

Morphology and Function of the Feeding Apparatus of the Lungfish, *Lepidosiren paradoxa* (Dipnoi)

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ABSTRACT The feeding mechanism of the South American lungfish, *Lepidosiren paradoxa* retains many primitive teleostome characteristics. In particular, the process of initial prey capture shares four salient functional features with other primitive vertebrates: 1) prey capture by suction feeding, 2) cranial elevation at the cranio-vertebral joint during the mouth opening phase of the strike, 3) the hyoid apparatus plays a major role in mediating expansion of the oral cavity and is one biomechanical pathway involved in depressing the mandible, and 4) peak hyoid excursion occurs after maximum gape is achieved.

Lepidosiren also possesses four key morphological and functional specializations of the feeding mechanism: 1) tooth plates, 2) an enlarged cranial rib serving as a site for the origin of muscles depressing the hyoid apparatus, 3) a depressor mandibulae muscle, apparently not homologous to that of amphibians, and 4) a complex sequence of manipulation and chewing of prey in the oral cavity prior to swallowing. The depressor mandibulae is always active during mouth opening, in contrast to some previous suggestions.

Chewing cycles include alternating adduction and transport phases. Between each adduction, food may be transported in or out of the buccal cavity to position it between the tooth plates. The depressor mandibulae muscle is active in a double-burst pattern during chewing, with the larger second burst serving to open the mouth during prey transport. Swallowing is characterized by prolonged activity in the hyoid constrictor musculature and the geniothoracicus.

Lepidosiren uses hydraulic transport achieved by movements of the hyoid apparatus to position prey within the oral cavity. This function is analogous to that of the tongue in many tetrapods.

A key event in the evolution of land vertebrates from fishes was the transition from aquatic suction feeding to terrestrial feeding with the tongue and jaws. This transition involved numerous major changes in the feeding mechanism, such as the evolution of novel mechanisms for acquiring food, handling it in the oral cavity, and swallowing the processed food (Bramble and Wake, '85).

Although much has been written about the fish-amphibian transition, little information exists on the feeding mechanics of the Dipnoi or lungfishes, one of the most important living groups relevant to the origin of terrestrial feeding (Thomson, '69). The phylogenetic position of lungfishes makes this group critical to our understanding of vertebrate evolution. Whether lungfishes are regarded as the sister group of tetrapods (Rosen et al., '81) or whether they are considered to be a

more distant outgroup (Schultze and Trueb, '81), lungfishes are the closest *living* outgroup of tetrapods, *Latimeria* probably being more distantly related to tetrapods than previously supposed (Miles, '77; Wiley, '79; Rosen et al., '81). As such, dipnoans are in a unique position to offer insight into the evolution of tetrapod feeding systems. At the same time, members of the radiation of lungfishes, distinct from its first appearance in the lower Devonian, have diverged extensively from the common primitive body plan that lungfishes and tetrapods shared. The extant lungfishes are in some respects poor models of phylogenetically primitive lung-

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fishes. We know this, because their excellent fossil record documents extensive morphological change during their evolution (Bemis, '84b). For example, early in their history, lungfishes exhibited much evolutionary diversification and specialization in the feeding apparatus, and two distinct groups—one with specialized tooth plates and the other showing periodic growth and shedding of denticles—can be recognized (Campbell and Barwick, '83).

In this paper, we provide the first cinematographic and electromyographic study of the feeding system of any dipnoan. The subject of our study is the South American lungfish, *Lepidosiren paradoxa*. *Lepidosiren* and the closely related African lungfishes, *Protopterus* spp., are members of a clade that is distinct in many respects from the Australian lungfish, *Neoceratodus*. Thomson ('69) provided a general discussion of dipnoan food and feeding systems, and concluded that the manner of feeding in all three genera is similar. In the only detailed examination of the feeding mechanics of living lungfishes, Perkins ('72) based conclusions about muscle function solely on dissections. Most research on the functional morphology of lungfishes has focused on respiration and circulation, such as the electromyography of respiration in *Protopterus* (McMahon, '69), cineradiography of air breathing in *Lepidosiren* (Bishop and Foxon, '68), and functional anatomy and physiology of lungfish hearts and circulatory systems (e.g., Johansen and Hanson, '68).

The major goal of our study was to describe and analyze the feeding of *Lepidosiren* and use this information to identify anatomical and functional features of the feeding system that are primitive to dipnoans. These primitive features are then compared to those previously identified in the feeding systems of other lower vertebrates (e.g., ray-finned fishes, coelacanths, and salamanders). Comparison allows us to recognize which features of the vertebrate feeding mechanism are phylogenetically common to all of these lineages, which features are common to tetrapods and lungfishes, and which are specializations limited to the Dipnoi. A second goal was to provide a detailed functional analysis of the tooth plates, which are the major specialization of the feeding mechanism of extant lungfishes. Unlike the vast majority of fishes, lungfishes process food items using a marginally placed dental apparatus, with movements that are broadly convergent to

mammalian mastication. A final goal of this paper will thus be to discuss some of these convergent features, particularly the lingual transport system typically used by tetrapods and the hydraulic transport system used by lungfishes.

MATERIALS AND METHODS

Animals and diet

Nine individual *Lepidosiren paradoxa* obtained from commercial dealers were available for this study. The five specimens used for experiments ranged in size from 15 cm total length (TL) to 20 cm TL. Individuals were housed in 20-liter aquaria held at approximately 26 degrees C. Animals were trained to feed readily on pieces of earthworm (*Lumbricus*). A special training period to accustom the animals to feeding under the bright movie lights began one month prior to the start of filming. Individuals were starved for up to two weeks prior to either filming sessions or electromyographic studies. Large African lungfish, *Protopterus aethiopicus*, are known to feed extensively on molluscs, although smaller individuals consume a diversity of both hard and soft bodied invertebrates (Corbet, '61). Sparse ecological data available for *Lepidosiren* is inconclusive concerning dietary specialization, though it seems likely that they are opportunistic omnivores. Carter and Beadle ('30) maintained young specimens on a diet of earthworms, prepared (presumably shell-less) snails, and plant material. They considered plant material to be especially important, and stated that only a few potential prey items—worms, insects, and insect larvae—were available in the water where the fish were taken. On the other hand, Lankester (1896), citing the field observations of Bohls, stated that *Lepidosiren* feeds chiefly on the hard-shelled snail *Ampullaria*, and that plant material may be ingested by accident.

Anatomy

Gross morphological features of the feeding apparatus (osteology, arthrology, and myology) were examined by dissection using a Zeiss IVb dissecting microscope. A camera lucida attachment was used to prepare the figures from dissected specimens and cleared and stained specimens. Three individuals were cleared and stained (for both cartilage and bone) following procedures modified from Wassersug ('76) and Dingerkus and Uhler ('77). In addition, prepared skeletons were

used to examine osteological details. Both dry and cleared and stained material was also available for *Protopterus*.

The serially sectioned head of a single individual prepared for an earlier study (Bemis, '84a) was available for analysis. Briefly, this specimen was fixed in 5% neutral buffered formalin, embedded in low viscosity nitrocellulose (type RS 1/2, Hercules Inc., Wilmington, Delaware), and serially cross sectioned at 30 μm . Most sections were stained with Ehrlich's hematoxylin or Verhoeff's elastin, counterstained with picroponceau or PAS, following methods outlined in Humason ('72). These sections were studied for details about the tooth plates, cranial bones, and muscles.

Cinematography and video

Kodak 4X Reversal Film was used in a Photosonics 16-1PL camera to record all phases of feeding. Filming rate varied from 200 frames per second (for suction feeding) to 100 frames per second (for extended slow chewing sequences). Three 600 Watt Smith-Victor filming lights provided illumination. We filmed more than 40 suction feeding sequences. Nineteen of these sequences met the minimum requirements for further kinematic analysis, in that the view was lateral throughout the sequence and the film was judged to be up to speed on the basis of timing light marks on the edge of the film. Ten chewing sequences were filmed, and the two longest usable sequences, each representing an entire "bout" of activity (see below), were analyzed in detail.

Kinematic patterns were analyzed by projecting every third frame of a sequence onto a Houston Instruments Hi-Pad digitizer and measuring a series of variables. Measuring every third frame provided more than adequate resolution of the extreme excursions, based on the frame-by-frame analysis of several test sequences. Variables measured in both the suction feeding and chewing sequences were: hyoid depression, gape distance, distance from the prey to the plane of the gape, and cranial elevation. Two additional variables were measured in the chewing sequences: adductor bulge and "crush." Adductor bulge is the perpendicular distance that the adductor mandibulae posterior muscle bulges above the surface of the head. The "crush" measurement is highly correlated with adductor bulge, but differs in that it incorporates information on the positions of

both the lower jaw and the hyoid apparatus. It was measured as the perpendicular distance from a line drawn between the hyoid and the lower lip to the most indented point on the lower jaw between the two end points. Together, these two measurements provide a qualitative estimate of both the length and strength of the adduction phase of chewing.

To aid in interpreting electromyographic recordings, simultaneous video recordings were made of feeding behavior. A Panasonic video cassette recorder, two Panasonic video cameras, and a special effects generator permitted the recording of the animal's behavior and the simultaneous electromyographic activity as displayed on the chart recorder. The time between frames on the videotape was .0333 sec, more than adequate for the analysis of prolonged chewing sequences. Overall, ten simultaneous video and electromyographic sequences were analyzed, each including sustained chewing bouts; because each feeding sequence involves one prey-capture suction and a small number of constrictions, we recorded fewer successful simultaneous recordings of these events.

Electromyography

Electromyograms were obtained using standard procedures (see Lauder, '83a,b). Briefly, the fish was anaesthetized with tricaine methane sulfonate, and fine wire steel alloy bipolar electrodes were implanted in as many as eight cranial muscles. We based our protocol for electrode placement on detailed study and measurement of dissected specimens. Owing to the small number of living specimens available, we did not kill individuals to check our electrode placements following successful recordings. Consistency of electromyographic patterns from implantation to implantation was accepted as evidence of consistent electrode placement.

