Correspondence concerning this manuscript should be addressed to Alexandra Seelig,
Department of Wildlife Ecology & Conservation, University of Florida, 110 Newins-Ziegler
Hall, PO Box 110430, Gainesville, FL 32611-0430.
Contact phone: 269-405-3025
Contact email: aseelig@ufl.edu

Running heading: Stable isotopes reveal seasonal trophic niche variation

Using stable isotope ratios to evaluate dietary breadth in *Oryzomys palustris sanibeli*

Alexandra L. Seelig*, Wesley W. Boone IV, and Robert A. McCleery

*Department of Wildlife Ecology and Conservation, University of Florida, Gainesville, FL 32611, USA (ALS, WWB, RAM)*

*Correspondent: aseelig@ufl.edu*

The Sanibel Island rice rat (SIRR; *Oryzomys palustris sanibeli*) is a subspecies marsh rice rat endemic to Sanibel Island, currently listed as a Species of Special Concern in the state of Florida. Rice rats inhabit Sanibel’s interior freshwater marshes, buttonwood shrublands [*Conocarpus erectus*], and exterior mangrove swamps. The population of SIRR is estimated to be well under 100 individuals and little is known about their ecological niche. We examined stable isotope ratios ($\delta^{13}$C and $\delta^{15}$N) of SIRR guard hair and samples of potential diet items to determine the
influence of seasonality (summer or winter) and habitat on trophic level and niche breadth. We
used Levene’s test for homogeneity of variance to determine if SIRR dietary niche breadth
varied seasonally. Analysis showed that dietary niche breadth varied between summer and winter
samples for $\delta^{15}$N ($p=0.018$) but not $\delta^{13}$C ($p=0.068$), showing that SIRR diet was more diverse
during summer than winter periods. We found that SIRR isotopic ratios, using separate models
for $\delta^{15}$N and $\delta^{13}$C, overlapped with the animal prey base for $\delta^{15}$N ($f$-value = 6.598, $p=0.014$) but
not $\delta^{13}$C ($f$-value = 3.682, $p=0.062$), and showed no overlap with a plant prey base ($\delta^{13}$C $f$-value
= 15.394, $p=0.0003$; $\delta^{15}$N $f$-value = 9.241, $p=0.004$). Seasonality did not explain variation in
isotopic $\delta^{15}$N ($t$-value = -0.328, $p = 0.745$) or $\delta^{13}$C ($t$-value = 1.573, $p = 0.127$). These findings
show that SIRR significantly prefers animal prey items over plant diet items when available. As
such, we can conclude a food web ranking above that of primary consumers.

Key words: Florida, diet selection, *Oryzomys palustris sanibeli*, Sanibel Island, stable isotopes
INTRODUCTION

The Sanibel Island Rice Rat (SIRR, *Oryzomys palustris sanibeli*) is a subspecies of marsh rice rat (*Oryzomys palustris*) endemic to Sanibel Island, Florida and listed by the State of Florida as a Species of Special Concern (Hipes et al. 2000). The marsh rice rat (*Oryzomys palustris*) is a semi-aquatic, medium-sized rat distributed throughout the Eastern United States, from Pennsylvania to Florida, and west to Texas (Svihla 1931; Hamilton 1946; Esher et al. 1978; Wolfe 1982). However, SIRR have evolved independently from other rice rat subspecies due to geographic isolation, increasing the threat of extinction to this population (Indorf and Gaines 2013). Though it morphologically resembles other *Oryzomys* subspecies, *O. p. sanibeli* is characterized by an amber brown pelage, an average body length of 263mm, tail length of 125mm, and 33mm hind foot (Hamilton 1955; Indorf and Gaines 2013).

