*nichthys cataractae* (Pisces: Cyprinidae) in Southeastern New York with comments on phylogeny and functional morphology. J. Freshwater Ecol. 2: 239–246.

YERGER, R. W., AND R. D. SUTTKUS. 1962. Records of freshwater fishes in Florida. Tulane Stud. Zool. 9:323–330.

DEPARTMENT OF ZOOLOGY, SOUTHERN ILLINOIS UNIVERSITY, CARBONDALE, ILLINOIS 62901. Accepted 14 May 1987.

# The Gular Glands of *Alligator mississippiensis*: Histology and Preliminary Analysis of Lipoidal Secretions

PAUL J. WELDON AND H. WAYNE SAMPSON

The histology of the gular glands of adult *Alligator mississippiensis* and a preliminary analysis of gular gland secretions are described. The capsule of the gland consists of an outer layer of striated muscle and an inner layer of connective tissue. Septa lined with melanocytes radiate from the connective tissue layer of the capsule to the border of a central conglomerate of cells, cell fragments, and secretions. Parenchymal cells containing lipid droplets degenerate as they move toward the central region of the gland. The polar and nonpolar lipid fractions of gular gland secretions were separated and subjected to thin-layer chromatography with known lipid standards to allow tentative identification of the gland constituents. Bands consistent with sterols, free fatty acids, triglycerides, steryl esters, and ceramides are among those observed. A carbon-13 nuclear magnetic resonance spectrum of whole gular gland secretions displays signals consistent with long-chain and esterified materials and provides additional evidence for some lipid classes indicated by thin-layer chromatography.

A LL crocodilians possess paired, evertible glands adjacent to each mandibular ramus on the ventral side of the lower jaw (Gadow, 1901). Neill (1971) refers to these organs as gular glands; other terms—mandibular glands, throat glands, musk glands, etc.—also have been used to denote these integumentary structures. Young crocodilians evert the gular glands when stressed by human handling (Gadow, 1901). Adults of some species are known to do so (and perhaps to spray secretions from the glands) while bellowing (McIlhenny, 1934; Garrick et al., 1978). Several authors suggest that gular glands produce pheromones, but this has not been investigated beyond Johnsen and Wellington's (1982) demonstration that yearling American alligators (*Alligator mississippiensis*) detect airborne gular gland odors from adult males, and Gorzula's (1985) observation that

young spectacled caimans (*Caiman c. crocodilus*) rapidly swim "to and fro" presumably in response to glandular secretions introduced into pond water. Bell (1827a, 1827b) suggested that gular gland secretions may be used by crocodilians to lure fish prey.

The histology and ultrastructure of crocodilian gular glands is best known in juvenile *Crocodylus porosus* (Wright and Moffat, 1985). The glands of this species possess a capsule of connective tissue containing numerous arteries and veins. Connective tissue septa containing capillaries extend from the capsule and divide the gland into lobules. Melanocytes scattered along the septa were observed in the glands of some individuals, but not in others. The presence of numerous spheroidal droplets within cells of the parenchyma of the gland and observations of disintegrating cells and cell prod-

ucts in the central glandular region indicate secretory activity. Descriptions of gular glands of embryonic and young *A. mississippiensis* (Reese, 1921; Schirner, 1931), embryos of an unidentified species of *Caiman* (Reese, 1921), and hatchlings of the Nile crocodile (*Crocodilus madagascariensis = Crocodylus niloticus*) (Voeltzkow, 1899) indicate a similar structural organization, including connective tissue septa laden with melanocytes, among these species.

No information is available on the chemicals in crocodilian gular gland secretions. We report here histological observations on gular glands of adult *A. mississippiensis* and describe the results of analyses of glandular secretions by thin-layer chromatography (TLC) and carbon-13 nuclear magnetic resonance (CMR).



Fig. 1. A gular gland is everted and a capillary tube containing secretions is withdrawn.

## METHODS

*Histology.*—Gular glands were obtained from four adult male alligators (total lengths = 1.9–3.1 m) from Port Arthur, Texas (Murphree Wildlife Management Area) during Sept. 1985. The glands were excised within 1 h following sacrifice and one of each pair was placed into 10% neutral buffered formalin or Carson's modified Millonig's phosphate-buffered formalin. The 10% neutral formalin-fixed glands were routinely embedded in paraffin, sectioned longitudinally or transversely at 4–7 $\mu$, stained with hematoxylin and eosin, and examined by light microscopy. Adjacent sections were stained using a modified alcian blue technique for proteoglycans (Thompson, 1966) or an ammonical silver carbonate technique for reticulum, collagen, and elastin (Humason, 1962).

