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Nutrient Composition of Whole Crayfish *Orconectes* and *Procambarus* species) Consumed by Hellbender (*Cryptobranchus alleganiensis*

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Hellbenders *Cryptobranchus alleganiensis* are one of the largest salamanders in North America, averaging 29–51 cm in total length, but reaching up to 70 cm (Nickerson and Mays 1973; Conant and Collins 1998; Johnson 2000). The Eastern Hellbender (C. a. alleganiensis) ranges from New York State south to Georgia, and west to Missouri, whereas the Ozark subspecies (C. a. bishopi) is only found in a few locations in south central Missouri and northern Arkansas (Firschein 1951; Pfingsten 1990; Petranka 1998). Serious population declines have targeted the Ozark Hellbender as a conservation priority species by numerous institutions (e.g. government agencies, universities, zoos, etc.). These institutions have spent considerable effort to recover the species in Missouri (Wheeler et al. 2003; Briggler et al. 2007). Due to the drastic decline in number of wild Hellbenders in Missouri, captive propagation was one necessary component to the recovery of the species in which the Ron Goellner Center for Hellbender Conservation at the Saint Louis Zoo was created. Numerous Hellbenders were removed from the wild and moved to their new artificial stream raceway to facitate long-term propagation efforts. As with many propagation efforts, various aspects of Hellbender biology are being investigated in detail at the St. Louis Zoo, including ecological nutrition and digestive physiology, to better address long-term health needs of this captive breeding stock. Hellbenders are fully aquatic, spending most of their life under flat rocks in stream bottoms (Nickerson and Mays 1973; Johnson 2000). Diet consists of a variety of aquatic prey including small fish and insects, but a majority of the diet comprises whole crayfish, consumed in their entirety (Nickerson and Mays 1973; Peterson et al. 1989; Petranka 1998).

The primary purpose of this study was two-fold: 1) to obtain details on a preferred food item of Hellbenders, for which no nutritional composition data exist (crayfish plus shell) as a basis for assessing nutritional status in the wild; and 2) to compare native food composition with substitute food items used in captivity. A secondary goal was to investigate possible factors that may underlie any differences noted in chemical composition. These initial data might provide useful information for optimizing nutritional health and dietary management of Hellbender and other crayfish-eating species (e.g., storks, Negro and Garrido-Fernandez 2000).

Samples.—Three species of native crayfish were used in this study. Long-pincered Crayfish *Orconectes longidigitus*), Spot-handed Crayfish *O. punctimanus*), and Ringed Crayfish *O. neglectus*) were opportunistically collected from two locations in close proximity on the North Fork of the White River, Missouri, USA in August 2006 and September 2007 N = 21 total samples). Crayfish were transported in buckets of stream water and maintained overnight before being identified to species, weighed, measured, and processed for nutrient analysis. Three individual crayfish were not positively identified and were included in samples as unidentified.

Feeder crayfish obtained locally in 2005 and 2006 comprised a single species, Northern Crayfish *Orconectes virilis*), from Paul's Bait Shop (PBS, N = 9; St. Louis, Missouri, USA) or directly from Ozark Fisheries (OF, N = 7; Stoutland, Missouri USA). In 2007, *Procambarus paeninsulanus* (Peninsula Crayfish) from Florida (FL, N = 9) were added as a substitute feeder species, and *O. virilis* were also collected for analysis from ponds on the grounds of the Saint Louis Zoo as part of a larger population study for potential sustainable harvest (SLZ, N = 21). SLZ crayfish of three body sizes (see below) were sampled from two sites (North and South Lakes) during either summer Jun–Aug) or fall (Sep–Oct) 2007. Purchased crayfish were shipped or transported in water overnight, and processed within 24 h of receipt. Crayfish acquired on zoo grounds were collected in the morning from traps set the previous day using canned cat food as bait, and sampled within 24 h. Because Hellbenders consume crayfish in toto, an additional subset of trapped crayfish from SLZ N = 24) were dissected to determine percent contribution of nutrients from shell vs. remainder of the body by weighing and analyzing portions separately, and water and substrate samples were taken for chemical analysis at the two sites.

