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DISCRIMINATION FACTORS FOR STABLE ISOTOPES OF CARBON AND NITROGEN IN BLOOD AND FEATHERS FROM CHICKS AND JUVENILES OF THE CALIFORNIA CONDOR

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Abstract. Stable-isotope ratios of carbon ($^{13}C/^{12}C$; $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$; $\delta^{15}N$) in animal tissues are analyzed to estimate animal foraging ecology because these ratios reflect those of an animal's diet. This reflection is generally indirect, as stable-isotope ratios change with trophic level. These differences, called discrimination factors (reported as Δ), vary considerably by species and tissue. Variations in discrimination factors used in stable-isotope mixing models can lead to inaccurate estimates of diets. Therefore, determining accurate discrimination factors specific to species and tissue is important. We established the $\Delta^{13}C$ and $\Delta^{15}N$ values between diet and blood and feathers from chicks and juveniles of the California Condor (*Gymnogyps californianus*). Hatchlings were fed rats for 76–119 days, whereas juveniles were fed dairy calves for 64 days. The mean $\Delta^{13}C$ and $\Delta^{15}N$ values (± SD) between chick feathers and rat muscle were 0.4 ± 0.4‰ and 3.1 ± 0.2‰, respectively; those between chicks' whole blood and rat muscle were –0.7 ± 0.1‰ and 1.7 ± 0.1‰, respectively. The mean $\Delta^{13}C$ and $\Delta^{15}N$ values between juvenile condors' plasma and calf muscle were 0.9 ± 0.2‰ and 3.3 ± 0.7‰, respectively; those between juveniles' red blood cells and calf muscle were 0.3 ± 0.3‰ and 1.8 ± 0.1‰, respectively; and those between juveniles' whole blood and calf muscle were 0.3 ± 0.3‰ and 1.9 ± 0.2‰, respectively. We report the first discrimination factors for the Cathartidae (New World vultures), and our findings will have important applications in studies of the critically endangered California Condor's foraging ecology.

Key words: *vulture, fractionation, isotope enrichment, captive-feeding experiment, stable-isotope analysis.*

Isótopos Estables de Nitrógeno y Carbono como Factores Discriminantes para Sangre y Plumas de Pichones y Juveniles de *Gymnogyps californianus*

Resumen. Los cocientes de isótopos estables de nitrógeno ($^{15}N/^{14}N$; $\delta^{15}N$) y carbono ($^{13}C/^{12}C$; $\delta^{13}C$) en los tejidos animales son analizados para estimar la ecología de forrajeo de los animales debido a que estos cocientes reflejan aquellos de la dieta de un animal. Este reflejo es generalmente indirecto, ya que los cocientes de isótopos estables cambian con el nivel trófico. Estas diferencias son llamadas factores discriminantes (reportadas como Δ) y pueden variar considerablemente por especie y tejido. Pequeñas variaciones en los factores discriminantes usadas en los modelos de mezcla de isótopos estables pueden llevar a estimaciones inexactas de la dieta. Por lo tanto, la determinación de factores discriminantes exactos específicos para las especies y los tejidos es importante. Establecimos los valores de $\Delta^{15}N$ y $\Delta^{13}C$ entre la dieta y los componentes de la sangre y las plumas de pichones y juveniles cautivos de *Gymnogyps californianus*. Los pichones fueron alimentados con ratas sólo por 76 a 119 días, mientras que los juveniles fueron alimentados exclusivamente con terneros lecheros por 64 días. Los valores medios de $\Delta^{13}C$ y $\Delta^{15}N$ entre las plumas de los pichones y el músculo de rata fueron 0.4‰ y 3.1‰; aquellos entre la sangre entera de los pichones y el músculo de rata fueron –0.7‰ y 1.7‰. Los valores medios de $\Delta^{13}C$ y $\Delta^{15}N$ entre el plasma de la sangre de los juveniles y el músculo de los terneros lecheros fueron 0.9‰ y 3.3‰; aquellos entre las células rojas de la sangre de los juveniles y el músculo de los terneros fueron 0.3‰ y 1.8‰; aquellos entre la sangre entera de los juveniles y el músculo de los terneros fueron 0.3‰ y 1.92‰, respectivamente. Presentamos los primeros factores discriminantes para los Cathartidae (buitres del Nuevo Mundo), y nuestros resultados tendrán aplicaciones importantes en el estudio de la ecología de forrajeo de la especie en peligro crítico *G. californianus*.

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INTRODUCTION

Natural variations in the stable-isotope ratios of carbon ($^{13}C/^{12}C$, reported as $\delta^{13}C$ values) and nitrogen ($^{15}N/^{14}N$, reported as $\delta^{15}N$ values) in animal tissues are used for many applications in ecology. Predictable enrichment of ^{15}N with increasing trophic level allows for estimation of an animal's relative trophic position (Gannes et al. 1998, Vanderklift and Ponsard 2003), whereas $\delta^{13}C$ values reflect sources of primary productivity and thus can be used to assess diet and habitat use (Gannes et al. 1998, Kurle and Worthy 2001, Rubenstein and Hobson 2004, Kurle and Gudmundson 2007).