Simultaneous signals from up to six muscles were amplified with Grass P511J preamplifiers (set for a 30 to 3000 Hz bandpass) and recorded on tape with a Bell and Howell 4020A FM tape recorder. Electromyograms were played back at one-eighth speed on a Gould 260 chart recorder, so that the effective frequency response of the myograms displayed on the chart recorder was nearly 1,000 Hz.

Electromyographic patterns obtained during feeding were analyzed in several ways. First, the overall pattern of muscle activity was summarized using the method outlined

in Lauder ('83a). For each of the three behaviorally discrete events discussed below, a summary diagram was constructed (e.g., Figs. 10, 13). This was done by choosing a reference muscle for each behavior, and using the onset of activity in this muscle as the point from which the onset and offset times for activity in other muscles were measured. Our method for determining onset and offset times is somewhat subjective, particularly in the case of muscles such as the adductor mandibulae, in which a train of isolated spikes typically precedes the major continuous burst of activity (e.g., Figs. 12, 14). In such cases, we accepted the first spike of an evenly spaced train of spikes as the onset of a muscle's activity period. A number of electromyograms was measured and, for each muscle, the mean onset and offset times, the standard errors of these means, and the percentage of experiments that the muscle was active were calculated. This information was combined into a summary diagram showing the overall pattern of activity for the behavior.

We also analyzed the amplitude and spike patterns of electromyograms by playing the signals back from the tape recorder into a 12 bit DAS analog to digital converter. The tape recorder speed was slowed down to give an effective sampling frequency of 8,000 Hz. The digitized myograms were stored on an IBM XT computer where the signals were processed using the spike counting algorithms developed by Beach et al. ('82) and Gorniak and Gans ('80). Both digitized myograms and the derived spike and amplitude analyses were plotted using an HP 7470A plotter (1000 points per inch resolution; Fig. 14).

To be sure that the sample rate of the analog to digital conversion was adequate to represent the information in the electromyograms, we sampled one channel of data at a rate of 31.4 KHz. A Fast Fourier Transform (FFT, calculated using the Cooley-Tukey algorithm) of these signals showed that virtually no power is present above 1000 Hz, and thus our sample rate of 8,000 Hz more than satisfies the Nyquist sample criterion.

Statistical analyses

Statistical procedures used in this study (analysis of variance, both nested and one-way, regression, paired t-tests) generally follow Sokal and Rohlf ('81). One of the statistical analyses in this paper, the nested analysis of variance (used to assess interindividual

differences, differences due to electrode implants and experimental days) follows procedures presented in Shaffer and Lauder ('85a), where this approach is discussed further.

RESULTS

Anatomy

Osteology and tooth plates

Figure 1 illustrates the cranial bones and cartilages of *Lepidosiren*. In comparison to the primitive condition for osteichthyans and amphibians, the skulls of living dipnoans show a reduction of many bony elements (Bridge, 1898; Miles, '77). For example, the maxillary-premaxillary arcade, which is only debatably present in any dipnoan (Rosen et al., '81; Campbell and Barwick, '83), is absent in all three living genera. Such extreme reduction and modification make it difficult to identify homologies of some cranial bones. Our terminology follows Bemis ('84a).

The largest and most important dermal elements from the standpoint of the feeding mechanism are the pterygoid and prearticular (Fig. 1: PT, P). These bones support the massive, three-bladed tooth plates characteristic of lepidosirenids. The right and left prearticular bones are immovably attached to each other at the broad mandibular symphysis; a similar symphysis binds the right and left pterygoids to each other. Because of the absence of the maxillary arcade and dentary, the prearticular and pterygoid tooth plates topologically make up the margins of the jaws, even though they are located well behind the lips and the oral opening (Fig. 1). During feeding, the anatomical limitations of the jaw joint and symphysis restrict the motion of the lower jaw to an up and down hinge-like motion, which in turn, causes the tooth plates to occlude precisely (Bemis, '84a). Anteriorly, a small pair of so-called vomerine teeth are embedded in cartilage beneath the dermal ethmoid (Fig. 1). The articulation of the dermal ethmoid with the supraorbital is not kinetic as believed by Berman ('79). The fossa for the large adductor mandibulae muscles are bounded above by the supraorbital, on its median surface by the frontal bone and braincase, and below by the squamosal. Beneath the squamosal are the two small elements of the opercular series, greatly reduced from their condition in fossil dipnoans (Fig. 1: OP, SOP).

The only element of the visceral skeleton exhibiting any ossification is the ceratohyal (Fig. 1:CH). The ceratohyals are suspended

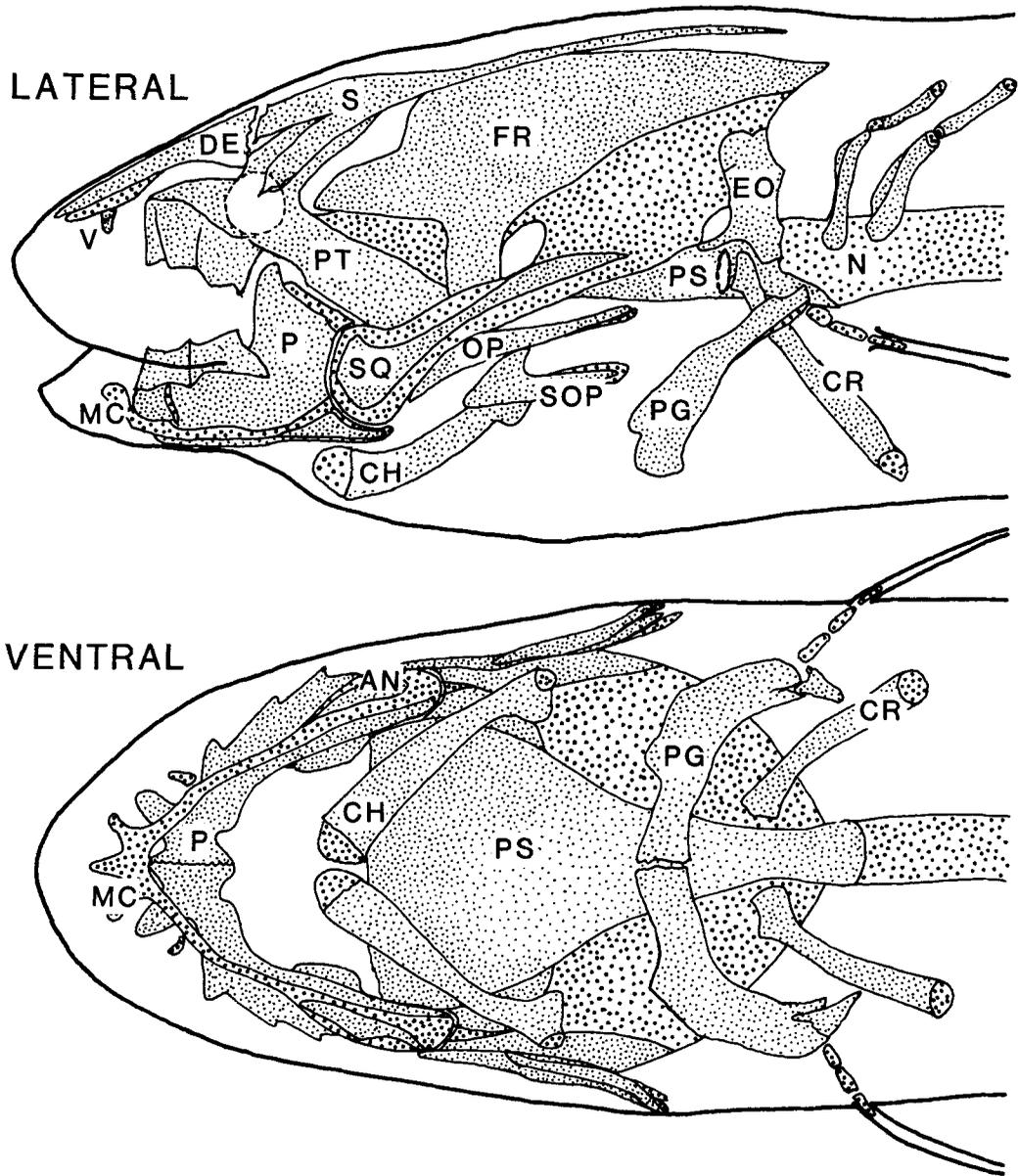


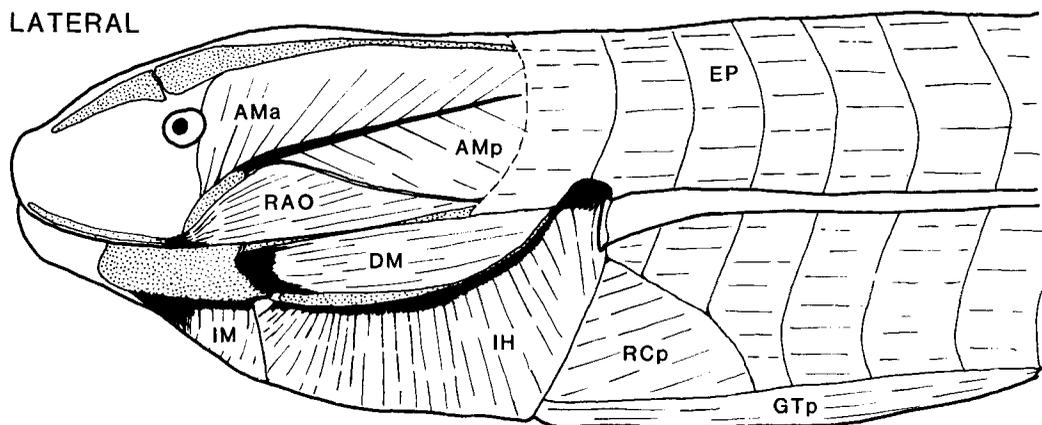
Fig. 1. Lateral and ventral views of the cranial skeleton of *Lepidosiren paradoxa*. Note the vomerine tooth (V), and the pterygoid (PT) and prearticular (P) bones, which support the tooth plates. Also, note the relationship of the ceratohyal (CH) to the pectoral girdle (PG)

and cranial rib (CR). Other labeled elements are: AN, angular; DE, dermal ethmoid; EO, exoccipital; FR, frontal; MC, Meckel's cartilage; N, notochord; OP, operculum; S, supraorbital; SOP, suboperculum; SQ, squamosal.

by hyoid suspensory ligaments from the ventral surface of the quadrate cartilage and squamosal bone. A second ligament, the mandibulohyoid ligament, ties each ceratohyal to the lower jaw. Cranial ribs (Fig. 1:CR) extend ventro-posteriorly from the posterior

corners of the skull. Anterior-posterior motion of the ribs is allowed by the well-developed synovial articulations with the skull. The pectoral girdle is embedded in the musculature halfway between the cranial ribs and the ceratohyal.

