

Food Web Linkages Demonstrate Importance of Terrestrial Prey for the Threatened California Red-Legged Frog

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ABSTRACT.—Restoration efforts are ongoing to protect the threatened California Red-Legged Frog (*Rana draytonii*) from further decline, but conserving species that have aquatic and terrestrial life stages can be challenging. For example, although it is clear that aquatic habitat must be protected for breeding, less is known about the importance of terrestrial habitat. Food web linkages remain largely unknown for this species yet would offer insight on the source of prey items and, thus, the importance of each type of habitat. We used three methods to analyze food web linkages for the California Red-Legged Frog: 1) stable isotopes collected from frogs and key species in their ecosystem; 2) stomach contents sampled from live frogs in the wild; and 3) stomach contents sampled from museum specimens. The stable isotope mixing model found 99.7% of *R. draytonii* diet came from terrestrial prey. Wet and dry season stomach content samples flushed from captured frogs had 90% terrestrial prey, and museum specimens contained 82% terrestrial prey. These data suggest that conservation efforts should protect riparian and upland habitats adjacent to aquatic habitats in addition to aquatic habitats.

Amphibians are the most threatened class of vertebrates with more than one-third of species threatened with extinction (Stuart et al., 2004; Hussain and Pandit, 2012). Factors contributing to declines include disease, habitat loss, climate change, introduced species, and pollution (Blaustein and Wake, 1990; Halliday, 1998; Wake and Vredenburg, 2008). Well-documented amphibian declines are prominent in California (Jennings and Hayes, 1994; Vredenburg et al., 2008; Davidson et al., 2012). Of particular interest among declining amphibians in California is the California Red-Legged Frog (*Rana draytonii*), which has been extirpated from at least 70% of its former range and became federally listed as threatened in 1996 (U.S. Fish and Wildlife Service, 1996). The California Red-Legged Frog is a focal species in habitat restoration projects and mitigation programs associated with conservation efforts throughout the San Francisco Bay area (Berkeley Economic Consulting, 2010). Understanding the ecology of *R. draytonii*, and more specifically its diet and role in food webs, is important in determining the most efficient and effective way to protect this species and its habitat.

Breeding habitat has received disproportionate management attention historically (U.S. Fish and Wildlife Service, 2002), but nonbreeding areas might be equally important because adult *R. draytonii* typically only spend a short time at breeding sites. Radio-tracking studies reveal that *R. draytonii* movement and migration in terrestrial habitat varies among sites and individual frogs (Bulger et al., 2003; Fellers and Kleeman, 2007). Understanding how frogs use the terrestrial habitat and its importance for foraging could help inform best management practices for current populations and optimum reserve design for restoration projects.

The California Red-Legged Frog Recovery Plan outlines the necessary actions for recovery, including to “conduct research to better understand the ecology of the California Red-Legged Frog” (U.S. Fish and Wildlife Service, 2002). Information on diet and food web linkages for *R. draytonii* is limited. More specifically, the relative importance of terrestrial prey in *R. draytonii* diet and the importance of upland terrestrial habitats for foraging are based on sparse data. Hayes and Tennant (1985) examined the gut contents of 35 *R. draytonii* from Santa Barbara,

San Bernardino, and Los Angeles Counties and found over 42 taxa consumed. Most prey items were invertebrates, but larger frogs also consumed vertebrates such as Pacific Chorus Frogs (*Pseudacris regilla*) and California Mice (*Peromyscus californicus*). Hayes and Tennant’s study (1985) is the only one to demonstrate that *R. draytonii* feed on terrestrial prey. Additionally, a few reports exist of *R. draytonii* feeding on other terrestrial and aquatic vertebrates including juvenile Common Garter Snakes (*Thamnophis sirtalis*), Western Toad tadpoles (*Bufo boreas*), California Voles (*Microtus californicus*), and Western Harvest Mice (*Reithrodontomys megalotis*) (Hayes et al., 2006; Davidson, 2010; Stitt and Seltenrich, 2010).