In general, animal tissues have an isotopic composition different from that of their diet because of isotopic fractionation (McCutchan et al. 2003). This difference between diet and consumer is called the trophic discrimination factor (Martínez del Rio et al. 2009) and is typically expressed with Δ notation ($\Delta^{13}C$ for C and $\Delta^{15}N$ for N), which is defined as $\Delta^{13}C_{tissue-diet} = \delta^{13}C_{tissue} - \delta^{13}C_{diet}$ (and similarly for N). Note that the sign of a Δ value is determined by the order of the subscripts, which should always be reported. In this paper, readers should assume that Δ values always have the subscript "tissue–diet."

Discrimination factors can change with many variables. These include taxon, type of tissue analyzed, differences in digestive physiology, nutritional or reproductive status, form of waste, macromolecular composition of the diet (i.e., proportions of protein, carbohydrate, and lipid), as well as the extent of routing of dietary macromolecules to the same macromolecules in body tissues (i.e., dietary protein to body protein or dietary fat to body fat) (Bearhop et al. 2002, Kurle 2002, McCutchan et al. 2003, Pearson et al. 2003, Vanderklift and Ponsard 2003, Robbins et al. 2005, 2010, Martínez del Rio et al. 2009, Lecomte et al. 2011, Poupin et al. 2011). Given the propensity for variation, the use of assumed discrimination factors in stable-isotope mixing models can result in significant errors in the estimation of wild animals' foraging ecology (Bond and Diamond 2011, Phillips 2012; Kurle, unpubl. data). Therefore, determination of discrimination factors by means of captive animals with a controlled diet can be very important for accurate modeling of animal diets in the wild.

The California Condor (*Gymnogyps californianus*) is the largest terrestrial bird in North America with a wingspan of almost 3 m and a weight of ~8.5 kg (Snyder and Schmitt 2002). In 1982 it was on the brink of extinction with only 22 birds left in existence (Walters et al. 2010). The species is currently designated as critically endangered by the International Union for the Conservation of Nature (BirdLife International 2012) as well as endangered under the United States Endangered Species Act. The success of the captive-breeding program for the California Condor, initiated in the early 1980s (Kiff et al. 1996), expanded the number of condors to 417 individuals by 30 April 2013, with over half of those birds free-flying (living in the wild) and released from sites within California (n = 138), Arizona (n = 73), and Baja California, Mexico (n = 29) (U.S. Fish and Wildlife Service 2013).

California Condors feed exclusively on carrion and depend primarily on large mammalian carcasses (Snyder and Schmitt 2002). Although a large portion of the condor's diet is currently from terrestrial sources, isotopic studies of historical materials and fossils show that from the Pleistocene to the 1700s, marine mammals were also a significant food source for condors on the Pacific coast (Chamberlain et al. 2005, Fox-Dobbs et al. 2006). Today, along the California coast, condors feed on beach-cast marine mammals with some regularity (Walters et al. 2010), but the extent to which this behavior occurs has not been established.

To determine important baseline information necessary for estimating the foraging ecology of free-flying California Condors, we analyzed the $\delta^{15}N$ and $\delta^{13}C$ values from blood components and feathers collected from captive condor chicks (birds with flight feathers not yet fully developed) and full-grown juveniles held on known diets, then compared those with the stable-isotope values from their diet items to establish values of $\Delta^{15}N$ and $\Delta^{13}C$. Analysis of the discrimination factors between the diet and multiple tissue types is useful for dietary analysis of wild animals because the $\delta^{15}N$ and $\delta^{13}C$ values from multiple tissues can provide data on foraging ecology over several time scales, depending on a tissue's turnover rate (Hobson and Clark 1992a, Kurle 2009), and, as mentioned above, discrimination factors can vary considerably among tissue types from the same animal.

We selected feathers and blood for determination of discrimination factors because they (1) can be collected nonlethally and (2) their stable-isotope values illustrate diet items incorporated into condors over three periods. Feathers represent diet eaten during the time when condors molt and grow new feathers (Hobson and Clark 1992a), whereas blood components reflect diet items incorporated one to two months (red blood cells [RBCs] and whole blood) and one to two weeks (plasma) prior and up to tissue sampling (Hobson and Clark 1992a, 1993, Bearhop et al. 2002, Kurle 2009).

The discrimination factors we quantified are especially important as stable-isotope analysis may prove to be a valuable tool for modeling the foraging ecology and habitat use patterns of the California Condor, including its potentially shifting reliance on marine versus terrestrial food sources.

METHODS

CONDORS SAMPLED: STUDY OF CHICKS' FEEDING

We analyzed feathers and whole blood from three California Condor chicks (identification numbers 507, 530, and 537; these numbers are assigned by California Condor studbook keeper, San Diego Wild Animal Park, and will follow the birds throughout their lifetimes) hatched and reared at the Los Angeles Zoo on a diet composed exclusively of adult laboratory

TABLE 1. Condor ID numbers, sex, place of origin, tissues collected for stable isotope analysis, hatch date, and number of days held on experimental diet. WB = whole blood, PF = primary feather, RBC = red blood cells.

