sequences were clustered into molecular operational taxonomic units (MOTUs) using the UPARSE algorithm (Edgar 2013) and the centroid sequence of each cluster was run against the USEARCH global alignment algorithm (Edgar 2010) using a database of high quality sequences derived from the National Center for Biotechnology Information (NCBI) that is maintained by RTL. This output was analyzed using an internally developed python program paired with USEARCH to assign taxonomic information to each sequence. The top six matches in the database for a given sequence were identified and from these sequences a confidence value to each taxonomic level was assigned for the read. Using this confidence value, two separate identification outputs were created, Full and Trimmed Taxa information. Full taxonomic identifications were made based on the taxonomic information for the top hit, regardless of how many of the top six matches agreed. Trimmed Taxa data retained the Full Taxa identification only if the taxonomy at each level agrees in at least four out of the top six hits. If multiple MOTUs were assigned to the same arthropod species based on the Full Taxa identifications, these MOTUs were combined (Van Den Bussche et al., in press). Each taxonomic level that was not documented from Big Bend National Park (Van Pelt, in litt.),

Texas, New Mexico, or northern Mexico was removed from the list (SCAN 2016) because the possibility of consumption was unlikely.

As a comparative approach, prey identities also were inferred by comparing a representative sequence from each MOTU with the standard nucleotide collection existing on GenBank in March 2016 using basic local alignment (BLAST) and the megablast program. Species level identifications were made if a match occurred under the following criteria modified from Clare et al. (2014): 1a = matched to one species with 100% similarity (query coverage ≥92%); 1b = matched to a species with ≥97% similarity (query coverage ≥92%) that is documented in the study region (southwestern US), but could belong to a congener not represented in the database; 2 = matched to multiple species with ≥97% similarity (query coverage >92%), but only one species is documented in the study region and this species is kept and considered a match; 3 = matched to multiple species of the same genus, where none of the species occur in the study area but the genus does and therefore the genus designation was used.

Variations between the capture rates of adults and juveniles of both sexes were assessed across the survey months using 2-tailed binomial tests in the statistical program R (R Core Team 2014) to determine if there was a significant difference across both sex and age-classes in each survey month. The number of unique prey sequences in each fecal sample was then determined by combining MOTUs that were assigned to the same species (pooling samples), and including any un-identified species that represented the only prey item of an identified upper level taxon. All resulting MOTU groupings were considered unique prey items and their assigned taxonomic identification was ignored. Statistical comparisons using permutational multivariate analysis of variance (PERMANOVA) and the Jaccard similarity

measure were made in the statistics program R (R Core Team 2014) using the function "adonis" (package vegan required) between each of the established age, sex and reproductive classes to determine if significant dietary differences or shifts were documented. The Holm P-value adjustment method was applied using the p.adjust method in R to account for elevated type I error in instances where multiple comparisons were made.

Using presence absence data from the above dataset, the frequency of occurrence (across samples) for each prey taxon occurring within the study area was determined at each taxonomic level. This frequency provided a standardized measure of common prey items (Clare et al. 2009) in the diet of parastrelles. To estimate species richness of arthropods included in the diet of *P. hesperus*, a rarefaction curve with 1,000 iterations was carried out using all individual samples in EstimateS version 9.1.0 (Colwell 2013).

Collection of insect references.— Insect sampling was conducted at each netting site during survey trips and corresponded with deployment of mist nets in an attempt to collect specimen references for potential prey items in the arthropod community at each location (Whitaker et al. 2009). Ultraviolet light was utilized as the method of collection. Each trap was hung at least 100 meters away from the mist nets and approximately 2 m above the ground in an attempt to target species occurring within the foraging range of *P. hesperus* (2-15m off of the ground; Mumford et al. 1964). Arthropod specimens that were collected were originally placed in collecting cups and upon sorting were transferred into individually labeled 2 ml cryotubes filled with 95% ethanol. Arthropod remains were identified to order, family, and when possible, genus and species.

Identified insect specimens were provided to RTL for DNA sequencing of the COI gene. All insect specimens were destroyed during DNA analysis leaving no reference

collection. Sequence data from identified arthropod taxa were added to the existing in-house library at RTL to serve as additional references for fecal analysis and to potentially narrow gaps in species identification during molecular analysis.

RESULTS

Capture rates.— Over nine nights (68 net hours) in Big Bend National Park, I captured a total of 149 parastrelles (2.19 bats/hour). Capture rates varied across survey site with 100 individuals captured at Ernst Tinaja over 41 net hours (2.4 bats/hour) and 49 individuals captured at Carlota Tinaja over 27 net hours (1.8 bats/hour). The overall sex ratio of captured parastrelles was analyzed using a 2-tailed binomial test and were not different from 1 (Table 1; $P_{adj} > 0.280$). However, a seasonal difference in the sex ratio was observed with 86.7% of the captures in May being male (Table 1; $P_{adj} < 0.001$) and 92.5% of the captures in June being female (Table 1; $P_{adj} < 0.001$). Although the sex ratio in July was not skewed ($P_{adj} > 0.499$), a difference in the capture of the two age classes was observed, with juveniles making up 65.8% (Table 1; $P_{adj} > 0.019$) of captures for the month.

Collection of insect references.— Eighty-three total insect specimens were collected in May and July and provided to RTL for sequencing. From these specimens a subset was identified to 7 orders, 25 families, 22 genera, and 10 species (Table 2). Insects were not collected in June due to a damaged ultraviolet light.

Sequence analysis of fecal samples.—Of the total 149 captured, 147 parastrelles provided fecal samples within 30 minutes of capture (Table 3). Two individuals, an adult male in May and a post-lactating adult female in July were released 30 minutes post-capture without producing a fecal sample. From these 147 bats, a total of 84 individual samples, consisting of 259 guano pellets, were selected for analysis (Table 3). Amplification and sequencing success varied across the samples. Five of the initial 84 samples had low

Table 1. — Captures of *Parastrellus hesperus* during summer 2015 in Big Bend National Park, Texas at Ernst Tinaja and Carlota Tinaja by sex and age classes.

Month	Captures of *P. hesperus*				
	Total	Males	Females	Adults	Juveniles
May	30	26	4	30	0
June	40	3	37	40	0
July	79	36	43	27	52
Total	149	65	85	97	52

Table 2. — List of invertebrate taxa collected by means of ultraviolet light during 2015 summer survey efforts at Ernst and Carlota Tinajas in Big Bend National Park, Texas. These specimens were sent to Research and Testing Laboratory in Lubbock, Texas to be added to their in-house reference library.

