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Morphological and Behavioral Aspects of Lateral Line Neuromast Topography in Amphibians

by

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Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy
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ABSTRACT

A major problem in biology is understanding how animals interact with their environment. A first step towards solving this problem is the elucidation and understanding of the sensory systems that animals employ to detect important environmental parameters. Many, if not most, aquatic salamanders are nocturnal. Here I show that nocturnal salamanders need not rely on vision or olfaction to detect prey, but can use the organs of their lateral line system. My thesis is that variation in neuromast organ topography reflects both the ecology and phylogeny of aquatic salamanders and frogs.

Primary neuromast numbers in larval amphibians do not vary with growth. Anurans have single rows of primary neuromasts, urodeles have multiple rows of neuromasts on their snouts. In urodeles neuromasts form transverse stitches in the families Ambystomatidae and Cryptobranchidae, and longitudinal stitches in the Proteidae and Salamandridae; the remaining salamander families do not form stitches. Larval pond forms in all anuran families have transverse stitches. In both urodeles and anurans, transverse stitches are characteristic of larval pond forms while an absence of stitches in both urodeles and anurans, and longitudinal stitches in urodeles are characteristic of larvae that live in flowing water. Species that live in flowing water also tend to have neuromasts sunken into their epidermis and, in urodeles, have a larger proportion of neuromasts anteriorly. In urodeles, stitch type is consistent within a family; species within anuran families show more variation. Anuran larvae can be divided into three groups -- generalized, obligate suspension feeding, and a mixed group of specialized larvae -- based on neuromast topography.

Based on these and other morphological studies I propose that the common amphibian ancestor had transverse stitches, single neuromast rows, and electroreceptors. Urodeles developed multiple neuromast rows, anurans lost electroreceptors, and caecilians lost the ability to form stitches. The fossil record indicates that this common ancestor probably occurred sometime after the Stegocephalia arose and neuromasts became superficial. Based upon correlations between morphology and ecology in modern forms this common ancestor was probably a pond-dwelling carnivore.

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Introduction: General Introduction and Literature Review

GENERAL INTRODUCTION

Animals use sensory systems outside of our own to interact with their environment. The eighth cranial nerve (CN VIII) systems of echolocation in bats and electrolocation in fishes are among the better known of these senses. If we are to understand how these animals sense their environment we must understand the nature of the information obtained through these senses and how animals use this information. Mechanoreception -- which is also associated with CN VIII -- is not as well known as echo- and electrolocation, but can also be used by some vertebrates (aquatic anamniotes) to receive environmental signals.

Aquatic salamanders feed at night on large numbers of small prey. In Chapter 1 "Size selective predation is not dependent on vision in aquatic salamanders" I show that, contrary to data on many fishes, salamanders do not necessarily use visual and olfactory cues to feed "normally" on natural prey. This strongly suggests that either one or both of the lateral line systems of mechanoreception and electroreception are being used by these animals for this behavior.

Given these results I focus my morphological studies on the ecological and phylogenetic relationships of mechanoreceptive neuromast organs in amphibians. There

are several reasons for this, among them being that this system is evolutionarily more primitive than electroreception (see below). Mechanoreceptive neuromasts are also believed to be the morphological precursors of the vertebrate organs of hearing and motion detection. Therefore the neuromast system may exhibit various CN VIII organizational and functional principles in their most primitive states. Additionally, there is less known about mechanoreception than electroreception, suggesting that new data on mechanpreceptors will increase our knowledge of the diversity of morphological, functional, and evolutionary aspects of CN VIII systems.

The second Chapter "Inter- and intraspecific variation in neuromast topography in Ambystoma larvae" is a prerequisite to my comparative morphological studies. Basically, it elucidates the neuromast topographical features that remain constant through ontogeny and which, therefore, can be compared across taxa. This paper also shows that neuromast topography can distinguish species within genera, but not morphs within species.

The third and fourth papers, "Neuromast topography in urodele amphibians" and "Neuromast topography in anuran amphibians" are surveys of neuromast topography in salamanders and frogs, respectively. In total I examine over 60 species from twenty families, primarily using scanning electron microscopy. In general I detail

several phylogenetic and ecological relationships both within and between these two amphibian orders.

In the fifth chapter "The evolution of the lateral line system in amphibians and its bearing on amphibian phylogeny" I combine my morphological results on the lateral line system of salamanders and frogs with published information on the lateral line system of caecilians (the remaining extant amphibian order) to refine our knowledge of amphibian evolution. In particular I suggest generalized and derived characteristics of neuromast topography, and propose the lateral line morphology for a common modern amphibian ancestor. Based upon ecological correlations in modern forms I suggest that this ancestor was a pond dwelling carnivore. Based on fossil evidence I propose that this ancestor existed after, but was probably derived from, the ichthyostegid-stegocephalian amphibians.

LITERATURE REVIEW

General reviews on aspects of the lateral line system in amphibians have been written by Wright (1951), Dijkgraaf (1963), Flock (1971), and Russell (1976). Here I review briefly some fundamental aspects of this system.

Hair Cell Structure and Properties

Lateral line hair cells are pear-shaped in longitudinal section (Fig. I-1); their basal portion is expanded and embedded in epidermis or dermis (Wright, 1951), their apical portion extends out, away from the animal's body. In salamanders hair cells are about 80 μm long and 20 μm wide (Chezar, 1930; Harris et al., 1970; Russell, 1976). The apical portion of the hair cell has numerous sensory hairs, or cilia, extending from it. Each hair cell has one static cilium (the kinocilium) and numerous microvilli (stereocilia) (Kalmijn, 1961 unpublished, in Dijkgraaf, 1963; Trujillo-Cenóz, 1961). The kinocilium is composed of a typical ciliary nine double-barrel peripheral and two single barrel central mictotubule arrangement that is easily discernible in cross section (Fig. I-2). Stereocilia are composed of actin microfilaments that are not orderly arranged (Jande, 1966).

Cilia arrangement within a hair cell is asymmetrical;

Figure I-1. Schematic diagram of a vertebrate hair cell in longitudinal section illustrating the kinocilium (k), stereocilia (s), cell components (not labelled), and afferent (a) and efferent (e) innervation.

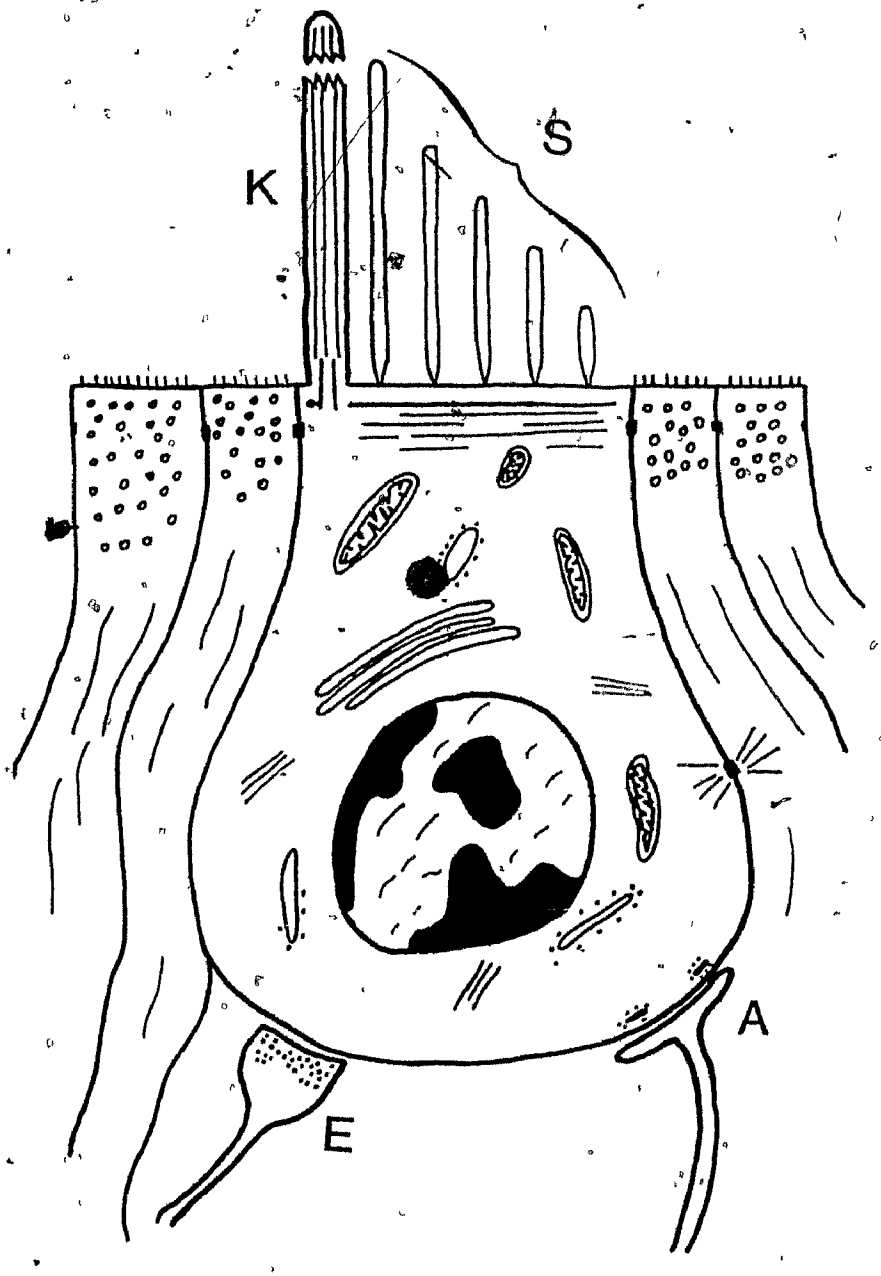
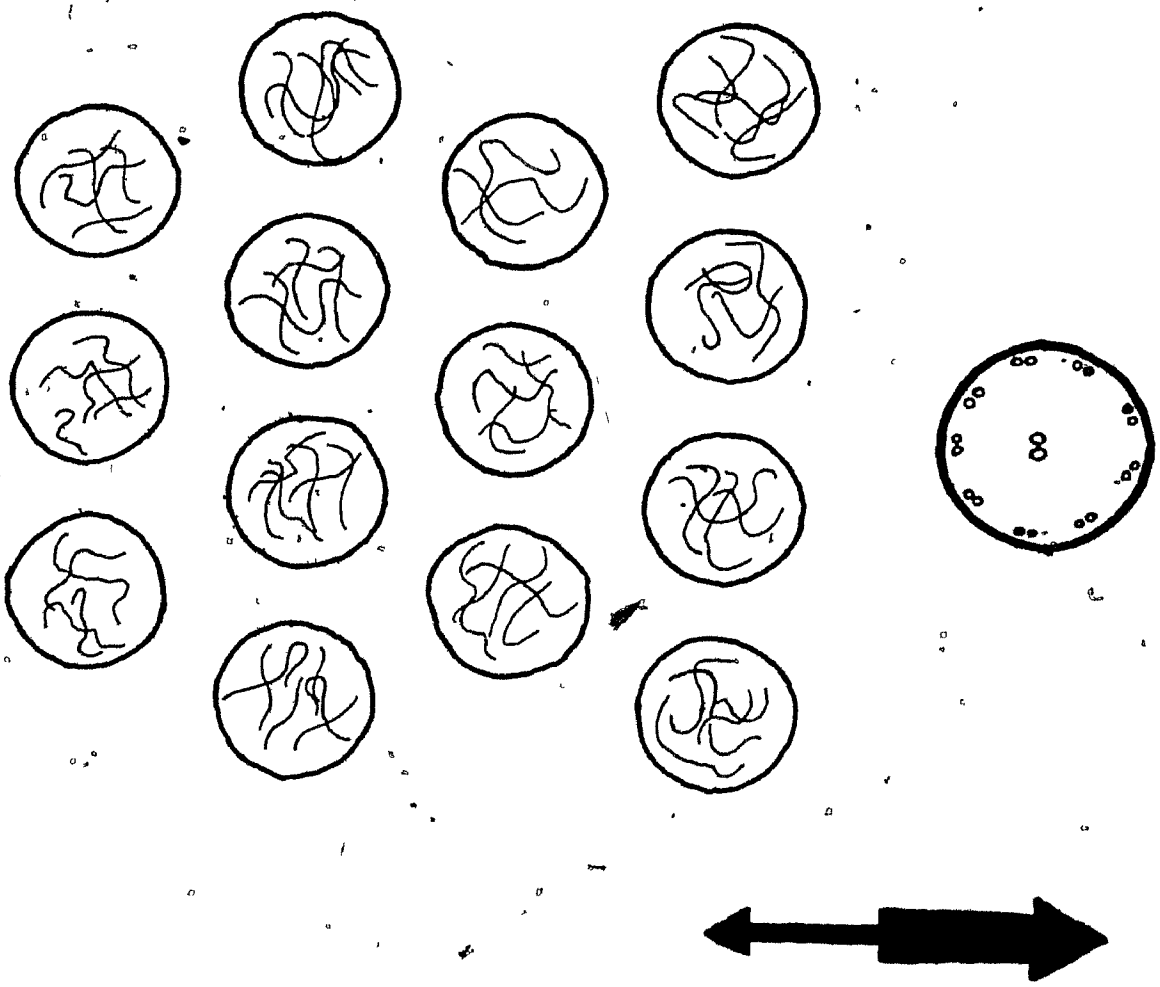

K
S
A
E

Figure I-2. Schematic diagram of a hair cell in cross section taken through the sensory hairs. The kinocilium has a typical ciliary 9 peripheral double barrel and two central barrel microtubule arrangement. Stereocilia are oriented to one side of the kinocilium and are composed in life of actin microfilaments. Arrow represents directions of maximum sensitivity. Large portion of arrow points in the direction that sensory hair movement results in hair cell depolarization, the opposite direction hyperpolarizes the cell.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the kinocilium is located at the periphery of the stereocilia bundle (Fig. I-2). The kinocilium is wider (0.23 μm) and taller (>5 μm) than stereocilia (0.10 - 0.13 μm wide, 5 μm tall for the tallest) (Jørgensen and Flock, 1973; Flock and Jørgensen, 1974). These dimensions vary by taxon. Stereocilia decrease in height with increasing distance from the kinocilium (Fig. I-1). Other histological features include apical aggregations of mitochondria, a basally-located nucleus and, below the nucleus, numerous presynaptic vesicles (Fig. I-1).

Hair cells are innervated by a single nerve fiber from a bipolar afferent neuron that synapses on their basal portion (Fig. I-1; Harris et al., 1970). At rest, afferents fire spontaneously in response to random presynaptic depolarizations (Hoagland, 1932; Harris and Milne, 1966).

Hair cells function by being sensitive to shearing motions in a direction parallel to a line drawn through the kinocilium and the center of the stereocilia bundle (Fig. I-2; e.g., Hudspeth and Corey, 1977; Hudspeth, 1983). When the kinocilium is moved in a direction away from the stereocilia bundle a depolarization occurs and the rate of firing increases; a displacement in the opposite direction hyperpolarizes the hair cell and afferent firing decreases below the spontaneous firing rate (Lowenstein and Wersall, 1959; Flock and Wersall, 1962).

If the stimulus is sinusoidal and low frequency, afferent firing may be phase locked to it (Hoagland, 1932; Katsuki et al., 1951). Hair cell sensitivity diminishes as a function of the cosine of the angle from the direction of maximum sensitivity (Flock, 1965; 1967).

In the salamander Necturus, the receptor potential of individual hair cells is about 800 μV (Flock, 1971), an order of magnitude smaller than those recorded in the retina (Russell, 1976). Hair cell receptor potential may control the rate of afferent transmitter release (Davis, 1965; Ishii et al., 1971; Strelioff and Honrubia, 1978). Several potential afferent transmitters have been proposed: GABA (δ-amino butyric acid) (Flock and Lam, 1974; Galindo, 1969), monoamines (Osborne and Thornhill, 1972), and glutamate (Steinbach and Bennett, 1971).

Hair cells receive an efferent innervation that, when stimulated, inhibits afferent nerve firing (Russell, 1968, 1976). Efferent nerves fire in response to motor nerve firing to muscles that cause animal locomotion (Russell, 1971a, b, 1976). Therefore, hair cells are essentially shut down during locomotion, presumably to prevent sensory overload. The efferent transmitter appears to be acetylcholine (Russell, 1971b).

Neuromast Structure and Properties

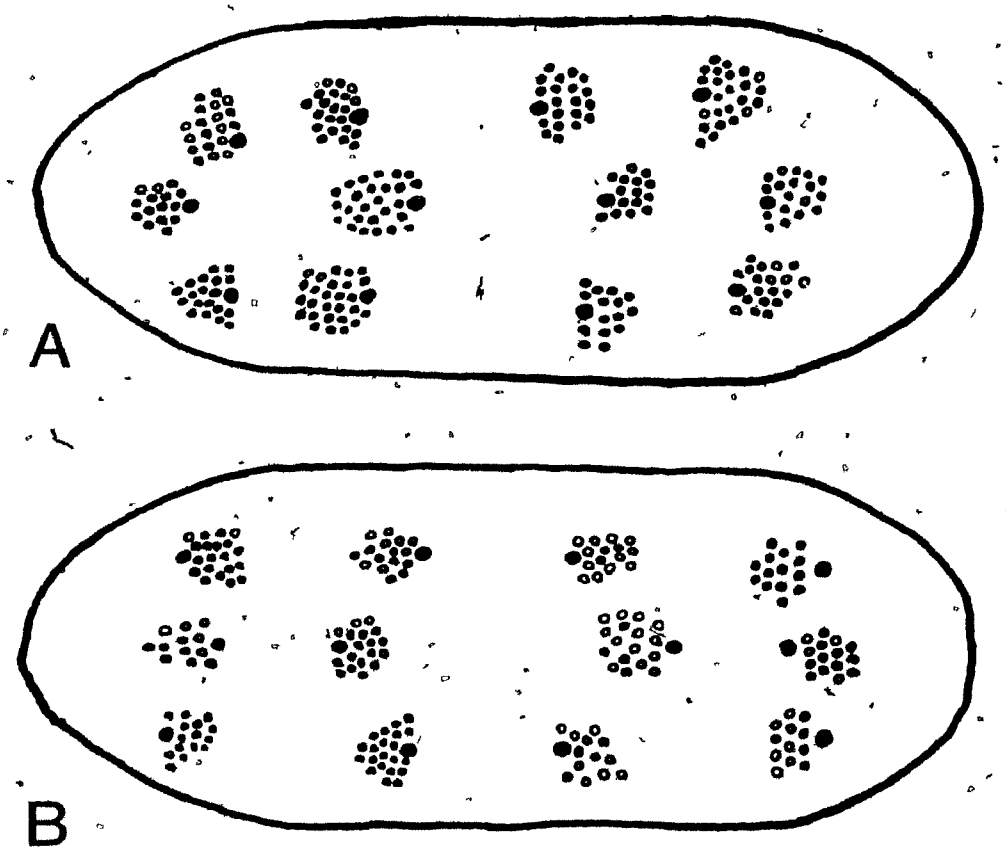
Hair cells are grouped to form neuromasts. In

amphibians, numbers of hair cells per neuromast vary within and between species: 5 - 10 in Rana (Jande, 1966), 6 - 10 in Necturus (Flock, 1971), and 12 - 20 in Ambystoma (Jørgensen and Flock, 1973). Hair cells may or may not be separated by supporting cells (Chezar, 1930; Wright, 1951; Russell, 1976) and it is the hair cells and their cupula plus these supporting cells that comprise the entire neuromast organ.

Within each neuromast the arrangement of hair cells is precise; hair cells are polarized so that a line drawn through the kinocilium and the middle of the stereocilia bundle is parallel to the neuromast long axis. Approximately one half of the hair cells in any neuromast have their kinocilium at one pole, in the remaining cells the kinocilium is at the opposite pole. Adjacent hair cells may be oppositely polarized, as in Ambystoma (Jørgensen and Flock, 1973), Xenopus (Shelton, 1970, 1971), and Necturus (Fig.I-3b; Flock, 1971) or hair cells may be clumped by polarity as in Rana (Fig. I-3a; Jande, 1966).

Each neuromast is innervated by fibers of two afferent nerves. Within each neuromast hair cells of the same polarity are innervated by the same nerve (Sand, 1937; Görner, 1963). Therefore, any given stimulus will depolarize one half of the hair cells while hyperpolarizing the other half. Hyperpolarization is

Figure I-3. Two schematics illustrating differences in hair cell arrangement within single neuromasts in amphibians. A) Hair cells clumped by orientation, perhaps typical of Rana, and B) hair cells alternating polarity, typical of all other amphibians.


A
B

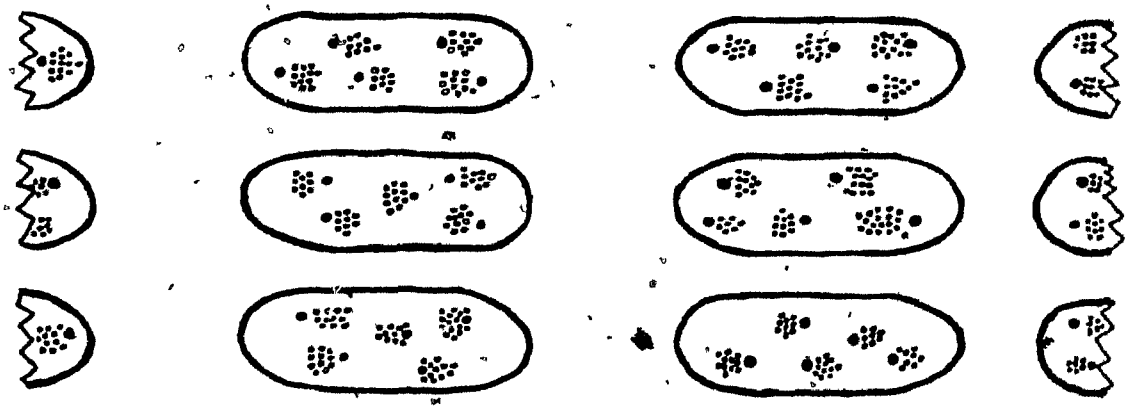
less pronounced (about one quarter to one eighth the response) than depolarization. This morphology explains why extracellular recordings of neuromasts stimulated sinusiodally at low frequencies record a diminished receptor potential twice the frequency of the stimulus (Dijkgraaf, 1963; Flock and Wersäll, 1962).

The cilia of all hair cells in a neuromast project into and connect to one gelatinous protein cupula. This cupula is believed to be secreted by the supporting cells (Russell, 1976) but attached only to the hair cells (Flock, 1967). The cupula shears the sensory epithelium with impinging water displacements. Because of the within-neuromast cupular yoking to hair cells and the specific neuromast innervation pattern, Dijkgraaf (1963) defined the neuromast as the functional unit of the mechanoreceptive system.

In amphibians, neuromasts are often organized into parallel groups called stitches (Fig. I-4; Harris and Milne, 1966; sometimes called plaques, Murray, 1955). Stitches are formed by neuromast growth and division in posthatching amphibians after the original, or primary, neuromast has been laid down embryonically (Stone, 1933; Winklbauer and Hausen, 1983a).

The stitch long axis is typically perpendicular to its component neuromasts' long axes and to the axis of maximum hair cell sensitivity (Fig. I-4; Jørgensen and

Figure I-4. Schematic digram showing the orientation of hair cells within neuromasts, and the orientation of neuromasts within transverse stitches. Note that hair cell and neuromast sensitivity are perpendicular to the long axis of each stitch.



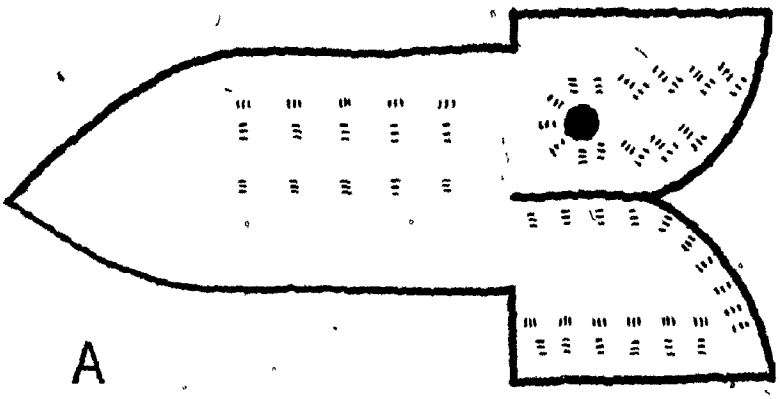
Flock, 1973; Flock and Jørgensen, 1974). This organization is important to investigators concerned with neuromast function. The direction of maximum hair cell sensitivity, which initially could only be determined under high-power microscopy, can now be deduced from stitch orientation, which is visible to the unaided eye or under low power magnification. The one exception to this rule may be Necturus, which are reported to have neuromasts organized into linear stitches (Harris, et al., 1971).

All hair cells of the same polarization within a stitch are innervated by fibers from the same neuron (Dijkgraaf, 1963) The receptor potentials of the neurons are therefore summed when a hair cell is excited (Dijkgraaf, 1963). Flock (1971) has pointed out that, in animals that have neuromasts organized into stitches, the stitch, rather than the neuromast, is the functional unit.

Neuromast Group Structure and Properties

Neuromasts and stitches are organized into lines (or fields, Reno and Middleton, 1973) that extend along the body and head (Fig. I-5). The generalized condition appears to be three body lines -- dorsal, middle, and ventral -- and three head lines -- supraorbital, infraorbital, and submandibular. Along the body adjacent

Figure I-5. Schematic diagrams showing two possible stitch formations in urodeles, assuming that neuromast long axes are always perpendicular to their stitch long axis. A) Known neuromast and stitch topography in Ambystoma, B) hypothesized neuromast topography based on stitch orientation in Necturus.



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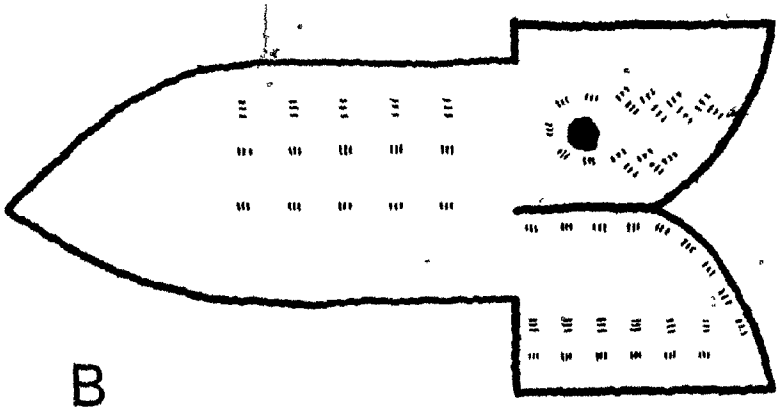

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stitches within a line may be parallel, and perpendicular to the long axis of the body (e.g.,middle body line, Fig. I-5a) or serial, in a line parallel to the body axis (e.g. middle body line, Fig. I-5b). Stitch orientation on the head is more complex. Supra- and infraorbital stitches near the eye may be oriented radially (Fig.I-5a) or tangentially (Fig.I-5b) to the eye. Entire lines or parts of lines may be duplicated; auxilliary lines occur in some taxa. Presumably, these variations are correlated with environmental parameters that affect fitness (Dijkgraaf, 1963). In fishes there appears to be a reduction in lines and neuromasts per line proceeding from generalized to derived forms (Branson and Moore, 1962). No comparable data have been collected for amphibians.

Surveys of amphibian neuromast group arrangement, or topography, have been conducted by Malbranc (1876), Kingsbury (1895), Escher (1925), and Hilton (1947). In general, these surveys involve few, but taxonomically diverse, species and emphasize urodeles. Malbranc and Escher provide the most detailed drawings and careful analysis. Kingsbury is also careful, and although his drawings are weak, much useful information may be obtained. Kingsbury is also the best work done in English. Hilton surveyed more species than the previous workers.

These early workers were at a disadvantage; before

Lowenstein and Wersäll (1959) and Flock and Wersäll (1962) the morphological polarization and directional sensitivity of neuromasts were not realized. This meant that while evolutionary scenarios could be made based on topographical comparisons (i.e., Escher, 1925), the functional significance of topographical differences could not be determined. For example, it is now known that a topography with a predominance of orthogonally-arranged neuromasts is specialized for detecting water displacements in all directions tangential to the body surface (Dijkgraaf, 1963).

Aquatic members of the third amphibian order, Gymnophiona (Caecilia), also possess lateral line organs. Hetherington and Wake (1979) describe the lateral line system of Ichthyophis sp. and, as well, give a brief literature review of this subject for caecilians. On the head, Ichthyophis neuromasts are arranged into lines that appear homologous with those of anurans and urodeles. On the body, however, Icthyophis has only one paired dorsal body line, as opposed to dorsal, middle, and ventral body lines in the other two orders. Like urodeles, caecilian larvae also have electroreceptive ampullary organs (Hetherington and Wake, 1979).

Neuromast Central Nervous System Connections

Lateral line afferent neurons are bipolar cells with

their cell bodies located in ganglia in the otic region. Afferent nerve bundles are divided into anterior and posterior divisions. The anterior lateral line nerve (NLLA) is divided into supraorbital, infraorbital, and postorbital branches that innervate supraorbital, infraorbital, and mandibular neuromasts, respectively (Escher, 1925). The supra- and infraorbital lines may fuse; their common ganglion is the trigeminal (CN V) lateral line ganglion; the postorbital nerve cell bodies are located in the facial (CN VII) lateral line ganglion (Fritzsch, 1981a; Fritzsch et al., 1984). The posterior lateral line nerve (NLLP) is divided into dorsal, middle, and ventral branches; their cell bodies are located in glossopharyngeal (CN IX) and vagal (CN X) lateral line ganglia. From these cranial nerve ganglia lateral line afferents travel centrally to the medullary alar plate and divide into ascending and descending fascicles (Fritzsch et al., 1984; Boord and McCormick, 1984).