Identification number	Sex	Origin	Date of hatching	Days on captive diet	Tissue collected
Chick[a]					
507	F	Boise, ID[b]	10 Apr 2009	119	WB, unknown PF
530	M	Big Sur, CA[c]	12 May 2009	87	WB, PF L3
537	F	Los Angeles, CA[d]	23 May 2009	76	WB, PF L10
Juvenile[e]					
525	M	Portland, OR[f]	NA	64	Plasma, RBC, WB
534	F	Portland, OR[f]	NA	64	Plasma, RBC, WB
543	F	Boise, ID[b]	NA	64	Plasma, RBC, WB
547	F	Boise, ID[b]	NA	64	Plasma, RBC, WB

[a]Chicks were raised from eggs transported to and hatched at the Los Angeles Zoo.
[b]Egg laid at the World Center for Birds of Prey.
[c]Egg laid in the wild.
[d]Egg laid at the Los Angeles Zoo.
[e]Juveniles were raised from hatchlings at their site of origin until moved to Pinnacles National Park for the feeding trial on 6 June 2010.
[f]Oregon Zoo.

feeder rats (Layne Laboratories, Inc., Arroyo Grande, CA) (Table 1). Zoo personnel collectively fed each chick and its parents 15 rats per day for the duration of the experiment, and the parents provided ~40 g of tissue per day during the first week of the chick's life, increasing to ~400–500 g per day by the end of the experiment. The condors did not receive supplemental vitamins or minerals.

Chicks grew throughout the experimental period. Chick 507 weighted 2.4 kg on 14 May 2009, 5.5 kg on 17 June 2009, and 6.1 kg on 7 August 2009, indicating she gained 3.7 kg over the 85 days prior to being sampled. Chicks 530 and 537 weighed 5.9 and 5.4 kg, respectively, on 7 August 2009. These chicks were targeted for release, so their handling time and exposure to humans was kept to a minimum. Therefore they were not weighed again, but their increase in size over the experimental period was similar to that of chick 507.

Zoo personnel cut feathers at the base of the vane; they were 32.5 cm (507), 12.0 cm (530), and 12.2 cm (537) in length and stored in polyethylene bags. Prior to analysis, we placed feather segments in labeled containers, rinsed them with HPLC-grade methanol and ultrapure water to remove surface contamination, then dried them in a vacuum desiccator for 15 hr. Using a 21-gauge × 0.75-inch BD Vacutainer blood-collection kit, zoo personnel drew blood samples from a leg vein into vacuum tubes containing sodium heparin, an anticoagulant that does not measurably alter the stable-isotope values in blood (Lemons et al. 2012). We also sampled muscle tissue from three rats representative of the diet fed the condor chicks at the Los Angeles Zoo. We froze all samples at –20° C until they were processed for stable-isotope analysis.

CONDORS SAMPLED: STUDY OF JUVENILES' FEEDING

During routine management for acclimating captive-reared California Condors prior to release to the wild, personnel with the condor-recovery team transported four fully grown juvenile condors (numbers 525, 534, 543, and 547; Table 1) on 6 June 2010 to a flight pen at Pinnacles National Park in western central California. Before arriving at Pinnacles, these birds were raised from hatchlings either at the Oregon Zoo, Portland, Oregon or at the Peregrine Fund's World Center for Birds of Prey, Boise, Idaho (Table 1).

The condors' diet was composed exclusively of dairy calves (that were stillborn or otherwise died of natural causes) obtained from organic dairy farms in Modesto, California. The Pinnacles' personnel fed the condors an average of two calves per week and provided no vitamin or mineral supplements. The juvenile condors were fully grown at the initiation of the controlled diet, as indicated by body length and feather development. Birds 525, 534, and 547 weighed the same at the beginning and end of the experiment; no beginning weight data exist for bird 543, but she weighed the same (~8 kg) as the other birds at the end of the experiment.

The calf-only diet began 12 July 2010, and on 14 September 2010, during standard monitoring, staff of Pinnacles National Park collected whole blood into Vacutainer tubes containing zero additives and RBCs and plasma into vacuum tubes containing sodium heparin. We centrifuged the blood samples at 1000× *g* for 15 min and collected plasma and RBC fractions for analysis. We also collected samples of muscle tissue from 17 calves that were fed to the juvenile condors

throughout the study period. We froze all samples at –20 °C until they were processed for stable-isotope analysis.

STABLE-ISOTOPE ANALYSIS

As chicks were held on the same diet from hatching and primary feathers do not grow in until a condor is at least 60 days old (Mike Clark, Los Angeles Zoo Condor Program, pers. comm.), we did not expect any diet-related changes influenced by egg material (and thus maternal diet) to be reflected in the stable-isotope ratios of the condor chicks' feathers. Nonetheless, to assess potential variability in stable-isotope values between feather segments, we checked our assumption by cutting from each chick's feather five segments ~2 cm in length, spaced roughly equally, with each segment reflecting a different period of feather growth. Segments closer to the base of the feather reflect the most recent growth (and diet), whereas segments closer to the tip reflect earlier growth.