LATERAL



VENTRAL

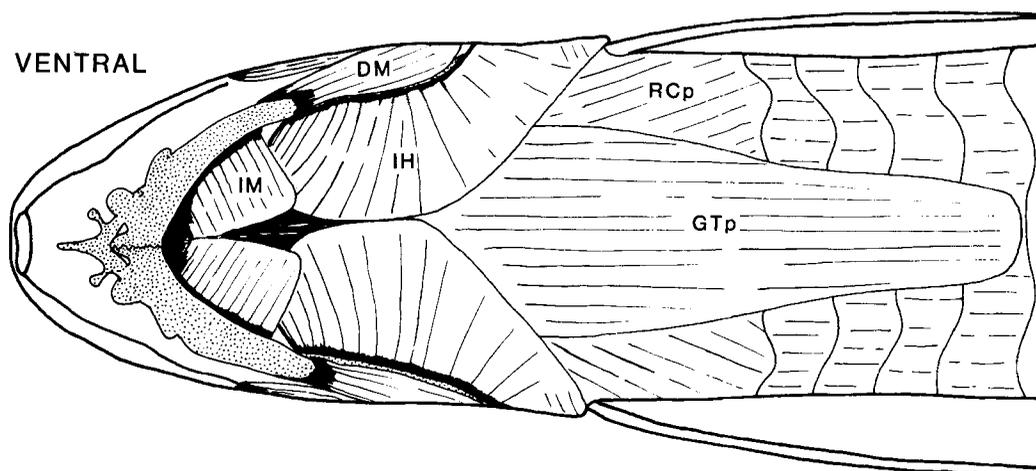


Fig. 2. Lateral and ventral views of the superficial cranial musculature of *Lepidosiren paradoxa*. AMa and AMp, adductor mandibulae anterior and posterior; DM, depressor mandibulae; EP, epaxialis; GTa and GTp, geniothoracicus (anterior and posterior subdivisions are for

electromyographic purposes); IH, interhyoideus; IM, intermandibularis; RAO, retractor anguli oris superficialis; RCa and RCp, rectus cervicis (anterior and posterior subdivisions are for electromyographic purposes).

Myology

The major cranial muscles of *Lepidosiren* are illustrated in Figure 2. Many authors have contributed to understanding the cranial musculature of dipnoans, including Owen (1839), Bridge (1898), Luther ('13), Edgeworth ('26, '35), Fox ('65), and McMahon ('69). However, not all authors agree on the details of cranial myology in the group (e.g., Wiley, '79; Jollie, '82). For the present purpose of clarifying function, it is simplest to divide the musculature into five functional categories based on origins and insertions, and then describe the individual muscles of each group. These groups are: 1) jaw closing; 2)

jaw opening; 3) jaw and hyoid compressing or constricting; 4) hyoid depressing; and 5) head raising.

There are four muscles in the first group (jaw closing muscles), and all are innervated by the trigeminal nerve: the adductor mandibulae anterior and posterior, and the retractor anguli oris superficialis and profundus (Fig. 2). The major jaw closing muscles are the pinnately fibered anterior and posterior divisions of the adductor mandibulae. Although these two portions are distinct, they arise developmentally from a common primordium (Edgeworth, '35), and insert on the coronoid process of the lower jaw via a common tendon. Both portions of the adduc-

tor mandibulae were studied electromyographically.

The remaining two muscles of the first group, the lip retractors, originate from the fascia covering the posterior portion of the adductor mandibulae as well as the squamosal bone. They insert on the connective tissue of the upper lip. The superficial lip retractor was studied electromyographically.

The three muscles in the second group are all potential jaw-opening muscles: the geniothoracicus, rectus cervicis, and depressor mandibulae. The geniothoracicus and rectus cervicis are hypobranchial muscles, while the depressor mandibulae is derived from the hyoid arch. The geniothoracicus originates on the fascia of the ventral trunk muscles and its straight, parallel fibers continue anteriorly to the area beneath the hyoid (Fig. 2). The muscle becomes a thin tendinous sheet by the point of its insertion near the symphysis. For the purposes of electromyography, we distinguished an anterior portion at the hyoid and a posterior portion in the belly of the muscle. The large recti cervicis muscles are described under group 4 below. Although hyoid depression is the main function of these muscles, they are potential jaw depressors due to the ligamentous connection between the hyoid and mandible.

The muscle we refer to here as the depressor mandibulae has been known by a variety of names, most commonly, retractor mandibulae (e.g., McMahon, '69; Edgeworth, '35). Thomson, ('69, pg. 98) refers to a posterodorsal depressor mandibulae muscle of dipnoans, presumably the same muscle we designate here. Our use of the name depressor mandibulae is intended to reflect the correct function of the muscle. The muscle originates from the surfaces of the opercular and subopercular bones and inserts via a tendon onto the posterior corner of the lower jaw. Due to the large diameter of the jaw joint, the muscle actually inserts quite far from the center of rotation of the joint. Thus, mechanically, the depressor mandibulae is well suited to depress the lower jaw. Functionally, the jaw joint cannot allow retraction. Electrodes were placed in the center of the muscle.

The two muscles of group three, the intermandibularis and interhyoideus, are derived from the mandibular and hyoid arch respectively. The muscles originate as thin sheets from the ventral borders of the lower jaw and suboperculum and meet in a tendinous sheet at the ventral midline. The posterior part of

the interhyoideus forms the muscle surrounding the opercular opening. The intermandibularis and interhyoideus muscles function as constrictors, acting to raise the hyoid apparatus or stiffen the opercular flap. Both were studied electromyographically.

In the fourth group is the major hyoid depressor, the rectus cervicis (= sternohyoideus). At its posterior end, the large paired rectus cervicis muscles are continuous with the ventral trunk musculature: some fibers originate from the cranial ribs, others at the pectoral girdle. The fibers of the recti cervicis run antero-medially to insert on the ceratohyals. The primary action of these muscles is to depress the hyoid; however, hyoid depression is coupled to lower jaw depression by the mandibulohyoid ligament. Anterior and posterior subdivisions of this muscle were recognized for electromyographic study. Anterior electrodes were placed near the muscle's insertion on the ceratohyal. Posterior electrodes were located in the main body of the muscle, ventral to the base of the pectoral fin.

The fifth group, the head-elevating muscles, consists of the epaxialis. The epaxial muscles continue from the trunk up over the posterior end of the adductor musculature, where they are attached both to the skull and to the fascia overlying the adductor muscles. Epaxial muscles were studied by placing electrodes about 0.5 cm posterior to the skull.

Histology

Parker (1892) is an excellent source on cranial histology of *Protopterus* and *Lepidosiren*. In cross section, the oral cavity is shaped like an inverted "U" (Fig. 3A,B). The palatal mucosa is underlain by the parasphenoid bone, ethmoid cartilage, and dense connective tissue, and the sides of the cavity are supported by the ventral wings of the pterygoid bones.

Anteriorly, the mucosal surface of the tongue is supported by a stiff, chondroid connective tissue "pad" (Fig. 3A,B). The tongue pad continues posteriorly, along the lateral margins of the tongue, where it supports a prominent ridge on each side. Posteriorly, the mucosal surface of the tongue is supported by a thin layer of connective tissue lying over the large recti cervicis muscles. There is no separate intrinsic musculature. The outer fibrous layer of the tongue pad is continuous with the periosteum of the ceratohyal bones. Thus, the tongue cannot move