Laboratory Processing/Analyses.—Animals were weighed to the nearest 0.1 g, and carapace and tail-to-rostrum length TR) measured to the nearest 0.01 cm with calipers. Crayfish were categorized as small (<2.5 cm total length), medium-sized 2.5 to 5 cm total length), or large (>5 cm). All crayfish were euthanized by decapitation, then head and body were homogenized together in a food processor (Toastmaster Chopster). Subsamples of homogenate (0.5 g in duplicate) were taken immediately and extracted via previously described methods for meat samples (Barker et al. 1998) to determine vitamin A (as retinol), E as α-tocopherol), and total carotenoids. Extracts were sealed in cryovials, stored in an ultracold freezer (-20°C), and sent to Arizona State University for analysis via HPLC following the methods of McGraw et al. (2006). Pigment extracts were resuspended in 200 µl mobile phase 42:42:16 (v/v/v) methanol : acetonitrile : dichloromethane) and injected into a Waters Alliance 2695 HPLC system (Waters Corporation, Milford, MA) fitted with a Waters YMC Carotenoid 5.0 µm column (4.6 mm × 250 mm) and a built-in column heater set at 30°C. A three-step gradient solvent system was used to analyze both xanthophylls and carotenes in a single run, at a constant flow rate of 1.2 ml min-1: first, isocratic elution with the aforementioned mobile phase for 11 min, followed by a linear gradient up to 42:23:35 (v/ v/v) methanol : acetonitrile : dichloromethane through 21 min, held isocratically at this condition until 26 min, and finishing with a return to the initial isocratic condition from 26 to 29.5 min. Data were collected from 250 to 600 nm using a Waters 2996 photodiode array detector. Pigments were identified by comparing their respective retention times and absorbance maxima λmax) to those of reference carotenoids run as external standards.

Subsamples (1.0 g) from 10 randomly-selected farmed crayfish *Orconectes virilis*) were frozen for determination of fatty acid content at the University of Missouri-Columbia; a frozen Pacific Krill *Euphasia pacifica* sample was also sent for comparison since krill are often used in feeding very small salamanders (J. Ettling, pers. comm.). Total lipids were extracted with chloroform: methanol (2:1 v/v) after samples were homogenized in 10 mM EDTA. Lipids were methylated with freshly made 5% methanolic HCl. Fatty acid methyl esters were extracted with hexane. Pigments and residual water were removed from each sample with the addition of 0.1 g each of anhydrous sodium sulfate and activated charcoal. Methyl esters were analyzed by gas chromatography using an HP 5890A instrument with a 30-meter capillary column (Omegawax 250; Supelco, Bellefonte, PA) according to the methods of Sukhija and Palmquist 1988. Individual fatty acids were identified using retention times of standards (i.e., Omegawax, PUFA-II, PUFA-I; Supelco).

Remaining sample homogenates were freeze-dried (Labconco Freezone 4.5) to determine water content, and dried, ground samples were sent for proximate analysis (crude protein, crude fat, fiber fractions, ash) and minerals to Dairy One Forage Lab (Ithaca, NY). Substrate mineral content was determined at the Amino Acid Laboratory, University of California (Davis, California; data not presented). Differences in nutrient composition by species (stream-caught) or source/location within species pond-reared) were evaluated with Mann-Whitney tests at a significance level of 0.05 (Snedecor and Cochran 1967). For the STZ crayfish samples only, percentage data were arcsine transformed to approximate normal distribution, and seasonal, body size, and sampling location differences in nutrient composition were compared by analysis of variance.

Wild-captured crayfish sampled from Hellbender habitats varied widely in size compared to those utilized as food for captive salamanders at the Saint Louis Zoo (Table 1). Regardless of species, size ranges encompassed the small and medium body size criteria preferred by zoo managers. Specific preferences of crayfish prey size have not been determined for Hellbenders; presumably crayfish must be suitably sized to trigger a feeding response, yet not too large to defend themselves against ingestion. Although similar in carapace length to those obtained from commercial suppliers, Northern Crayfish from the zoo ponds in this study contained ash-free dry weight (AFDW) similar to that reported from wild-caught Virginia *O. virilis* (~3.7 g vs. 3.8 g, respectively; Mitchell and Smock 1991) and almost twice the AFDW calculated for the fisheries-reared crayfish (~2.0 g from PBS, ~1.5 g from OF).

Body sizes did not differ between *O. virilis* samples collected for dissection at the two STZ sites, hence data were combined (mean weight = 25.2 ± 5.4 g). Of total body composition, shell comprised 61.5 ± 5.7% of whole body mass, whereas meat and body fluids reflected the remaining 38.5 ± 5.7% (wet basis). Shells averaged 31.2 ± 4.3% dry matter DM) (or 68.8% water), and meat averaged 17.8 ± 2.1% DM, approximately 82.2% water.