Primary afferents of the octavolateralis system travel in distinct fascicles and terminate on the dorsolateral wall of the medulla in one of three nuclei: electrosensory afferents terminate in the dorsal nucleus, mechanosensory afferents in the intermediate nucleus, and octavo afferents in the lateral nucleus (Boord and McCormick, 1984). In urodeles the NLLA is composed of two long and one short fascicle, the NLLP of two long

fascicles (Fritzsch, 1981a). Long fascicles are composed of mechanoreceptive afferents, the short fascicle is composed of electroreceptive afferents (Fritzsch, 1981a). Anurans have one, two, or several long fascicles, they have no short fascicles and no dorsal nucleus, and therefore are not thought to be electrosensitive (Fritzsch et al., 1984).

The arrangement of mechanoreceptive afferents into long fascicles in urodeles corresponds with the peripheral innervation pattern of neuromasts. As stated previously, neuromasts are innervated by fibers of two afferents; each afferent only innervates hair cells of the same polarity. For both the NLLA and NLLP, afferents from hair cells of the same polarity run in the same fascicle (Fritzsch, 1981a). In anurans, this division is not as distinct. Because neuromasts are structurally, and presumably functionally, the same between these two groups, the urodele separation of afferents into fascicles may not be important as initially thought (Fritzsch, 1981a; Fritzsch et al., 1984).

In Xenopus secondary afferent neurons emerging from the intermediate or lateral line nucleus project either to the contralateral intermediate nucleus, the cerebellum, or the contra- or ipsilateral torus semicircularis (Plassmann, 1980). Secondary, or higher level connections to the telencephalon via thalamic

connections probably also exist (Boord and McCormick, 1984).

The NLLA and NLLP also contain lateral line efferent neurons. Efferents originate in the medullary reticular formation and appear to be nonspecific; one neuron may go both to the labyrinth and to neuromasts (Fritzsch, 1981b), and, within the neuromast division, probably supply more than one stitch (Will, 1982).

Neuromast Development and Evolution

Neuromasts are unique in that they develop embryonically from several migrating pre- and postotic ectodermal placodes (Harrison, 1903; Stone, 1933; Knouff, 1935; Winklbauer and Hausen, 1983a,b, 1985a,b). These placodes arise anterior and posterior to the otic placode, which gives rise to inner ear structures. As the otic placode invaginates, preotic placodes migrate rostrally to from head neuromasts (and electroreceptors), postotic placodes migrate caudally to form trunk and tail neuromasts.

Each pre- and postotic placode can be divided into a proximal and distal portion relative to the otic capsule. The proximal portion eventually develops into the lateral line nerves and ganglia, the distal portion forms the primary neuromasts.

Lateral line placode formation, development, and

migration appears to be the same in all amphibians and fish (Stensio, 1947; Holmgren and Pehrson, 1949; Jarvik, 1980). These developmental similarities combined with the morphological, physiological, and neuronal similarities of hair cells, neuromasts, and neuromast groupings leave no doubt that these structures are homologous within amphibians, and between fishes and amphibians (Stensio, 1947; Holmgren and Pehrson, 1949). Likewise, their is little doubt that hair cells throughout the acousticolateralis system are homologous (Dijkgraaf, 1963; van Bergeijk, 1967; Northcutt and Gans, 1983).

The incomplete fossil record and lack of available embryological cues has limited speculation on how neuromasts first evolved. Neuromasts appear to be present in the oldest vertebrate fossils (ostracoderms, Schmalhausen, 1968; Romer, 1971). Because of the structural similarity of kinocilia to ordinary cilia (a 9 + 2 microtubule arrangement) most evolutionary scenarios favor a ciliary origin of hair cells and neuromasts (Denison, 1966; Northcutt and Gans, 1983). Northcutt and Gans (1983) speculate that in prevertebrates epidermal cilia were largely responsible for locomotion. The subsequent development of a notocord coupled with axial musculature provided a more efficient method of locomotion, and replaced cilia. With the ciliary system

free to perform other functions, and being generally sensitive, ciliary patches and their associated sensory and motor nerve plexuses specialized to form specific types of sensory receptors, including mechanoreceptors, electroreceptors, and taste buds.

Neuromasts and Behavior

Scharrer (1932) made the first observations on the role neuromasts play in determining amphibian behavior. He first enucleated Ambystoma embryos then removed the preotic lateralis placode unilaterally from either side of these animals. The placodal surgery removed all of the supra- and infraorbital neuromasts and most submandibular ones. Animals then stimulated with a water jet from a small pipette on the ablated side failed to respond in any way, while contralateral stimulation on the intact side almost always elicited a snapping response, as if the water from the pipette was food.

Xenopus adults respond to surface waves by turning and swimming towards the stimulus (e.g., Görner, 1973; Elepfandt, 1982; Görner et al., 1984). This response is still present, but less accurate, after neuromast ablation with a few bilateral stitches remaining intact, but is lost with only ipsilateral stitches remaining (Görner et al., 1984; Elepfandt, 1982). Apparently, comparative bilateral input is necessary for the

orientation response, and more neuromasts means a more accurate response. Xenopus may also use ventral neuromasts to detect surface waves when dorsal neuromasts are ablated (Elepfandt, 1984); this contrasts with surface-feeding fish that depend on dorsal neuromasts exclusively for this behavior (Schwartz, 1971).

Wassersug et al., (1981) show that streptomycin, an inhibitor of hair cell function, adversely affects schooling behavior in Bufo tadpoles. These data agree well with the findings of Partridge and Pitcher (1980) who show that neuromasts play a role in the maintenance of fish schools. Neuromasts are generally implicated as being important in other behaviors such as avoiding predators and seeking mates. This, however, has not been proven for any amphibians.

Chapter 1: Vision is Not Necessary for Size-Selective Zooplanktivory in Aquatic Salamanders

INTRODUCTION

Aquatic vertebrates feed on zooplankton by one of two methods: they either take zooplankters individually and are size selective (normally taking the largest individuals available) or they filter feed and take a broad range of zooplankton sizes (Zaret, 1980; Greene, 1985). The former method is virtually always associated with diel activity patterns, the latter frequently with nocturnality.

Salamander larvae prey heavily on zooplankton (e.g., Dineen, 1955; Lannoo and Bachmann, 1984a), feeding on them individually and taking the largest animals available (Dodson and Dodson, 1971; Branch and Altig, 1981). It is commonly assumed that vision mediates this predation pattern (Anderson, 1968; Dodson and Dodson, 1971; Sprules, 1972; Zaret, 1980). However for this to be true salamanders should exhibit diel feeding patterns. This, in fact, is not the case. It is well known that many aquatic salamanders are nocturnal (e.g., Noble, 1931; Anderson and Graham, 1967; Joly and Caillère, 1983). The exceptions are in areas where diel predators are scarce (Dodson and Dodson, 1971; Sprules, 1972) or where nocturnal predation is especially heavy (Holomuzki, 1984).

How, then, do nocturnal salamanders detect

zooplankton? Can salamanders be size selective if they cannot use vision? Or, conversely, if salamanders are size selective nocturnally, can the assumption be justified that size selection in diel populations is visually mediated?

If visual cues are essential, or even most important, to size selective predation three predictions should hold true for sighted salamanders feeding in light conditions compared to enucleated salamanders and dark conditions: 1) their feeding rates should be higher (Peckarsky, 1982), 2) they should select larger prey (Dodson and Dodson, 1971), and 3) they should select the darker, more visible prey (Sprules, 1972). To test these predictions I offer sighted and enucleated larval Ambystoma maculatum in light or dark conditions a choice of large and small or normal and coloured Daphnia (a natural prey), and record prey type and number taken.

METHODS

Ambystoma maculatum eggs and larvae were collected from Heart-shaped Pond in Halifax Co., Nova Scotia, Canada (44° 40'N, 93° 40'W). Field-collected eggs were hatched in the laboratory. Larvae were raised on a mixture of live Daphnia magna and frozen brine shrimp (Artemia salina) and in accordance with guidelines set by the Canadian Council on Animal Care (1984). Daphnia were

cultured in my laboratory from stocks maintained at the Bedford Institute of Oceanography, Dartmouth, Nova Scotia.

A total of 40 Ambystoma maculatum larvae were tested. Laboratory-raised salamanders were kept on a 12:12 L:D cycle centered at 1400 h at 21±1° C. Field-collected larvae (SVL 21.0 - 26.0 mm) were tested within 24 h of capture and therefore not fed (Test 1, below). Laboratory-raised larvae (SVL 10.5 - 13.0 mm) were not fed a minimum of 16 h before testing (Tests 2, and 3, below). In each experiment only one larva per container was used, to remove competitive effects. Containers were rectangular (10 x 8 x 6 cm), made of clear glass and filled with 200 ml of previously-aerated, aged tap water to a depth of approximately 25 mm (Test 1), or opaque plastic (13.5 x 10 x 7 cm) filled with 250 ml of water to a depth of approximately 20 mm (Tests 2, and 3). All salamanders were tested between 1000 and 1600 h at 21±1° C and were large enough to ingest the largest Daphnia offered to them. The "light" condition was normal laboratory fluorescent light, the "dark" condition was complete darkness in a photographic darkroom. Salamanders were allowed to acclimate to darkness for at least 20 min before testing began. I judged this acclimation time to be sufficient because in nature most salamanders in this population begin feeding

each day at dusk or immediately after darkness sets in. Daphnia concentrations offered to salamanders were within the range found in nature (Janssen, 1980; Lannoo and Bachmann, 1984b).

Salamanders were enucleated after first being anesthetized in 0.03% MS 222 (tricaine methanesulfonate). Enucleation was done with forceps and iridectomy scissors. The wounds were cauterized with a Hyfrefractor cauterizing unit set at low voltage. Enucleated salamanders were tested a minimum of 24 h after surgery. Two of the 16 enucleated larvae did not subsequently feed and were excluded from the tests. During experiments in light conditions I made additional behavioural observations on both sighted and enucleated salamanders. These observations caused no visible disturbance to the animals. Data collected approximated normal distributions and parametric statistics were used (pooled t-test, MINITAB; Ryan et al., 1976).

TEST 1: Sighted A. maculatum feeding on Daphnia in a range of sizes in light and dark conditions.

The following questions were addressed in this test: 1) Are salamanders feeding in light size selective? 2) Are salamanders in dark size selective? 3) Are there selectivity differences between salamanders feeding in light and dark conditions? and 4) Are there feeding-rate

differences between salamanders feeding in light and dark conditions?

Twelve field-collected A. maculatum were tested, six each in light and dark conditions. One trial was conducted per salamander and all salamanders were tested simultaneously. About equal numbers of variously-sized Daphnia (range 0.8 - 2.4 mm carapace length) were placed in twelve containers. Containers were randomly assigned to light or dark conditions and salamanders were randomly assigned to containers. Salamanders fed for 30 min, after which they were immediately killed and preserved in 10% formalin (which did not cause regurgitation of stomach contents). Daphnia that had not been eaten remained in each container and were filtered from the water and killed and preserved in 10% formalin. Salamander stomachs were removed, opened, and Daphnia ingested counted and their sizes measured (carapace length) with a calibrated ocular micrometer. Likewise, Daphnia not ingested were counted and measured.

TEST 2: Sighted and enucleated A. maculatum feeding on large and small Daphnia in light and dark conditions.

The following questions were addressed in this test: 1) Is there a difference in sizes and total numbers of Daphnia ingested between the sighted-light treatment and the other treatments? 2) Is there a difference in sizes

and total numbers of Daphnia ingested between sighted-dark and enucleated-dark treatments (surgical control)?, and 3) Is there a difference in sizes and total numbers of Daphnia ingested between enucleated-light and enucleated-dark treatments (prey behaviour control)?

The experimental design was a 2 x 2 factorial test comparing sighted and enucleated salamanders in light and dark conditions. Sixteen A. maculatum larvae, four per treatment, were used. Salamanders were each fed 20 large (2.0 - 2.4 mm carapace length) and 20 small (0.8 - 1.5 mm carapace length) Daphnia. Salamanders were tested in two trials, twenty minutes per trial. After each trial large and small Daphnia remaining were removed and counted; numbers ingested for each prey group were obtained by subtraction from 20 (original prey number). Totals for the two trials were added and these single numbers used in the statistical analyses. To compare size selectivity between treatments, for each salamander numbers of large Daphnia ingested were divided by total numbers of Daphnia ingested (large plus small Daphnia) to create a size index. The greater the size index value the more large Daphnia were taken. This size index produces values between 0 and 1 and was used in the between-treatments statistical analyses.

Because sham-operated enucleation controls are

difficult, if not impossible to construct, I decided a priori to determine the effect of enucleation on feeding performance by comparing data from sighted-dark treatments with enucleated-dark treatments. To test for possible light-dark differences in Daphnia swimming or predator avoidance behaviour, data from enucleated-light treatments were compared to enucleated-dark treatments.

TEST 3: Sighted and enucleated A. maculatum feeding on normally-coloured and artificially-darkened Daphnia in light conditions.

In this test prey-colour preferences and feeding rates were compared for sighted and enucleated salamanders. This test then addressed the role of vision in diel feeding in these salamanders.

Six sighted and six enucleated A. maculatum larvae were each allowed to feed for 20 min on ten large (2.0 - 2.4 mm carapace length) normally-coloured Daphnia and ten large Daphnia kept overnight in a suspension of India ink particles. India ink has been frequently used to darken Daphnia in tests involving visual predation on zooplankton by fish (eg., Zaret, 1980). To the human eye ink-exposed Daphnia were considerably darker than normal Daphnia and appeared to behave normally. Because Test 2 results showed no deleterious effects of enucleation surgery on feeding performance (see RESULTS) and

enucleation surgical controls are difficult if not impossible to construct, no sham surgical controls were done. A colour index was calculated in the manner of the size index in Test 2 -- by dividing numbers of ink-darkened Daphnia ingested by total Daphnia ingested. Prey-colour preferences and feeding rates were compared for sighted and enucleated salamanders.

RESULTS

Test 1.

A. maculatum larvae in light conditions were size selective (Table 1-1 a). Salamanders in dark conditions were also size selective (Table 1-1, b). Salamanders in dark conditions took larger prey than those in light conditions -- a surprising result -- but this difference was not significant when ingested prey sizes were corrected for available prey sizes (Table 1-1, c). Feeding rates were nearly identical for salamanders in light and dark conditions both in terms of absolute numbers of prey ingested (Table 1-1, d) and percent available prey ingested (Table 1-1, e).

Test 2.

All salamanders, sighted and enucleated, in light and dark conditions, fed similarly. Most importantly to the questions addressed in this test, there was no

Table 1-1. Feeding performance of Ambystoma maculatum larvae in light and dark conditions fed Daphnia in a range of sizes (Test 1). Statistical comparisons A and B test Daphnia sizes ingested against Daphnia available in light and dark treatments, respectively. Test C compares sizes of Daphnia ingested in light and dark treatments after correcting for sizes of Daphnia available (by subtracting sizes of prey available from prey ingested). Tests D and E compare feeding rates between light and dark treatments in terms of number and percent of available Daphnia ingested. Means and standard errors are given; statistical comparisons were done using pooled t-tests (Snedecor and Cochran, 1967). Asterisks indicate probability values less than 0.05.

COMPARISON: Treatment/Measure	n	$\bar{x}$	SE	Probability
A. Light/Size				
ingested	6	1.45 mm	± 0.02	
available	6	1.23 mm	± 0.01	<0.0001*
B. Dark/Size				
ingested	6	1.63 mm	± 0.02	
available	6	1.33 mm	± 0.01	0.003*
C. Light vs. Dark/Ingested-Available				
light	6	0.20 mm	± 0.04	
dark	6	0.32 mm	± 0.05	0.20

Table 1-1 (cont.)

D. Light vs. Dark/Number Ingested[a]				
light	6	25.3	± 7.19	
dark	6	25.5	± 5.12	0.99
E. Light vs. Dark/Percent Ingested[a]				
light	6	37.2	± 6.99	
dark	6	33.9	± 6.35	0.75

[a] Feeding rates for a 30 min period.

significant difference in size index between the sighted-light treatment and the other treatments (Table 1-2, a). Feeding rates were higher for animals in the sighted-light treatment although these differences were not significant (Table 1-2, b).

There was no significant difference in size index between sighted-dark and enucleated-dark treatments (Table 1-3, a). Feeding rates were unexpectedly higher for enucleated than sighted salamanders (Table 1-3, b). These results indicate that enucleation surgery had no deleterious effects on feeding performance.

There was no significant difference between enucleated-light and enucleated-dark treatments in size index (Table 1-4, a) or feeding rate (Table 1-4, b). These results indicate no light-dark differences in salamander feeding performance due to diurnal differences in zooplankton swimming or predator avoidance behaviour.

Test 3.

Sighted and enucleated salamanders fed similarly on normally-coloured and artificially-darkened Daphnia. The most important result in terms of the questions addressed in this test was that there was no significant difference in colour preference between sighted and enucleated salamanders (Table 1-5, a). Feeding rates were higher for sighted animals but this difference was not significant

Table 1-2. A comparison of the feeding performance of *A. maculatum* larvae with visual cues available to them (i.e., sighted animals in light conditions) to larvae unable to use vision (enucleated animals and dark conditions) feeding on large and small *Daphnia* (Test 2). Statistical test A compares size indices (formula in text) between visual and nonvisual treatments; test B compares total numbers of *Daphnia* ingested. Comparisons were made using (A) Mann-Whitney U-tests and (B) pooled t-tests. In A, ranges are given for the values, which were not normally distributed.

Comparison	n	$\bar{x}$	SE	Prob.
A. Size index				
sighted light	4	0.78 (0.73 - 0.86)		
enucleated or dark	12	0.76 (0.62 - 1.0)		>0.05
B. Numbers ingested[a]				
sighted light	4	22.8	± 5.9	
enucleated or dark	12	14.9	± 2.6	0.18

[a]Feeding rates adjusted for a 30 min period.

Table 1-3. Results of control testing the effect of enucleation surgery on feeding performance (Test 2). In this test sighted versus enucleated A. maculatum larvae fed in dark conditions on large and small Daphnia. Statistical test A compares size indices of prey taken between sighted and enucleated salamanders; test B compares total numbers of Daphnia ingested for both groups. Comparisons were made using (A) Mann-Whitney U-tests and (B) pooled t-tests. In A, ranges are given for the values, which were not normally distributed.

Comparison:	n	$\bar{x}$	SE	Prob.
A. Size index				
sighted dark	4	0.82 (0.71 - 1.0)		
enucleated dark	4	0.79 (0.67 - 0.92)		>0.05
B. Numbers ingested[a]				
sighted dark	4	9.0	± 1.2	
enucleated dark	4	16.8	± 6.6	0.29

[a] Feedings rates adjusted for a 30 min period.

Table 1-4. Results of control testing for possible diurnal differences in Daphnia swimming or predator avoidance behaviour that could affect salamander feeding performance (Test 2). In this test enucleated salamanders fed in light and dark conditions on large versus small Daphnia. Test A compares size indices between salamanders in light and dark treatments; comparison B tests total numbers of Daphnia ingested for both groups. Comparisons were made using (A) Mann-Whitney U-tests and (B) pooled t-tests. In A, ranges are given for the values, which were not normally distributed.

Comparison:	n	$\bar{x}$	SE	Prob.
A. Size index				
enucleated light	4	0.70 (0.62 - 0.75)		
enucleated dark	4	0.79 (0.6[illegible] 0.92)		>0.05
B. Numbers ingested[a]				
enucleated light	4	19.0	± 3.3	
enucleated dark	4	16.8	± 6.6	0.77

[a] Feeding rates adjusted for a 30 min period.

Table 1-5. Results of a test designed to assess the role of prey visibility in prey detection and ingestion by A. maculatum larvae (Test 3). In this test sighted and enucleated salamanders in light conditions were given a choice between normally-coloured and artificially-darkened Daphnia. Comparison A considers colour index (calculated similar to the size index; see text) between sighted and enucleated groups; test B considers numbers of Daphnia ingested. Comparisons were made using (A) Mann-Whitney U-tests or (B) pooled t-tests.

Measure/Treatment	n	$\bar{x}$	SE	Prob.
A. Colour index				
sighted	6	0.45	(0.25 - 0.58)	
enucleated	6	0.44	(0.33 - 0.57)	>0.05
B. Numbers ingested[a]				
sighted	6	9.5	± 2.0	
enucleated	6	6.0	± 2.0	0.25

[a] Feeding rates adjusted for a 30 min period.

(Table 1-5, b).

DISCUSSION

If visual cues are essential, or even most important, to size selective predation in aquatic salamanders three predictions should have held true for sighted animals in light compared to other treatments: 1) their feeding rates should have been higher (Peckarsky, 1982), 2) they should have selected larger prey (Dodson and Dodson, 1971), and 3) they should have selected the darker, more visible prey (Sprules, 1972). The data here support none of these predictions. Feeding rates were similar whether salamanders used vision or not (Tables 1-1 - 1-5); salamanders always fed on the largest Daphnia available (Tables 1-1 - 1-4); and salamanders fed on normal and coloured prey in a 50:50 ratio (Table 1-5). From these results I conclude that not only is vision not being used in nocturnal feeding by these salamanders, but that vision may not be as important in diel feeding as initially suspected (Nicholas, 1922; Anderson, 1968; Dodson and Dodson, 1971; Sprules, 1972; Zaret, 1980). This conclusion agrees with Ambystoma growth data collected by Detwiler and Copenhaver (1949) who observe: "...the absence of eyes or of light both fail to affect...growth, ...larvae feed just as well without eyes in the dark as they do in the light with

eyes" (p. 245).

During the course of my light condition experiments I observed three behavioural tendencies of salamanders feeding on Daphnia that provide further insights into these results. Firstly, sighted animals oriented towards and approached prey up to approximately three to four cm (~1.5 - 2.0 body lengths) away (see also Hoff et al., 1985) whereas enucleated salamanders oriented towards and approached prey only within approximately two cm. Secondly, both sighted and enucleated salamanders ignored small prey and chose large over small prey when both were near. Thirdly, rectangular experimental enclosures caused prey to aggregate in corners (see also Bovbjerg, 1975), where both sighted and enucleated salamanders fed most successfully.

From the first observation it appears that vision plays a role in far-field prey detection by these animals, but in my experimental enclosures this did not translate into more prey, or more visible prey, being selected. Prey were taken in the near field and visual cues did not greatly affect this behaviour. This observation does indicate visual cues may become more important as prey density decreases and prey become less numerous and more difficult to find. The second observation, that both sighted and enucleated salamanders chose large over small Daphnia, confirms that in the near

field large prey are selected over small prey. This observation is, however, equivocal regarding which sensory systems are employed. The third observation suggests that enucleated salamanders are as good as sighted animals in locating prey aggregations. However, salamanders, like Daphnia, may simply be following container edges to their corners rather than following the Daphnia per se.

The conclusions I draw here about the potential unimportance of vision in salamander predation conflict with those of Nicholas (1922), who conducted experiments similar to mine. Nicholas fed sighted, enucleated, olfactory-deprived, and enucleated and olfactory-deprived A. tigrinum larvae earthworm pieces and concluded that visual cues are most important in prey detection, followed by olfactory cues, followed by mechanical cues. (Before Hetherington and Wake, 1979, and Fritzsch, 1981a it was generally not known that amphibians have electroreceptors.) However, while laboratory Ambystoma will readily feed on worm pieces, liver pieces, frozen brine shrimp, and other dead prey, in the field they feed predominantly if not exclusively on live prey. The differences between Nicholas' results and mine probably reflect the fact that salamanders use different senses to feed on intact-alive vs. wounded or dead prey.

The role of olfaction in detecting zooplankton is

also questionable. Salamanders tend to be sit-and-wait predators and take zooplankters individually (Anderson and Graham, 1967; Hassinger et al., 1970; Branch and Altig, 1981; Lannoo and Bachmann, 1984b; Hoff et al., 1985). Because molecules diffuse through water slower than zooplankton usually swim, any scent given off by a zooplankter will often reach a stationary salamander after the zooplankter is past the salamander and out of striking distance (Peckarsky, 1982). I ran a preliminary test examining the role of olfaction in zooplanktivory. I offered three enucleated A. maculatum larvae each ten large and ten small Daphnia that had been heat killed then cooled to room temperature immediately prior to testing. This treatment eliminated all prey motion but retained olfactory cues. Larvae were allowed to feed for twenty minutes and I recorded prey size and number ingested. Numbers of Daphnia ingested were greatly reduced; salamanders took a total of only six prey, a feeding rate 4.2 times lower than the feeding rates of animals in Test 2, where live large and small Daphnia were prey. Keeping in mind this small sample size, salamanders were also not size selective -- taking three large and three small prey. These results suggest that olfaction alone is not sufficient for the observed feeding rates and size selection in salamanders. Indeed, Detwiler and Copenhaver (1940) found that Ambystoma

larvae deprived of both eyes and nasal placodes as embryos, responded by snapping at food and inanimate objects in motion. They state (p. 243): "We wish to emphasize the fact that in the absence of both eyes and the nasal placodes the larvae feed as well as do normal animals."

Other factors besides vision correlate positively with prey body size, such as mechanical water perturbances prey make as they swim, and electrical field changes around prey due to their muscle contractions, and may be sensed by salamanders. Scharrer (1932), Görner et al. (1984), and Elepfandt (1982, 1984) have shown that neuromasts, and Himstedt et al. (1982) have shown that ampullary organs, are used by amphibians to detect prey. Perhaps these lateral line systems, either singly or in combination, are used by nocturnal salamanders to detect zooplankton.

By suggesting that lateral line organs are being used by nocturnal salamanders to detect zooplankton I do not wish to diminish the importance of vision and olfaction in detecting other prey types or for determining other behaviours. While salamanders feed predominantly on zooplankton (Dineen, 1955; Freda, 1983), and exhibit a specialized floating behavior to do so (Anderson and Graham, 1967; Branch and Altig, 1981; Lannoo and Bachmann, 1984b) they also feed on snails,

oligochaetes (Dodson and Dodson, 1971; Brophy, 1980; Lannoo and Bachmann, 1984a) and amphibian eggs (Grusser-Cornehls and Himstedt, 1976) for which olfactory and visual cues would likely be most important (Joly and Caillère, 1983). In addition aquatic adults exhibit other behaviours such as courtship and mating that probably depend on vision and olfaction (e.g., Halliday and Sweatman, 1976). However, to generalize, given the numerical and volumetric predominance of zooplankton and other small prey found in the stomachs of salamander larvae and the salamander tendency towards nocturnality it may well be that visual and olfactory cues are of secondary importance to prey detection in these animals.

Chapter 2: Intra- and Interspecific Variation in Neuromast Topography In *Ambystoma* larvae

INTRODUCTION

It is well known that lateral line neuromast topography may be used to distinguish genera and higher level taxa in fishes (e.g., elasmobranchs, Chu and Wen, 1979; holosteans, Jarvik, 1980 and references therein; teleosts, Parvin and Astakhov, 1982) and amphibians (Kingsbury, 1895; Escher, 1925: Hilton, 1947). However, few studies have examined differences between species within a genus (Jollie, 1984 considers this for Lepisosteus). Before such intrageneric comparisons can be made, it is necessary to determine the degree of normal intraspecific variation in neuromast parameters, as well as the nature of ontogenetic differences.

In amphibians neuromasts are grouped to form stitches; stitches, in turn, are clustered to form groups, or lines. I examined cephalic neuromast groups, stitches per group, neuromasts per stitch, and neuromast density for complete larval developmental series of Ambystoma maculatum and Ambystoma tigrinum, including typical and cannibal morphs. The specific questions addressed are: 1) how do these neuromast parameters change with growth within a species, 2) do species differ in these parameters, and 3) can they be used to distinguish trophic morphs within a species?

MATERIALS AND METHODS

General Methods

Ambystoma tigrinum and *Ambystoma maculatum* developmental series were obtained by field collecting larvae or by hatching field-collected eggs and raising larvae in the laboratory. Larval *A. tigrinum* were collected in northwestern Iowa (Dickinson Co., 43° 23'N, 95° 11'W) and included both typical (N = 25, 10 - 62 mm standard snout vent length (SVL)) and cannibal (N = 2, 61, 62 mm SVL) morphs. Animals were immediately killed and preserved in 10% formalin (see Lannoo and Bachmann, 1984a for further details of collecting methods, population parameters, and cannibal morphs). Larval *A. maculatum* (N = 17, 8 - 23 mm SVL) were collected and preserved from Halifax Co., Nova Scotia (44° 40'N, 63° 40'W).

To visualize neuromasts, preserved animals were first placed in 0.5% trypsin for 12 - 24 h. This insured separation of the epidermis from the underlying dermis and later allowed neuromasts to be peeled away from the animal's body with the epidermis. After trypsin treatment, animals were placed in 30 - 35% hydrogen peroxide until skin pigments were bleached (12 - 72 h). Bleaching insured that no neuromasts were masked by pigment granules. Bleaching with the epidermis still on

the animal avoided tissue curling and subsequent difficulty in tissue mounting. After bleaching, the cephalic epidermis (i.e., the tissue immediately anterior to the gill rami dorsally and gular fold ventrally) was removed. Mid-dorsal and mid-ventral incisions were made along the entire length of the head, and the epidermis removed in left and right sections that included both dorsal and ventral skin (Fig. 2-1) These incisions insured that no neuromasts or stitches were bisected and yielded two tissues that could be flattened easily for microscopic examination. Tissues were placed in water between two glass microscope slides and viewed with a dissecting microscope at 10 - 40x under darkfield illumination.