Month	Location	Order	Family	Genus
May	Ernst	Blattodea	Blattidae	*Pseudomops**
May	Carlota	Blattodea	Ectobiidae	*Ectobius*
May	Ernst	Coleoptera	Braconidae	
May	Ernst	Coleoptera	Carabidae	*Colliuris*
July	Carlota	Coleoptera	Chrysomelidae	*Alticini*
May	Carlota	Coleoptera	Chrysomelidae	*Diabrotica*
May	Ernst	Coleoptera	Chrysomelidae	*Altica*
May	Ernst	Coleoptera	Chrysomelidae	
May	Ernst	Coleoptera	Chrysomelidae	
May	Ernst	Coleoptera	Chrysomelidae	
July	Carlota	Coleoptera	Chrysomelidae	
May	Carlota	Coleoptera	Cleridae	*Cymatodera*
May	Ernst	Coleoptera	Coccinellidae	*Olla*
July	Ernst	Coleoptera	Coccinellidae	
May	Ernst	Coleoptera	Curculionidae	
July	Carlota	Coleoptera	Elateridae	
May	Ernst	Coleoptera	Elateridae	
July	Carlota	Coleoptera	Lampyridae	
July	Ernst	Coleoptera	Meloidae	*Epicauta*
July	Ernst	Coleoptera	Meloidae	*Epicauta*
May	Carlota	Coleoptera	Oedemeridae	*Oxycopis*
May	Ernst	Coleoptera	Pentatomidae	*Acrosternum*
July	Carlota	Coleoptera	Scarabaeidae	*Phyllophaga*
May	Carlota	Coleoptera	Scarabaeidae	*Phyllophaga*
May	Ernst	Coleoptera	Scarabaeidae	*Phyllophaga*
May	Ernst	Coleoptera	Scarabaeidae	*Phyllophaga*
July	Ernst	Coleoptera	Scarabaeidae	*Phyllophaga*
July	Ernst	Coleoptera	Scarabaeidae	*Phyllophaga*
July	Ernst	Coleoptera	Scarabaeidae	*Onthophagus*
July	Ernst	Coleoptera	Tenebrionidae	
July	Ernst	Coleoptera	Tenebrionidae	
July	Ernst	Diptera		
July	Carlota	Diptera		
July	Carlota	Diptera		
May	Carlota	Hemiptera	Cicadellidae	

Table 2. — Continued.

Month	Location	Order	Family	Genus
May	Ernst	Hemiptera	Cicadellidae	
May	Ernst	Hemiptera	Lygaeidae	*Neacoryphus*
July	Ernst	Hemiptera	Lygaeidae	
May	Carlota	Hemiptera	Miridae	*Phytocoris*
July	Carlota	Hemiptera	Pentatomidae	
July	Ernst	Hymenoptera	Braconidae	
May	Carlota	Hymenoptera	Tiphiidae	
May	Ernst	Hymenoptera	Tiphiidae	
July	Carlota	Hymenoptera		
May	Carlota	Lepidoptera	Erebidae*	*Cisseps**
May	Carlota	Lepidoptera	Geometridae	*Chlorospilates*
May	Carlota	Lepidoptera	Geometridae	*Scopula*
May	Carlota	Lepidoptera	Noctuidae	*Papaipema**
May	Carlota	Lepidoptera	Noctuidae	
May	Carlota	Lepidoptera	Noctuidae	*Euxoa*
May	Carlota	Lepidoptera	Noctuidae	
May	Carlota	Lepidoptera	Noctuidae	
May	Carlota	Lepidoptera	Noctuidae	*Acontia*
May	Carlota	Lepidoptera	Noctuidae	
May	Carlota	Lepidoptera	Noctuidae	
July	Carlota	Lepidoptera	Noctuidae	
July	Carlota	Lepidoptera	Noctuidae	
July	Carlota	Lepidoptera	Noctuidae	
July	Carlota	Lepidoptera	Noctuidae	
May	Ernst	Lepidoptera	Noctuidae	
May	Ernst	Lepidoptera	Noctuidae	
May	Ernst	Lepidoptera	Noctuidae	
May	Ernst	Lepidoptera	Noctuidae	
July	Ernst	Lepidoptera	Noctuidae	
July	Ernst	Lepidoptera	Noctuidae	
July	Ernst	Lepidoptera	Noctuidae	
July	Ernst	Lepidoptera	Noctuidae	
July	Ernst	Lepidoptera	Noctuidae	
July	Ernst	Lepidoptera	Noctuidae	
July	Ernst	Lepidoptera	Noctuidae	
July	Ernst	Lepidoptera	Noctuidae	
July	Ernst	Lepidoptera	Noctuidae	
May	Carlota	Lepidoptera	Pterophoridae	
May	Carlota	Lepidoptera		

Table 2. — Continued.

Month	Location	Order	Family	Genus
July	Carlota	Lepidoptera		
May	Ernst	Lepidoptera		
July	Ernst	Lepidoptera		
July	Ernst	Lepidoptera		
July	Ernst	Lepidoptera		
July	Ernst	Lepidoptera		
July	Ernst	Lepidoptera		
May	Carlota	Neuroptera	Chrysopidae	
May	Carlota	Neuroptera	Myrmeleontidae	*Myrmeleon*

* Taxon has not been previously documented in Big Bend National Park

Table 3. — Number of fecal samples collected from American parastrelles (*Parastrellus hesperus*) captured during summer 2015 in Big Bend National Park, Texas and the number of subset samples selected for analysis and sequenced in each of the established categories.

		Fecal samples of *P. hesperus*		
Category	Condition	Collected	Selected for analysis	Sequenced
Adult male	Non-reproductive	45	18	17
Adult female	Pregnant	3	3	3
	Lactating	38	23	22
	Post-lactating	8	8	7
	Non-reproductive	1	0	0
Juvenile male	Non-reproductive	19	15	13
Juvenile female	Non-reproductive	33	17	17
Totals		147	84	79

amplification success and were dropped prior to sequencing and an additional 12 samples produced low sequence coverage (read range: 5-763). Molecular analysis of the 79 sequenced samples identified 144,473 sequences among 329 MOTUs. The number of MOTUs found in each fecal sample ranged from 1 to 59 with an average of 16.3 MOTUs per sample (Fig. 1).

Diet of American parastrelles.— In total, 133 prey items were able to be considered unique identifications. With 53% of these prey items identified only once, a species rarefaction curve indicated that an asymptote had not been reached (Fig. 2). The number of dietary prey items in each fecal sample revealed that individual bats on average consumed 7.6 (range = 1-26) unique prey items (Fig. 3). Taxonomic identification of these MOTUs varied across technique.

Full Taxon analysis under USEARCH global alignment assigned 275 MOTUs to 99 identified species of 118 genera, 72 families, 10 orders, and 3 classes. In addition to identified prey items, 70 MOTUs produced unclassified results at various taxonomic levels and 54 MOTUs were not matched to a reference sequence at any level. After confirming the geographic occurrence of prey species in the deserts of the southwestern United States, the number of acceptable identifications at each taxonomic level was reduced (Table 4). In total, 37 of the 99 species (Table 5) were identified as plausible dietary items for parastrelles.

