NUTRIENT COMPOSITION OF WHOLE CRAYFISH EATEN BY HELLBENDER SALAMANDERS (*CRYPTOBRANCHUS ALLEGANIENSIS SPP.*)

Ellen S. Dierenfeld PhD,[1]* Kevin McGraw,[2] Kevin Fritsche,[3] and Katherine R. Kerr[1,4]

[1]Department of Animal Health and Nutrition, Saint Louis Zoo, St. Louis, MO; [2]School of Life Sciences, Arizona State University, Tempe, AZ; [3]Department of Animal Science, University of Missouri – Columbia, Columbia, MO; [4]Department of Nutritional Sciences, University of Illinois, Champaign, IL

Abstract

Four species of native Missouri crayfish identified as foods of the Ozark hellbender salamander (*Cryptobranchus alleganiensis bishopi*), were analyzed for nutrient content including water, proximate composition (crude protein, crude fat, crude fiber, and ash), as well as vitamin A, vitamin E, total carotenoids, and select mineral concentrations. Additionally, fatty acids in crayfish were examined and compared with krill as a possible substitute food item for captive and/or larval salamanders. Crayfish collected from running streams (n=5 samples) contained less water than those collected in standing water (n=11 samples); 62 – 65% vs. 74 – 80%, respectively. On a dry matter (DM) basis, crayfish collected in native streams contained less protein (34 to 41%) than "farmed" crayfish (54 to 60%) and more ash (43 to 51% vs. 31 to 36%, respectively. Fat content in whole crayfish ranged from 2 to about 4.5% of DM. Overall, vitamin A concentrations in whole crayfish were low (755 to 2951 IU/g of DM, whereas vitamin E concentrations were relatively high (140 to 808 IU/g DM); crayfish sampled from "farmed" areas contained about twice the concentration of these nutrients as stream-living crayfish. Total carotenoids were also considerably higher in farm-raised crayfish, 55 to 75 mg/kg compared to 12 to 34 mg/kg in stream-living animals. All crayfish displayed high calcium content, 10-20% of DM, likely due to incorporation of this mineral within chitinous exoskeletons, whereas phosphorus levels ranged from 0.8 to 1.2% of DM. Both macromineral and trace element concentrations varied by habitat from which the crayfish were sampled. These nutrient differences are likely the result of nutrients in commercial diets fed to fish in the managed fisheries operations from which the crayfish were sampled. The comparison of molar ratios of fatty acids in crayfish compared with marine krill suggest that krill are not an equivalent nutritional substitute; krill contain higher levels of saturated fatty acids, and a very different distribution of n-3 to n-6 fatty acids than do crayfish. These initial data provide some baseline information for developing more optimal feeding programs for endangered species that consume crayfish, including the Ozark hellbender salamander.

Introduction

Hellbenders are the largest salamanders in North America, averaging 24 to 44 cm in length, but reaching up to 64 cm.[4,6] The Eastern hellbender (*Cryptobranchus alleganiensis 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.[3,8] Serious population and habitat declines have targeted the Ozark hellbender as a conservation priority species of the Saint Louis Zoo,[4,9] and various aspects of its biology are being investigated in detail, including ecological nutrition and digestive physiology.

Hellbenders are fully aquatic, spending most of their life under flat rocks in stream bottoms.[4,6] Diet consists of a variety of aquatic prey including small fish and insects, but a majority of the diet comprises whole crayfish.[4,6,7] The nutrient composition of whole crayfish consumed by hellbenders was examined, and data from species found in native habitat with those available commercially (either as by-product of fisheries and/or from bait shops) were compared, as well as with some whole prey often fed to hellbenders in captivity. These initial data may provide useful information for optimizing nutritional health and dietary management of hellbender salamanders and other crayfish-eating species.