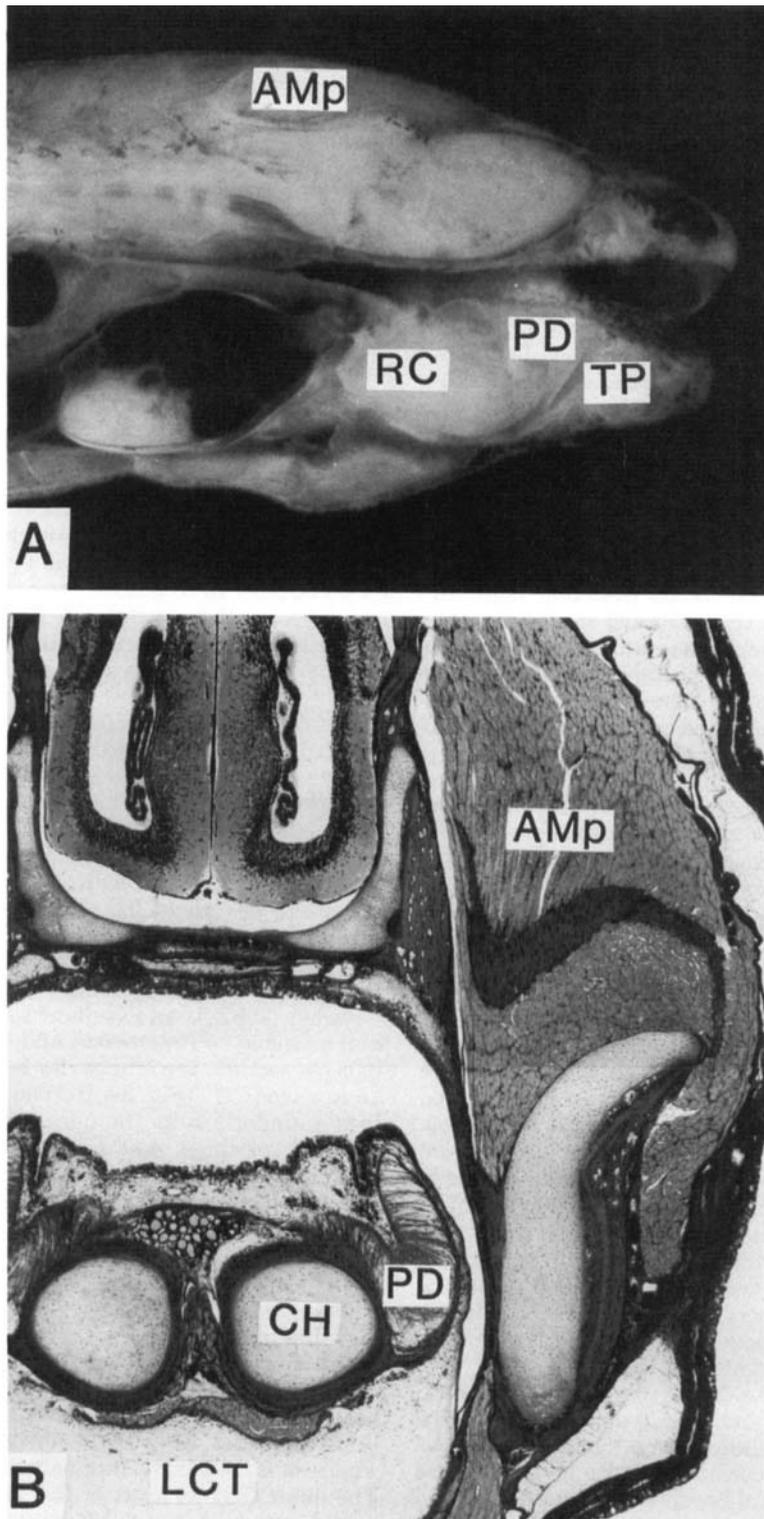


Fig. 3. A) Mid-sagittal view of *Lepidosiren* head, showing oral cavity, large rectus cervicis muscle (RC) and tongue pad (PD) forming the body of the tongue, position of prearticular tooth plates (TP), and margins of lips extending anteriorly. B) Cross section through the

head of *Lepidosiren*, showing the shape of the oral cavity, ceratohyal bones (CH), lateral wings of the tongue pad (PD), loose connective tissue beneath hyoid and tongue (LCT), and the large adductor mandibulae posterior muscle (AMp).

independently of the hyoid apparatus. When the hyoid is in a raised position, the tongue nearly fills the oral cavity, so that there is little dead volume prior to mouth opening.

Strong ventral movements of the hyoid apparatus and its attached tongue occur during feeding and respiration. The sublingual region is filled with a very loose connective tissue, providing an easily deformed cushion into which the hyoid apparatus can be moved (Fig. 3). Scalation of the skin in this region is suppressed, presumably facilitating distension.

Anteriorly, the mouth is constricted to an approximately circular opening. The lips extend out, away from the underlying bones. They are supported by various cartilaginous derivatives of the nasal capsule and Meckel's cartilage as well as abundant chondroid tissue. The margin of the upper lip curls laterally around the margin of the lower lip. The upper lip is supported by a rod of chondroid tissue that contains elastic fibers (Bertmar, '66, believed this rod to be homologous with an upper labial cartilage). The lower lip does not contain a comparable support bar, though its medial edge is well defined, with a slight curl that mates with the upper lip.

The tooth plates are surrounded by fleshy pads of tissue so that only the apical ridges of the tooth plates appear to project from the epithelium (Parker, 1892). Like the tongue pad, these tooth plate pads are supported by a core of chondroid connective tissue. The pads are easily deformed or moved when pressed upon. Thus, food items being chewed presumably contact more than just the exposed ridges of the tooth plates.

Overview of events during feeding

The process of prey capture and swallowing in lungfishes may be divided into four components: 1) approach to the prey; 2) the strike; 3) chewing; and 4) swallowing. The approach to prey located on the bottom often occurs with the head depressed ventrally and the anterior body arched. Prolonged searching may occur before prey are located, and during this time the head is usually maintained at an angle to the substrate.

After a food item is located, the entire feeding sequence may last from 10 seconds to several minutes, with the average in this study being 36 seconds. Figure 5 summarizes the events and terminology used here to describe feeding. The strike is usually initiated only when the prey is either touching the

snout or within several millimeters of it (Fig. 4: frame 1). Strikes are characterized by a relatively short duration (between 50 and 200 ms), during which the prey is engulfed and carried into the buccal cavity with the flow of water created by expansion of the mouth cavity (Fig. 4). There is usually a pause of from one to several seconds following the strike before chewing begins. This is shown in Figure 5 by the absence of any electromyographic activity immediately following the strike. Chewing of the prey between the tooth plates occurs in several stages that we refer to as *chewing bouts*, with successive bouts separated by pauses (Fig. 5). From one to five or more chewing bouts may occur during a feeding (mean = 2.5, based on 16 feedings).

Each chewing bout consists of a series of chewing or adduction cycles (mean number of cycles per bout = 7.2, based on 40 bouts). Each chewing cycle in turn consists of a period of jaw adduction in which the tooth plates are adducted and the prey crushed (= adduction phase), and a period during which the prey is transported within the oral cavity and repositioned between the tooth plates (= transport phase). Alternation of these two phases produces the key characteristic of the chewing bouts, which is the movement of prey into and out of the oral cavity, with crushing of the prey occurring between each transport phase. Figure 6 illustrates a chewing sequence in which the prey is being transported into the mouth cavity (frames a, b, and c), crushed (frames d to k), and then again transported into the mouth (frames l to o) before being crushed again.

After the final chewing bout has ended, the prey is located within the mouth cavity and swallowing occurs. Swallowing may be initiated by rapid hyoid depression that creates a strong flow of water through the oral cavity and moves the prey posteriorly to the pharyngeal region. Subsequent contraction of the hyoid musculature constricts the posterior part of the buccal cavity and forces the food into the esophagus. We term this behavior a constriction or buccal compression.

The strike: initial suction

Photographs of a complete strike are given in Figure 4. Considerable variation was found both within and between individuals in the time course of prey capture by suction feeding. Gape profiles, for example, can be either unimodal or have multiple peaks and can vary significantly in duration (Fig. 7A).

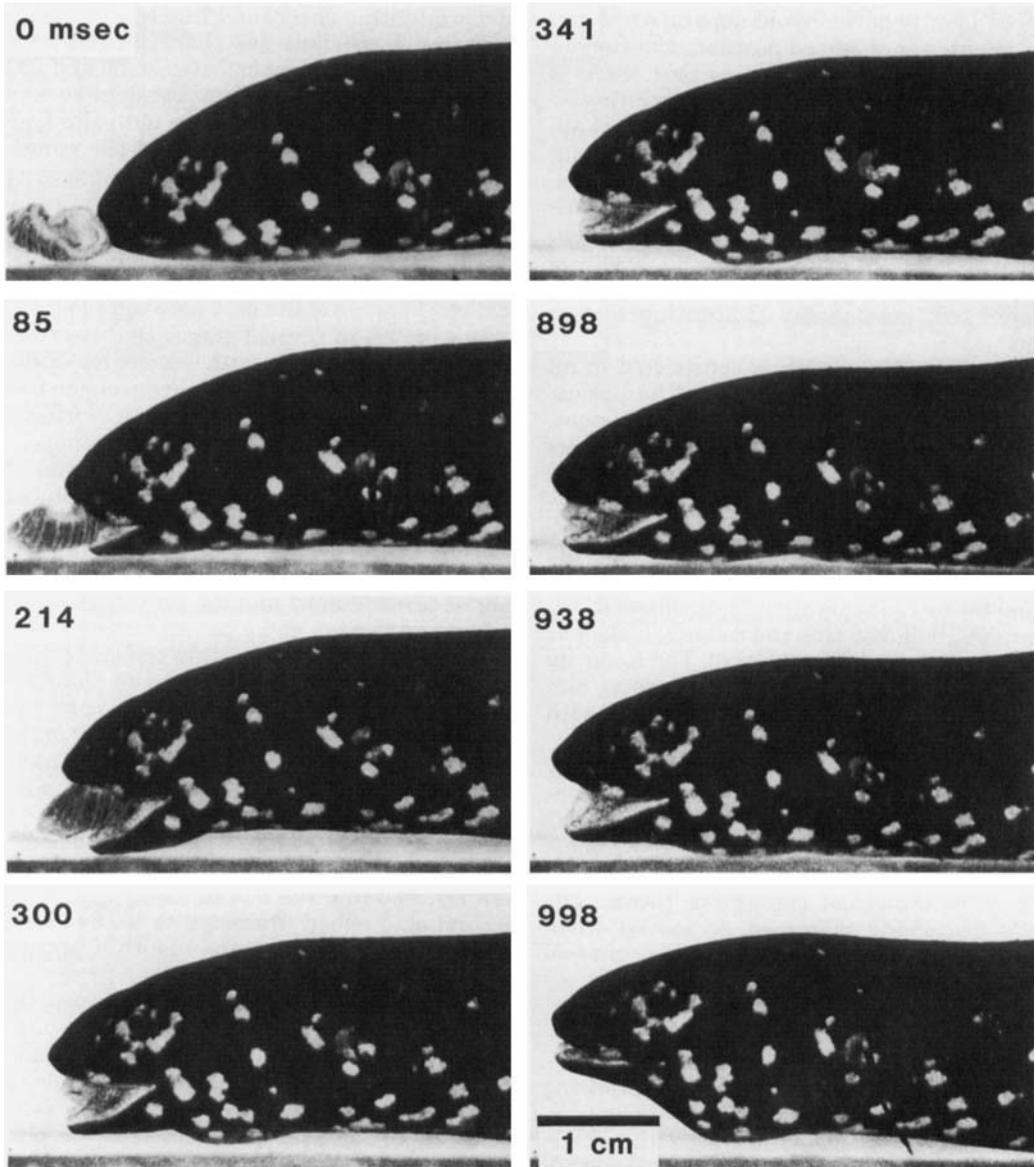


Fig. 4. Eight frames from a film taken at 200 frames/second to show the pattern of head movement that occurs during a strike at a piece of worm located on the

bottom of the tank. Peak hyoid depression occurs in the last frame.

