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# Nutrient Composition of Prey Items Consumed by Free-Ranging *Drymarchon couperi* (Eastern Indigo Snakes)

Ellen S. Dierenfeld[1,*], Terry M. Norton[2,3], Natalie L. Hyslop[4], and Dirk J. Stevenson[5]

**Abstract.** *Drymarchon couperi* (Eastern Indigo Snake) has a threatened conservation status and restricted range in the southeastern US. Evidence suggests it mainly consumes other reptile species. Dietary nutrient analysis is a component of habitat/resource quality and species health assessments, and the results provide guidelines for optimal captive-feeding protocols. Native prey items (7 snakes, 1 tortoise, 1 rodent) had higher protein and lower fat content, considerably higher concentrations of vitamins A and E, and variable mineral content (high Ca, P, Na; low Cu, Mn) compared to the diets of commercially reared and captive-fed rodents. Data suggest that diets for captive snakes may require modification to better duplicate natural food sources. Further investigation of captive diets is warranted to understand possible health implications for wild Indigo Snake populations.

## Introduction

*Drymarchon couperi* (Holbrook) (Eastern Indigo Snake) is a large (up to 2.6 m total length [TL]; Conant 1998) colubrid currently federally listed as a threatened species throughout its restricted range of Florida and the Coastal Plain of Georgia (USFWS 2008). In southern Georgia, Eastern Indigo Snakes use the burrows of *Gopherus polyphemus* (Gopher Tortoise) for protection from temperature extremes and fire, for foraging, and potentially as nesting sites (Hyslop 2007, 2009; Hyslop et al. 2014; Newberry 2009; Speake and McGlincy 1981; Speake et al. 1978). Adult Eastern Indigo Snakes have been documented feeding on a wide variety (48 spp.) of vertebrates in Georgia and Florida including: amphibians (anurans, 14% of prey records), mammals (rodents, 15%), other snakes (45%), and reptiles—notably juvenile Gopher Tortoises (15%) (Stevenson et al. 2010).

As a component of health assessments conducted simultaneously with population monitoring and spatial ecology studies of Eastern Indigo Snakes on Fort Stewart, GA, and adjacent private lands between 2001–2005 (Hyslop 2007; Stevenson et al. 2003, 2009), we opportunistically collected Eastern Indigo Snake prey samples for nutrient analysis. Chemical information on prey items offers details that can be interpreted in terms of habitat/resource quality for wildlife-management purposes, provide useful guidelines for development of captive-feeding protocols, and contribute to evaluation of biochemical health parameters.

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## Materials and Methods

We obtained prey specimens either as fresh road-killed snakes of species known or suspected to be eaten by Eastern Indigo Snakes (Stevenson et al. 2010), or from the gastrointestinal tracts of Eastern Indigo Snakes during postmortem examination. We placed all samples on ice in the field and stored them frozen at -20 °C (for less than 1 year) until transferred to the St. Louis Zoo Animal Nutrition Laboratory, St. Louis, MO. We then partially thawed, sectioned into smaller segments using either a band saw or meat cleaver, and ground whole prey items in a commercial meat grinder. In the case of the 3 viperids *Crotalus horridus* (Eastern Timber Rattlesnake), *Agkistrodon contortrix* (Southern Copperhead), and *A. piscivorus* (Eastern Cottonmouth; *n* = 4 individuals), we removed and discarded heads and venom glands prior to grinding. We used only the head and tail from 1 *Farancia abacura* (Eastern Mudsnake) and only the front half from a second Mudsnake due to extensive tissue damage. We also analyzed 2 intact Gopher Tortoise neonates obtained from the stomach of 1 adult Eastern Indigo Snake and a partially digested rodent and snake sample from the stomach contents of another Eastern Indigo Snake.

