Estimating Diet And Food Selectivity Of The Lower Keys Marsh Rabbit Using Stable Isotope Analysis

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ESTIMATING DIET AND FOOD SELECTIVITY OF THE LOWER KEYS MARSH RABBIT USING STABLE ISOTOPE ANALYSIS

by

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ABSTRACT

Understanding the effect of food abundance on feeding behavior can benefit conservation efforts in many ways, such as to determine whether impacted environments need food supplementation, whether different locations of threatened species contain different food abundances, or whether reintroduction sites are missing key components of a species' diet. I studied the relationship between feeding behavior and food abundance in the Lower Keys marsh rabbit (*Sylvilagus palustris hefneri*), an endangered subspecies endemic to the lower Florida Keys. Specifically, my study set out to measure the relative abundance of the primary plants within the natural habitat of the Lower Keys marsh rabbit and estimate the proportion of each of these plants within the rabbit's diet. With this information, I tested the following hypotheses: first, the Lower Keys marsh rabbit selectively feeds on specific plants; second, that diet does not differ among sites; and third, that diet is not affected by food abundance. Using stable isotope analysis, I determined that two plants were prominent in the rabbit's diet: a shrub, *Borrichia frutescens*, and a grass, *Spartina spartinae*. These two species were prominent in the rabbit's diet in most patches, even where they were relatively rare, suggesting the rabbits are indeed selectively feeding on these species. In addition, although diet did differ among patches, selective feeding was apparent in all cases. Overall, this study determined that certain food types are important food sources for the federally endangered Lower Keys marsh rabbit and that these rabbits do not feed on plants based on plant abundance. This knowledge can be directly applied to reintroduction and restoration efforts for the Lower Keys marsh rabbit. More generally, the methods used in this study can be applied to other species of concern in order to address questions associated with diet requirements and foraging behavior.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ACRONYMS/ABBREVIATIONS

AG	*Andropogon glomeratus*
AV	*Andropogon virginicus*
BCK	Boca Chica Key
BF	*Borrichia frutescnes*
BM	*Batis maritinus*
CE	*Conocarpus erecta*
ERK	East Rockland Keys
FC	*Fimbristylis castanea*
GGK	Geiger Key
GPS	Global Positioning System
LKMR	Lower Keys marsh rabbit
LPK	Little Pine Key
LR	*Languncularia racemosa*
ML	*Monanthochloe littorais*
SLK	Sugarloaf Key
SS	*Spartina spartinae*
SV	*Sporobolus virginicus*
USFWS	United States Fish and Wildlife Service

CHAPTER 1: INTRODUCTION

A fundamental goal of ecology is to understand how food types and abundance influence foraging behavior of an animal in a given ecosystem. Optimal foraging theory, for example, splits foraging behavior into a set of decisions dealing with food choice and abundance that optimizes the energy or nutrient intake of an individual (Stephens and Krebs 1987). In simple models, the optimal diet of an individual will depend on the average value of each food type, the handling times for each food, and encounter rate of different food types (Pyke 1984). The energetic value of available foods affects the net rate of energy intake, while handling times and encounter rates act as constraints on the rate of intake (Krebs and Davies 1993). Food abundance and distribution are major factors that can affect an animal's optimal diet, altering the encounter rate with different food types. For example, Parsons et al. (1994) observed foraging behavior in sheep when two available foods, grass and clover, were present at varying abundances. They found that sheep fed on a mixture of the two plants, even though a strict clover diet would have increased their energy intake, because an effort to search for clover would have been more expensive than eating more abundant, yet less nutritious grass (Parsons et al. 1994). Because food abundance can vary over time and space, an animal's diet can undergo change between seasons (Popa-Lisseaunu et al. 2007) or sites where food abundances are different (Kohler 1984).

Understanding optimal foraging is increasingly important for species of conservation concern, especially in ecosystems that are fragmented or naturally limited in size (e.g. islands). Michel et al. (2009) found that the movement and habitat selection in two reintroduced bird

populations were affected by the abundances of preferred food sources. Differences in food sources between habitat types can also affect foraging success, as shown in flying squirrels, whose dispersal and feeding are limited by fragmented landscapes (Flaherty et al. 2010). Studies on habitat selection in butterflies (Schtickzelle et al. 2006) and foraging behavior in insect pollinators (Goverde et al. 2002) also show how movement and feeding behavior are altered by fragmented ecosystems. Because feeding behavior and habitat selection are partially dependent on the food sources of a habitat, knowing food preferences can help researchers choose suitable locations for conservation sites for threatened species. One example where understanding diet and its constraints have aided the management of a species is the cinerous vulture (*Aegypius monachus*), in which preferred foods were used to create supplementary feeding sites (Moreno-Opo et al. 2010).

The reintroduction of a species requires an understanding of that particular species ecological requirements, such as food sources and abundances of those foods in order to ensure persistence (Hirzel et al. 2004). Reintroductions involve establishing a species in an area of its historic range from which it has become extirpated (IUCN 1998). Reintroductions have been used as a conservation method since the early 1900s and have become increasingly common in recent years (Seddon et al. 2007). The success of this approach depends on multiple factors, including habitat preferences, home range, social behavior, and feeding preferences of the focal species (IUCN 1998). Overall habitat quality, including food type and abundance (Johnson 2007), is one of the main factors in determining whether a reintroduction will be successful or not (Griffith et al. 1989, Ewen and Armstrong 2007), but few studies have specifically looked into the affect of food abundances on reintroduction success. Because different potential

reintroduction sites may contain differing food abundances, it is vital that we understand how food abundance can affect the feeding behavior and survival of an organism before it is released into a habitat.

The Lower Keys marsh rabbit (*Sylvilagus palustris hefneri;* hereafter LKMR) is an endangered subspecies of marsh rabbit that lives in fragmented metapopulations in the lower Florida Keys (Forys 1999). In 1990, the LKMR was listed as federally endangered by the United States Fish and Wildlife Service (USFWS 1990). Since then, the population has continued to decline due mainly to habitat fragmentation (USFWS 2006), as well as predation by feral and domestic cats (Forys and Humphrey 1999). As of 2006, the population size was estimated to be only 100 to 300 individuals (USFWS 2006). Individuals of this species live in habitat patches of brackish and freshwater wetlands (Faulhaber et al. 2008). Interpatch dispersal in this species typically occurs when subadult males leave their natal patch upon reaching maturity. Each male then remains in his new patch for the rest of his lifetime (Forys and Humphrey 1996). Past reintroduction projects with this subspecies have been successful, but are not yet common due to the limited amount of land available that can be acquired for reintroduction sites (Faulhaber et al. 2006). Therefore, it is important to understand how food availability affects this species and to test if there is any selectivity towards specific habitats so that the best available land can be chosen for reintroductions.