The dominant kinematic pattern for suction feeding is shown in Figure 7B. The prey moves toward the jaws as the mouth opens and usually enters the mouth at or near peak gape. Initial depression of the hyoid occurs at the start of mouth opening, but a slow hyoid elevation typically follows. The major ventral movement of the hyoid does not be-

gin until the mouth nears maximum gape, and peak hyoid excursion occurs after peak gape (Fig. 7B). The cranial angle trace is similar to that of the gape, achieving its peak value at about the same time as peak gape.

Variation in the four kinematic measurements as well as the total gape cycle time in nineteen feedings by four individuals is

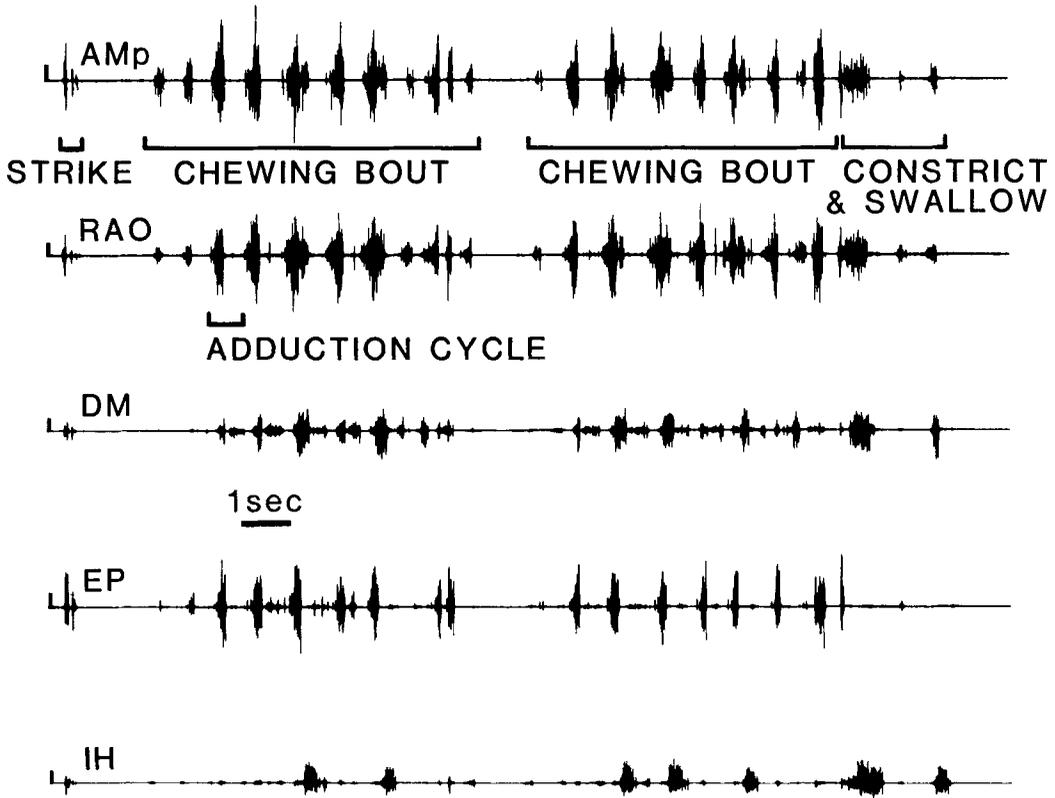


Fig. 5. Five electromyograms recorded simultaneously to show the behavioral events in a feeding sequence and the terminology used in this paper. Calibration bars on left side of each trace = 200 microvolts. Initial prey capture occurs at the strike, and is followed by a one second pause with no muscle activity.

Two chewing bouts then occur, with about a one second pause between bouts. Within a chewing bout are several adduction cycles (= chewing cycle), each consisting of an adduction phase and a transport phase. At the end of the last bout, two constrictions (= buccal compressions) occur.

shown in Figure 8, and the results of pairwise analyses of variance (ANOVA) among these variables is given in Table 1. In all feedings, the hyoid reaches its maximum ventral excursion significantly after peak gape (Table 1). Cranial angle, however, can reach its peak either before or after gape (Fig. 8), and the times to maximum gape and cranial angle are not significantly different (Table 1).

The electromyographic patterns during suction feeding differed greatly with the speed of the strike (Fig. 9). In slow strikes, only the depressor mandibulae and adductor mandibulae muscles showed activity, and there was a 100 to 200 ms delay between the offset of depressor activity and the onset of the adductor muscles (Fig. 9A). In the slowest strikes (gape cycles of a second or more), no activity was recorded from the adductor mandibulae muscles. When the strike was

more rapid, a decrease in time between the onset of the depressor mandibulae and the onset of the adductor was seen (Fig. 9B). Other muscles, such as the interhyoideus and the geniothoracicus become active also. The fastest strikes (gape cycle times of 40 ms) display considerable overlap between mouth opening and closing muscles (Fig. 9C): the jaw adductors and depressors become active within 20 ms of each other.

A summary pattern for $N = 34$ strikes is shown in Figure 10. The depressor mandibulae was active in all strikes as was the rectus cervicis posterior (first burst). In at least two-thirds of all feedings analyzed, the anterior rectus cervicis also was active with the onset of depressor activity. The epaxial and interhyoideus muscles become active, on the average, during the first half of the activity period of the depressor mandibulae. The activity period of the interhyoideus muscle cor-

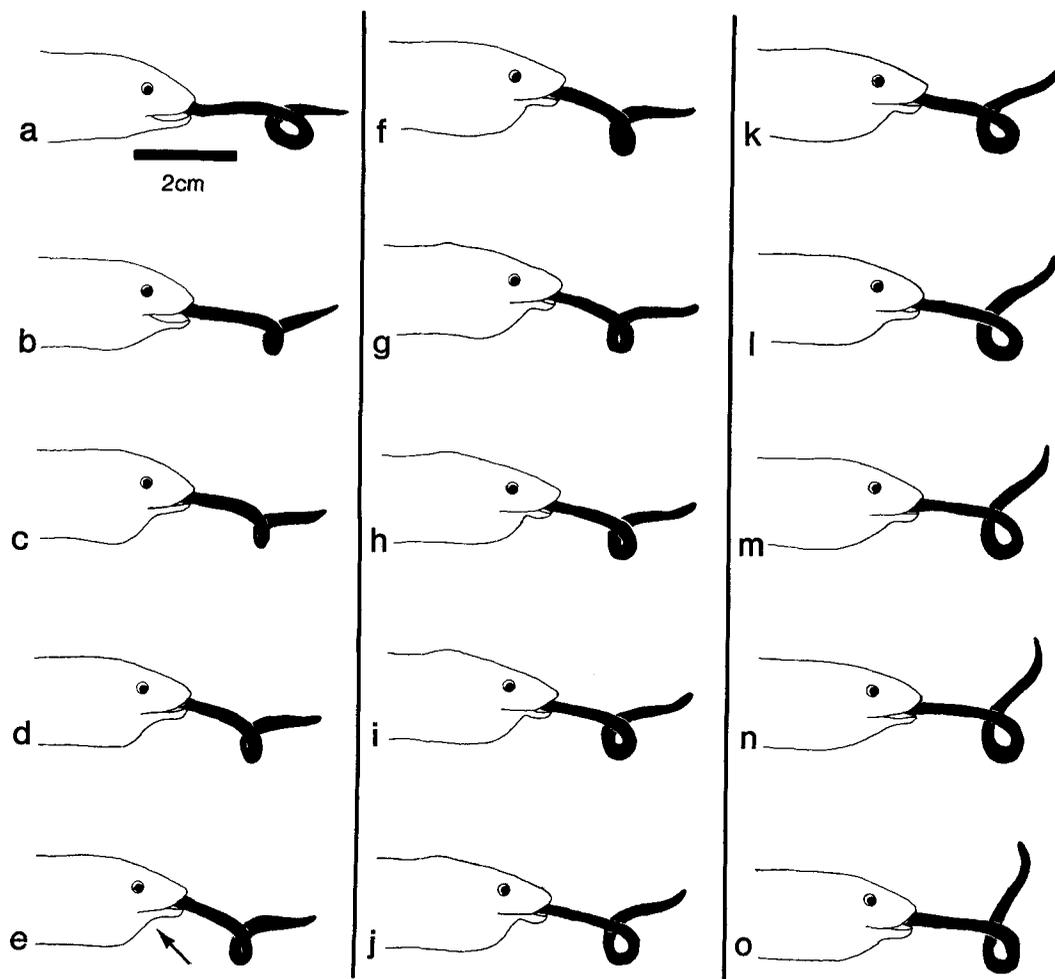


Fig. 6. Tracings from a film taken at 100 frames per second (every fifth frame traced) of *Lepidosiren* chewing on an earthworm. Arrow in frame e points to the characteristic dimple that forms under the mandible during strong crushing movements. The perpendicular depth of this dimple is the "crush" measurement described in the text. See text for further discussion.

