Using stable isotope analysis to infer diet differentiation between cryptic rails

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ABSTRACT

The California Black Rail (*Laterallus jamaicensis coturniculus*) is a wetland specialist found in isolated patches of freshwater and marine wetlands in California. Because the Black Rail tends to be found in the same habitats as the larger and more common Virginia Rail (*Rallus limicola*), a key question is how the two related species partition diet resources. The quantification of naturally occurring isotopes of carbon, nitrogen and sulfur has proven useful in answering otherwise difficult ecological questions. In this study, I compared the concentrations of stable isotopes of carbon and nitrogen in the feathers of Black and Virginia Rails from the Sierra Nevada foothills with those of diet sources collected from the Rails' habitat. I used SIAR, a Bayesian mixing model, to evaluate the relative dietary contributions of three isotopically distinct groups of diet sources. The data support the hypothesis that the Virginia Rail is better able to utilize aquatic prey than the California Black Rail.

KEYWORDS

Black Rail, *Laterallus jamaicensis*, Virginia Rail, *Rallus limicola*, stable isotope, wetlands

INTRODUCTION

The California Black Rail (*Laterallus jamaicensis coturniculus*) is a wetland specialist found in isolated patches of freshwater and marine wetlands in California. It is listed as threatened under the California Endangered Species Act (State of California Department of Fish and Game, 2010) and as a species of concern under the federal endangered species act (U.S. Fish and Wildlife Service, 2010). The primary threat to its survival is likely habitat loss: 91% of California's wetlands have been drained or filled since European settlement (Dahl, 1990) and an estimated 80% of the remaining population is in protected salt marsh in the northern San Francisco bay (Evens, Page, Laymon, & Stallcup, 1991). An additional small population was discovered in the Sierra Nevada foothills in 1994 (Aigner, Jerrry Tecklin, & Koehler, 1995). Tsao's study of the home range and habitat selection of Black Rails in the San Francisco Bay area and Richmond's study of the Sierra Nevada Foothills population demonstrate the birds' dependence on dense, tall vegetation emerging from shallow water (Orien M.W. Richmond, Chen, Risk, Jerry Tecklin, & Steven R. Beissinger, 2010; Tsao, Takekawa, Woo, Yee, & Evens, 2009). The same habitat requirements that threaten the California Black Rail also make it difficult to study; little is known about its biology, population dynamics or interspecies relationships (Eddleman, Flores, & Legare, 1994), particularly in the smaller, more isolated habitat patches of the Sierra Nevada Foothills.

Because the Black Rail tends to be found in the same habitats as the larger and more common Virginia Rail (*Rallus limicola*) (O. M.W Richmond, Hines, & S. R Beissinger, 2010a), a key question is how the two related species partition diet resources. Standard ecological niche theory states that two species with identical habitat and dietary requirements cannot coexist in a resource-limited environment. Although the Virginia Rail could outcompete and exclude the smaller Black Rail by its size and numbers, the two coexist in relatively small habitat patches (O. M.W Richmond, Hines, & S. R Beissinger, 2010b). Current work under Dr. Steven Beissinger at U.C. Berkeley is assessing home range overlap to assess their ecological relationship, but the degree of diet overlap between the two remains an open question. Because these birds are difficult to observe directly, and because traditional methods of diet study, such as collecting stomach contents, are too invasive to use on a threatened population, a more indirect method is needed.

The quantification of naturally occurring isotopes of carbon, nitrogen and sulfur has proven useful in answering otherwise difficult ecological questions (Peterson & Fry, 1987). Organisms with different diets, such as predators and herbivores, or with different metabolic pathways, such as C3 and C4 plants, integrate these isotopes into tissues in varying concentrations, allowing us to reconstruct diet inputs based on their relative abundance. The field of stable isotope analysis is developing rapidly: new mathematical models show promise for distinguishing among varied and unpredictable food sources (Phillips & Gregg, 2003) and multiple-isotope analyses, which may provide better resolution, are becoming available (Moreno, Jover, Munilla, Velando, & Sanpera, 2009). Feathers are nonliving tissue and are replaced seasonally. Thus, their stable isotope composition reflects the diet of the bird when they were grown (Hobson & Clark, 1992). By analyzing stable isotope ratios in feathers, we can compare the feeding behavior of the two species.

I compare the concentrations of stable isotopes of carbon and nitrogen in the feathers of Black and Virginia Rails collected during the breeding season in the Sierra Nevada foothills to answer the following questions: 1. what is the extent of dietary overlap between the two species? 2. Can we discern a significant difference in prey type or trophic level that would explain the coexistence of the two species in limited habitat patches? I hypothesize that Virginia Rail's longer bill and legs allow it to utilize food resources associated with deeper water and that its feather isotope signature will be indicate a greater dependence on aquatic taxa.