Stitches per group and neuromasts per stitch were counted. Only neuromasts in well-defined stitches, and stitches in well-defined groups were considered, thereby avoiding confusion with ampullary lateral line organs (Fritzsch and Wahnschaft, 1983; Münz et al., 1982, 1984) and/or skin glands (see Hetherington and Wake, 1979). Stitches were assigned to groups based on criteria discussed in the following section.

Surface area of each tissue was determined using a Zeiss IBAS Image Analyzer. For each tissue three values were obtained and averaged. Average values for right and left tissues of the same animal were then summed.

Neuromast Group Definitions

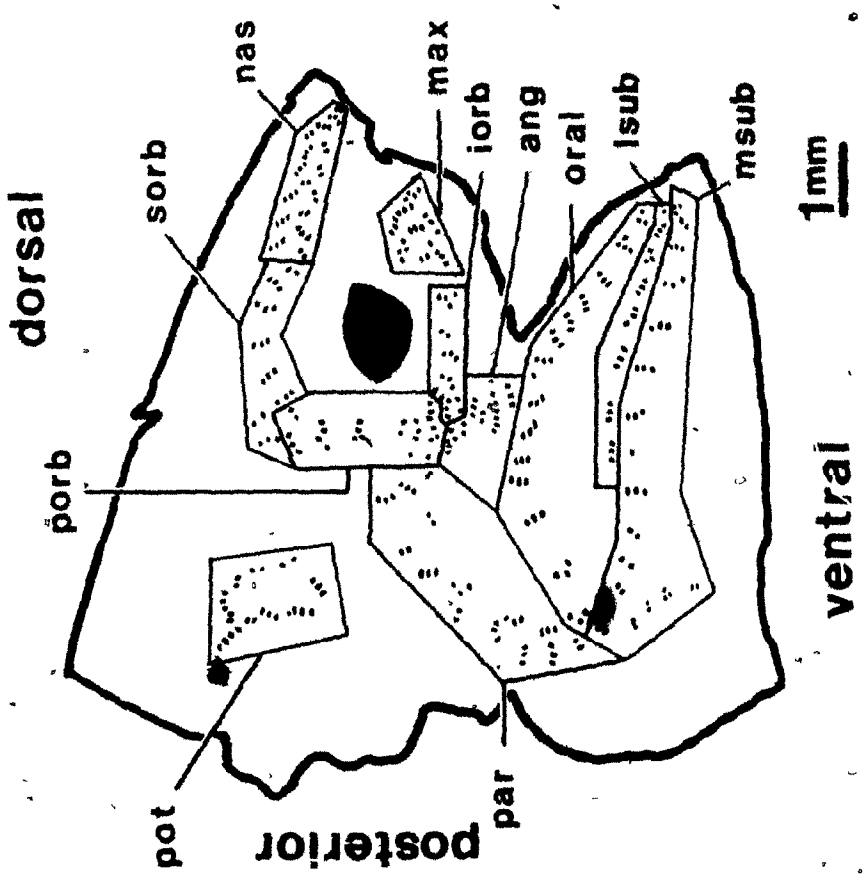
Ambystoma neuromasts, stitches and stitch groupings are illustrated in Fig. 2-1. In Fig. 2-1 (left), neuromasts appear as light ovals against the darkfield illumination. Neuromasts are grouped into clearly defined stitches, composed in this specimen of predominantly three neuromasts per stitch. Stitch orientation differs. These differences are critical to the functioning of neuromasts as the maximum sensitivity of each neuromast is perpendicular to the long axis of its stitch (Flock, 1967).

Figure 2-1 (right) illustrates stitch groupings which I base here on stitch orientation. These divisions differ slightly from those used by previous authors (Kingsbury, 1895; Escher, 1925; Hilton, 1947), who based their nomenclature on common nerve pathways. In most cases my groupings simply subdivide traditional groups. The groups identified here are:

Supraorbital Group -- single row of stitches; long axis of each stitch oriented approximately transverse to long axis of body, radial with respect to the eye.

Nasal Group -- anterior extension of supraorbital group; two stitch rows, one medial and one lateral; medial row of stitches oriented anterolaterally to posteromedially,

Figure 2-1. A darkfield photograph and tracing showing neuromast location and group organization in an Ambystoma maculatum larva (SVL 23 mm). The upper border of the tissue is the dorsal midline, the lower border the ventral midline, the dark oval the eye, and the anterior notch the mouth. Postotic neuromasts are difficult to discern in the photograph. Group abbreviations: ang = angular; iorb = infraorbital; lmax = lateral maxillary; lnas = lateral nasal; lpar = lateral parietal; lsub = lateral submandibular; mmax = medial maxillary; mnas = medial nasal; mpar = medial parietal; msub = medial submandibular; oral = oral; porb = postorbital; pot = postotic; sorb = supraorbital.


nas
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lateral row of stitches oriented anteromedially to posterolaterally; adjacent medial and lateral stitches perpendicular, as illustrated in Escher (1925; fig. 1e).

Postorbital Group -- single row of stitches; long axis of each stitch approximately parallel to long axis of body and radial to eye.

Infraorbital Group -- single stitch row; each stitch with long axis approximately transverse to long axis of body and radial to eye; an anterior extension of postorbital stitches.

Maxillary Group -- anterior extension of infraorbital group; two stitch rows, one medial, one lateral; medial row of stitches oriented anterolaterally to posteromedially, lateral row of stitches anteromedially to posterolaterally; adjacent medial and lateral stitches perpendicular.

Parietal Group -- stitches arc back from postorbital group to submandibular group; two stitch rows, one medial and one lateral; anterior portion of medial row oriented anterolaterally to posteromedially, posterior portion of medial row oriented anterior to posterior; anterior portion of lateral row oriented anteromedially to posterolaterally, posterior portion of lateral row oriented dorsoventrally; adjacent medial-lateral stitches

perpendicular.

Oral Group -- single row of stitches; stitches follow rim of mandible from midline anteriorly to junction of parietal and submandibular groups posteriorly; stitches approximately transverse to body axis.

Submandibular Group -- two stitch rows, one lateral and one medial; anterior extensions of lateral and medial parietal rows; lateral submandibular stitches parallel to long axis of body, medial stitches transverse.

Angular Group -- single diffuse row; located posterior to jaw angle; stitches oriented parallel to body axis.

Postotic Group -- stitches loosely organized; located caudally and dorsally; perhaps extension of dorsal (or medial?) body groups; develop from at least one postotic placode; the postotic group is innervated by a branch of posterior lateral line nerve.

All cephalic stitches other than postotic develop from preotic placodes and are innervated by anterior lateral line nerve branches. Supraorbital and nasal stitches are innervated by the supraorbital nerve; postorbital, infraorbital, and maxillary groups are innervated by the infraorbital nerve; and parietal, oral, submandibular, and angular stitches are innervated by the postorbital nerve (Escher, 1925).

RESULTS

Stitch number remains constant with growth for larval Ambystoma ($p > f = 0.25$ for A. tigrinum; $p > f = 0.10$ for A. maculatum). In A. tigrinum there are significantly more total cephalic stitches ($\bar{x} = 284.1$; range 221 - 344) than in A. maculatum ($\bar{x} = 242.1$; range 189 - 288) ($p > f < 0.001$; Table 2-1), thus this character can be used to distinguish populations of these species. However, intraspecific variation is too great to assign unidentified individuals to species based on this character alone. In seven of the ten neuromast groups: supraorbital, postorbital, parietal, oral, angular, submandibular, and postotic (Table 2-1), A. tigrinum have significantly more stitches than A. maculatum. Within individuals, contralateral stitch counts vary. The greatest variation observed in A. maculatum was 9.2% (120 vs. 137 stitches) and in A. tigrinum 11.5% (139 vs. 155 stitches). There were no differences in stitch number between cannibal and typical morph A. tigrinum (cannibal stitch numbers were 275 and 319).

Average number of neuromasts per stitch increases with growth, from one to three in A. maculatum and one to seven in A. tigrinum. For A. tigrinum neuromasts per stitch = 0.4 + 0.1 SVL ($r^2 = 0.87$; $p > f < 0.001$). For

Table 2-1. A comparison of mean number of cephalic neuromast stitches by group in 27 Ambystoma tigrinum and 17 Ambystoma maculatum larvae. Asterisks indicate significant species differences (t-test). See Figure 1 for group location and orientation. Contralateral values were summed. Totals do not add precisely due to rounding during data compilation.

	A. tigrinum		A. maculatum			
Stitch groups	$\bar{x}$	SE	$\bar{x}$	SE	t	P
Nasal	35.2	2.0	36.4	1.8	0.41	0.68
Maxillary	23.0	1.0	22.2	0.8	0.57	0.57
Supraorbital	23.4	0.6	19.8	0.8	3.52	0.01*
Postorbital	15.2	0.4	9.0	0.4	3.91	0.01*
Infraorbital	9.1	0.4	8.4	0.4	0.95	0.35
Angular	12.4	0.6	10.0	0.4	3.28	0.01*
Parietal	41.0	1.4	31.4	1.8	4.00	0.01*
Submandibular	56.7	1.3	46.2	1.4	4.89	0.01*
Oral	41.4	1.4	35.4	1.6	2.81	0.01*
Postotic	27.8	1.6	20.6	1.8	2.90	0.01*
Total	284.1	6.7	242.1	7.0	3.64	0.01*

A. maculatum neuromasts per stitch = 0.2 + 0.1 SVL (r^2 = 0.82; p > f < 0.001). Same-sized larvae of both species have similar numbers of neuromasts per stitch. Cannibal morphs averaged about seven neuromasts per stitch. Total numbers of neuromasts increased from 258 to 690 in A. maculatum and from 283 to 2168 in A. tigrinum, including cannibal morphs.

Despite increases in neuromast number, neuromast density decreases with growth from 12.1 to 2.4 neuromasts per mm^2 in A. tigrinum, and from 13.3 to 4.8 neuromasts per mm^2 in A. maculatum. The regressions for neuromast density vs. SVL are: neuromast density = 12.1 - 0.2 SVL for A. tigrinum and neuromast density = 16.7 - 0.6 SVL for A. maculatum. Same-sized heterospecific larvae have similar neuromast densities. Cannibal morphs had the lowests neuromast densities (2.4 neuromasts per mm^2).

DISCUSSION

Neuromast stitch number remains constant with growth and distinguishes larval populations of Ambystoma tigrinum from those of Ambystoma maculatum. It appears, therefore, that stitch number may be a useful taxonomic character for distinguishing other closely-related amphibian species. Stitch number is not useful for distinguishing cannibal morph A. tigrinum from typical

animals.

Neuromasts per stitch and neuromast density change with growth and are not useful taxonomic characters. Unexpectedly, while neuromasts per stitch increase with growth, neuromast density decreases. Cannibal morphs, being the largest members of a population, usually have the most neuromasts per stitch and the lowest neuromast densities. Cannibal morphs do not differ from typical morphs of the same size in these neuromast parameters.

Neuromast number affects the mechanosensory ability of the neuromast system as a whole; the more neuromasts an animal has, the greater will be its ability to perceive water displacements. However, there is no evidence that A. tigrinum has better mechanosensory perception than A. maculatum. Indeed, it is difficult to assess what the interspecific difference in average total stitches of 14.3% (240 vs. 280 stitches) means, given that there can be at least an 11.5% difference between left and right sides of the same animal. Görner et al. (1984) have shown that Xenopus adults with all but two (out of approximately 100) stitches ablated on either side still orient towards a stimulus, although their precision is greatly reduced. The large left-right within-individual variance in stitch number certainly argues against finely-tuned functional differences in this neuromast parameter.

Neuromast orientation affects mechanosensory ability (Flock, 1967). In Ambystoma, some neuromast groups are composed of parallel stitches, while in other groups, adjacent stitches are perpendicular. Because neuromasts are directionally sensitive, a perpendicular stitch arrangement enables those animals with only two stitches to be sensitive to stimuli through 360^{o}. A series of these perpendicular stitch couplets implies high discriminatory ability. Three of the four groups containing perpendicular stitches (nasal, maxillary, and submandibular) are near the snout and are presumably involved in prey detection. Both A. tigrinum and A. maculatum feed on many of the same prey species (Branch and Altig, 1981). There are no significant interspecific differences in nasal and maxillary stitch number dorsally, and ventrally, there are no differences in perpendicular stitch number as measured by lateral submandibular stitches (Fig. 2-1; $\bar{x}$ = 18.6, s.e. = 0.6, A. tigrinum; $\bar{x}$ = 17.6, s.e. = 0.8, A. maculatum; $p > t = 0.30$).

Typical A. tigrinum morphs tend to be microphagic feeding predominantly on zooplankton -- while cannibal morphs tend to be macrophagic (Collins and Holomuzki, 1984; Lannoo and Bachmann, 1984a). I predicted that the gross changes in cannibal head morphology (i.e., broadening of the head and enhanced vomerine teeth

development) may correlate with concomitant changes in sensory input mediated by neuromast topography. This prediction, however, was not supported. Cannibal morphs develop from typical-looking larvae; they retain typical morph neuromast topographies.

With the exception of the Ambystoma data presented here, quantitative aspects of amphibian neuromast topography have been considered only for Xenopus laevis (Shelton, 1970). Neuromast topography may be of systematic use in amphibians, as it has been for fishes. Because neuromasts are polarized, some functional information may be also derived from topography. However, because the precise role of neuromasts in the behavioral ecology of an animal is yet unknown, it is difficult at this time to assess the functional significance of topography and topographical differences.

Chapter 3: Neuromast Topography in Urodele Amphibians

INTRODUCTION

With few exceptions, fishes and aquatic amphibians possess mechanoreceptive neuromast organs, which either singly or in combination with electroreceptors comprise their lateral line system. The function of neuromasts is to detect environmental water displacements in the range of a few to a few hundred Hertz, such as those produced by predators, prey, or conspecifics (Harris and van Bergeijk, 1962; Russell, 1976).

Neuromasts course across the body surface and are directionally sensitive (e.g., Flock, 1965). Therefore, their arrangement, or topography, is crucial to the functioning of the mechanoreceptive system: an animal's neuromast topography in combination with the number and sensitivity of its neuromasts determines its ability to detect and distinguish water displacements.

Neuromast topography may also contain phylogenetic information. Chu and Wen (1979) used the lateral line system to reinterpret the systematics of Western Pacific elasmobranchs. In teleosts, Branson and Moore (1962) and Page (1977) have shown intrafamilial trends in the arrangement of neuromast bony canals in sunfish (Centrarchidae) and darters (Etheostomatini), respectively.

Despite the potential for functional and phylogenetic

information from neuromast topography, no studies have examined these structures from this perspective in amphibians. The overall purpose of the present study is to elucidate the functional and phylogenetic patterns of neuromast topography in aquatic urodeles. Before I list the more specific goals of this study, I will briefly describe how neuromasts are constructed in urodeles, and review what has been previously discovered about their topography.

Neuromast Organization and Arrangement in Amphibians

The amphibian neuromast system, like that of fishes, is organized into a hierarchy: hair cells form neuromasts; neuromasts, in turn, form lines or groups that course across the animal's body (e.g., Gorner, 1963; Flock, 1971).

Hair cells are directionally sensitive and polarized (oriented alternately in opposite directions, i.e., 0^{o}, 180^{o}, 0^{o}, along the same axis). This hair cell polarization imparts an axial sensitivity to neuromasts (e.g., Flock, 1971). The axis of maximum sensitivity of any particular neuromast can be inferred from the gross morphology of its sensory epithelium.

The sensory epithelium of a neuromast is usually oval; its axis of maximum sensitivity is parallel to the long axis of this oval (Flock and Wersall, 1962; Flock,

1971). The neuromast sensory epithelium reflects the shape of the neuromast cupula, which is also oval. The cupula is the structure that receives water displacements and transfers them to the hair cells. Because of their oval shape, cupulae are most sensitive to viscous drag forces, and therefore to water displacements, along their long axis (e.g., Harris and Milne, 1966).

In larval amphibians, unlike in fishes, neuromasts can form stitches. Stitch formation is a function of ontogeny; stitches are not present at hatching, they develop with growth (Lannoo, 1985). At hatching only one neuromast, termed the primary neuromast (Winklbauer and Hausen, 1983a), is present. Primary neuromasts are laid down embryonically by migrating, ectodermally derived placodes (e.g., Stone, 1933; Winklbauer and Hausen, 1983a; Northcutt and Gans, 1983).

During larval growth in some amphibians these primary neuromasts divide, usually along their long axes, to form secondary neuromasts. Secondary neuromasts are parallel to each other and together are called a stitch (Harris and Milne, 1966). With larval growth, at least in Xenopus and Ambystoma, more secondary neuromasts are added to the stitch and the stitch becomes longer (Winklbauer and Hausen, 1983a,b; Lannoo, 1985). Typically, the long axis of each stitch is oriented transverse to the long axes of its component neuromasts, and therefore transverse to the

stitches' axis of maximum sensitivity (JØrgensen and Flock, 1973; Flock and JØrgensen, 1974; Harris and Flock, 1967). In Necturus, however, Kingsbury (1895), Harris, et al. (1970), and Flock (1971) have observed that the stitch long axis may be oriented parallel to its component neuromasts. This potential difference in stitch formation has never been assessed either functionally or phylogenetically.

Neuromasts and stitches are arranged into lines that course across the animal's body. Three lines are present along each side of the trunk of most amphibians (e.g., Kingsbury, 1895; Wright, 1951; JØrgensen and Flock, 1973). Basically, three lines are also present on each side of the head: one along the mandible, one along the maxilla, and one dorsal medial to the eye and nostril. All three head lines meet behind the eye. (See Lannoo, 1985 and Chapter 2 for a photograph of this pattern in Ambystoma.)

The previous studies on urodele neuromast topography include Malbranc (1876), Kingsbury (1895), Escher (1925), Hilton (1947, 1950), Wickham (1972), Reno and Middleton (1973), and Lannoo (1985). In Table 3-1 I summarize the contributions of these authors by taxon. The list serves to illustrate which taxa were emphasized in these previous studies, and also elucidates familial trends. In particular, stitch formation is restricted to a few families and can be of two types: transverse and

Table 3-1. An annotated list of the species of urodeles that have published information available about their lateral line topography. Authors are numbered and footnoted. Information included under 'Comments' is based either on text descriptions or illustrations provided by original authors.

Species	Comments
Hynobiidae	
Batrachuperus pinchonii	Neuromasts present[1]
Ranodon sibiricus	Neuromasts present, Nasal neuromasts form single line[2]
Crytobranchidae	
Cryptobranchus alleganiensis	Neuromasts form transverse stitches[3]
	Neuromasts present[4]
	Neuromasts form stitches[1]
Andrias japonicus	Neuromasts present[3]
Ambystomatidae	
Ambystoma annulatum	Neuromasts form stitches[1]
A. gracile	Neuromasts on head[1]
A. jeffersonianum	Neuromasts not observed[1]
A. macrodactylum	Neuromasts form stitches[1]
	Neuromasts form stitches[5]

Table 3-1 (cont.)

A. maculatum	Neuromasts form transverse stitches[4]
	Neuromasts form stitches[1]
	Neuromasts form transverse stitches, fewer neuromasts present than in *A. tigrinum*[6]
A. mexicanum	Neuromasts form transverse stitches[3]
A. opacum	Neuromasts not observed[1]
A. talpoideum	Neuromasts not observed[1]
A. texanum	Neuromasts on head[1]
A. tigrinum	Neuromasts form stitches[1]
	Neuromasts form transverse stitches[6]
A. t. californiense	Neuromasts not observed[1]
Rhyacosiredon sp.	Neuromasts present[1]
Dicamptodontidae	
Rhyacotriton olympicus	Neuromasts present[1]
Dicamptodon ensatus	Neuromasts present[1]
	Neuromasts present, do not form stitches[5]
Plethodontidae	
Desmognathus fuscus	Neuromasts present[1]
D. quadramaculatus	Neuromasts present[1]
Eurycea (several species)	Neuromasts present[1]
Gyrinophilus porphyriticus	
	Neuromasts present[4]
	Neuromasts present[1]
Leurognathus marmoratus	Neuromasts present[1]

Table 3-1 (cont.)

Stereochilus marginatus	Neuromasts present[1]
	Neuromasts present[7]
Typhlomolge rathbuni	Neuromasts present[1]
Typhlotriton spelaeus	Neuromasts present[1]
Salamandridae	
Cynops pyrrhogaster	Neuromasts present[1]
Euproctus platycephalus	Neuromasts not observed[1]
Notophthalmus viridescens	Neuromasts form linear stitches[4]
	Neuromasts present[1]
Pachytriton breviceps	Neuromasts present[1]
Pleurodeles waltl	Neuromasts form linear stitches[8]
	Neuromasts present[1]
Salamandra sp.	Neuromasts present[3]
Salamandra atra	Neuromasts not observed[1]
Salamandra salamandra	Neuromasts not observed[1]
Triturus sp.	Neuromasts present[3]
Triturus alpestris	Neuromasts form linear stitches[8]
	Neuromasts present[1]
T. cristatus	Neuromasts present[1]
T. granulosus (?)	Neuromasts present[1]
T. klauberi (?)	Neuromasts not observed[1]
T. marmoratus	Neuromasts not observed[1]
T. torosus (?)	Neuromasts present[1]
T. vulgaris	Neuromasts present[1]

Table 3-1 (cont.)

Amphiumidae	
Amphiuma means	Neuromasts present[4]
Proteidae	
Necturus maculosus	Neuromasts form linear stitches[4]
	Neuromasts sunken into epidermis[1]
	Neuromasts form linear stitches[9]
Proteus anguinus	Neuromasts form linear stitches[3]
	Neuromasts located in grooves[1]
Sirenidae	
Pseudobranchus striatus	Neuromasts present[1]
Siren sp.	Neuromasts in epidermal grooves[1]
	Neuromasts form "fields"[10]

[1]Hilton (1947); [2]Schmalhausen (1968); [3]Malbranc (1876); [4]Kingsbury (1895); [5]Wickham (1972); [6]Lannoo (1985); [7]Hilton (1950); [8]Escher (1925); [9]Harris et al. (1970); [10] Reno and Middleton (1970).

longitudinal. From this review it appears that transverse stitches are restricted to the families Ambystomatidae and Cryptobranchidae, and that longitudinal stitches are present in the Proteidae and Salamandridae. These results should be accepted with caution, however, because: 1) all but Wickham (1972) and Lannoo (1985) used conventional light microscopy on intact specimens, making neuromast visualization difficult; 2) workers prior to Flock and Wersäll (1962) could not know neuromasts were sensitive to water displacements along one axis, and could therefore not determine the functional importance of differences in neuromast orientation; and 3) workers prior to Fritzsch (1981) did not know that urodeles have both neuromasts and ampullary organs -- structures which earlier workers confused (e.g., Kingsbury noted ampullary organs and called them developing neuromasts).

In a previous study, I photographed and described the neuromast topography of Ambystoma maculatum and A. tigrinum larvae using a trypsin, hydrogen peroxide, skinning technique and light microscopy (Lannoo, 1985; Chapter 2). I found that the pattern of neuromasts on the anterior dorsal surface of the head in these animals is characterized by orthogonally oriented neuromast or stitch couplets. From drawings in the literature, especially those of Malbranc (1876), Kingsbury (1895), and Escher (1925), there are hints that this pattern is common among

urodeles. In fact, Malbranc (1895) noted the tendency of neuromasts to occur in orthogonal couplets and speculated that if neuromasts were directionally mechanoreceptive this arrangement would be advantageous. In addition to having functional importance, orthogonal couplets could have systematic value; they are not known to exist in other amphibians or fishes.

Specific Goals

The goals of the present paper are to: 1) describe the basic neuromast topographical pattern on the dorsal surface of the head in urodeles; 2) describe variations in this basic pattern among urodele taxa; and 3) correlate these variations with ontogenetic, phylogenetic, and ecological factors. The topographical parameters that I examine are neuromast number and relative orientation, neuromast sensory epithelial surface area, hair cell numbers per neuromast, hair cell sizes, stitch formation, and the position of the neuromast sensory epithelium relative to the epidermal surface.

In terms of general neuromast and stitch trends, I confirm the the findings of the previous workers using scanning electron microscopy (SEM), and extend these observations to new species. Additionally, I quantify neuromast, stitch, and hair cell parameters; something the early workers did not or, for technical reasons, could not

do. The ultimate goal of this study is to achieve a deeper understanding of the functional significance of the neuromast system, and its avenues of evolutionary response.

MATERIALS AND METHODS

I sampled salamander larvae from all nine extant urodele families and attempted to obtain more than one individual per species -- preferably of a different size (age) -- and where applicable, more than one species per genus, and more than one genus per family. Table 3-2 lists species and sizes of specimens that I sampled. In some cases I specifically tried to obtain species already examined by other workers, to confirm their conclusions.

I obtained specimens either by capturing animals alive and preserving them in 10% buffered formalin, or from private collections (see ACKNOWLEDGMENTS), museum collections, and biological supply companies. Generally, formalin-fixed and stored animals were preferable for the following preparations because they did not slough skin to the same degree that alcoholic specimens did.

Specimens were viewed with a Cambridge S 150 scanning electron microscope (SEM). Formalin-fixed specimens were prepared for SEM viewing by dehydrating them in a graded ethanol series (70%, 90%, 95%, 100%, 100% dry) with 20 min

Table 3-2. A listing of the specimens examined in the present study and their snout vent lengths (SVL, to the nearest 0.5 mm). Also listed are the sizes of individual neuromast sensory epithelia, the numbers of hair cells per neuromast, and the range of diameters of individual hair cells in these specimens. Sizes are in microns (μm), areas are in square microns (μm^2).

		Neuromast			Hair Cell	
Species	SVL	Number	Size	Area	Number	Size
Hynobiidae						
Hynobius nebulosus #1	14.5	90	7x17	119	15.1	2-2.5
#2	16.0	100	7x20	140	15.1	3.0
#3	18.0	92	7x18	126	15.0	2.0
Cryptobranchidae						
Cryptobranchus alleganiensis	84.0	-	36x66	2376	-	-
Andrias davidianus	155.0	-	27x53	1431	-	-
Ambystomatidae						
Ambystoma laterale	10.5	-	4x19	76	13.8	2.0
A. maculatum #1	8.0	105	4x12	48	11.0	1.5-2.5
#2	11.0	119	4x16	64	14.6	1-2.0
#3	24.0	87	4x14	56	12.1	1-2.0

Table 3-2 (cont.)

A. mexicanum	#1	30.0	112	7x26	182	20.4	1.5-3.0
	#2	60.0	106	6x27	162	21.5	1-3.0
A. tigrinum	#1	14.0	-	5x19	95	11.0	2-3.0
	#2	28.5	116	6x31	186	15.7	2-3.0
	#3	60.0	108	5x36	180	17.0	1.5-2.5
Dicamptodontidae							
Dicamptodon ensatus		70.0	134	13x41	533	33.0	1.5
Rhyacotriton olympicus	#1	29.0	-	-	-	-	-
	#2	34.0	-	-	-	-	-
Plethodontidae							
Eurycea bislineata	#1	28.0	122	5x13	65	11.5	1.5
	#2	31.5	120	9x24	216	9.4	1-1.5
Gyrinophilus porphyriticus	#1	47.5	117	9x18	162	16.0	1-2.0
	#2	56.0	124	7x20	140	15.9	1-2.0
Haideotriton wallacei		22.0	-	-	-	-	-
Pseudotriton montanus		32.0	-	13x33	416	-	1-1.5
P. ruber	#1	26.0	130	9x24	216	22.6	1-2.5
	#2	50.5	148	8x18	144	17.0	1-2.5
Typhlotriton spelaeus	#1	29.5	154	9x24	216	-	1-2.5
	#2	39.0	116	16x25	400	16.2	1-2.0

Table 3-2 (cont.)

Salamandridae							
Notophthalmus							
viridescens	#1	6.5	119	3x7	21	8.2	1.0
	#2	17.5	126	5x12	60	11.2	1-1.5
	#3	19.0	123	5x15	75	11.5	1-1.5
Amphiumidae							
Amphiuma means		430.0	120	56x91	5096	-	-
Proteidae							
Necturus maculosus		210.0	150	19x36	684	-	-
Sirenidae							
Pseudobranchus							
striatus		61.5	-	-	-	-	-
Siren intermedia		250.0		11x27	297	-	-

at each step, drying them in a Sorvall critical point drier, and sputter coating them with gold. This procedure differs from standardly-employed methods for preparing amphibian epithelium for SEM viewing (e.g., Wickham, 1972; Wassersug and Rosenberg, 1979) by eliminating the osmium fixation step. I found that neuromasts of osmium-fixed specimens retained their cupulae, and that these cupulae collapsed during specimen dehydration and drying, covering the neuromasts and hair cells, and preventing the visualization of these structures (see also Wickham, 1972). By not using osmium, specimens were undoubtedly more susceptible to drying artifact. While this artifact could have affected my measurements of neuromast sensory epithelial area and hair cell size, neuromast and hair cell counts were not affected.

Other specimens were viewed using a dissecting microscope (10-40x) undissected, or dissected using the trypsin-hydrogen peroxide, skinning technique of Lannoo (1985; Chapter 2). Light microscopy was most useful for counting neuromasts and, where stitches were present, determining stitch orientation.