Glands placed into Carson's fixative were postfixed in osmium tetroxide for electron microscopy. They were dehydrated in a graded series of ethanol and embedded in Spurr's low-viscosity resin for ultramicrotomy. Sections exhibiting gold to silver interference colors were viewed on a Phillips 420 or a Hitachi H500 electron microscope.

*Chemistry.*—Gular gland secretions were obtained within 1 h after sacrifice from seven adult males (total lengths = 1.7–3.2 m) and one female (2.1 m) from Port Arthur, Texas during Sept. 1986. Secretions from the males were pooled in two collecting vials; materials from the female were kept separate. Secretions were collected by manually everting each gland and inserting a glass capillary tube into the gland's
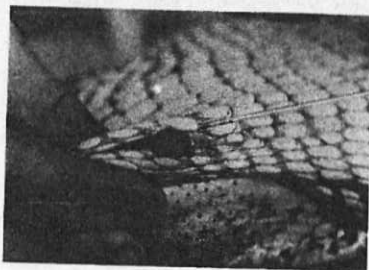
single opening (Fig. 1). The tube was withdrawn and the end packed with secretions was broken off into a glass vial containing 5 ml of chloroform. Secretions for TLC from three males (pooled) and one female were filtered and the solvent was removed by rotary evaporation under reduced pressure. The residues were transferred in a chloroform : methanol solution (1:1 v/v), evaporated to dryness under a stream of nitrogen, desiccated in vacuo, and weighed. A total of 5.2 mg of secretions was obtained from the males; the female yielded 0.6 mg.

The dried lipids were dissolved in chloroform and applied to a silicic acid column (ca 3 g in a 30 ml cylindrical filter tube) to separate polar from nonpolar lipids. The column was washed with 75 ml of chloroform to elute the nonpolar lipids and 75 ml of methanol to elute the polar lipids, according to the procedure of Borgstrom (1952). The polar and nonpolar lipids each were dried and weighed. Secretions from the males gave 4.6 and 0.4 mg of nonpolar and polar lipids, respectively. Secretions from the female gave 0.5 and 0.1 mg of nonpolar and polar lipids, respectively. The lipid residues were redissolved in chloroform and aliquots of these solutions (150 $\mu$g for nonpolar lipids and 225 $\mu$g for polar lipids) were applied to 8 mm lanes on a 0.5 mm layer of silica gel G on 5 × 20 cm glass plates. Mixed lipid standards (Nu-Chek-Prep, Elysian, Minnesota, for nonpolar lipids and phospholipids; Supelco, Bellefonte, Pennsylvania, for ceramides) were developed on adjacent lanes of each chromatoplate. The nonpolar lipids were resolved by successive development with hexane (to 15 cm), toluene (to 15 cm), and hexane : ether : acetic acid (80:20:1, v/v/v, twice to 8 cm). The polar lipids
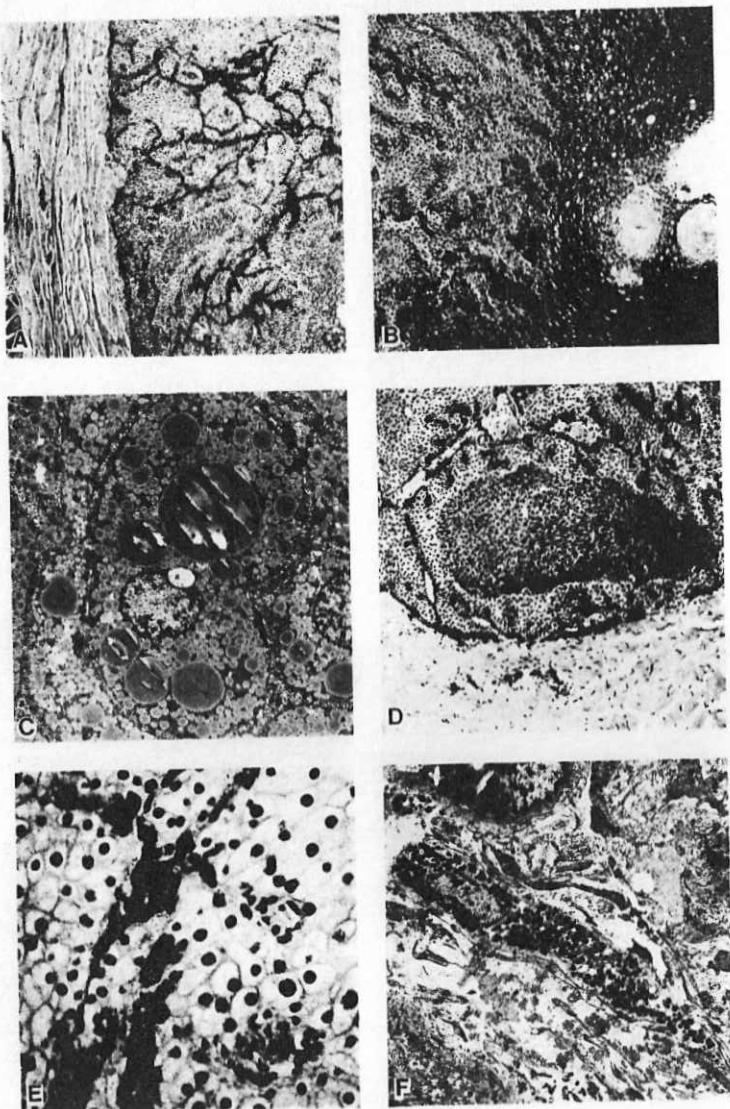
were resolved by development in chloroform : methanol : water (60:10:1, v/v/v, to 15 cm). The TLC plates were sprayed with 50% $H_2SO_4$ and heated on a hotplate to char the lipids.

