FEEDING HABITS OF ENDANGERED PYGMY RACCOONS (*PROCYON PYGMAEUS*) BASED ON STABLE ISOTOPE AND FECAL ANALYSES

Katherine W. McFadden,* Raymond N. Sambrotto, Rodrigo A. Medellín, and Matthew E. Gompper

Department of Ecology, Evolution and Environmental Biology, Columbia University, 1200 Amsterdam Avenue, MC 5557, New York, NY 10027, USA (KWM)
Lamont-Doherty Earth Observatory of Columbia University, Route 9W, Palisades, NY 10694, USA (RNS)
Instituto de Ecología, Universidad Nacional Autónoma de Mexico, Apartado Postal 70-275 Ciudad Universitaria, Distrito Federal 04510, Mexico (RAM)
Department of Fisheries and Wildlife Sciences, University of Missouri, Columbia, MO 65211, USA (MEG)

Knowledge of foraging ecology of endangered mammals is often based on limited data because of logistical constraints of accessing animals, their stomach contents, or fecal samples. Here we use a stable isotope approach to examine feeding habits of a rare mammal, gaining insights over a greater temporal scale than a traditional fecal analysis would allow, and ameliorating some of the constraints of reduced sample sizes that can limit studies of mammalian foraging ecology. We focus on the endangered pygmy raccoon (*Procyon pygmaeus*), an endemic species from Cozumel Island, Mexico. Raccoons are thought to be omnivorous based on studies in the temperate zone, yet few dietary analyses have been conducted on raccoons in the tropics. Using hair and blood samples obtained from trapping over 3 years (2001–2003) and at 3 localities, the feeding habits of this species were examined based on the isotopic ratios of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$). Feces also were collected to supplement and compare to isotopic data. Both isotopic data and scat analyses suggest an omnivorous diet specialized on crabs, which constituted >50% of the diet, followed by fruits and insects. Hair and blood samples did not significantly differ in carbon or nitrogen isotopic ratios and we found no age- or sex-related variation. Although we observed subtle spatial and temporal variation in diet, both stable isotope and fecal analyses emphasize the dominance of crabs across these scales.

Key words: carnivores, Cozumel Island, diet, fecal analysis, *Procyon pygmaeus*, raccoons, stable isotope ratios

Insights into limiting resources (e.g., dietary needs) are critical to population management and identification and protection of important habitats. Problematically, for rare or threatened taxa, lack of access to organisms may hinder the ability to obtain this information. For instance, an understanding of mammalian carnivore dietary habits is generally obtained via analyses of feces or stomach contents. For species of conservation concern, obtaining these samples can be hampered by limitations in obtaining permits, ethical concerns associated with capturing the last remaining individuals of a taxon, and sample size concerns because of the limited number of capture events (when possible). Stable isotope techniques offer the opportunity to overcome some of these constraints

by increasing the amount of information that can be obtained from relatively rare capture events (Hilderbrand et al. 1996; Kelly 2000). Here we demonstrate the practicality of this approach with an examination of the pygmy raccoon (*Procyon pygmaeus*), an endangered species endemic to Cozumel Island, Mexico (Cuarón et al. 2004; Hilton-Taylor 2001; SEMARNAT 2002).

Common raccoons (*Procyon lotor*) usually are considered opportunistic and generalist omnivores whose diets typically vary seasonally (Carrillo et al. 2001; Gehrt 2003; Lotze and Anderson 1979). However, the feeding ecology of tropical and island-dwelling raccoons such as *P. pygmaeus* is poorly understood. Navarro and Suarez (1989) examined 7 fecal samples of *P. pygmaeus* and concluded that their diet consisted mainly of crabs, insects, and some plant material. Aside from this brief study, feeding habits of this species are unknown.

We collected fecal samples from trapped *P. pygmaeus* to gain baseline insights on their diet and undertook an assessment of stable isotope ratios to determine longer-term

---

* Correspondent: katherine.mcfadden@noaa.gov

feeding strategies and address variation among individuals and subpopulations. Assessments made by capturing animals and obtaining fecal samples are limited because *P. pygmaeus* do not always defecate in traps, because identifying dietary components from feces is constrained by differential digestibility of food items (especially plant materials), and because understanding long-term feeding habits (e.g., annual patterns) would necessitate a long-term capture commitment, which is logistically difficult to carry out for this rare endemic animal. Isotopic diet studies offer advantages over other methods in that isotopic ratios reflect nutrients assimilated over extended periods of time and not simply those recently ingested. Also, because the metabolic rate of different tissues determines the turnover of stable isotopes in tissues, one can glean dietary information on varying timescales (Tieszen et al. 1983) by sampling multiple tissue types. Applying stable carbon and nitrogen isotopic ratios to tissues enables us to investigate the temporal and spatial variation in feeding habits in *P. pygmaeus*. Examination of pilot data showed that *P. pygmaeus* primarily use mangrove forest and wetlands (Cuarón et al. 2004; McFadden 2004). Therefore, we hypothesized that diet of *P. pygmaeus* would be dominated by mangrove crabs, which are common invertebrates in these communities on Cozumel.

MATERIALS AND METHODS

Study sites and sample collection.—Cozumel (20°16′–20°26′N, 86°44′–87°02′W) is a 486-km² island (population ∼75,000) covered by a variety of terrestrial habitats including dry deciduous forests, mangrove stands, sandy palm areas, and multistratal tropical semievergreen forests (Téllez and Cabrera 1987; Cabrera et al. 1982). Seventy-five percent of Cozumel Island is covered by vegetation (Martinez-Morales 1999); the majority of dry forest on the island is secondary succession.

During two 3-month sampling periods in 2001 and 2002, 10 sites throughout the island were sampled for a minimum of 2 weeks at each site, with 3 sites in the northwestern mangrove-dominated habitats chosen for further study based on the identification of substantial raccoon populations (McFadden 2004). All 3 sites were north of San Miguel, the largest city on Cozumel. Site 1 was located on the most northwestern tip of the island in mangrove forests. Cozumel's primary water-treatment plant is located 1 km southeast of site 1. Site 3 was located approximately 2 km southwest of site 1, and site 2 was approximately 2.5 km southeast of sites 1 and 3. Site 2 was in the closest proximity to anthropogenic influences, such as access to garbage areas, and included dry forest and mangroves that bordered the golfing greens of Cozumel Country Club. Despite the proximity of these sites to one another, individuals from one site were never trapped in any other site, and therefore we treat the sites as being independent.