Proximate Composition.—Overall, crayfish provided crude protein concentrations in excess of the requirements determined for domestic carnivores, fish and shellfish (ca. 15 to ~40% of DM; Zootrition 2006). Protein and fat, as fractions of whole body mass, are typically inversely related when evaluating composition of whole vertebrate prey. This pattern was not apparent in crayfish, possibly due to the much greater contribution of both ash and carbohydrate fractions (from shells) in crayfish compared to vertebrate prey species. Total ash reported in whole vertebrate prey DM ranges from 7.5 (juvenile hamsters) to 17.5% (snowshoe hare) as opposed to 30–45% in crayfish, whereas carbohydrate content is generally negligible (Dierenfeld et al. 2002). Additionally, the crude fat content of most whole prey (range 5–45% of DM, but typically 15–35%, depending on species and age of prey; Dierenfeld et al. 2002) is considerably higher than recommended minimum dietary levels (~5 to 10% of dry matter, DM) for domestic carnivores (NRC 2006). Crayfish contained some of the lowest fat concentrations measured across a wide variety of whole prey consumed by carnivores, with both wild-caught and farmed crayfish averaging <5% fat (DM basis, Tables 2 and 3). This may be important for overall energetics and nutritional balance, as amphibians and other poikilotherms have a lower metabolic rate than homeotherms, hence require fewer calories per unit body mass. Thus low-fat, lower nutrient density dietary ingredients may be entirely appropriate for Hellbenders, but actual nutritional requirements remain uncharacterized for amphibians and most reptiles. As with all species—but perhaps particularly so for low-energy amphibian species—the feeding of higher-fat dietary ingredients in captive management programs should be carefully controlled to minimize risk of obesity or health conditions associated with excess body condition.

Nonetheless, some fat, and particularly essential fatty acids (EFAs) are required in the diet, hence our analysis provides baseline information on fatty acid content of whole crayfish (Table 4). Fatty acids are the primary constituent of most lipids, and can be deposited in animal tissues with minimal modification from diets; fatty acid signatures are often used to differentiate and understand trophic interactions in aquatic ecosystems (Iverson et al. 2002). Although the essential fatty acid (EFA) requirements of salamanders have not been determined to our knowledge, minimum dietary recommendations of domestic carnivores for two of the omega-6 EFAs, linoleic acid (18:2n6) and arachidonic 20:4n6), have been set at 0.5 and 0.2% of dietary DM, respectively NRC 2006). Crayfish in this study contained 9% of total FA as linoleic, and 6.5% as arachidonic acid (see Table 4). Converting FA data reported here to a comparable dietary DM basis yields 0.3%

Table 1. Size (mean ± SD; range in parentheses) parameters of crayfish sampled to determine nutrient composition of whole crayfish available as food to Ozark Hellbender (*Cryptobranchus allegeniensis bishopi* . nd = not determined.

Species	N	Mass (g)	Carapace Length (cm)	Tail–Rostrum Length (cm)
Wild-Caught				
Longpincered Crayfish	7	53.20 ± 20.33	8.00 ± 3.79	11.22 ± 2.04
(*Orconectes longidigitus*		14.54–75.14)	4.6–13.6)	8.00–13.40)
Ringed Crayfish	7	10.80 ± 3.45	3.00 ± 0.48	6.19 ± 1.01
(*Orconectes neglectus*		3.18–14.56)	2.23–3.37)	4.53–7.17)
Spothanded Crayfish	4	23.12 ± 6.31	5.96 ± 2.91	7.97 ± 0.47
(*Orconectes punctimanus*		13.62–31.04)	3.57–9.70)	7.60–8.50)
Unidentified	3	8.35 ± 5.33	2.69 ± 0.55	5.46 ± 1.11
(*Orconectes* species		3.95–14.27)	(2.11–3.20)	4.26–6.43)
Farmed				
Northern Crayfish - Local bait shop	9	12.11 ± 3.56	7.68 ± 0.82	nd
(*Orconectes virilis*		6.19–16.69)	6.0–8.9)	
Northern Crayfish - Ozark Fisheries	7	9.64 ± 2.13	7.14 ± 0.50	nd
(*Orconectes virilis*		5.42–16.88)	6.0–8.4)	
Northern Crayfish - SLZ ponds	21	21.04 ± 8.77	7.39 ± 2.27	10.41 ± 2.71
(*Orconectes virilis*		7.74–38.64)	3.93–18.19)	6.88–15.00)
Peninsula Crayfish - Florida	9	6.13 ± 2.85	2.94 ± 0.83	5.55 ± 0.79
(*Procambarus paenenisulanus*		2.92–9.66)	2.10–4.83)	4.26–6.43)