Gular gland secretions for CMR analysis were pooled from four males, filtered, placed under reduced pressure to remove the chloroform, and dried. A total of 8.4 mg of secretions was recovered and dissolved in deuterated chloroform ($CDCl_3$). A spectrum was obtained at ambient temperature using a Varian XL-200E NMR spectrometer equipped with a 5 mm dual probe.

## RESULTS

*Histology.*—The oval-shaped capsule of the gland is comprised of a thin layer of connective tissue surrounded by striated muscle (Fig. 2A). Projections or septa arising from the connective tissue portion of the capsule extend into the gland, but are absent in the central region. These septa, in which we have observed capillaries, divide the parenchyma of the gland into irregular lobules. No distinct lumen or secretory duct is observed.

The central region of the gland is composed of a conglomerate of cells, cell fragments, and secretions (Fig. 2B). Cells of the central region have a darkly staining cytoplasm, as do cells extending from this region into the center of the parenchymal lobules (Fig. 2A–B, D). The surrounding parenchymal cells (near the septa) possess lightly staining cytoplasm and a large, round nucleus. Cross-sections through the more peripheral regions of the gland give the appearance of lymphoid tissue due to this arrangement in the lobules of a region of dark cells surrounded by a region of lighter staining cells (Fig. 2D).

Electron microscopy indicates that cells in the parenchyma of the gland contain numerous large lipid droplets (Fig. 2C). Cells distant from the septa undergo karyolyses and, toward the center of the gland, they degenerate into a colorless coagulation of secretion product. Observations by light microscopy indicate darkly staining "grains" along the length of the septa (Fig. 2E). Electron microscopy reveals that this material is comprised of elongate melanocytes, each with an elongate nucleus and a predominance of round or ellipsoid, fully melanized melanosomes. Few other cytoplasmic organelles are apparent (Fig. 2F).

*Chemistry.*—The chromatograms of both male and female nonpolar gular gland secretions display bands consistent with sterols, free fatty acids, triglycerides, and steryl esters (Fig. 3A). The chromatogram of the males' displays several bands that are indistinct in the female's chromatogram (Rf's = 0.6, 0.7, and 0.9); a band in the female's chromatogram (Rf = 0.8) is unclear in that of the males'. A band close to the solvent front (Rf > 0.9), especially prominent in the males' chromatogram, likely denotes hydrocarbons, but these may be contaminants. A faint band (Rf = 0.1), above that attributed to sterols, occurs in both male and female chromatograms in the region to which alcohols migrate.