BLAST methodology and identification criteria produced a more conservative list of identified prey items (Table 6) across each taxonomic level (Table 4). This dietary list was chosen for further analysis of taxonomic breakdown when determining the frequency of occurrence for prey items at the various taxonomic levels. The most commonly consumed arthropod orders (based on frequency of occurrence across fecal samples) were Coleoptera,

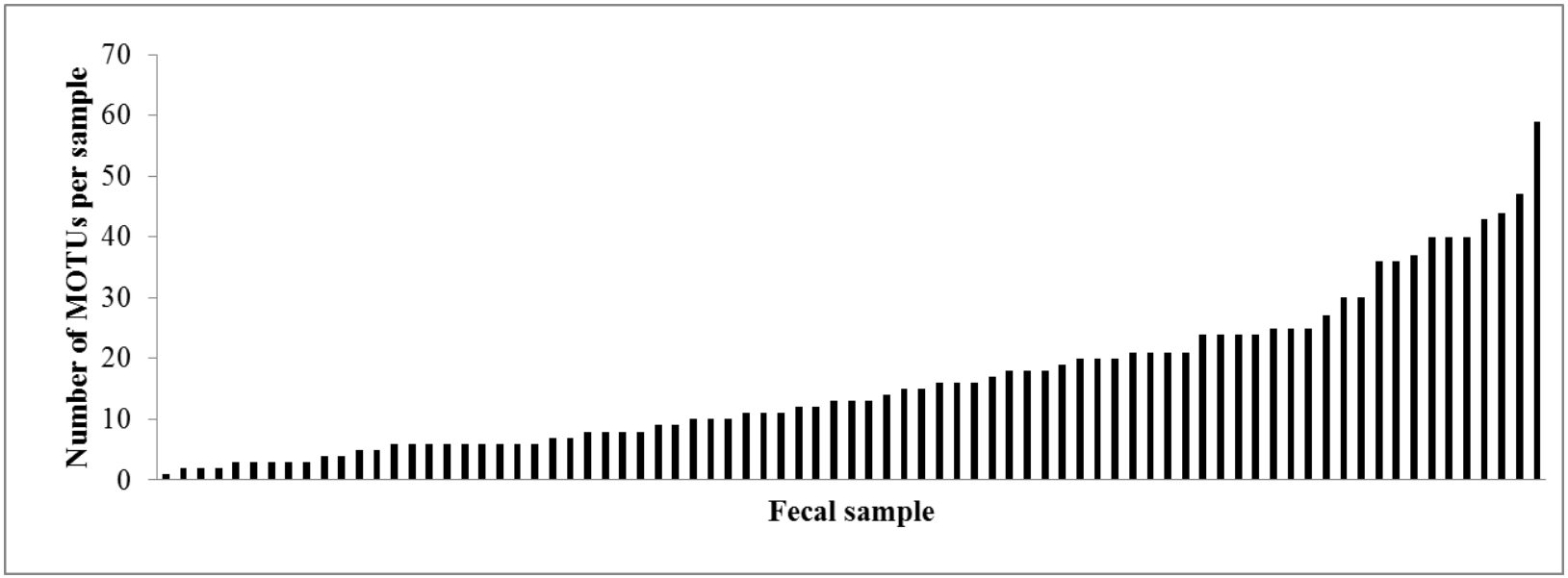

Fig. 1. — Number of unique MOTUs identified in each of the 79 fecal samples collected from individual *Parastrellus hesperus* during the 2015 summer in Big Bend National Park, Texas.

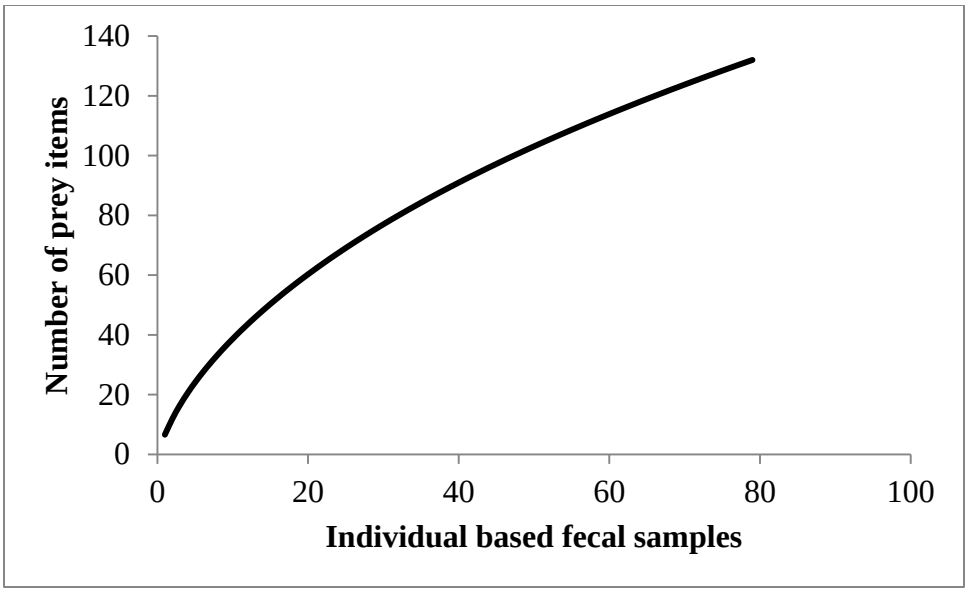

Fig. 2. —A rarefaction curve for operational taxonomic units identified to 133 unique prey items found in the fecal pellets of 79 *Parastrellus hesperus* captured during the 2015 summer, Big Bend National Park. Lacking a clear asymptote, the detection of species continues to increase with added fecal samples.