Stable isotope analysis is an important tool used in food web studies to help identify the primary sources and trophic levels of particular species (Szepanski et al., 1999; Kupfer et al., 2006; Najera-Hillman et al., 2009; Ikeda et al., 2010). Differences exist among the carbon stable isotope signatures of different types of primary producers (Fry and Sherr, 1984; Peterson and Fry, 1987). The isotopic differences in the primary food sources continue to be reflected in the animals that feed on the primary sources as well as the animals at the top of the food web (DeNiro and Epstein, 1978, 1981). Carbon stable isotopes have been used in a number of studies to separate aquatic and terrestrial energy (Szepanski et al., 1999; Kupfer et al., 2006; Zeug and Winemiller, 2008; Willson et al., 2010). Post (2002) showed that littoral and pelagic primary sources have lower carbon signatures as lake size decreases. The ponds used in this study are smaller than 1 ha, which would indicate that the aquatic primary sources would have lower carbon signatures than the terrestrial sources because of the sources of dissolved organic carbon that fuel production in small lakes (Post, 2002). Additionally, Peterson and Fry (1987) found that soil organic matter and leaves from terrestrial plants have an average $\delta^{13}\text{C}$ signatures of -26% and -28% , respectively, and particulate organic matter in lakes has an average carbon signature of -35% $\delta^{13}\text{C}$. Therefore, in this study terrestrial sources are expected to have higher carbon signatures than aquatic signatures. While carbon isotopic signatures can be used to identify the sources of primary production supporting consumers, nitrogen stable isotopes can be used to estimate the trophic level at which an animal feeds (Kupfer et al., 2006; Najera-Hillman et al., 2009). For each trophic level increase, the $\delta^{15}\text{N}$ signatures are expected to increase on average by 3.4‰, and the $\delta^{13}\text{C}$ signatures are

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DOI: 10.1670/12-288

expected to increase on average by 0.5‰ because of fractionation (Vander Zanden and Rasmussen, 2001; Post, 2002; McCutchan et al., 2003). Stable isotope fractionation is the alteration of the distribution of stable isotopes based on a chemical or physical process (Peterson and Fry, 1987).

The focus of this study was to elucidate the food web for the California Red-Legged Frog using stable isotopes, and stomach contents from live and preserved specimens. The study was also aimed at answering the following questions: 1) How important is terrestrial prey? and 2) Does frog diet differ seasonally? We used stable isotopes to investigate food webs of the aquatic and terrestrial habitats associated with *R. draytonii*. We also stomach flushed live frogs and dissected museum specimens to determine the specific prey that *R. draytonii* consume during different times of the year (Solé et al., 2005; Mahan and Johnson, 2007). We used these complimentary techniques of stable isotope analysis and stomach flushing, to determine the long-term composition of the diet and provide snapshot representations of prey to demonstrate the relative importance of terrestrial and aquatic habitats for *R. draytonii*.

MATERIALS AND METHODS

Study Site.—Golden Gate National Recreation Area is a National Park unit in Marin, San Francisco, and San Mateo Counties. Mori Point and Milagra Ridge are within Golden Gate National Recreation Area in the city of Pacifica, San Mateo County. We collected samples from a human-made pond at Milagra Ridge (Milagra Pond), two human-made restoration ponds (Willow Pond and Wetland Pond), and one creek (Sanchez Creek) at Mori Point. We selected the sites on the bases of accessibility and known presence of relatively large populations of *R. draytonii*. We collected samples between November 2009 and August 2010.

Stable Isotope Collections.—Samples collected from all four sites were used to conduct food web analyses. We used stable carbon isotopes ($\delta^{13}\text{C}$) to assess energy sources and stable nitrogen isotopes ($\delta^{15}\text{N}$) to measure trophic position.

Muscle tissue is most often used in stable isotope food web studies, but removing muscle tissue from amphibians can be lethal. Because of the federally threatened status of the California Red-Legged Frog (U.S. Fish and Wildlife Service, 1996), we used tissue from toe clips, a standard technique for obtaining tissue from anurans (Leyse et al., 2003). Isotopic analysis of toe versus muscle tissues has revealed no significant differences in isotopic analysis in another anuran species, *Lithobates catesbeianus* (Finlay and Vredenburg, 2007). One phalange of digit IV from one of the forefeet was collected from 32 different adult and subadult *R. draytonii* caught in the dry season after which the frogs were immediately released. Tail tissue from larvae was also collected from 30 *P. regilla*, 7 California Newt (*Taricha torosa*), and 27 *R. draytonii* in June 2010. Tissue from other species, in addition to *R. draytonii*, was collected to validate the ability of stable isotopes in discriminating among trophic groups and terrestrial and aquatic origins. Larvae were caught with a dipnet, and approximately 5 mm² of their tails was removed after which they were immediately released. We kept all toe and tail tissues chilled in a cooler in the field before freezing them at -29°C within 6 h of collection.

We used a sweepnet and dipnet to collect terrestrial and aquatic invertebrates at, and surrounding, each pond. All invertebrates were separated to family or order and kept alive

for 24 h before samples were frozen. The invertebrate samples were collected from April through July 2010. We could not determine the stable isotope signature for 18 of the 37 taxa found in *R. draytonii* stomachs, including *P. regilla* adults, because these species were not present during the sample collection period of April through July.