METHODS

Study organisms and populations

The California Black Rail (BLRA) is limited to small regions of the San Francisco bay, the Sierra Nevada foothills, and along the southern Colorado river (Conway, 1995). We know little about its migration or dispersal. The Virginia Rail (VIRA) is common and widely distributed across North America, with year-round resident populations along the west coast. Migratory populations winter in Mexico, along the gulf coast and in Florida and breed across the northern United States and Canada (Conway, 1995). The ranges of both species are shown in

Figure 1, but the California subspecies of BLRA, *Laterallus jamaicensis coturniculus* only occurs in the far west portion of the range map.

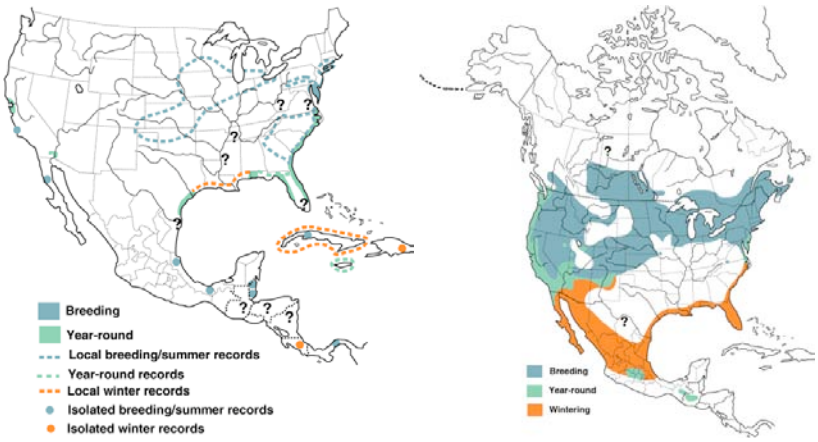

Figure 1. Ranges of *Laterallus Jamaicensis* (left) and *Rallus limicola* (right). The subspecies *L. j. coturniculus* is limited to the western region of the range map (Figure: Eddleman et al., 1994).

The BLRA and the VIRA have similar habitat requirements and life histories, but vary in distribution, size, and bill shape. Morphological differences potentially affect the prey available to each species: the VIRA is 25-27 cm long with a bill length of 32-46 mm, while the BLRA is only 10-15 cm long with a 12-16 mm bill (Pyle, 2008, p. 480). Both are wetland specialists that prefer permanently flooded areas with dense vegetation over 0.5m tall. In the study area, bulrushes (*Scirpus spp.*), rushes (*Juncus spp.*) cattails (*Typha spp.*) and sedges (Family *Cyperaceae*) are the dominant vegetation (Orien M.W. Richmond et al., 2010)

Study area

Wetland habitat patches in the northern Sierra Nevada foothills tend to be small, isolated and ephemeral, making the region ideal for studies of population dynamics and interspecies competition. Richmond et al. (2010) have surveyed BLRA and VIRA populations at 218 sites over an area of 400 square miles in Butte, Nevada, Placer and Yuba counties. They found that the two species co-occur more often than expected and that home ranges overlap more than they do in the San Francisco bay area. To maximize sampling efficiency, we (Professor Steven

Beissinger's Black Rail Project team) selected the sites with the highest estimated populations of both species.

Feather Collection

We located rails by listening for their responses to recorded calls. Once rails were located, we set up a twelve meter mist net near the source of the response and staked the bottom edge to the ground. We used a variety of calls to elicit a response, including contact calls, aggressive calls, and songs, prompting the rails to run into the net. Once captured, we cut two secondary feathers and plucked several breast feathers and then released the bird. Feather samples from 52 BLRA and 11 VIRA that were either adult or of unknown age were collected between 2004 and 2009. I did not include known hatch-year birds in this study because of differences in diet and feather structure.

Prey collection

Because I am interested in areas where BLRA and VIRA ranges overlap, I collected potential food items at sites where we had previously tracked both species using radio telemetry. My goal was to collect a broad range of available prey taxa (i.e., taxa richness) rather than to assess abundance. I used vegetation shakes, net sweeps to capture insects and seeds in vegetation, 10 cm deep mud cores, and used a dip net to capture aquatic and benthic insects and small vertebrates. I placed the specimens on ice in a portable cooler to sedate them and then froze them in the laboratory at -4° C. I thawed and identified them to family. I collected 72 samples of potential food items from three sites with breeding populations of both rail species in August 2010. Of these, I selected 27 to represent a range of functional classes including small fish, tadpoles, crayfish, terrestrial and aquatic insects and plant material. The taxa and their $\delta^{13}C$ and $\delta^{15}N$ values are listed in appendix I.