I chose to focus this study on dorsal cephalic neuromasts. The previous workers showed that the amphibian neuromast system is relatively simple and generalized everywhere except on the dorsal cephalic surface, which contains numerous neuromasts in a complex

pattern. Lannoo (1985; Chapter 2) dissected out this pattern for Ambystoma and found that on each side of the head the pattern was basically a "U" shape with the closed portion arranged around the eye and the open portion directed anteriorly along the snout.

I quantified the number of neuromasts per group, numbers of hair cells per neuromast, the sizes of neuromasts and hair cells, and if stitches were present the number of neuromasts per stitch. I also noted the orientation of neuromasts, and any peculiarities such as position of the neuromast sensory epithelium with reference to the epidermal surface, and the correspondence of pigmentless patches of skin with neuromasts. Every neuromast visible on the SEM preparations was measured and its hair cells counted; averages given for neuromast parameters are based on counts ranging from a dozen to hundreds of neuromasts observed per specimen.

Unfortunately, not all information could be gathered for every individual and taxon. In particular complete neuromast counts were often impossible to obtain for certain specimens because of damage during preservation or drying.

RESULTS

All urodeles that I examined had neuromasts and

presumptive ampullary organs present. Here I present the results of my neuromast data.

Neuromast parameters

All aquatic urodeles examined exhibited a pattern of neuromast organization on the dorsal surface of their heads similar to that described for Ambystoma (Figs. 3-1, 3-2). Neuromasts on the head were divided into supraorbital, infraorbital, nasal, and maxillary groups on the basis of their position and number of neuromast rows. Supraorbital neuromasts were located dorsal and medial to the eyes. Infraorbital neuromasts were located posterior and ventral to the eyes. The nasal group was an anterior extension of the supraorbital group. The maxillary group was an anterior extension of the infraorbital group.

Across urodele taxa, neuromasts in the same location on the head were oriented in the same direction (Figs. 3-1, 3-2). Examples of infraorbital neuromasts of species in four urodele families are given in Fig. 3-3; note their oval or rectangular shapes. Individual supra- and infraorbital neuromasts were oriented with their long axes tangential to the eye (Fig. 3-2a), and were therefore sensitive to water displacements along these axes (Fig. 3-2b). In some species an accessory supraorbital group was located medial to, and towards the posterior end of, the supraorbital group. Neuromasts in this accessory

Figure 3-1. SEM micrographs of whole heads of aquatic larvae from four urodele families illustrating neuromasts. A). Hynobius nebulosus (Hynobiidae), B) Ambystoma laterale (Ambystomatidae), C) Eurycea bislineata (Plethodontidae), D) Notophthalmus viridescens (Salamandridae). Each micrograph illustrates the left side of the head; the nostril is in the lower left corner, the eye is in the oval structure in the upper center or right. In each micrograph arrows indicate single neuromasts although other neuromasts are visible. Neuromast group abbreviations: m = maxillary, i = infraorbital, s = supraorbital, n = nasal. At this low magnification neuromasts can be difficult to visualize. Neuromasts are most easily visualized when they are either raised above the epidermal surface (B, D) or sunken below the epidermal surface (C). Scale lines = 500 μm.

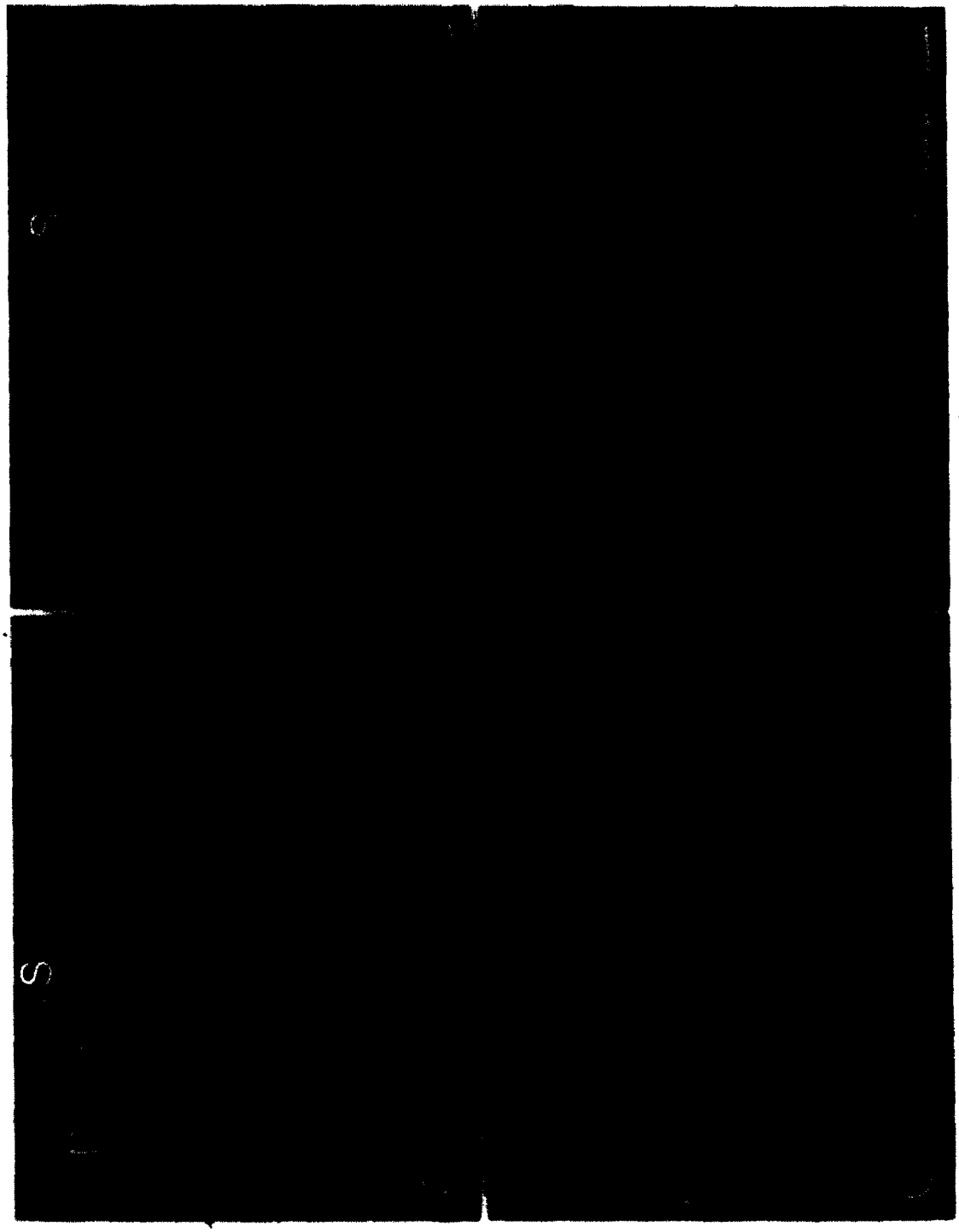
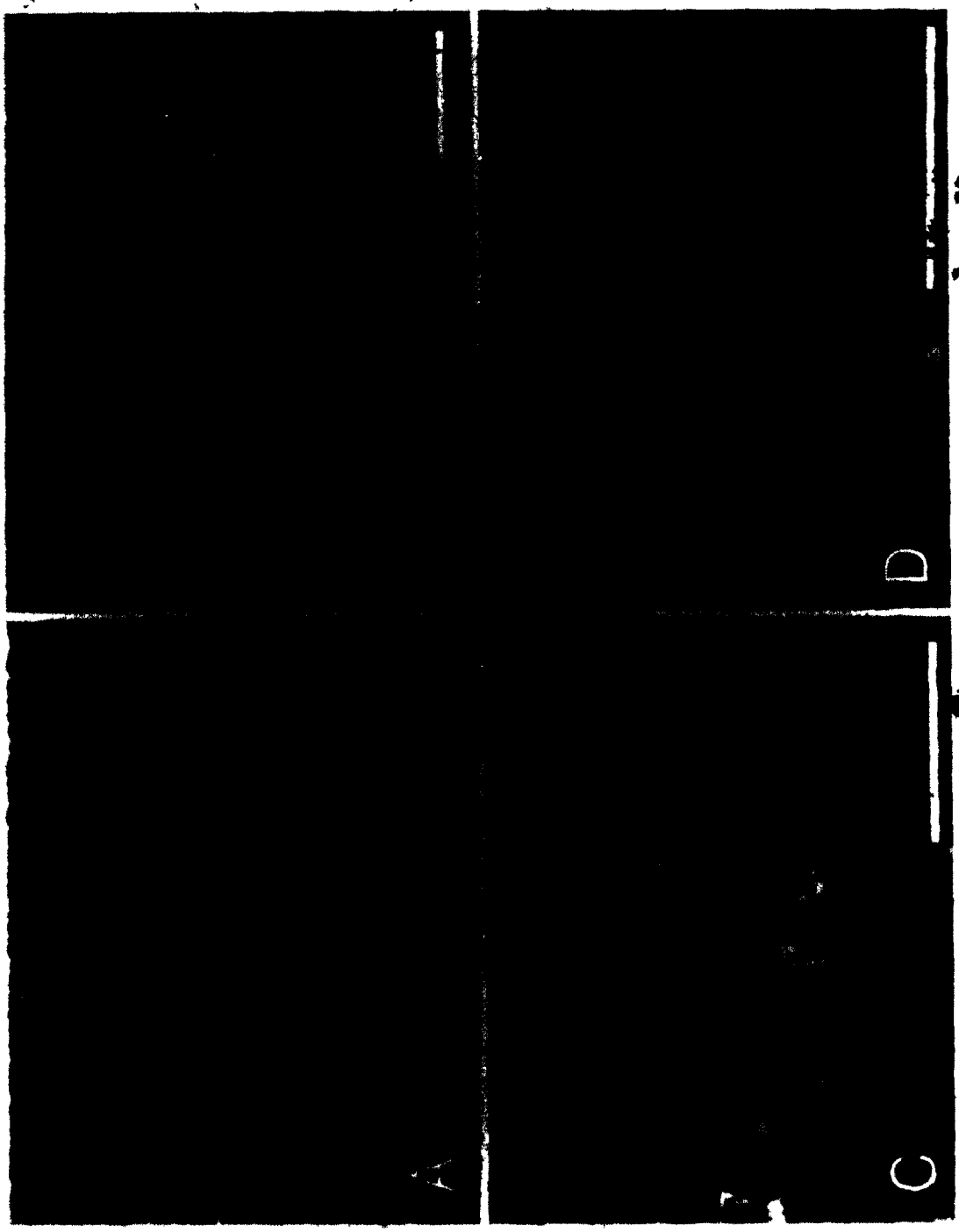


Figure 3-2. Schematic drawings of the heads of aquatic urodeles illustrating the three neuromast patterns found in these animals (A, C, D) and the direction of neuromast maximum sensitivity (B), which is the same no matter which neuromast pattern is present. Neuromasts are illustrated as ovals, with a long and a short axis, which correspond to their appearance in life. Figure A is drawn to a 30% smaller scale to show the entire head. A) Primary neuromasts: this condition is present in posthatching larvae in all families, and older larvae in the Hynobiidae, Dicamptodontidae, Plethodontidae and Amphiumidae. B) Arrows indicate the direction of maximum sensitivity of neuromasts in all urodeles; note in particular the sensitivity of circumorbital neuromasts and that nasal and maxillary neuromasts are sensitive to water displacements from all directions. C) Transverse stitches: note that the long axis of the stitch is perpendicular to the long axes of its component neuromasts. This method of stitch formation is characteristic of Ambystomatidae and perhaps the Cryptobranchidae. D) Longitudinal stitches: note that the stitch long axis is parallel to the long axes of its component neuromasts. This method of stitch formation is characteristic of the Proteidae and the Salamandridae.

B
D
A
C

Figure 3-3. SEM micrographs showing the sensory epithelium of infraorbital neuromasts in the four specimens pictured in Figure 1. A) Hynobius nebulosus; B) Ambystoma laterale; C) Eurycea bislineata; D) Notophthalmus viridescens. Hair cells or their aggregate cilia show up as lighter, round structures. Note the linear nature of the neuromast in each species, although this is not as apparent in Eurycea bislineata (C). The axis of maximum neuromast sensitivity is parallel to the neuromast long axis. Scale lines = 10 µm.


D
C

group were always parallel to the supraorbital neuromasts.

Nasal neuromasts usually occurred in two rows (Fig. 3-1, 3-2). Individual nasal neuromasts had their long axes oriented oblique to the body axis of the salamander; neuromasts in one row were oriented perpendicular to adjacent neuromasts in the second row (Fig. 3-2a; Figs. 3-4, 3-5 and 3-6 in combination show orthogonal neuromasts in the nasal groups of species in all urodele families). These orthogonal neuromast couplets are theoretically sensitive to water displacements coming from all directions in a plane across the animal's body surface (Fig. 3-2b).

Maxillary neuromasts usually occurred in three rows (Fig. 3-1, 3-2). Neuromasts in the middle maxillary row were oriented as if they were an anterior extension of the infraorbital group (Fig. 3-2). Anterior portions of neuromasts in the lateral and medial rows of the maxillary group were rotated away from the middle row about 45^{0}, making neuromasts in the lateral and medial maxillary rows perpendicular to each other (Fig. 3-2a). Taken together, maxillary neuromasts, like nasal neuromasts, should be sensitive to water displacements in all directions along a plane across the body surface (Fig. 3-2b). Variations in nasal and maxillary group row numbers occurred, but tended to be the result of one or a few aberrant neuromasts and did not appear to be consistent within a

Figure 3-4. SEM micrographs showing orthogonal neuromast couplets in the nasal lines of aquatic urodeles. A) Hynobius nebulosus; B) Cryptobranchus alligheniensis; C) Eurycea bislineata; D) Notophthalmus viridescens; E) Amphiuma means; F) Siren intermedia. Light bars indicate the neuromast long axis, and the axis of neuromast sensitivity. In each micrograph anterior is to the left. Scale line = 100 μm.

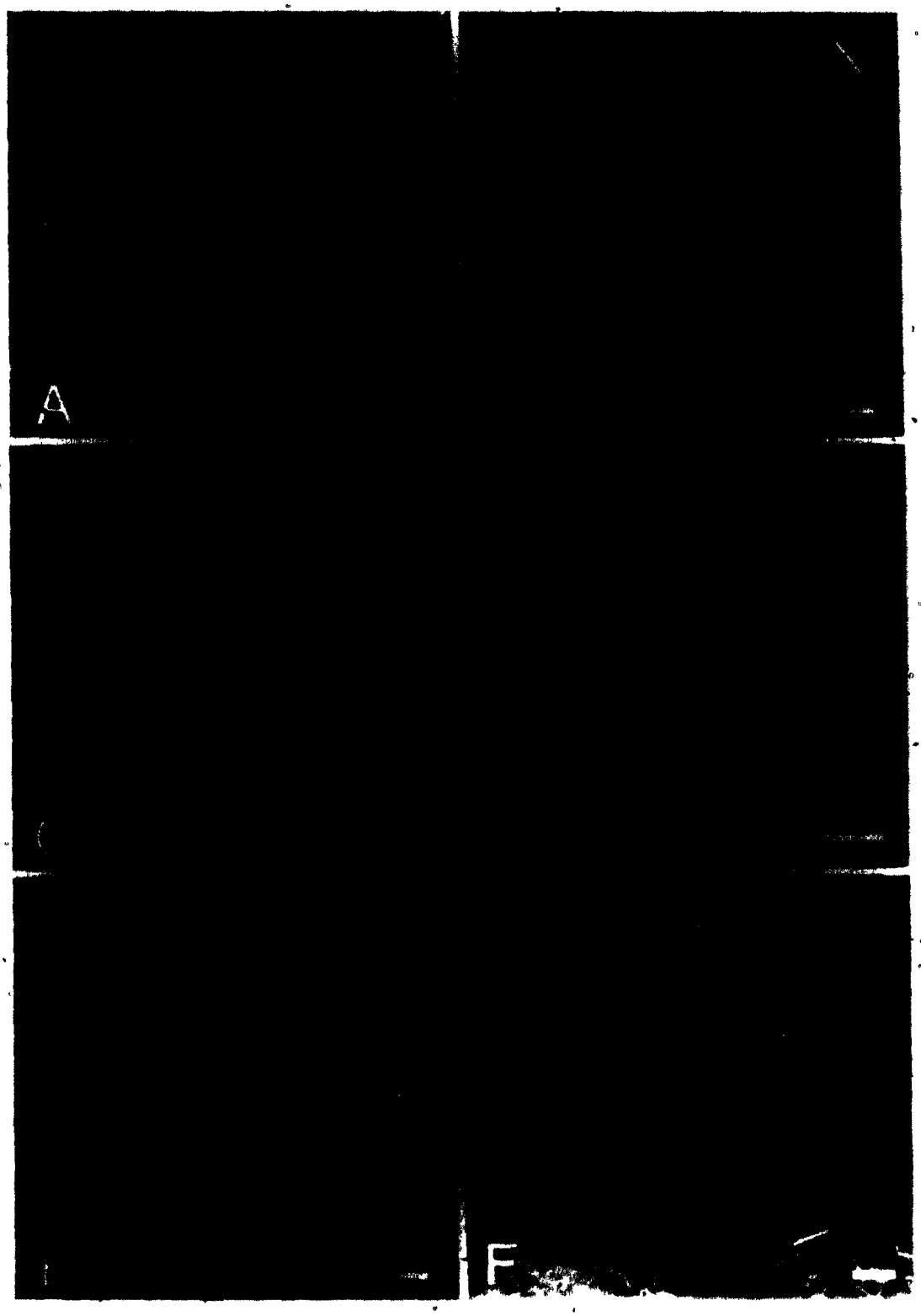

A
F

Figure 3-5. SEM micrographs of neuromast features in Dicamptodon ensatus. A) A view of the left side of the head; the nostril is located in the lower left corner, the eye is the round structure in the upper right. Neuromasts are located in epidermal grooves, several of which have been noted with arrows. Scale line = 1 mm. B) Close-up of a single neuromast located in one of the infraorbital grooves. Note its linear organization, and that a part of the cupula has remained attached. Scale line = 100 μm.

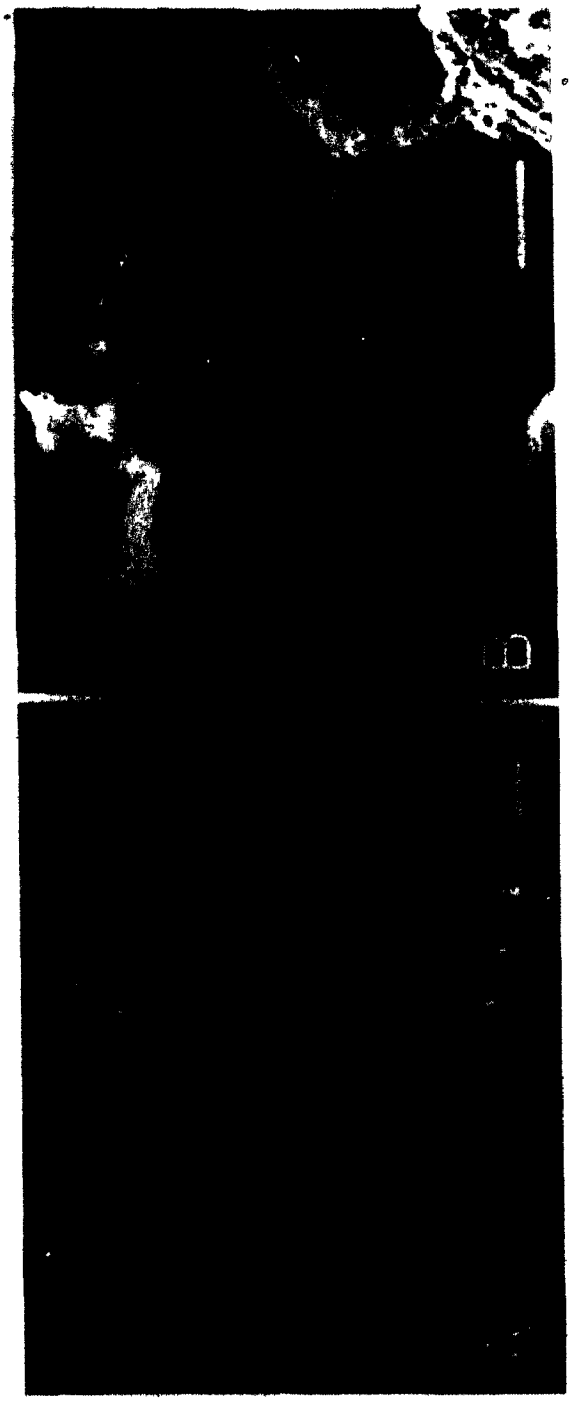
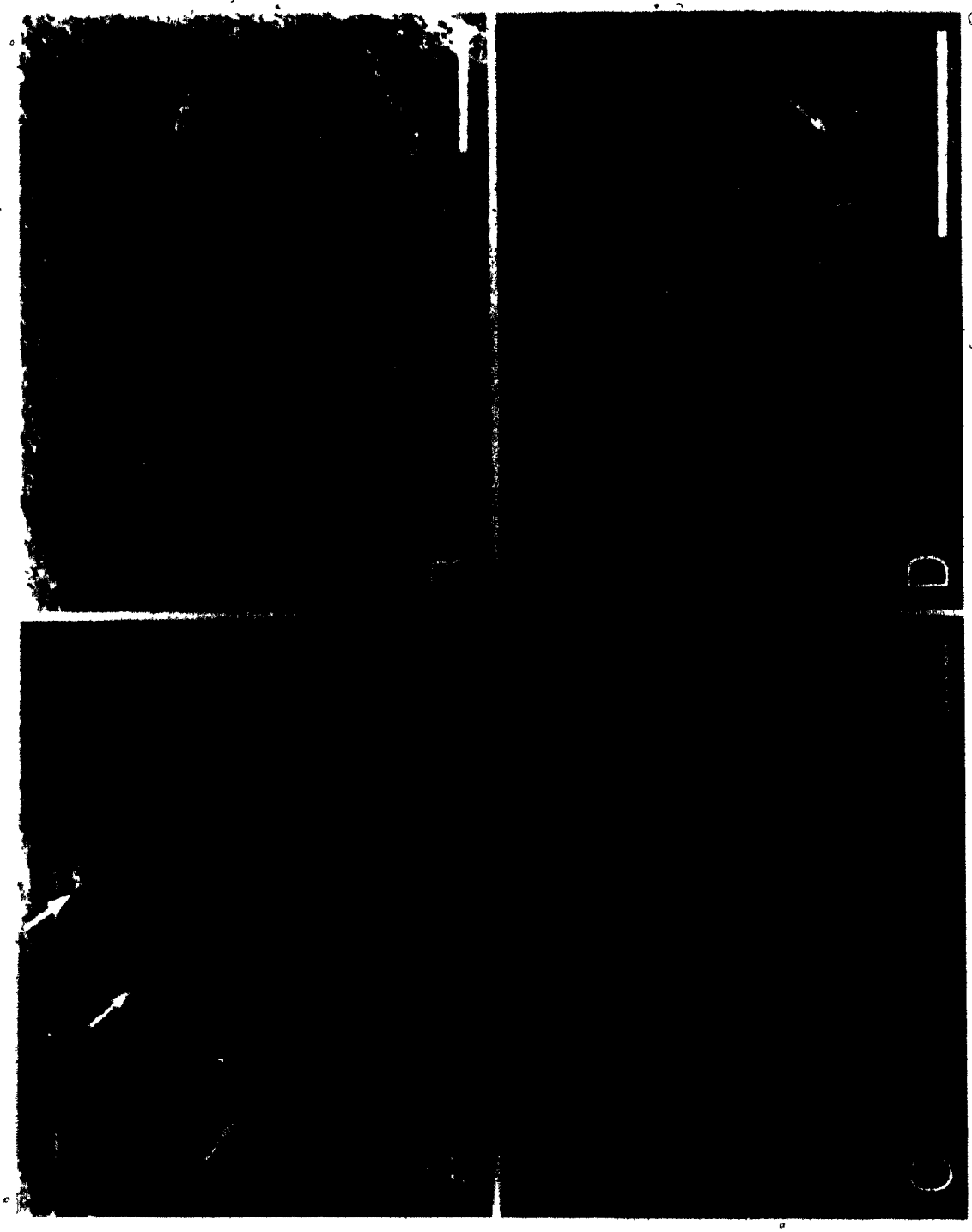


Figure 3-6. SEM micrographs illustrating differences in stitch orientation in Ambystoma and Necturus. A) and B) Several stitches in the nasal group of Ambystoma and Necturus, respectively. In each micrograph anterior is to the left. Note that in Ambystoma neuromasts are located flush with or slightly raised above the epidermis, while in Necturus neuromasts are sunken into epidermal grooves. Note in particular that in Ambystoma, two stitches form a "y" that faces anteriorly, while in Necturus stitches form a "v" that faces posteriorly. Homologous neuromasts in both species face approximately the same direction. It may be useful to compare these micrographs to Fig. 3-2c and Fig. 3-2d. Scale lines in A, B = 300 μm. C) and D) Close ups of individual stitches in Ambystoma and Necturus, respectively. Neuromasts in Necturus are noted with arrows. Scale lines in C, D = 100 μm.



species.

Numbers of neuromasts, considered either by group or in total, varied among taxa, within species, and even between sides of the same individual. Taxonomic variation in neuromast numbers is illustrated in Tables 3-2 and 3-3. Total numbers of dorsal head neuromasts ranged from a mean of 94 in Hynobius nebulosus to 150 in Necturus maculosus. Plethodontids tended to have more primary neuromasts than did the other families (Tables 3-2, 3-3).

Neuromast number did not vary with SVL of the animal ($p > 0.25$). An example of the intraspecific and intra-individual variation in neuromast numbers is given in Table 4 for Hynobius nebulosus. Despite this intraspecific variation, neuromast counts fall within a narrow enough range for many species to allow unidentified individuals to be assigned to a species based on this character alone (Table 3-3).

The sensory epithelium of neuromasts was positioned flush with or raised slightly above the epidermal surface, or sunken into the epidermis in pits or grooves in a species specific way. In Hynobius, the cryptobranchids, the ambystomatids, Notophthalmus, Amphiuma, and the sirenids the sensory epithelium was flush with the epidermal surface (Figs. 3-1, 3-5, 3-6). In Dicamptodon, single neuromasts were sunken into grooves (Fig. 3-4). In the plethodontids, neuromasts were sunken into pits;

Table 3-3. A listing of the mean numbers of neuromasts on the dorsal surface of the head of urodeles. Neuromasts are divided by group. SO = supraorbital, IO = infraorbital; MAX = maxillary; NAS = nasal. Also given are numbers of circumorbital (CIRCUM) neuromasts (SO + IO neuromasts), numbers of anterior (ANT) neuromasts (MAX + NAS neuromasts), total numbers of neuromasts, and the ratio of ANT to CIRCUM neuromasts (A/C).

Species	SO	IO	(CIRCUM)	MAX	NAS	(ANT)	Total	A/C
Hynobiidae								
Hynobius nebolosus	17.0	25.6	(42.6)	22.3	29.3	(51.6)	94.2	1.2
Ambystomatidae								
Ambystoma lateŕale	18.0	30.0	(48.0)	-	-	-	-	-
A. maculatum	19.7	16.3	(36.0)	28.7	39.0	(67.7)	103.7	1.9
A. mexicanum	16.0	19.0	(35.0)	22.0	52.0	(74.0)	109.0	2.1
A. tigrinum	18.0	30.0	(48.0)	24.0	40.0	(64.0)	112.0	1.3
Dicamptodontidae								
Dicamptodon ensatus	16.0	24.0	(40.0)	36.0	58.0	(94.0)	134.0	2.4
Plethodontidae								
Eurycea bislineata	18.5	24.0	(42.5)	36.5	42.0	(78.5)	121.0	1.8
Gyrinophilus porphyriticus	13.5	15.0	(28.5)	46.0	46.0	(92.0)	120.5	3.2
Pseudotriton ruber	17.0	15.0	(32.0)	55.0	52.0	(107.0)	139.0	3.3

Table 3-3 (cont.)

Typhlotriton spelaeus	15.5	9.5	(25.0)	55.0	55.0	(110.0)	135	0 4.4
Salamandridae								
Notophthalmus viridescens	18.3	28.0	(46.3)	32.7	43.7	(76.4)	122.7	1.7
Amphiumidae								
Amphiuma means	12.0	14.0	(26.0)	66 0	28.0	(94.0)	120.0	3.6
Proteidae								
Necturus maculosus	12.0	26.0	(38.0)	66.0	46.0	(112.0)	150.0	2.9

Table3-4. A list of the numbers of neuromasts per group on the heads of three Hynobius nebulosus larvae. Ipsilateral values are given (left and right sides). Group abbreviations: SO = supraorbital; IO = infraorbital; MAX = maxillary: NAS = nasal. Note both intraspecific and intraindividual variation.

SVL		SO	IO	MAX	NAS	TOTAL
14.5						
	Left	9	13	11	12	45
	Right	9	-	-	12	-
16.0						
	Left	7	15	12	15	49
	Right	9	14	13	15	51
18.0						
	Left	7	11	10	18	46
	Right	10	-	10	16	-

Figure 3-7 illustrates these pits and their variation in depth between plethodontid species. Necturus neuromasts formed stitches that were located in epidermal grooves (Fig. 3-6).

There were correlations between neuromast number, neuromast position with reference to the epidermal surface, and the habitat of a species; in general lotic (flowing water) forms had more primary neuromasts, a higher proportion of anterior neuromasts, and had neuromasts that were sunken into the epidermis (Tables 3-3 and 3-5).

In aquatic plethodontids pigmentless patches of skin corresponded to neuromasts.