While the life history of SIRR is not yet well-documented, much is known about the ecology of other *O. palustris* populations. *O. palustris* has been suggested to be largely carnivorous (Sharp 1967), while other sources have recorded a diet high in seeds and succulent plant parts (Svihla 1931; Lowery 1974; Hamilton and Whitaker 1979). Negus et al. (1961) recorded a diet ranging from 85% vegetation and seeds to 75% arthropods, with significant seasonal variation. Insects, fiddler crabs, and snails are other common food items, as well as fish, clams, and the carcasses of small birds or mammals (Lowery 1974; Hamilton and Whitaker 1979). Little is known in comparison about plant diet items, but consumption of *Spartina alterniflora*, *Spartina glabra*, *Salicornia europea*, *Tripsacum* sp., and *Elymus* sp. is presumed (Hamilton 1946).

These differences may be dependent on what foods are available. Presumably, flooded periods offer increased carnivorous food sources because aquatic macroinvertebrates would be in higher abundance, while dry periods may result in shifts towards plant food sources. This is of
particular interest on Sanibel Island which experiences distinct rainy, flooded (summer) and dry, non-flooded (winter) seasons.

Due to these conflicting reports and the lack of specific knowledge on the SIRR, we investigated the dietary breadth and diet item selection. We measured stable isotope ratios ($\delta^{13}$C and $\delta^{15}$N), which are commonly used for evaluating trophic relationships and diet base, to compare samples of SIRR guard hair samples in summer and winter with potential diet items. Specifically, we tested four predictions: 1) SIRR would feed on a wider variety of diet items in summer months compared to winter months due to greater availability of macroinvertebrates, 2) SIRR feeds primarily on animal prey, in accordance with the findings of Sharp (1967) for Oryzomys palustris, 3) SIRR found in freshwater grasslands would have isotopic signatures more closely related to grass samples than those found in mangrove swamps, and 4) SIRR would consume a greater amount of animal prey in mangrove wetlands than elsewhere due to the limited diversity of plant species present.

**Materials and Methods**

*Study area.*—Sanibel Island is a small (<4,900 ha) barrier island in southwest Florida (City of Sanibel 2013). Conservation lands account for ~50% of the island, with remaining lands used for residential development (City of Sanibel 2013). Remnant sand ridges ~1-2 m above mean sea level trapped rainwater within the island’s interior, creating freshwater wetlands with seasonally variable water depths (Boggess 1974). A series of low ridges and swales occurred within the island’s interior creating a diversity of freshwater plant communities (City of Sanibel 2013). Although historic accounts document nearly-continuous grasslands within the island’s interior (Hammond 1970), woody species have become dominant on many ridges. The freshwater
hydrology on Sanibel Island has been greatly altered by the construction of ponds and drainage
canals, elevated roads, and the filling of wetlands (Boggess 1974). Sanibel’s mangrove forests,
while largely intact, have also experienced degradation due to mosquito control de-watering
projects. Historically, rice rats have been known to occur within these freshwater wetlands
(Humphrey et al. 1986), while recent research has uncovered their use of mangrove forests on
Sanibel Island (Boone, Unpublished data).

Sanibel Island lies within a tropical climate, with summers being significantly wetter than
winters, with fall to late spring only containing 15% of annual rainfall (Kushlan 1987; Duever et
al. 1994). Summers are characterized by frequent thunderstorms and occasional tropical cyclones
(Duever et al. 1994). Occasionally, winter frontal systems result in higher than average winter
rainfall (Duever et al. 1994). However, human alteration of the islands may significantly alter
hydroperiods of Sanibel’s wetlands in comparison to other naturally-occurring wetlands (City of
Sanibel 2013). It is currently unknown how these hydroperiod variations impact SIRR
distribution (Abuzeineh et al. 2007; van der Merwe 2016).