Previous studies have investigated the composition of fecal pellets in this species and found that the LKMR is a generalist feeder, with approximately twenty plant species found as part of its diet. However, more than 70% of the LKMR's diet consisted of four plant species: two grasses (*Sporobolus virginicus* and *Spartina spartinae*), a succulent shrub (*Borrichia frutescens*),

and a mangrove (*Laguncularia racemosa*) (Forys 1999). In the same study, Forys (1999) measured the availability of food items and found that ground cover of the food items correlated with their density in fecal pellets. Forys (1999) suggested that the LKMR may selectively choose habitats where these food items are abundant because LKMR diets did not vary between sites. However, the study was limited to six sites, five of which were on a single island, and food abundances did not significantly differ among these sites (Forys 1999). Thus, whether changes in food abundance influence diet remains untested. In addition, fecal analysis can be prone to human error (Westoby et al. 1976) and since different plants may have different digestive rates (Wallage-Drees et al. 1986), the proportion of a plant species in the fecal pellets may not represent its importance in the diet.

One method that can provide a more accurate investigation into the feeding behavior of the LKMR is stable isotope analysis. Stable isotope analysis can be used to estimate the relative proportion of isotopically different foods in an animal's diet (Crawford et al. 2008). Due to differences in photosynthesis pathways and resource use, different plant species typically have different stable isotope ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) (Kelly 2000). The isotopic signatures of a plant are incorporated into an animal's cells when it consumes the plant, so diet (the type and relative proportion of foods eaten) will alter the isotopic signatures of the herbivore (Kelly 2000). By measuring the ratios of stable carbon and nitrogen isotopes ($\delta^{13}C$ and $\delta^{15}N$, respectively—measured in parts per thousand [‰]) of the animal and of its food sources, it is possible to estimate how much each food source contributes to the animal's diet (Moore and Semmens 2008).

I used stable isotope analysis to investigate the relationship between feeding behavior and food abundance in the Lower Keys marsh rabbit by testing for possible selective feeding (i.e. despite a variety of potential food sources, the animal mainly feeds on one or a few sources) and also to relate rabbit density with plant abundance. I measured the isotopic signatures of LKMR and its potential food sources and used these data to compare diets among sites and to test how diet changes with changing food abundance. Specifically, I sought to test the following hypotheses: first, the Lower Keys marsh rabbit selectively feeds on specific plants; second, that diet does not differ among sites; and third, that diet is not affected by food abundance. If LKMR feeds selectively, choosing preferred foods despite the food items relative abundance, estimated diet should not differ among sites and plant abundance will not influence the rabbit's diet. If LKMR does not feed selectively, I predict that estimated diet will differ among sites and food abundance will affect diet. Understanding the foraging behavior of this species and how it is affected by potential changes in food abundances is critical given LKMR's endangered status because proposed reintroduction sites may vary in their food abundance and these sites may need to contain certain food sources in order for reintroductions to be successful.

CHAPTER 2: MATERIALS AND METHODS

Study Area

I measured plant abundance and reconstructed the diet of rabbits in 10 habitat patches from five islands in the lower Florida Keys (Figure 1, Table 1): one habitat patch in each of Little Pine Key (LPK), East Rockland Key (ERK), and Sugarloaf Key (SLK), two patches in Geiger Key (GGK), and five patches in Boca Chica Key (BCK). Previous studies in conjunction with the United States Fish and Wildlife Service (USFWS) have found LKMRs in these habitat patches (USFWS 2006). A habitat patch is defined as an area of transition-zone between saltwater marsh and grassland isolated from another patch by a large body of water or a road (Forys and Humphrey 1996). In general, habitat patches on the same island were separated from each other by roads or other human development.

Figure 1: Lower Florida Keys: **(A)** Boca Chica Key; **(B)** East Rockland Key; **(C)** Geiger Key; **(D)** Sugarloaf Key; **(E)** Little Pine Key. Dots represent habitat patches.

Table 1: Features of the habitat patches studied, including patch number (designated by USFWS), location and size.

Patch Number	Island	Latitude (°)	Longitude (°)	Patch Area (ha)
2	ERK	24.586	-81.664	0.915
5	GGK	24.575	-81.662	1.084
10	GGK	24.574	-81.666	0.443
36	SLK	24.632	-81.536	10.623
99	LPK	24.720	-81.304	10.469
14	BCK	24.571	-81.674	1.383
157	BCK	24.583	-81.696	1.913
160	BCK	24.580	-81.678	2.820
161	BCK	24.584	-81.696	0.310
170	BCK	24.569	-81.709	0.887

Stable Isotope Analysis

To estimate diet from stable isotope analysis, I collected tissue samples from LKMR and the plants found in its habitat. For stable isotope analysis of the LKMR, I used hair samples, which are common in mammal stable isotope studies (Crawford et al. 2008) and are noninvasive to collect. The USFWS collected hair samples during the summer of 2008 from 88 individual rabbits in 10 habitat patches (Figure 1, Table 1) in conjunction with other research being conducted on this species. To prepare the samples for stable isotope analysis, I washed them with soap and water to remove dirt and oils, placed them in a drying oven at 90ºC for 24 hours and then chopped them finely with scissors Roth et al. (2007). Approximately 1.0 mg subsamples were weighed for measurement of $\delta^{13}C$ and $\delta^{15}N$. Stable isotope ratios were measured using an isotope ratio mass spectrometer (Finnigan MAT Delta Plus XL) at the University of Georgia Institute of Ecology Stable Isotope Laboratory, calibrated with internal standards.

For stable isotope analysis of food sources, I used samples of plants found in the habitat patches where the LKMR samples were collected. In June-July 2009, I revisited the 10 habitat patches where the LKMR hair samples had been collected the previous year. In each patch, I picked three to five locations using GPS coordinates randomly selected by ArcGIS. In smaller patches (< 1 ha), I used three or four locations, whereas in larger patches (> 1 ha), I used five locations. At each location, I randomly picked a direction and marked a 15m transect line (also used for measuring plant abundance, see below). I collected a small amount of leaf material from one individual of each plant species along the transect lines. For each patch, this left me with

one to five samples of each species encountered (one sample for species encountered along a single line, five for species encountered along all five lines).

To reduce the number of sources in my mixing model, I only used samples from plants that previous studies suggested were likely to contribute a significant proportion to the LKMRs diet, rather than using all samples I collected. Specifically, I only used samples from the four major food sources found by Forys (1999) and from species that had a frequency of 10% or greater in the patch where they were collected. In total, I analyzed isotopic signatures for a total of 10 plant species. I reduced the number of sources to 10 species because mixing models become less precise as the number of sources is increased (Phillips and Gregg 2003). I rinsed each plant sample with water in order to remove any dirt, then I freeze-dried samples for 48 hours and ground plants individually with a mortar and pestle or cut samples with scissors (Hannan et al. 2007). Approximately 3.0 mg of each sample was sent to the University of Georgia Institute of Ecology Stable Isotope Laboratory for measurement of $\delta^{13}C$ and $\delta^{15}N$ values.

Plant Abundances

In each patch, I determined relative abundance of each plant species by measuring species frequency using the line-intercept method (Bonham 1989). The line-intercept method involves laying down a transect line and noting how often each plant species falls under this line. Plants with higher frequencies will appear under the line more often than rarer plants (Bonham 1989), so this method is effective and often used for determining plant abundance (Warren et al 2008, Godinez-Alvarez et al. 2009).