Lentic forms, such as Ambystoma and Notophthalmus had patches of epidermal cilia located between neuromast lines (Fig. 3-1b, d). These cilia were never present between neuromasts within a line; cilia were never present on stream species.

Hair cell parameters

In this study the surface area of the sensory epithelium of a single neuromast ranged from 21 μm^2 in Notophthalmus to 5096 μm^2 in Amphiuma (Table 3-2). Variation in neuromast size was accounted for by changes in hair cell number [(Neuromast size in μm^2 = -110.0 + 15.8 Hair Cell #) r^2 = 59.4; $p < 0.001$] rather than hair

Figure 3-7. SEM micrographs illustrating variation in the depth of neuromast pits. A) Hynobius nebulosus, B) Eurycea bislineata, C) Gyrinophilus porphyriticus, D) Pseudotriton ruber. Each micrograph shows the dorsal surface of the head, anterior is down. Scale lines = 1 mm.

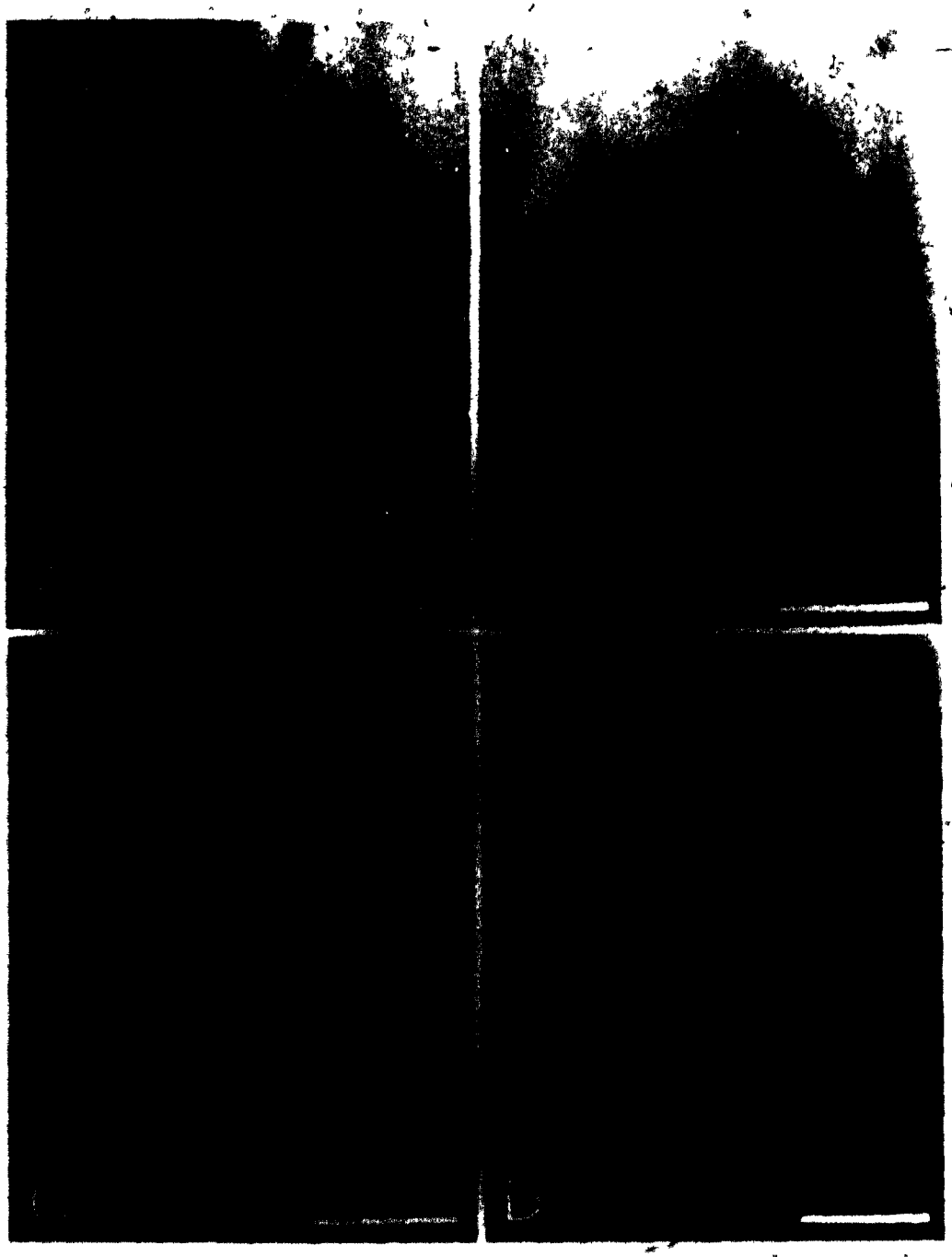


Table 3-5. The correspondence between neuromast features and habitat parameters for the species considered here. In particular, note the close association between stitch formation and lentic (still water) habitats, and the association of neuromasts located in pits or grooves with lotic (flowing water) habitats. Animals that live in lotic habitats tended to have more than 120 neuromasts and anterior:circumorbital neuromast ratios > 2. Habitat information for most species was obtained from Stebbins (1966) or Conant (1975). Habitat information for Hynobius nebulosus was obtained from Kusano (1985); for Ambystoma mexicanum from Shaffer (1984). Species are sorted by habitat and by family within habitat.

SPECIES	HABITAT		NEUROMAST FEATURES			
	Lentic	Lotic	Stitches	Pits/Grooves	#>120	A/C>2
Hynobius nebulosus	X					
Ambystoma laterale	X		X			
Ambystoma maculatum	X		X			
Ambystoma mexicanum	X		X			X
Ambystoma tigrinum	X		X			
Notophthalmus viridescens	X					
Necturus maculosus	X	X	X	X	X	X
Cryptobranchus alleganiensis		X				
Dicamptodon ensatus		X		X	X	X
Eurycea bislineata		X		X		
Gyrinophilus porphyriticus		X		X	X	X
Pseudotriton montanus		X		X	?	?
Pseudotriton ruber		X		X	X	X
Typhlotriton spelaeus		X		X	X	X
Amphiuma means		X			X	X

cell size ($p > 0.25$). Neuromast size increased with SVL across taxa [(Neuromast size in μm^2 = -94.7 + 8.96 SVL) r^2 = 65.8; $p < 0.001$]. Consequently, hair cell number also increased with SVL across taxa (Figure 3-8). This was true even when I eliminated the highest value (for Dicamptodon) and the lowest value (for Notophthalmus; Fig. 3-8).

Within a species there appeared to be no increase in numbers of hair cells with size (Table 3-2). There also appeared to be no increase in numbers of primary neuromasts, or hair cell size, with growth (Table 3-2).

Stitch formation

Among the specimens that I examined stitch formation was limited to Ambystoma and Necturus. In Ambystoma, numbers of secondary neuromasts, and therefore numbers of neuromasts per stitch, increased with growth (Fig. 3-9). The rate of secondary neuromast formation varied by species, with A. laterale apparently having the highest rate, A. tigrinum and A. maculatum having intermediate rates, and A. mexicanum having the lowest rate (Fig. 3-9). I could not determine the rate of neuromast addition in Necturus stitches, although the animal I examined had a mean of four neuromasts per stitch with a range of three to five.

Two types of stitches were present, those with their long axis oriented transverse to the long axis of their

Figure 3-8. A plot showing the increase in hair cell numbers per neuromast with snout vent length across taxa in larval urodeles. The line is a least squares regression fit to the data. The equation of the line is: Hair cell # = 12.0 + 0.103 SVL; r^2 = 23.7; $p < 0.005$. The regression remains significant ($p < 0.05$) when the two extreme values for Dicamptodon and Notophthalmus are removed. Abbreviations: Al = Ambystoma laterale; Ama = Ambystoma maculatum ; Ame = Ambystoma mexicanum; At = Ambystoma tigrinum; D = Dicamptodon ensatus; E = Eurycea bislineata; G = Gyrinophilus porphyriticus; H = Hynobius nebulosus; N = Notophthalmus viridescens; P = Pseudotriton ruber; T = Typhlotriton spelaeus.

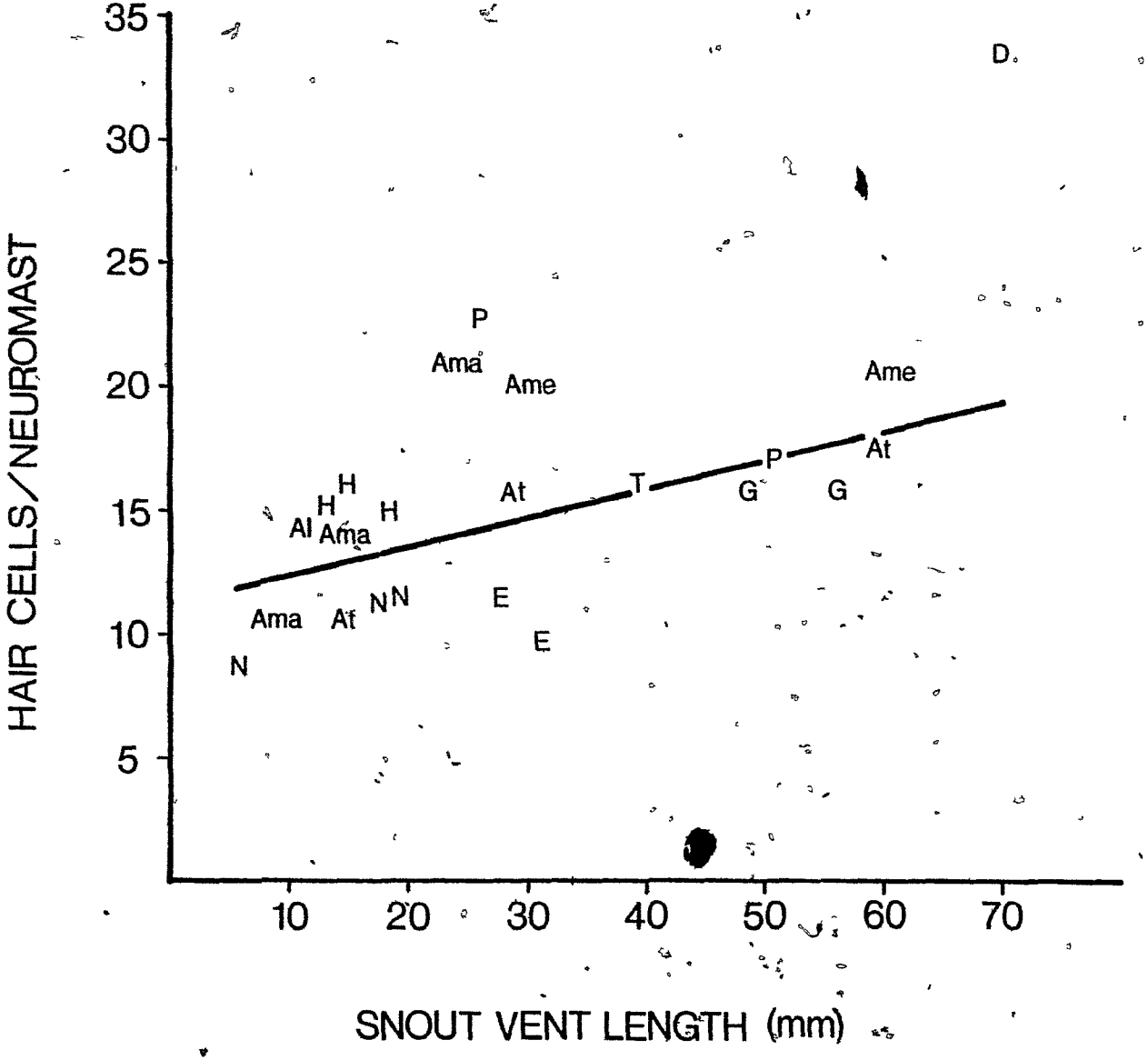
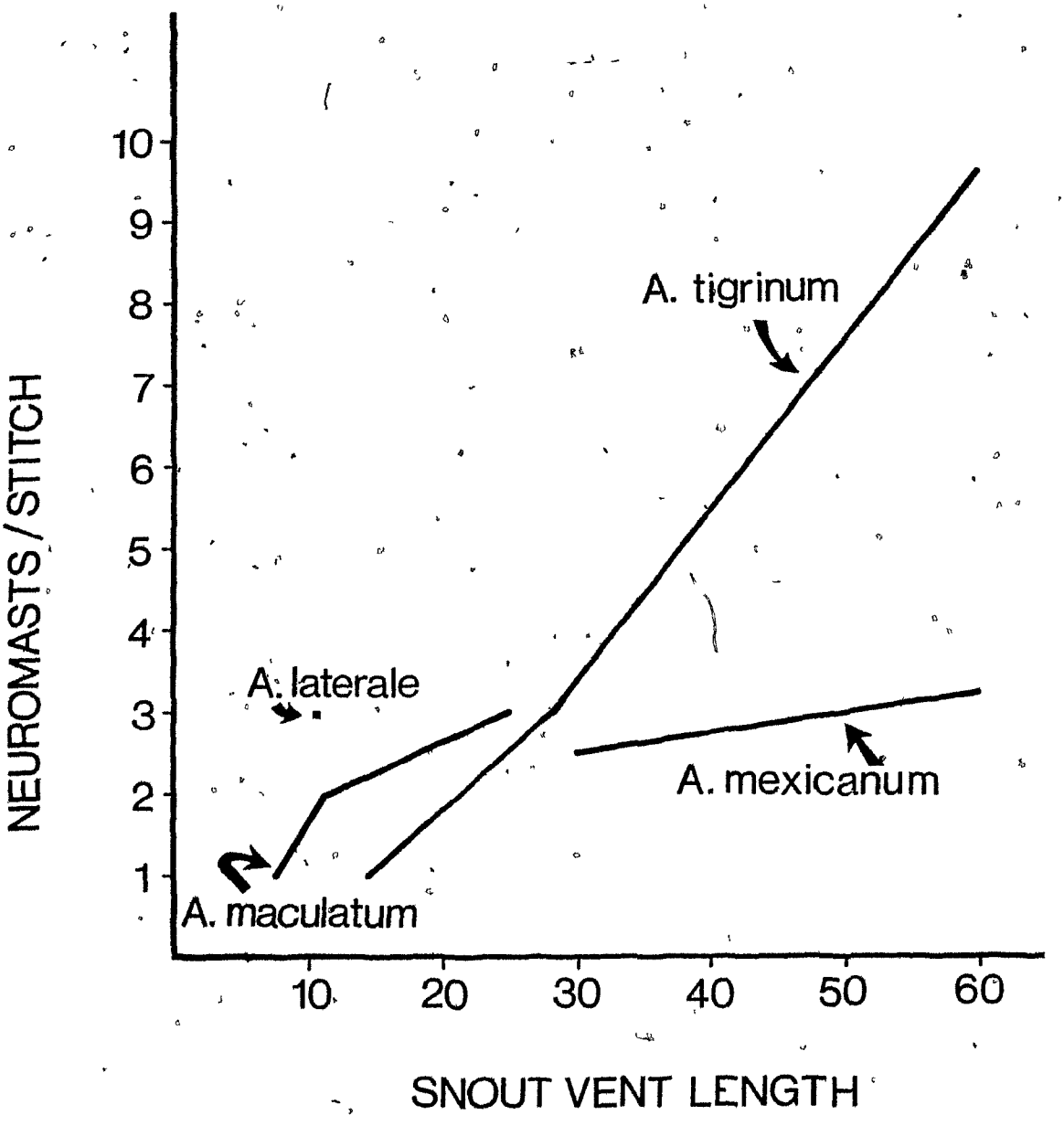

HAIR CELLS/NEUROMAST
35
30
25
20
15
10
5
10
20
30
40
50
60
70
SNOUT VENT LENGTH (mm)
D
P
Ama
Ame
Ame
At
H
H
H
Al
Ama
At
T
P
G
G
At
Ama
At
N
N
E
E
N

Figure 3-9. A plot illustrating interspecific variation in numbers of neuromasts per stitch with size in four species of Ambystoma. Note that for their sizes, A. laterale have more, and A. mexicanum have fewer, neuromasts than either A. tigrinum or A. maculatum.


NEUROMASTS / STITCH
10
9
8
7
6
5
4
3
2
1
A. tigrinum
A. laterale
A. mexicanum
A. maculatum
10
20
30
40
50
60
SNOUT VENT LENGTH

component neuromasts, and those with their long axis oriented longitudinally (Fig. 3-6). Transverse stitches were characteristic of all Ambystoma. Longitudinal stitches were characteristic of Necturus. Transverse stitches were formed from neuromasts positioned flush with the epidermal surface. Longitudinal stitches were located in epidermal grooves (Table 3-5).

DISCUSSION

A critique of the literature

The results obtained here support many of the results and conclusions obtained by previous workers (which are summarized in Table 3-1); they also question or refute some of these earlier findings. In agreement with the earlier studies I found transverse stitches in Ambystoma and longitudinal stitches in Necturus. I found no stitches in the hynobiids, dicamptodontids, plethodontids, and amphiumids. Transverse stitches may be present in cryptobranchids: the Andrias specimen that I examined may have had transverse stitches, but if so, within-stitch neuromasts were spread as far apart as neuromasts between stitches. This condition in Andrias resembled the "neuromast fields" described for Siren by Reno and Middleton (1973) rather than Ambystoma stitches. It may be that in both cryptobranchids and sirenids neuromasts

are formed into multiple lines, as is the case for some fishes (Branson and Moore, 1962; Webb, 1985). It will require an analysis of developmental series to resolve this question.

There is good evidence from the literature that Proteus has longitudinal stitches: the illustrations of Malbranc (1876) indicating this condition are detailed and supported by Hilton's (1947) written description. The support for longitudinal stitches in salamandrids is also good. It is based on observations on three genera by two authors (Kingsbury, 1895; Escher, 1925).

Early workers did not observe neuromasts in a large proportion of aquatic urodeles that they examined (Table 3-1). I feel that neuromasts are present in all these species, but for various reasons are difficult to observe using light microscopy on unskinned specimens. Several plethodontids that I examined had neuromasts indicated by pigmentless patches, making their visualization easy. In other species, however, neuromasts were pigmented and blended into the background colors of the skin, making standard light microscopic visualization difficult, if not impossible. Another factor contributing to neuromast visibility is the method of preservation and subsequent amount of specimen distortion and skin sloughing. Neuromasts may also be more easily viewed depending on where on the body they occur. I think it is unlikely that

there are any aquatic urodeles without neuromasts and, in contrast to the findings of Hilton (1947; Table 3-1), that neuromasts may be absent on one area of the body (i.e., the head) while present on another (i.e., the trunk).

Neuromast parameters

The predominant, if not universal, pattern of neuromast topography on each side of the dorsal cephalic surface of urodeles takes the form of a "U", with the closed portion located around the eye and the open portion tracking anteriorly along the snout (Fig. 3-1; Fig. 3-2; Lannoo, 1985; Chapter 2). Circumorbital neuromasts are organized into one row; anterior neuromasts are organized into multiple rows, with neuromasts between these rows oriented orthogonally. Ranodon may be exceptional in having only one row of nasal neuromasts, however its maxillary neuromasts are in multiple rows (Schmalhausen, 1968). This organization of neuromast lines appears unique to urodeles; fishes, anurans, and caecilians do not show this pattern (Jarvik, 1980; Escher, 1925; Hetherington and Wake, 1979). Lannoo (1985; Chapter 2) erred in describing only two neuromast lines in the maxillary row of Ambystoma, three lines are usually present.

Because neuromasts are directionally sensitive, the orthogonal arrangement of anterior neuromasts in urodeles

(Fig. 3-2b) may be extremely important for locating sources of water displacements. While workers as early as Malbranc (1876) realized that orthogonally oriented neuromasts could be important, more recent workers (e.g., Wickham, 1972; Reno and Middleton, 1973) have either not recognized this arrangement or not realized its functional importance.

Homologous neuromasts across taxa are oriented in the same direction. This conclusion confirms the light microscopical findings of Malbranc (1876) and Kingsbury (1895), and expands their findings to taxa that they did not consider.

There is a considerable amount of variation in neuromast numbers (Table 3-2, 3-3). Neuromasts are formed from primordia laid down by migrating, ectodermally derived placodes (see Winklbauer and Hausen, 1983a,b; 1985a,b for discussions and an elucidation of this process). The number of neuromast primordia laid down by these migrating placodes is variable but may be within a narrow enough range to detect differences between species (Table 3-2; Lannoo, 1985; Chapter 2). I did not examine enough individuals within species to determine species or familial differences with statistical confidence. From my limited sample, however, it appears that consistent taxonomic differences exist.

Hair cell considerations

Surface area of the neuromast sensory epithelium also varies. Differences were due primarily to variations in hair cell number, rather than hair cell size. Numbers of hair cells per neuromast increased with SVL across taxa (Table 3-2; Fig. 3-8). Perhaps hair cells per neuromast increase to compensate for the greater epidermal surface area of larger animals.

Hair cell size varied between one and three micrometers. These values agree with values cited for Ambystoma and Dicamptodon by Wickham (1972) and confirm that the light and more recent electron microscopical techniques are comparable in the type or amount of distortion that they introduce into the specimens.

I could detect no increase in hair cell numbers with individual size (Table 3-2; Fig. 3-8). Additionally, neuromast numbers also did not increase with growth. This latter result agrees with the conclusions of Winklbauer and Hausen (1983a, b, 1985a, b) and Lannoo (1985; Chapter 2). Apparently the only means by which larval urodeles can increase their numbers of hair cells with growth is for primary neuromasts to divide to form stitches. In Table 3-6 I provide estimates of hair cell numbers on the dorsal surface of the head in urodeles. In general, for animals that did not form stitches, hair cell numbers were approximately 1000 - 1500 (although Dicamptodon ensatus

and Pseudotriton ruber were outliers with over three thousand hair cells in their primary neuromasts alone). With stitch formation, however, hair cell numbers increase in direct proportion to the number of neuromasts per stitch (see data for Ambystoma maculatum in Table 3-6). In fact, the largest Ambystoma tigrinum larva I examined with ten neuromasts per stitch had almost 20,000 hair cells on the dorsal surface of its head alone.

Stitch formation

Stitch formation is limited to the Ambystomatidae, Cryptobranchidae, Proteidae and Salamandridae. Two types of stitch formation occur that fall out along family lines: division along the long axis of neuromasts to form transverse stitches (characteristic of Ambystoma and perhaps Cryptobranchus; Malbranc, 1876), and division along the neuromast short-axis to from longitudinal stitches (characteristic of Necturus, Proteus, and Notophthalmus Malbranc, 1876; Kingsbury, 1895).

The value of stitch orientation in determining urodele family associations and evolutionary history is contingent upon how easily the plane of division in primary neuromasts can be altered. If this process is simple -- perhaps the result of one or a few gene mutations affecting development -- stitch orientation may represent convergence and will have no systematic value.

Table 3-6 Estimates of the number of dorsal cephalic hair cells of urodeles (right column). Species names are given in the left column (column 1). The numbers adjacent to the species names (column 2) refer to the specimens examined and are given in Table 3-2. Hair cell estimates were obtained by multiplying together the mean number of hair cells per neuromast (column 3), the numbers of primary neuromasts present (column 4), and the mean numbers of secondary neuromasts present per stitch (column 5). The number of neuromasts in parentheses for Ambystoma tigrinum #3 is an estimate from A. tigrinum #2 based on primary neuromast counts, which do not vary with size

Species		#Haircells/ neuromast	#Neuromasts	Neuromasts/ stitch	Estimated # hair cells
Hynobius nebulosus	#1	15.1	90	1	1,359
	#2	16.1	100	1	1,610
	#3	15.0	93	1	1,395
Ambystoma maculatum	#1	11.0	105	1	1,155
	#2	14.6	120	2	3,504
	#3	12.1	87	3	3,158
Ambystoma mexicanum	#2	21.5	106	4.5	10,255
Ambystoma tigrinum	#2	15.7	116	3	5,464
	#3	17.0	(116)	10	19,720
Dicamptodon ensatus		33.0	134	1	4,422
Eurycea bislineata	#1	11.5	122	1	1,403
	#2	9.5	135	1	1,282

Table 3-6 (cont.)

Gyrinophilus					
porphyriticus	#1	15.9	117	1	1,860
	#2	16.0	114	1	1,824
Pseudotriton ruber	#1	22.6	144	1	3,254
Notophthalmus					
viridescens	#1	8.2	119	1	976
	#2	11.2	126	1	1,411
	#3	11.3	122	1	1,403

If a shift in stitch orientation is an evolutionarily difficult (and thus conservative) process, however, stitch formation may be a useful systematic character.

Stitch orientation is correlated with environmental parameters. Transverse stitches are characteristic of animals found in lentic habitats, and animals with their neuromast sensory epithelia located flush with the epidermal surface (Table 3-6). Longitudinal stitches in Necturus, an animal that occurs in rivers (Conant, 1975), are located in epidermal grooves (Table 3-6). This correlation between neuromast position and habitat is not absolute, however one invariant pattern is that transverse stitches do not occur in epidermal grooves; to accommodate the stitch, a groove would have a bore greater than its length, which would expose the sensory epithelia.

Rate of stitch formation varies with species (Fig. 3-9), indicating a genetic component to this process. However, Winklbauer and Hausen (1985a, b) reported that stitch formation is retarded in starved tadpoles (Xenopus laevis), indicating that environmental conditions may also affect stitch development.

Stitch formation separates Ambystoma from Dicamptodon and Rhyacotriton (Table 3-1; Wickham, 1972). This character provides support for Edwards (1976) division of these two groups into the families Ambystomatidae and Dicamptodontidae, respectively. There has also been some

controversy about whether Necturus and Proteus should comprise one or two families (e.g., Hecht and Edwards, 1977). Both taxa have longitudinal neuromasts. This feature alone does not support the view that they should be grouped into one family, because some species of salamandrids also have longitudinal stitches. However, these two genera cannot be separated based on this character.

Additional functional considerations.

In general, lotic forms tend to have more primary neuromasts, relatively more anterior neuromasts, and have their neuromasts located in epidermal pits or grooves (Table 3-5). It is difficult to envision why lotic forms should have more primary neuromasts and more anterior neuromasts. Perhaps the need to have neuromasts in epidermal pits has eliminated transverse stitch formation, and primary neuromast increases are in partial compensation for this loss. The function of epidermal pits and grooves may be to protect the neuromasts from continuous stimulation, or damage due to sudden or large displacements caused by water currents. A similar correlation between lotic habitats and neuromasts located in bony canals occurs in teleosts (Branson and Moore, 1962).

Cave salamanders do not have more neuromasts or hair

cells per neuromast than larvae with more generalized ecologies. The statement is frequently made that these animals -- because they live in the dark or are blind -- must be more dependent on other senses, particularly lateral line organs, than more generalized forms. This statement ignores the fact that most aquatic urodele larvae are nocturnally active (see references in Chapter 1).

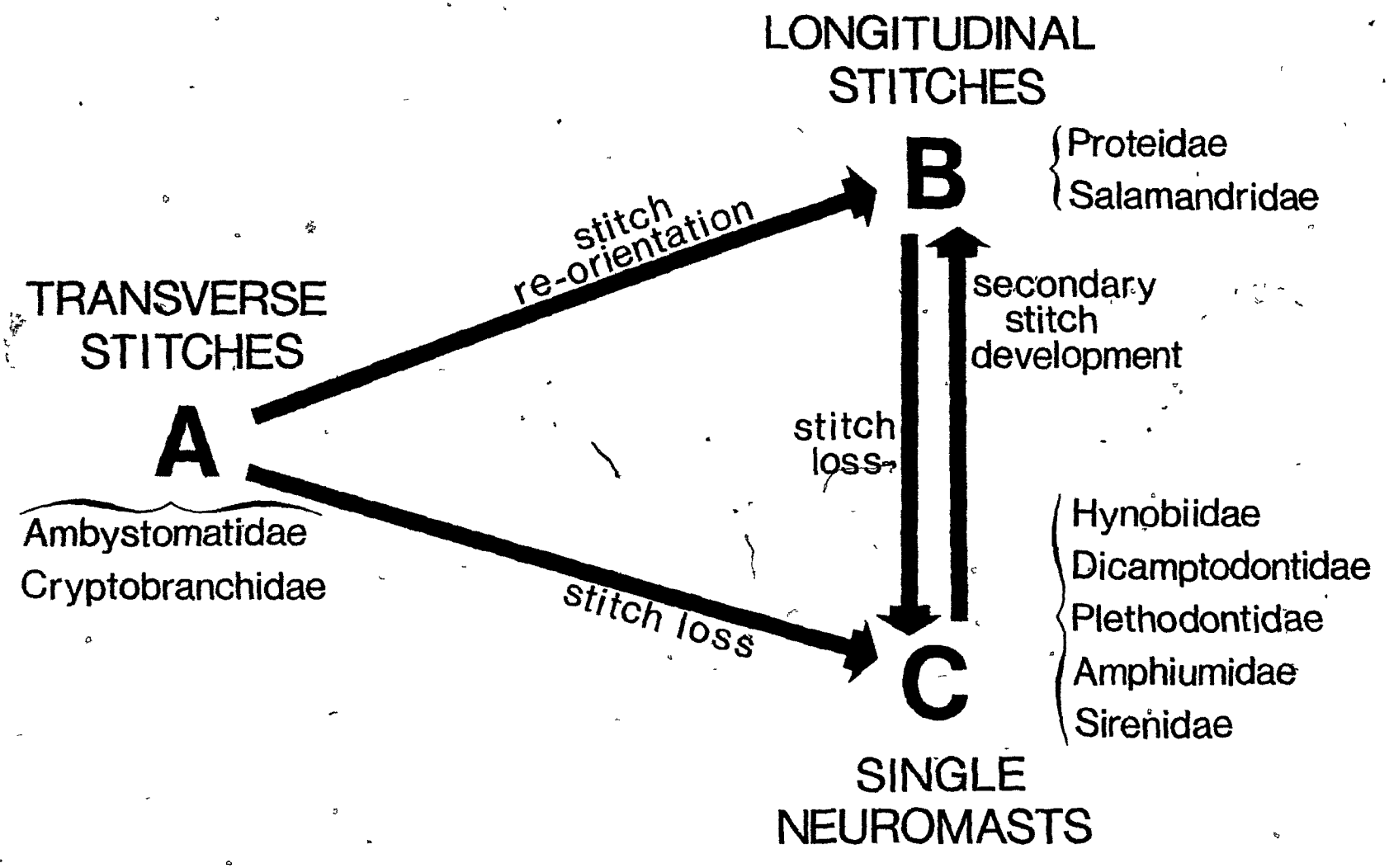
The combination of large hair cell numbers and orthogonal neuromast couplets should provide aquatic urodeles with the means to detect and locate sources of water displacements. Many aquatic urodeles are sit-and-wait predators. This hunting strategy is most effective if extrinsic water displacment detectors (i.e., neuromasts) are sensitive and discriminative over the whole striking range of the animal.