linoleic acid (3% crude fat (Table 3) * 0.09), and 0.2% arachidonic (3% fat *0.065). Assuming that amphibian carnivores have similar fatty acid requirements as domestic animals, and since fat has ~2 times the caloric value of carbohydrates or protein, crayfish would provide about 0.5% of dietary energy as linoleic acid, a value that would be considered borderline for meeting EFA requirements in the absence of any preformed arachidonic acid (AA). However, the presence of 0.2% AA determined in the crayfish should be sufficient to meet the requirements for omega-6 polyunsaturated FA for most animals that might be consuming crayfish as a primary food source. Marine krill, however, appear to provide insufficient levels of both EFAs (Table 4). Both whole crayfish and whole krill contained relatively high concentrations of polyunsaturated fatty acids (PUFA) (22–32% of total fatty acids as PUFA). PUFA can be more prone to oxidative degradation compared with more saturated fats, thus may require higher levels of dietary antioxidants. By comparison, saturated fats were much higher in the marine krill (45.4%) compared with fresh water crayfish (32.4%), comprising almost half of total fatty acids quantified. Additionally, the omega3:omega6 fatty acid ratios measured in crayfish compared with krill vary considerably (Table 4), with omega-6 fatty acids about 10-fold higher in crayfish compared with krill; these two feed types are not nutritional equivalents. In particular, omega3:omega 6 ratios/composition may have health implications for captive feeding programs of Hellbenders, particularly regarding immune function (Fritsche 2006), which should be investigated further. Interestingly, meat from farmed crayfish contained higher concentrations of all fatty acids compared with meat from wild crayfish reported in the USDA Nutrient Database (USDA 2009), further suggesting that captive dietary management may certainly influence composition, and should be considered when raising species as food for other animals.

Regarding carbohydrate (CHO), fractions, while most vertebrate whole prey are not considered to contain substantial concentrations of simple sugars, dietary fiber (measured as crude fiber, acid detergent fiber, and/or neutral detergent fiber), may be measurable in gastrointestinal contents of herbivorous prey species. In the case of crayfish, the chitinous exoskeleton is a chemical matrix comprising polysaccharide units (similar to plant cellulose) and protein. It is unclear whether the exoskeleton provides a source of dietary nutrients to Hellbenders; chitinase enzyme activity has not been reported, and digestibility trials have not been performed. Hellbenders have the ability to regurgitate exoskeleton fragments—identification of such samples is a technique routinely used for feeding ecology studies (Peterson et al. 1989). The dietary fiber (CHO) measured in crayfish samples (~14 to 24–30% of DM, depending on fraction considered from Tables 2 and 3) derives from the exoskeleton, and likely provides physical fill and/or a source of mineral nutrition to Hellbenders consuming whole crayfish, rather than readily available calories.

Mineral Content.—In general, the wild-caught crayfish (Table 2) contained more inorganic constituents (ash) compared with commercially-reared crayfish (Table 3; 47.16 versus 36.81%, respectively) and thus may provide better dietary mineral levels. The shell contributed 79.6% of measured ash, ~90% of Ca, and 31–64% of other minerals (with the exception of S, where 80% was present in meat). Northern Crayfish sampled from SLZ were more similar to wild-captured crayfish than were those sampled from either PBS or OF, and differed significantly ($P < 0.05$) in total ash, Ca, Cu, and Fe from those groups (Table 3). Other

TABLE 2. Nutrient composition (mean ± SD) of wild captured crayfish collected from two locations in close proximity on the North Fork of the White River, Missouri, Aug 2006 and Sept 2007. Data represent whole crayfish; all nutrients except water presented on a dry matter basis.