To measure plant frequencies in each habitat patch where hair samples were collected, I used the same 15m transect lines used to collect plant samples. Plant cover was relatively high in habitat patches (pers. obs.), so a 15m line was sufficient to estimate frequency accurately for the patch (Bonham 1989). Starting at 0m, I walked along the line and noted which plant species occurred under the line at each half-meter mark. I identified plant species using multiple field guides and with assistance by local biologists. In cases where two species overlapped on a point (such as grass beneath a tree), I only recorded the lower species, as these species are the more likely food source given LKMR's small size (350-400 mm [Lazell 1984]). I also noted any points where there was bare ground and included these points in the analyses. To calculate plant (or bare ground) frequency in a patch, I used the following formula (Bonham 1989):

$$frequency\ of\ species\ n = \frac{counts\ of\ species\ n}{total\ counts} \times 100\%$$

Data Analyses

All statistical analyses were preformed with the statistical software program R (version 2.11.1).

Before using the stable isotope values for mixing models, I tested for variation in the isotope values of LKMR and the plant species. Because $\delta^{13}C$ and $\delta^{15}N$ values represent the rabbit's diet, any variation in the rabbit's isotopic values should indicate variation in diet. Variation in the stable isotope values among plant species indicate that the species are isotopically different from one another, which is important for the mixing models. For LKMR stable isotope values (measured from the hair samples), I used one-way ANOVAs to test for variation of the stable isotope values among habitat patches. For plant stable isotope values, I

used one-way ANOVAs to test for variation among plant species across all patches. For all ANOVAs, stable isotope ratio was the response variable and habitat patch or plant species was the predictor.

Plants staple isotope ratios may vary spatially (Guest et al. 2004), which could contribute to spatial variation in the LKMR signatures. To determine if plants differ in their isotopic signatures among patches, I used one-way ANOVAs for each plant species found in multiple patches. Due to the large number of tests, I used a sequential Bonferroni correction (Rice 1989) to determine the appropriate p-value.

With the stable isotope values from the LKMR hair samples and the plant samples, I quantified the rabbit's diet in each habitat patch using an isotopic source partitioning model, MixSIR (Moore and Semmens 2008). This model uses the isotopic values of source samples (the plant samples from a patch) to estimate how much each source contributes to a mixture (the LKMR hair samples from that same patch). Since the isotopic values of the hair depend on the rabbit's diet, this model will estimate how much each plant contributes to the diet of the Lower Keys marsh rabbit. Unlike older mixing models, MixSIR accounts for any variability in the isotope values of the plants (Moore and Semmens 2008). This model also takes into account the enrichment (or increase) of $\delta^{13}C$ and $\delta^{15}N$ values between source and mixture. The enrichment value for LKMR is not specifically known; therefore I assumed average enrichment values between plants and primary consumers—and increase of 0.5‰ for $\delta^{13}C$ and 3‰ for $\delta^{15}N$ (estimated from a variety of species, McCutchan et al. 2003).

To determine if diet differs among patches, I used the diet estimates from MixSIR, which gives a range of possible proportions for each food source, created from thousands of iterations

(each with its own estimate). For each patch, I randomly selected the estimates from 100 iterations. To test for variation among patches, I used a non-parametric multivariate ANOVA (Anderson 2001), which includes the diet proportions of all plant species in all patches in a single model.

I also used a non-parametric multivariate ANOVA to determine if patches differed in their plant composition. The analysis tests for differences among patches using the frequencies of all plant species with a single model. If the analysis indicated that patches were significantly different in plant frequencies, I used similar analyses to perform pair wise comparisons between two patches at a time. Because there were 10 patches being compared, there were 45 comparisons overall. To account for the large number of test, I again used a sequential Bonferroni correction (Rice 1989). These comparisons enabled me to estimate which patches differed in their plant composition.

To determine if diet was affected by plant abundances I used linear regressions with the average proportion of each plant species in the diet (estimated from MixSIR) as the response variable and the relative frequency in the patches as the predictor. This analysis was limited to plants that were measured in at least three habitat patches. Both diet proportion and relative frequency were measured as percentages, therefore I arcsine transformed the values before performing the regression (Gotelli and Ellison 2004). In addition, I performed a sequential Bonferonni correction to determine the appropriate p-value.

Finally, I tested for a relationship between the abundances of food sources and the estimated densities of the LKMR. The estimates of LKMR densities came from surveys done by the USFWS (Forys and Humphrey 1997, Phillip Hughes, pers. comm.). These surveys represent

samplings done from 1990-2010 and involve detection of LKMR fecal pellets. In the surveys, each habitat patch was rated from 0-3 depending on the abundance of fecal pellets found (0 for none, 1 for low abundance, 2 for medium abundance and 3 for high abundance). An average of these numbers from multiple years was used to give a rough estimate of the LKMR density in each habitat patch, with a higher number representing a higher density. To test for a relationship between these estimates and food abundances, I used linear regressions for each plant species with rabbit density as the response variable and plant abundances as the predictors, again only using plant species that were found in three or more patches.

CHAPTER 3: RESULTS

Stable Isotope Analysis

Overall, I measured the $\delta^{13}C$ and $\delta^{15}N$ values of 88 LKMR hair samples and 122 plant samples, collected across ten habitat patches (Figure 2). Plant samples consisted of ten species, six of which were found on multiple patches. $\delta^{13}C$ values for LKMR ranged from -22.41 to -16.10‰, but did not vary significantly among patches ($F_{1,55}$=0.537, p=0.47). $\delta^{15}N$ values for LKMR ranged from 3.01 to 6.12‰, but also did not vary among patches ($F_{1,55}$=0.007, p=0.93). There was large variation in $\delta^{15}N$ values in plants overall, but this is expected as terrestrial plants tend to vary highly in their $\delta^{15}N$ (Kelly 2000), particularly in coastal areas where water inputs can vary (Hannan et al. 2007). Individual plant stable isotope values likewise did not differ significantly among patches, except for *Borrichia frutescens* (Table 2). However, different plant species differed significantly in both $\delta^{13}C$ ($F_{9,111}$=221, p<0.001) and $\delta^{15}N$ ($F_{9,111}$=5.32, p<0.001).

I was able to estimate diet proportions from eight of the ten habitat patches (Appendix A), but was not able to obtain diet estimates for rabbits from GGK 10 and LPK 99 due to inconsistencies in the $\delta^{15}N$ values between plant samples and rabbit samples. Specifically, the $\delta^{15}N$ values of the plant samples were too high for MixSIR to determine how much each source contributed to the mixture, even after correcting for trophic enrichment. On average, *Borrichia frutescens* was the most common food source, making up 52% of the rabbit's diet. *Spartina spartinae* was also prominent, with an average proportion of 19%. All other plant species had an average proportion estimated to make up 10% or less of the diet. Diets differed significantly among patches ($F_{7,792}$=464, p<0.001).