For our research, we categorized SIRR habitats on the island into three distinct communities:
buttonwood shrublands, mangrove swamps, and inland grass marshes. The freshwater wetlands
make up much of the inland habitat for SIRR and consist of both ridges with short hydroperiods
and swales which remain dry for much of the year (City of Sanibel 2013). Swales are
characterized by water-dependent plant species and ridges contain more mesic species (Boone,
Unpublished data). Common freshwater wetland species in the region include cordgrasses
[Spartina sp.], sawgrasses [Cladium sp.], and leather ferns [Acrostichum aureum] (Boone,
Unpublished data). The buttonwood shrublands consist of wetland ridges that have transitioned
from grassy vegetation to woody species, especially buttonwood [Conocarpus erectus], in the
last eighty years (Hammond 1970). These buttonwood ridges are seasonally flooded, leading to a transitional hydrologic profile (FNAI 2015). Coastal mangrove swamps are abundant on the island, but SIRR has not been observed in these areas in the past (Florida Fish and Wildlife Commission 2013). These swamps are characterized by red [Rhizophora mangle], black [Avicennia germinans], and white mangroves [Avicennia marina] (Boone, Unpublished data).

We can refer to these communities as freshwater, transitional, and saltwater, respectively. We classified collection sites into vegetative communities using vegetative data from the Florida Natural Areas Inventory (Florida Natural Areas Inventory 2015) in ArcGIS.

**Data collection**—To understand the relationship between SIRR diet selection, seasonality, and plant community we took hair samples from SIRR found in interior freshwater marshes, buttonwood shrublands, and exterior mangrove swamps in summer and winter. We conducted small mammal trapping on 54 grids located on conservation lands on Sanibel Island, Florida. We placed 54 grids total, 18 grids in each of 3 communities; freshwater marshes, buttonwood shrublands, and mangrove swamps. Each grid consisted of 25 Sherman box traps (8 cm × 9 cm × 23 cm; H.B. Sherman Traps, Tallahassee, Florida, USA) in a 5x5 arrangement with 15m between traps. We baited traps with birdseed and ran them for four consecutive nights at a time. We collected approximately 1 cm² of dorsal guard hair to allow adequate material for stable isotope analysis (Darimont & Reimchen 2002; van der Merwe and Hellgren 2016). To do this, we scruffed each animal upon trapping and cut dorsal guard hair with scissors before releasing the animal. We placed the hair in sterile plastic vials and froze until processing. We collected hair samples during 2 trapping seasons (June-August 2017 and December 2017-February 2018). All samples were collected from conservation lands in J.N. “Ding” Darling National Wildlife Refuge on Sanibel Island, Florida as part of an ongoing study.
We chose hair for our stable isotope analysis to reflect a seasonal dietary turnover (Schwertl et al. 2003; Sponheimer et al. 2003b; Cerling et al. 2006). Though SIRR hair molting frequencies are currently unrecorded, we assumed a similar molting cycle to golden mice (Ochrotomys nuttali) and deer mice (Peromyscus spp.). We only caught adult rats, allowing us to operate on the assumption of biannual adult molts in the spring and fall (Linzey and Linzey 1967; Miller et al. 2008). By these assumptions and because we sampled in summer and winter, hair samples can be used to estimate diet over the prior 2-4 months, since the most recent spring/winter molt.

We collected potential food items from each area including vegetation, epifauna, fish, and macroinvertebrates. Due to increased availability in both macroinvertebrate and plant diet items, we chose to collect diet items in the summer season. We clipped vegetation and used mesh dip nets to sample for macroinvertebrates, fish, and insects in each of 3 sampling communities because these items are known components of SIRR diets (van der Merwe and Hellgren 2016). Invertebrates included insects (mostly dragonflies, damselflies, aquatic beetles and all within the orders Hemiptera, Coleoptera and Odonata), gastropods (from families Littorinidae, Ellobiidae, Thiaridae and Mytilidae) and crustaceans (mangrove crabs, crayfish, and fiddler crabs from families Sesarmidae, Cambaridae, and Ocypodidae, respectively). Vertebrates included fish (Gambusia and Poecilia) and anoles (Anolis sagrei). Vegetation samples included mangrove shoots [Avicennia germinans; A. marina; Rhizophora mangle], vines or propagules, buttonwood [Conocarpus erectus], sedges [family Cyperaceae], cordgrasses [Spartina sp.], purslane [family Portulacaceae], sawgrass [Cladium sp.], and flowering plants including Tillandsia recurvata, Eustoma exaltatum, Bacopa monnieri and Parthenocissus quinquefolia.