Stomach Flushing.—We collected California Red-Legged Frog specimens by hand, dipnet, or pit fall traps during two main sampling periods: November 2009 to February 2010 (wet) and June 2010 to August 2010 (dry). In the wet season, 14 frogs were captured at Mori Point and 47 at Milagra Ridge. In the dry season, 34 frogs were captured at Mori Point and 3 at Milagra Ridge. Frogs were caught both during the day and at night depending on their site-specific visibility. Pitfall traps were left open overnight and checked in the morning. For each frog caught, their location, mass, and snout–urostyle length (SUL) were recorded. We stomach flushed each frog immediately after capture using a 60-ml syringe attached to tubing and filled with distilled water (Solé et al., 2005). Individuals were flushed two to five times to ensure the entire stomach contents were collected. If a frog continued to regurgitate stomach contents after two flushes, we continued to flush the frog until no contents were visible, up to a maximum of five flushes. Frogs were then held in a bucket of water and observed for approximately 5 min before being released at the location of capture. We observed all frogs to jump or swim away after being released, and no injuries or deaths were observed as a result of stomach flushing.

Museum Collections.—Sixty California Red-Legged Frogs from the collections at the California Academy of Sciences (San Francisco) were dissected to remove their stomach contents (Appendix 1). Only frogs from the San Francisco Bay area were used; most of the specimens were from San Mateo County. The specimens were collected from 1935–2008. Specimens were divided into wet season (November through May) and dry season (June through October) samples for analysis.

Analysis of Stable Isotope Samples.—We thawed all samples, rinsed with milliQ distilled water, dried in an oven at 45°C for 48 h, ground into a fine powder with a mortar and pestle, and then packed into 5×9 mm tin boats. Each boat contained 1–2 mg; if there was <1 mg, then multiple individuals of the same taxon were pooled to achieve at least 1 mg. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were calculated for each sample at the University of California, Berkeley with a Vario ISOTOPE cube elemental analyzer (Elementar, Hanau, Germany) interfaced with an IsoPrime100 mass spectrometer (IsoPrime Ltd., Manchester, UK). Isotopic results are expressed as δ values in per mil (‰), $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = 1,000 ([R_{\text{sample}}/R_{\text{standard}}] - 1)$. R_{sample} and R_{standard} are the $^{13}\text{C} : ^{12}\text{C}$ or $^{15}\text{N} : ^{14}\text{N}$ ratios of the sample and standard. The standards are Vienna-Pee Dee Belemnite limestone (V-PDB) for carbon and atmospheric N_2 for nitrogen.

The data were combined into four prey groups of terrestrial herbivores, terrestrial detritivores, terrestrial carnivores, and aquatic invertebrates, and standard errors were calculated. The invertebrates were grouped according to where they were collected and what their diet is thought to be (Table 1). Only invertebrate taxa found in frog stomachs during flushing were included in these prey groups. We grouped aquatic invertebrates together because the herbivores, detritivores, and carnivores had similar $\delta^{15}\text{N}$ signatures; thus, separate trophic levels could not be distinguished. The prey groups were corrected for fractionation by adding 0.5‰ to the $\delta^{13}\text{C}$ signatures (McCutchan et al., 2003) and 3.4‰ to the $\delta^{15}\text{N}$ signatures (Vander Zanden and Rasmussen, 2001; Post, 2002)

TABLE 1. Nitrogen and carbon stable isotope signatures.

Feeding group	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	Sample size	SE $\delta^{15}\text{N}$	SE $\delta^{13}\text{C}$
Terrestrial carnivore	10.11	-26.54	10	1.32	0.3
Araneae	8.61	-27.39	6		
Carabidae	10.82	-26.36	1		
Coccinellidae	7.53	-26.44	2		
Ichneumonidae	13.49	-25.98	1		
Terrestrial herbivore	7.08	-28.18	12	0.98	0.59
Aphidae	6.58	-28.90	3		
Cercopidae	8.77	-28.47	3		
Cicadellidae	8.45	-26.44	2		
Lepidoptera larvae	4.52	-28.92	4		
Terrestrial detritivore	5.51	-26.08	8	0.84	0.45
Formicidae	4.27	-26.40	1		
Isopod	3.15	-24.29	1		
Muscidae	9.71	-27.50	1		
Oligochaeta	5.25	-26.15	1		
Pulmonata	4.52	-26.13	1		
Rhaphidophoridae	4.47	-27.30	1		
Tipulidae	7.19	-24.79	2		
Aquatic invertebrates	9.49	-32.69	7	0.29	1.31
Amphipoda	9.13	-34.24	1		
Gerridae	10.09	-28.78	3		
Notonectidae	9.85	-33.80	1		
Aquatic Pulmonata	8.88	-33.93	2		
<i>R. draytonii</i> post-metamorphs	11.36	-25.81	21	0.30	0.25
<i>P. regilla</i> tadpoles	8.13	-35.2	5	0.56	1.56
<i>R. draytonii</i> tadpoles	10.55	-34.85	4	1.58	0.56
<i>T. torosa</i> tadpoles	11.48	-31.11	2	0.19	0.11