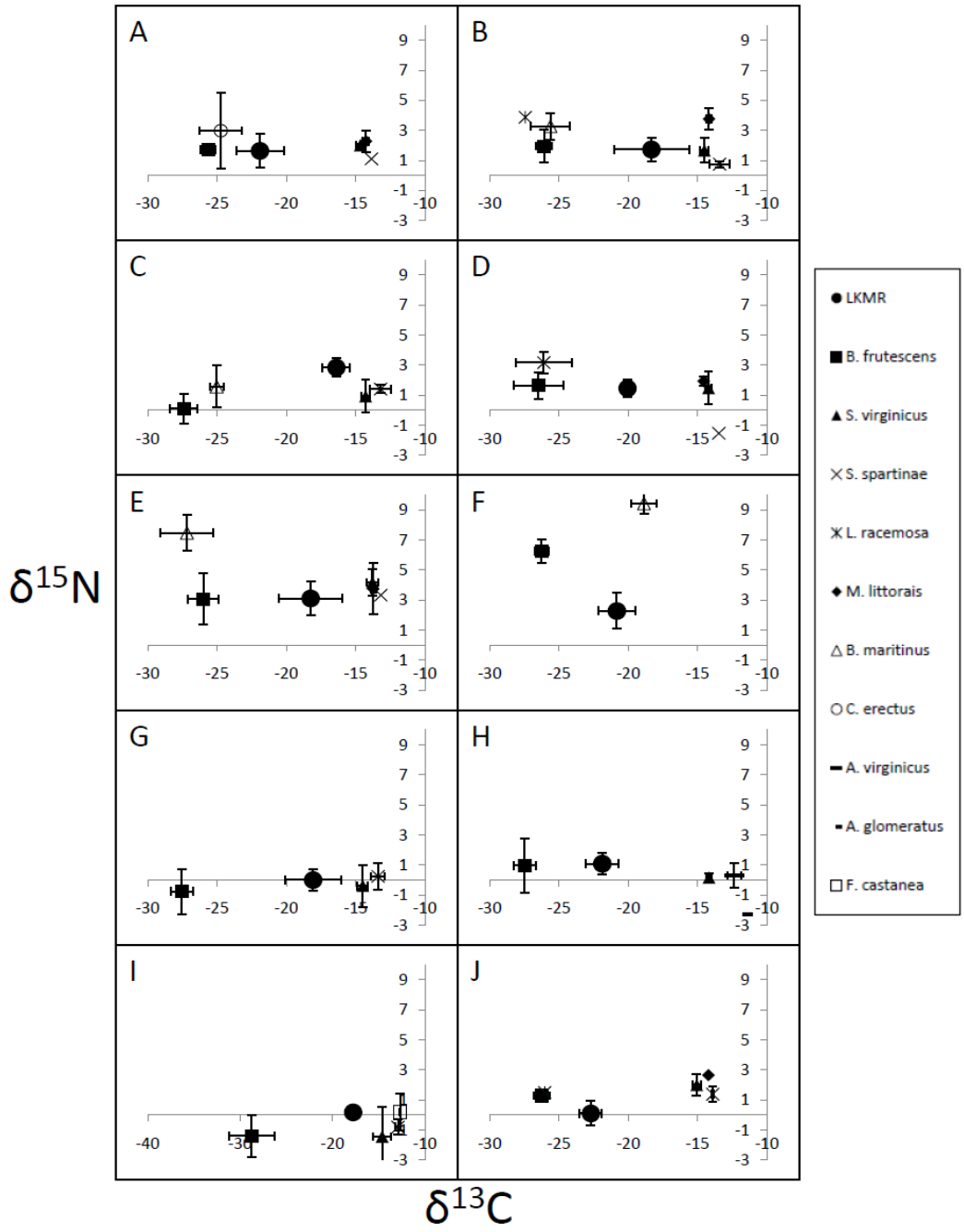


Figure 2: Plot of average $\delta^{13}C$ and $\delta^{15}N$ values for LKMR and ten plant species in each of ten habitat patches. Rabbit values have been corrected for trophic enrichment. **(A)** ERK2, **(B)** GGK5, **(C)** GGK10, **(D)** SLK36, **(E)** LPK99, **(F)** BCK14, **(G)** BCK157, **(H)** BCK160, **(I)** BCK161, **(J)** BCK170.

Table 2: Overall mean stable isotope values of ten plant species (in decreasing order of number of samples) and variation in staple isotope values among habitat patches. Bold indicates significance after a sequential Bonferroni correction (number of tests = 12).

Species	**n**	**Patches sampled**	**df**	**δ^{15}N mean ± std. dev.**	**F-value**	**p-value**	**δ^{13}C mean ± std. dev.**	**F-value**	**p-value**
Borrichia frutescens	34	10	1, 32	1.50 ± 2.42	5.26	0.028	-26.82 ± 1.35	10.53	**0.0027**
Sporobolus virginicus	31	9	1, 29	1.14 ± 1.95	0.793	0.38	-14.41 ± 0.55	4.31	0.047
Spartina spartinae	16	8	1, 14	0.60 ± 1.29	0.001	0.98	-13.40 ± 0.46	0.419	0.53
Monanthocloe littorais	14	7	1, 12	2.92 ± 1.20	1.70	0.22	-14.13 ± 0.35	0.385	0.55
Batis maritinus	13	7	1, 11	4.79 ± 3.13	1.20	0.30	-24.80 ± 3.76	4.20	0.065
Languncularia racemosa	4	3	1, 2	2.93 ± 1.10	0.165	0.72	-26.07 ± 1.42	10.16	0.086
Conocarpus erectus	3	1		2.97 ± 2.53			-24.74 ± 1.54		
Andropogon virginicus	3	1		0.31 ± 0.89			-12.37 ±0.51		
Fimbristylis castanea	2	1		0.20 ± 1.25			-12.70 ± 0.02		
Andropogon glomeratus	1	1		-2.29 ± 0			-11.75 ± 0		

Plant Abundances

I measured the relative frequency of twenty-one plant species in the habitat patches (Appendix B). *Borrichia frutescens*, *Sporobolus virginicus*, *Monanthochloe littorais*, and *Batis maritinus* were the most abundant plant species found, with other species less abundant or only found on one or two habitat patches. Overall, patches differed in their plant frequencies ($F_{9,35}$=2.55, p<0.0001). Pair wise comparisons of plant frequencies between habitat patches show differences in plant abundances in 22 of the 45 comparisons before a Bonferroni correction, but in only 6 comparisons after the correction (Table 3). However with 45 comparisons, a sequential Bonferroni correction may be too conservative (Roback and Askins 2005), so it is likely that more patches are different from one another than what is shown after the correction.

Five plant species were tested for a relationship between plant frequency and diet proportion: *B. frutescens*, *S. virginicus*, *S. spartinae*, *L. racemosa* and *M. littorais*. After a sequential Bonferonni correction, there was no significant relation between the two variables in all five plant species measured (Figure 3), suggesting that the frequency of the plants in the habitat did not significantly affect their consumption by LKMR's.