Isotope analysis

Different types of sample material required different treatments to prepare them for

analysis. I washed feathers in a solution of 2 parts ethanol to 1 part chloroform for 24 hours to remove surface dirt and oils. Whereas feathers are relatively uniform in composition and represent a single tissue type, the diet samples were mostly whole animals. Isotope ratios can vary widely between tissue types, introducing the risk of sampling error if only a part of the organism is analyzed. Therefore, I oven dried the specimens (Gravity Oven 1340a, Lindberg/MPH, Riverside, MI) at 50ºC for one week and ground them to a homogenous powder in a mortar and pestle. Because trophic level, as indicated by $^{\delta 15}$N concentration, is a key metric of diet partitioning, I did not remove lipids from the diet samples because this treatment can alter δ^{15}N levels (Mintenbeck, Brey, Jacob, Knust, & Struck, 2008). The samples were analyzed at the Center for Stable Isotope Geochemistry at UC Berkeley with an IsoPrime 100 IRMS gas source stable isotope ratio mass spectrometer. The isotope values are reported in parts per thousand relative to an international standard for each isotope.

Data analysis

I performed all statistical tests with the R statistics package (R Development Core Team, 2010), using R Commander (Fox et al., 2011) as a graphical interface. To test for broad interspecies differences, I first compared mean feather isotope ratios without reference to prey sample data. I pooled all individuals of each species and performed one Kruskal-Wallis test (values were not normally distributed) for each of the two isotopes: ^{13}C and ^{15}N. This larger sample would highlight broad patterns, such as overall trophic level differences between species. Because a difference in variances could have statistical and ecological implications, I used Levene's test for homogeneity of variances to test for differences in variance between species. I centered the test on the median to minimize the effect of outliers in the data.

To produce a meaningful estimate of the relative contributions of diet components, a mixing model needs to work with relatively few inputs. Because each sample is represented in two dimensions (C and N), and there is high variability between samples, I used k-means cluster analysis to classify diet samples into three groups. Cluster analysis enabled me to group sources based on their measured trophic differences rather than on predictions based on life history. A k-means analysis using three clusters provides a feasible grouping of the diet samples, placing most aquatic taxa together.

Stable Isotope Analysis in R (SIAR) (Parnell, Inger, Bearhop, & Jackson, 2010) is a mathematical model that compares the proportions of isotopes in consumer tissues with those in food items to estimate the relative importance of each food source. SIAR uses Bayesian statistics to produce a weighted range of possible inputs. I calculated means and standard deviations for the clusters given by k-means and used these as the class inputs to SIAR.

RESULTS

Feather Analysis

The combined feather samples (breast and secondary) from 2004-2009 show a significant difference in $\delta^{13}C$ between the BLRA and VIRA using the nonparametric Kruskal-Wallis test ($p=0.002$), but not in $\delta^{15}N$ ($p=0.09$). Mean $\delta^{13}C$ was -22.57 ± 2.57 (n=52) in BLRA and -24.59 ± 1.69 (n=11) in VIRA. Mean $\delta^{15}N$ was 9.41 ± 1.69 in BLRA and 10.34 ± 1.54 in VIRA. Virginia Rail feathers are more depleted in $\delta^{13}C$ and more enriched in $\delta^{15}N$. Although standard deviations for $\delta^{13}C$ were 2.52 for BLRA and 1.69 for VIRA, Levene's test for homogeneity of variances did not show a significant difference when centered on the median.