and entered into IsoSource, a mixing model (Phillips and Greg, 2003). The IsoSource mixing model uses the stable isotope signatures of the study organism and prey groups (sources) to determine the importance of each prey group in the study organism's diet by calculating a percent range of each prey group's contribution. All possible combinations of each source contribution (0–100%) are examined by the mixing model in small increments (1%). Combinations of that sum to *R. draytonii* isotopic signature within a small tolerance are considered to be feasible solutions, from which the frequency and range of potential source contributions can be determined. The number of combinations to examine increases with more sources, according to the formula: #combinations = $([100/i] + (s - 1))$,

where i = increment (%) and s = number of sources.

Analysis of Stomach Contents.—We used the same procedure to analyze stomach contents obtained by stomach flushing or dissection. Each whole or partial prey item in a sample was identified to family or the most specific possible taxonomic level. The frequency of occurrence (F) (number of individuals that have a particular taxon in their stomach) and the number of prey (N) were determined for wet and dry season samples. We performed a chi-square test to determine whether the higher proportion of terrestrial prey was significantly different than the proportion expected by chance.

RESULTS

Stable Isotope Analysis.—Aquatic and terrestrial prey had distinct $\delta^{13}\text{C}$ values, with aquatic samples consistently lower or less enriched in their $\delta^{13}\text{C}$ values than terrestrial samples (Table 1, Fig. 1). *Rana draytonii* tadpoles were much lower in $\delta^{13}\text{C}$ values (-34.9 ± 0.6) than post-metamorphic *R. draytonii* (-25.8 ± 0.3). *Rana draytonii* post-metamorphic samples (11.4 ± 0.3) had higher $\delta^{15}\text{N}$ signatures than the terrestrial prey groups. *Taricha torosa* larvae are carnivorous and had the most enriched nitrogen signature for the aquatic samples (11.5 ± 0.2) (Petranka, 1998).

Terrestrial carnivores had the most significant contribution to post-metamorphic *R. draytonii* diet in the mixing model (range: 50–55%, avg: $52.4\% \pm 1.4$). Terrestrial detritivores had the next most significant contribution to post-metamorphic *R. draytonii* diet (range: 43–48%, avg: $45.7\% \pm 1.4$). Terrestrial herbivores and aquatic invertebrates had very small contributions to post-metamorphic *R. draytonii* diet (range: 0–4%, avg: $1.6\% \pm 1.4$; and range: 0–1%, avg: $0.3\% \pm 0.4$, respectively).

Stomach Flushing.—We captured and stomach flushed 98 *R. draytonii* from November 2009 to August 2010 (61 wet, 37 dry). From these individuals, 14 either had empty stomachs, or a diet sample could not be obtained. Of the 84 diet samples (53 wet, 31 dry), 59 had identifiable prey items (36 wet, 23 dry), and the other 25 samples (17 wet, 8 dry) had digested unidentifiable material, plant material, or both. In the 59 samples, we identified 349 total individual prey items (118 wet, 231 dry, mean = 5.9 per frog, SE = 1.3; wet season mean = 3.3 per frog, SE = 0.5, range = 1–11; dry season mean = 10.0 per frog, SE = 3.2, range = 1–66, Table 2). Terrestrial prey comprised 87.3% of the wet season prey items, 91.3% of the dry season prey items, and 90.0% of all prey items.

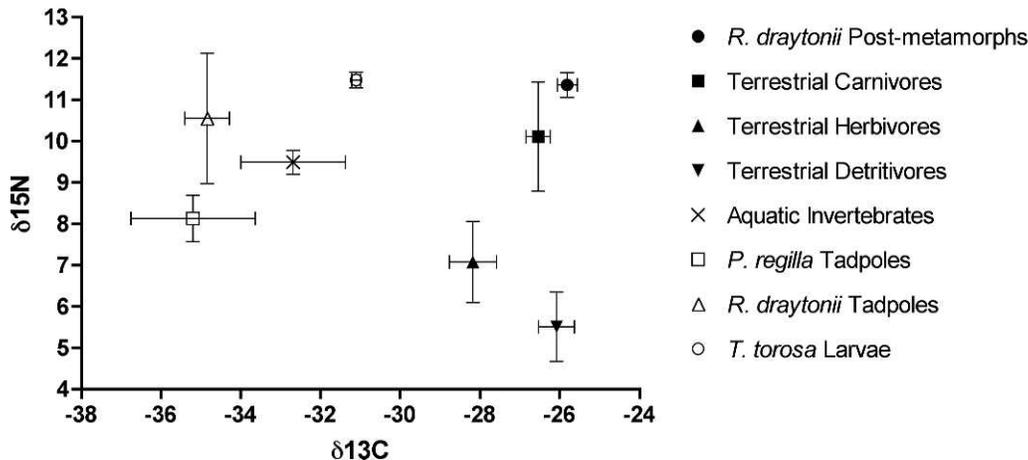


FIG. 1. Stable isotope signatures (\pm SE) of possible prey groups, *Rana draytonii* post-metamorphs, and three species of amphibian larvae.