Table 3: P-values of plant abundance comparisons between patches. Bold indicates a comparison that was significantly different after a sequential Bonferroni correction (number of tests = 45). * indicates a comparison that was significantly different before the correction (α=0.05).

Patch	**ERK2**	**GGK5**	**GGK10**	**BCK14**	**SLK36**	**LPK99**	**BCK157**	**BCK160**	**BCK161**	**BCK170**
ERK2		0.079	0.058	**<0.0001***	0.17	0.065	0.024*	**<0.0001***	0.0036*	0.37
GGK5			0.21	0.0079*	0.078	0.63	0.034*	0.0086*	0.0095*	0.22
GGK10				0.0082*	0.23	0.22	0.20	**<0.0001***	0.011*	0.62
BCK14					0.084	**<0.0001***	0.25	0.10	**<0.0001***	0.0084*
SLK36						0.51	0.37	0.0086*	0.0114*	0.58
LPK99							0.045*	0.0035*	0.0082*	0.66
BCK157								0.084	0.11	0.39
BCK160									**<0.0001***	0.019*
BCK161										0.020*
BCK170										

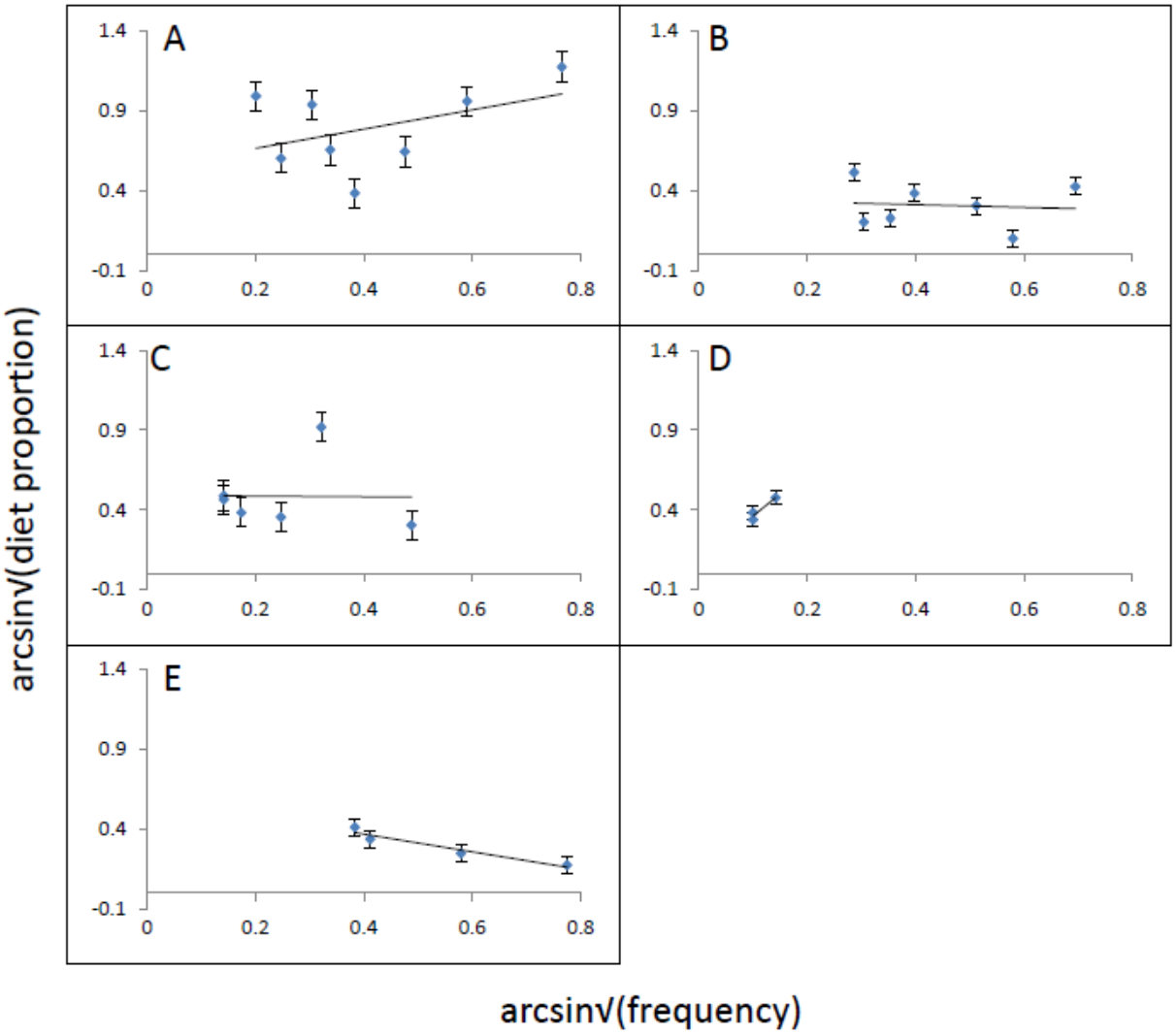


Figure 3: Linear regressions between arcsine transformed plant frequency and diet proportion (including best-fit line and standard error bars) of: **(A)** *Borrichia frutescens* (adjusted R^2=0.054, $F_{1,6}$=1.40, p=0.28); **(B)** *Sporobolus virginicus* (adjusted R^2=-0.19, $F_{1,5}$=0.044, p=0.84); **(C)** *Spartina spartinae* (adjusted R^2=-0.25, $F_{1,4}$=0.001, p=0.98); **(D)** *Languncularia racemosa* (adjusted R^2=0.79, $F_{1,4}$=8.6, p=0.21); **(E)** *Monanthocloe littorais* (adjusted R^2=0.89, $F_{1,4}$=25, p=0.037)

LKMR Densities

Most habitat patches had a medium density of LKMR (average 1.6 among ten patches) (Table 4), yet enough variation existed to test for a correlation between rabbit abundance and plant frequencies. The regressions between LKMR densities and four plant species—*B. frutescens, S. virginicus, S. spartinae* and *M. littorais*—were not significant (Figure 4), suggesting that increasing abundances of these plants do not affect LKMR. On the other hand, there was a negative relationship between LKMR densities and the abundance of *L. racemosa* (Figure 4, D).

Table 4: Densities of LKMR (on a 0-3 scale) and relative frequencies of five plant species used for a multiple regression between rabbit abundance and plant abundance.