As an aside, all aquatic urodeles examined here appeared to have electroreceptive ampullary organs present on their dorsal cephalic surface. Although I did not quantify ampullary organs, they appeared to be scattered among neuromasts and around neuromasts lines. Electroreceptors and mechanoreceptors may complement each other as far field and near field receptors, or work in concert in the near field (Fritzsch, et al., 1984).

Additional systematic considerations

The transverse stitch formation characteristic of Ambystoma and perhaps Cryptobranchus is identical to the published descriptions of stitch formation in anurans: specifically Rana (Malbranc, 1876; Kingsbury, 1895; Escher, 1925) and Xenopus (Görner, 1963; Shelton, 1970). Based on these data the assumption can be made that transverse stitches are the generalized condition for this character in urodeles and that this condition was also characteristic of the anuran-urodele common ancestor. This interpretation infers that both the absence of stitches and the linear stitch pattern in urodeles are derived (Fig. 3-10). This interpretation is in direct opposition to Moodie (1908) and Hilton (1947), who considered the condition in Necturus to be primitive. This interpretation also suggests that pond dwelling hynobiids, salamandrids, and plethodontids evolved from stream dwelling ancestors. In the plethodontids there is support for this from other structures, i.e., the absence of lungs, Wake (1966). Less support is available for this in hynobiids and salamandrids, although Schmalhausen (1968) argues that urodeles in general evolved from stream forms.

Figure 10. An illustration of the directions and mechanisms of phylogenetic changes in stitch patterns in aquatic urodeles, along with the families that exhibit each pattern. In this scenario I assume transverse stitches are the generalized condition based on outgroup comparison to anurans. The transverse stitch pattern could have led to the longitudinal stitch pattern if the direction of secondary neuromast growth was altered 90^{o} (A → B). Transverse or longitudinal stitches could have formed single neuromasts if animals exhibiting these patterns eliminated their secondary neuromast formation (A → C or B → C). Conversely, longitudinal stitches may have been formed from animals with single neuromasts if these animals re-invented stitch formation (C → B).


LONGITUDINAL
STITCHES
B
Proteidae
Salamandridae
stitch
re-orientation
TRANSVERSE
STITCHES
secondary
stitch
development
stitch
loss
A
Ambystomatidae
Cryptobranchidae
stitch loss
Hynobiidae
Dicamptodontidae
Plethodontidae
Amphiumidae
Sirenidae
C
SINGLE
NEUROMASTS

Chapter 4: Neuromast Topography in Anuran Amphibians

Introduction

In the present paper I describe the ecological correlations and taxonomic patterns of neuromast topography in anuran larvae. I provide data on the same neuromast parameters in anurans that were examined previously for urodeles (Chapter 3). As was the case for urodeles, there have been few papers published on neuromast topography and its diversity in anuran larvae. In fact, in the most recent review of the anuran lateral line system (by Russell, 1976) the diversity of neuromast topography in anurans is not even considered.

The lateral line system of anurans is simpler than in urodeles; the anuran system is composed of mechanoreceptive neuromast organs only, while urodeles have both neuromasts and electroreceptive ampullary organs (Fritzsch et al., 1984).

Among anurans, neuromast anatomy is best known for the pipid frog Xenopus (see Winklbauer and Hausen, 1983a,b; 1985 a,b for embryology, and Shelton 1970, 1971 for larval neuromast topography and metamorphic changes in neuromast organization). Data are also available for the ranid, Rana (see Knouff, 1935 and Wright, 1947 for developmental data; Malbranc, 1876 and Kingsbury, 1895 for descriptions of larvae and metamorphic individuals). Escher (1925) compared neuromast topography in anuran

larvae and adults from several families and proposed evolutionary scenarios for the derivation of certain neuromast features, such as shifts in line positions and the addition of auxillary lines. In Table 4-1 I summarize the species that have information published on neuromast topography.

Methods

I examined 86 anuran specimens from 36 species in 19 genera and 11 anuran families (Table 4-2). Where possible I examined more than one individual per species, more than one species per genus, and more than one genus per family. Both light microscopy and scanning electron microscopy (SEM) were used. Light microscopy was used to establish the presence of lines, to count neuromasts per stitch, and to count neuromasts or stitches per line. SEM was used to count and measure neuromasts, stitches, and hair cells. Stitch formation is dependent upon ontogeny; larger (older) tadpoles tend to have more neuromasts per stitch (Stephens, 1981). I therefore examined the largest premetamorphic tadpoles available to me for each species to assess stitch formation.

Specimens were prepared for light microscopy by the skinning, hydrogen peroxide technique of Lannoo (1985; Chapter 2). Because of the globose nature of most

Table 4-1. A summary of the literature on neuromast topography in anuran larvae. I restrict this table to papers specifically designed to examine neuromast topography; several tadpole descriptions in the herpetological literature show neuromast lines, but give no specific neuromast counts.

Species	Life History Stage	Source
PIPIDAE		
Xenopus laevis	Tadpole	Shelton, 1970
	Adult	Escher, 1925
		Gorner, 1963
		Shelton, 1971
	Embryo	Winklbauer and Hausen, 1983a,b;1985a,b
Hymenochirus boettgeri	Adult	Escher, 1925
Pipa sp.	Juvenile	Escher, 1925
DISCOGLOSSIDAE		
Alytes obstetricans	Tadpole	Wintrebert, 1904
Bombina orientalis	Tadpole, Juvenile	Malbranc, 1876
*Bombina variegata**	Tadpole, Juvenile	Escher, 1925
PELOBATIDAE		
Pelobates cultripes	Tadpole	Escher, 1925

Table 4-1 (cont.)

PELODYTIDAE		
Pelodytes punctatus	Tadpole	Escher, 1925
RANIDAE		
Rana catesbeiana	Tadpole	Kingsbury, 1895
Rana clamitans	Tadpole	Escher, 1925
Rana palustris	Embryo	Wright, 1947
Rana pipiens	Embryo	Wright, 1947
Rana sylvatica	Embryo	Wright, 1947

* See Elepfandt and Simm (1985) for the correction of Escher's (1925) nomenclature.

Table 4-2. A list of the anuran larvae examined here, and the microscopical techniques (light, SEM) used to examine them. Specimen sizes (SVL) in mm, and developmental stages (Gosner, 1960) are given.

	LIGHT		SEM	
FAMILY, Species	SVL(mm)	Gosner	SVL(mm)	Gosner
ASCAPHIDAE				
Ascaphus truei	14.5	25	14.0	25
DISCOGLOSSIDAE				
Discoglossus pictus	7.0	28	8.0	28
			6.0	28
Alytes obstetricans	-	-	11.0	28
PIPIDAE				
Xenopus laevis	16.0	33	11.0	29
	16.0	34	19.0	40
			17.5	35-36
Hymenochirus boettgeri	6.0	36	3.5	28
			5.0	36
RHINOPHRYNIDAE				
Rhinophrynus dorsalis	15.0	37	13.0	35
			18.0	36

Table 4-2 (cont.)

PELOBATIDAE				
Scaphiopus bombifrons	12.0	27	-	-
Scaphiopus holbrookii	9.0	27	9.0	25
			8.0	25
MICROHYLIDAE				
Gastrophryne carolinensis	8.5	33	9.0	31
	12.0	40	-	-
Chiasmocleis ventrimaculata	7.0	39-40	8.0	38
Phrynomerus annectens	21.0	41	21.0	41
RANIDAE				
Amolops sp.	12.5	35	12.5	35
Rana aurora	27.5	39-40	-	-
	25.0	38	-	-
Rana boylii	17.0	25	-	-
	18.0	31	-	-
Rana catesbeiana	13.0	27	12.5	27
	15.0	25	-	-
Rana clamitans	32.0	38	-	-
Rana fuscigula	17.0	36	-	-
	28.5	31	-	-
	16.0	37	-	-
Rana hecksheri	60.0	36		
Rana magna	16.5	38-39	-	-
	-	39		

Table 4-2 (cont.)

Rana microdisca		34-35	-	-
	13.0	25-26	-	-
Rana palmipes	16.5	25	-	-
	17.9	25	-	-
	15.5	25	-	-
	17.0	26-27	-	-
Rana palustris	8.0	-	-	-
	7.2	26	-	-
Rana pipiens	19.0	41	15.0	41-42
Rana septentrionalis	20.0	35		
Rana sylvatica	15.0	38	-	-
	16.0	38	-	-
	15.0	38		
	14.5	37-38		-
	13.5	31	16.0	38
	11.5	31	15.0	28
MYOBATRACHIDAE				
Limnodynastes tasmaniensis	19.0	36	19.5	37
	21.0	38	-	-
	22.0	36	-	-
	21.0	36	-	-
LEPTODACTYLIDAE				
Odontophrynus occidentalis	15.0	28	-	-
Heleophryne nebulosus	16.5	25	-	-

Table 4-2 (cont.)

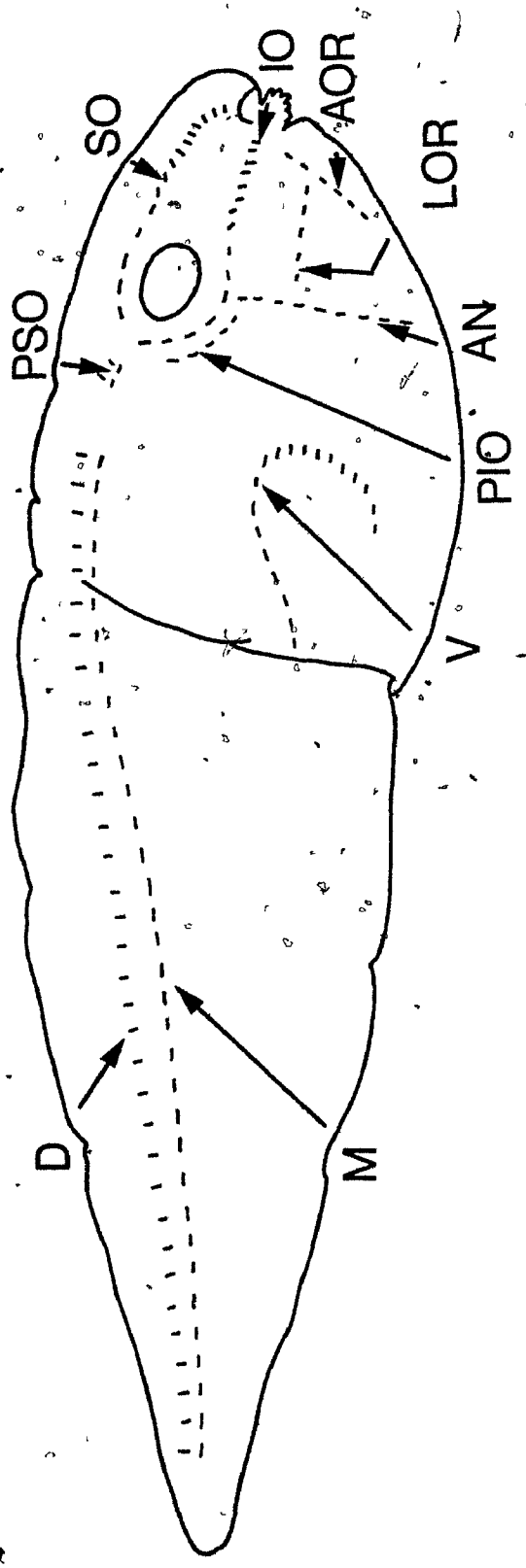
Heleophryne purcelli	15.0	25	16.5	25
BUFONIDAE				
Bufo terrestris	12.0	39	9.5	38
	10.0	36	-	-
Bufo valliceps	-	-	8.0	39
	-	-	7.0	35
HYLIDAE				
Acris gyrillis	12.5	32-33	-	-
Hyla crucifer	5.5	26-27	5.0	26-27
Hyla regilla	13.0	41	11.5	41
Osteopilus brunneus	13.0	-	12.5	41
	-	-	8.5	29
Osteopilus septentrionalis	12.5	33	-	-

tadpoles, their skins had to be cut radially to flatten them without wrinkling. Specimens were prepared for SEM viewing using the technique of Lannoo (Chapter 3). Neuromast parameters were counted and measured either directly under the microscope or from micrographs of the preparations.

In this study I focused on neuromast lines of the head and trunk region, because it is here that neuromast organization is most complex and likely to reflect phylogenetic and ecological relationships. Neuromast line nomenclature is based on Noble (1931) with a few modifications. My nomenclature and definitions of neuromast lines are illustrated in Fig. 4-1 and are as follows:

The supraorbital line begins posterior and medial to the eye and courses forward along the dorsolateral aspect of the snout, dorsal or medial to the nares. A posterior supraorbital line is present in most species near the posterior portion of the supraorbital line. The infraorbital line begins behind the eye, curves around below the eye, and courses along the lateral aspect of the snout, ventral or lateral to the nares. A posterior infraorbital line is present in most species. The angular line may be continuous with the posterior infraorbital line in some species and can begin at a point from anywhere behind the eye to below the eye; it then usually

Figure 4-1. A schematic drawing illustrating the pattern of neuromasts on the right side of a generalized tadpole. Neuromasts are drawn larger than scale for clarity. Single neuromasts are illustrated, rather than stitches. The long axis of each neuromast represents the axis of maximum sensitivity of that particular neuromast. Notice the orientation changes in SO and IO neuromasts as they course anteriorly onto the snout. Abbreviations: SO = supraorbital, IO = infraorbital, PSO = posterior supraorbital, PIO = posterior infraorbital, AN = angular, LOR = longitudinal oral, AOR = anterior oral, and D, M, and V = dorsal, middle, and ventral body lines, respectively. Body lines are not considered in detail in the present paper except to note one peculiarity of the ventral line on tadpoles with sinestral spiracles. On the left side of these tadpoles this line forms a semicircle that wraps around the spiracle. On the right side (shown here) of these tadpoles this line takes the same form, despite the absence of the spiracle.


IO
AOR
SO
LOR
PSO
AN
PIO
V
D
M

courses ventrally. The oral line is anterior to the angular line. I divide the oral line where possible into two distinct components, the anterior oral line near the oral disc and the longitudinal oral line (Escher's 1925, jugular line) that courses between the vertical oral line and the angular line.

This nomenclature is simpler than that proposed for anurans by Holmgren and Pehrson (1949), who base their nomenclature on embryonic placodal derivations. However, as they point out, the embryonic sources of neuromast lines are not always clear when examined in larval stages. Because Holmgren and Pehrson (1949) did not examine a wide range of larvae and because I did not collect embryonic information, I have attempted to make my nomenclature here as clear and unambiguous as possible, bearing in mind that neuromast lines assigned the same names might not be strictly homologous.

Results

Neuromast Lines

The four basic neuromast lines -- supraorbital, infraorbital, angular, and oral -- were present in all anuran larvae I examined (e.g., Figs. 4-2, 4-3), and did not vary greatly in their placement.

Of these four lines, the oral line appeared to be the

Figure 4-2. Light micrographs of the right sides of the trunk and head of four anuran larvae comparing neuromast topography in different families. A) Ascaphus truei (Ascaphidae), B) Alytes obstetricans (Discoglossidae), C) Rana pipiens (Ranidae), D) Hyla regilla (Hylidae). Neuromasts are visible as small light circles arranged into lines. Neuromast line abbreviations: SO = supraorbital, IO = infraorbital, PSO = posterior supraorbital, PIO = posterior infraorbital, ANG = angular, AOR = anterior oral, LOR = longitudinal oral. Note in Ascaphus the obliquely oriented angular line and the oral line along the oral margin. In Alytes note the ventrally located posterior infraorbital line. In Hyla note the absence of a horizontal oral line. Magnification approximately 7 - 15 x.

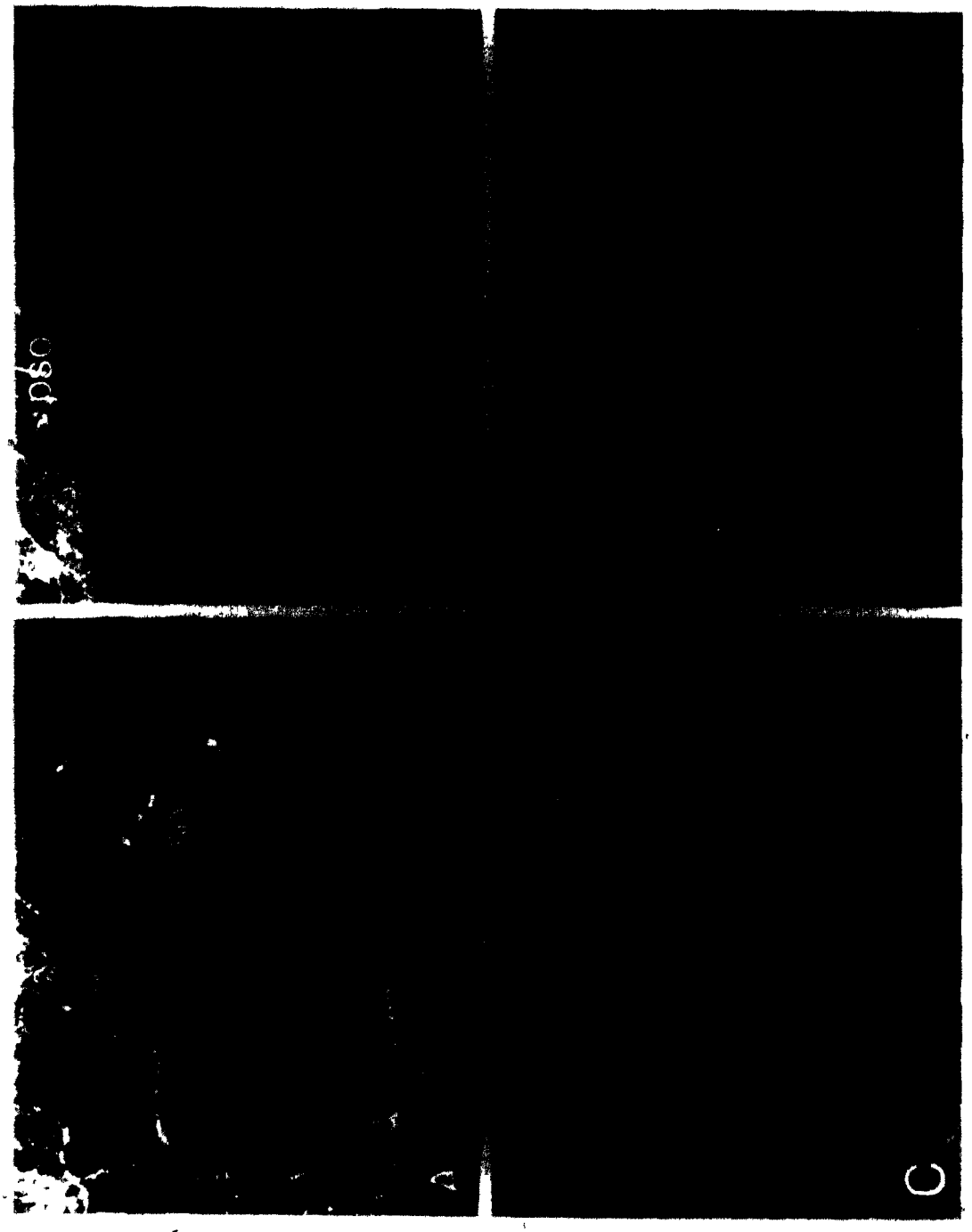
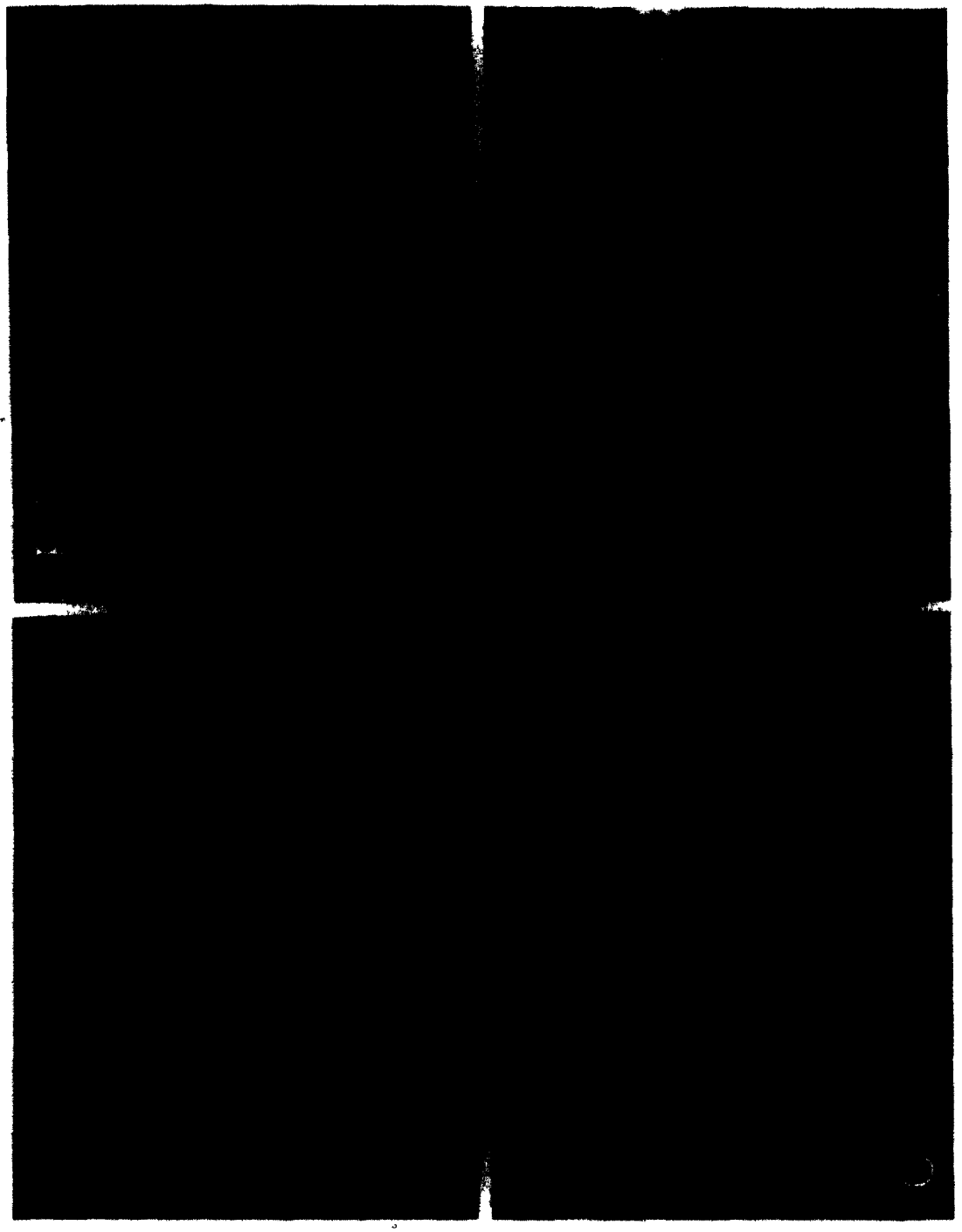

C

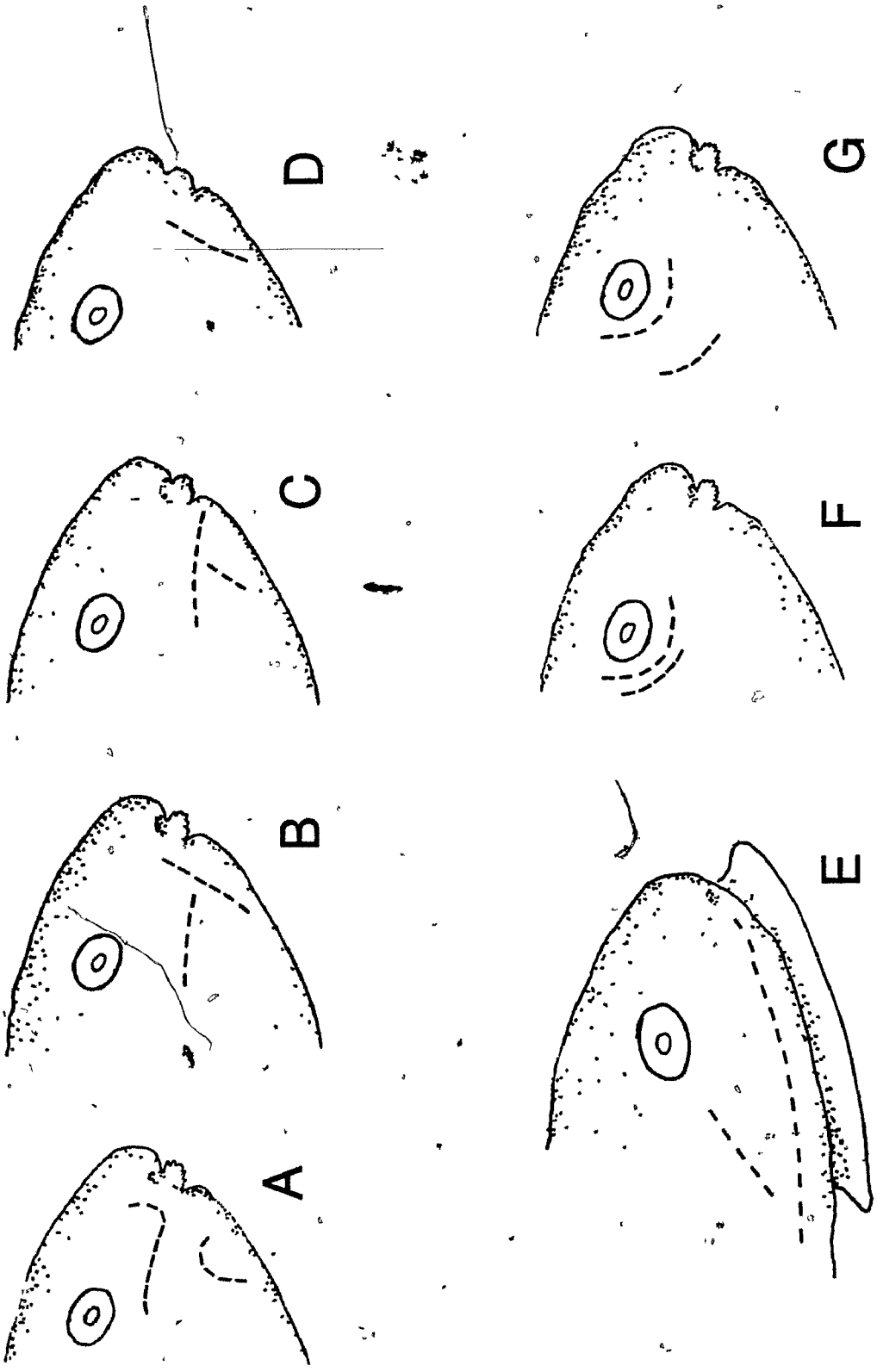
Figure 4-3. Light micrographs illustrating interspecific and interindividual variation in neuromast topography within the Ranidae. A) Rana palustris right side, B) Rana sylvatica right side, C), D) Rana microdisca right and left sides respectively. Neuromasts are visible as small light circles arranged into lines. Note in particular differences in the otic and oral regions. Neuromast nomenclature the same as in Fig. 4-1. Magnification approximately 7 - 10 x.



most variable (Fig. 4-4). This variation included the presence of only one straight line, which in Ascaphus truei (Figs. 4-2a, 4-4e) coursed longitudinally along the margin of its enlarged oral disc, and which coursed nearly vertically in Hyla regilla (Figs. 4-2d, 4-4d). The predominant general pattern, however, was for the oral line to be in the form of an inverted "U" or "V". This pattern is shown in Rana pipiens (Figs. 4-2c, 4-4b,c). Several other Ranas extend the anterior leg of their oral line up along the snout to form a "T" pattern (or in conjunction with the angular line, an "H" pattern); these variations do not appear to fall out along species groups. In Alytes obstetricans the longitudinal oral line connects with the dorsal portion of the angular line, while the anterior oral line by itself forms an inverted "U" (Figs. 4-2b, 4-4a).

There were other minor variations in the locations of neuromast lines peculiar to species (Figs. 4-2, 4-4). The angular line in Ascaphus differed in orientation from all the other anurans I examined (Figs. 4-2a, 4-4e). In Ascaphus the angular line slanted posteriorly and ventrally back from the eye, rather than nearly vertically as in all other tadpoles. In Discoglossus the posterior infraorbital line is shifted ventrally, in a position posterior and parallel to the angular line (Figs. 4-2b, 4-4g).