### Fat-soluble components

Following homogenous grinding and mixing, we saponified duplicate 1-g subsamples and extracted fat-soluble components for analysis using the methodology of Taylor (1976) as detailed in Douglas et al. (1994). Instead of conducting analyses immediately, we drew 1-mL extracts drawn from the hexane layer (top), evaporated the samples under nitrogen, then reconstituted with 0.25 mL ETOH containing 0.2% BHT and stored them at -20 °C until shipment to Arizona State University, Tempe, AZ, where they were analyzed using high-performance liquid chromatography (HPLC, McGraw et al. 2006) to quantify vitamin E (as α-tocopherol) and total carotenoids. We measured vitamin A (as all-trans retinol) with absorbance spectrophotometry (in hexane, using quartz cuvettes and the extinction coefficient of 1600) at λ max = 277 nm. We calculated vitamin E activity as 1 mg α-tocopherol = 1.49 IU vitamin E and vitamin A activity as 0.3 µg all-trans retinol = 1 IU vitamin A.

### Proximate nutrients and mineral content

We placed remaining homogenates in aluminum pans, and weighed and freeze-dried them to determine water content. We ground freeze-dried samples in a coffee grinder, placed the products in individual plastic bags, and sent them for proximate composition (crude protein, crude fat, ash) and mineral analysis using standardized methodology (AOAC 2004) for animal products (Dairy One Forage Lab, Ithaca, NY). Minerals were not analyzed in the partially digested rodent prey as the sample was too small.

## Results

We analyzed 7 known and 1 unidentified snake species, along with 2 Gopher Tortoises and an unidentified rodent species eaten by Eastern Indigo Snakes. Proximate nutrient composition, calculated vitamins A and E, and total carotenoids in prey



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items are listed in Table 1. *Pituophis melanoleucus mugitus* (Florida Pinesnake) appeared to be unique in fat content within the snake species examined, with fat levels measuring 1.7 to 8-fold higher than other species (Table 1). The partially digested snake prey (Table 1) displayed the lowest protein content, likely due to digestive processes. Further substantiation of digested tissue (primarily protein, possibly fat) was also evident from the relatively higher ash (bone) content in the partially digested snake sample: 42% ash compared with 16–24% in all other potential prey items. Mineral contents of whole prey are listed in Table 2, where higher levels of Ca, Mg, P, and Mo are apparent in the partially digested snake-prey sample; this is again indicative of tissues other than bone being digested.

## Discussion

Proximate nutrient composition of prey eaten by Eastern Indigo Snakes is similar in many aspects to values reported in a wide variety of other vertebrate prey items. Water content ranges from ~60 to 85% in most whole vertebrate prey, with median values averaging 70–80% (Dierenfeld et al. 2002a), similar to most whole-prey snakes analyzed (Table 1). Gopher Tortoise neonates and Florida Pinesnakes—the latter species a previously undocumented but suspected prey of Eastern Indigo Snakes (Stevenson et al. 2010)—could provide as much as 20% less water in a meal, which may be physiologically important to Eastern Indigo Snakes in dry areas or during hot seasons. Additionally, obligate carnivores can meet their water needs through metabolic breakdown of dietary fats; however, the primary food items of Eastern Indigo Snakes appear limited to those low in fat content. Hence, the free water itself could be critical in maintaining hydration and electrolyte balance.

Across the prey-snake items analyzed, crude fat content ranged from about 2% to 17% of dry matter (DM); by comparison, fat content in laboratory rodents (primarily mice and rats) fed to captive snakes typically ranges from 20 to 30% (or higher, depending on husbandry and species; Dierenfeld et al. 2002a). Snake-prey items contained some of the lowest fat concentrations measured across a wide variety of vertebrate prey analyzed; only anurans display whole-body fat content consistently lower than 15% of DM (Dierenfeld et al. 2002a). This finding may be important for overall energetics and nutritional balance in the Eastern Indigo Snake because poikilotherms have a lower metabolic rate than homeotherms, and hence require fewer calories per unit body mass. High-fat diets can be linked with obesity and associated health problems of captive-managed snakes (Frye 1984). Our data suggest that lower-fat, higher-protein diets may be more characteristic of foods consumed by the free-ranging Eastern Indigo Snake. Lower nutrient-density dietary components may be entirely appropriate for this species, but actual nutritional requirements remain unknown.