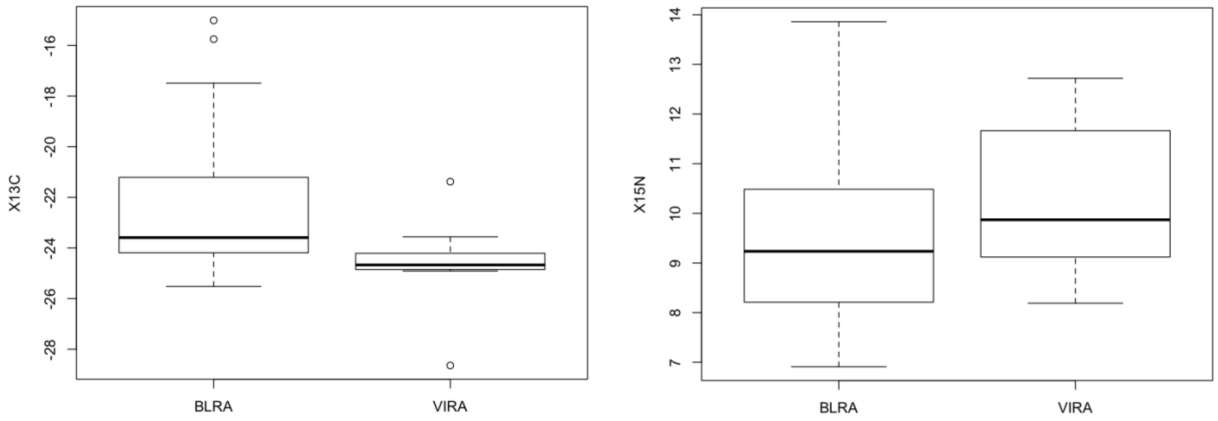

Figure 1. Feather isotope ratios for BLRA and VIRA compared.

Diet source analysis

There was strong variation in isotope ratios within taxa and between those with similar ecological factions. A three-group cluster analysis produced the most informative classes, placing aquatic taxa together in one group. The isotope data and the cluster assignments are given in Appendix 1. As shown in Figure 1, the three clusters are broad, but distinct enough to be informative as inputs to the mixing model.

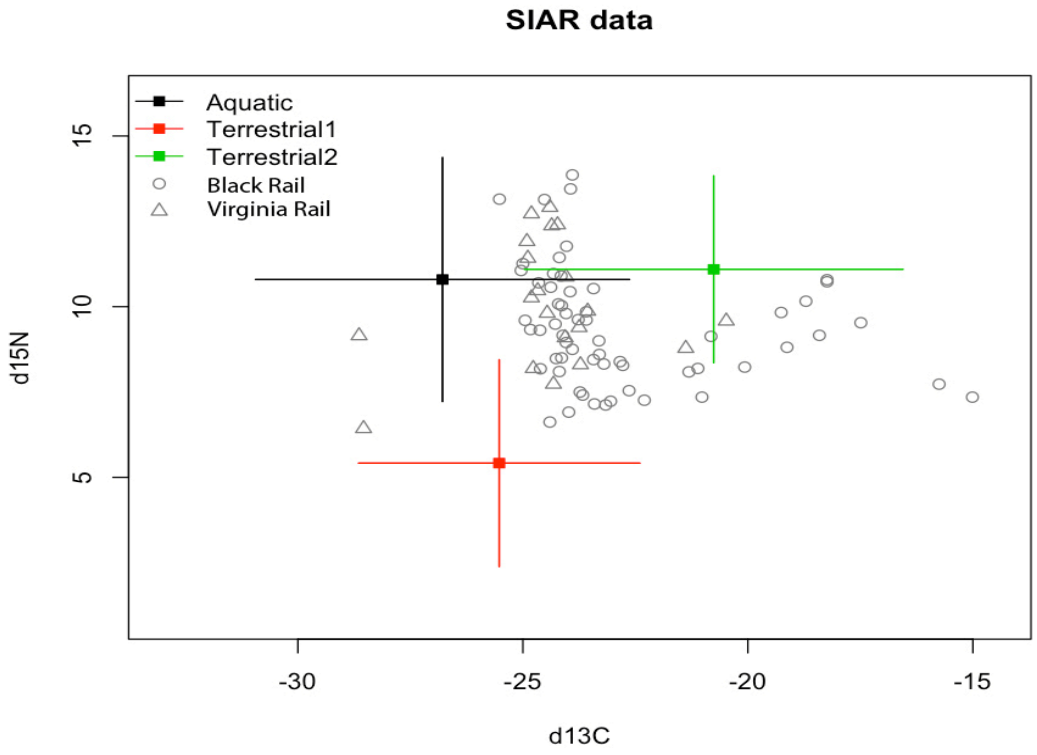

Figure 2. The three prey clusters as they relate to feather isotope values.

Mixing model

SIAR estimates a higher dietary contribution from the 'Aquatic' group in VIRA, as shown in Figure 3. It estimates a 0-20% contribution in BLRA versus 52-72% in VIRA (95% confidence interval). For group 'Terrestrial1', the model estimates a 20-37% input in BLRA compared with 0-28% in VIRA. Finally, it reports contributions of 52-72% and 19-52% from

group ‘Terrestrial2’ in BLRA and VIRA respectively.