Fieldwork was conducted at 3 primary intervals over 3 years. The 1st field season was conducted from July through September 2001 (wet season, heavier rains), the 2nd field season spanned from April (late dry season) to July 2002 (mid–wet season), whereas the 3rd season was February–March 2003 (dry season). Animals were captured using Tomahawk 207 box traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin) checked at least once daily, and trapped animals were immobilized with ketamine hydrochloride (10–12 mg/kg) and xylazine (2 mg/kg) before handling, ear-tagging (Hasco Tag company, Dayton, Kentucky), and tissue and blood sample collection. Age was approximated as juvenile (0–12 months), subadult (13–21 months), or adult (>21 months) from tooth wear (Grau et al. 1970),

body size, reproductive status, and recapture history. Blood samples were collected by jugular venipuncture in both additive-free (for the separation of serum and clotted cells) and heparin-containing (for the separation of plasma and red blood cell components) Vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, New Jersey). Blood was centrifuged, and blood components were frozen (−20°C on Cozumel, and then −80°C in the laboratory). Hair samples (∼20 hairs per individual) were obtained by plucking a small patch from the root shaft and stored dry in a paper envelope to avoid mildew growth. Research on live animals was performed following guidelines of the American Society of Mammalogy (Animal Care and Use Committee 1998), and all research protocols were approved by Columbia University's Institutional Animal Care and Use Committee.

Potential prey items (fruit, reptiles, crabs, etc.) in varying habitats throughout the island were collected in each field season (Appendix I). Vertebrate and invertebrate samples were stored frozen until analysis. Fruit and vegetation samples were oven dried and stored at room temperature.

Carbon and nitrogen isotope analyses.—All samples were prepared and analyzed at Columbia University in the laboratory of RNS. Hair, blood, fruit, and insect samples were dried at 60°C for 24 h and then powdered with a mortar and pestle. Carbon isotopic values of lipids may differ significantly from those of other tissues (DeNiro and Epstein 1978; McConnaughey and McRoy 1979; Vogel 1978); therefore, lipids were removed from blood and prey specimens using a modified methanol–chloroform extraction technique (Bligh and Dyer 1959). Samples were then dried at 60°C for at least 24 h. Oils from hair samples were removed by cleaning samples with 90% ethanol followed by soaking in chloroform–methanol.

Carbon and nitrogen stable isotope ratios in hair and blood are expressed in delta notation (difference between sample and standard) as parts per thousand (‰):

$$\delta = [(R_{sample}/R_{standard}) - 1] \times 1,000,$$

where R is the ratio of the minor to major isotope (i.e., $\delta^{13}C/\delta^{12}C$ or $\delta^{15}N/\delta^{14}N$) as measured by mass spectrometry. All results are reported relative to atmospheric nitrogen as the standard for $\delta^{15}N$, and to Cretaceous belemnite (*Belemnitella americana*) from the Peedee formation of South Carolina for $\delta^{13}C$ (Craig 1957). Samples were prepared for mass spectroscopy via an automated Dumas procedure (Knowles and Blackburn 1993) and analyzed with a Europa 20-20 isotope ratio mass spectrometer (PDZ-Europa, Cheshire, United Kingdom) in continuous-flow mode. The resultant CO_2 and N_2 gases were separated chromatographically and introduced sequentially to the source of the mass spectrometer for isotopic analysis. Analytical precision of the system is 0.12‰ for $\delta^{13}C$ and 0.20‰ for $\delta^{15}N$. In continuous-flow mode, the samples were bracketed by National Institute of Standards and Technology (NIST) primary or secondary isotopic standards that were calibrated against the NIST material. A minimum of 3 replicates was analyzed from each sample per individual. The quantitative assessment of feeding ecology from stable isotopic ratios is based on the breakdown between source material and metabolic factors:

$$\delta_{tissue} = \delta_{diet} + \Delta_{dt},$$

where Δ_{dt} represents the isotopic fractionation or trophic enrichment factor between dietary and consumer tissue (DeNiro and Epstein 1978, 1981).

In the absence of estimates for procyonids, we assume fractionation values of 2‰ for carbon when fruit was consumed, and 1‰ when invertebrates were consumed, and nitrogen fractionation values of 3.4‰ for all noninvertebrate prey items. These fractionation values

were based on results from captive feeding experiments on American mink (*Mustela vison*) and American black bears (*Ursus americanus*— Ben-David et al. 1996; Hilderbrand et al. 1996). We also used alternative fractionation values from red foxes (*Vulpes vulpes*) based on Roth and Hobson (2000), whose work found hair carbon to be enriched by 2.6‰ and hair nitrogen enriched approximately 3.4‰.

Mixing models and statistical analyses.—Diet sources for *P. pygmaeus* were based on a dual-isotope, multiple-source mixing model that took into account both δ^{13}C and δ^{15}N. A Euclidean mixing model (Ben-David et al. 1997) was used to estimate the contribution of each potential prey item to the diet of the predator. The mean δ^{13}C and δ^{15}N of each type of prey was used to estimate the proportion of food items most commonly used as prey. The mixing model requires that isotopic values of all prey be significantly different from each other, which was tested using a K nearest-neighbor randomization test (Ben-David et al. 1996; Schilling 1986). The model assumes that predators consume all possible prey types (Ben-David and Schell 2001) and thus tends to overestimate the proportion of food items that are rarely consumed and underestimate the proportion of commonly consumed prey (Ben-David et al. 1997). As such, the use of a mixing model serves as an index of the relative contributions of prey items to the diet, rather than as a predictor of exact proportions.

Because of its availability in our captures, and to maximize sample sizes, hair was selected as the consumer tissue for our mixing models when geographic and temporal differences were examined. Average blood (red blood cells [RBCs] and clotted cells) and hair isotopic values were used when calculating the overall mixing model proportion of each food item. Mean δ^{13}C or δ^{15}N of each isotopically distinct prey type was used in this model. Potential prey items found on Cozumel were supplemented by isotopic data of other plausible prey items from the literature (Herrera et al. 2001, 2002; Hobson et al. 2000; Appendix I). Mean prey values were corrected for the enrichment due to fractionation in predator ratios compared with diet (Ben-David et al. 1997; DeNiro and Epstein 1981; Kline et al. 1993; Tieszen and Boutton 1989). The Euclidean distance between the average corrected isotopic values of predator and prey was then calculated as:

$$z = \sqrt{x^2 + y^2},$$

where x and y represent the Euclidean distance of carbon or nitrogen. The relative contribution (% composition) of each prey to the diet of the raccoon is inversely related to the distance between the corrected isotopic signature of the prey (A′, B′, and C′) and the predator (P′) and is calculated as:

$$\% \text{ X in diet} = PX'^{-1}/(PA'^{-1} + PB'^{-1} + PC'^{-1}) \times 100,$$

where X′ is A′, B′, or C′ and P is the predator's isotopic ratio. One assumption made by this model is that partitioning among food sources is the same for both carbon and nitrogen.