TABLE 2. List of total number of prey items (*N*) and frequency of occurrence (*F*) from stomach flushing. The sites where each prey item was observed are listed as M (Milagra Pond), S (Sanchez Creek), We (Wetland Pond), and Wi (Willow Pond).

Order or subclass	Family	<i>N</i>	<i>F</i>	Summer <i>N</i>	Summer <i>F</i>	Winter <i>N</i>	Winter <i>F</i>	Site	
Archaeognatha	Machilidae	3	2	0	0	3	2	M	
Coleoptera	Dermestidae	1	1	1	1	0	0	Wi	
	Coccinellidae	1	1	1	1	0	0	Wi	
	Cantharidae	14	1	14	1	0	0	S	
	Carabidae	3	2	1	1	2	1	S, We	
	Staphylinidae	2	2	1	1	1	1	M, S	
	Unidentified Coleoptera larvae	7	4	4	1	3	3	M, Wi	
	Unidentified Coleoptera	7	7	3	3	4	4	M, S, We, Wi	
	Collembola	Sminthuridae	1	1	0	0	1	1	M
	Isotomidae	5	5	0	0	5	5	M, We	
	Entomobryidae	3	2	0	0	3	2	M	
Dermaptera	Forficulidae	2	2	1	1	1	1	S, We	
Diptera	Drosophilidae	2	2	1	1	1	1	M, S	
	Anthomyiidae	1	1	1	1	0	0	S	
	Culicidae	2	2	2	2	0	0	S, Wi	
	Sepsidae	1	1	1	1	0	0	Wi	
	Muscidae	10	6	10	6	0	0	M, S, We, Wi	
	Chironomidae	8	8	5	5	3	3	M, We, Wi	
	Tipulidae	7	5	7	5	0	0	M, We, Wi	
	Calliphoridae	2	2	2	2	0	0	S	
	Culicidae larvae	2	2	2	2	0	0	M, Wi	
	Tipulidae larvae	3	3	1	1	2	2	M, We	
	Unidentified Diptera larvae	2	2	1	1	1	1	M, We	
	Unidentified Diptera	9	8	7	6	2	2	M, S, We, Wi	
	Hemiptera	Cercopidae	1	1	1	1	0	0	Wi
		Cicadellidae	2	2	2	2	0	0	We
		Aphidae	107	5	106	4	1	1	S, We
Notonectidae		1	1	0	0	1	1	We	
Gerridae		1	1	1	1	0	0	M	
Unidentified Hemiptera		1	1	1	1	0	0	S	
Hymenoptera		Formicidae	7	2	6	1	1	1	M, S
	Ichneumonidae	18	2	18	2	0	0	S, Wi	
	Unidentified Hymenoptera	2	2	2	2	0	0	Wi	
Lepidoptera	Noctuidae	1	1	0	0	1	1	S	
	Lepidoptera larvae	11	5	0	0	11	5	M	
Orthoptera	Rhaphidophoridae	1	1	0	0	1	1	M	
	Unidentified Orthoptera	1	1	0	0	1	1	M	
Amphipoda	Unidentified Amphipoda	14	2	14	2	0	0	Wi	
Isopoda	Unidentified Isopoda	4	4	0	0	4	4	M	
Araneae	Unidentified Araneae	17	14	10	9	7	5	M, S, We, Wi	
Chilopoda	Unidentified Chilopoda	3	3	0	0	3	3	M	
Diplopoda	Unidentified Diplopoda	6	5	0	0	6	5	M, We	
Pulmonata	Unidentified Pulmonata	7	5	1	1	6	4	M, We, Wi	
Hirudinea	Unidentified Hirudinea	1	1	1	1	0	0	S	
Oligochaeta	Unidentified Oligochaeta	31	17	0	0	31	17	M, We	
Anura	<i>P. regilla</i>	5	5	0	0	5	5	M, We	
Unidentified		9	9	2	2	7	7	M, S, We, Wi	
Total		349	162	231	72	118	90		

The other prey items were aquatic. The identifiable prey consisted of significantly more terrestrial prey than aquatic prey ($P < 0.001$). The average SUL of the 59 *R. draytonii* was 73.0 mm (range: 47.4–90 mm). Five of the prey items were vertebrates (*P. regilla*); the average SUL of the frogs that ate vertebrates was 78.4 mm (range: 69.5–86 mm). The most numerous prey order for all samples was Hemiptera (true bugs) with 32.4% of prey items. Hemiptera was also the most numerous prey order in the dry season samples (48.1%), whereas Oligochaeta (earthworms) was the most numerous taxon in wet season samples (26.3%). The order with the highest frequency of occurrence for all samples regardless of season was Diptera (flies, 25.9%). Diptera also had the highest frequency of occurrence in dry season samples (48.5%), whereas Oligochaeta had the highest frequency of occurrence in wet season samples (18.9%).