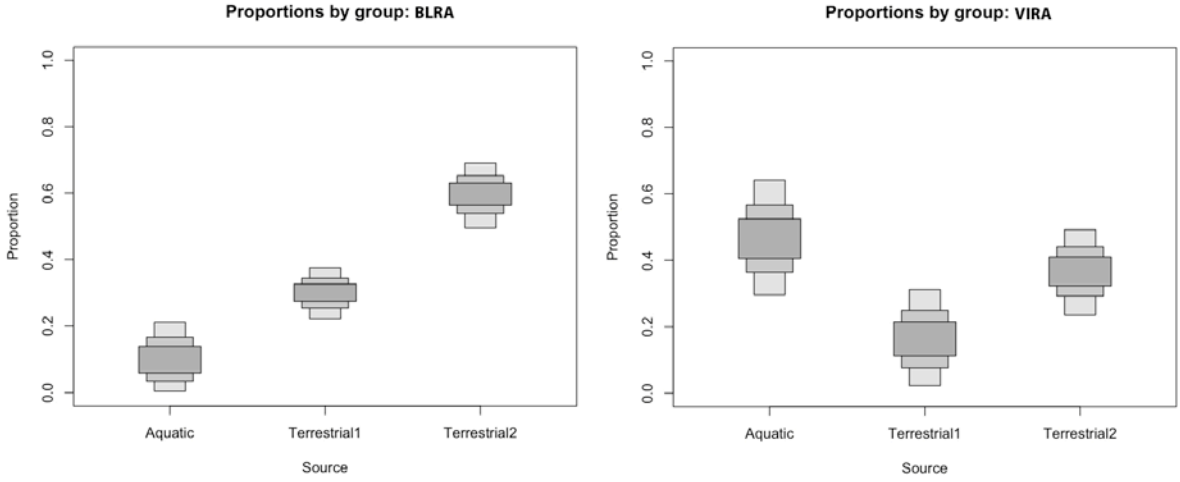

Figure 3: SIAR estimates of the relative importance of three prey classes.

DISCUSSION

The data support the hypothesis that BLRA and VIRA are utilizing different food resources, even in small, heterogeneous habitat patches. VIRA feathers are more depleted in the heavy isotope of carbon, possibly indicating a greater contribution from aquatic primary producers. The mixing model gives better resolution, indicating that taxa associated with deeper water are a more important component in the VIRA’s diet than the BLRA’s diet. The high variability in isotope ratios in both feathers and food sources, however, make interspecies differences difficult to quantify.

Isotope differences between species

Despite the wide variability in isotope values, patterns exist both in mean values and in mixing model outputs. Although the relative depletion in $\delta^{13}C$ in Virginia Rail feathers is consistent with a greater usage of aquatic habitat, the source of variation is unclear. Particulate organic matter found in wetlands is depleted in the heavy carbon isotope relative to terrestrial plants by about 8‰, but a similar difference occurs between plants with C3 (-22 to -27‰) and C4 (-9 to -14‰) photosynthetic pathways (Teeri & Schoeller, 1979). The VIRA feather values

are still consistent with inputs from C3 primary producers, and the relative $\delta^{13}C$ enrichment found in BLRA could indicate a larger input from C4 plants. The tendency toward higher $\delta^{15}N$ values in the Virginia Rail (10.3‰ in VIRA vs. 9.4 in BLRA) suggest that it is able to take a wider range of prey, particularly small vertebrates and aquatic insects, consistent with Richmond's finding that it favors deeper water habitat (Orien M.W. Richmond et al., 2010). Although the difference is not statistically significant, the mixing model supports to this interpretation.

Diet sample clusters

In using cluster analysis to categorize diet samples, I assume that a difference in isotope composition represents a real ecological difference. Specifically, I assume that samples that have similar isotope signatures to aquatic taxa, such as fish and frogs, rely on, and were collected in, deeper-water habitats. I labeled the three groups Aquatic, Terrestrial1 and Terrestrial2 but note that none of the groups divides taxa strictly according to ecological niche. Spiders, for example, fall into more than one group, presumably based on individual habitat and feeding habits. The Aquatic group includes *Gambusia*, a 3.5cm fish, Bullfrog (*Rana catesbeiana*) tadpoles, juvenile crayfish and aquatic insect larvae as well as terrestrial taxa, such as leafhoppers and spiders. The Terrestrial1 group is comprised of herbivorous terrestrial insects and one plant sample. The two plant-sucking *Pentatomidae* are both included in this group. With the exception of the diving beetle (*Hydrophylidae*), Terrestrial2 is comprised of terrestrial insects and spiders. More predatory and scavenging taxa are represented in Terrestrial2, but some herbivores are also included.