Paired *t*-tests were used to test for differences in isotope ratio values for different tissue types (i.e., blood and hair) and between the different blood components (serum, RBCs, etc.). Tukey's post hoc tests were performed on blood components to identify if some components could be pooled for further analysis. A 1-way analysis of variance (ANOVA) was used to individually test for differences between stable isotope ratios of different age classes, sexes, the 3 trapping sites, and the 3 field seasons. A Tukey test for multiple comparisons was used to determine the origin of significant results of univariate tests (Zar 1999). If no differences existed, data were pooled for further analyses. Two-way ANOVAs were used to test for possible interactions between trap sites and field seasons on stable isotope

ratios. Three-way ANOVAs to examine interactions between site, season, and other variables such as age class or sex could not be used because of sample size constraints.

After calculating the relative contribution of each food item to the diet of each individual using the dual isotope, multiple-source mixing-model, the mean ($\pm SE$) for the entire sample was calculated. A 1-way ANOVA was used to test for differences in the relative contribution in different food items by season and site.

Fecal analysis.—Scat samples were collected using a fecal loop (plastic loop with slotted ends for insertion into the rectum to obtain fecal material) after the animal was anesthetized and were stored frozen or in 10% formalin until analysis. Given the limitations of assessing diet from the small quantities of feces obtained with a fecal loop, these samples were used as complementary independent measurements to substantiate or further constrain interpretations of the isotopic evidence. Scats collected from recaptured individuals on different days were considered separate samples. Before examination, the scats were emulsified for 12–24 h in a mixture of 10 parts ethyl alcohol (95%), 3 parts water, and 1 part general detergent and then sorted manually using a sieve with mesh size of 500 μm. Scats were then dried at 60°C overnight and prey items were identified using a dissecting microscope. Prey items were classified in general categories such as fruit, crabs, vegetation (grasses, plants, and woody forbs), banana (trap bait), vertebrates, and insects. It was not always possible to discriminate between fruit and other plant material. Generally, if fruit was only partially digested, or seeds were present, we categorized this as fruit, whereas leaflike material was categorized as plant material. Data were recorded for each scat as presence or absence of individual food categories.

Prey items are expressed in percentage of occurrence. Chi-square tests were used to examine differences in the percentage occurrence of food items in scats as well as to compare distributions of food items between sites and during the entire study. Frequency of occurrence data were converted to proportional frequency to calculate Levins' index (Levins 1968):

$$B = \left(\sum p_i^2\right)^{-1},$$

where p_i is the proportional use of a food item relative to other food items. B ranges from 1 to n (n = total number of food item categories) and was used to calculate diversity of diets seasonally and at each site. Using Hurlbert's method (Krebs 1989), diversity was standardized (B_s) to a scale of 0.0–1.0:

$$B_s = (B - 1)/(n - 1).$$

Food diversity was calculated using all prey items found in the scat, excluding banana (i.e., bait). Levins' index gives an indication of trophic niche breadth and increases as food habits become more generalized, reaching a value of 1 when all food types are exploited equally (Krebs 1989). The same number of possible prey categories n was used for each site. Values are presented as mean ± SE.

RESULTS

Hair samples.—No significant differences in δ^{13}C and δ^{15}N existed between males (n = 30) and females (n = 33; 1-way ANOVA; P = 0.595 and P = 0.623, respectively) or between age classes of males and females (P = 0.131 and P = 0.543, respectively). Significant differences were found in both δ^{15}N and δ^{13}C isotopic ratios among the 3 trapping sites (P = 0.007 and P < 0.001, respectively; Fig. 1a). Individuals from site 3 had significantly lower δ^{15}N isotope ratios (6.91‰ ± 0.21‰)

than those from sites 1 (8.03‰ ± 0.23‰) and 2 (8.69‰ ± 0.34‰; Tukey's post hoc test; $P = 0.050$ and $P = 0.003$, respectively). All 3 sites differed from one another in $\delta^{13}C$ ratios (Fig. 1a). As with nitrogen, individuals from site 3 had the lowest isotopic ratios (−22.09‰ ± 0.49‰), followed by site 2 (−19.60‰ ± 0.38‰) and site 1 (−17.93‰ ± 0.29‰).

Significant differences were found between $\delta^{15}N$ and $\delta^{13}C$ isotope ratios between the 3 field seasons ($P = 0.001$ and $P < 0.001$, respectively; Fig. 1b). Individual annual differences in nitrogen and carbon isotope ratios from 2001 and 2002 (both wet seasons) did not differ, whereas 2003 (dry season) was significantly lower compared to 2001 (Tukey's post hoc test; $P = 0.001$ and $P = 0.05$, respectively). A 2-way ANOVA indicated a significant interaction between trap site and field season isotope ratios in $\delta^{15}N$ ($F = 4.13$, $d.f. = 7, 63$, $P = 0.021$) and $\delta^{13}C$ ($F = 16.91$, $d.f. = 2, 64$, $P = 0.001$).

Blood samples.—Only blood samples from the 2003 season were used for statistical analyses because of a freezer breakdown. We tested the effect of extended time at room temperature on 7 unaffected blood samples from 2003 by comparing the carbon and nitrogen isotopic values of fresh blood with a subsample that was left at room temperature for a 2-week period. No significant change in $\delta^{15}N$ was found between the fresh and thawed samples (paired t-test; $P = 0.429$), but $\delta^{13}C$ was significantly lower ($P = 0.047$). Therefore, results from only the nitrogen isotopic analysis are presented for 2001–2002 blood samples ($n = 15$). Blood from 28 individuals were sampled for the 2003 field season.

Comparisons between plasma and serum, and between RBCs and clotted cells identified no significant differences in isotopic signatures (paired t-tests; $P > 0.05$). Therefore, plasma and serum were pooled for all further analysis, as were RBCs and clotted cells. Blood plasma–serum $\delta^{15}N$ and $\delta^{13}C$ were significantly lower compared to the cellular fraction by 0.72‰ and 1.45‰, respectively (paired t-tests; $t = -4.03$, $d.f = 19$, $P = 0.001$ and $t = -3.94$, $d.f. = 19$, $P = 0.001$, respectively).

No significant differences were found between males and females for $\delta^{15}N$ and $\delta^{13}C$ isotopes (1-way ANOVA; $P > 0.05$). Differences in $\delta^{15}N$ isotope ratios between the 3 age classes approached statistical significance (1-way ANOVA; $F = 2.96$, $d.f. = 2, 61$, $P = 0.060$); juveniles had slightly higher $\delta^{15}N$ isotope ratios than did older animals.

Significant differences existed between the 3 sites for both $\delta^{15}N$ and $\delta^{13}C$ isotopic ratios (1-way ANOVA; $F = 4.93$, $d.f. = 2, 58$, $P = 0.010$ and $F = 4.55$, $d.f. = 2, 41$, $P = 0.017$, respectively; Fig. 1). A Tukey's post hoc test indicated that site 3 had the lightest (6.88 ± 0.206, $P = 0.050$), and site 2 the heaviest $\delta^{15}N$ isotopic ratios (7.90 ± 0.202, $P = 0.032$). In contrast, site 1 (−18.77 ± 0.674) was significantly heavier in $\delta^{13}C$ isotope ratios (1-way ANOVA; $F = 4.55$, $d.f. = 2, 41$, $P = 0.017$) compared to the other sites (site 2 = −20.99 ± 0.373, site 3 = −21.51 ± 0.742).