Museum Specimens.—We dissected 60 *R. draytonii* from the collections of California Academy of Science. Seven of the frogs had empty stomachs (5 wet, 2 dry), and 15 other frogs (10 wet, 5

dry) had digested unidentifiable material, plant material, or both. We found identifiable prey in 38 of 60 stomachs (28 wet, 10 dry). In the 38 samples, 240 total individual prey items were extracted (mean = 6.3 per frog, SE = 1.1, range = 1–29; 176 wet season prey items, wet season mean = 6.3 per frog, SE = 1.3; 64 dry season prey items, dry season mean = 6.4 per frog, SE = 2.3, Table 3). Terrestrial prey comprised 78.4% of the wet season prey items, 92.2% of the dry season prey items, and 82.1% of the total number of prey items. The other prey items were aquatic. The identifiable prey consisted of significantly more terrestrial prey than aquatic prey ($P < 0.001$). The average SUL of the 38 *R. draytonii* was 62.9 mm (range: 31–108.5 mm). Four of the prey items were vertebrates (*P. regilla*); the three *R. draytonii* with vertebrates in their stomachs had an average SUL of 83.9 mm (range: 69.5–96.6 mm). The most numerous prey order for all samples was Diptera (26.3% of prey items). Diptera and Coleoptera (beetles) each comprised 25% of the wet season prey items. Hymenoptera comprised 32.3% and Diptera 30.6% of the

TABLE 3. List of total number of prey items (N) and frequency of occurrence (F) from museum specimens.

Order or subclass	Family	N	F	Summer N	Summer F	Winter N	Winter F	
Coleoptera	Dermestidae	3	3	0	0	3	3	
	Coccinellidae	8	2	4	1	4	1	
	Attelabidae	1	1	0	0	1	1	
	Cantharidae	1	1	0	0	1	1	
	Carabidae	27	10	5	3	22	7	
	Staphylinidae	1	1	0	0	1	1	
	Tenebrionidae	1	1	0	0	1	1	
	Larvae	9	4	2	2	7	2	
	Unknown	6	5	2	1	4	4	
	Dermaptera	Forficulidae	1	1	1	1	0	0
Diptera	Drosophilidae	1	1	1	1	0	0	
	Syrphidae	10	1	10	1	0	0	
	Muscidae	10	8	2	2	8	6	
	Chironomidae	10	3	0	0	10	3	
	Tipulidae	1	1	0	0	1	1	
	Stratiomyidae larvae	23	1	0	0	23	1	
	Tipulidae larvae	1	1	0	0	1	1	
	Unknown	7	4	6	3	1	1	
	Hemiptera	Cicadellidae	4	3	0	0	4	3
	Hymenoptera	Formicidae	4	3	2	1	2	2
Vespidae		16	4	15	3	1	1	
Chrysididae		2	1	2	1	0	0	
Ichneumonidae		2	1	0	0	2	1	
Pompilidae		1	1	1	1	0	0	
Apidae		1	1	0	0	1	1	
Lepidoptera	Noctuidae	1	1	0	0	1	1	
Odonata	Zygoptera	4	2	0	0	4	2	
Orthoptera	Rhaphidophoridae	4	1	0	0	4	1	
	Gryllidae	2	1	0	0	2	1	
Trichoptera	Caddisfly larvae	4	1	4	1	0	0	
Isopoda	Unidentified Isopoda	31	10	3	1	28	9	
Araneae	Aranea	29	13	3	3	26	10	
Chilopoda	Unidentified Chilopoda	1	1	0	0	1	1	
Pulmonata	Unidentified Pulmonata	7	6	0	0	7	6	
Oligochaeta	Unidentified Oligochaeta	1	1	0	0	1	1	
Anura	<i>P. regilla</i>	4	3	0	0	4	3	
Unknown	Unknown	1	1	1	1	0	0	
Total:		240	104	64	27	176	77	

dry season prey items. The order with the total highest frequency of occurrence was Coleoptera (26.9%). Coleoptera also had the highest frequency of occurrence in the wet season samples (27.3%). Coleoptera and Diptera had the highest frequency of occurrence in the dry season samples (25.9%).