Mixing model

The strength of using SIAR to examine diet is in its ability to compute likely dietary contributions using data widely variable data. It would be difficult to discern a meaningful pattern by looking at Figure 3, and impossible using a plot of the individual diet samples. The output of the mixing model supports the hypothesis that VIRA is making more use of deeper-water prey, indicating that more than half of its diet comes from the 'Aquatic' group.

Conversely, BLRA appears to depend most on the 'Terrestrial2' group, which is comprised of small, near-ground-dwelling taxa. Flying insects are notably absent. There is great variability within each of the diet classes, however, and it is important to remember that SIAR will calculate a solution regardless of the relevance of the data.

Limitations

The primary limitation of this study, and the reason that little is known about Rail biology, relates to the cryptic lifestyle of these birds. Rails tend to live in deep cover and at low density. Intraspecies territoriality played out in small habitat patches keeps sample sizes low. Our study sites are smaller than ten hectares (Orien M.W. Richmond et al., 2010), and Tsao et al. (2009) estimate mean Black Rail home range area at .59 ha, even in the much larger habitats around the San Francisco Bay. We do not yet have a measure of defended territory size or of population density in the Sierra Foothills due to the difficulty of making direct observations, but we trapped no more that eight birds at any given site. Three was the average daily take at any one site. Because wetland habitat is easily damaged and any trapping operation has some impact, we did not attempt to trap all the birds at any site, and we usually only trapped for one day. Furthermore, because the BLRA is the primary focus of the Black Rail Project, more effort goes into trapping BLRA than VIRA and we therefore have fewer VIRA feather samples.

Although feather $\delta^{13}C$ differed significantly and SIAR supported differences in diet, it is important to be cautious in attributing the variation purely to differing food requirements. The type and abundance of nutrients may vary spatially and temporally, and the isotope composition of invertebrate prey may vary significantly within a season based on changes in primary producers (Taylor & Batzer, 2010). In this study, the feathers we sampled were likely molted the year before the diet samples were collected. I did not find a significant difference between feathers sampled in different years, but the small sample sizes could conceal a real difference. Boon and Bunn that a single wetland plant species could vary by 10ppt between seasons and sites within the same geographic region (1994), but found that most of the variation was seasonal. Because our sampling efforts are timed to the rail breeding season, this variation is likely minimal.

Future directions and broader implications

Continued sample collection over subsequent years would help assess the degree of annual variation in feather chemistry and track the sources of variability in the feather isotope values of the two species. Based on the mixing model results, a diet sampling plan focusing on tying samples directly to the microhabitat where they were collected would make the prey clusters more statistically robust. Additional vegetation and aquatic organic matter sampling would help trace the sources of dietary carbon.

The difference in variation between the two species could indicate a difference in niche width or foraging area, suggesting that the Virginia Rail is foraging over a wider area or on a more varied diet (Bearhop, Adams, Waldron, Fuller, & Macleod, 2004; Flaherty & Ben-David, 2010). The reduced variation in the VIRA's $\delta^{13}C$ feather chemistry suggests that it may be able to forage over a wider area or a broader range of food sources. Symes, for example, sampled a range of possible diet sources in forest plots and modeled the feeding behavior of a "perfect generalist" consumer, comparing isotope values of various species to it and inferring feeding specificity rather than diet composition (2009).

Following up on Richmond and Tsao's (Tsao et al., 2009) work, this study sheds some new light on the habitat use of these two species. The low density of rails detected at individual sites in Richmond's studies, combined with the high productivity of wetlands suggests that minimal resource partitioning is required. The species' apparent lack of competition for food resources helps explain their frequent co-occupancy and suggests that preservation and expansion of available habitat will support larger populations of both birds. Mixing model estimates, however, suggest some degree of diet and habitat differentiation, and support the hypothesis that deeper water favors the larger VIRA.

As new tools and analytical methods are developed, they can best be validated and refined by testing them in real-world situations. The field of stable isotope analysis shows great promise for helping us understand complex food webs in general, and is an excellent tool for understanding system-wide nutrient flow in complex habitats. By using SIAR in this context, where small sample sizes and high variability make interpretation difficult, I have sketched the trophic landscape in the rails' habitat. This sketch, developed further, could inform land use and management decisions that would help conserve the California Black Rail.

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REFERENCES

Aigner, P. A., Tecklin, Jerry, & Koehler, C. E. (1995). Probable breeding population of the black rail in Yuba County, California. *Western Birds*, *26*(3), 157-160.

Bearhop, S., Adams, C. E., Waldron, S., Fuller, R. A., & Macleod, H. (2004). Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of Animal Ecology*, *73*(5), 1007–1012.