We transferred subsamples of ~100–200 μL of each blood component (whole blood, plasma, or RBCs) into cryovials, freeze-dried them for 12 hr, then held them in a desiccator until analysis. We removed subsamples of rat or calf muscle from the center of the muscle and split them into two samples; we extracted lipid from one with petroleum ether (Dobush et al. 1985, Martínez del Rio and Anderson-Sprecher 2008) in a Dionex ASE-200 Accelerated Solvent Extractor but did not extract lipid from the other sample. We chose to analyze muscle samples rather than homogenized prey carcasses for two reasons: fully homogenizing whole rat and calf bodies was logistically impossible and muscle tissue represents the bulk of digestible and assimilated material in these carcasses (Therrien et al. 2011).

We freeze-dried all muscle samples. We did not extract lipid from the blood components because whole blood has very low lipid content (Bearhop et al. 2002) and the C:N ratios of the blood components were all ≤3.2, below the level recommended for tissues requiring lipid extraction (Post et al. 2007). We cut the feather segments into ~1-mm pieces and ground all other tissue samples to a powder by hand. We sealed ~0.7 mg of each tissue into 5 × 9-mm tin capsules and analyzed them for stable C and N isotope ratios with a Carlo Erba CE1108 elemental analyzer interfaced via a ConFlo III device to a Thermo-Electron Delta Plus XP mass spectrometer at the Department of Earth and Marine Sciences, University of California, Santa Cruz. We calculated the average precision of these data as the SD of the $\delta^{15}N$ and $\delta^{13}C$ values from a set of standards (acetanilide), and it was 0.03‰ for nitrogen and 0.07‰ for carbon.

STATISTICAL ANALYSIS

To determine the $\Delta^{13}C$ and $\Delta^{15}N$ values, we subtracted the mean $\delta^{13}C$ and $\delta^{15}N$ values of the prey items (rats or dairy calves, lipid not extracted) from these values in the tissues from each individual condor. We report mean differences ± SD as the mean $\Delta^{13}C$ and $\Delta^{15}N$ values for each tissue from the chicks and juveniles.

We used Systat 13 (Cranes Software International) for all statistical tests and used parametric methods, as all data met the assumptions for parametric tests. We report values as means ± SD and tested significance at the $\alpha = 0.05$ level.

RESULTS

CHICKS

There were no differences in the mean $\delta^{13}C$ or $\delta^{15}N$ values among the five feather segments measured for each bird (ANOVAs, $F_{1,13} = 1.4$, $P = 0.25$, and $F_{1,13} = 0.4$, $P = 0.52$, respectively [Table 2]). This was expected as the chicks were held on a controlled diet throughout the time of feather growth. The overall mean $\delta^{13}C$ and $\delta^{15}N$ values from the chicks' feathers were –18.2 ± 0.4‰ and 8.4 ± 0.2‰, respectively (Table 2). The mean $\delta^{13}C$ and $\delta^{15}N$ values from the whole blood collected from the chicks were –19.4 ± 0.0‰ and 7.0 ± 0.1‰, respectively (Table 2). The mean $\delta^{13}C$ and $\delta^{15}N$ values from the whole blood were significantly lower than those from the feathers (*t*-tests, $t = –5.0$, df = 16, $P < 0.01$ and $t = –13.8$, df = 16, $P < 0.01$, respectively).

There were no differences in the $\delta^{13}C$ and $\delta^{15}N$ values between rat muscle from which lipid was extracted (–19.0 ± 0.0‰ and 5.7 ± 0.5‰, respectively) and that from which it was not (NLE; –18.7 ± 0.2‰ and 5.3 ± 0.3‰, respectively) ($n = 3$; paired *t*-tests, $t = –2.67$, df = 2, $P = 0.11$ and $t = 3.25$, df = 2, $P = 0.08$, respectively). Therefore, we used the stable-isotope values from NLE rat muscle for the calculations of the chicks' discrimination factors.

The mean $\Delta^{13}C$ and $\Delta^{15}N$ values measured between feathers and NLE rat muscle were 0.4 ± 0.4‰ and 3.1 ± 0.2‰, respectively (Fig. 1; Table 2). The mean $\Delta^{13}C$ and $\Delta^{15}N$ values between whole blood and NLE rat muscle were –0.7 ± 0.1‰ and 1.7 ± 0.1‰, respectively (Fig. 1; Table 2).

JUVENILES

The mean $\delta^{13}C$ and $\delta^{15}N$ values of the plasma (–21.0 ± 0.2‰ and 10.8 ± 0.7‰, respectively), RBCs (–21.6 ± 0.3‰ and 9.4 ± 0.1‰, respectively), and whole blood (–21.6 ± 0.3‰ and 9.5 ± 0.2‰, respectively) from the juvenile condors were significantly different from one another (ANOVAs, $F_{2,9} = 14.38$, $P < 0.01$ and $F_{2,9} = 5.76$, $P = 0.03$) (Table 2). The $\delta^{13}C$ values from plasma were higher than those from the RBCs and whole blood (Tukey's, $P = 0.04$ for both), whereas they were the same between the RBCs and whole blood (Tukey's, $P = 1.0$). The $\delta^{15}N$ values from plasma were also higher than those from the RBCs and whole blood (Tukey's, both $P < 0.01$), whereas they were the same between the RBCs and whole blood (Tukey's, $P = 0.91$) (Table 2).