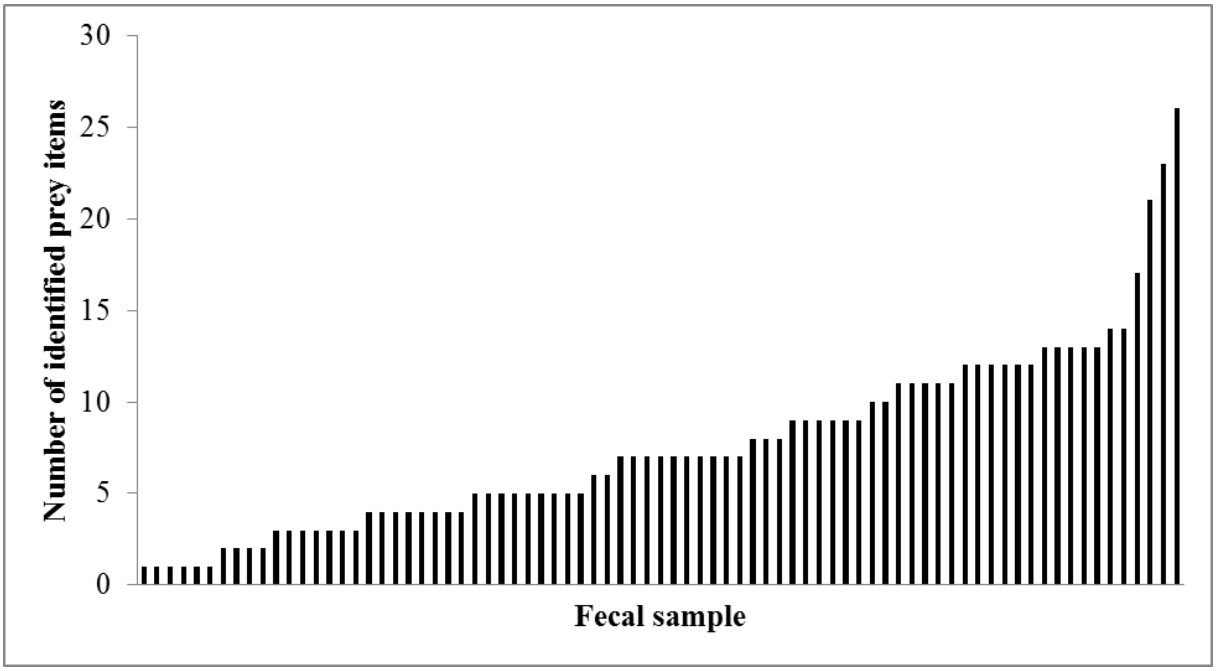

Fig. 3. — Number of uniquely identified prey items in each of the fecal samples collected from individual *Parastrellus hesperus* during the 2015 summer in Big Bend National Park, Texas.

Table 4. —The total number of identified taxa, and assumed prey items of *Parastrellus hesperus* at each taxonomic level reported from the USEARCH global alignment and BLAST methodologies.

	USEARCH global alignment		BLAST	
Taxonomic level	Identified	Assumed Prey	Identified	Assumed Prey
Class	3	2	1	1
Order	10	9	8	8
Family	68	67	28	28
Genus	73	72	36	36
Species	38	37	27	27

Table 5. — List of the molecular identification of 38 species found in the fecal pellets of American parastrelles using DNA sequence analysis of a fragment of the cytochrome c oxidase subunit I gene (COI) as compared to a database of high quality sequences derived from the National Center for Biotechnology Information (NCBI) and maintained by Research and Testing Laboratory, Lubbock, Texas.

Class	Order	Family	Genus	Species
Arachnida	Araneae	Araneidae*	*Metepeira**	
		Philodromidae	*Philodromus*	*Philodromus rufus*
		Salticidae	*Pelegrina*	*Pelegrina flaviceps*
		Theridiidae	*Latrodectus*	*Latrodectus hesperus*
Insecta	Blattodea	Blattidae	*Shelfordella*	*Shelfordella lateralis*
	Coleoptera	Brentidae*	*Apion**	
		Carabidae	*Amara**	
			*Discoderus**	
			Harpalus	*Harpalus caliginosus**
				*Harpalus reversus**
			*Platynus**	
			*Pterostichus**	
			Selenophorus	*Selenophorus opalinus*
				*Selenophorus planipennis**
		Cerambycidae*	*Xylotrechus**	
		Chrysomelidae	*Chaetocnema**	
			Mimosestes	*Mimosestes acaciestes*
			Scymnus	
		Coccinellidae		

Table 5. — Continued.

Class	Order	Family	Genus	Species
		Curculionidae *		
		Dermestidae	*Dermestes*	*Dermestes maculatus*
		Lucanidae*		
		Ptinidae*		
		Scarabaeidae*		
		Staphylinidae*	*Tachinus**	
	Diptera	Anthomyiidae*		
		Aulacigastridae	*Aulacigaster*	
		Ceratopogonidae*		
		Chironomidae	*Glyptotendipes*	*Glyptotendipes meridionalis*
			*Parachironomus**	
			*Procladius**	
		Chloropidae*		
		Culicidae*	*Anopheles**	
		Drosophilidae	*Drosophila*	*Drosophila suzukii*
			Scaptomyza	*Scaptomyza frustulifera**
		Ephydridae*		
		Fanniidae*	*Fannia**	
		Lauxaniidae*		
		Muscidae*		
		Pipunculidae*		
		Sarcophagidae*	*Ravinia**	
		Simuliidae	*Simulium*	

Table 5. — Continued.

Class	Order	Family	Genus	Species
		Tachinidae*		
		Tephritidae*		
	Hemiptera	Berytidae	*Jalysus*	*Jalysus wickhami*
		Cicadellidae	*Balclutha*	
			*Empoasca**	
			*Ollarianus**	
			Xerophloea	*Xerophloea viridis*
		Cixiidae*	*Oliarus**	
		Coreidae*		
		Delphacidae*	*Delphacodes**	
		Lygaeidae	*Neortholomus*	*Neortholomus scolopax*
			Nysius	*Nysius raphanus*
			Xyonysius	*Xyonysius californicus*
		Miridae	*Lygus*	*Lygus lineolaris*
			Melanotrichus	*Melanotrichus coagulatus*
			*Phytocoris**	*Phytocoris sulcatus**
				*Phytocoris neglectus**
			*Prepops**	
		Pentatomidae	*Acrosternum**	*Acrosternum hilare**
			*Podisus**	*Podisus maculiventris**
			Thyanta	*Thyanta custator accerra*
				*Thyanta pallidovirens**
		Rhopalidae	*Arhyssus*	

Table 5. — Continued.

Class	Order	Family	Genus	Species
		Rhyparochromidae	*Peritrechus**	
			Pseudopachybrachius	*Pseudopachybrachius basalis*
		Tingidae*		
	Hymenoptera	Braconidae*	*Meteorus**	
		Formicidae	*Solenopsis*	*Solenopsis xyloni*
		Vespidae	*Polistes*	*Polistes bellicosus*
	Lepidoptera	Acrolophidae (Tineidae)	*Acrolophus*	*Acrolophus variabilis*
		Bucculatricidae*	*Bucculatrix**	
		Cosmopterigidae*		
		Erebidae (Noctuidae)*	*Lesmone**	
			*Toxonprucha**	
		Gelechiidae*	*Aristotelia*	
			*Chionodes**	
			*Deltophora**	
			Filatima	
			*Scrobipalpula**	
		Geometridae*	*Eois**	
			*Eupithecia**	
			*Scopula**	*Scopula ancellata**
		Gracillariidae*	*Phyllonorycter**	

Table 5. — Continued.