A 1-way ANOVA indicated significant differences in $\delta^{15}N$ isotopic ratios ($F = 3.63$, $d.f. = 2, 58$, $P = 0.031$) between years; 2002 was significantly higher in $\delta^{15}N$ isotope ratios (8.88 ± 1.150) compared to 2001 (7.40 ± 0.185; Tukey's post hoc test; $P = 0.031$) and between 2003 (7.50 ± 0.1449; $P =$
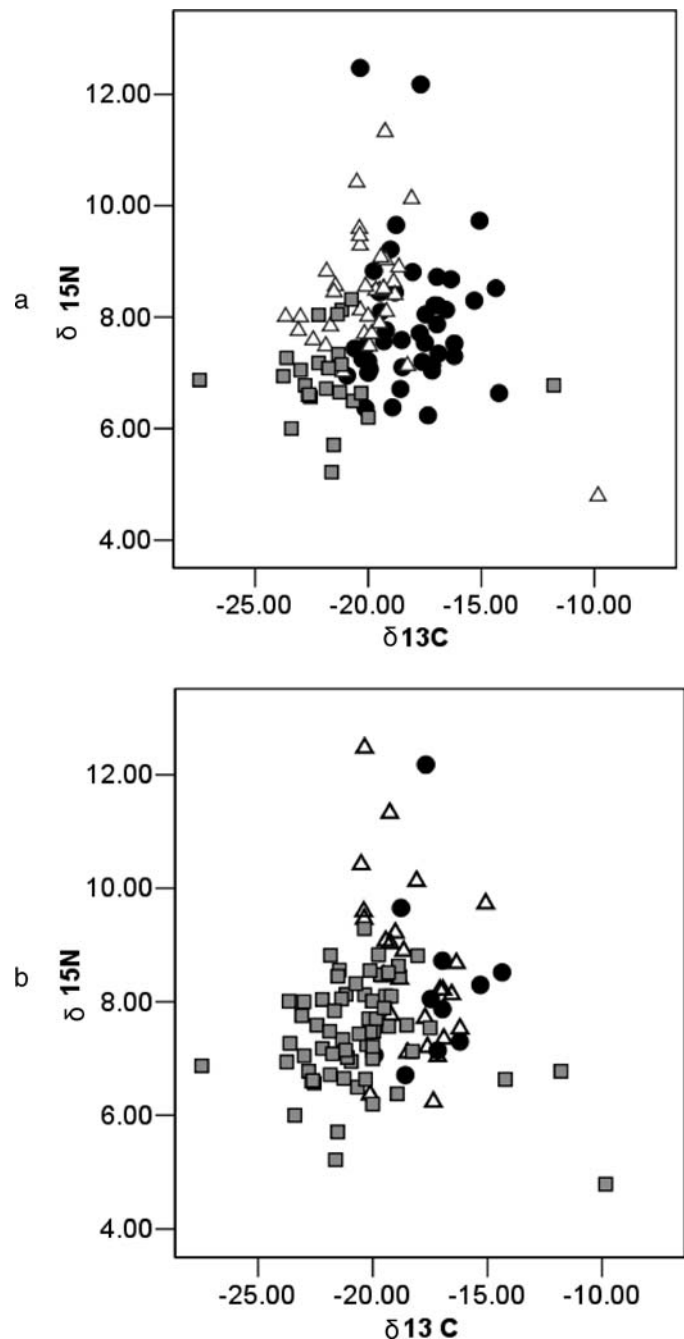

FIG. 1.—Stable carbon and nitrogen isotope ratios in blood or hair samples for *Procyon pygmaeus* sorted by a) 3 sites (site 1 = circles, site 2 = triangles, site 3 = squares) on Cozumel Island, Mexico, 2001–2003, and b) seasons (2001 = wet season, circles; 2002 = wet season, triangles; 2003 = dry season, squares). Each symbol represents mean isotope ratio for the group.

0.030; Fig. 1). A 2-way ANOVA indicated that the interaction between the variables of trap site and year significantly affected $\delta^{15}N$ isotopic ratios ($F = 4.18$, $d.f. = 4, 61$, $P = 0.020$).

Blood versus hair.—Hair had significantly higher $\delta^{15}N$ and $\delta^{13}C$ isotope ratio values compared to plasma–serum (paired t-test; $t = -2.45$, $d.f. = 22$, $P = 0.023$ and $t = -3.05$, $d.f. = 11$, $P = 0.008$, respectively; Table 1). In contrast, $\delta^{15}N$ and $\delta^{13}C$ for hair and RBCs–clotted cells did not significantly differ

TABLE 1.—Values of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) for blood and hair samples of *Procyon pygmaeus* on Cozumel Island, Mexico, over 3 field seasons (2001–2003).

| | Blood | | | | | | Hair | | | | | |
| | $\delta^{15}N$ (‰) | | | $\delta^{13}C$ (‰) | | | $\delta^{15}N$ (‰) | | | $\delta^{13}C$ (‰) | | |
Year	$\bar{X} \pm SE$	n		$\bar{X} \pm SE$	n		$\bar{X} \pm SE$	n		$\bar{X} \pm SE$	n	
2001	7.400 ± 0.185	15					8.318 ± 0.344	11		−17.217 ± 0.572	11	
2002	8.883 ± 1.150	4					8.598 ± 0.352	28		−18.368 ± 0.350	28	
2003	7.501 ± 0.144	42		−21.295 ± 0.840	41		8.105 ± 0.420	62		−18.921 ± 0.438	62	
$\bar{X}$	7.568 ± 0.135	61		−21.295 ± 0.321	24		8.130 ± 0.243	64		−18.910 ± 0.257	62	

($P > 0.05$). To maximize sample size, the average values ($\pm SE$) for $\delta^{15}N$ and $\delta^{13}C$ for hair and RBCs–clotted cells (8.13‰ ± 0.13‰ and −19.11‰ ± 0.28‰) were used for the predator values relating to the mixing models.

Potential prey items.—A total of 52 potential prey items were collected (Appendix I). Fruit and crabs were collected from multiple sites on the island. Stable isotope ratios of immature and mature crabs and those collected in different seasons did not differ significantly (K nearest-neighbor randomization test, $P = 0.381$). Because of small sample size, isotopic differences between fruit species and between crab species were not examined. Therefore, all crab isotopic data were pooled and the mean was used in mixing models and other analyses.