There were no differences in the $\delta^{13}C$ or $\delta^{15}N$ values between calf muscle from which lipid was extracted (–21.9 ± 2.0‰ and 7.4 ± 0.7‰, respectively) and that from which it was not (–21.9 ± 2.1‰ and 7.5 ± 0.6‰, respectively) ($n = 17$; paired *t*-tests, $t = –0.87$, df = 16, $P = 0.40$ and $t = –1.35$, df = 16, $P = 0.20$, respectively) (Table 2). Therefore, we used the

TABLE 2. The mean $\delta^{13}C$ and $\delta^{15}N$ values (‰; ± SD) from chicks and juveniles of the California Condor and their food, and the mean $\Delta^{13}C$ and $\Delta^{15}N$ values (‰; ± SD) between condor tissues and diet.

	$\delta^{13}C$	$\delta^{15}N$	$\Delta^{13}C$	$\Delta^{15}N$
Chicks (n = 3)				
Feather segment				
1 (tip)	–18.3 ± 0.1	8.4 ± 0.1		
2	–18.3 ± 0.3	8.4 ± 0.2		
3	–18.5 ± 0.8	8.3 ± 0.3		
4	–18.1 ± 0.3	8.4 ± 0.1		
5 (base)	–18.0 ± 0.2	8.3 ± 0.0		
Mean	–18.2 ± 0.4	8.4 ± 0.2	0.4 ± 0.4	3.1 ± 0.2
Whole blood	–19.4 ± 0.0	7.0 ± 0.1	–0.7 ± 0.1	1.7 ± 0.1
Juveniles (n =4)				
Plasma	–21.0 ± 0.2	10.8 ± 0.7	0.9 ± 0.2	3.3 ± 0.7
Red blood cells	–21.6 ± 0.3	9.4 ± 0.1	0.3 ± 0.3	1.8 ± 0.1
Whole blood	–21.6 ± 0.3	9.5 ± 0.2	0.3 ± 0.3	1.9 ± 0.2
Food				
Lipid extracted				
Rat muscle (n = 3)	–19.0 ± 0.0	5.7 ± 0.5		
Dairy calf muscle (n = 17)	–21.9 ± 2.0	7.4 ± 0.7		
Lipid not extracted				
Rat muscle (n = 3)	–18.7 ± 0.2	5.3 ± 0.3		
Dairy calf muscle (n = 17)	–21.9 ± 2.1	7.5 ± 0.6		

stable-isotope values from NLE calf muscle for the calculations of the discrimination factors.

The mean $\Delta^{13}C$ and $\Delta^{15}N$ measured between the blood components and NLE calf muscle were 0.9 ± 0.2‰ and 3.3 ± 0.7‰, respectively, for plasma, 0.3 ± 0.3‰ and 1.8 ± 0.1‰, respectively, for RBCs, and 0.3 ± 0.3‰ and 1.9 ± 0.2‰, respectively, for whole blood (Fig. 1; Table 2).

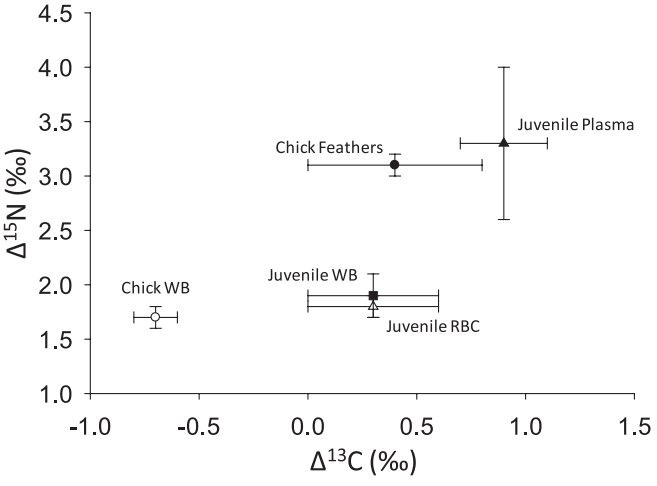

Figure 1. Mean (± SD) stable C and N isotope discrimination factors between diet and five tissues (WB, whole blood; RBC, red blood cells) from captive chicks and fully grown juveniles of the California Condor.

The mean $\Delta^{13}C$ values for whole blood from chicks (–0.7‰) and juveniles (0.3‰) were significantly different (t-test, df = 5, P = 0.01) (Fig. 1; Table 2). The mean $\Delta^{15}N$ value for whole blood from chicks trended lower than that from juvenile condors (1.7‰ vs. 1.9‰; t-test, df = 5, P = 0.07) (Fig. 1; Table 2). Whole blood is the only tissue in which we could compare chicks and juveniles because the condor's molt pattern is such that the juveniles did not have feathers growing during the period in which they were held on the controlled diet (Snyder et al. 1987).