We developed sampling processing protocol in accordance with van der Merwe and Hellgren (2016). We froze samples within 6 hours of collection except for hair samples which we stored at
ambient temperature. We stored macroinvertebrate samples in ethanol for identification before being processed. We soaked hair in an acetone bath for 2 hours and we rinsed all samples for 10 minutes in deionized water before drying them for 72 hours at 60°C. Following oven drying, we also dried and ground some samples in a cryogenic freezer mill. We homogenized and ground the remainder of the samples with a mortar and pestle. We stored subsamples (~2.0mg for vegetation; ~0.35–0.45 mg for hair and invertebrates) in glass vials while waiting for isotopic analysis. This work was authorized by the University of Florida’s Institutional Animal Care and Use Committee (IACUC; Protocol 201709811). We then analyzed stable isotope ratios of hair and food samples (δ13C and δ15N) to test seasonal niche breadth variation and to investigate typical trophic level of SIRR in each community.

Stable isotope analysis.— To understand relationships between rice rats, diet items, and season, we applied δ15N and δ13C values to our samples. Values of δ15N from animal tissue samples reveal the nitrogen isotopic composition of the animal’s diet items with 3-4% enrichment, with values increasing as trophic level increases (DeNiro and Epstein 1981; Minagawa and Wada 1984; Peterson and Fry 1987). The δ15N values also serve to elucidate complex food web interactions that otherwise are difficult to quantify (Kling et al. 1992). Conversely, δ13C levels remain stable between trophic positions but vary among primary producers, and by extension, organisms feeding on primary producers (Rounick and Winterbourn 1986; Peterson and Fry 1987; France and Peters 1997). In terrestrial systems, δ13C can also be used to discriminate between vegetation characterized by different photosynthetic pathways (C3 vs. C4 vs. CAM) and therefore, is useful for further characterizing the nature of plants in a diet (Rounick and Winterbourn 1986; Peterson and Fry 1987; O’Leary et al. 1992). Because C3 plants discriminate against 13C more than C4 plants, a higher ratio of 13C to 12C (denoted as δ13C)
in hair samples would indicate a diet higher in C4 plants like grasses and sedges (Sponheimer et al. 2003a). However, we cannot use these values alone to determine trophic level without appropriate isotopic baselines for comparison purposes (Post 2002).

Samples were analyzed at the University of Florida Light Stable Isotope Mass Spectrometry Lab using a Thermo Electron DeltaV Advantage isotope ratio mass spectrometer coupled with a ConFlo II interface linked to a Carlo Erba NA 1500 CNHS Elemental Analyzer. Samples were loaded into tin capsules and placed in a 50-position automated Zero Blank sample carousel on a Carlo Erba NA1500 CNS elemental analyzer. After combustion in a quartz column at 1020°C in an oxygen-rich atmosphere, the sample gas was transported in a He carrier stream and passed through a hot reduction column (650°C) consisting of elemental copper to remove oxygen. The effluent stream then passed through a chemical (magnesium perchlorate) trap to remove water followed by a 0.7 meter GC column at 120°C to separate N2 from CO2. The sample gas next passed into a ConFlo II preparation system and into the inlet of a Thermo Electron Delta V Advantage isotope ratio mass spectrometer running in continuous flow mode where the sample gas was measured relative to laboratory reference N2 and CO2 gases. All carbon isotopic results are expressed in standard delta notation relative to VPDB. All nitrogen isotopic results are expressed in standard delta notation relative to AIR (Curtis, personal communication).

