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Growth Patterns, Behavior and Food Items Fed to Nestling Great Horned Owls (Bubo virginianus)

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GROWTH PATTERNS, BEHAVIOR AND FOOD ITEMS FED TO NESTLING GREAT HORNED OWLS (*BUBO VIRGINIANUS*)

Two nestling Great Horned Owls *(Bubo virginianus)* were observed for 47 days from 10 March to 25 April, 1979. The nest was located three miles south of Batesville, Independence County, Arkansas. The habitat surrounding the nest site consisted of an open rocky meadow three to five acres in size. It was bordered by an open and broken oak woods with a mixing of red cedar *(Juniperus virginiana,* Linn.). The nest was in an old fence row lined mainly with red cedar, with the nest situated near the trunk of a red cedar (15 m in height) in branches approximately ten m above the ground. The nest was an abandoned crow's nest constructed of sticks with a diameter of 45 - 60 cm and a depth of six cm.

Observations began when the two nestlings were approximately three days old. Growth and behavior patterns are summarized in Tables 1 and 2. Territory, courtship and nesting of the Great Horned Owl previously have been reported by Miller (1930), Errington (1930, 1932), and Baumgartner (1938, 1939). According to Bent (1938), Great Horned Owls lay their eggs in February and sometimes in January. The incubation period is about 28 days, the young remain in the nest six or seven weeks, and they are unable to fly before they are ten or 12 weeks old. Egg laying for this pair took place around 7 February.

The Great Horned Owl is a ravenous feeder on a variety of animal life and is a generous provider for its young (Bent, 1938). Food items observed in the Batesville nest with the two nestlings included numerous Common Grackles *(Quiscalus quiscula),* one Blue Jay *(Cyanocitta cristata),* one Mockingbird *(Mimus polyglottos),* one Common Flicker *(Colaptes auratus),* two young cottontails *(Sylvilagus floridanus)* and one adult cottontail with hind quarters only and one mole *(Scalopus aquaticus).* All of the passerine birds observed in the nest had their heads removed, apparently being brought to the young owls in this condition. Baumgartner and Baumgartner (1944) summarized food items analyzed from 67 Great Horned Owl pellets in which they recorded 71 food items, collected at Lake Carl Blackwell near Stillwater, Oklahoma. They found the cotton rat *(Sigmodon hispidus)* to be the major food item follows by the cottontail *(Sylvilagus floridanus).* Other food items mentioned in their study included species of shrews, moles, mice and several avian species. Also found were several beetles.

The adult Great Horned Owls apparently abandoned their young or were killed as they did not appear at the nest after 9 April. The nestlings then were removed on the following day and housed in an out-door wire cage. Both nestlings died on 25 April, apparently from the annoyance of large numbers of black flies (Simulidae) which are known to kill turkeys, chickens, pigeons and apparently nestling birds. These flies attack young birds especially around the head region in large numbers.

Table 1. Observations on Growth Patterns of Nestling Great Horned Owls.

Age	
3 days old	All white down feathers. Large beaks, weight 4-6 ounces, eyes barely open.
6 days old	All white down feathers. Little growth, one bird slightly larger than the other.
10 days old	Rapid growth. Brown contour feathers beginning to appear with down feathers.
12 days old	Body weight doubled. Considerable more contour feathers.
14 days old	No change.
16 days old	Contour feathers growing rapidly. Down still present to some extent. Body weight 3 times that when hatched.
20 days old	Contour feathers now cover most of body. Quill feathers developing. Primary coverts showing on wings.
24 days old	Ear tuffs (Horns) now appear.
27 days old	Quill feathers developed on wings. Tail developing rapidly. Weight 12-16 oz.
29 days old	Brown contour feathers developed. Primary coverts and tail feathers developing. Breast bars dark.
36 days old	Eye lashes now appearing. Definite face pattern. Most contour feathers developed.
38 days old	Wing feathers now well developed. Secondary coverts and tail developed.
40 days old	Full feather coloration with wings almost fully developed.
47 days old	Almost ready to fly with body well feathered.

Table 2. Behavioral Patterns of Nestling Great Horned Owls.

Age	Behavior
3-4 days old	Peeping and chirping.
5-6 days old	Chirping loudly. Aware of observer.
9-10 days old	Pair lay close together for support.
10-11 days old	No response to touching.
14 days old	Clapping of beaks first noticed and continuous peeping.
15 days old	Young owls cannot stand by themselves but remain close to each other.
17 days old	Owls standing for first time. Some clapping of beaks.
21 days old	Owls very alert. Movement of head 180 degrees. Owls are able to stand but remain close together. Much clapping of beaks.
24 days old	Smaller of the two owls is less aggressive. The larger one very aggressive and tries to bite.
26 days old	Both birds becomming aggressive. Hissing and both try to bite. If hand fed the aggressiveness stops.
31 days old	Adults have quit feeding young. No adult owls observed in area and no additional food brought to nest.
32 days old	Both very aggressive and hungry.
35 days old	Young remove from nest. Clucking and snapping of beaks.
37 days old	Both are very aggressive in cage.
40 days old	Both attack hand upon feeding. Clapping of beaks when one approaches cage. Hissing and spreading of wings and tail.