Boon, P. I., & Bunn, S. E. (1994). Variations in the stable isotope composition of aquatic plants and their implications for food web analysis. *Aquatic Botany*, *48*(2), 99–108.

Conway, C. J. (1995). Virginia Rail (*Rallus Limicola*). In *The Birds of North America Online*. Ithaca: Cornell Lab of Ornithology. Retrieved from http://bna.birds.cornell.edu/bna/species/173

Dahl, T. E. (1990). *Wetlands losses in the United States 1780's to 1980's* (Report to congress). U.S. Department of the Interior, Fish and Wildlife Service, Washington, D.C.: Northern Prairie Wildlife Research Center Online. Retrieved from http://www.npwrc.usgs.gov/resource/wetlands/wetloss/index.htm

Eddleman, W. R., Flores, R. E., & Legare, M. (1994). Black Rail (*Laterallus jamaicensis*). In A. Poole & F. Gill (Eds.), *The Birds of North America Online*. Retrieved from http://bna.birds.cornell.edu/bna/species/123

Evens, J. G., Page, G. W., Laymon, S. A., & Stallcup, R. W. (1991). Distribution, Relative Abundance and Status of the California Black Rail in Western North America. *The Condor*, *93*(4), 952–966.

Flaherty, E. A., & Ben-David, M. (2010). Overlap and partitioning of the ecological and isotopic niches. *Oikos*, *119*(9), 1409-1416.

Fox, J., Andronic, with contributions from L., Ash, M., Boye, T., Calza, S., Chang, A., Grosjean, P., et al. (2011). *Rcmdr: R Commander*. Retrieved from http://CRAN.R-project.org/package=Rcmdr

Hobson, K. A., & Clark, R. G. (1992). Assessing Avian Diets Using Stable Isotopes I: Turnover of 13C in Tissues. *The Condor*, *94*(1), 181–188.

Mintenbeck, K., Brey, T., Jacob, U., Knust, R., & Struck, U. (2008). How to account for the lipid effect on carbon stable-isotope ratio ($?lta$13C): Sample treatment effects and model bias. *Journal of Fish Biology*, *72*(4), 815–830.

Moreno, R., Jover, L., Munilla, I., Velando, A., & Sanpera, C. (2009). A three-isotope approach to disentangling the diet of a generalist consumer: the yellow-legged gull in northwest Spain. *Marine Biology*, *157*(3), 545-553.

Parnell, A. C., Inger, R., Bearhop, S., & Jackson, A. L. (2010). Source partitioning using stable isotopes: coping with too much variation.

Peterson, B. J., & Fry, B. (1987). Stable Isotopes in Ecosystem Studies. *Annual Review of Ecology and Systematics*, *18*, 293–320.

Phillips, D. L., & Gregg, J. W. (2003). Source partitioning using stable isotopes: coping with too many sources. *Oecologia*, *136*(2), 261-269.

Pyle, P. (2008). *Identification Guide to North American Birds Part II*. Point Reyes Station, CA: Slate Creek Press.

R Development Core Team. (2010). *R: A Language and Environment for Statistical Computing*. Vienna, Austria. Retrieved from http://www.R-project.org/

Richmond, O. M.W, Hines, J. E., & Beissinger, S. R. (2010a). Two-species occupancy models: a new parameterization applied to co-occurrence of secretive rails. *Ecological Applications*, *20*(7), 2036–2046.

Richmond, O. M.W, Hines, J. E., & Beissinger, S. R. (2010b). Two-species occupancy models: a new parameterization applied to co-occurrence of secretive rails. *Ecological Applications*, *20*(7), 2036–2046.

Richmond, O. M., Chen, S. K., Risk, B. B., Tecklin, J., & Beissinger, S. R. (2010). California black rails depend on irrigation-fed wetlands in the Sierra Nevada foothills. *California Agricultire*, *64*(2), 85-93.

State of California Department of Fish and Game. (2010). *State & Federally Listed Endangered & Threatened Animals of California*. Retrieved from http://www.dfg.ca.gov/biogeodata/cnddb/plants_and_animals.asp

Symes, C. T., & Woodborne, S. M. (2009). Trophic level delineation and resource partitioning in a South African afromontane forest bird community using carbon and nitrogen stable isotopes. *African Journal of Ecology, 48,* 984-993.

Taylor, A. N., & Batzer, D. P. (2010). Spatial and temporal variation in invertebrate consumer diets in forested and herbaceous wetlands. *Hydrobiologia*, *651*(1), 145-159.

Teeri, J. A., & Schoeller, D. A. (1979). Delta 13 C values of an herbivore and the ratio of C 3 to C 4 plant carbon in its diet. *Oecologia*, *39*(2), 197–200.