Methods

Three species of native crayfish found in the North Fork of the White River, Missouri, were collected in August, 2006. Ringed crayfish (*Orconectes neglectus*) ranged from 6.6 to 7.7 cm in body length, weighing 11.5 ± 1.9 g. Six individuals were measured, five were sampled individually for vitamins and carotenoids; remaining purees of three specimens each were pooled for further nutritional analysis (n = two samples). Spot-handed crayfish (*O. puntimanus*) were medium-sized, ranging from 8.5 to 9.7 cm in length, weighing 25.1 ± 2.7 g; three animals were individually sampled for vitamin and carotenoids, then the purees pooled to comprise one sample for further analysis. The largest species, long-pincered crayfish (*O. longidigitus*), ranged from 13.3 to 13.6 cm in length and weighed 63.5 ± 1.6 g; two animals were individually sampled (n = two). Northern crayfish (*O. virilis*) were collected either from a local bait shop (n = nine samples) or recovered from commercial fishery ponds (n = two samples). Body lengths varied from 6.5 to 13.5 cm, and weights ranged from 12 to 43 g, thus encompassed the range of the free-swimming crayfish. Northern crayfish would have been exposed to commercial fish foods and fish wastes in confined ponds, whereas the three other species were living in rapidly-flowing streams.

All crayfish were euthanized by decapitation; heads and remainder of body were then homogenized in a food processor. Subsamples were taken immediately and one set extracted via previously described methods for meat samples to determine vitamin A (as retinol), E (as α-tocopherol), and total carotenoids.[1] Extracts were sealed, stored in a freezer, and sent to Arizona State University for analysis via HPLC following the methods of McGraw et al.[5] Pigment extracts were 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 X 250 mm) and a built-in column heater set at 30°C. A three-step gradient solvent system was used to analyse both xanthophylls and carotenes in a single run, at a constant flow rate of 1.2 ml min^{-1}: first, isocratic elution with 42:42:16 (v/v/v) methanol : acetonitrile : dichloromethane 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 30 min, and finishing with a return to the initial isocratic condition from 30 to 48 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 (λ_{max}) to those of reference carotenoids run as external standards.

Subsamples from ten crayfish (*Orconectes spp.*) were frozen for determination of fatty acid content at the University of Missouri-Columbia; a frozen whole krill (*Euphasia pacifica*) sample was also sent for comparison since krill are often used in feeding very small salamanders. Total lipids were extracted with chloroform:methanol (two:one v/v) after samples were homogenized in ten mM EDTA. Lipids were methylated with freshly made five % methanolic HCl. Fatty acid methyl esters were extracted with benzene. Pigments and residual water were removed with the addition of 0.1 g each of anhydrous sodium sulfate and activated charcoal to each sample. Methyl esters were analyzed by gas chromatography using an HP 5890A instrument with a 30-meter capillary column (Omegawax 250; Supelco, Bellefonte, PA). Individual fatty acids were identified using retention times of standards (i.e., Omegawax, PUFA-II, PUFA-I; Supelco). Fatty acid data are expressed as a percentage of total fatty acids in the sample (i.e., g/100g).

Remaining samples were freeze-dried to determine water content, and dried, ground samples were send for proximate analysis (crude protein, crude fat, crude fiber, ash) and for calcium (Ca), chloride (Cl-), potassium (K), phosphorus (P), magnesium (Mg), sodium (Na), sulfur (S), copper (Cu), cobalt (Co), iron (Fe), manganese (Mn), molybdenum (Mo), and zinc (Zn) to Dairy One Forage Lab (Ithaca, NY).

Results and Discussion

Water content, proximate composition, and detergent fiber values of different species of crayfish from varying environments are found in Table 1. Crayfish from the native habitat of hellbenders (North Fork of the White River) were drier than the farmed crayfish, and contained substantially less protein and much more mineral (ash) compared to crayfish found in the fishery or the baitshop. Fat content was low among all species of crayfish, true difference would need to be confirmed with further studies involving larger sample sizes. The dietary "fiber" content of the crayfish (NDF and ADF – neutral and acid detergent fiber, as well as crude fiber), appears to vary considerably depending on the analytical assay used, as well as possibly by species or habitat. Again, further assays are necessary with larger sample sizes to differentiate real variation. If one pools data from the northern crayfish in the bait shop vs. the fishery pond, there is no difference among crude fiber content of the various species. However, it is possible that the difference seen between the pond-reared (11% crude fiber) and bait-shop northern crayfish (20% crude fiber) is a function of habitat, diet and/or gut fill and requires further investigation.