Data analysis.— We investigated a metric of trophic niche breadth by quantifying the variance of stable isotopic values among-individuals (Bearhop et al. 2004). We compared stable isotopic niche breadth of δ15N and δ13C between SIRR summer and winter samples, and between samples collected in each of the 3 vegetative communities, using Levene’s test of homogeneity of variance (Flaherty and Ben-David 2010). We used Levene’s test of homogeneity to infer whether δ15N and δ13C isotopic ratios overlapped sampled plant and animal prey items. We
assumed that a smaller breadth of variance was the result of specialization on a limited number of food sources, and larger variance resulted from a more generalist diet. For all analyses, we used hair-to-diet discrimination factors of 1‰ C and 3‰ N (Steenweg et al. 2011; Weiser and Powell 2011; Osterback et al. 2015). We also investigated whether vegetative community explained variation in isotopic δ¹⁵N and δ¹³C between samples (separate models for δ¹⁵N and δ¹³C) using a Tukey-Kramer Pairwise Multiple Comparison Test to account for unequal sample sizes between groups (Sokal and Rohlf 1995). We also used generalized linear models, separately for δ¹⁵N and δ¹³C (van der Merwe and Hellgren 2016), in program R (R version 3.4.2, www.r-project.org, accessed 4 March 2017) to determine if seasonality explained variation in isotopic δ¹⁵N and δ¹³C sample values.

Results

We collected 15 SIRR hair samples in summer and 15 in winter. Of these, we collected 23, 5, and 2 hair samples from freshwater marshes, buttonwood shrublands, and mangrove swamps, respectively. We collected 28 plant and 19 animal specimens for isotopic analysis. We averaged isotopic values when multiple samples of a single species were available so that only 1 value was used in the statistical analyses. This yielded 25 plant and 15 animal specimens included in statistical analyses.

Rice rat fur isotopic ratios were found to overlap with that of an animal prey base for δ¹³C (f-value = 3.682, p=0.062), but differed significantly for δ¹⁵N (f-value = 6.598, p=0.014).

Fur isotopic ratios differed significantly from a plant food base (δ¹³C f-value = 15.394, p=0.0003; δ¹⁵N f-value = 9.241, p=0.004; Fig. 1). Hair sample δ¹⁵N varied significantly between summer (mean = 5.37) and winter (mean = 5.51, p=0.018), while δ¹³C did not (summer
mean = -23.06, winter mean = -23.86, p=0.068; Fig. 2). Vegetative community modeled using a
Tukey-Kramer Pairwise Multiple Comparison Test was significantly correlated with $\delta^{13}$C
isotopic ratios for pairwise comparisons because the 95% confidence interval (CI) was positive
and did not pass through zero for freshwater marsh-buttonwood shrubland (mean = 1.604, CI =
0.3-2.909) and buttonwood shrubland-mangrove swamp (mean = 4.375, CI = 2.164-6.587), but
not for mangrove swamp-freshwater marsh whose CI contained zero (mean = -2.771, CI = -
4.720—0.822; Fig. 3). There was no significant effect for $\delta^{15}$N isotopic ratio pairwise
comparisons between any vegetative communities because all comparisons crossed zero
(freshwater marsh-buttonwood shrubland mean = -0.566, 95 CI = -1.963-0.831; buttonwood
shrubland-mangrove swamp mean = -1.526, CI = -3.895-0.842; mangrove swamp-freshwater
marsh mean = 0.96, CI = -1.127-3.047). Seasonality did not explain variation in isotopic $\delta^{15}$N (t-
value = -0.328, p = 0.745) or $\delta^{13}$C (t-value = 1.573, p = 0.127).