Species N	*Orconectes longidigitus* 7	*Orconectes neglectus* 7	*Orconectes punctimanus* 4	*Orconectes* species 3
Nutrient				
Water, %	67.40 ± 10.07	65.84 ± 10.31	64.25 ± 3.59	71.59 ± 1.57
Crude Protein, %	41.53 ± 3.95	38.33 ± 4.17	38.08 ± 1.83	34.45 ± 1.77
Crude Fat, %	4.47 ± 1.51	4.44 ± 1.32	3.38 ± 1.33	2.75 ± 1.20
Crude Fiber, %	14.25 ± 0.49	15.20 ± 0.14	14.20 (N = 1)	na
Neutral Detergent Fiber, %	23.36 ± 4.00	23.02 ± 5.33	24.42 ± 2.78	20.95 ± 2.62
Acid Detergent Fiber, %	14.70 ± 2.28	14.41 ± 2.47	16.01 ± 3.08	15.42 ± 0.73
Ash, %	42.77 ± 5.09	46.11 ± 5.15	47.43 ± 4.02	52.33 ± 1.59
Minerals				
Calcium, %	15.65 ± 2.23	20.27 ± 5.07	15.96 ± 4.51	19.49 ± 0.59
Chloride, %	0.73 ± 0.01	0.57 ± 0.10	0.64 (N = 1)	na
Magnesium, %	0.33 ± 0.05	0.26 ± 0.02	0.30 ± 0.03	0.28 ± 0.02
Phosphorus, %	0.96 ± 0.15	0.93 ± 0.14	0.77 ± 0.08	0.78 ± 0.04
Potassium, %	0.52 ± 0.05	0.51 ± 0.09	0.53 ± 0.07	0.54 ± 0.07
Sodium, %	0.50 ± 0.04	0.55 ± 0.15	0.47 ± 0.08	0.53 ± 0.11
Sulfur, %	0.35 ± 0.05	0.31 ± 0.07	0.30 ± 0.05	0.24 ± 0.01
Cobalt, mg/kg	0.49 ± 0.03	0.58 ± 0.05	0.41 (N = 1)	na
Copper, mg/kg	38.86 ± 8.40	27.57 ± 5.09	27.00 ± 7.70	39.00 ± 11.31
Iron, mg/kg	59.29 ± 26.31	71.57 ± 34.98	73.00 ± 37.72	38.50 ± 6.36
Manganese, mg/kg	82.29 ± 23.94	85.57 ± 15.13	93.50 ± 38.52	86.50 ± 23.33
Molybdenum, mg/kg	0.47 ± 0.28	0.40 ± 0.35	0.20 ± 0.14	0.20 ± 0.00
Zinc, mg/kg	75.86 ± 8.30	78.57 ± 6.32	65.25 ± 11.09	84.50 ± 3.54
Fat-Soluble Vitamins				
Vitamin A, µg/g, as retinol	95.57 ± 143.25	123.43 ± 124.61	160.14 ± 164.89	10.36 ± 5.34
Vitamin A, IU/kg	318,567± 477,500	411,433 ± 415,367	533,800 ± 549,633	34,533 ± 17,800
Vitamin E, mg/kg, α-tocopherol	267.72 ± 129.08	261.98 ± 141.30	248.26 ± 83.33	162.21 ± 66.62
Total Carotenoids, mg/kg	30.90 ± 12.96 ^{ab}	26.43 ± 19.35 ^{ab}	43.52 ± 11.74^{a}	21.14 ± 3.08^{b}

^{ab} Means in the same row without a common superscript letter differ (Mann-Whitney test; $P < 0.05$).

significant differences between the SLZ-pond and fishery-pond reared crayfish (Mg, K, S levels) may reflect differences in water or substrate quality, and/or result from unspecified holding and feeding schedules at the bait shop. Macromineral requirements (as a percentage of dietary DM) for growing mammal and bird species (Ca, 0.4 to 1.2%; Mg, 0.03 to 0.1%; P, 0.3 to 0.6%; K, 0.2 to 1.4%; and Na, 0.05 to 0.4%; Zootrition 2006) appear to be met by any of the crayfish analyzed in this study.

Wide variability among and within samples in trace element composition is evident and likely due to multiple factors including limited sample size, differing original dietary trace mineral levels, and possibly habitat/substrate variability. Mineral concentrations in water and substrate did not differ between sampled sites at SLZ (data not shown), but neither water, substrate, nor food samples were obtained from any other crayfish group, so source of variation is unknown at this time. Dietary requirements for Cu range from about 3 to 5 mg/kg DM for domestic carnivores (NRC 2006); and high levels can be toxic. In particular, the high Cu level documented in some crayfish (>100 mg/kg) is of potential health concern. Although LC50 is not directly comparable to LD50, toxicity (based on survival) has been determined between 40 and ~800 µg/L for *Ambystoma* spp. salamanders (Sparling et al. 2000), a magnitude lower than dietary levels measured in this study. While Cu LD50 has not been reported for salamanders, dietary tolerance of this nutrient is 15 and 40 mg/kg for sheep and cattle, respectively, and 100 mg/kg for fish (NRC 2005). Northern Crayfish could have been exposed to copper from commercial fish foods and fish wastes in confined ponds, which may have affected their overall composition. Copper sulfate is often added as an algaecide and may contribute to crayfish body composition (38% of Cu was found in the shell); hence, aquatic environmental quality is critical both directly and indirectly to the health of

TABLE 3. Nutrient composition (mean ± SD) of pond-reared crayfish consumed by Ozark Hellbender *Cryptobranchus alleganiensis bishopi* at the Saint Louis Zoo (2005–2007). Data represent whole crayfish; all nutrients except water presented on a dry matter basis.