The chromatograms of the polar lipids display several bands consistent with ceramides (Fig. 3B). Phospholipids are suggested most clearly in the chromatogram of the female.

A CMR spectrum of gular gland secretions is shown (Fig. 4). Chemical shifts are reported in values (ppm) downfield from internal tetramethylsilane. Signals are assigned on the basis of values reported in Schell and Weldon (1985) and references cited therein.

Central non-perturbed methylenes of long carbon chains are indicated by a large peak at 29.7 ppm. Other signals in the 29.1–30.5 ppm region reflect methylenes perturbed by unsaturation. Long-chain unsaturation also is indicated by absorptions in the 127.9–130.2 ppm region. Resonances for the ω and ω-1 positions

Fig. 2. (A) Hematoxylin- and eosin-stained section of periphery of gland showing capsule (×25). (B) Photomicrograph of center of gland with degenerating cells. Note termination of septa to left of degeneration region (×30). (C) Electron micrograph of parenchymal cell. Note prominent nucleus and abundant cytoplasmic lipid droplets (×2600). (D) Cross-section through the compartmentalized peripheral region of gland. The more centrally located cells have a denser cytoplasm. These dense cells are continuous with degenerating cells in the center of the gland (×40). (E) Light micrograph of oval parenchymal cells with prominent round nuclei. The dark "flecks" in the septa are melanocytes (×300). (F) Electron micrograph of septal melanocyte (×2800).
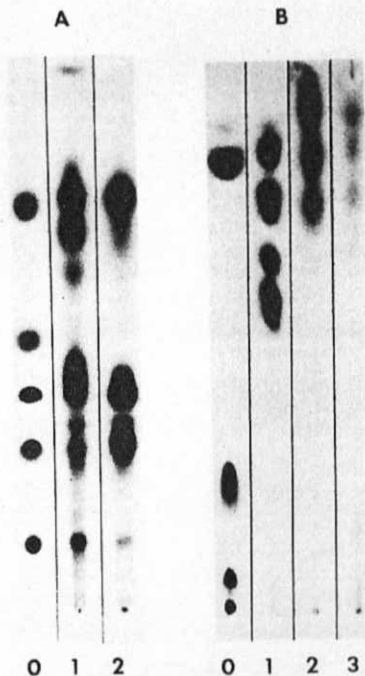
A      B

0   1   2     0   1   2   3

Fig. 3. (A) Thin-layer chromatograms of nonpolar gular gland lipids. Lane 0 contains the reference lipids (from bottom): sterol (cholesterol), fatty acid (oleic acid), triglyceride (triolein), methyl ester (methyl oleate), and steryl ester (cholesteryl oleate). Lane 1 contains gular gland lipids pooled from three males. Lane 2 contains lipids from one female. (B) Thin-layer chromatograms of polar gular gland lipids. Lane 0 contains the reference lipids (from bottom): phosphatidylcholine, phosphatidylethanolamine, lysophosphatidylcholine, and cholesterol. Lane 1 contains a mixture of ceramides. Lane 2 contains gular gland lipids pooled from three males. Lane 3 contains lipids from one female.

of long chains appear at 14.2 and 22.7 ppm, respectively. A resonance at 31.9 ppm is consistent with the $\omega$-2 position.

Absorptions consistent with free and esterified fatty acids are indicated. A signal at 179.6 ppm likely denotes the carboxyl group of free fatty acids. Peaks at 34.0 and 34.2 ppm corre-

spond to $\alpha$-methylenes of esterified fatty acids. Peaks in the 34.8–35.7 ppm region may represent other esters. A signal at 24.6 ppm is consistent with $\beta$-methylene carbons. Signals consistent with the glycerol moiety and esterified fatty acids of triglycerides are observed. The glycerol methylene and methine carbons are indicated at 62.1 and 68.8 ppm, respectively. Resonances at 173.2 and 172.9 ppm are consistent with the carboxyl carbons of esterified fatty acids. Signals consistent with most carbons of cholesterol, the chief sterol expected, are observed, except for that of C-3 at ca 71.6 ppm. Signals by which cholesterol and cholesteryl esters can be distinguished are indicated, again except for that of the cholesteryl ester C-3 which should occur at ca 73.7 ppm.