DISCUSSION

VARIATION IN $\Delta^{13}C$ AND $\Delta^{15}N$ VALUES AMONG DIFFERENT TISSUES

Our study provides the first diet–tissue discrimination factors reported for New World vultures (family Cathartidae) from multiple tissues, ages, and experimental diets. The stable-isotope discrimination factors from animals held on consistent diets vary significantly by tissue (Kurle 2002, 2009, Florin et al. 2011), and our study supports these findings. Differences in the stable-isotope values among the tissue types we studied (between whole blood and feathers for chicks, and among plasma, RBCs, and whole blood for juveniles) were likely due to differences in the primary proteins and subsequent amino acids present in the different blood components and feathers because values of $\delta^{15}N$ and $\delta^{13}C$ for specific amino acids vary widely (Lorrain et al. 2009, Martínez del Rio et al. 2009, Dale et al. 2011).

TABLE 3. Stable nitrogen and carbon isotope discrimination factors (‰, ± SD when available) for different tissue types from omnivorous and carnivorous birds.

Species and diet	Tissue	$\Delta^{15}N$	$\Delta^{13}C$	Reference
American Crow				
Perch	Feather	~2.0	~1.4	Hobson and Clark (1992b)
Perch	Plasma	NA	0.5 ± 0.3	Hobson and Clark (1993)
Perch	Whole blood	~1.9	~3.0	Hobson and Clark (1992b)
California Condor (chick)				
Rat	Feather	3.1 ± 0.1	0.4 ± 0.4	This study
Rat	Whole blood	1.7 ± 0.1	–0.7 ± 0.1	This study
California Condor (juvenile)				
Dairy calf	Plasma	3.3 ± 0.7	0.9 ± 0.2	This study
Dairy calf	RBC	1.8 ± 0.1	0.3 ± 0.3	This study
Dairy calf	Whole blood	1.9 ± 0.2	0.3 ± 0.3	This study
Dunlin				
Wheat-based poultry grower, chicken eggs, blood meal, canola oil	Plasma	3.3	0.5	Evans Ogden et al. (2004)
See above	RBC	3.0	1.5	Evans Ogden et al. (2004)
See above	Whole blood	2.9	1.3	Evans Ogden et al. (2004)
Great Skua				
Sprat and beef (lipid and lipid not extracted)	Feather	4.4, 4.6, 4.8, 5.0	2.1, 2.2, 5.3, 7.0	Bearhop et al. (2002)
See above	Whole blood	2.6, 2.8, 4.0, 4.2	1.1, 2.3, 4.3, 7.1	Bearhop et al. (2002)
Peregrine Falcon				
Quail	Feather	2.7 ± 0.5	2.1 ± 0.1	Hobson and Clark (1992b)
Quail	Whole blood	3.3 ± 0.4	0.2 ± 0.0	Hobson and Clark (1992b)
Snowy Owl				
Mice	Whole blood	1.6 to 2.1	–0.3 to 0.5	Therrien et al. (2011)
Spectacled Eider				
Mazuri sea duck diet, Atlantic silverside	Feather	5.6 ± 0.3	3.2 ± 0.2	Federer et al. (2010)
See above	Plasma	4.9 ± 0.2	0 ± 0.2	Federer et al. (2010)
See above	RBC	4.0 ± 0.2	2.0 ± 0.2	Federer et al. (2010)
Yellow-rumped Warbler				
Insects	Plasma	3.0	0.6	Pearson et al. (2003)
Insects	Whole blood	2.7	2.2	Pearson et al. (2003)
Insects/mashed banana	Plasma	2.5 to 2.9	–1.5 to 0.2	Pearson et al. (2003)
Insects/mashed banana	Whole blood	1.7 to 1.8	–1.2 to 1.8	Pearson et al. (2003)

The mean $\Delta^{13}C$ and $\Delta^{15}N$ values from the feathers (0.4‰ and 3.1‰, respectively) collected from condor chicks were higher than those from the chicks' whole blood (–0.7‰ and 1.7‰, respectively). Our findings are similar to the pattern observed for $\Delta^{15}N$ and two of four $\Delta^{13}C$ values from captive juveniles of the Great Skua (*Stercorarius skua*), a large, piscivorous seabird (Bearhop et al. 2002) (Table 3), and for $\Delta^{13}C$ values (but not $\Delta^{15}N$ values; they showed the opposite trend) from captive adults of the Peregrine Falcon (*Falco peregrinus*), a large bird of prey (Hobson and Clark 1992b) (Table 3). American Crows (*Corvus brachyrhynchos*), large, omnivorous, carrion-eating birds, held on a fish diet of perch, had $\Delta^{15}N$ values (~2.0‰) from their feathers that were nearly identical to those from their whole blood (~1.9‰), and $\Delta^{13}C$ values from their feathers (~1.4‰) that were lower than those from their whole blood (~3.0‰) (Hobson and Clark 1992b) (Table 3), not following the pattern we observed in the condor.