Isotope ratios of all other potential prey types (C_3–C_4 fruit, frogs, lizards, and insects) were distinct (K nearest-neighbor randomization test, $P = 0.045$; Fig. 2). Average carbon and

nitrogen isotope ratios for fruit samples collected from Cozumel were combined with the average $\delta^{15}N$ and $\delta^{13}C$ values for fruit taken from other isotopic studies in Mexico (Herrera et al. 2001, 2002, 2003; Hobson et al. 2000) and these average C and N values were used for the mixing model.

Isotopic mixing models.—Using the dual-isotope, 3-source (crab, insect, and fruit) mixing model, we estimated that crab was consistently a major contribution in the diet of *P. pygmaeus*. Estimated contribution varied from 45% to 54% depending upon season and site (Table 2). Fruit generally made up 25–31% of the diet and the corresponding variance usually was reflected in a change in the proportion of insects. Differences in proportion of fruit in the diet did not significantly vary between the wet and dry seasons. Significant differences were found between the estimated proportion of crab (1-way ANOVA; $F = 6.01$, $d.f. = 2, 20$, $P = 0.004$) and fruit ($F =$
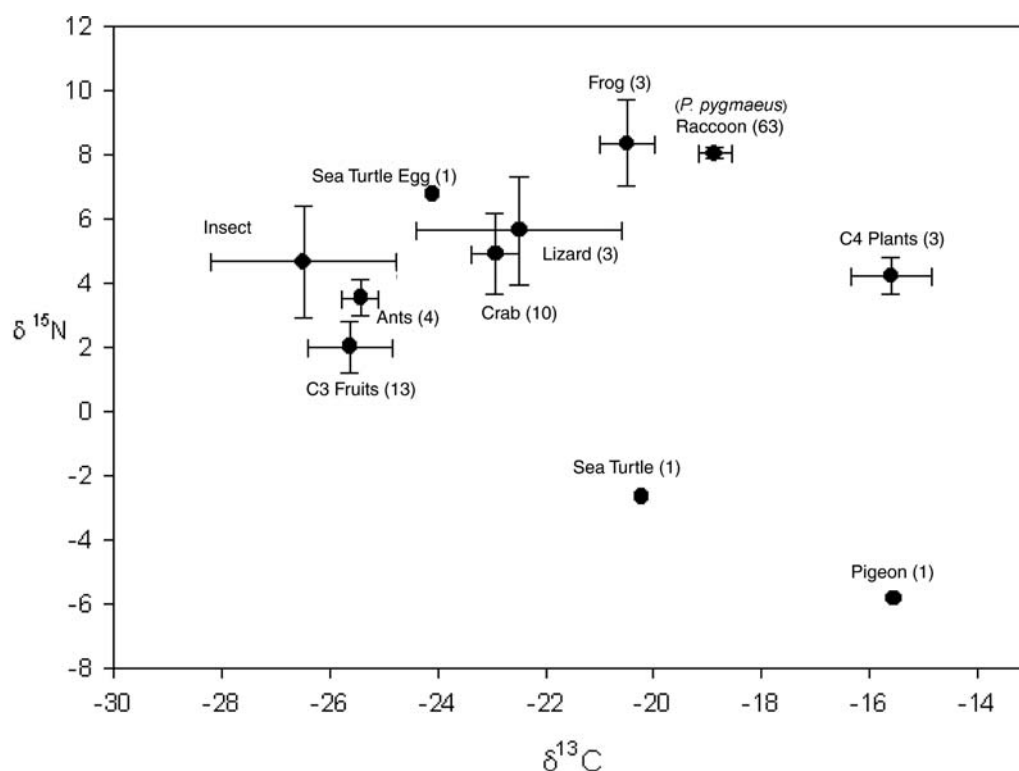

FIG. 2.—Isotopic ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) for pygmy raccoon (*Procyon pygmaeus*) hair samples and potential prey items collected on Cozumel Island, Mexico, 2001–2003, supplemented by values from the literature. Sample size is indicated in parentheses except where data were from the literature. Data for insects are from Herrera et al. (2001), for C_4 plants are from Herrera et al. (2003), and for ants are from Hobson et al. (2000). Symbols indicate mean ± SE.

TABLE 2.—Percentage contribution (mean ± *SE*) of different prey in the diet of *Procyon pygmaeus* on Cozumel Island, Mexico, in different wet and dry seasons of 2001–2003 and at different sites based on stable isotope ratios of raccoon hair, and red blood cells and clotted cells, and derived from mixing models.

	n	Crab	Fruit	Insect
Overall red blood cells and hair	65	48 ± 1.00	29 ± 0.59	23 ± 0.67
Overall plasma and serum	33	51 ± 2.36	29 ± 1.86	19 ± 0.88
2001, wet season	11	45 ± 0.89	30 ± 1.64	25 ± 0.64
2002, wet season	27	48 ± 0.97	28 ± 0.68	23 ± 0.43
2003, dry season	24	50 ± 2.33	29 ± 1.34	21 ± 1.63
Site 1	35	47 ± 0.70	30 ± 0.41	23 ± 0.43
Site 2	17	54 ± 2.12	25 ± 1.19	21 ± 1.00
Site 3	10	47 ± 4.10	31 ± 2.27	22 ± 3.69

10.02, $d.f. = 2, 20, P < 0.001$) consumed at site 2 compared to the other 2 sites (Tukey's post hoc test; site 1–2, $P = 0.001$; site 2–3, $P = 0.017$). When examining the predicted proportion of food items in the diet by year, however, no significant differences were found between years (1-way ANOVA; $P > 0.05$). A 2-way ANOVA was not used to examine for interaction between site and year because of low sample sizes. Using alternative fractionation values (carbon fractionation for invertebrates and insects increased from 1‰ to 2‰, and fruit increased from 2‰ to 2.5‰), we found no significant differences in the proportions of the 3 major food items.

Composition of fecal samples.—Scat analyses ($n = 50$) indicated that the 3 dominant food components (% occurrence) in pygmy raccoon diet were vegetative material (54%), crab (44%), and insects (36%). Fruit and other plant material also were found in a substantial number of scats (Table 3).

Males and females did not vary in proportion of food items found in their scats (χ^2 test; $P > 0.05$). Among age groups, however, subadults consumed fewer insects than did juveniles and adults ($\chi^2 = 8.33, d.f. = 1, P = 0.015$). Percentage occurrences of food also varied by season; insects were more prevalent in scats collected from the wet season (2002) than the dry season (2003; $\chi^2 = 4.66, d.f. = 1, P = 0.031$; Table 2). The other most prevalent food items found in fecal matter, crab and fruit, did not differ significantly between the 2 seasons ($\chi^2 = 0.298, d.f. = 1, P = 0.582$ and $\chi^2 = 0.810, d.f. = 1, P = 0.348$, respectively). The prevalence of fruit and crab in scats varied

between the 3 sites ($\chi^2 = 8.81, d.f. = 1, P = 0.012$ and $\chi^2 = 6.21, d.f. = 1, P = 0.048$, respectively), with fruit being more prevalent at site 3 and crab most prevalent at site 1 (Table 2).