**DISCUSSION**

As predicted, SIRR isotopic values indicated a diet higher in animal prey than vegetation,
regardless of season or community. These findings were in agreement with those in Sharp (1967)
that rice rats are primarily carnivorous when animal prey items are available. However, animal
prey items found in mangrove areas (crabs, snails, etc) had high $\delta^{13}$C values (> -18), in line with
those of grassland vegetation, that were not detected in the hair of rodents from mangrove areas
indicating a potential avoidance of animal food sources in mangrove areas. Therefore, it appears
that most reliance on animal prey occurs in Sanibel’s freshwater wetlands, not in mangrove
wetlands as we had predicted. This emphasis on freshwater animal prey is worrisome for a
barrier island frequently impacted by hurricanes and that is vulnerable to rising sea levels (Titus 1990).

We also found that SIRR diet breadth variance does indeed vary seasonally as we predicted. Variation of winter isotopic ratios (both $\delta^{13}$C and $\delta^{15}$N) in hair samples was less than that of summer samples. This wide summer scatter confirms that SIRR diet is much broader and more diverse in summer months than in winter months. This may indicate seasonal variation in their reliance on macroinvertebrates (high $\delta^{15}$N values) which are presumably more abundant during wet summer months, and a greater abundance and diversity of plant food sources in summer (low $\delta^{15}$N values) (Neckels et al. 1990). If macroinvertebrate populations decrease in drier months, SIRR would be forced to consume more plants, especially grasses and sedges, leading to a more consistent diet profile across all sites (Neckels et al. 1990).

Finally, we found that hair isotopic $\delta^{13}$C composition varied significantly depending on the community in which they were caught (Fig. 3.). Because C4 plants like grasses and sedges favor the heavier $^{13}$C compared to C3 plants, C4 plants will exhibit a higher $\delta^{13}$C ratio in the hair samples (Sponheimer et al. 2003a). The lower values of $\delta^{13}$C came from hair samples collected in the mangrove swamps, so we can conclude that that population shows the least signature of a grass-based diet. SIRR caught in grassy freshwater marshes show a comparatively stronger grass diet signature as the $\delta^{13}$C are higher than those in mangrove swamps. However, SIRR trapped in the buttonwood shrubland (transitional) areas exhibited higher $\delta^{13}$C values and therefore had a larger signature of a grass diet than those caught in the grasslands. This may be because grasslands are flooded more frequently, and therefore likely support a greater abundance and diversity of macroinvertebrate and fish prey. Therefore, SIRR occupying grasslands eat more animals and less grass than those in the buttonwood shrubland. Furthermore, this may indicate
that SIRR selectively choose C4 plants (grasses over other vegetation types) in the shrublands that may be more prevalent. Additionally, the lack of overlap between SIRR hair isotopic ratios in different vegetative communities implies that movement between these areas may be limited. This finding is noteworthy because it indicates that multiple populations of SIRR may occur separately on Sanibel Island and maintain foraging area fidelity, increasing the likelihood of local extirpation. It is not known if movements are restricted as a result of anthropogenic alteration of the island (i.e. isolation by roads, development, canals, etc).

Our findings suggest that SIRR are capable of exploiting seasonally available food sources, with dietary breadth maximized during summer months when much of the island is inundated with fresh water. Animal prey is likely an important dietary component during all seasons, in accordance with Sharp (1967). Further research regarding the effects of water level modification on aquatic invertebrate diversity and abundance are needed to ensure adequate prey levels are maintained. Finally, the apparent lack of movement of rice rats between various habitat types, at least over time spans of 3-4 months, requires further investigation to determine if dispersal barriers have been induced by anthropogenic change.

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LITERATURE CITED


Florida Natural Areas Inventory. 2015. Cooperative land cover map. Tallahassee, Florida.


Fig. 1. Isotopic ratios of all samples (animal, hair, and plant) from both seasons.

Fig. 2. Hair sample isotopic ratios sorted by the season in which they were collected.
Fig. 3. Isotopic ratios of hair samples sorted by the vegetative community in which they were captured.