## DISCUSSION

Our histological observations of the gular glands of adult *A. mississippiensis* indicate that they are anatomically similar to those described in other crocodilians (Reese, 1921; Schirner, 1931; Wright and Moffat, 1985). These integumentary organs are unique to the order Crocodilia. A comparable but non-homologous pair of macroscopic glands, called mental glands, occurs on the ventral aspect of the lower jaw of some turtles. Winokur and Legler (1975) observed mental glands among 21 genera in the families Emydidae, Platysternidae, and Testudinidae and they suggest that these organs are homologous among these taxa.

Unlike turtle mental glands, crocodilian gular glands are surrounded by a layer of striated muscle. Bell (1827a) suggested that the circular muscle surrounding alligator gular glands extends posteriad to attach to the hyoid apparatus. Schirner (1931) suggested that this muscle is an extension of M. constrictor III and that it is innervated by the glossopharyngeal nerve.

Our histological observations on *A. mississippiensis* and those reported on other crocodilians indicate that gular glands exhibit holocrine secretion. This mode of secretion is documented in numerous other reptilian skin glands (Quay, 1972). Cells of the parenchyma move toward the center of the gland, degenerate, and release their secretions. Cells in the peripheral region of the gland likely receive nutrients and oxygen from nearby capillaries within the septa. Wright and Moffat (1985) observed capillaries in the gular glands of *C. porosus* that extend beyond the septa into the glandular tissue.

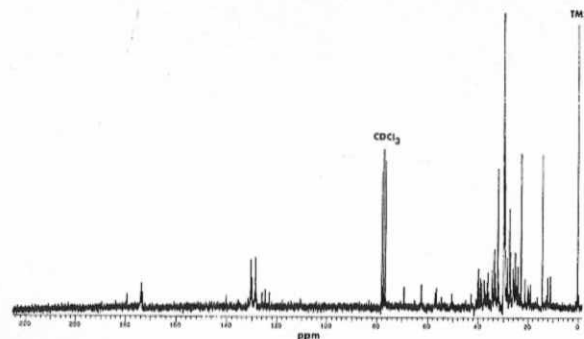Numerous elongate melanocytes line the in-

Fig. 4. CMR spectrum of *Alligator mississippiensis* gular gland secretions. Signals due to the solvent (CDCl₃) and the internal standard, trimethylsilane (TMS), are indicated.

ternal septa of the gular glands of *A. mississippiensis*. These cells are similar morphologically to those in the dermis of vertebrates known as continent melanocytes (Seiji, 1981). Unlike secretory melanocytes of the epidermis, continent melanocytes do not discharge their melanosomes to keratinocytes and they exhibit little pigment synthesis. Wright and Moffat (1985) observed that processes of melanocytes in *C. porosus* gular glands extend into the spaces between the secretory cells, but because the secretions were not pigmented it is unlikely that melanosomes are discharged from these cells.

Some authors suggest that continent melanocytes function to obstruct the penetration of ultraviolet radiation into tissues (Fitzpatrick et al., 1981; Quevado, 1981). The melanocytes of crocodilian gular glands likely prevent the photodestruction of glandular secretions. This function could be examined by comparing the exudates of normal vs albinistic individuals after exposure to radiation.

Madison (1977) suggested that gular gland secretions are lipoidal since observations by McIlhenny (1934) on the release of these substances in the field indicate that they are not miscible with water. Electron microscopic observations indicate lipid droplets in the parenchymal cells of the glands, and preliminary chemical analysis points to several general classes of lipids in the secretions.

Sterols, free fatty acids, triglycerides, and steryl esters are indicated among the nonpolar lipids by TLC. The presence of minor bands in

some regions, e.g., free fatty acid, likely denotes constituents within these classes with different chain lengths, branching patterns or degrees of unsaturation. Compounds less polar than lysophosphatidylcholine comprise the bulk of the polar lipids indicated. Ceramides appear to be the predominant class of polar substances of those for which chemical standards were available in our study. Phospholipids are suggested in small amounts in the secretions.

Some differences in the males' and female's TLC profiles are evident. Obviously, little can be deduced from these chromatograms regarding sexual differences in gular gland chemistry given the small sample sizes. Surveys comparing the gular gland secretions of males and females need to be done, preferably using materials obtained during the mating season, when these glands presumably are most active. Alligators in the populations we sampled mate during April and May (J. Dixon, pers. comm.; T. Joanen, pers. comm.).