Levins' index (Levins 1968) for food diversity for scats across all seasons was $B_s = 0.75$. Food diversity was higher for scats collected during the wet season (2002; $\bar{X} = 0.725$) than for 2003 ($\bar{X} = 0.60$; Table 2). In addition, site 1 had slightly higher food diversity than site 2.

DISCUSSION

It is widely accepted that generalist foragers cope with changes in food availability by changing their diet to include alternative resources, with these changes in raccoons typically observed on a seasonal basis (Lotze and Anderson 1979). Although we observed some differences in the diet of *P. pygmaeus* in different seasons and locations, the isotopic budget suggests that crabs always provided most of the assimilated protein. We might then classify *P. pygmaeus* as a generalist omnivore that has specialized on feeding on crabs, because this food item represents approximately 50% of the diet. The importance of this prey item is further confirmed by its high rate of occurrence in fecal samples. The occurrence of crab, insect, and fruit as major prey items in our study concurs with previous feeding habit analyses in this species (Navarro and Suarez 1989). The feeding habits of *P. pygmaeus* appear to fall within the range of food items consumed by Central American *P. lotor* (Carrillo et al. 2001).

Different turnover and accretion rates resulted in slightly differing isotopic signatures for plasma–serum (days), RBCs–clotted cells (weeks–months), and hair (up to a year). This likely reflects differences in isotopic fractionation between diet and these tissues. (In carnivores such as dogs [Benson et al. 2000] and bears [Gau and Case 2002], the amino acid composition of erythrocytes and cellular portions of the blood vary.) Although no protein compositional studies have examined differences in amino acid in the blood of *P. lotor*, we expect that these components vary in their composition and consequently their stable isotope signatures in a similar manner.

The molting patterns of *P. pygmaeus* are unknown, but based on the molting patterns of other tropical procyonids (Gompper 1995), *P. pygmaeus* likely molts in summer.

TABLE 3.—Percentage occurrence of food items in *Procyon pygmaeus* scats, by season and site. Levins' index for food diversity for pygmy raccoon scats is indicated in square brackets.

Scat item	2002[a] [0.73]		2003[b] [0.60]		
	Site 1 ($n = 7$) [0.75]	Site 2 ($n = 11$) [0.70]	Site 1 ($n = 9$) [0.57]	Site 2 ($n = 10$) [0.53]	Site 3 ($n = 13$) [0.69]
Crab	57	27	67	20	54
Insect	57	55	11	30	31
Fruit	29	18	18	10	54
Vertebrate	0	9	0	0	0
Leaf or plant	0	0	18	10	23
Woody material	43	73	44	60	46
Grooming hair	0	18	18	30	8

[a] Summer–wet season = April–July.
[b] Winter–dry season = February–March.

Therefore, hair samples collected during the late dry season (February–March) hypothetically represent feeding habits approximately 6–9 months before sampling, whereas samples collected in late summer (July–August) may only reflect recent feeding habits. Because of the discrepancy in the period hair samples reflect, blood (which reflects a period of days to weeks) serves as an independent indicator of diet patterns with relation to time. The relative constancy of the hair and RBC stable isotope ratios suggests that the diet of this species does not radically shift over the course of the year. Pairwise differences between sites and years indicate similar patterns of enrichment (i.e., higher isotopic values) for hair and blood, indicating no large shift in feeding habits over the course of the study. The Levins' food diversity indices (Levins 1968), although constrained by low sample size and broad categorization of food items, also suggest that no significant shifts in feeding habits occur over time or space.

Areas where *P. pygmaeus* were captured were exclusively mangrove forest and wetlands, although some of these areas also bordered sandy beach and deciduous forest. Our expectation that crab would be an important food component in the diet of *P. pygmaeus* was supported by isotopic and fecal data. When developing our isotopic mixing models, we aimed to use ecologically plausible source material. Although our selection of prey items used in this study likely does not represent the absolute breadth of prey items *P. pygmaeus* consumes, the general agreement of 2 independent analytical techniques, stable isotopes and fecal analyses, strengthens our findings and emphasizes the value of a stable isotope approach for assessing dietary habits, especially when access to dietary information via alternative techniques such as scat analyses alone is logistically infeasible.

Stable isotopes from hair and blood revealed a pattern of subtle seasonal and geographic differences in diet (Fig. 1). For instance, carbon isotopic ratio values for the dry season in 2003 were lower compared to ratios from the other 2 field seasons (both wet season). Of the prey examined, higher carbon isotopic ratios correspond to fruit as a prey item. Therefore, the lower carbon during the dry season may correspond to seasonal availability of vegetation (fruit or plant) biomass, which is lower in the dry season in regions of similar landscape (Estrada and Coates-Estrada 2001). Additionally, *P. pygmaeus* may have preferentially consumed other more abundant, yet isotopically lower, prey items during the dry season, when fruit is presumably less abundant. In contrast, nitrogen isotope ratios from 2002 were higher than the other field seasons. Nitrogen isotopes generally reflect the trophic level at which an organism feeds, whereas carbon tends to reflect habitat usage (DeNiro and Epstein 1981). The discrepancy seen between differences in carbon and nitrogen may represent a pulse in use of prey from higher trophic levels (e.g., vertebrates).

Although all *P. pygmaeus* were trapped in and around mangrove forest, we found subtle differences in isotopic ratio values and scat data for each site, and these differences may have influenced results of the mixing models. Differences may be due to proximity to anthropogenic influencing factors. For example, site 1 was within 1 km of a wastewater-treatment facility and site 2 was located on the perimeter of a golf course. Either of these could be sources of isotopically enriched (i.e., higher) nitrogen (Hansson et al. 1997; Rau et al. 1981). Some fraction of the presumably enriched wastewater (site 1) or runoff from the treated greens and proximity to garbage areas (which typically contain enriched forms of carbon and nitrogen [Macko and Ostrom 1994]; site 2) may have found its way into the food chain of the pygmy raccoon. Thus, contribution from any of these sources may have contributed to an overestimate of crab consumption for site 2, particularly in light of the fact that the available scat data do not reflect a similar increase. The change in average isotopic values for this site may have skewed our mixing model toward falsely predicting higher levels of crab consumption because this prey item had the greatest carbon and nitrogen values of the 3 prey items used in our model. Some uncertainty around the proportion estimates from the mixing model is expected because of random measurement errors, variation in isotopic composition among prey individuals, and variation in fractionation and assimilation, and consequently the composition among predator individuals. The mixing models predicted only slight changes in feeding habits in the different years. These differences were not statistically significant and differ from the isotopic and scat analyses, which indicated subtle differences by season and site. Such discrepancy is not unexpected because mixing models serve as a gauge of prey consumption and not the precise proportions of the diet.