TABLE 1. Pairwise analyses of variance on five kinematic variables from the initial strike by *Lepidosiren**

	Time to peak cranial angle (1)	Prey distance (2)	Time to peak gape (3)	Time to peak hyoid depression (4)	Gape cycle time (5)
(1)	—	0.11	2.54	22.95 ¹	25.81 ¹
(2)	1, 17	—	1.24	28.30 ¹	37.61 ¹
(3)	1, 18	1, 17	—	43.29 ¹	63.51 ¹
(4)	1, 18	1, 17	1, 18	—	8.79 ¹
(5)	1, 18	1, 17	1, 18	1, 18	—

*All variables measured in milliseconds. Values above the diagonal are the F statistic for the ANOVA, while values below the diagonal are the degrees of freedom used in the test.

¹Significant at the $P < .01$ level.

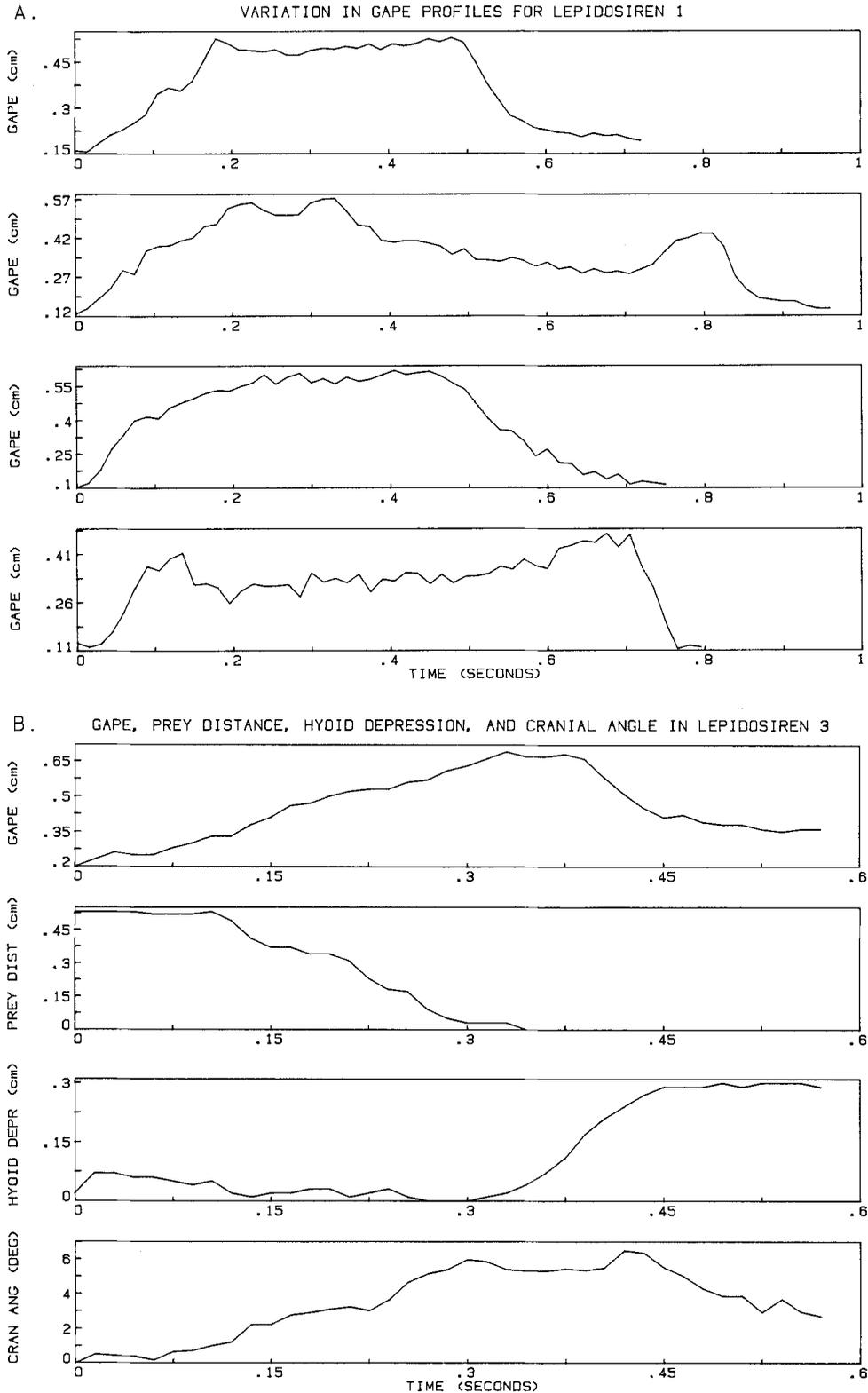


Fig. 7. A) Variation in the profile of gape distance during four feedings by one individual. B) Four kinematic measurements plotted against time to show the kinematic pattern during capture of a worm. Note that peak excursion of the hyoid is reached after peak gape, and that maximum cranial elevation is nearly coincident

with maximum gape. The four kinematic measurements are: Gape, the distance between the upper and lower jaw; Prey Dist, the distance from the prey to the plane of the gape; Hyoid Depr, the distance of hyoid depression; Cran Ang, the angle of the head with respect to the body.

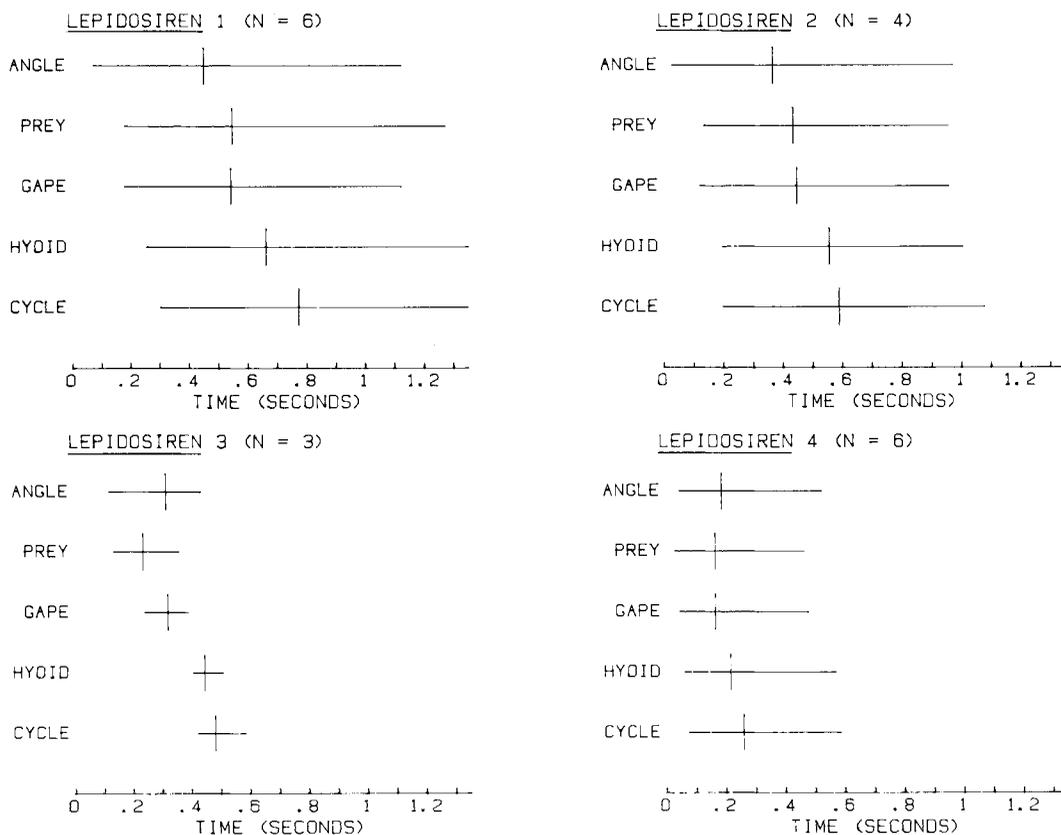


Fig. 8. Mean (vertical bar) and ranges (horizontal lines) for five kinematic variables in four individual lungfishes measured during the initial strike. Each panel represents a different individual. The four individuals differ in the relative time of maximum gape, prey entrance, and cranial angle, but show the same pattern of hyoid and gape timing. Pooled data for all four individuals shown are analyzed in a pairwise fashion in Table

1. The five variables are: Angle, time from the start of mouth opening to maximum angle of the head on the vertebral column; Prey, time from the start of mouth opening until the prey has completely entered the mouth; Gape, time from the start of mouth opening to peak gape; Hyoid, time from the start of mouth opening to peak hyoid depression; Cycle, total duration of the gape cycle, from start of mouth opening to closing.

relates with the slow and slight hyoid elevation seen in Figure 7B. Two muscles are active during the mouth-closing phase of the gape cycle: the adductor mandibulae and the rectus cervicis. The muscle with the most variable pattern of activity during the strike was the geniothoracicus posterior (Fig. 10: GTp). No well defined period of activity could be identified, and this muscle was active in less than 33% of all experiments.

Chewing and constriction

Figure 6 illustrates the pattern of events in a single chewing cycle. This sequence is 750 milliseconds long and the frames are 50 milliseconds apart. The prey transport phase occurs first, and mouth opening, in frames a

and b, is followed by depression of the hyoid that moves the worm further into the mouth. By frame d adduction of the jaws has started. The strongest adduction occurred in frames g through j, which show the posterior part of the adductor mandibulae muscle bulging above the posterior surface of the head.

Two entire bouts of chewing by two different individuals are shown in Figure 11. Figure 11A includes a prey capture, followed almost immediately by a chewing bout consisting of seven chewing cycles. Figure 11B shows a longer chewing bout consisting of nine chewing cycles, followed by two constrictions and swallowing.