CMR signals of whole gular gland secretions indicate the presence of high concentrations of esterified and long-chain materials which, of course, are typical of a wide range of lipid classes. Signals providing additional evidence for some classes of nonpolar lipids—fatty acids and triglycerides—are observed. Absorptions specifically attributable to polar lipids, e.g., the lecithin moiety of phospholipids, are not clear in our spectrum. Polar lipids comprise about 15% of the whole gular gland secretions, as indicated by weights obtained after the separation of non-

polar and polar lipids for TLC. The quantities of polar lipids in the whole extract we examined likely were insufficient for CMR analysis. Further detailed studies of gular gland substances are needed.

## LITERATURE CITED

BELL, T. 1827a. On the structure and use of the submaxillary odoriferous gland in the genus *Crocodilus.* Phil. Trans. Roy. Soc. Lond. 1827:1–8.
———. 1827b. On the use of the odoriferous gland of the alligator as a bait. Edinburgh J. Sci. 7:162–163.
BORGSTROM, B. 1952. Investigation on lipid separation methods. Acta Phys. Scand. 25:101–110.
FITZPATRICK, T. B., A. B. LERNER, J. J. NORDLUND, R. R. ANDERSON, R. I. GARCIA, G. SZABO AND G. PROTA. 1981. Introduction to dermal pigment biology and dermal pigmentary disorders (ceruloderma): significance, physical basis, cytologic and biochemical basis, p. 3–18. *In:* Biology and diseases of dermal pigmentation. T. B. Fitzpatrick (ed.). University of Tokyo Press, Tokyo, Japan.
GADOW, H. 1901. Cambridge natural history, amphibians and reptiles. Macmillan and Co., London, England.
GARRICK, L. D., J. W. LANG AND H. A. HERZOG, JR. 1978. Social signals of adult American alligators. Bull. Am. Mus. Nat. Hist. 160:153–192.
GORZULA, S. 1985. Are caimans always in distress? Biotropica 17:343–344.
JOHNSEN, P. B., AND J. L. WELLINGTON. 1982. Detection of glandular secretions by yearling alligators. Copeia 1982:705–708.
HUMASON, G. L. 1962. Animal tissue techniques. W. H. Freeman, San Francisco, California.
MADISON, D. M. 1977. Chemical communication in amphibians and reptiles, p. 135–168. *In:* Chemical signals in vertebrates. D. Muller-Schwarze and M. M. Mozell (eds.). Plenum Press, New York, New York.
MCILHENNY, E. A. 1934. The alligator's life history. Christopher Publ. House, Boston, Massachusetts.
NEILL, W. T. 1971. The last of the ruling reptiles: alligators, crocodiles, and their kin. Columbia University Press, New York, New York.
QUAY, W. B. 1972. Integument and the environment: glandular composition, function, and evolution. Am. Zool. 12:95–108.
QUEVADO, W. C., JR. 1981. Physiology of vertebrate dermal pigmentation, p. 39–50. *In:* Biology and diseases of dermal pigmentation. T. B. Fitzpatrick (ed.). University of Tokyo Press, Tokyo, Japan.
REESE, A. M. 1921. The structure and development of the integumental glands of the Crocodilia. J. Morphol. 35:581–611.
SCHELL, F. M., AND P. J. WELDON. 1985. ¹³C-NMR analysis of snake skin lipids. Agric. Biol. Chem. 49: 3597–3600.
SCHIRNER, G. 1931. Uber die Kieferdrüsen der Krokodile. Zeit. Anat. Entwicklung. 94:802–821.
SEIJI, M. 1981. Biology of dermal melanin, p. 21–38. *In:* Biology and diseases of dermal pigmentation. T. B. Fitzpatrick (ed.). University of Tokyo Press, Tokyo, Japan.
THOMPSON, S. W. 1966. Selected histochemical and histopathological methods. Charles B. Thomas Publisher, Springfield, Illinois.
VOELTZKOW, A. 1899. Biologie und Entwicklung der ausseren Korperform von *Crocodilus madagascariensis.* Grand. Abh. Senckenb. Nat. Ges. 26:1–149.
WINOKUR, R. M., AND J. M. LEGLER. 1975. Chelonian mental glands. J. Morph. 147:275–292.
WRIGHT, D. E., AND L. A. MOFFAT. 1985. Morphology and ultrastructure of the chin and cloacal glands of juvenile *Crocodylus porosus* (Reptilia, Crocodilia), p. 411–422. *In:* Biology of Australasian frogs and reptiles. G. Grigg, R. Shine and H. Ehmann (eds.). Surrey Beatty and Sons, Chipping, New South Wales, Australia.