Results of stable isotope analyses generally correspond to data found in fecal matter, examination of which indicates that the 3 most prominent food items were crab, fruit, and insects. Because of the highly digested nature of food items in scats, a certain level of error is possible in our categorization of vegetation or fruit found in scats, as it was not possible to ascertain if these remains represent undigested remnants of a whole fruit (i.e., seed or outer shell), or truly woody plant material. The high level of woody and fragmentary plant debris in scats may be due to captured animals consuming such materials while in traps. Nonetheless, examination of both fecal and isotopic data indicates that crabs play an important role in the diet of *P. pygmaeus* and are a major food source during all times of the year. Examination of our data indicates that the diet of *P. pygmaeus* reflects what appears to be a typical generalist procyonid predator that opportunistically uses prey items in its primary habitat and whose dietary preferences are within the range of prey preferences found in other species within this genus (Gehrt 2003; Lotze and Anderson 1979). Although few studies have reported the diet of raccoons in the tropics, one such study from Costa Rica reported an overwhelming dominance in the diet by crabs (up to 94%) and a much lower representation of fruits (about 10%—Carrillo et al. 2001). Seasonal variation in Costa Rica was much greater than that on Cozumel, and this may reflect differences in food item availability on an island compared to the Central American mainland.

Given the great relevance of mangroves not only as habitat for Cozumel's dwarf raccoon and the Central American raccoon, as well as the environmental services provided by this vegetation type, Cozumel, and the tropics in general, would benefit from a robust mangrove conservation policy. This

would result in effective conservation for many species such as the endemic, endangered dwarf raccoon, and in the maintenance of the ecosystem services.

RESUMEN

El conocimiento de la ecología de forrajeo de mamíferos amenazados frecuentemente está basado en datos muy limitados, debido a restricciones logísticas o acceso a los animales, sus contenidos estomacales, o muestras fecales. En este estudio usamos una técnica de isótopos estables para examinar los hábitos alimenticios de un mamífero raro, obteniendo información durante una escala temporal mayor que lo que permitiría un análisis fecal tradicional, y atenuando algunas de las restricciones de tamaños de muestra que pueden limitar estas investigaciones. Estudiamos al mapache pigmeo (*Procyon pygmaeus*), una especie en peligro y endémica de la Isla Cozumel, Mexico. Con base en estudios realizados en zonas templadas, se considera que los mapaches son animales omnívoros, pero se han realizado muy pocos estudios sobre su dieta en los trópicos. Usando muestras de pelo y de sangre obtenidas a lo largo de 3 años (2001–2003) en 3 localidades de la Isla, examinamos los hábitos alimenticios de esta especie usando las proporciones isotópicas de nitrógeno (δ^{15}N) y carbono (δ^{13}C). También analizamos excretas directamente para suplementar y comparar los datos isotópicos. Tanto los datos isotópicos como el análisis de las excretas sugieren una dieta omnívora especializada en cangrejos, que constituyeron >50% de la dieta, seguidos de frutas e insectos. Las muestras de pelo y sangre no mostraron diferencias significativas en las proporciones isotópicas de carbono o nitrógeno y tampoco encontramos variaciones relacionadas con la edad o el sexo. Aunque observamos variaciones sutiles espaciales y temporales, tanto los análisis de isótopos estables como los de las muestras fecales enfatizaron la dominancia de los cangrejos en la dieta a través de ambas escalas.

LITERATURE CITED

ANIMAL CARE AND USE COMMITTEE. 1998. Guidelines for the capture, handling, and care of mammals as approved by the American Society of Mammalogists. Journal of Mammalogy 79:1416–1431.

BEN-DAVID, M., R. T. BOWYER, AND J. B. FARO. 1996. Niche separation by mink and river otters: coexistence in a marine environment. Oikos 75:41–48.

BEN-DAVID, M., R. W. FLYNN, AND D. M. SCHELL. 1997. Annual and seasonal changes in diets of martens: evidence from stable isotope analysis. Oecologia 111:280–291.

BEN-DAVID, M., AND D. M. SCHELL. 2001. Mixing models in analyses of diet using multiple stable isotopes: a response. Oecologia 127:180–184.

BENSON, D. A., I. KARSCH-MIZRACHI, D. J. LIPMAN, J. OSTELL, B. A. RAPP, AND D. L. WHEELER. 2000. GenBank. Nucleic Acids Research Supplement 28:15–18.

BLIGH, E. G., AND W. J. DYER. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37:911–917.

CABRERA, C. E., S. SOUSA, AND V. O. TÉLLEZ. 1982. Imagenes de la flora Quintanarroense. CIQRO-UNAM Press, Puerto Morelos, Quintana Roo, Mexico.

CARRILLO, E., G. WONG, AND M. A. RODRIGUEZ. 2001. Feeding habits of the raccoon (*Procyon lotor*) (Carnivora: Procyonidae) in a coastal, tropical wet forest of Costa Rica. Revista de Biología Tropical 49:1193–1197.

CRAIG, H. 1957. Isotopic standards for carbon and oxygen and correction factors for mass spectrometric analysis of carbon dioxide. Geochimica et Cosmochimica Acta 12:133–149.

CUARÓN, A. D., M. A. MARTÍNEZ-MORALES, K. W. MCFADDEN, D. VALENZUELA, AND M. E. GOMPPER. 2004. The status of dwarf carnivores on Cozumel Island, Mexico. Biodiversity and Conservation 13:317–331.

DENIRO, M. J., AND S. EPSTEIN. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochemica Cosmochimica Acta 42:496–506.

DENIRO, M. J., AND S. EPSTEIN. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochemica Cosmochimica Acta 45:341–351.

ESTRADA, A., AND R. COATES-ESTRADA. 2001. Species composition and reproductive phenology of bats in a tropical landscape at Los Tuxtlas, Mexico. Journal of Tropical Ecology 17:627–646.

GAU, R. J., AND R. CASE. 2002. Grizzly bear studies in the Northwest Territories: nutritional condition of grizzly bears via stable nitrogen signatures and insulin-like growth factor-1. Ursus 13:285–291.

GEHRT, S. D. 2003. Raccoon (*Procyon lotor* and allies). Pp. 661–634 in Wild mammals of North America (G. A. Feldhamer, B. C. Thompson, and J. A. Chapman, eds.). Johns Hopkins University Press, Baltimore, Maryland.

GOMPPER, M. 1995. *Nasua narica*. Mammalian Species 487:1–10.

GRAU, G. A., G. C. SANDERSON, AND J. P. ROGERS. 1970. Age determination of raccoons. Journal of Wildlife Management 34:364–372.

HANSSON, S., J. E. HOBBIE, R. ELMGREN, AND U. LARSSON. 1997. The stable nitrogen isotope ratio as a marker of food-web interaction and fish migrations. Ecology 78:2249–2257.