Importantly, low dietary fat may impact the uptake of fat-soluble nutrients such as vitamins A and E in this species. To our knowledge, these are the first data on the vitamin A and E content of whole snakes utilized as prey. Vitamin A levels tend to increase with age/maturity in vertebrate-prey species through accumulation in



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Table 1. Water, crude protein, crude fat, ash, calculated vitamins A and E, and total carotenoids in prey (or potential prey) items consumed by Eastern Indigo Snakes (*Drymarchon couperi*). Crude protein, crude fat, and ash are presented as % DM (mean ± SD), vitamin A as IU/g DM, vitamin E as IU/kg DM, and carotenoids as µg/g DM. *denotes heads not included in analyses; na = not analyzed.

| Sample | *n* | Water (%) | Crude protein | Crude fat | Ash | Vitamin A | Vitamin E | Total carotenoids |
|---|---|---|---|---|---|---|---|---|
| Snakes | | | | | | | | |
| *Heterodon platirhinos* (Latrielle) (Eastern Hognose) | 2 | 77.07 ± 2.40 | 69.85 ± 5.02 | 9.60 ± 0.57 | 18.11 ± 3.15 | na | na | na |
| *Pituophis melanoleucus* Barbour (Florida Pinesnake) | 2 | 63.43 ± 0.60 | 63.75 ± 1.48 | 16.65 ± 0.78 | 20.70 ± 1.01 | 888.74 ± 116.82 | 179.15 ± 49.83 | 0.26 ± 0.05 |
| *Farancia abacura* (Holbrook) (Eastern Mudsnake) | 3 | 77.87 ± 4.27 | 83.03 ± 6.82 | 3.60 ± 3.21 | 15.83 ± 3.93 | 1592.88 ± 356.38 | 163.81 ± 147.55 | 0.06 ± 0.08 |
| *Agkistrodon contortrix* (L.)* (Southern Copperhead) | 2 | 71.51 ± 0.49 | 71.05 ± 1.77 | 4.90 ± 1.41 | 22.88 ± 0.66 | 1371.66 ± 261.91 | 203.61 ± 94.37 | 0.01 ± 0.02 |
| *Coluber constrictor* L. (Southern Black Racer) | 1 | 72.05 | 75.30 | 3.00 | 24.16 | 1343.20 | 118.74 | 0.73 |
| *Crotalus horridus* L.* (Timber Rattlesnake) | 1 | 76.20 | 73.20 | 2.10 | 22.88 | 1908.33 | 593.85 | 0.40 |
| *Agkistrodon piscivorus* Lacepede* (Eastern Cottonmouth) | 1 | 73.73 | 67.80 | 9.20 | 19.89 | 2150.20 | 1041.81 | 0.07 |
| Snake Average ± SD | | 73.30 ± 5.60 | 72.89 ± 7.73 | 7.28 ± 5.31 | 19.81 ± 3.62 | 1475.97 ± 430.61 | 306.02 ± 301.03 | 0.39 ± 0.69 |
| Tortoises | | | | | | | | |
| *Gopherus polyphemus* (Daudin) (Gopher Tortoise) neonates | 2 | 67.85 ± 2.51 | 67.80 ± 0.85 | 15.15 ± 0.78 | 20.44 ± 3.97 | 1062.04 ± 31.33 | 148.99 ± 39.41 | 3.68 ± 0.19 |
| Partially digested prey | | | | | | | | |
| Rodent (species unknown) | 1 | 70.00 | na | 11.90 | na | 1059.85 | 182.95 | 1.66 |
| Snake (species unknown) | 1 | 71.42 | 57.80 | 2.90 | 41.57 | 947.30 | 227.97 | 2.24 |