Patch	**LKMR density**	***Borrichia frutescnes***	***Sporobolus virginicus***	***Spartina spartinae***	***Languncularia racemosa***	***Monanthocloe littorais***
ERK2	1.2	70%	5%	20%	0%	3%
GGK5	0.5	14%	24%	22%	14%	16%
GGK10	1.6	9%	21%	42%	0%	6%
SLK36	0.6	37%	17%	14%	21%	11%
LPK99	2.4	5%	25%	1%	0%	41%
BCK14	2.1	85%	0%	0%	0%	0%
BCK157	2.3	36%	1%	63%	0%	0%
BCK160	2.4	67%	4%	0%	0%	0%
BCK161	2.4	34%	14%	12%	0%	0%
BCK170	1.0	9%	24%	22%	1%	30%

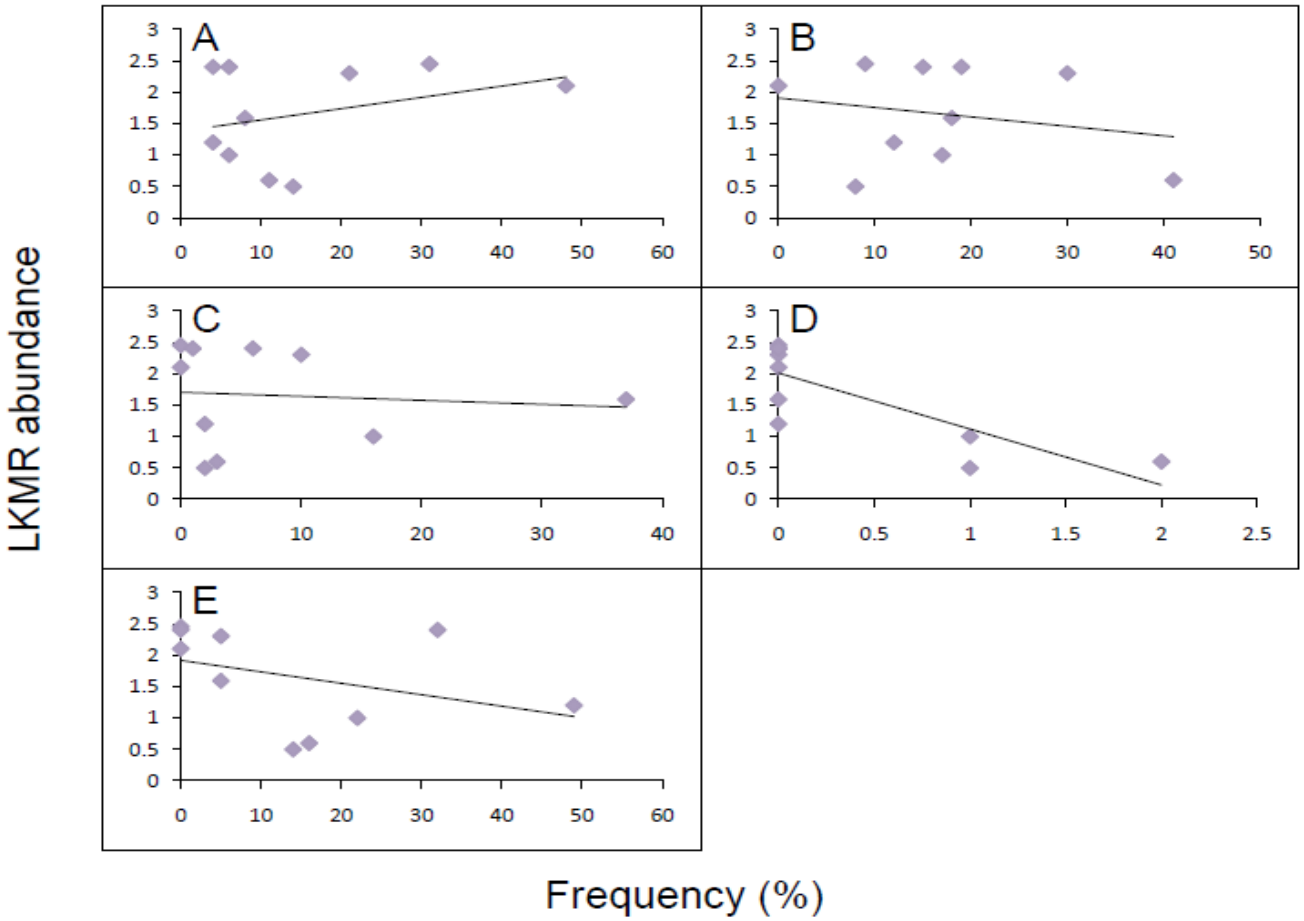


Figure 4: Linear regressions between LKMR density and frequency of five plant species: **(A)** *Borrichia frutescens* (adjusted R^2=-0.0026, $F_{1,8}$=0.97, p=0.35); **(B)** *Sporobolus virginicus* (adjusted R^2=-0.068, $F_{1,8}$=0.42, p=0.53); **(C)** *Spartina spartinae* (adjusted R^2=-0.12, $F_{1,4}$=0.071, p=0.80); **(D)** *Languncularia racemosa* (adjusted R^2=0.59, $F_{1,8}$=14, p=0.0054); **(E)** *Monanthocloe littorais* (adjusted R^2=0.036, $F_{1,4}$=1.32, p=0.28)

CHAPTER 4: DISCUSSION

Selective and Optimal Feeding

The Lower Keys marsh rabbit has been thought to be a generalist feeder, with a large variety of possible food sources. However, LKMR should likely be considered a specialist feeder; despite being able to feed on many plant species (Forys 1999), I have shown that LKMR selectively feeds on only a few plant species, specifically *Borrichia frutescens* and *Spartina spartinae*, even when these two species were relatively rare in the habitat. Other potential food sources, such as *Sporobolus virginicus* and *Batis maritinus* were less prominent in the rabbit's diet, even in areas where the plants were abundant. If the rabbits were not feeding selectively, differences in plant abundances should have significantly affected the proportions of those plants in the rabbit's diet, but this was not the case.

In herbivores, optimal diet can be important as plants can have relatively low nutritional content (Belovsky 1984). For this reason, herbivores' diets tend to optimize energy and nutritional intake over digestive capacity or feeding time (Belovksy 1986). LKMR's selective feeding of *B. frutescens* and *S. spartinae* provided strong evidence for optimal foraging theory, as these plants can optimize the intake of important nutrients. Previous studies have identified nutrients that are important to rabbits, including proteins, phosphorus, and nitrogen (Lindlof et al. 1974, Somers et al. 2008). *S. spartinae* is known to have high protein production (Garza et al. 1994) and *B. frutescens* is high in nitrogen content (Moon and Stiling 2000), which would make these plants optimal to LKMR in terms of nutritional content. A higher nutritional content would explain why the rabbit's diet consists mostly of these two species, even if their encounter rate

(abundance) is lower than other potential food sources. These results are consistent with studies on other rabbit species, which have shown selective feeding on plants that optimize energy intake or nutritional value. Miller (1968) found that hares and rabbits selectively fed on heather (*Calluna vulagirs*), which has high nitrogen content, while Seccombe-Hett and Turkington (2008) found that snowshoe hares (*Lepus americanus*) selected foods that were high in protein and energy content.

Optimal foraging can also affect habitat patch use and selection. An individual will select an optimal patch that maximizes foraging benefits (such as energy and nutrition gain) while minimizing costs (such as predation risk) (Meyer and Valone 1999). In small mammals, such as rabbits, habitat patches are selected for plants that provide energy and nutrition benefits (Somers et al. 2008) and plants that provide cover from predators (Marin et al. 2003). Here, LKMR provides evidence of this theory as the rabbit's selection of *S. spartinae* is due to the plant's use as cover (Faulhaber et al. 2008) as well as its use as a food source. To maximize energy and nutrient intake and minimize predation, LKMR is likely selecting patches that contain *S. spartinae* as well as *B. frutescens*.