HERRERA, L. G., E. GUTIERREZ, K. A. HOBSON, B. ALTUBE, W. G. DIAZ, AND V. SANCHEZ. 2002. Sources of assimilated protein in five species of New World frugivorous bats. Oecologia 133:280–287.

HERRERA, L. G., K. A. HOBSON, D. ESTRADA, A. MANZO, G. MENDEZ, AND V. SANCHEZ-CORDERO. 2001. The role of fruits and insects in the nutrition of frugivorous bats: evaluating the use of stable isotope models. Biotropica 33:520–528.

HERRERA, L. G., K. A. HOBSON, M. RODRIGUEZ, AND P. HERNANDEZ. 2003. Trophic partitioning in trophic rain forest birds: insights from stable isotope analysis. Oecologia 136:439–444.

HILDERBRAND, G. V., S. D. FARLEY, C. T. ROBBINS, T. A. HANLEY, K. TITUS, AND C. SERVHEEN. 1996. Use of stable isotopes to determine diets of living and extinct bears. Canadian Journal of Zoology 74: 2080–2088.

HILTON-TAYLOR, C. 2001. IUCN Red List categories and criteria: version 3.1 IUCN. International Union for the Conservation of Nature and Natural Resources, Gland, Switzerland.

HOBSON, K. A., B. MCLELLAN, AND J. WOODS. 2000. Using stable carbon and nitrogen isotopes to infer trophic relationships among black and grizzly bears in the Upper Columbia River basin, British Columbia. Canadian Journal of Zoology 78:1332–1339.

KELLY, J. F. 2000. Stable isotope of carbon and nitrogen in the study of avian and mammalian trophic ecology. Canadian Journal of Zoology 78:1–27.

KLINE, T. C., J. J. GOERING, O. A. MATHISEN, P. H. POE, P. L. PARKER, AND R. S. SCALAN. 1993. Recycling of elements transported upstream by runs of Pacific salmon: II. Stable carbon and nitrogen evidence in the Kvichak River watershed, Bristol Bay, southwestern Alaska. Canadian Journal of Fisheries and Aquatic Science 50: 2350–2365.

KNOWLES, R., AND T. H. BLACKBURN. 1993. Nitrogen isotope techniques. Academic Press, London, United Kingdom.

KREBS, C. J. 1989. Niche overlap and diet analysis. Pp. 654–670 in Ecological methodology (C. J. Krebs, ed.). Harper & Row, New York.

LEVINS, R. 1968. Evolution in changing environments. Princeton University Press, Princeton, New Jersey.

LOTZE, H. H., AND S. ANDERSON. 1979. Procyon lotor. Mammalian Species 119:1–8.

MACKO, S. A., AND N. E. OSTROM. 1994. Pollution studies using stable isotopes. Pp. 45–62 in Stable isotopes in ecology and environmental science (K. Lajitha and M. Michener, eds.). Blackwell Scientific Publications, Oxford, United Kingdom.

MARTINEZ-MORALES, M. 1999. Conservation status and habitat preferences of the Cozumel curassow. Condor 101:14–20.

MCCONNAUGHEY, T., AND C. P. MCROY. 1979. Food-web structure and the fractionation of carbon isotopes in the Bering Sea. Marine Biology 53:257–262.

MCFADDEN, K. W. 2004. The ecology, evolution, and natural history of the dwarf carnivores of Cozumel Island, Mexico. Ph.D. dissertation, Columbia University, New York.

NAVARRO, D. L., AND M. SUAREZ. 1989. A survey of the pygmy raccoon (Procyon pygmaeus) of Cozumel, Mexico. Mammalia 53: 458–461.

RAU, G. H., R. E. SWEENEY, A. J. MEARNS, AND D. R. YOUNG. 1981. Differences in animal ^{13}C, ^{15}N and D abundance between a polluted site and an unpolluted site: likely indicators of sewage uptake by a marine food web. Estuarine Coastal and Shelf Science 13: 701–707.

ROTH, J. D., AND K. A. HOBSON. 2000. Stable carbon and nitrogen isotopic fractionation between diet and tissue of captive red fox: implications for dietary reconstruction. Canadian Journal of Zoology 78:848–852.

SCHILLING, M. F. 1986. Multivariate two-sample tests based on nearest neighbors. Journal of the American Statistical Association 81: 799–805.

SEMARNAT. 2002. Normal Oficial Mexicana NOM-059-ECOL-2001, protección ambiental—especies nativas de México de flora y fauna silvestres—categorías de riesgo y especificaciones para su inclusión, exclusión o cambio. Pp. 1–81 in Lista de especies en riesgo. Secretaría de Medio Ambiente y Recursos Naturales, México City, México.

TÉLLEZ, V. O., AND E. F. CABRERA. 1987. Listados floristicos de Mexico—VI. Instituto de Biología, Universidad Nacional Autónoma de México, México City, Distrito Federal.

TIESZEN, L. L., AND T. W. BOUTTON. 1989. Stable carbon isotopes in terrestrial ecosystem research. Pp. 167–195 in Stable isotopes in ecological research (P. W. Rundel, J. R. Ehleringer, and K. A. Nagy, eds.). Springer-Verlag, New York.

TIESZEN, L. L., T. W. BOUTTON, K. G. TESDAHL, AND N. A. SLADE. 1983. ^{13}C analysis of diet. Oecologia 57:32–37.

VOGEL, J. C. 1978. Isotopic assessment of the dietary habits of ungulates. South African Journal of Science 74:298–459.

ZAR, J. 1999. Biostatistical analysis. 4th ed. Prentice Hall, Inc., Upper Saddle River, New Jersey.

APPENDIX I

Potential prey items analyzed from 3 sites on Cozumel Island, over 3 field seasons (2001–2003). Isotopic values for prey item were also taken from the literature, as indicated by superscripts: [1]Herrera et al. (2001), [2]Hobson et al. (2000), and [3]Herrera et al. (2003).

Prey item	Taxon	n
Crab	Coenobita clypeatus	4
	Uca	3
	Ocypode quadrata	3
Sea turtle hatchling	Caretta caretta	1
Sea turtle egg	Unknown	1
Frog	Bufo marinus	3
Lizard	Ctenosaura similis	2
	Iguana iguana	1
Fruit	Alibertia edulis	3
	Jacquinia	4
	Terminalia catappa	4
	Coccoloba	2
Pigeon	Columba	1
Insects[1]	Blattaria	3
	Coleoptera	31
	Diptera	14
	Hemiptera	8
	Homoptera	3
	Hymenoptera	7
	Lepidoptera	56
	Mantodea	1
	Megaloptera	1
	Orthoptera	6
	Trichoptera	20
Ants[2]	Formicidae	4
Fruits[3]	Piper pletata	16
	Piper auritum	16
	Ficus yoponensis	16
	Cecropia obtusifolia	16