Class	Order	Family	Genus	Species
		Momphidae*	*Mompha**	
				Cropia templada
		Noctuidae	*Cropia*	
		Notodontidae	*Datana*	*Datana perspicua*
		Prodoxidae*	*Prodoxus**	*Prodoxus coloradensis**
		Pyralidae*		
		Thyrididae*		
		Xyloryctidae		
	Neuroptera*	Chrysopidae*	*Eremochrysa**	*Eremochrysa punctinervis**
		Hemerobiidae*		
	Orthoptera	Gryllidae	*Gryllus*	
Bdelloidea*	Adinetida*	Adinetidae*	*Adineta**	*Adineta vaga**

* Taxonomic assignment was not documented using the BLAST criteria.

Table 6. — List of invertebrate prey taxa identified by comparing cytochrome c oxidase subunit I gene (COI) sequences extracted from the fecal pellets of American parastrelles to reference sequences in the National Center for Biotechnology Information (NCBI) database. Identity refers to the percent sequence identity match between the sequence used to represent the MOTU and the match in NCBI.

Class	Order	Family	Genus	Species	Identity
Arachnida	Araneae	Philodromidae	*Philodromus*	*Philodromus rufus*	100%
		Theridiidae	*Latrodectus*	*Latrodectus hesperus*	100%
Insecta	Blattodea	Blattidae	*Shelfordella**	*Shelfordella lateralis**	99%
	Coleoptera	Carabidae	*Harpalus*	Unidentified	98%
			Selenophorus	*Selenophorus opalinus**	97%
		Chrysomelidae	*Mimosestes*	*Mimosestes acaciestes*	99%
		Coccinellidae	*Scymnus*	Unidentified	97%
		Dermestidae	*Dermestes*	*Dermestes maculatus**	100%
	Diptera	Aulacigastridae*	*Aulacigaster**	Unidentified	97%
		Chironomidae	*Glyptotendipes**	*Glyptotendipes meridionalis**	100%
		Drosophilidae	*Drosophila*	*Drosophila suzukii**	100%
			*Scaptomyza**	Unidentified	97%
		Simuliidae	*Simulium*	Unidentified	99%
	Hemiptera	Berytidae	*Jalysus*	*Jalysus wickhami*	99%
		Cicadellidae	*Balclutha*	Unidentified	100%
			*Xerophloea**	*Xerophloea viridis**	98%
		Cydnidae	*Microporus**	*Microporus obliquus**	98%
		Lygaeidae	*Neortholomus**	*Neortholomus scolopax**	100%

Table 6. — Continued.

Class	Order	Family	Genus	Species	Identity
			Nysius	*Nysius raphanus**	100%
			Xyonysius	*Xyonysius californicus*	100%
					100%
		Miridae	*Lygus*	*Lygus lineolaris*	
			*Melanotrichus**	*Melanotrichus coagulatus**	100%
		Pentatomidae	*Thyanta**	*Thyanta custator acerra**	100%
		Rhopalidae	*Arhyssus*	*Arhyssus lateralis*	97%
			*Liorhyssus**	*Liorhyssus hyalinus**	100%
	Hymenoptera	Formicidae	*Solenopsis*	*Solenopsis xyloni*	99%
		Vespidae	*Polistes*	*Polistes bellicosus**	100%
	Lepidoptera	Tineidae	*Acrolophus*	*Acrolophus variabilis*	99%
		Gelechiidae	*Aristotelia*	Unidentified	97%
			Filatima	*Filatima abactella**	98%
		Noctuidae	*Cropia*	*Cropia templada*	97%
		Notodontidae	*Datana*	*Datana perspicua*	98%
		Xyloryctidae	*Crypsicharis*	Unidentified	97%
	Orthoptera	Gryllidae	*Gryllus*	Unidentified	99%

*Taxon has not been documented in Big Bend National Park but occurs in the deserts of the southwest.

Hemiptera, Diptera, and Lepidoptera (Fig. 4). Subsequent analysis revealed high frequencies of consumption for prey items belonging to three genera *Harpalus*, *Selenophorus* and *Nysius* (Fig. 5) within two families, Carabidae and Lygaeidae (Fig. 6).

Dietary variations.— Considering all 133 unique prey items, PERMANOVA analyses revealed the diet of adult parastrelles varied significantly across the three surveyed months ($F = 1.50$, $P < 0.03$). No significant difference was documented when comparing the dietary items consumed in May to those of June or July ($F = 1.02$, $P_{adj} < 0.50$; $F = 1.32$, $P_{adj} < 0.50$). However, a dietary difference was observed in adults between June and July ($F = 1.93$, $P_{adj} < 0.05$). When both age classes were evaluated for the month of July, prey consumption between adult and juvenile *P. hesperus* was not significantly different ($F = 1.07$, $P < 0.5$).

The dietary items of adults differed between the sexes across all three survey months ($F = 2.15$, $P < 0.01$). This sex-based distinction was not documented among juveniles in July ($F = 0.91$, $P_{adj} < 0.60$). Further analysis of adult *P. hesperus* revealed that the sex-based distinction remained ($F = 1.67$, $P < 0.05$) when evaluating the sexes across a combined subset of the months May and July. However, a significant dietary difference was not documented when males and females in May ($F = 1.40$, $P < 0.50$), and July ($F = 1.22$, $P < 0.50$) were analyzed separately. No male samples from June were selected for analysis; therefore no statistical comparison between the sexes was made for this month. When considering the reproductive condition of adult females throughout the survey season, no significant difference was observed in diet between pregnant, lactating and post-lactating females ($F = 1.31$, $P < 0.10$).

To evaluate potential effects on the temporal availability of prey item, the diet of adult parastrelles was compared across all months. A difference in the consumed dietary items of adults was documented when May, June and July ($F = 1.50$, $P < 0.05$) were compared. After pairing these months into three unique combinations, subsequent analyses revealed that there was no significant dietary difference between May and June ($F = 1.02$, $P < 0.50$), or May and July ($F = 1.32$, $P < 0.50$). However, a significant dietary difference was observed between June and July ($F = 1.93$, $P < 0.01$).