Figure 4-4. Schematic drawings illustrating variations in the positions of neuromast lines in tadpoles. A) Oral neuromasts in Alytes; B, C) Oral neuromasts in Rana; D) The single vertical oral neuromast line in Hyla regilla; E) The single oral line in Ascaphus; F) The typical position of the posterior infraorbital line in tadpoles; and G) its more ventral position in Alytes.


D
G
C
F
B
E
A

Unlike neuromast line placements, which were conservative, there was considerable intrafamilial, intrageneric, and intraindividual variation in neuromast counts (Table 4-3; Fig. 4-3). Fig. 4-3 illustrates some of this variation within the Ranidae; note in particular the otic and oral areas. In the otic area it was often difficult to assign neuromasts to lines with complete confidence.

Stitch Formation

In all anuran families I examined except Ascaphidae, primary neuromasts are divided to form secondary neuromasts and stitches. A stitch always has its long axis oriented transversely to the long axes of its component neuromasts (see Fig. 4-5 for examples of anuran neuromast morphology).

Stitches vary with respect to the number of neuromasts they contain and the organization of their neuromasts (Fig. 4-6). Within families, stitches were not present in stream forms. This was true of Ascaphus truei, the ranid Amolops sp., and the leptodactylid Heleophryne purcelli (systematics according to Frost, 1985). Other specialized larvae also did not form stitches, including the carnivorous Hymenochirus boettgeri, the desert pond-dwelling, omnivorous Scaphiopus holbrookii and S. bombifrons, and the arboreal, oophagous Osteopilus

Figure 4-5. Scanning electron micrographs showing single neuromasts in anuran larvae. A) Ascaphus truei (Ascaphidae), B) Rhinophrynus dorsalis (Rhinophrynidae), C) Xenopus laevis (Pipidae), D) Bufo valliceps (Bufonidae), E) Heleophryne purcelli (Leptodactylidae), F) Hyla crucifer (Hylidae). Scale lines; A, C, E, F = 5 μm; B = 25 μm; D = 2.5 μm.

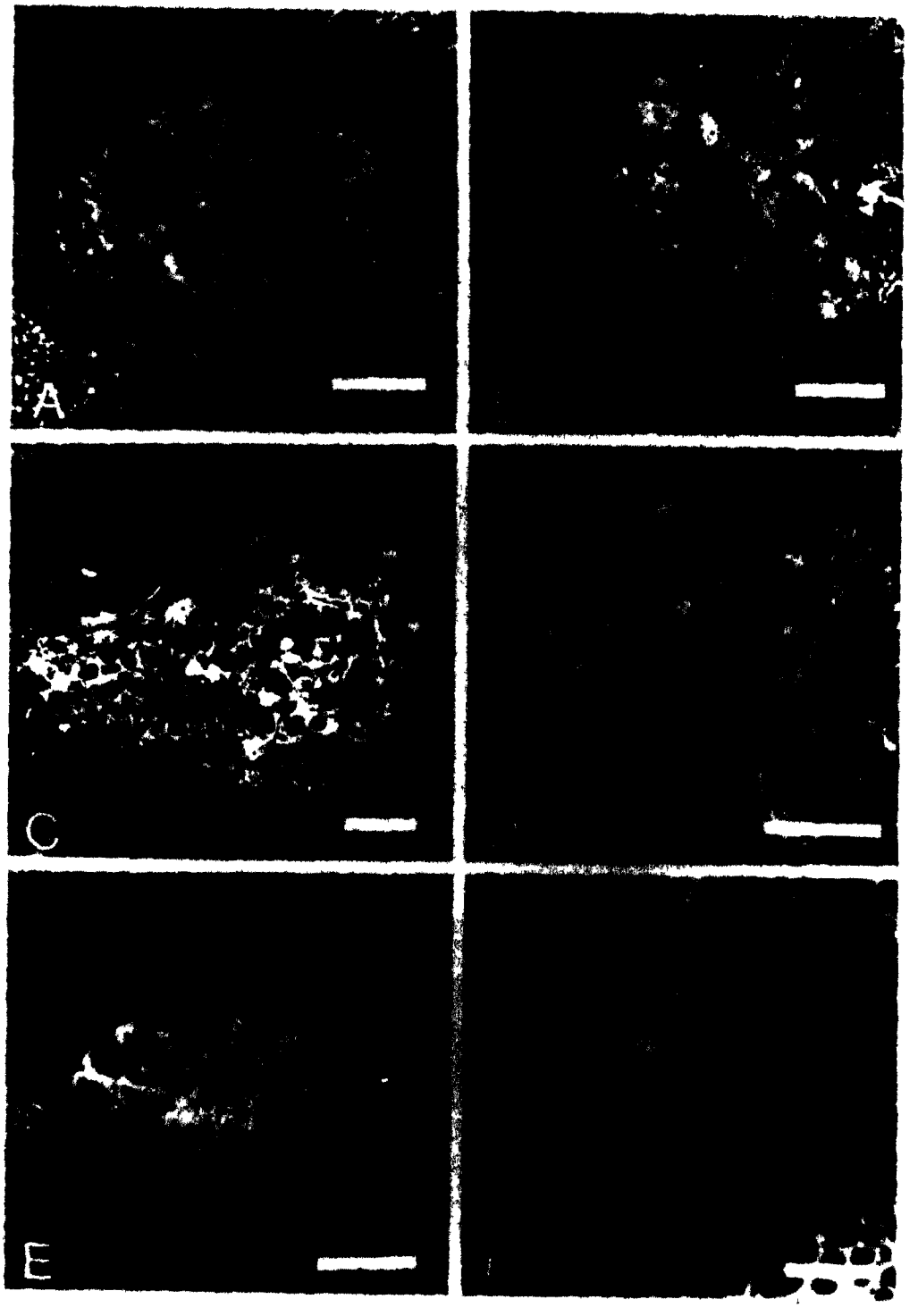
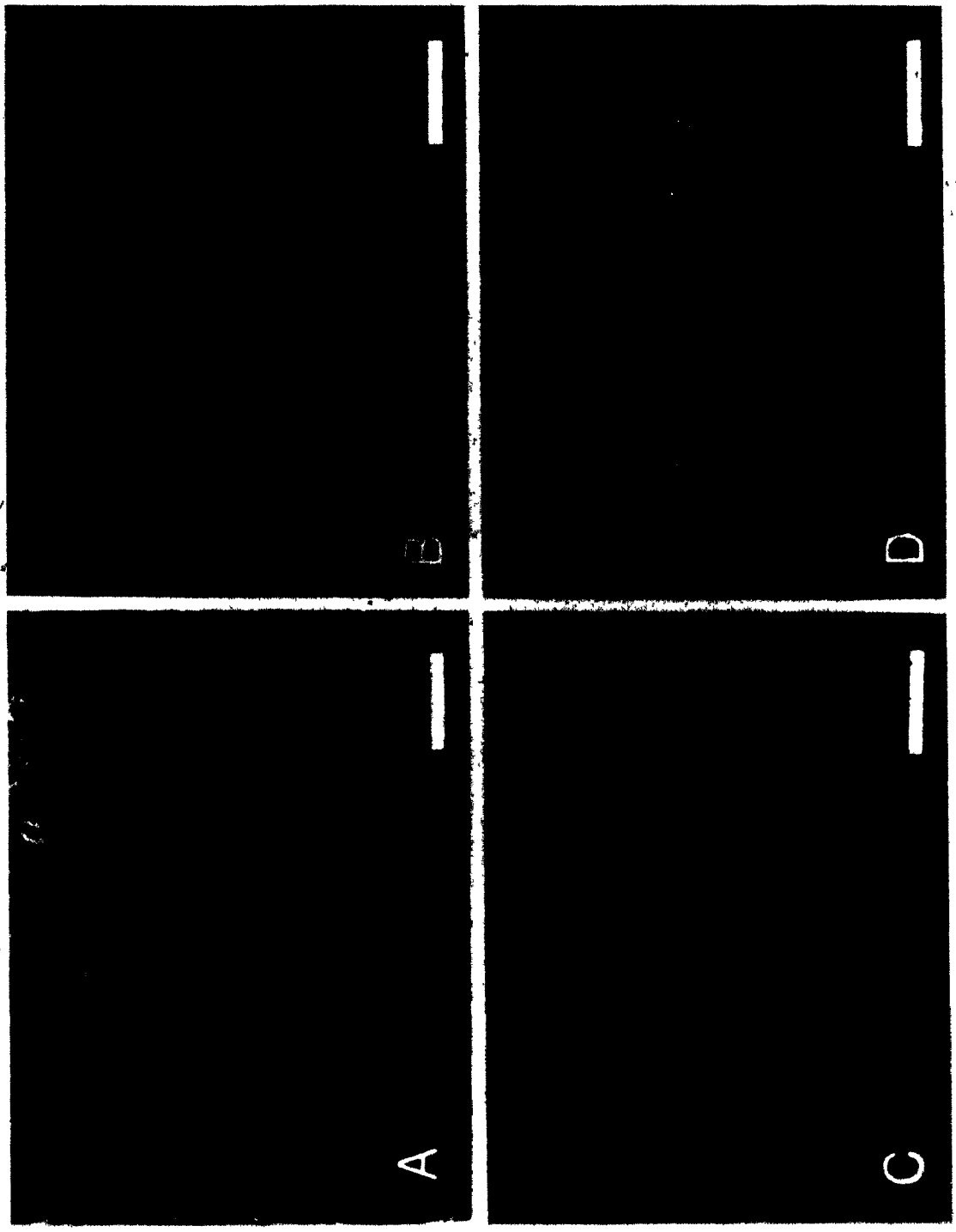

A
C
E

Figure 4-6. Scanning electron micrographs showing neuromasts comprising stitches in anuran larvae. A) Xenopus laevis (Pipidae), B) Rhinophrynus dorsalis (Rhynophrynidae), C) Chiasmocleis ventrimaculata (Microhylidae), and D. Bufo valliceps (Bufonidae). In particular note that neuromasts in Xenopus and Rhynophrynus are clumped while Chaismocleis and Bufo are linear. Also notice the numerous neuromasts per stitch in Xenopus, Rhinophrynus, and Chiasmocleis, and the few neuromasts per stitch in Bufo. Linaer stitches in Bufo containing two neuromasts are representative of stitches in typical generalized tadpoles in the families Discoglossidae, Ranidae, Leptodactylidae, Bufonidae, and Hylidae, which usually contain two or three neuromasts. Scale lines: A = 25 μm; B = 100 μm; C = 50 μm; D =10 μm.


B
D
A
C

brunneus.

There is probably no single common ecological factor causing this morphological convergence. One possibility is that several of these species have tadpoles that live in crowded conditions. This might result in tadpoles jostling each other and abrading each other's skin, which in turn would result in neuromast damage (admittedly, this is an odd concept, however this may be important in tadpoles such as Osteopilus brunneus which occur in large numbers in small arboreal habitats, and Scaphiopus which occupy small pools subject to drying).

As an aside, neuromast counts for stream forms were more difficult to obtain than for pond forms. Stream animals appeared to have a relatively thicker epidermis that reduced the amount of light transmitted in flattened microscopic preparations and made neuromasts more difficult to observe.

All generalized tadpoles that I examined, including species in the families Discoglossidae, Ranidae, Leptodactylidae, Bufonidae, and Hylidae, had stitches with fewer than five (and more often only two or three) neuromasts arranged linearly (Figs. 4-2, 4-3, 4-6). On the other hand stitches in Xenopus (Pipidae), Rhinophrynus (Rhynophrynidae), Chiasmocleis, and Phrynomerus (Microhylidae) contained more than six neuromasts. In Xenopus, Rhinophrynus, and Phrynomerus, neuromasts within

each stitch were clumped into loose groups. In Chiasmocleis neuromasts were organized into straight lines (Fig. 4-6).

Neuromast orientation

Around the eye, supraorbital neuromasts are oriented with their long axis directed rostral-caudally (Fig. 4-1). As the supraorbital line courses onto the snout, however, successive neuromasts undergo a rotation (Fig. 4-1; seen as a stitch rotation in Figs. 4-2, 4-3). As neuromasts proceed anteriorly onto the snout, the anterior ends of these rostral-caudally oriented neuromasts swing laterally or ventrally to become transversely oriented. In mirror image fashion, the infraorbital neuromasts, which at the eye were also rostro-caudally oriented, rotate their anterior edges medially or dorsally to become transversely oriented along the snout. Angular neuromasts are usually transversely oriented throughout the line. If oral neuromasts abut angular neuromasts (as they do in Rana and more ventrally in Hyla; Figs. 4-2, 4-3), oral neuromasts are rostro-caudally oriented and neuromasts in the two groups are perpendicular to each other. Likewise, within the oral group, longitudinal and anterior neuromasts are usually perpendicularly oriented.

The oral lines together with the angular line form an "H" pattern on the cheek of many tadpoles (Figs. 4-1, 4-2,

4-3). Together, with neuromasts in the supra- and infraorbital lines, cranial neuromasts constitute an orthogonal array of directionally sensitive water displacement detectors that provide the animal with the capability of sensing water movements from all directions.

Neuromast and Stitch Numbers

The total number of bilateral primary neuromasts, or stitches, on the head region of the tadpoles that I examined varied from 136+ in Rhinophryus dorsalis to 332 in Rana aurora (Table 4-3). There were most commonly between 250 and 320 primary neuromasts or stitches across taxa.

Intraspecific variation in stitch counts differed between species. Stitch count variation was low in the genus Rana, ranging from one to eight percent, depending on the species. On the other hand, variation in Limnodynastes tasmaniensis counts was high, at 30% for the four species examined.

There may be some tendency for species that do not form stitches to have reduced numbers of neuromasts. Both Ascaphus truei and Heleophryne purcelli had low numbers of neuromasts (Table 4-3). On the other hand both Xenopus and Rhinophrynus had low numbers of primary neuromasts and formed stitches.

Size does not appear to have a general affect on

Table 4-3. Numbers of primary neuromasts or stitches for anuran larvae. Counts are given by neuromast line for each side of the animal (R = right, L = left). Line abbreviations: SO = supraorbital, IO = infraorbital, PSO = posterior supraorbital, PIO = posterior infraorbital, ANG = angular, LOR = longitudinal oral, AOR = anterior oral, IP TOT = ipsilateral total, BI TOT = bilateral total. Columns between the headings PIO and ANG and between HOR and VOR are totals for both groups and are given where groups could not be distinguished.

Species		SO	IO	PSO	PIO	ANG	HOR	VOR	IP TOT	BI TOT
ASCAPHIDAE										
Ascaphus truei	R	20	11	4	3	12	23		73	146+
	L	20	16	2	-	14	21+		73+	
DISCOGLOSSIDAE										
Discoglossus pictus										
	R	39	41	6	2	29	23	19	159	318
Alytes obstetricans										
	R	35	35	6	20	28	23	12	159	318
PIPIDAE										
Xenopus laevis*										
RHINOPHRYNIDAE										
Rhinophrynus dorsalis										
	R	21+	15	?	?	13	4	15?	68+	136+

Table 4-3 (cont.)

PELOBATIDAE										
Scaphiopus bombifrons		R	30+	32	?	?	30	21	26	139+ 289+
		L	35	37	?	?	27+	26+	25+	150+
RANIDAE										
Amolops sp.		R	52+	30+	?	?	21	6+		109+
Rana aurora		R	45	31	8	?	38	15	29	166 332
Rana boylii	#1	R	30	30	7	7	32	21	16	143 286
	#2	R	33	32	5	6	37	12	15	140 281
		L	33	34	6	9	32	12	15	141
Rana catesbeiana										
	#1	R	28	28	14	12	25	20	30	157 301
		L	32	23	5	8	29	21	26	144
	#2	R	29	33	4	10	26	21	19	142 289
		L	33	26	6	7	31	22	22	147
Rana fusciqula	#1	R	27	26	6	5	19	7	16	106 230
		L	29	24	6	10	21	17	17	124
	#2	R	26	24	11	8	21	13	10	113 226
Rana hecksheri		R	24	17	4	4	11	21	6	87 177
		L	26	17	4	6	10	20	7	90
Rana magna		R	36	29	6	5	30	16	18	140 287
		L	35	35	5	9	32	15	16	147

Table 4-3 (cont.)

Rana microdisca									
#1	R	36	35	7	8	19	28	10	143 292
	L	36	26	8	9	23	33	14	149
#2	R	30	32	6	8	31	17	11	135 280
	L	32	30	5	7	33	19	19	145
#3	R	38	35	6	10	32	21	14	156 303
	L	38	28	9	8	31	14	19	147
Rana palustris	R	46	32	7	9	34	24	17	169 311
	L	24	35	8	7	31	24	13	142
Rana pipiens #1	R	45	33	6	7	29	19	7	146 305
	L	42	32	7	20	25	18	15	159
#2	R	49	36	13	5	30	12+	18	163+ 325+
	L	46	31	12	13	26	14	20	162
Rana septentrionalis									
	R	30	30	6	10	28	19	23	146 292
Rana sylvatica	R	40	32	4	11	23	15	19	144 277
	L	43	23	4	6	27	12	18	133
#2	R	38	32	6	6	26	21	15	144 288

Table 4-3 (cont.)

MYOBATRACHIDAE									
Limnodynastes tasmaniensis									
#1	R	29	21	15	6	23	10	15	119 242
	L	27	23	25		24	12	12	123
#2	R	27	25	4	6	20	10	12	104 208
	L	28	18	4	7	24	9	14	104
#3	R	22	21	3	3	18	11	12	90 184
	L	23	18	16		17	8	12	94
#4	R	25	18	6	8	18	9	16	100 196
	L	26	19	2	2	21	9	17	96
LEPTODACTYLIDAE									
Odontophrynus occidentalis									
	R	33	46	?	?	50	28	5	162+ 324+
Heleophryne purcelli									
	R	15	25	3	?	9	11	7+	70+ 140+
HYLIDAE									
Hyla regilla	R	38	23	11	12	24	-	21	129 258

* Shelton (1970) reports neuromast counts range from 136 - 190 in *Xenopus laevis* tadpoles.

stitch counts among species, but the huge tadpole of Rana hecksheri had a relatively low 177 stitches.

Neuromast and Hair Cell Characteristics

Neuromasts varied in size among species (Fig. 4-5, Table 4-4). Neuromasts in Xenopus and Rhinophrynus were large, about 10 x 30 µm on average (although the variation here is high), compared to the ≤ 10 µm linear neuromast dimensions of most other generalized tadpoles.

Neuromast size was correlated with the numbers of hair cells they contained (Table 4-4). The large neuromasts of Xenopus and Rhinophrynus contained 20 or 30 hair cells; the smaller neuromasts of most other species contained fewer than 15 hair cells. This pattern did not hold for the stream-dwelling Amolops and Heleophryne which had small neuromasts but 25 and ≤ 28 hair cells per neuromast respectively (Table 4-4). Hyla regilla also had small neuromasts but a high number of hair cells (20) per neuromast.

Hair cell sizes were fairly constant across taxa, generally ranging in diameter from 0.75 to 2.0 µm, although Xenopus had hair cells that were 3 µm in diameter (Table 4-4).

Distance between primary neuromasts varied between species, with an average distance of about 200 µm (Table 4-4). Within a species these distances could vary up to

Table 4-4. Neuromast and hair cell parameters for anuran larvae. Neuromast size, distance between neuromasts (or stitches), hair cells per neuromast, hair cell sizes (as they appear on the surface), and stitch formation including numbers of hair cells per stitch. All measurements are given in microns (μm).

FAMILY, Species	Neuromast size	Distance	Hair Cells/ Neuromast	Hair Cell Size	Stitches
ASCAPHIDAE					
Ascaphus truei	7x15-8x18	50-60, 200	11-14	0.75-1.0	No
DISCOGLOSSIDAE					
Discoglossus pictus	2x4-4x8	60-120	7-12	1.5	No
Alytes obstetricans	6x8-10x18	30-200	8-14	1.0-1.5	2
PIPIDAE					
Xenopus laevis	10x15-15x40	150-200	8-28	1.0-3.0	≤18
Hymenochirus boettgeri	8x8	40	10	1.0-2.0	No

Table 4-4 (cont.)

RHINOPHRYNIDAE					
Rhinophrynus dorsalis	8x20-12x24	200-300	18-44	1.5	6-20
PELOBATIDAE					
Scaphiopus holbrookii	4x6-5x8	80-120	6-10	0.75-1.0	No
MICROHYLIDAE					
Chiasmocleis ventrimaculata	3x3-4x5	60,150-200	7-10	?	7-10
Gastrophryne carolinensis	4x4-4x5	175-200	≥9	1.0	2
Phrymomerus annectens	4x4-4x6	150	?	?	2-7
RANIDAE					
Amolops sp.	10x20-10x25	70-200	25	1.5-2.0	No
Rana pipiens	3x3-7x12	75-200	4-12	0.75-1.0	1-3

Table 4-4 (cont.)

MYOBATRACHIDAE					
Limnodynastes tasmaniensis	3x4-7x12	140-350	7-10	1.5	No
LEPTODACTYLIDAE					
Heleophryne purcelli	6x15-8x25	300-600	12-28	1.0-2.0	No
BUFONIDAE					
Bufo terrestris	4x6-5x8	100-200	2-20	1.0	2
Bufo valliceps	4x8-5x12	100-175	4-13	0.75-1.0	1-3
HYLIDAE					
Hyla crucifer	3x8-4x18	100-175	7-15	1.5-2.0	2
Hyla femoris	4x6-4x8	200	7-20	1.0-1.75	2
Osteopilus brunneus	2x4	100-220	?	0.5	No

six fold depending on where neuromasts were located on the body. Not surprisingly, there was some tendency for species with fewer neuromasts (e.g., Rhinophrynus, Limnodynastes, and Heleophryne) to have greater distances between their neuromasts.

DISCUSSION

Several authors (e.g., Kingsbury, 1895: Noble, 1931; Holmgren and Pehrson, 1949) state or imply that the anuran lateral line system is morphologically conservative. The data that I present here, however, do not support this general assertion. Variation in neuromast lines, stitch formation, and neuromast parameters including hair cell number and neuromast size, is substantial and of systematic and functional importance.

In Fig. 4-7, I outline three morphological groups of tadpoles -- generalized forms, midwater suspension feeders, and a mixed bag including stream, arboreal, carnivorous, and desert-pond forms -- as determined by their neuromast topography.

In generalized tadpoles across anuran families, neuromast features tend to be conservative (Fig. 4-2b,c,d). In these tadpoles all neuromast lines are present, although their position and extent may vary (Fig. 4-4). Neuromasts form stitches containing two to three

Figure 4-7. A diagram illustrating the three groups of tadpoles based on neuromast topography, that I discuss. In the generalized morphology tadpoles have linear stitches composed of fewer than five neuromasts, small neuromasts, and tend to have fewer than 15 hair cells. Numbers of primary neuromasts (and therefore stitches) range between 250 and 320. Midwater suspension feeders also have stitches, but with large numbers of clumped neuromasts. neuromasts are large and have large numbers of hair cells. The third group is composed of a mixture of stream, arboreal, carnivorous, and desert-pool forms. These tadpoles do not form stitches and have various-sized neuromasts that tend to have large numbers of hair cells.

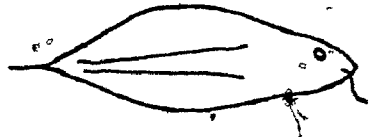
Stream, Arboreal, Carnivorous, Desert-Pond Forms

- stitches absent
- primary neuromasts < 200

Generalized Pond Forms

- stitches present, linearly arranged, with < 5 neuromasts
- primary neuromast counts 250 - 320
- neuromasts small, contain ≤ 15 hair cells

Midwater Suspension Feeders



- stitches present, loosely clumped, with > 5 neuromasts
- neuromasts large, > 15 hair cells

(but sometimes up to five) neuromasts in older larvae. Distance between stitches in older larvae is about 100 µm. There are about 200 - 300 stitches present. Individual neuromasts contain $\leq$ 15 hair cells; hair cells have a surface diameter of 1 - 2 µm.

In generalized tadpoles subtle differences exist in the positioning of neuromast lines between families (Figs. 4-2, 4-4). In the discoglossids the posterior infraorbital line is shifted ventrally away from the eye but still follows the contour of the eye; the longitudinal oral line connects the angular line at its most dorsal extent, and the anterior oral line forms an inverted "U". The hylids may have a reduced longitudinal oral line (Figs. 4-2, 4-4), but not enough species have been examined to know whether this is truly a familial characteristic. Within the Ranidae, there is interspecific variation in the relative extent and position of the oral lines (Fig. 4-3). This variation may reflect slight differences in some related parameter such as tadpole shape.

On the other hand intraindividual variation on the position of neuromast lines (see oral lines in Rana microdisca, Fig. 4-3c,d) suggests that these minor differences in line placements are relatively unimportant (from a functional perspective), perhaps resulting from minor differences in developmental processes. In fact

there is some evidence for developmental influences in the ventral body line of tadpoles (Figs. 4-1, 4-2, 4-3). In tadpoles with a sinistral spiracle the anterior portion of the left ventral body line begins near the ventral midline, courses dorsally, then curves around the spiracle before coursing posteriorly (Fig. 4-3d). On the right side of these tadpoles this line courses the same way even though a spiracle is absent (Fig. 4-3c). In the discoglossid Alytes (Fig. 4-2), which has a ventral medial spiracle, both left and right ventral body lines course in a similar fashion identical to that described above; they appear to "avoid" spiracles that are not present. It therefore appears that the lateral line placode(s) that form these body lines migrate around the gills which, at this developmental stage, protrude from the body. The gills later become covered with an opercular flap. However, the embryonic morphology is maintained in larval Alytes and on the right side of tadpoles with a sinistral spiracle, even though the spiracle does not present an obstacle to neuromast lines.

The stream forms Ascaphus (Ascaphidae), Amolops (Ranidae), and Heleophryne (Leptodactylidae) have primary neuromasts that do not form stitches. They also have reduced numbers of neuromasts, but increased numbers of hair cells per neuromast. The carnivorous Hymenochirus (Pipidae), the arboreal, oophagous Osteopilus brunneus

(Hylidae), and the desert-pond dwelling, omnivorous Scaphiopus (Pelobatidae) also have single neuromasts and reduced neuromast numbers. Together, these tadpoles constitute a morphological grouping based upon neuromast topography (Fig. 4-7). I do not propose one common cause for this convergent morphology, however the result of this morphology may be that these animals are less sensitive to minute water displacements that, given their environments, would constitute background noise.

Xenopus (Pipidae) Rhinophrynus (Rhinophrynidae), and Phrynomerus (Microhylidae) constitute a third morphological grouping based on neuromast parameters (Fig. 4-7). Unlike my second morphological grouping, however, these tadpoles all have a common ecology, they are obligate midwater suspension feeders. All three genera form stitches with more than six neuromasts (up to eighteen in Rhinophrynus), which tend to be clumped rather than linearly arranged (in fact, Murray, 1955 termed them "plaques"). In Xenopus and Rhinophrynus, neuromasts also have large numbers of hair cells (20 - 40 in this study; Shelton, 1971, cites an average of 24 hair cells in Xenopus). The neuromast topography of Xenopus, Rhinophrynus, and Phrynomerus is convergent and is probably specialized (for some as yet unknown reason). Adult Xenopus have linear stitches with many fewer neuromasts (Shelton, 1970), a neuromast topography typical

of tadpoles in other anuran families.

Chiasmocleis (Microhylidae) have stitches with large numbers of linearly arranged neuromasts (Fig. 4-6c). Holmgren and Pehrson, (1949) show the same condition for the midwater, suspension-feeding ranoid Rhacophorus cruciger.

Comparisons with Urodeles

The most striking difference in the organization of neuromasts between anurans and urodeles is that anuran neuromast lines are composed of only one row of neuromasts, while the nasal (anterior supraorbital) and maxillary (anterior infraorbital) lines of urodeles consist of two or three orthogonally oriented rows of neuromasts. The tendency for orthogonal neuromast groupings is, however, maintained in anurans if the oral and angular lines are considered together.

A larger proportion of anurans form stitches than do urodeles. Additionally, when stitch formation occurs in urodeles, it is characteristic of a whole family. In anurans, stitch loss generally occurs within, rather than between, families and appears derived. The family Ascaphidae was the only anuran family that I examined that did not form stitches. This family is monotypic, however, and the tadpole is stream dwelling, which is usually related to stitch reduction.

All anuran stitches are oriented transverse to the axis of maximum neuromast sensitivity. In this regard anurans are like ambystomatid and cryptobranchid urodeles (Chapter 3). Based on this evidence I consider transverse stitches to be the generalized stitch condition for extant amphibians [there is at present no information on stitch formation in caecilians, although Hetherington and Wake, 1979 report no increases in neuromast counts with size in Ichthyophis].

Several aspects of neuromast morphometry vary between anurans and urodeles. While on average, hair cells per neuromast are about equal between the two groups, anuran neuromasts are smaller and tend to be less rectangular than those of urodeles. Hair cell diameters were similarly constant between urodeles and anurans, and among families within these orders (Chapter 5; Fig. 4-5). These values appear to have been unaffected or only slightly affected by specimen preparation techniques: values for hair cell diameters I present here for Xenopus agree with Shelton (1971, fig. 1), who used different preparation methods.

The position of the neuromast sensory epithelium relative to the epidermis varies in urodeles; stream forms have neuromasts sunken into pits or grooves. Sunken neuromasts do not occur to the same extent in anurans; only Heleophryne purcelli and Osteopilus brunneus of the

anurans that I examined had neuromasts sunken into the epidermis. In urodeles, hair cells per neuromast tend to increase with increasing SVL across taxa; there is no evidence for this in anurans.