The gape profiles shown in Figure 11 are the best guide to interpreting these se-

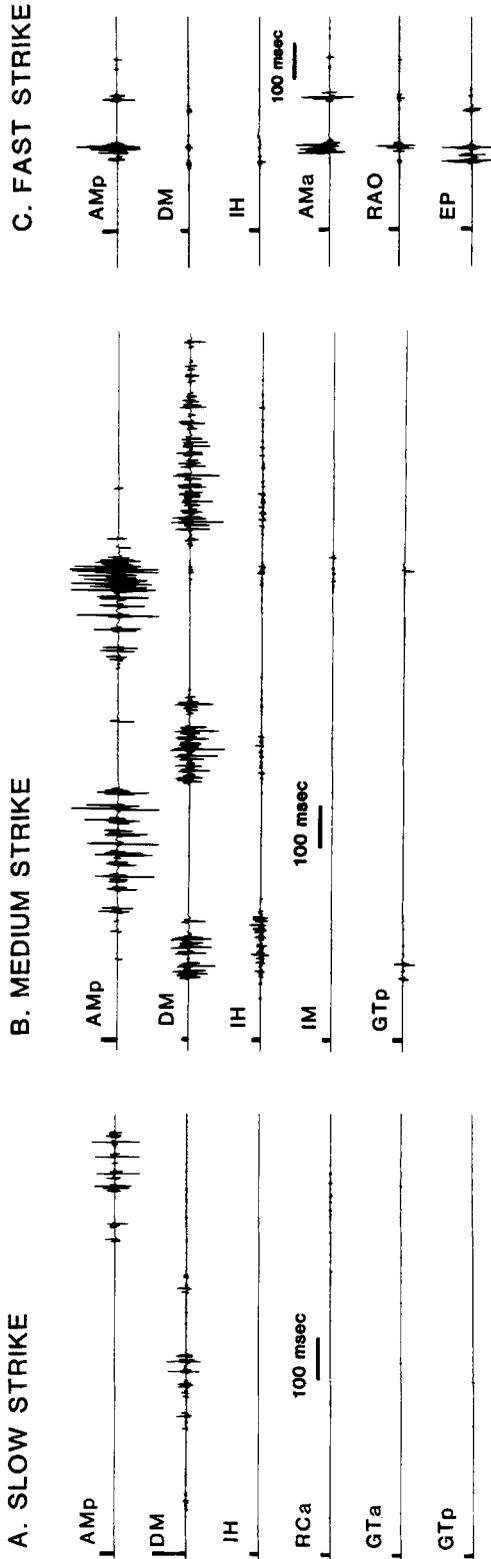


Fig. 9. Differences in EMG patterns for different strike speeds. A) Slow strike; B) moderately rapid strike; C) rapid strike. In B, chewing on the prey begins just after the offset of activity in the first adductor mandibulae posterior muscle burst. EMG calibration bar = 200 microvolts.

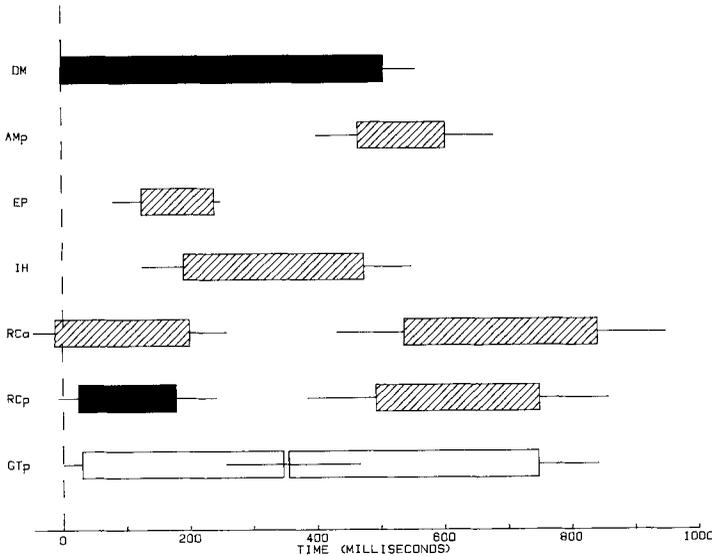


Fig. 10. Summary block diagram illustrating the pattern of muscle activity during suction feeding. In this and subsequent block diagrams, black bars represent activity that occurred in more than 66% of all experiments, shaded bars represent activity in more than 33% but less than 66% of all experiments, and white bars indicate that a muscle was active in less than 33% of all experiments. This figure summarizes data from 34

strikes. The ends of the bars indicate the mean onset and offset times, while the thin lines extending from the ends indicate the standard error of that mean. The overlapping error bars for the geniothoracicus posterior muscle indicate that a pattern we believed to consist of two bursts is in fact not distinguishable from one highly variable burst of activity. See text for further discussion.

quences. For each chewing cycle, the mouth is opened and closed rapidly. A large gape distance after mouth closing means that the tooth plates are in contact with the food. Thus, in the gape profile in Figure 11B, the lungfish made two chewing cycles before the prey was transported to a position between the tooth plates. In the next seven chewing cycles (C3-C9), the prey was crushed between the tooth plates. In Figure 11A, there are fewer "successful" chews, that is, in some cases, the food was not between the tooth plates during peak adduction.

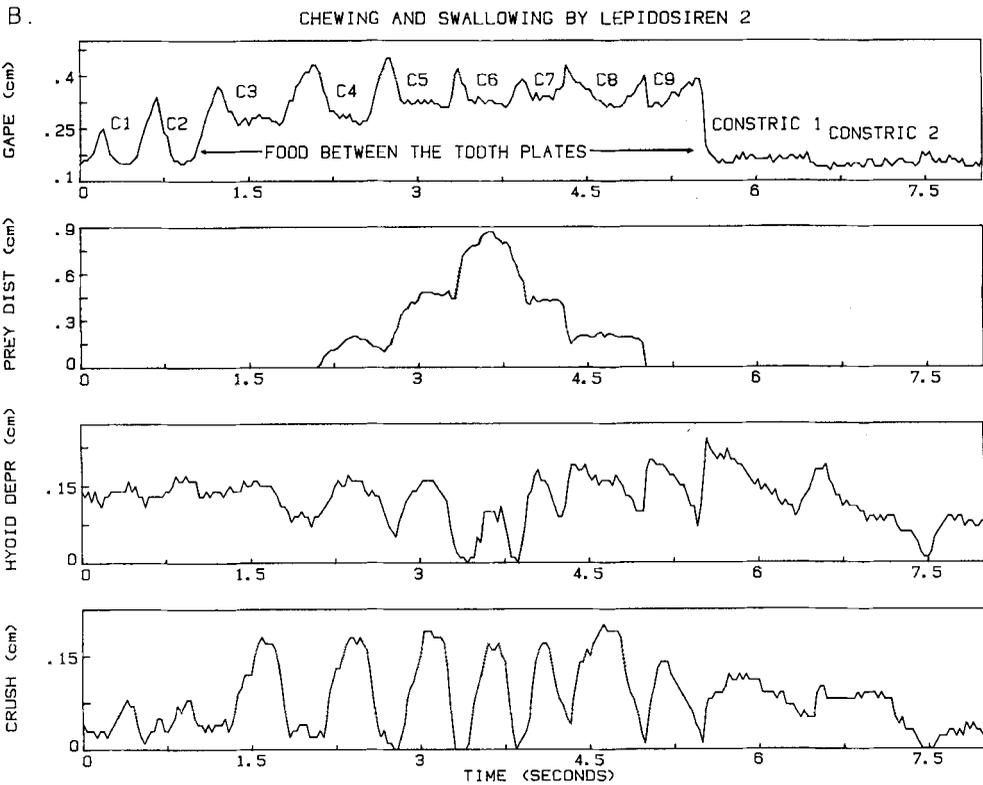
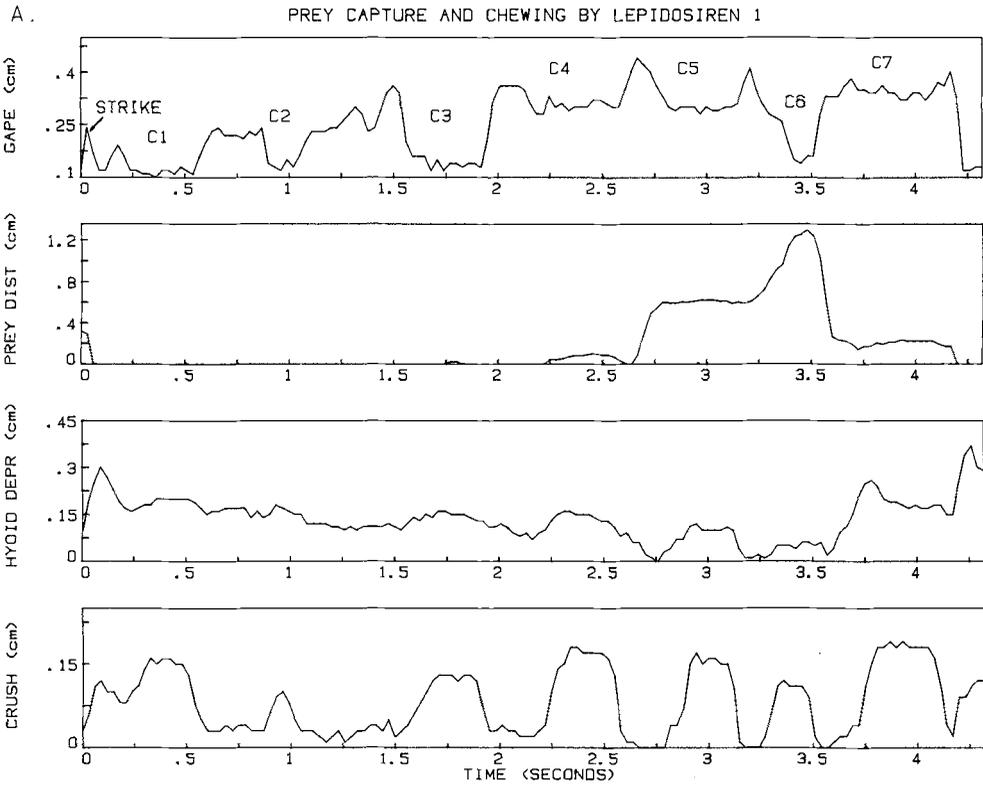
Figure 11 also documents the tendency of *Lepidosiren* to transport prey items part way out of the mouth during a chewing bout. This is shown by the traces of prey distance. In Figure 11B, the worm is expelled partially in the fourth chewing cycle. It is transported out a few more millimeters before each of the next two adduction phases. Then, the pattern reverses, and the worm is transported back into the mouth for the next two crushes. A similar pattern of partially expelling the prey item during transport phases is shown in Figure 11A.