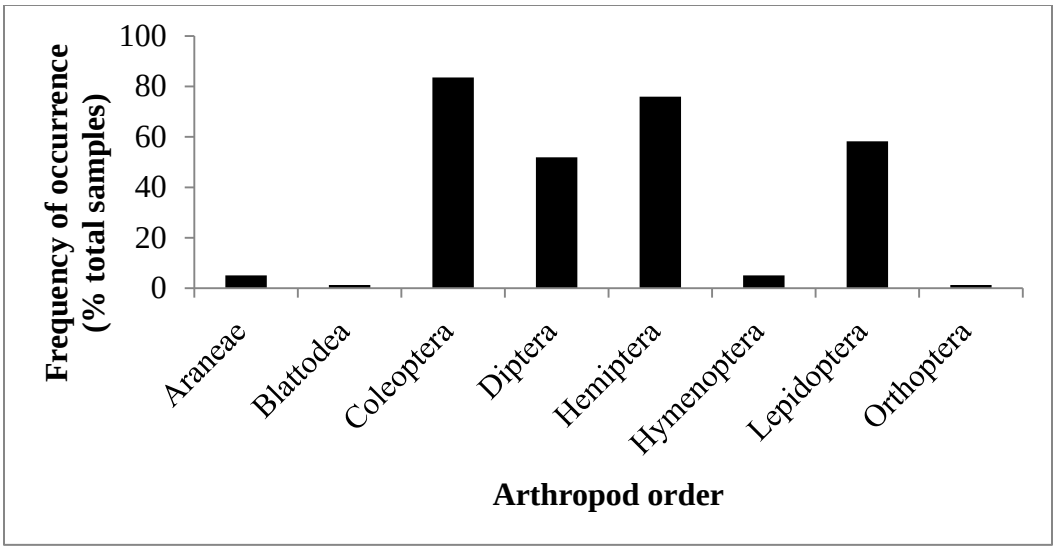

Fig. 4. — Occurrence of arthropod orders, identified by the BLAST methodology, in fecal samples of *Parastrellus hesperus* captured in Big Bend National Park, Texas in summer 2015.

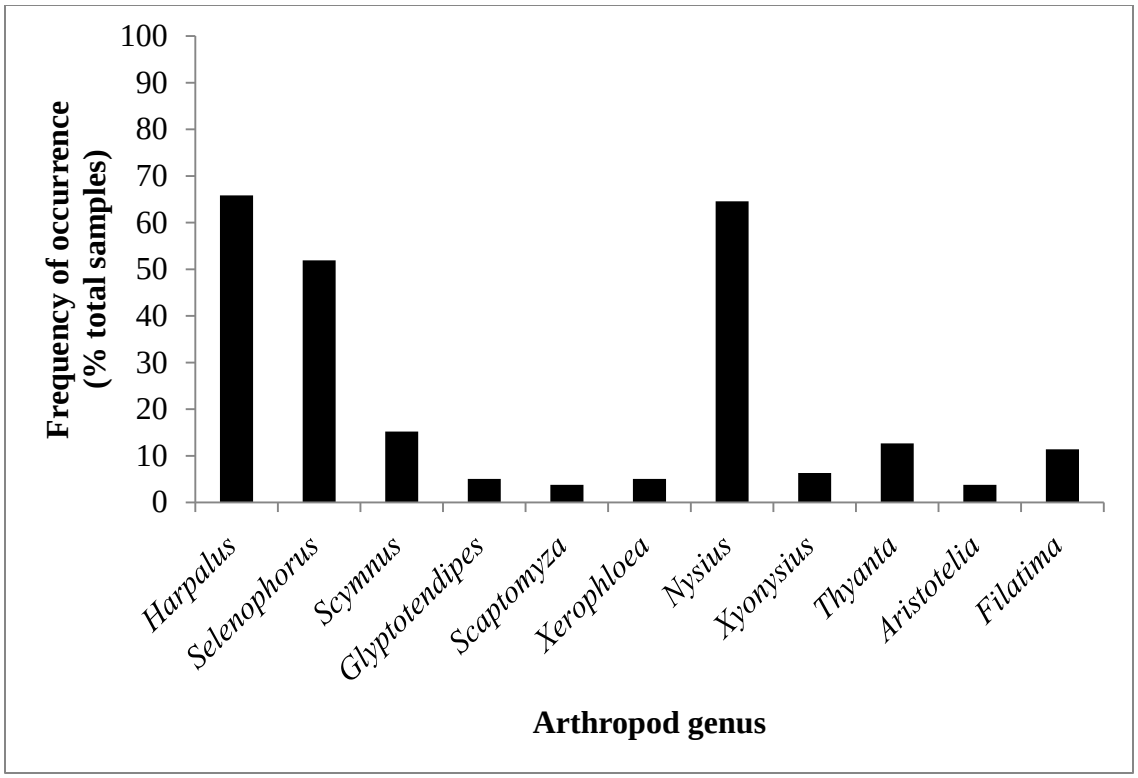

Fig. 5. — Occurrence of arthropod genera, identified by the BLAST methodology, that were found in the fecal pellets of three or more *Parastrellus hesperus* captured in Big Bend National Park, Texas in summer 2015.

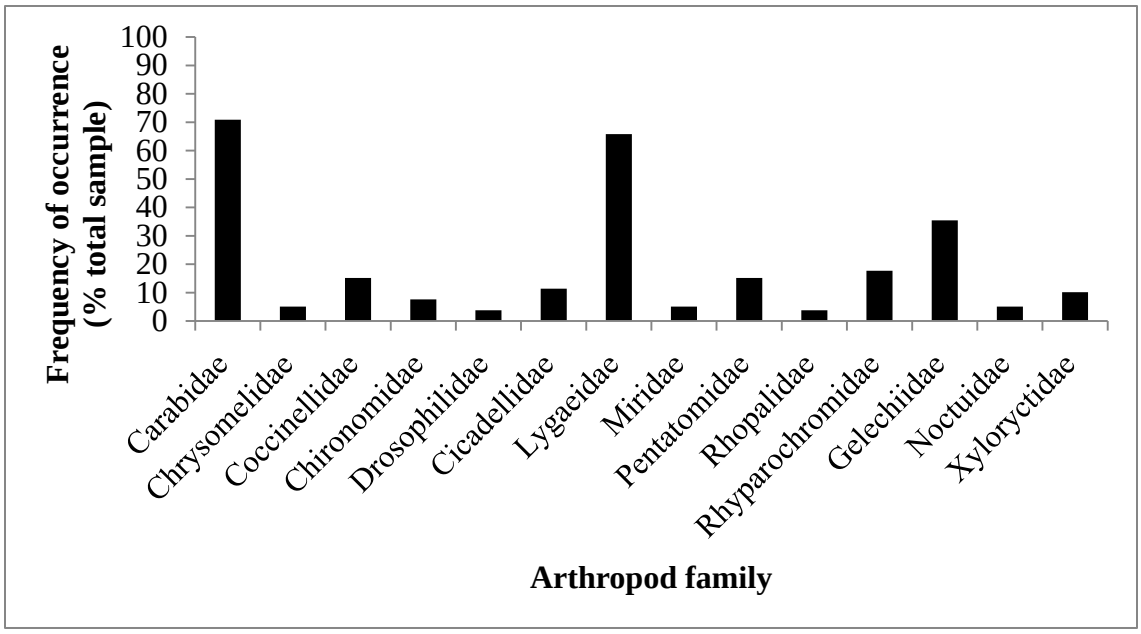

Fig. 6.— Occurrence of arthropod families, identified by the BLAST methodology, in fecal pellets of three or more *Parastrellus hesperus* captured in Big Bend National Park, Texas in summer 2015.