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body stores, particularly the liver (Dierenfeld et al. 2002a). Calculated vitamin A values measured in this study, regardless of species, should be considered exceptionally high in foods eaten by the Eastern Indigo Snake compared with other assayed whole-prey reported in the literature. Five rodent species fed to captive carnivores had whole-body vitamin A concentrations ranging from ~16,000 IU/kg to ~600,000 IU/kg DM, 2 bird species contained ~36,000 IU/kg DM to 70,000 IU/kg DM, and 2 lizard species contained ~5000 IU/kg DM to ~39,000 IU/kg DM, whereas 2 anurans displayed vitamin A levels ranging from ~16,000 IU/kg DM to 38,000 IU/kg DM (Dierenfeld et al. 2002a). By comparison, the 7 snake species we analyzed in this study averaged about 1,475,000 IU/kg DM vitamin A; Gopher Tortoise neonates also contained vitamin A levels >1,000,000 IU/kg DM. It may be that these seemingly excessive levels of vitamin A in whole prey are necessary due to the low dietary-fat content, but this speculation remains to be investigated.

All whole prey analyzed would appear not only to exceed the vitamin A dietary requirements established for obligate carnivores or domestic carnivores (cats, ~4000 IU/kg DM, dogs: 11,000 IU/kg DM; NRC 2006), but even exceed presumed upper safe limits for this nutrient (33,000 IU/kg for canids, up to 100,000 IU/kg for felids; NRC 1987). These data suggest possible unique, perhaps seasonal cycling of this nutrient in Eastern Indigo Snakes and further studies to better understand species requirements are warranted. Furthermore, to avoid potential toxicities, it is possible that snakes, like other carnivores, store vitamin A in specialized hepatic cells (Leighton et al. 1988) and/or mobilize it throughout body tissues safely packaged in retinyl esters, a phenomenon particularly associated with sporadic feeding (Schweigert et al. 1991).

We found no references in the literature on the ability of snakes to convert dietary carotenoids to active forms of vitamin A; given the quite low levels of total carotenoids detected in whole prey consumed by Eastern Indigo Snakes along with the high preformed vitamin A detected, we suspect that this metabolic route is of little consequence to overall nutritional health of this species. However, total carotenoid concentrations, in the Gopher Tortoises, even though they were neonates, were about 10-fold higher than in the snake-prey items, reflecting the more herbivorous habits of the tortoises, and subsequent deposition of carotenoids into eggs and developing embryos (Dierenfeld et al. 2002b).

Vitamin E concentrations for all prey (except viperid snakes), whether intact or partially digested, averaged ~200 IU/kg DM, and were generally higher than most captive-reared rodent or avian prey (25–174 IU/kg DM, excluding neonatal rats) but similar to values reported from amphibian prey sampled from a comparable locale (82–370 IU/kg DM) (Dierenfeld 2002a). While there was some variability across species, the 3 viperids (in which heads were removed prior to grinding/analysis) contained the highest vitamin E concentrations (3- to 5-fold higher for the Cottonmouth and Timber Rattlesnake, respectively). There may be species-specific differences in vitamin E need, utilization, or metabolism in snakes. Reduced hemolytic action of viper venoms has been correlated with human vitamin E supplementation (Mukherjee et al. 1998). Speculatively, perhaps an affiliated endogenous protective



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mechanism exists in some snakes, such as the Eastern Indigo Snake, that consume venomous prey. Average vitamin E concentrations in the prey items of the Eastern Indigo Snakes consistently exceeded dietary vitamin E recommendations established for domestic carnivores (30–50 IU/kg DM for canids, up to ~80 IU/kg DM for felids; NRC 2006). Recommendations vary depending upon amounts and types of dietary fats, as well as physiologic stage; high dietary polyunsaturated fats, for example, may increase the dietary vitamin E requirement up to 5-fold due to high oxidative potential of these fats. Vitamin E deficiency, manifested as steatitis, has been only occasionally reported in captive snakes (Dierenfeld 1989).