Chapter 5, A Discussion: The Evolution of the Lateral Line System in Amphibians and its Bearing on Amphibian Phylogeny

Introduction

There is no consensus concerning the origin of modern amphibians. Controversy centers around the relationships of the three extant orders -- Anura, Urodela, and Gymnophiona -- to each other, and the relationships of these modern orders to ancient forms. This debate is due in part to a shortage of reliable systematic characters that allow solid judgements to be made about the relationships among and within these extant orders (e.g., Carroll and Holmes, 1980). One set of structures that has the potential to resolve some phylogenetic issues has, to date, not been extensively considered in amphibian systematics -- the organs of the lateral line system.

I recently examined the lateral line system in approximately sixty amphibian species from nine urodele and eleven anuran families (Lannoo, 1985; Chapters 2, 3, 4). Here I use these data in combination with data collected on the caecilian lateral line system (Taylor, 1950; Hetherington and Wake, 1979; Wahnschaffe et al., 1985) to derive several conclusions about: 1) the use of lateral line organs as systematic characters within amphibians; 2) the evolution of the lateral line system within this group; and 3) amphibian evolution.

Lateral Line Homologies

The lateral line system of modern vertebrates may have developed from the cilia and other structures of the early ostracoderm pore canal system (Denison, 1966; Northcutt and Gans, 1983). This primitive lateral line system appears to have been the precursor of the vertebrate auditory and vestibular systems (e.g., Denison, 1966; van Bergeijk, 1966).

There are two types of lateral line receptors in anamniotic vertebrates -- mechanoreceptive neuromasts and electroreceptive ampullary organs. Ampullary organs are considered to be derived from neuromasts (e.g., Northcutt and Gans, 1983). Ampullary organs probably originated very early in vertebrate evolution; both systems appear to be present and fully formed in the Ostracodermi, the earliest vertebrates with known fossils (e.g., Romer, 1971; Schmalhausen, 1968; Northcutt and Gans, 1983; Boord and McCormick, 1984).

In fossils the lateral line system is preserved as a series of canals, grooves, or pits that are homologous with similar structures in living fishes (Denison, 1947; Stensio 1947; Moy-Thomas and Miles, 1971; Thomson, 1977; Jarvik, 1980). The primary use of lateral line structures in amphibian fossils has been to resolve skull bone homologies (e.g., Thomson, 1967; Schmalhausen, 1968; although see Woodie, 1908; Stensio, 1947). In addition to

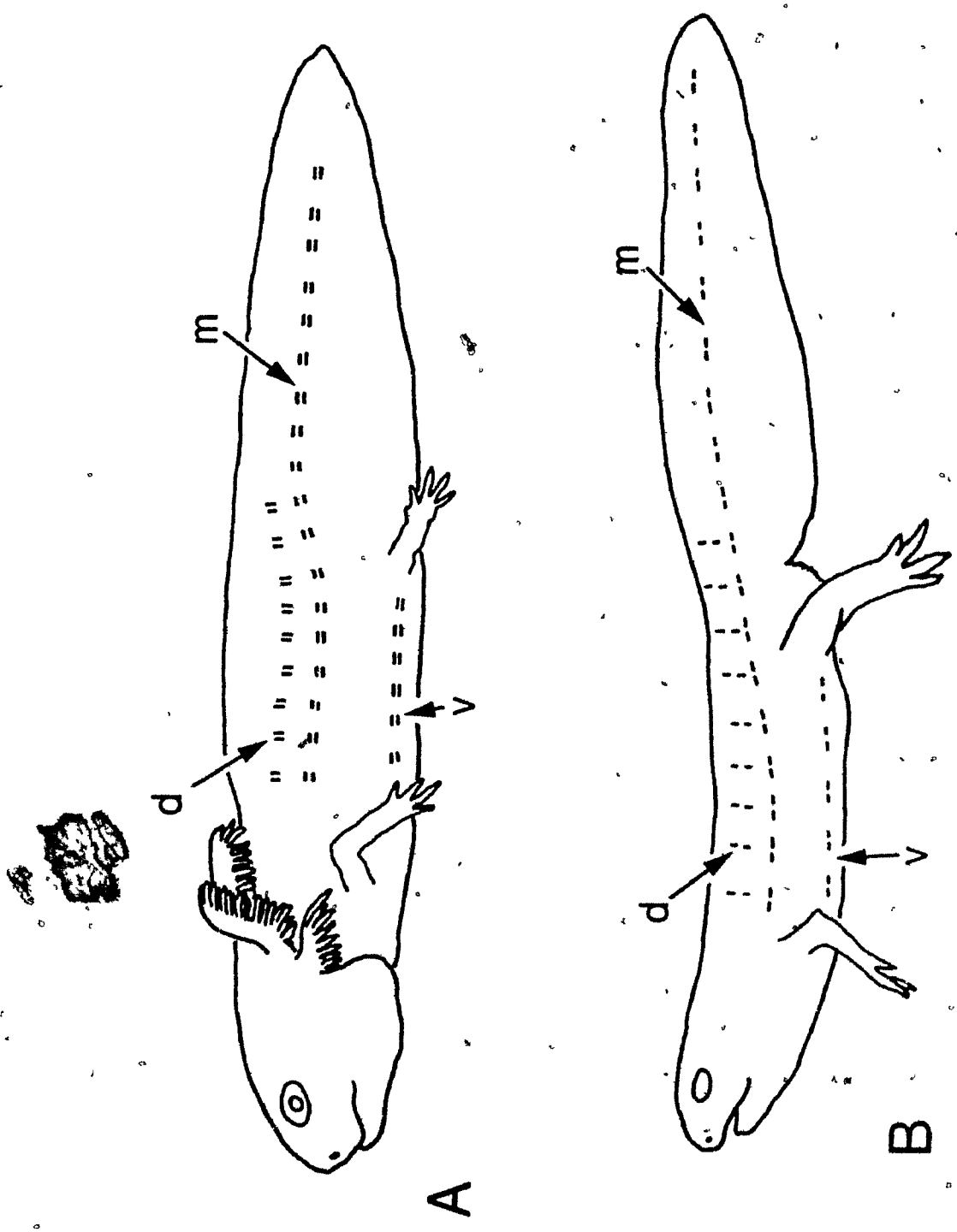
the fossil evidence, lateral line homologies have been proposed on the basis of embryology (Holmgren and Pehrson, 1949) and central nervous system connections (Boord and McCormick, 1984).

Neuromast Orientation and Stitch Formation

Most aquatic amphibians have neuromasts organized into three head lines (supraorbital, infraorbital, and mandibular) and three body lines (dorsal, middle, and ventral). In urodeles and caecilians head lines contain both ampullary organs and neuromasts; body lines contain only neuromasts (Münz, Claas, and Fritzsch, 1982; 1984). Anurans are not known to have ampullary organs (Fritzsch et al., 1984). In all anamniotes, neuromasts are elongated and most sensitive to water displacements along their long axis (Flock, 1965; JØrgensen and Flock, 1973; Flock and JØrgensen, 1974).

Amphibians differ from fishes in two aspects of the gross morphology of their neuromast lines. First, all amphibian neuromasts in each line except the dorsal body line are oriented with their long axis directed rostrocaudally; dorsal body line neuromasts are oriented transversely or dorsoventrally (Fig. 5-1). In fish with bony canals neuromasts are predominantly oriented rostocaudally because canals run in this direction and neuromasts must be most sensitive to fluid displacements

Figure 5-1. Schematic illustrations of stitches along the bodies of salamanders in the genera A) Ambystoma and B) Pleurodeles. (Head neuromasts are not illustrated.) Drawings are modified from Kingsbury (1895). Abbreviations: D = dorsal, M = middle and V = ventral body lines I have drawn two neuromasts per stitch in each drawing; note that the neuromasts are linear. In life, the long axis of each neuromast is parallel to its axis of maximum sensitivity Note that in both genera middle and ventral body line neuromasts are oriented longitudinally, while dorsal neuromasts are perpendicular. Note also that stitch formation in Ambystoma is perpendicular to the long axis of its component neuromasts while in Pleurodeles stitch axes parallel neuromast axes


m
d
v
A
m
d
v
B

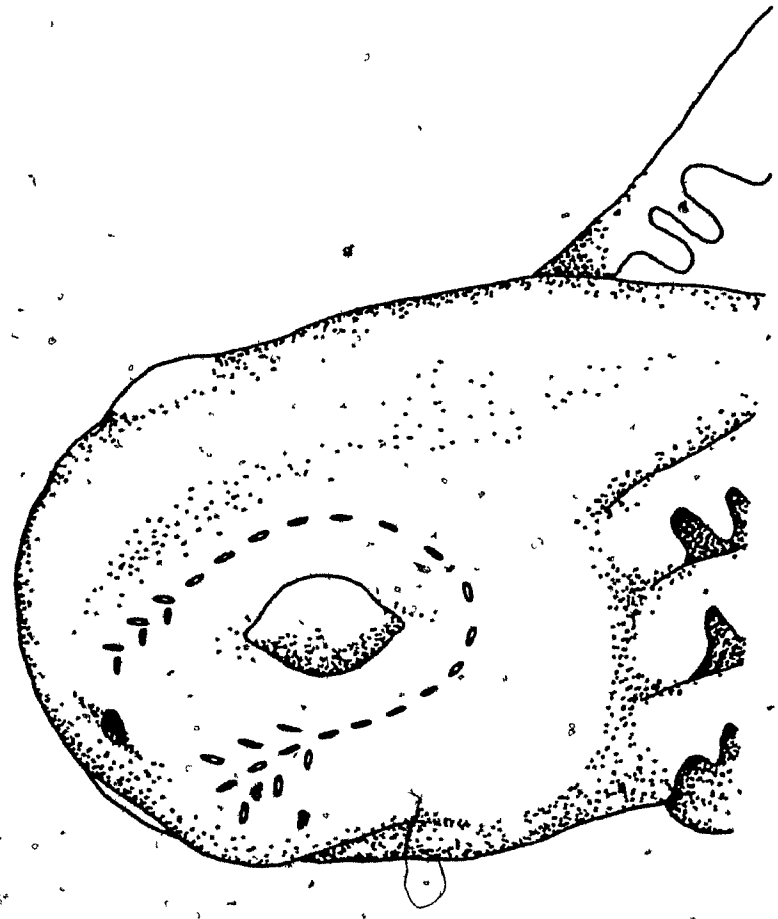
within each canal.

Secondly, amphibian neuromasts may form stitches. In many larval amphibians primary neuromasts divide to form secondary neuromasts that together are termed a stitch (illustrated in Figs. 5-1, 5-2). Stitches may form along one of two axes, either transverse to (Fig. 5-1a), or parallel with (Fig. 5-1b), the neuromast long axis (Chapter 3). Stitch formation in amphibian larvae appears to be a separate developmental event from the embryonic formation of primary neuromasts (Harrison, 1903; Stone, 1933; Winklbauer and Hausen, 1983a,b; 1985a,b). Fish may form neuromast "fields" with epidermally located neuromasts (e.g., Aphredoderus, Branson and Moore, 1962) or have multiple neuromast rows (Webb, 1985), but do not form stitches. The unique patterns of post-embryonic stitch formation suggest that this morphology is derived and homologous within amphibians.

The formation of transverse stitches requires the division of a primary neuromast along its long axis: a direction usually perpendicular to the body axis and to the migration pathway of the neuromast primordia (e.g., Winklbauer and Hausen, 1983a; Fig. 5-1a). In the formation of longitudinal stitches, a primary neuromast divides along its short axis and, in essence, retraces the migration pathway of the neuromast primordia (Fig. 5-1b).

The single major exception to these orientation rules

Figure 5-2. Schematic illustration of the left side of the head of a salamander larva showing the characteristic primary neuromast arrangement of urodeles. Neuromasts are illustrated as open ovals. Note that neuromasts course in a single row around the eye, form two rows on the nasal snout, and three rows on the maxillary snout.



provides an additional insight into the mechanisms of stitch formation. Regardless of whether transverse or longitudinal stitches are formed, primary neuromasts in the dorsal body line of amphibians are oriented transverse to the migration pathway of the neuromast primordia (Fig. 5-1). However, even in this line the rules of stitch formation hold: transverse stitches are oriented perpendicular to the neuromast long axis, but along the migration pathway (Fig. 5-1a); longitudinal stitches are oriented along the neuromast long axis, perpendicular to the migration pathway (Fig. 5-1b). This morphology strongly suggests that the axis of secondary neuromast formation is programmed into each primary neuromast and polarization is not the result of induction by some factor or factors in the surrounding epidermis.

Transverse stitches are present in all anurans that form stitches (Chapter 4), and in the more generalized urodele families Ambystomatidae (Chapter 3) and Cryptobranchidae (Malbranc, 1876). Longitudinal stitches are present in the urodele families Proteidae and Salamandridae (Malbranc, 1876; Harris et al., 1970; Chapter 3). Therefore, I consider transverse stitches to be an ancestral (or plesiomorphic) trait within the modern amphibians, and consistent with the hypothesis of a common amphibian ancestor at least for anurans and urodeles (a view supported on the basis of other characters by Carroll

and Holmes, 1980 and others cited by them).

In both anurans and urodeles, lotic (flowing water) forms do not have transverse stitches; in fact, most lotic forms do not form stitches at all (Chapters 3, 4). However, Necturus (Proteidae), which is found in rivers, forms longitudinal stitches (Chapter 3). I consider longitudinal stitches to be a derived (or apomorphic) trait, and indicative of a close phylogenetic relationship between the proteids and salamandrids. Longitudinal stitch formation also suggests that such an ancestor might have been stream dwelling (Chapter 3).

Both Moodie (1908) and Hilton (1947) have suggested separate, unconventional urodele relationships based on lateral line topography. Moodie (1908) observed a similarity in the origin of the single neuromast line on the tail tip in Necturus and one of the ancient microsaurs, and therefore considered these two groups to be related. Hilton (1947) considered the genera Necturus, Proteus, Siren, Amphiuma, and Dicamptodon to be the most generalized forms based upon neuromast type (i.e., his "elongate, short line type"). I feel that both authors have erred in their interpretations of neuromast characteristics: Moodie (1908) based his phylogeny on a plesiomorphic trait, Hilton (1947) used convergent character states. Additionally, the interpretations of these two authors form unnatural taxonomic groupings based

on other urodele characters.

If transverse stitch formation is a plesiomorphic trait in amphibians, the common ancestor of the modern forms must have had transverse stitches. Here, the fossil record is of some help; it allows an estimate of the period of time in which that ancestor existed.

Fossil Evidence

In ostracoderms the lateral line system is contained within narrow, roofed bony canals. Rhipidistian crossopterygians, the fish group that gave rise to the amphibians, retain this condition, as do the Ichthyostegidae (Stegocephalia), the earliest amphibians. Other, later stegocephalians, however, have neuromasts located in wide, open bony grooves positioned in the same relative locations as the earlier bony canals (Moodie, 1908; Schmalhausen, 1968). Later labyrinthodont and lepospondyl amphibians retained neuromast grooves (Schmalhausen, 1968; Romer, 1971).

Transverse stitches must have been invented in amphibians after neuromasts became freed from the physical constraints of narrow, bony canals. These stitches may have first formed in the wide bony grooves of the non-ichthyostegid stegocephalians (Schmalhausen, 1968) or at any time after these forms evolved (about 350 million years ago), suggesting that epidermally located neuromasts

(the condition in modern forms) are not a prerequisite for transverse stitch formation. Unfortunately, stitch formation does not give many clues as to which early amphibians might have been the ancestors of modern forms; both labryrinthodonts and lepospondyls appear to have had wide neuromast grooves (Schmalhausen, 1968; Romer, 1971).

In looking for a connection between ancient and modern amphibians almost every possibility has been explored (e.g., see review in Gardiner, 1983). Estes (1965), Romer (1971), and Gardiner (1983) prefer a labyrinthodont origin; Schmalhausen (1968) and Carroll and Holmes (1980) prefer a lepospondyl urodele origin and a labyrinthodont anuran origin; Parsons and Williams (1963) prefer a lepospondyl origin. Jarvik (1942) proposes separate anuran and urodele origins from rhipidistians. This latter hypothesis is unlikely if transverse stitches are a plesiomorphic amphibian trait.

Superficial neuromasts may have had some functional advantage to these early amphibians. In modern fishes, lotic forms have neuromasts in canals, while lentic forms have neuromasts located on the epidermal surface (Branson and Moore, 1962). Among amphibians, lentic forms have superficial neuromasts while lotic forms have neuromasts sunken into epidermal pits (Chapter 3, 4). Presumably a sunken position affords neuromasts some protection from currents, while a superficial position allows a greater

sensitivity to water displacements. A second explanation for the migration of roofed neuromast canals to the surface to form open grooves is that it may have occurred as the secondary result of bone loss for other reasons (i.e., to increase the speed or agility of these animals). Bone loss is a trend that has continued up to the present in most modern amphibians.

These two ideas on direct and indirect selection for superficial neuromasts need not be mutually exclusive. Modern urodele larvae frequently feed off the bottom on zooplankton (e.g., Anderson and Graham, 1967; Branch and Altig, 1981; Lannoo and Bachmann, 1984a). Ancient vertebrates are also thought to have been zooplanktonic suspension feeders (e.g., Mallatt, 1986). If ancient amphibians behaved similarly, bone reduction would facilitate floating behavior, and superficial neuromasts would aid in the detection of small pelagic prey.

Evolution within the Amphibians

In addition to transverse stitches, there were perhaps two other features of the lateral line system present in early amphibians that later became modified. Ancestrally, stitches or neuromasts were present in only one row (see figures in Schmalhausen, 1968; Romer, 1971), and the evidence suggests that electroreceptive ampullary

organs were present.

Unlike anurans and known caecilians, urodeles have developed multiple neuromast rows. All urodele larvae have anterior neuromasts arranged in multiple rows, with neuromasts or stitches in adjacent rows orthogonally oriented (Fig. 5-2; Lannoo, 1985; Chapter 2, 3). These orthogonal couplets are oriented similarly across urodele families and are not only unique among amphibians (pending the examination of additional caecilians), but unique among anamniotic vertebrates (although fishes may have orthogonally oriented canals, e.g., Harris and van Bergeijk, 1962). These orthogonal stitch couplets must have arisen after neuromasts became epidermally located, there is no fossil evidence that either bony canals or grooves were arranged in this pattern.

Ampullary organs are present in fossil fishes, most generalized fish classes, and in urodele and caecilian, but not anuran, amphibians (e.g., Northcutt and Gans, 1983; Bullock et al., 1983; Fritzsch et al., 1984). Therefore, we can assume that electroreceptors were present in ancestral amphibians and that anurans have lost this sensory system (Fritzsch et al., 1984). This interpretation is reinforced by the anatomical and physiological similarities between the ampullary organs of amphibians and generalized fishes. These similarities include ampullary organ peripheral anatomy, polarity, and

central nervous system organization. The independently and separately derived gymnotiform and mormyriform teleosts, which have secondarily developed electroreception, differ from both amphibians and non-teleost fishes in their ampullary organ characteristics (Heiligenberg, 1977; Bullock et al., 1983; Fritzsch et al., 1984).

If one accepts the above arguments, it becomes possible to define the modern orders of amphibians based solely on their combinations of lateral line structures, and to outline potential evolutionary pathways based on these morphologies.

Based solely on lateral line structures, urodeles are characterized primitively as having stitches, ampullary organs, and multiple neuromast rows. Anurans are characterized by having stitches, a loss of ampullary organs, and single neuromast rows. Caecilians do not form stitches but have ampullary organs and single neuromast rows. The unique features of each of these three orders are that 1) urodeles have developed multiple stitch rows, 2) anurans have lost ampullary organs, and 3) caecilians have lost the ability to form stitches. Two of these three ordinal traits are based on secondarily lost characters, which must be considered poor phylogenetic indicators (because losses may have occurred independently in widely separate lineages, see Hoyt and Fisher, [illegible]

Nevertheless, in anurans ampullary organ loss probably occurred early in their evolution, perhaps in the Jurassic and concommitantly with the development of the herbivorous tadpole (see Nauthcutt and Gans, 1983; Wassersug, 1975). In amphibians, ampullary organ presence is correlated with carnivorous feeding habits (i.e., the carnivorous urodeles and caecilians posses these organs); it may be that once anurans developed the herbivorous tadpole they no longer needed electroreception and lost ampullary organs. Secondarily carnivorous anuran larvae (e.g., the pipid Hymenochirus boettgeri), which are ecologically similar to urodele and caecilian larvae, do not have ampullary organs.

A second anuran lateral line feature may vary phylogenetically. While the neuromasts of fishes, urodeles and caecilians, and even Xenopus consist of adjacent hair cells that are oppositely polarized, Rana hair cells are reportedly grouped according to their polarities (Jande, 1966, fig. 2 caption). This observation must be confirmed, but if true may be a useful systematic character within the anurans. Neuromast function should not be affected by these differences in hair cell arrangement, because both hair cell orientations are present (e.g., Flock, 1965).

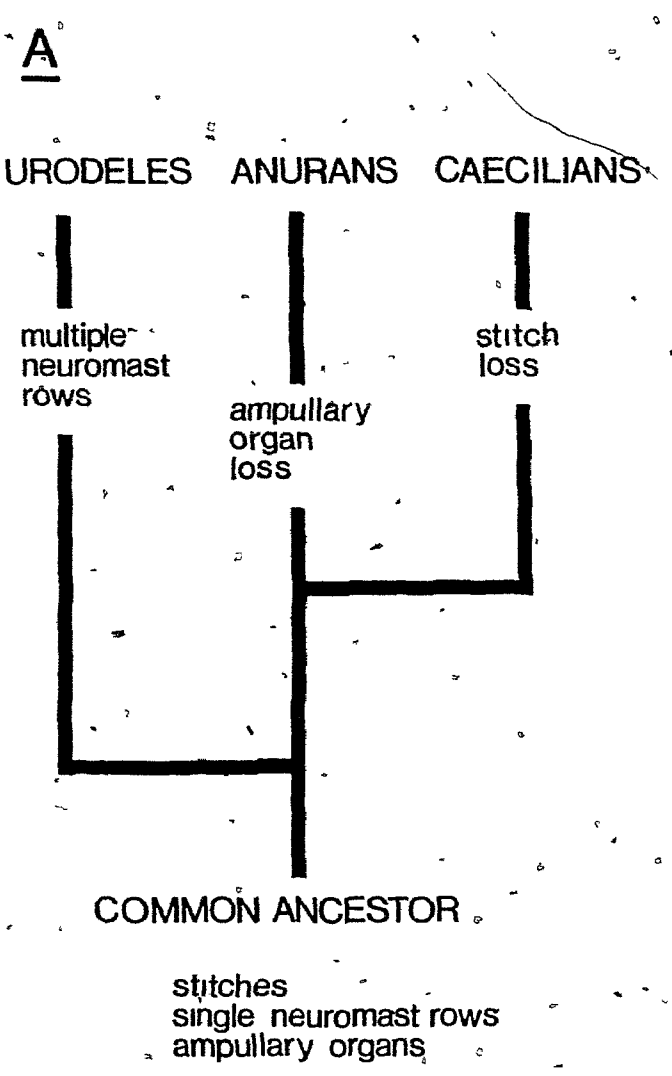
Too few caecilians have been examined to assess whether stitch loss is characteristic of all species. It

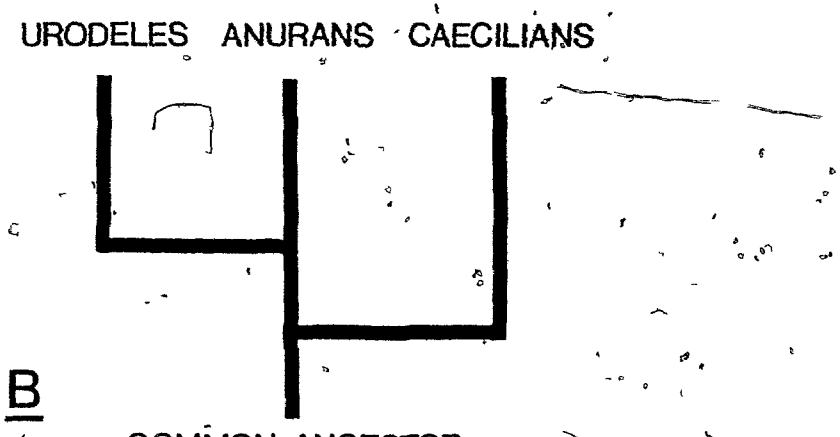
is certainly not an exclusive characteristic of this order because stream dwelling anurans and urodeles also do not form stitches. However, unlike these other two orders, stitch loss may have occurred primitively in caecilians. An additional lateral line character in caecilians may prove to be the presence of only two dorsal body lines (seen in the genus Ichthyophis by Hetherington and Wake, 1979). There is no evidence that aquatic urodeles and anurans ever have fewer than three trunk lines.

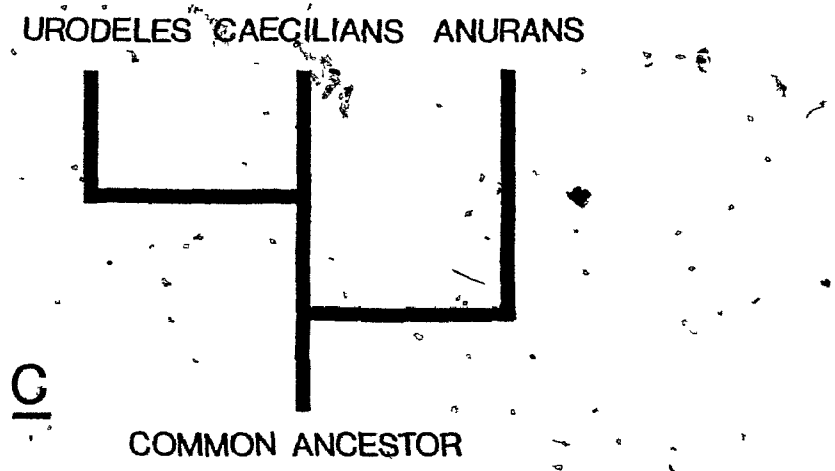
I consider the formation of orthogonal neuromast couplets in urodeles and the loss of ampullary organs in anurans to be fundamental within each of these orders and indicative of an early separation not only between these two groups, but between these groups and caecilians as well. Accepting this, there are three possible phylogenies, assuming that modern amphibians are derived from a common ancestor and that there is not a trichotomy (Fig. 5-3).

Following the arguments proposed here, I suggest that the common amphibian ancestor (at least its larval form) had transverse stitches arranged into single lines, and ampullary organs (Fig. 5-3a). No living forms provide a good model for this ancestor. Caecilians have single neuromast lines and ampullary organs, but do not form stitches (Hetherington and Wake, 1979). Aquatic adult Xenopus and Hymenochirus anurans (Pipidae) have single

Figure 5-3. Three possible phylogenies based upon lateral line characteristics in amphibians. The characteristics of each extant order, as well as the hypothesized common ancestor are given in A. In A, urodeles split off first from the common anuran-caecilian line; in B, caecilians spilt first from the common urodele-anuran line; and in C, anurans split first from the common urodele-caecilian line.


A
URODELES
ANURANS
CAECILIANS
multiple neuromast rows
ampullary organ loss
stitch loss
COMMON ANCESTOR
stitches
single neuromast rows
ampullary organs


URODELES
ANURANS
CAECILIANS
B
COMMON ANCESTOR


URODELES
CAECILIANS
ANURANS
C
COMMON ANCESTOR

neuromast lines and transverse stitches (Escher, 1925) but no ampullary organs (Fritzsch, et al., 1984).

In the first phylogeny (Fig. 5-3a) urodeles split off first from the common amphibian stock and developed multiple neuromast rows, leaving the common anuran-caecilian ancestor with transverse stitches, ampullary organs, and single neuromast rows. Anurans later lost ampullary organs and caecilians lost stitches, both groups retained single neuromast rows.

In the second phylogeny (Fig. 5-3b) caecilians split off first and lost their ability to form stitches, while the common anuran-urodele stock continued on. Urodeles later developed multiple neuromast rows, anurans lost their ampullary organs, and both groups retained stitches.

In the third phylogeny (Fig. 5-3c) anurans split off first from the common amphibian stock and lost their ampullary organs. Urodeles then split from the common urodele-caecilian line, urodeles formed multiple neuromast rows and caecilians lost stitches, both groups retained their ampullary organs.

Because the development of secondary neuromast rows in urodeles is the only independently derived character state among these various phylogenetic scenarios it is impossible to decide among these three possible phylogenies. Perhaps with further information on caecilians we will be able to reject two of these

hypotheses in favor of the third.

Finally, two ecological aspects of the proposed amphibian ancestor can be inferred based upon ecological correlations with lateral line morphology in modern forms. First, in both urodeles and anurans, transverse stitches are characteristic of lentic forms; therefore if this ancestor had transverse stitches it was likely pond or lake dwelling. Secondly, urodeles and gymnophions are carnivorous and posses ampullary organs; if this common ancestor had ampullary organs, it was probably also carnivorous. A similar argument has been proposed for the feeding habits of the earliest vertebrates by Jollie (1982) and Northcutt and Gans (1983). (See also Mallatt, 1984, 1986, for discussions of these arguments.) These ecological scenarios are in line with most current views on early amphibian ecology made independently of lateral line considerations (e.g., Nussbaum, 1985 for urodeles; Wassersug, 1975 for anurans), although they contradict Schmalhausen's (1968) ideas of stream origins for modern forms.

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