Species	*Orconectes virilis*	*Orconectes virilis*	*Orconectes virilis*	*Procambarus paeninsulanus*
N	9	7	21	9
	Paul's Bait Shop	Ozark Fisheries	St. Louis Zoo	Florida
Nutrient				
Water, %	74.31 ± 4.16	77.58 ± 3.23	68.02 ± 5.08	70.71 ± 4.57
Crude Protein, %	54.08 ± 4.14	54.80 ± 5.68	42.00 ± 4.98	47.84 ± 5.29
Crude Fat, %	2.96 ± 1.47	3.70 ± 0.80	3.03 ± 1.41	4.90 ± 1.62
Crude Fiber, %	20.04 ± 7.38a	13.78 ± 2.79b	na	na
Neutral Detergent Fiber, %	17.27 ± 0.95	21.70 ± 4.70	28.98 ± 3.31	31.94 ± 14.19
Acid Detergent Fiber, %	13.70 ± 0.66a	11.55 ± 0.64b	13.79 ± 1.53 ab	15.44 ± 3.84
Ash, %	35.69 ± 4.42a	31.90 ± 3.69a	45.36 ± 5.71b	34.27 ± 3.20
Minerals				
Calcium, %	12.00 ± 1.79a	11.85 ± 1.87a	16.31 ± 2.31b	12.25 ± 0.88
Chloride, %	1.22 ± 0.20	1.29 ± 0.01	na	na
Magnesium, %	0.40 ± 0.04a	0.28 ± 0.07b	0.37 ± 0.04a	0.35 ± 0.02
Phosphorus, %	1.16 ± 0.04	1.11 ± 0.06	1.07 ± 0.16	0.92 ± 0.06
Potassium, %	0.68 ± 0.11a	0.96 ± 0.15b	0.64 ± 0.14a	0.81 ± 0.08
Sodium, %	0.73 ± 0.06	0.78 ± 0.10	0.62 ± 0.09	0.60 ± 0.09
Sulfur, %	0.44 ± 0.06ab	0.47 ± 0.03a	0.33 ± 0.07b	0.40 ± 0.08
Cobalt, mg/kg	0.99 ± 0.29	1.22 ± 0.12	na	na
Copper, mg/kg	107.44 ± 12.19a	110.57 ± 18.38a	34.24 ± 13.51b	46.75 ± 9.74
Iron, mg/kg	133.78 ± 53.60a	104.00 ± 34.79a	315.71 ± 134.60b	89.25 ± 84.13
Manganese, mg/kg	108.44 ± 101.15	55.29 ± 13.34	43.19 ± 23.56	9.88 ± 2.17
Molybdenum, mg/kg	0.17 ± 0.08a	0.53 ± 0.23b	0.67 ± 0.46b	0.20 ± 0.08
Zinc, mg/kg	87.78 ± 6.04	84.86 ± 11.32	71.81 ± 9.45	66.38 ± 4.27
Fat-Soluble Vitamins				
Vitamin A, µg/g, as retinol	482.23 ± 70.95a	878.22 ± 206.11b	7.97 ± 2.51c	8.30 ± 2.69
Vitamin A, IU/kg	1,607,433 ± 236,500 a	2,927,400 ± 687,033 b	26,567 ± 8,367c	27,667 ± 8,967
Vitamin E, mg/kg, α-tocopherol	320.16 ± 118.80a	485.65 ± 190.76b	353.84 ± 136.49ab	252.07 ± 116.61
Total Carotenoids, mg/kg	55.37 ± 15.29	56.18 ± 38.79	32.35 ± 15.32	48.75 ± 17.41

^{ab} Means in the same row without a common superscript letter differ (Mann-Whitney test; P < 0.05).

the Hellbender. Similarly, iron requirements for carnivores range from ~30 to ~100 mg/kg DM (NRC 2006), and excessive levels may interfere with bioavailability of other trace minerals. Recommended levels of bioavailable Mn and Zn (5 mg/kg, and 10 to 50 mg/kg, for cats and dogs, respectively; NRC 2006) would likely be met by crayfish as a dietary staple, but nutrient requirements and dietary interactions in salamander nutrition remain to be investigated.