Of more interest are the differences among vitamin and carotenoid concentrations between the free-living (stream) crayfish and the farmed/pond-living individuals (Table 2). Diets fed to the fish in the fisheries pond quite likely impacted the higher levels of vitamins and carotenoids measured; it remains unclear, however, whether these higher levels of nutrient concentrations are of benefit to the salamanders consuming these crayfish. Overall, vitamin A levels are quite low in all crayfish sampled in comparison to other whole prey, but nutrient requirements for salamanders are unknown. The very high vitamin E and total carotenoids in these commercial crayfish sources may have a further antagonistic effect on vitamin A status in hellbenders; controlled feeding trials should be conducted to determine nutrient interactions.

Minerals concentrations are found in Table 3. All crayfish samples show extraordinarily high calcium content, particularly relative to the phosphorus content (Ca:P ratios of 10-20:1), most likely due to incorporation of this element into the chitinous exoskeleton. Stream-living crayfish displayed higher calcium content compared to pond-reared animals.

Conversely, pond-reared crayfish (both from the fishery as well as the bait shop) contained approximately twice the concentrations of electrolyte minerals Na, Cl, and K as stream-living crayfish. Microminerals Cu, Fe, and Zn were also very high in the pond-reared animals, with Cu at levels considered excessive for some species (including fish). These differences are likely due to the diets consumed by crayfish in the different habitats, with the pond-reared animals eating a much higher proportion of commercial fish diets and/or exposed to higher nutrient loads due to water stability.

Molar percentages of fatty acids in *Orconectes spp.* crayfish (n = ten) compared with krill (n = one) are found in Table 4. By comparison, saturated fats were much higher in the marine krill compared with fresh water crayfish, comprising almost half of total fatty acids quantified. PUFAs (polyunsaturated fatty acids) were high in both crayfish and krill, and likely meet essential (yet totally unknown) fatty acid requirements of hellbenders. Omega-6 fatty acids were about 10X higher (as a molar %) in crayfish compared with krill, and distribution of omega-3 and omega-6 fatty acids differs substantially between krill and crayfish, suggesting hat these two feed types are not nutritional equivalents.

These data provide preliminary detail on the nutrient composition of whole crayfish eaten by hellbender salamanders, but clearly species, size, and habitat variables influence the composition of this whole prey item in ways that require much further investigation. Hellbenders also eat small fish, lamprey, worms, insects, snails, mollusks, tadpoles, and fish entrails in nature; in captivity, they are fed black worms, krill, and fish along with crayfish (Collister, personal communication[2]). Intake, utilization, and growth response trials 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.

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Table 1. Proximate nutrient composition (x ± SD) of *Orconectes* crayfish species sampled in Missouri August – October 2006. All data (except water) are reported on percent dry matter basis. OF = Ozark Fisheries; PBS = Paul's Bait Shop; NFWR = North Fork of White River.

Sample	Source	Sample Size*	Water	Crude Protein	Crude Fat	NDF	ADF	Crude Fiber	Ash
				<------------------------------	--% dry matter	-----------------	------------	----------------	---->
Northern *O. virilis*	OF	2	79.97 ±1.08 (n=7)	60 ±0.71	4.1 ±0.42			10.9 ±0	31.4 ±0.38
Northern *O. virilis*	PBS	9	74.31 ±4.16	54.1 ±4.14	2.96 ±1.47	17.27 ±0.95	13.7 ±0.66	20.04 ±7.38	35.69 ±4.42
Ringed *O. neglectus*	NFWR	2	64.42 ±5.61 (n=5)	34.3 ±2.83	4.55 ±0.78			15.2 ±0.14	51.18 ±2.55
Spot-Handed *O. puntimanus*	NFWR	1	61.52	37.1	1.9	27.2	19.7	14.2	49.29
Long-Pincered *O. longidigitus*	NFWR	2	64.62 ±0.71	41.15 ±5.16	3.55 ±1.77	23.2 ±2.83	17.2 ±0.85	14.25 ±0.49	43.11 ±4.57

*Unless otherwise noted with data set. NDF = Neutral Detergent Fiber; ADF = Acid Detergent Fiber