We found no differences in the $\Delta^{13}C$ and $\Delta^{15}N$ values from RBCs and whole blood from juvenile condors. However, the $\Delta^{13}C$ and $\Delta^{15}N$ values of plasma from juvenile condors were higher than those from the RBCs and whole blood. Our previous work demonstrated a similar pattern for $\delta^{13}C$ and $\delta^{15}N$ values from plasma or serum over RBCs in captive rats (*Rattus norvegicus*) (Kurle 2009, unpubl. data) and for $\delta^{15}N$ values from captive northern fur seals (*Callorhinus ursinus*) (Kurle 2002).

Results from avian studies show a similar variation in discrimination factors between blood components. As we found in the condor, the $\Delta^{15}N$ values from plasma are higher than those from RBCs and whole blood of Dunlins (*Calidris alpina*) (Evans Ogden et al. 2004) held on a mixed wheat/animal protein diet, whole blood of Yellow-rumped Warblers (*Setophaga coronata*) held on a mixed banana/insect or 100% insect diet (Pearson et al. 2003), and RBCs of Spectacled Eiders (*Somateria fischeri*) (Federer et al. 2010) held on a

commercial duck diet mixed with fish (Table 3). The $\Delta^{13}C$ values from plasma were lower than those from whole blood for the warblers (Pearson et al. 2003), whole blood from American Crows held on an all-fish diet (Hobson and Clark 1992b, 1993), and RBCs from the Spectacled Eider (Federer et al. 2010) and Dunlin (Evans Ogden et al. 2004) (Table 3). These variations in the patterns of $\Delta^{13}C$ and $\Delta^{15}N$ values observed among blood components of various species further underscore the need for studies determining stable-isotope discrimination factors across multiple tissues.

VARIATION IN $\Delta^{13}C$ AND $\Delta^{15}N$ VALUES AMONG BIRD TAXA

The mean $\Delta^{15}N$ values from the chicks' feathers (3.1) fell within the range of those from other carnivorous birds (2.0 to 5.6‰; Hobson and Clark 1992b, Bearhop et al. 2002, Federer et al. 2010), but the mean $\Delta^{13}C$ values (0.4‰) for these feathers were well below those from other birds (1.4 to 7.0‰; Hobson and Clark 1992b, Bearhop et al. 2002, Federer et al. 2010) (Table 3). We do not know the cause of the difference between our values and other published $\Delta^{13}C$ values from feathers, but our feathers were from growing chicks and the feathers in other studies were from adults. Further studies comparing the $\Delta^{13}C$ values in the feathers of chicks and adults would clarify whether this finding is unique to the condor or because the chicks were growing.

The mean $\Delta^{13}C$ (0.9‰) and $\Delta^{15}N$ (3.3‰) values from plasma collected from juvenile condors were slightly higher (C) or lower (N) than the range of values for plasma reported in other studies in which the birds were held on all animal protein (0 to 0.5‰ for C and 4.9‰ for N; Hobson and Clark 1993, Federer et al. 2010) (Table 3). The mean $\Delta^{13}C$ (0.3‰) and $\Delta^{15}N$ (1.8‰) values from RBCs from the fully grown juvenile condors we studied were well below the range observed in other carnivorous birds (2.0 for C, 4.0 for N; Federer et al. 2010) (Table 3), and the mean $\Delta^{13}C$ and $\Delta^{15}N$ values from whole blood from chicks (1.7‰ and –0.7‰, respectively) and juvenile condors (1.9‰ and 0.3‰, respectively) were within the range observed in other birds (1.1–7.1‰ for C, 1.9–4.2‰ for N; Hobson and Clark 1992b, Bearhop et al. 2002) (Table 3).

We also compared the discrimination factors between diet and whole blood from the fully grown birds we studied with those from adults of two other species of large predator birds that may be more comparable to the California Condor. The mean $\Delta^{13}C$ (0.3‰) and $\Delta^{15}N$ (1.9‰) values from whole blood from the condors we studied fall within the range of values (–0.3 to 0.2‰ for $\Delta^{13}C$ and 1.6 to 2.7‰ for $\Delta^{15}N$) found for whole blood from captive adult Snowy Owls (*Bubo scandiacus*; Therrien et al. 2011) and the $\Delta^{13}C$ value (0.2) for whole blood from captive adult Peregrine Falcons (Hobson and Clark 1992b) (Table 3). However, the mean $\Delta^{15}N$ value (3.3) for whole blood from the falcons was higher than that from our condors. Unfortunately, these studies analyzed whole blood only, so we cannot compare our values for plasma or RBCs with those from other large carnivorous birds.

Therrien et al. (2011) speculated that the owls they studied may have had $\Delta^{15}N$ values lower than those of the falcons because of owls' better-developed cecum (Clench and Mathias 1995, Clench 1999) and the potential for different rates of nitrogen assimilation and excretion in birds with ceca of varying sizes. Vultures have the same small or vestigial ceca as members of the order Falconiformes (Clench 1999), so the lower $\Delta^{15}N$ values observed in our condors cannot be attributed to differences between condors and falcons in the size of the ceca.