Fat-Soluble Nutrients.—Vitamin A levels tend to increase with age/maturity in vertebrate prey through accumulation in body stores, particularly liver. Body size and vitamin A content were uncorrelated in our study; however, vitamin A levels were exceptionally high in farmed (OF) and baitshop (PBS) sourced crayfish (Table 3; 1.6 to 2.9 million IU/kg DM (0.3 µg retinol = 1 IU)) as well as in 3 of 4 wild crayfish species (~300,000 to 500,000 IU vitamin A/kg DM). The unidentified species and the SLZ pond-caught crayfish contained vitamin A concentrations ranging between ~26,000 and 40,000 IU/kg DM. Dietary requirements for vitamin A in domestic carnivores are approximately 4000 to 11,000 IU/kg DM (NRC 2006); presumed upper safe limits of this nutrient range from 33,000 IU/kg (dogs) to 100,000 IU/kg DM cats) (NRC 1987). Clearly more information is needed on safe levels of this nutrient for salamanders (and crayfish), but it appears likely that diets fed to the farmed animals may have contributed to the high body levels found in this study.

Vitamin E concentrations were quite high in all crayfish sampled; crayfish meat has been previously shown to be a good source of this nutrient (USDA 2009) and may provide an important dietary antioxidant. Vitamin E levels in crayfish 160–486 mg/kg DM, calculated 240–729 IU/kg) consistently exceeded

TABLE 4. Fatty acid composition (mean ± SD) of whole Missouri native crayfish (Northern Crayfish, *Orconectes virilis*), compared with whole Pacific Krill (*Euphasia pacifica* .

Fatty Acid Name	Fatty Acid*	Crayfish N = 10)	Krill N = 1)
		% of total fatty acids	
Lauric acid	12:0	4.8 ± 1.0	2.5
Myristic acid	14:0	3.6 ± 0.9	15.6
Palmitic acid	16:0	19.0 ± 3.6	25.6
Palmitoleic acid	16:1	6.6 ± 3.2	10.6
Stearic acid	18:0	5.0 ± 1.3	1.7
Oleic acid	18:1n9	24.8 ± 3.8	20.3
Linoleic acid	18:2n6	9.0 ± 2.9	1.9
γ-linolenic acid	18:3n6	0.7 ± 0.3	0.3
α-linolenic acid	18:3n3	4.0 ± 2.6	0
Dihomo- γ -linolenic acid	20:3n6	1.0 ± 1.0	0
Arachidonic acid	20:4n6	6.5 ± 1.9	0.3
Eicosapentaenoic acid (EPA)	20:5n3	8.1 ± 1.9	13.5
Docosahexaenoic acid (DHA)	22:6n3	2.6 ± 1.3	6
	%PUFA	31.9	22.0
	n3:n6	0.85:1	7.8:1

* First number denotes the # of carbons in the fatty acid; 2nd # denotes the total # of double bonds; the number following "n" refers to the position of the first double bond relative to the methyl- or "omega"–end of the fatty acid.

dietary requirements established for domestic carnivores 30–50 IU/kg; 1 mg natural source vitamin E = ~ 1.5 IU) fed diets containing moderate levels of polyunsaturated fats (PUFAs). High dietary PUFA (as found in crayfish), however, may increase the vitamin E requirement up to 5-fold due to the high oxidative potential of these fats; vitamin E can be depleted as it is utilized as a biological antioxidant. Vitamin E deficiency has been shown to adversely impact reproduction and immune function in other species (Dierenfeld and Traber 1992). Hence these high concentrations of vitamin E may be beneficial to health of Hellbenders, but require further investigation.

The only nutrient category that differed significantly among wild-caught crayfish species was total carotenoid concentration (Table 2), and unfortunately, this was compared with the species that remains unidentified. Carotenoid content measured is likely an artifact of small sample size, but may reflect real differences in feeding habits among crayfish species. *O. punctimanus* appears much broader in its habitat and possibly feeding requirements compared to other species, (MOFWIS 2008) and thus may be able to consume a diet containing higher levels of carotenoid pigments, but must be quantified further in more controlled studies. Diets fed in the fisheries pond(s) quite likely also contributed to the high levels of vitamins and carotenoids measured in the crayfish. It remains unclear, however, whether these higher nutrient levels are beneficial or detrimental to the salamanders consuming crayfish, as both vitamin A toxicity and deficiency can negatively impact health and reproduction. Vitamin A concentrations are lower in native crayfish or pond-captured crayfish compared with those sampled from farm-reared populations, but ranges varied by levels of magnitude, and, in general were higher than documented dietary requirements for carnivorous species in all samples – regardless of origin. Some of these concentrations are in ranges considered toxic for other species; to date, salamander dietary vitamin A nutrient requirements are unknown. Controlled feeding trials should be conducted and/or tissue levels of fat-soluble nutrients should be monitored to determine nutrient interactions and evaluate status. One potentially negative factor associated with low-fat diets (as found in crayfish) may be lower absorption of fat-soluble vitamins; this possibility should be investigated in future studies with Hellbenders.