Even though *B. frutescens* and *S. spartinae* are important food sources, their densities did not affect LKMR densities. These results may be due to other factors in the habitat patches that are affecting LKMR densities. The five patches on Boca Chica Key, for example, are located on an U.S. Air Force base, where predators such as feral cats and raccoons have mostly been removed. The presence or absence of predators is an important factor in regulating rabbit abundances (Trout et al. 2000). Second, some patches are less affected by human related factors, such as habitat fragmentation and vehicular mortalities. Little Pine Key, which also contains a

population with relatively high density, is undeveloped and located away from developed islands. The other sites are located near residential areas, where anthropomorphic factors would be more significant. The negative relationship between LKMR densities and *L. racemosa* may be due to the habitat the plant grows in rather than its abundance in that habitat. Further research will be necessary to determine what effect *L. racemosa* or its habitat has on LKMR.

Though stable isotope analysis was effective at estimating diet in most patches, the diets in GGK10 and LPK99 could not be determined. There are a few possible causes for the lack of fit between sampled plant and rabbit isotopic signatures. First, it is possible that LKMR is feeding on unsampled food sources that were isotopically different from the ones measured in these patches, so the LKMR isotope values would be different from those of the plants measured. Second, it is also possible there could have been changes in $\delta^{15}N$ values over time. The plant samples were not collected until a year after the LKMR samples, so changes in the stable isotope values in these sites over the year could explain the differences in rabbit and plant values. Given that there are a number of plant species that did not get measured for stable isotopes, discrepancies in the stable isotope values are more likely due to additional food sources. Despite difficulties with two of the patches, however, stable isotope analysis was still an effective method for noninvasively estimating diet

Another potential problem with diet estimates is that enrichment values assumed here (0.5‰ for $\delta^{13}C$ and 3‰ for $\delta^{15}N$) may not be the actual values, given that isotope enrichment can vary among species and even tissue types (DeNiro and Epstein 1981). The enrichment values for rabbits are unknown, though studies with mice show similar values as the ones used here (DeNiro and Epstein 1981, Minigawa and Wada 1984), so it is likely that LKMR has similar

values. In addition, leporids such as the marsh rabbit commonly perform coprophagy, or the consumption of feces, specifically its own (Hirakawa 2001), which leads to a host of bacteria and other parasites in the rabbit's gut (Neilson et al. 2005). The reingestion of their own feces and the host-parasite relationship may lead to another trophic level (Neilson et al. 2005) that could result in higher $\delta^{15}N$ enrichment values than assumed. However, Boag et al. (1998) suggest that coprophagy in rabbits has little effect on the metabolism of nitrogen, so it is unlikely that $\delta^{15}N$ enrichment values are affected.

Conservation implications

This study has immediate value to the conservation of the Lower Keys marsh rabbit. Here I have shown that LKMR feeds mainly on *B. frutescens* and *S. spartinae* and that increasing abundances of these plants correlate with increasing rabbit abundance. Future reintroduction sites should contain *B. frutescens* and *S. spartinae*. In addition, any efforts to restore or enhance current LKMR habitats should consider increasing the abundance of these plant species. To a lesser extent, *S. virginicus* and *L. racemosa* should also be considered important because these plants may bolster LKMR abundance. However, reintroductions should be only part of the conservation strategy for LKMR. Because habitat fragmentation is a major threat to LKMR (USFWS 2006) and rabbit species in general (e.g. Virgos et al. 2003), the conservation or restoration of areas between existing populations should be a priority. Predator removal will also be important, as predation can greatly impact rabbit populations (Trout et al. 2000).

This study and the general effort to conserve the Lower Keys marsh rabbit can also have conservation implications for other rabbit species of concern. For example, the Amami rabbit (*Pentalagus furnessi*), an endangered species endemic to southern Japan, exists in similar

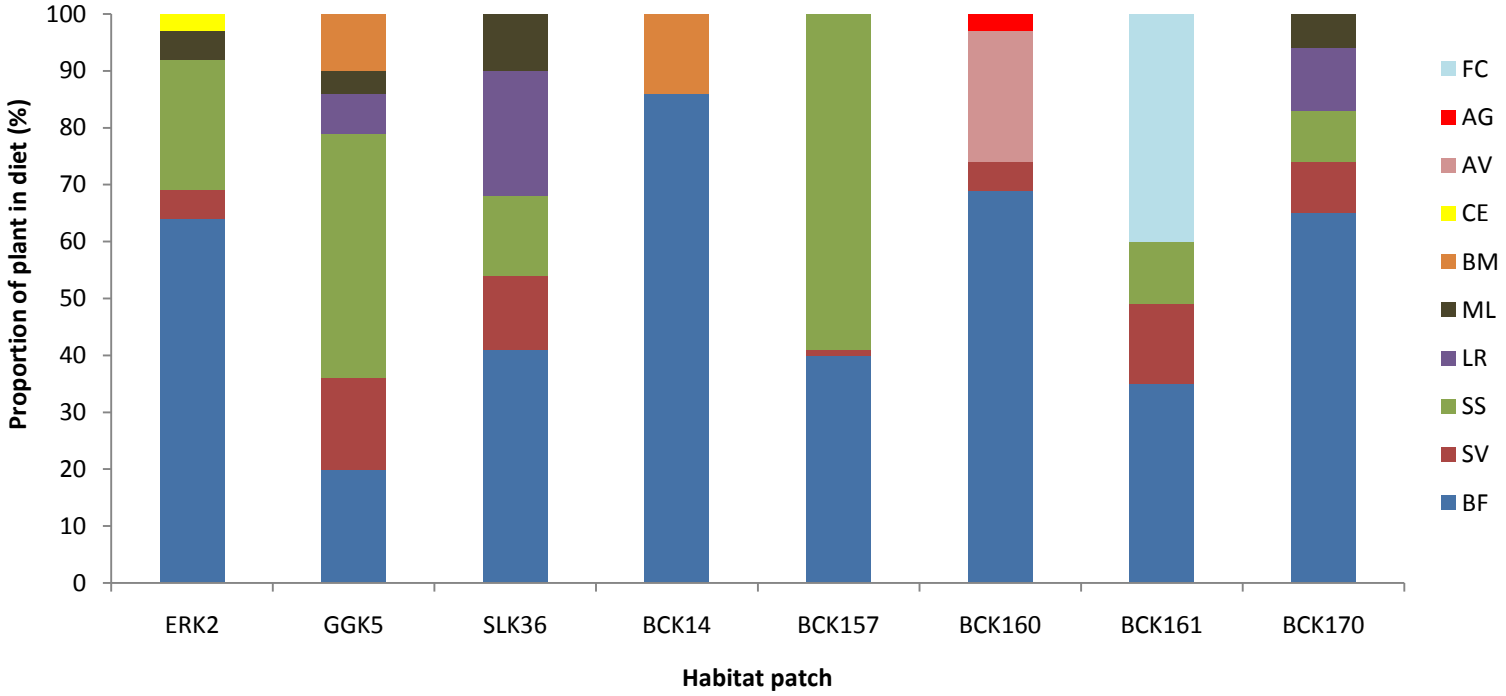
conditions to LKMR. Like LKMR, the Amami rabbit is only found on subtropical islands and has declined due to habitat loss and the introduction of non-native predators (Yamada 2008). The methods provided here could be useful for estimating the optimal diet of the Amami rabbit, which would help in choosing optimal habitat to conserve. Other threatened rabbits, such as the riverine rabbit (*Bunolagus monticularis*), the Tehuantepec jackrabbit (*Lepus flavigularis*), and the volcano rabbit (*Romerolagus diazi*), are in decline mainly due to fragmented habitats (Smith 2008). Knowledge of these species' optimal food sources (particularly those high in protein or nitrogen content) could be used for conserving or restoring habitats between the fragmented populations. Conservation studies of these species can also highlight factors that would be important for the conservations of LKMR. For example, Velazquez and Heil's (1996) study shows how habitat suitability is a major factor to the reintroduction of the volcano rabbit.