Table 2. Fat-soluble Vitamin A (Vit A as retinol) and Vitamin E (Vit E as α-tocopherol), and total carotenoid content (x ± SD) of *Orconectes* crayfish species sampled in Missouri August – October 2006. All data are reported on a dry matter basis. OF = Ozark Fisheries; PBS = Paul's Bait Shop; NFWR = North Fork of White River

Sample	Source	Sample Size	Vit A IU/g	Vit E IU/kg	Carotenoid mg/kg
Northern *O. virilis*	OF	7	2951.09 ±889.53	808.06 ±518.94	75.04 ±58.39
Northern *O. virilis*	PBS	9	1619.26 ±237.06	478.24 ±220.34	54.67 ±16.88
Ringed *O. neglectus*	NFWR	5	755.66 ±304.13	263.35 ±78.63	12.33 ±5.39
Spot-Handed *O. puntimanus*	NFWR	3	1034.73 ±98.91	277.28 ±80.37	34.39 ±7.53
Long-Pincered *O. longidigitus*	NFWR	2	1000.34 ±305.77	139.91 ±51.34	19.14 ±5.79

Table 3. Calcium (Ca), chloride (Cl-), potassium (K), phosphorus (P), magnesium (Mg), sodium (Na), sulfur (S), copper (Cu), cobalt (Co), iron (Fe), manganese (Mn), molybdenum (Mo), and zinc (Zn) composition (x ± SD) of *Orconectes* crayfish species sampled in Missouri August – October 2006. All data are reported on a dry matter (DM) basis. OF = Ozark Fisheries; PBS = Paul's Bait Shop; NFWR = North Fork of White River.

Sample	Source	Sample Size	Ca	Cl-	K	P	Mg	Na	S	Cu	Co	Fe	Mn	Mo	Zn
			←			% DM			→	←		mg/kg DM			→
Northern	OF	2	10.9	1.29	0.79	1.15	0.39	0.92	0.47	119	1.22	141.5	66		94.5
O. virilis			±0.47	±0.01	±0	±0.01	±0.04	±0.01	±0.03	±8.49	±0.12	±24.75	±12.73		±2.12
Northern	PBS	9	12	1.22	0.68	1.16	0.4	0.73	0.44	107.44	0.99	133.78	108.44		87.78
O. virilis			±1.79	±0.2	±0.11	±0.04	±0.04	±0.06	±0.06	±12.19	±0.29	±53.6	±101.15		±6.04
Ringed	NFWR	2	20.35	0.57	0.39	0.93	0.26	0.5	0.23	24	0.58	56.5	81		81.5
O. neglectus			±0.44	±0.1	±0.02	±0.04	±0.01	±0.07	±0.02	±4.24	±0.05	±7.78	±7.07		±13.44
Spot-Handed *O. puntimanus*	NFWR	1	17.06	0.64	0.45	0.86	0.31	0.519	0.31	34	0.41	108	89	0.1	69
Long-Pincered	NFWR	2	15.37	0.73	0.46	0.82	0.27	0.54	0.36	35.5	0.49	91.5	71	0.2	70
O. longidigitus			±2.35	±0.01	±0.04	±0.04	±0.01	±0.04	±0.06	±0.71	±0.03	±7.78	±8.49	±0.14	±1.41

Table 4. Fatty acid composition of whole Missouri native crayfish (*Orconectes spp.*) compared with whole marine krill (*Euphasia superba*).

Fatty acids	Crayfish n=10		Krill (n=1)
	mean	SD	*mol%*
12:0	4.8	1.0	2.5
14:0	3.6	0.9	15.6
16:0	19.0	3.6	25.6
16:1	6.6	3.2	10.6
18:0	5.0	1.3	1.7
18:1n7&9	24.8	3.8	20.3
18:2n6	9.0	2.9	1.9
18:3n6	0.7	0.3	0.3
18:3n3	4.0	2.6	0.0
20:3n6	1.0	1.0	0.0
20:4n6	6.5	1.9	0.3
20:5n3	8.1	1.9	13.5
22:6n3	2.6	1.3	6.0
Total	95.6		98.3