Sources of variability in nutrient parameters measured in the SLZ crayfish found season (summer vs. fall) to be significant only in crude fat content $P < 0.01$); location significantly impacted Cu ($P < 0.01$), Zn ($P < 0.01$), and P $P < 0.001$) levels, even though trace mineral and water phosphate levels did not vary between the two sites (data not shown). Body size (hence, age/particular molt) had the greatest impact on both proximate and mineral nutrient composition of Northern crayfish at the Saint Louis Zoo, affecting crude protein ($P < 0.001$), ash ($P < 0.001$), Ca ($P < 0.05$), K ($P < 0.001$), Na $P < 0.05$), P $P < 0.05$), Cu $P < 0.001$), Mn ($P < 0.05$), and Zn ($P < 0.01$) content. Larger crayfish contained higher levels of proximate nutrients (protein, fat, and ash) as well as macrominerals Ca, K, and Na compared to smaller animals. Phosphorus content, however, was higher in smaller crayfish, as were levels of trace minerals (Cu, Mn, and Zn). Locally harvested crayfish represented a nutritionally superior food for captive Hellbender compared to fisheries-reared individuals, and were closer in chemical composition to species found in native habitats. Sustainable local harvest should be explored as a viable alternative feeding option and as a way of reducing fossil fuel transportation) costs.

These data provide preliminary detail on the nutrient composition of whole crayfish eaten by Hellbender, but clearly species, size, and habitat of origin impact the nutritional content of crayfish (and likely other aquatic prey species) eaten by Hellbenders. The chemical composition of native, as well as substitute, food items must be considered integral to the development of optimal diets for this species, and monitoring prey composition can provide strong bioindicators of habitat quality. In addition to crayfish, Hellbenders also eat small fish, lamprey, worms, insects, snails, mollusks, tadpoles, and fish entrails in nature Nickerson and Mays 1973; Peterson et al. 1989; Petranka 1998); in captivity, they are fed black worms, krill, and fish along with crayfish (J. Ettling, pers. comm.). Intake, utilization, growth response trials, and health assessments to these varied whole prey items, and mixed diets, should provide valuable management guidelines that can assist in captive propagation, habitat evaluation, conservation and ultimate recovery of these unique and threatened salamanders. Characterization of both ex situ and in situ diets remains a critical component underlying health, reproduction, and recovery program management.

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NATURAL HISTORY NOTES

The Natural History Notes section is analogous to Geographic Distribution. Preferred notes should 1) focus on observations in the field, with little human intrusion; 2) represent more than the isolated documentation of developmental aberrations; and 3) possess a natural history perspective. Individual notes should, with few exceptions, concern only one species, and authors are requested to choose a keyword or short phrase which best describes the nature of their note e.g., Reproduction, Morphology, Habitat, etc.). Use of figures to illustrate any data is encouraged, but should replace words rather than embellish them. The section's intent is to convey information rather than demonstrate prose. Articles submitted to this section will be reviewed and edited prior to acceptance.

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Standard format for this section is as follows: SCIENTIFIC NAME, STANDARD ENGLISH NAME if available, for the United States and Canada as it appears in Crother [ed.] 2008. *Scientific and Standard English Names of Amphibians and Reptiles of North America North of Mexico*. SSAR Herpetol. Circ. 37:1–84, available from SSAR Publications Secretary, ssar@herplit.com; for Mexico as it appears in Liner and Casas-Andreu 2008, *Standard Spanish, English and Scientific Names of the Amphibians and Reptiles of Mexico.* Herpetol. Circ. 38:1–162), KEYWORD. DATA on the animal. Place of deposition or intended deposition of specimen(s), and catalog number(s). Then skip a line and close with SUBMITTED BY give name and address in full—spell out state names—no abbreviations). NCN) should be used for common name where none is recognized. References may be briefly cited in text refer to this issue for citation format). One additional note about the names list Crother 2008) developed and adopted by ASIH-HL-SSAR: The role of the list is to standardize English names and comment on the current scientific names. Scientific