Regarding mineral nutrition, the generally elevated levels of Ca and P in the whole prey eaten by Eastern Indigo Snakes likely reflects a high proportion of skeleton to body mass in the prey-snake species. Although the Ca:P ratio remains approximately 1.7–1.8:1 in both snake and tortoise prey-items, the concentrations of Ca and P (ranging from an average of 4.1% to 7.3% DM, and 2.4% to 4.0% DM, respectively, in snakes) are considerably higher than Ca (0.9–5.9% DM) or P (1.1–3.4%) reported in other vertebrate prey (Dierenfeld et al. 2002a). These high levels of Ca and P may be needed to properly support the skeletal tissues or oogenesis of Eastern Indigo Snakes, or may pose an excessive load requiring mineral excretion bound to urates (Shoemaker and Nagy 1977); quantitative Ca requirements have not been determined. Evaluation of dietary seasonality, particularly relative to reproduction, may elucidate some of the physiology involved with Ca metabolism in this species. Presumably, whole prey contains adequate stores of preformed vitamin D to support uptake and utilization of both Ca and P, but we did not quantify vitamin D levels in this study. *Pantherophis guttatus* (Corn Snake) exposed to artificial UV lighting for 28 days demonstrated increased circulating 25-OH vitamin D levels (from 57 to 196 nmol/L, Acierno et al. 2008). Environmental as well as dietary sources of vitamin D may well be required for optimal nutritional status. Macromineral requirements established for growing mammal and bird species (Ca, 0.4–1.1%; Mg, 0.04–0.1%; P, 0.4–0.8%; K, 0.3–1.4%; and Na, 0.1–0.2%; AAFCO 2012, NRC 1994) appear to be met by any of the whole prey items described in this study, but the actual requirements of Eastern Indigo Snakes remain unknown.

Wide variability in trace-mineral composition within samples was evident and likely due to multiple factors including limited sample size and possible habitat and/or primary-prey item (dietary) differences that impacted the whole body (Table 2). Dietary requirements for Cu range from about 3 to 5 mg/kg DM for domestic carnivores (AAFCO 2012, NRC 2006). Snake species, in particular, appear to contain low body levels of Cu (all 7 spp. had ≤10 mg/kg), as opposed to the Gopher Tortoise neonates at 22 mg/kg DM. By contrast, mammalian whole prey (9 spp.) Cu concentrations ranged from 2 to 62 mg/kg, 4 avian and 4 amphibian/reptile species exhibited levels of ≤11 mg/kg DM, and 1 anuran species contained >100 mg/kg Cu (Dierenfeld et al. 2002a). It appears that a mixed prey diet may optimize Cu intake for Eastern Indigo Snakes. In contrast, iron concentrations ranged more than 5-fold across the prey species analyzed (87–535 mg/kg DM).



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Table 2. Mineral concentrations in prey (or potential prey) items consumed by Eastern Indigo Snakes (*Drymarchon couperi*). All nutrients presented on a dry-matter basis (mean ± SD). *denotes heads not included in analyses. na = not analyzed.