POTENTIAL DIETARY AND GROWTH INFLUENCES ON ISOTOPE DISCRIMINATION FACTORS

It has been suggested that the $\delta^{15}N$ value of an animal's diet affects its diet–tissue nitrogen discrimination factors (Caut et al. 2008, 2009, Therrien et al. 2011). However, we are not aware of any physical or biochemical processes that could lead to different trophic fractionations based solely on the minuscule absolute differences in isotope concentration between different types of food (Robbins et al. 2010).

Nonetheless, there may be a relationship between $\Delta^{15}N$ values and quantity and quality of protein in the diet. Protein quantity is the amount of nitrogen available to a consumer from its diet, whereas protein quality is measured by how well an animal's amino acid requirements are met by the amino acid profile of its diet. For example, if a diet is a good match, then it is considered to be of high protein quality (Florin et al. 2011). For animals on an all-meat diet, high quality and lower quantity of protein both reduce $\Delta^{15}N$ values (Florin et al. 2011). Dietary protein quality can be correlated with the process of nitrogen discrimination in that the preferential retention of ^{15}N during nitrogen metabolism and excretion decreases with an increase in dietary protein quality. This decrease leads to smaller $\Delta^{15}N$ values between diet and tissue for animals on diets of higher protein quality. Variations in the quality and quantity of dietary protein could account for the differences in $\Delta^{15}N$ values observed among blood components from carnivorous birds in our and other studies. For example, Bearhop et al. (2002) reported that skuas fed fish (sprat) had $\Delta^{15}N$ values considerably lower (2.6–2.8‰) than did those fed beef (4.0–4.2‰) (Table 3). Since skuas are piscivorous seabirds, one would expect fish to match their amino acid needs more accurately (be of higher protein quality) than beef, accounting for the observed differences in $\Delta^{15}N$ values.

The $\Delta^{15}N$ values from whole blood collected from condor chicks were slightly lower than the values from whole blood collected from juveniles. We did not measure the amino acid content of rats or dairy calves, nor do we know how well the two diets matched the needs of the condors, so we cannot judge whether the quality of dietary protein affected the $\Delta^{15}N$ and $\Delta^{13}C$ values for whole blood collected from chicks and juveniles.

However, the condor chicks we studied grew throughout the experiment, whereas the juveniles were full grown and remained at a steady weight. Our data follow the pattern

observed by others, of $\Delta^{15}N$ values being lower in growing animals (see review in Martínez del Rio et al. 2009) because growing animals decrease their rate of excretion of nitrogenous waste. As this waste is ^{15}N-depleted relative to the body, reduction of this waste stream allows animals to assimilate more of the lighter isotope of nitrogen into their growing tissue (Martínez del Rio et al. 2009). We do not know why the $\Delta^{13}C$ values for whole blood of chicks and juveniles differed, as to our knowledge there are no data demonstrating that a difference in growth rates affects $\delta^{13}C$ and thus $\Delta^{13}C$ values.

CONCLUSIONS

Problems can arise in studies of stable-isotope discrimination when captive animals are not held on the experimental diet for a time sufficient to allow influences of the previous diet to be cleared from the targeted tissues. We are confident the blood we sampled from juveniles reflects the stable isotopes incorporated from our control diet because we held the birds on the experimental diet for 64 days, well over the time needed for blood to fully equilibrate to the control diet (Hobson and Clark 1992a, 1993, Bearhop et al. 2002, Evans Ogden et al. 2004, Kurle 2009). We are also confident that equilibration was not a concern for chicks, as the primary feathers we analyzed emerged ~60 days after hatching and reflect the control diet on which the chicks were held from hatching.

We found no significant differences between the $\delta^{15}N$ and $\delta^{13}C$ values from rats and calves from which we extracted lipids or not. Our finding was expected, as the C:N ratios were identical in both rats (3.3‰) and calves (3.2‰) regardless of lipid extraction, and lipid extraction is unnecessary for tissues from terrestrial animals that contain around 10% lipid or have a C:N ratio of ~4.0 or less (Post et al. 2007). We observed a fairly high degree of variability around the mean $\delta^{13}C$ values from the dairy calves (± SD 2.1‰), which is expected from animals eating a mix of C_3 and C_4 grasses and grains.

Animal diets are routinely estimated with stable-isotope mixing models, and the precision of these models relies on the input of accurate discrimination factors (Bond and Diamond 2011, Phillips 2012), which, as described above, can vary considerably by species and by tissue from the same species. In addition, the newer Bayesian mixing models such as MIXSIR, SIAR, and MIXSIAR allow for the incorporation of error around calculated discrimination factors, making their estimates of animals' foraging ecology even more powerful. For this reason, studies of captives such as ours that analyze and report the mean and variance around calculated discrimination factors are important for the most accurate assessments of wild animals' diets. The long-term sustainability of a free-flying California Condor population depends on reliable natural food sources, and our reported discrimination factors obtained via a controlled-feeding experiment are an important contribution to deciphering the species' foraging ecology.

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