DISCUSSION

In this study I performed the first molecular analysis of the diet of *P. hesperus* in a desert of the southwest and was able to test two predictions regarding potential dietary shifts across reproductive condition, sex and age class, while extending the list of known arthropod prey items for the species. I hypothesized that the diet items of female *P. hesperus* would vary significantly with reproductive status (pregnant, lactating, post-lactating, and non-reproductive) and that prey consumption of newly volant juvenile *P. hesperus* would vary significantly from that of adults feeding in the same general area during the same time of year. I was able to document significant dietary differences between the sexes of adult *P. hesperus*. However, this sex based difference could be a consequence of the temporal availability of prey items, as a significant difference in the diet across months was documented. The prey composition in the diet of juveniles and adults was not significantly different, nor was the diet between reproductive conditions of adult females. Further, I hypothesized that a molecular approach to diet analysis would expand the known prey items of this species. I successfully applied molecular-based criteria and extended the taxonomic identification of prey items to the species level while also documenting new arthropod genera, families, and orders.

Capture rates.— Capture rates of adult *P. hesperus* exhibited a temporal fluctuation between the sexes. The male-biased capture rates (86.7%) in May could indicate a large presence of bachelors within the area during the early summer season. Similarly, the predominant shift to females in June could represent a temporal increase in the area as females could be roosting near water sources in an attempt to balance daily water intake with

the reproductive demands of lactation (Kunz et al. 1995). If males remain in the area, low capture rates could be explained by intersexual differences in foraging and ecological segregation between the sexes. In other species, males and females differ not only in their utilized foraging area but also in the size of the area. Lactating females have exhibited shorter foraging distances (Swift 1980; Racey and Swift 1985; Wilkinson and Barclay 1997) and seem to use foraging areas that are familiar, reliable and close to young (Wilkinson and Barclay 1997). Adult male *Eptesicus fuscus* in contrast, forage over relatively larger outlying areas (Wilkinson and Barclay 1997). This ecological segregation may reduce intersexual competition, provide females with the optimal opportunity to provide for the young, and explain the female-biased capture rates in June.

Intraspecific competition could also play a role in ecological segregation once young are weaned and begin to forage on their own. Adult *M. lucifugus* have displayed shifts in foraging patterns during July, when juveniles have become volant (Adams 1997). Dispersal of adults away from prominent roost sites, foraging sites and resources could potentially reduce intraspecific competition among the age classes and reduce the distance that juveniles have to travel to find adequate resources (Kunz 1974; Adams 1996; Adam 1997). This potential partitioning of space may explain why such a high capture rate of juveniles (65.8%) was documented in July.

Variation in diet by sex and age.— The prey items consumed by American parastrelles varied across the surveyed months. Seasonal variations in the diet of *P. hesperus* have previously been documented by Ross (1967), and were attributed to the seasonal availability of prominent prey items throughout the year (Hayward and Cross 1979). However, variations in the diet occurring specifically within reproductive months were not

isolated for analysis of potential intersexual or reproductively driven dietary implications. Within this study a significant temporal difference in the diet of parastrelles was documented. However, due to low sample sizes and biased sex-ratios in May and June, it is unclear whether the significance is driven by a true temporal difference.

In this study, a significant intersexual difference in diet was observed in adult parastrelles. This sex-based dietary distinction was present across May and July when the months were combined, but did not display a significant difference when the months were analyzed separately. This statistical difference could be attributed to the relatively low number of fecal samples analyzed for adults in May (3 males and 3 females) and July (14 males and 7 females). The possible occurrence of sex-based monthly differences could be more clearly understood in the future by including more males from June in the study. Currently, with the exclusion of male samples from the month of June and relatively small sample sizes for May and July, I cannot determine if the dietary difference is driven by a sex-based factor or if it is a result of seasonal variability in insects.

Dietary differences between the sexes have been well documented across the reproductive season of other species of bats (Belwood and Fenton 1976; Wilkinson and Barclay 1997; Clare et al. 2011). These differences have been attributed to elevated energy demands placed on females during pregnancy and lactation influencing foraging behavior and diet (Barclay 1989; Adams 1997; Wilkinson and Barclay 1997). Analysis of parastrelle fecal samples by female reproductive condition suggests that the diet of adult females did not significantly shift across the reproductive season. Low sample sizes from pregnant and post-lactating females may have reduced the ability to detect any dietary differences that exist at the population level. If larger sample sizes from both pregnant and post-lactating individuals

are collected in the future, a dietary difference across reproductive condition could be documented.

Within other species, the maximum daily consumption by pregnant females was less than that of lactating females (Kunz 1974) and females with older young have been observed foraging longer and spending less time with the young as they became independent (Barclay 1989). In addition to longer foraging trips, lactating and postlactating *Myotis* females have been documented exhibiting more selectivity, and increasing their food consumption when feeding from late June into July after the birth of young (Kunz 1974; Anthony and Kunz 1977). These possibilities might help to decipher dietary distinctions between the sexes of parastrelles and should be revisited if future sampling efforts can obtain samples that are evenly distributed between males and females instead of the extreme sex bias observed in this study.

Although dietary differences between adult and newly volant bats have been documented in several species of Vespertilionidae (Rolseth et al. 1994; Adams 1996; Hamilton and Barclay 1998), no differences in prey item consumption were documented for adult and juvenile parastrelles. It has been suggested that morphological characteristics such as aspect ratio and wing-loading (mass support per unit of wing) are important determinants of flight maneuverability and therefore are predictors of potential foraging ecology (Norberg 1995; Adams 1996). Based on the average forearm length and weight at the time of capture, the *P. hesperus* juveniles I captured between 16-18 July were already experiencing wing-loading similar to that of adults. Existing literature suggests these juveniles could have been volant up to 3 weeks at the time of capture (Ammerman et al. 2012), an age at which juveniles of other species have exhibited flight patterns similar to adults (Kunz 1974; Racey

and Swift 1985). Therefore, at this age it is unlikely that adults are out-maneuvering and out-competing their offspring for preferred prey items and that any age-class dietary differences would not be observed. I suggest that future efforts should concentrate on acquiring fecal samples from newly volant juveniles beginning the last week in June and continuing through the second week in July to more adequately test potential ontogenetic dietary shifts occurring from a purely milk diet to an arthropod diet. However, the lack of tall, cluttered vegetation in this habitat may nullify any limitations due to maneuverability that have shaped the foraging behavior of juveniles of other species (Adams 1997).

Documented prey items for Parastrellus hesperus.— Using two molecular-based identification methods, I was able to go beyond the limitations of previous morphological assessments and establish species-level taxonomic assignments of prey. The identification of DNA sequences using this methodology required no prior knowledge of potential prey items and the ability to compare sequences to an existing reference database. DNA sequences from insect prey regularly survive digestion (Clare et al. 2009) and eliminates the need to analyze prey items solely by the surviving taxonomically distinguishable prey parts like previous morphological methods.