DISCUSSION

All evidence in this study points to the importance of terrestrial prey in the diet of California Red-Legged Frogs and suggests the importance of conserving terrestrial as well as aquatic habitats to sustain threatened populations of *R. draytonii*. The stable isotope mixing model revealed that almost all contributions to the diet of *R. draytonii* come from terrestrial sources. Frogs sampled in the field and the museum specimens also showed that terrestrial prey is significantly more prevalent in their diet than aquatic prey. Moreover, our stomach content analysis showed similar results in the amount of frog prey that was terrestrially derived in present-day frogs as those in the museum collections that were collected from 1935–2008.

The $\delta^{13}\text{C}$ value of *R. draytonii* post-metamorphs corresponded closely to the $\delta^{13}\text{C}$ values of the terrestrial prey groups (Fig. 1). The aquatic samples' carbon signatures in this study, including *R. draytonii* tadpoles, corresponded closely to the expected $-35 \delta^{13}\text{C}$ (Peterson and Fry, 1987). The mixing model estimated terrestrial carnivores, and terrestrial detritivores contributed approximately 50% each to *R. draytonii* diet according to the

mixing model, and *R. draytonii* is enriched 3.55‰ compared to the average of these two prey groups. This value is very close to the expected 3.4‰ enrichment per trophic level from fractionation (Vander Zanden and Rasmussen, 2001; Post, 2002). Post-metamorphic *R. draytonii* are known to be insectivores; thus, it would be expected that they would have the highest $\delta^{15}\text{N}$ signature for the terrestrial food web. Although we were not able to analyze all potential prey items, the strong terrestrial carbon signature of *R. draytonii* shows that very little of their diet is coming from aquatic sources. In addition, the very small standard error of the average carbon and nitrogen signatures (Table 1; Fig. 1) for post-metamorph *R. draytonii* proves that the variation in trophic level and amount of terrestrial prey between individuals is minimal despite the size range of frogs sampled (range: 43.6–101.9 mm SUL; avg SUL: 70.3 mm).

The type of prey items and number of items differed by season in the field and in the museum samples. This could be attributable to the differential availability of prey at different times of the year but could equally be attributable to between site differences in prey availability. *Rana draytonii* are thought to recognize prey by movement, rather than by discrimination of prey type (Hayes and Tennant, 1985). It is probable that *R. draytonii* prefer larger prey to maximize their energy expenditure while foraging (Pyke, 1984). Also, it may be more difficult for *R. draytonii* to detect movement of smaller prey. Larger prey items (*P. regilla*, Oligochaeta) were found in *R. draytonii* stomachs in the wet season and might account for the average

number of prey items in their stomachs being lower in the wet season than in the dry season.

A previous study emphasized that vertebrates are an important part of adult *R. draytonii* diet (Hayes and Tennant, 1985). We found 9 *P. regilla* individuals in 59 stomach-flush samples and 38 museum specimen stomach samples. Although there were very few vertebrate prey items, they are energetically much more important than other prey items because of their size. Mori Point and Milagra Ridge are near urban areas and have been disturbed in the past; thus, these areas might have lower vertebrate prey abundance, particularly small mammals, than the sites studied by Hayes and Tennant. In addition, Hayes and Tennant state that frogs that had eaten vertebrates were over 100 mm SUL. The largest frog that we stomach flushed in the field was 90 mm SUL; only one of the museum specimens in our study was over 100 mm SUL, and only one toe clip was from a frog over 100 mm SUL. Therefore, the differences between our studies may be reflective of site condition but, perhaps also, may demonstrate that *R. draytonii* diet will vary depending on frog size and the prey availability at each site.

Pseudacris regilla was the only vertebrate found in the field and museum stomach samples, and all were found in specimens collected during the wet season. Hayes and Tennant (1985) found *P. regilla* in two of 35 frogs, but all of the frogs were collected in January and February. It is likely that *P. regilla* is a rare prey item in the dry season but a more common prey item in the wet season because of availability. *Pseudacris regilla* were abundant at the study sites during the wet season but were not observed at any of the study ponds during the dry season. Oligochaeta was also very abundant and frequent in the wet season stomach-flush samples but was not present in the dry season samples. The majority of wet season stomach samples were collected from frogs caught during or just after heavy rains, which cause Oligochaeta to emerge from the soil and be available prey for *R. draytonii*. These observations suggest that *R. draytonii* have seasonal changes in their diet related to prey availability at different times of the year.