| Sample | *n* | Ca (%) | K (%) | Mg (%) | Na (%) | P (%) | Ca:P | Cu (mg/kg) | Fe (mg/kg) | Mn (mg/kg) | Mo (mg/kg) | Zn (mg/kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Snakes | | | | | | | | | | | | |
| Eastern Hognose | 2 | 5.12 ± 1.49 | 0.96 ± 0.08 | 0.12 ± 0.01 | 0.72 ± 0.20 | 2.86 ± 0.64 | 1.79 | 5.50 ± 0.71 | 163.00 ± 33.94 | 2.00 ± 0.00 | 0.40 (*n* = 1) | 135.00 ± 16.97 |
| Florida Pinesnake | 2 | 4.15 ± 0.56 | 0.99 ± 0.07 | 0.11 ± 0.01 | 0.48 ± 0.02 | 2.58 ± 0.28 | 1.61 | 2.00 ± 0.00 | 535.00 ± 258.80 | 11.00 ± 1.41 | 0.30 ± 0.28 | 108.50 ± 3.54 |
| Eastern Mudsnake | 3 | 4.26 ± 0.97 | 1.03 ± 0.08 | 0.10 ± 0.01 | 0.57 ± 0.10 | 2.38 ± 0.40 | 1.79 | 3.00 ± 1.73 | 482.67 ± 434.37 | 3.33 ± 2.31 | 0.27 ± 0.06 | 144.00 ± 21.79 |
| Southern Copperhead* | 2 | 7.01 ± 0.01 | 0.70 ± 0.01 | 0.15 ± 0.01 | 0.63 ± 0.03 | 3.75 ± 0.03 | 1.87 | 8.00 ± 1.41 | 156.00 ± 32.53 | 7.00 ± 1.41 | 0.55 ± 0.21 | 113.50 ± 6.36 |
| Southern Black Racer | 1 | 7.27 | 0.99 | 0.16 | 0.53 | 3.98 | 1.83 | 3.00 | 87.00 | 7.00 | 0.40 | 158.00 |
| Timber Rattlesnake* | 1 | 5.73 | 0.75 | 0.14 | 0.84 | 3.38 | 1.70 | 10.00 | 254.00 | 11.00 | 0.70 | 117.00 |
| Eastern Cottonmouth* | 1 | 5.74 | 0.75 | 0.13 | 0.62 | 3.15 | 1.82 | 9.00 | 410.00 | 4.00 | 0.60 | 104.00 |
| Snake Average ± SD | | 5.34 ± 1.37 | 0.91 ± 0.15 | 0.12 ± 0.02 | 0.61 ± 0.13 | 3.00 ± 0.65 | 1.77 ± 0.09 | 5.17 ± 3.04 | 325.58 ± 267.62 | 6.00 ± 3.69 | 0.42 ± 0.19 | 127.08 ± 21.16 |
| Tortoises | | | | | | | | | | | | |
| Gopher tortoise neonates | 2 | 3.29 ± 1.16 | 0.62 ± 0.05 | 0.08 ± 0.02 | 0.48 ± 0.06 | 1.87 ± 0.60 | 1.76 | 22.00 ± 5.66 | 365.50 ± 123.74 | 10.00 ± 0.00 | 1.00 ± 0.00 | 109.50 ± 16.26 |
| Partially digested prey | | | | | | | | | | | | |
| Rodent (species unknown) | 1 | na | na | na | na | na | na | na | na | na | na | na |
| Snake (species unknown) | 1 | 12.83 | 0.68 | 0.18 | 0.53 | 6.48 | 1.98 | <1 | 82.00 | 4.00 | 2.90 | 146.00 |



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Iron requirements for carnivorous species range from ~30 to ~100 mg/kg DM (AAFCO 2012, NRC 2006) depending on physiologic stage of development, and excessive levels may interfere with absorption and utilization of other trace minerals, including Cu (Puls 1988). Again, we noted some species-specific differences in Fe content that might reflect habitat/substrate or diet differences among prey-snake species. Recommended levels of dietary Mn and Zn (5–7.5 mg/kg, and 50–75 mg/kg, for dogs and cats, respectively; AAFCO 2012, NRC 2006) could be achieved through consumption of various individual food items or combinations. However, 71% (5 of 7) of the prey-snake species contained marginal or deficient levels of Mn, if consumed as sole diet items.

Until further studies are conducted to clarify the nutrient requirements of Eastern Indigo Snakes, the ranges measured in local prey items from native habitats can provide guidelines for nutritional assessment. Based on body composition analyses, snakes and Gopher Tortoise neonates eaten by Eastern Indigo Snakes represent less calorically dense (low fat, high ash) prey items compared with the chicks, rodents, or rabbits commonly fed to snakes in captivity. In a review of Eastern Indigo Snake prey items observed in the field from 1940–2008, 46% of 185 recorded prey items were snakes (Stevenson et al. 2010); these primary prey items may better represent optimal diets and nutritional profiles for Eastern Indigo Snakes. In contrast to energy, fat-soluble vitamin A and E concentrations, as well as Ca, P, and possibly Na contents in native prey were higher than expected based on published values for commercially reared whole-prey species. Of the trace minerals, Cu and Mn levels in some native whole prey appeared deficient. These data suggest that the diets of captive Eastern Indigo Snakes may need to be modified to better duplicate the diets of this species in the wild. Additionally, these observations may warrant further investigation to understand any underlying health implications for Indigo Snake populations. Future studies could examine seasonal variation in prey composition consumed by Eastern Indigo Snakes, not only due to a choice of species consumed but also variability within prey species, and correlate findings with natural history, behaviors, and physiologic status.

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