Outside of LKMR and other rabbit species, species thought to be generalist feeders may show similar patterns of selective feeding. Despite having many potential food sources, LKMR showed an optimal diet consisting of only a few plant species, and is likely more of a specialist than previously thought. It has been proposed that almost all herbivorous mammals are generalist feeders (Freeland and Jansen 1974); however this classification may be incorrect for many species. To determine if a species is a specialist feeder, stable isotope analysis can be used effectively to test a hypothesis of selective feeding. Knowledge of feeding behavior and diet is important for the conservation efforts of many animal species. If a species is a specialist, it will be very important that reintroduction and restoration sites contain specific food sources for that species.

CHAPTER 5: CONCLUSIONS

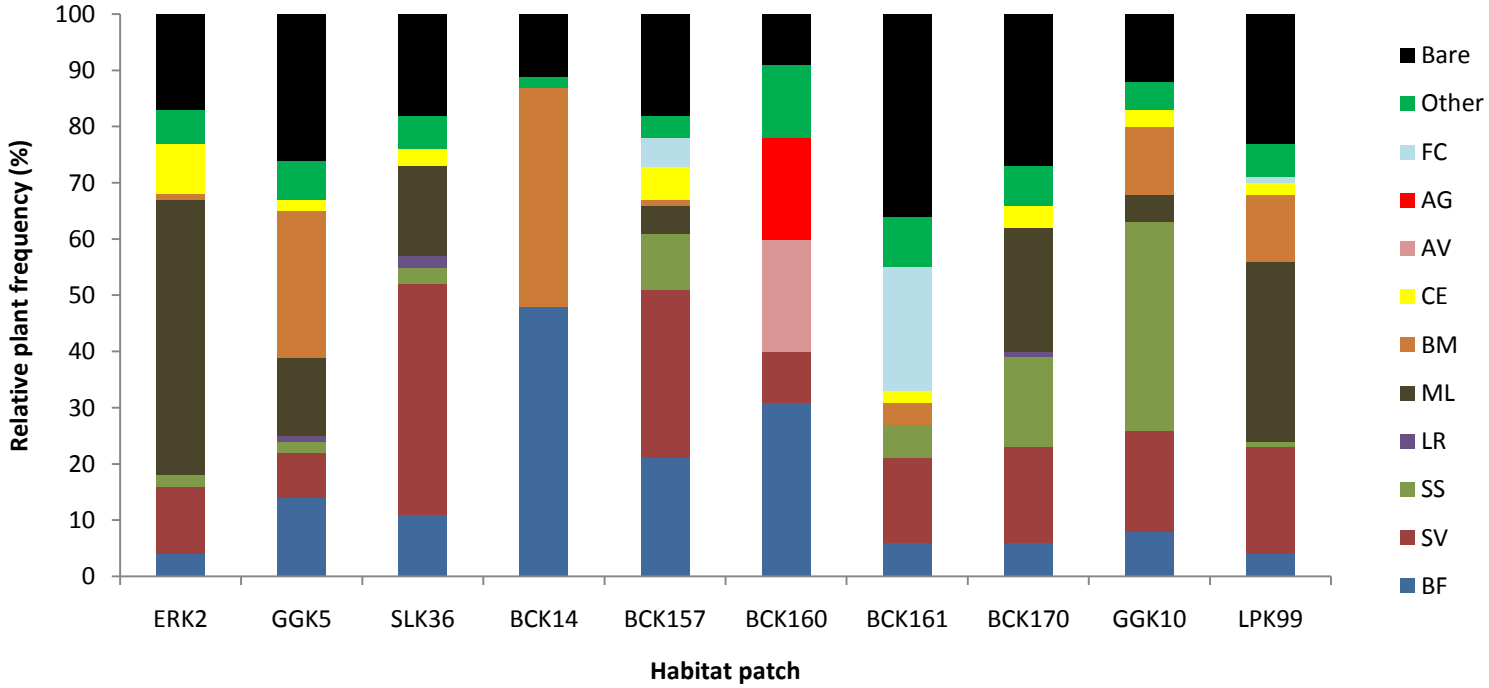
This study identified an example of optimal foraging and selective feeding, where an optimal diet consists of food sources that maximize nutritional intake (Pyke 1984, Stephen and Krebs 1987). Here, I showed an herbivore that, when presented with a variety of possible food sources, selectively feeds on only a few sources. Further studies will have to be done on a species that exists in a wider range of habitats so that we can test how large changes in habitat and food abundances affect diet; however, at a small scale, changes in food abundances do not affect diet due to this food selectivity. This knowledge of optimal diet and food selectivity has applications in conservation, as different areas will contain different food sources in different abundances, and this variation in turn can affect the feeding behavior of an animal. Finally, the methods provided here can form a template for estimating the diet of herbivores that have a large variety of potential food sources, but may be feeding selectively.

APPENDIX A: DIET ESTIMATES



Appendix A: Estimated proportions of ten plant species in the diets of LKMR among seven habitat patches and average diet proportions among all habitat patches. BF = *Borrichia frutescens*, SV = *Sporobolus virginicus*, SS = *Spartina spartinae*, LR = *Languncularia racemosa*, ML = *Monanthocloe littorais*, BM = *Batis maritinus*, CE = *Conocarpus erectus*, AV =*Andropogon virginicus*, AG = *Andropogon glomeratus*, FC = *Fimbristylis castanea*.

APPENDIX B: PLANT FREQUENCIES



Appendix B: Relative frequencies of plants species and bare ground among ten habitat patches. BF = *Borrichia frutescens*, SV = *Sporobolus virginicus*, SS = *Spartina spartinae*, LR = *Languncularia racemosa*, ML = *Monanthocloe littorais*, BM = *Batis maritinus*, CE = *Conocarpus erectus*, AV =*Andropogon virginicus*, AG = *Andropogon glomeratus*, FC = *Fimbristylis castanea*.

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