(PJW): DEPARTMENT OF BIOLOGY, TEXAS A&M UNIVERSITY, COLLEGE STATION, TEXAS 77843, and (HWS): DEPARTMENT OF ANATOMY, COLLEGE OF MEDICINE, TEXAS A&M UNIVERSITY, COLLEGE STATION, TEXAS 77843. Accepted 15 May 1987.

---

# Ontogeny of Aquatic Feeding Performance in the Eastern Newt, *Notophthalmus viridescens* (Salamandridae)

STEPHEN M. REILLY AND GEORGE V. LAUDER

Ontogenetic changes in feeding performance on four prey types were studied in the eastern newt, *Notophthalmus viridescens,* to test two hypotheses about functional changes in aquatic feeding during development. The hypothesis that the ontogenetic transformation from a unidirectional to bidirectional feeding system results in a decrease in feeding performance is corroborated by comparing the aquatic feeding ability of larvae and efts. Feeding performance in fully transformed and branchiate adults does not support the hypothesis that retention of larval components of hyobranchial morphology in branchiates results in increased aquatic feeding performance.

THE relationship between behavioral performance (the ability of animals to execute behaviors) and the morphological and functional bases of performance has been the subject of increasing discussion in the literature (Emerson, 1978; Emerson and Diehl, 1980; Arnold, 1983). The process of metamorphosis in urodeles involves a dramatic reorganization of skull morphology and function (Duellman and Trueb, 1986) and provides an excellent case study of the relationships among morphology, function, and behavioral performance.

The purpose of this paper is to provide an ontogenetic analysis of feeding performance in the eastern newt (*Notophthalmus viridescens*) to test two functional hypotheses. Our general goal is to test for changes in feeding ability during ontogeny predicted on the basis of previously documented functional and morphological changes that occur during development.

Our first hypothesis is based on the work of Lauder and Shaffer (1986). Larval salamanders possess an aquatic feeding system in which prey are captured by suction (Lauder and Shaffer, 1985): water enters the front of the mouth as pressure drops within the mouth cavity and exits posteriorly through the gill slits. There is thus a unidirectional flow through the mouth cavity. In metamorphosed individuals, the gill slits are closed, and individuals that feed in the water possess bidirectional water flow: water that enters the mouth as prey are captured also exits through the mouth as the jaws close (Lauder and Shaffer, 1986). This ontogenetic transformation from a unidirectional to a bidirectional water-flow system represents a fundamental change in feeding hydrodynamics and design

within a vertebrate species and provides a unique opportunity to examine the relationship between performance and major changes in functional design. Lauder and Shaffer (1986) suggested that the ontogenetic transition from larval to metamorphosed morphology in *Ambystoma* is accompanied by a significant decrease in feeding performance. They indicated that this performance decrease may be due to the change from unidirectional to bidirectional water flow and metamorphic changes in the head muscles and structure of the hyoid. Therefore, we tested the hypothesis that the transformation from a unidirectional to a bidirectional water-flow system during feeding results in a decrease in feeding performance.

Our second hypothesis is based on the work of Reilly (1986, 1987). He has documented ontogenetic changes during metamorphosis in the cranium and hyobranchial apparatus in fully transforming and branchiate *N. viridescens.* Branchiate *N. viridescens* are, in fact, completely metamorphosed except for the variable retention of external gills and ceratobranchials 2–4. Reilly (1987) proposed that, although the retention of gill structures is not necessary for survival in aquatic adult newts, the retention of hyobranchial elements may improve aquatic feeding performance in branchiate newts which remain entirely aquatic. Thus, we tested the hypothesis that the structure of the hyobranchial apparatus affects feeding performance such that postmetamorphic animals that retain more of the hyobranchial apparatus have increased feeding performance relative to individuals that retain only one ceratobranchial (Reilly, 1986, 1987).