For optimal success, this molecular methodology requires comprehensive sequence data from many species to serve as a reference. However, for many geographic regions where large quantities of invertebrate species have not been sequenced and deposited in GenBank, a comprehensive database is currently an unrealistic expectation. In this study, I discovered that seventy-five MOTUs were assigned to 19 genera that have been documented in Big Bend National Park, but to a species that has not. Across these genera a total of 87 species have been documented in the park, but only 22 species have reference sequences in Genbank.

Eight of the 19 genera lacked COI gene sequence references for any of the documented species at the time of this study. Therefore, centroid sequences assigned to MOTUs not identified to species, identified to a species at < 97% similarity, or identified to a species not documented from the southwestern United States are presumably from arthropods that were consumed but are currently underrepresented or lack references in the DNA databases as of the date of this study.

Understanding the limitation of current references libraries, I suggest that the species-level data generated using the BLAST method is the most likely to be correct, yet serves as an incomplete view of the actual prey items consumed by *P. hesperus.* Acceptance of these species as the only identified prey items is likely conservative and may limit type II statistical errors. However, by including the USEARCH global alignment identifications that met the distribution criteria, I have chosen to reduce the possibility of type I statistical errors and report these species as possible prey items. This decision was further verified after determining that the taxonomic identifications under the USEARCH global alignment criteria overlapped more with previously-documented prey items than did those identified using BLAST. Therefore, through the use of molecular-based methodology, I documented the presence of 27 different species, 36 genera, 28 families, and 8 orders of prey items in the fecal pellets of *P. hesperus* and propose the potential inclusion of an additional 12 species, 37 genera, 40 families, and 1 order identified by USEARCH global alignment criteria and species distributions. The number of newly-identified prey items using only the BLAST method was as follows: 2 orders, 20 families, 35 genera, 27 species. If extending the count to include the additionally-proposed prey items from USEARCH global alignment, these newly-identified prey numbers increase to 3 orders, 52 families, 71 genera and 37 species.

All identified prey species, including the spiders that are likely young ballooning through the air on silks (Fries 1981), could have been caught by parastrelles while in flight. One notable deviation from previously reported parastrelle prey was the inclusion of large dietary items in the orders Orthoptera and Blattodea. *Gryllus*, a genus of field cricket (Orthoptera, Gryllidae, Gryllinae) includes members that range between 15-31 mm in length. *Shelfordella lateralis* (Blattodea), the Turkistan roach, can range in length from 22 to 28 mm. The handling of large prey items such as these could be cumbersome and would most likely require parastrelles to return to a feeding roost. To my knowledge, this feeding behavior has not been observed for this species. It is important to note that both of these prey items were documented in the diet of all 79 parastrelles only once.

The rarefaction curve for operational taxonomic units at the species level illustrated the species richness found in the diet of *P. hesperus*. The large number of prey items that were identified only once could illuminate an incomplete sampling effort. Additionally, our results could support previous claims that of all the bats in the southwest, parastrelles are best adapted to feed wholly on opportunistic prey (Ross 1967) and are a prime example of a dietary generalist. I would suggest that future research efforts involve collecting fecal samples from a larger number of *P. hesperus* and across a seasonally diverse timeframe. Until the time that an asymptote is reached, the high diversity of prey items consumed across individuals of this generalist insectivore may reduce the ability to quantitatively document any dietary shifts or patterns as the dietary extent of each group has not been adequately documented.

Several identified species, including the arachnids, are known predatory insects. The possibility of secondary predation represents a potential source of error for molecular-based

diet analyses. It is possible, although probably rare, that some of the COI sequences that were generated in this analysis were from prey items of these predatory insects – although this problem has not been addressed in the literature. To avoid potential issues with secondary predation and the possibility of sample contamination, I would advise eliminating rare prey items occurring in only one sample because problematic sequences should be rare in the population. This step should be taken only after securing a large enough sample size to reach an asymptote for dietary richness. To my knowledge there currently are no data to address this issue and future investigations should explore methods for distinguishing primary prey from secondary prey.

Using a qualitative scale of hardness for invertebrate prey items (Freeman 1981), I determined that 9 of the 20 (45%) newly identified families reported by BLAST methods were arthropods that ranked either a 1 (softest) or a 2 on a 5 (hardest) point scale. Of the 52 new families reported under the USEARCH method, 32 (62%) represent arthropods that rank either a 1 (softest) or a 2. Based on this percentage of soft, newly-identified taxa, the use of DNA-based molecular analysis appeared to limit identification bias between preserved hard-bodied species and easily degraded soft-bodied species (Whitaker et al. 2009), thus providing a more complete dietary analysis. Among previously documented families of prey, arthropods ranking either a 1 or a 2 on the hardness scale (prey with softest bodies) constituted only 38% of the prey items.

Molecular analysis cannot estimate the abundance or volume of a prey item within a sample but documents both rare and common prey items as 'present'. Therefore, in an attempt to infer commonality, the frequency across samples was determined for all prey items (Clare et al. 2014). To limit potential bias in the commonality of consumed prey items,

the frequency of occurrence for families and orders supported by at least one species with 97% or greater similarity was determined using all subsequent taxonomic assignments for genera and species regardless of their sequence similarity or distribution. Although assigning a sequence to a higher taxonomic level is error prone (Clare et al. 2011) , removing potential prey items at lower taxonomic levels that are underrepresented or lack reference sequences in the database will result in a biased underrepresentation of the consumed family or order. Without taking this approach, the most commonly consumed prey item (66% of the samples), an unidentified *Harpalus*, would not have been represented for the family Carabidae or the order Coleoptera. Similarly, the number of *Selenophorus* reported across the diet of 79 *P. hesperus* would have been reduced from the reported 53% to 1%. When these effects are considered together, the frequency of Carabidae in the diet of parastrelles is drastically underestimated. Although the approach used in this study has the possibility of overinflating the frequency of any given taxon, the degree of inflation should be much less than the degree to which frequency would be underestimated using the alternative approach.

Compared to the morphological analysis of Ross (1967), that reported the three primary food sources of *P. hesperus* as microlepidoptera, leafhoppers and flying ants, my efforts have identified the False Chinch Bug (*Nysius raphanus*), and two ground beetles (*Harpalus* and *Selenophorous*) as the primary food sources for *P. hesperus*. This distinctive standing continues until the taxonomic level of order, when the pooling of Diptera and Lepidoptera families reveal them as proportionally similar dietary components.

The data from this study indicated that dietary differences in adult *P. hesperus* were present between the sexes, but that this difference could be affected by the temporal availability of prey items. A difference in prey consumption across age-classes or

reproductive conditions was not documented for this species Additional collection efforts are needed in order to more adequately test hypotheses on how sex and age affect diet in parastrelles. However, the use of DNA-based molecular methodology was a successful approach to documenting numerous previously-unreported prey items of an opportunistic dietary generalist in a desert of the southwestern United States.

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