Tsao, D. C., Takekawa, J. Y., Woo, I., Yee, J. L., & Evens, J. G. (2009). Home Range, Habitat Selection, and Movements of California Black Rails at Tidal Marshes at San Francisco Bay, California. *The Condor*, *111*(4), 599-610.

U.S. Fish and Wildlife Service. (2010, May 6). Species Profile for California Black rail (laterallus jamaicensis coturniculus). *Environmental Conservation Online System*. Retrieved May 6, 2010, from http://www.fws.gov/ecos/ajax/speciesProfile/profile/speciesProfile.action?spcode=B0EE

APPENDIX 1

Table 1. Isotope values of diet source samples. Cluster assignments are given in column KMeans 3.

Sample ID	Description	Common name	Functional class	d.15N	d.13C	Group
169.13	Neuroptera	Lacewing	Nectar feeder	2.79	-26.6	Terrestrial1
169.07	Gryllidae	Cricket	Herbivore	2.6	-26.21	Terrestrial1
Y-26.01	Pentatomidae	Shield bug	Plant sucker	0.07	-25.76	Terrestrial1
169.05	Pentatomidae	Shield bug	Plant sucker	1.56	-25.52	Terrestrial1
169.18	Juncus	Rush	Plant	2.56	-28.54	Terrestrial1
161.1	Gambusia	Mosquitofish	Fish	9.49	-27.28	Aquatic
161.17	Decapoda	Crayfish	Scavenger	9.56	-28.19	Aquatic
161.18	Odonata	Dragonfly larva	Aq. predator	5.97	-28.66	Aquatic
161.09	Rana	Bulfrog tadpole	Aq. herbivore	6.22	-28.81	Aquatic
161.12	Annelida	Worm	Aq. worm	6.46	-26.77	Aquatic
Y-26.02	Coenagrionidae	Damselfly	Predator	6.77	-30.32	Aquatic
218.12	Delicopodidae	Long-legged fly	Predator	9.32	-26.36	Aquatic
169.15	Seeds – no ID	Wetland plant	Plant	6.41	-27.53	Aquatic
169.04	Aranidae	Spider	Predator	6.01	-26.38	Aquatic
169.12	Aranidae	Spider	Predator	5.79	-26.72	Aquatic
218.23	Aranidae	Spider	Predator	5.25	-25.98	Aquatic
218.08	Cicidellidae	leaf hopper	Herbivore	7.83	-27.67	Aquatic
218.1	Cicidellidae	leaf hopper	Herbivore	8.38	-26.57	Aquatic
161.31	Macromiidae	Dragonfly	Predator	6.18	-26.18	Aquatic
218.13	Scirpus	Sedge	Plant	8.16	-28.14	Aquatic
169.02	gambusia	Mosquitofish	Fish	8.92	-33.02	Aquatic
161.33	Coenagrionidae	Sedge sprite	Predator	7.23	-24.38	Terrestrial2
161.27	Drosophilidae	Fruit fly	Herbivore	8.72	-22.6	Terrestrial2
161.34	Aranidae	Spider	Predator	8.48	-19.24	Terrestrial2
161.28	Elateridae	Click beetle	Herbivore	7.55	-21.66	Terrestrial2
161.35	Gryllidae	Cricket	Herbivore	6.12	-22.53	Terrestrial2
161.19	Hydrophylidae	Diving beetle	Scavenger	7.47	-20.15	Terrestrial2

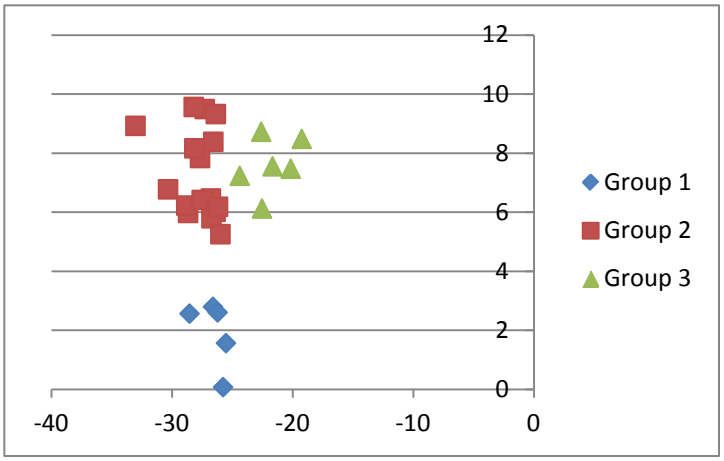

Figure 4. Clustering of prey isotope values.