This study demonstrates the importance of two techniques in studying amphibian diet: stomach flushing and stable isotopes. Past studies sacrificed amphibians to dissect their stomach contents (Hayes and Tennant, 1985; Licht, 1986; Arujo et al., 2007; Hothem et al., 2009) or used muscle tissue for stable isotope analysis (Yi et al., 2006; Verburg et al., 2007). Sacrificing individuals can be harmful for populations of threatened or endangered amphibian species; therefore, this study illustrates how alternative methods can still extract a cohesive picture of amphibian diet.

Restoration projects and habitat protection programs for *R. draytonii* have historically tended to focus on restoring and protecting breeding ponds (U.S. Fish and Wildlife Service, 2002). Breeding ponds are clearly important, but previous studies have shown that *R. draytonii* spend considerable time in terrestrial habitat and can move large distances away from breeding ponds (Bulger et al. 2003; Fellers and Kleeman, 2007). Although the frogs in our study were all caught on pond margins, most of their prey was terrestrial in origin. Moreover, radio-tracking studies indicate that *R. draytonii* forage several meters into dense riparian areas (G. Rathbun pers. comm. 1993, as cited in U.S. Fish and Wildlife Service, 1996), which presumably provide moisture and cover (Fellers and Kleeman, 2007), but further research is clearly needed to understand the specific microhabitats where frogs feed. Our study concludes that conservation efforts for this species must extend to terrestrial habitats around

aquatic breeding sites that either are being used for foraging directly or are important for the production of the predominantly terrestrial prey of *R. draytonii*.

Acknowledgments.—We thank E. Bueno, S. Bishop, J. Mitchell, C. Singer, and R. Townsend for assistance with fieldwork. We thank E. Bueno for assistance with invertebrate identification. We thank D. Fong for providing useful guidance on the project and C. Davidson, N. Reeder, S. Bishop, and anonymous reviewers for offering useful comments on the manuscript. We thank the California Academy of Sciences for use of their specimens. Stable isotope analysis and field equipment were funded by Golden Gate National Parks Conservancy. VTV was funded by National Science Foundation grants EF-0723563 and 1120283 and the College of Science and Engineering at San Francisco State University (SFSU). All research was conducted under U.S. Fish and Wildlife Service Permit TE036499-6, National Park Service permit GOGA-2009-SCI-0011, and SFSU Animal Care and Use Protocol A9-009.

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APPENDIX 1.—Museum material examined from California Academy of Sciences.

Museum number	Collection date	Collection location (County)
1844	1/1/2009	Santa Clara
3558	7/26/1928	Santa Clara
3923	3/10/1935	Marin
8256	8/21/1938	San Mateo
8258	8/21/1938	San Mateo
8259	12/23/1938	San Mateo
8260	July 1938	San Mateo
8261	July 1938	San Mateo
8263	12/24/1942	San Mateo
8947	4/8/1939	Santa Clara
13394	7/15/1935	San Mateo
13395	7/15/1935	San Mateo
13396	7/15/1935	San Mateo
13397	7/15/1935	San Mateo
13398	7/15/1935	San Mateo
13857	April–May 1949	San Mateo
13858	April–May 1949	San Mateo
13860	April–May 1949	San Mateo
13861	April–May 1949	San Mateo
13862	April–May 1949	San Mateo
13863	April–May 1949	San Mateo
20178	1/12/1960	Santa Clara
85333	1/2/1962	San Mateo
93357	3/30/1962	San Mateo
173688	8/27/1989	Sonoma
173728	3/11/1989	San Mateo
173729	3/11/1989	San Mateo
178492	2/28/1986	San Mateo
178500	7/25/1985	San Mateo
195063	10/2/1964	Santa Clara
203690	11/9/1997	Alameda
203719	3/24/2001	Santa Clara
203835	Spring 1956	Santa Clara
203836	3/5/1939	Santa Clara
203837	2/25/1939	Santa Clara
203838	2/25/1939	Santa Clara
203839	1/1/1939	Santa Clara
203840	2/18/1939	Santa Clara
203843	5/17/1950	Santa Clara
203845	8/8/1966	Santa Clara
203846	4/20/1976	Santa Clara
203847	4/25/1950	Santa Clara
203848	3/5/1955	Santa Clara
207121	8/2/1998	San Mateo
210380	4/30/1998	Santa Clara
211720	2/11/2000	Santa Clara
225271	1/1/2003	San Mateo
225310	2/7/2002	San Mateo
228056	3/14/2003	San Mateo
228057	3/14/2003	San Mateo
228058	3/14/2003	San Mateo
228068	11/8/2008	San Mateo
228071	11/30/2003	San Mateo
228076	11/30/2003	San Mateo
233397	1/1/2004	San Mateo
233401	1/1/2005	San Mateo
233402	7/24/2005	San Mateo
238596	6/6/2003	Santa Clara
238607	4/19/1999	Santa Clara
241751	12/19/2008	San Mateo