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ISOLATION OF PHOSPHOLIPASE A_2 FROM *AGKISTRODON BILINEATUS* VENOM

Venom produced by the Mexican mocassin, *A. bilineatus*, contains phosphomonoesterase, phosphodiesterase, 5'-nucleotidase, esterases, thrombin-like, L-amino acid oxidase, protease, phospholipase A_2, and NAD nucleosidase activities (Tu et al., 1967; Denson et al., 1972; Sifford and Johnson, 1978; Brunson et al., 1978). Of these enzymes, phospholipase A_2 (PhL-A_2) was chosen in this work for possible isolation. This choice of PhL-A_2 for isolation was due primarily to its heat stability and to its distribution in the eluates obtained by ion exchange chromatography of the crude venom as evidenced previously (Sifford and Johnson, 1978).

Assay procedures with minor modifications (Sifford and Johnson, 1978) included phospholipase A_2 using the clearing of an egg yolk suspension (Marinetti, 1965), phosphomonoesterase and phosphodiesterase (Richards et al., 1965), esterase (Tu et al., 1965), 5'-nucleosidase (Lo et al., 1966; Ging, 1956), and L-amino acid oxidase (Paik and Kim, 1965). Hyaluronidase was assayed according to the turbidimetric procedures of Kass and Seastone (1944).

A 450 mg sample of crude venom (Sigma) was separated on Concanavalin A covalently bound to Sepharose 4B gel (Con A) into glycoproteins (anthrone reagent positive) and nonglycoproteins (anthrone reagent negative) by employing the methods of Iscove et al. (1974) and Aspberg and Porath (1970). In fractionations by ion exchange chromatography employing DEAE Sephadex A-50, the methods of Cheng and Ouyang (1967), Ouyang et al. (1971), and Johnson and Sifford (1978) were used. Proteins were desalted by using Sephadex G-10 columns at 4°C. Sephadex G-75 and G-50 columns were used to separate PhL-A_2 from higher molecular weight molecules.

An immunizing schedule was prepared according to Ownby et al. (1979). Preimmune serum was obtained from approximately 12 month old New Zealand white rabbits. An immunizing dose was prepared by dissolving 17 mg of lyophilized *A. bilineatus* crude venom in 20 ml of sterile physiological saline. A 0.5 ml aliquot of this solution was then mixed with 0.5 ml of Freund's complete adjuvant. Injections of 0.5 ml then were made subcutaneously into each thigh. Booster injections were prepared by mixing 0.5 ml of *A. bilineatus* venom (0.8 mg/ml) and 0.5 ml of Freund's complete adjuvant. One week later, subcutaneous injections of 0.5 ml of the solution were made in each shoulder. Four weeks after the booster injections, antiserum via heart puncture was collected and stored at −20°C.

Rabbit antiserum for the purified phospholipase A_2 fraction was prepared by injecting an immunizing dose containing 0.3 ml of purified enzyme (0.1 mg/ml) and 1.0 ml Freund's complete adjuvant. Subcutaneous injections of 0.65 ml of this solution were made into each thigh. One week later, booster injections of the same dose were administered into each shoulder. Four weeks later, antiserum was collected and stored at −20°C.

Immunoelectrophoresis methods outlined by Campbell et al. (1963) and Garvey et al. (1977) were employed to determine PhL-A_2 purity. Dodecyl Sulfate-Polyacrylamide gel electrophoresis procedures of Weber and Osborn (1969) were used by Dr. Collis Geren (University of Arkansas at Fayetteville) to assay crude venom and fraction samples.

A. bilineatus crude venom contains nonglycoprotein and glycoprotein enzymes (Fig. 1). The larger nonglycoprotein fraction (Fraction I), comprising approximately 80% of the crude venom proteins, contained numerous enzyme activities. These included PhL-A_1, phosphomonoesterase, phosphodiesterase, 5'-nucleosidase, hyaluronidase, TAMEase, BAEEase, and L-amino acid oxidase activities.

Fraction I, obtained by Con A chromatography, was pooled, lyophilized, and then desalted with Sephadex G-10. Fractionation of this desalted nonglycoprotein fraction by ion exchange chromatography (DEAE Sephadex A-50) yielded three large fractions and several minor fractions. PhL-A_2 activity was concentrated in the second major fraction (Fig. 2). PhL-A_2 activity (14,000 units/mg) in this fraction was much higher than that of the crude venom (234 units/mg).

The PhL-A_1-containing fraction obtained by chromatography with DEAE Sephadex A-50 was divided into two samples. Even-numbered tubes (42 through 66) were pooled to from one sample while odd-numbered tubes (41 through 67) formed the other sample. These samples, after lyophilization and desalting, were fractionated with Sephadex G-75. In both instances, PhL-A_2 activity (24,400 units/mg) was observed only in the low molecular weight fraction (Fig. 3).

The low molecular weight PhL-A_2 fraction (tubes 16-22) obtained by Sephadex G-75 chromatography was pooled, lyophilized, desalted, and applied to a Sephadex G-50 column. This fractionation yielded a fraction free of the larger molecules (Fig. 4).

Immunoelectrophoresis and disc electrophoresis of crude venom samples indicated a complex mixture of proteins although the PhL-A_2 fraction obtained by the sequence of Con A-Sepharose 4B, DEAE A-50, Sephadex G-75, and Sephadex G-50 chromatography procedures was greatly purified (Figs. 5-8). Close examinations of both types of electrophoresis patterns, however, indicated trace contaminations. These contaminations could be due, in part, to TAMEase and L-amino acid oxidase since the distributions of these enzymes in *A. bilineatus* venom overlap PhL-A_2 after DEAE Sephadex A-50 chromatography (Sifford and Johnson, 1978). At present, work is directed toward purification of large amounts of PhL-A_2 in order that more enzyme characteristics may be obtained.

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