The underlying basis for the patterns of prey transport is apparent if the record for

the hyoid movement is studied. During the first half of the sequence shown in Figure 11B, each time the mouth is opened, the hyoid is raised, expelling the prey slightly out of the mouth. During the second half, this pattern reverses, and the worm is brought back into the mouth.

The approximate strength of an adduction is reflected by the tracings for the crush variable. The first two chewing cycles in Figure 11B show weak adductions, but the subsequent seven adductions are larger. This is comparable to patterns of electromyographic activity in the adductor muscles, in which

Fig. 11. Representative kinematic patterns for extended chewing bouts in two individuals. The measurement labelled "crush" provides an indication of the time the prey is being crushed between the tooth plates; further discussion is given in the text. A) A successful strike is followed by seven chews; C1-C7 indicate the adduction phases of the chewing cycles. B) This record is the final chewing bout of a longer feeding sequence. Nine adduction phases (C1-C9) are indicated, during most of which, the worm was successfully located between the tooth plates. The "stepped" appearance of the prey distance measurement clearly shows the consequences of the intra-oral prey transport system, serving to move the prey in and out of the mouth during a chewing bout and expose it to the full action of the tooth plates.



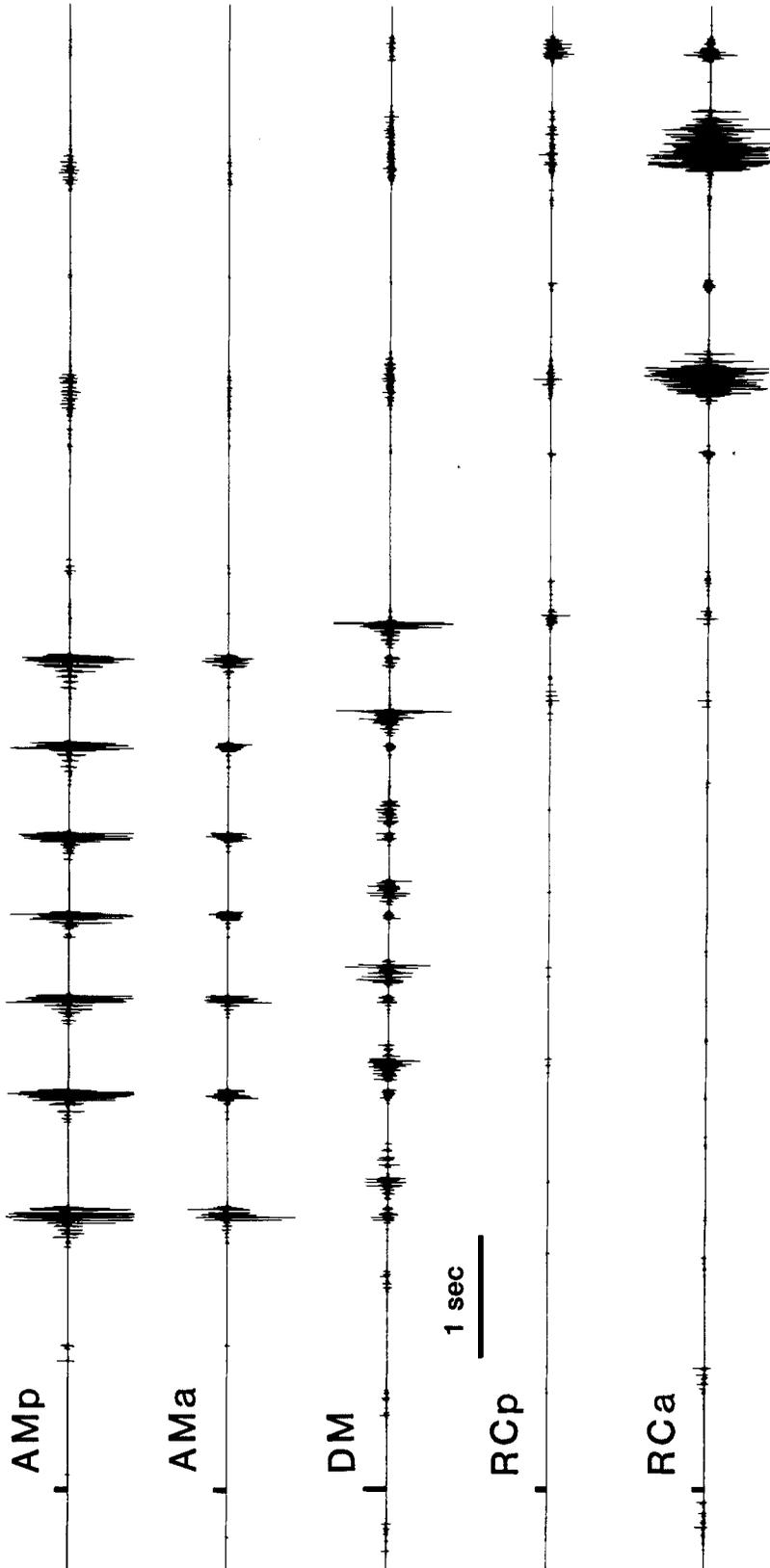


Fig. 12. Pattern of EMG activity in five muscles (simultaneously recorded) during a chewing bout and the subsequent constrictions or buccal compressions. Note the virtual absence of activity in the rectus cervicis posterior during chewing, and the presence of strong activity during constriction. The reverse is true of the other muscles. EMG calibration bar = 200 microvolts.

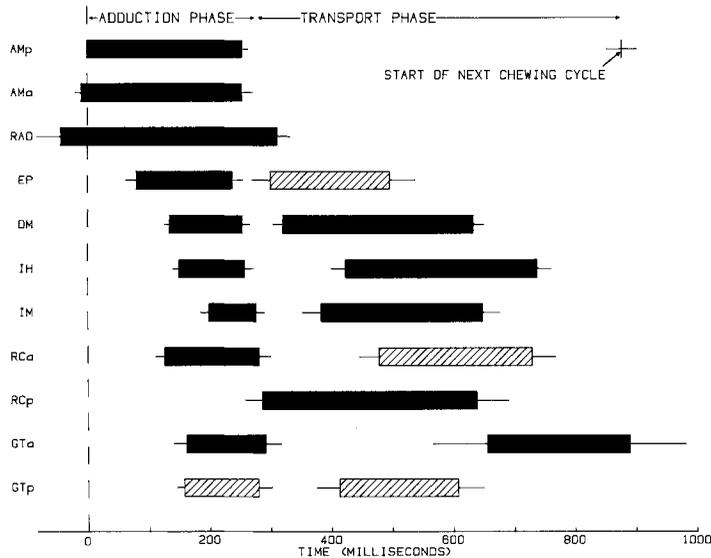


Fig. 13. Summary diagram of electromyographic activity during a chewing cycle. Conventions as in Figure 10. This figure summarizes data from 89 chewing cycles. The adduction phase and the transport phase of chewing cycles are definable both kinematically and electromyographically. The adduction phase indicates crushing of

food between the tooth plates, while the transport phase reflects intra-oral movement of food. The three adductor muscles (AMp, AMa, RAO) are active only during the adduction phase, while many muscles, including in particular the depressor mandibulae (DM), have a double-burst pattern.

the amplitude of adductor bursts in a given chewing bout starts out low, and builds up to a much larger level as the chewing bout progresses (e.g., Fig. 5).

The kinematics of constriction movements are shown at the end of the sequence in Figure 11B. During constrictions, the mouth is kept tightly closed, the prey is located within the buccal cavity, and the hyoid is moved sharply down and then slowly elevated. In this sequence, there are two constrictions. During each, the amount of hyoid depression is steadily reduced. After these two constrictions, swallowing occurred.

Figure 12 shows an electromyogram for a chewing bout consisting of eight chewing cycles. The start of this bout is indicated by slight activity in the depressor mandibulae muscle. This activity indicates mouth opening and the beginning of a transport phase. After two such transport phases, during which the food is positioned between the tooth plates, there is a small burst in the adductor muscles. The subsequent, much larger adductor bursts are correlated with crushing of the food. These larger adductor bursts occurred regularly, with a period of about one second. There is some tendency (described below) for this rhythm to speed up

during a chewing bout. The depressor mandibulae shows two periods of activity in each chewing cycle (Fig. 13: DM). A synchronous burst of activity in the depressor accompanies the adductor burst. The second, and usually larger, period of activity in the depressor mandibulae occurs between successive adductor bursts. This activity indicates mouth opening associated with the transport phase.

The summary block diagram (Fig. 13) for electromyographic activity at all 11 recording sites shows an obvious break between adduction and transport phases. During the adduction phase, the three adductor muscles (AMp, AMa, RAO) are active. During transport, these muscles are silent. Many muscles exhibit two bursts of activity comparable to the synchronous and asynchronous bursts of the depressor mandibulae. However, it is usually the second burst that is the stronger of the two. The most consistently active muscle during transport is the depressor mandibulae, because mouth opening occurs in every transport phase. Several muscles act directly or indirectly on the hyoid, tending to depress or raise it. Those such as the recti cervicis tend to depress the hyoid, bring the food item farther into the mouth, and make the transport appear kinematically similar to initial

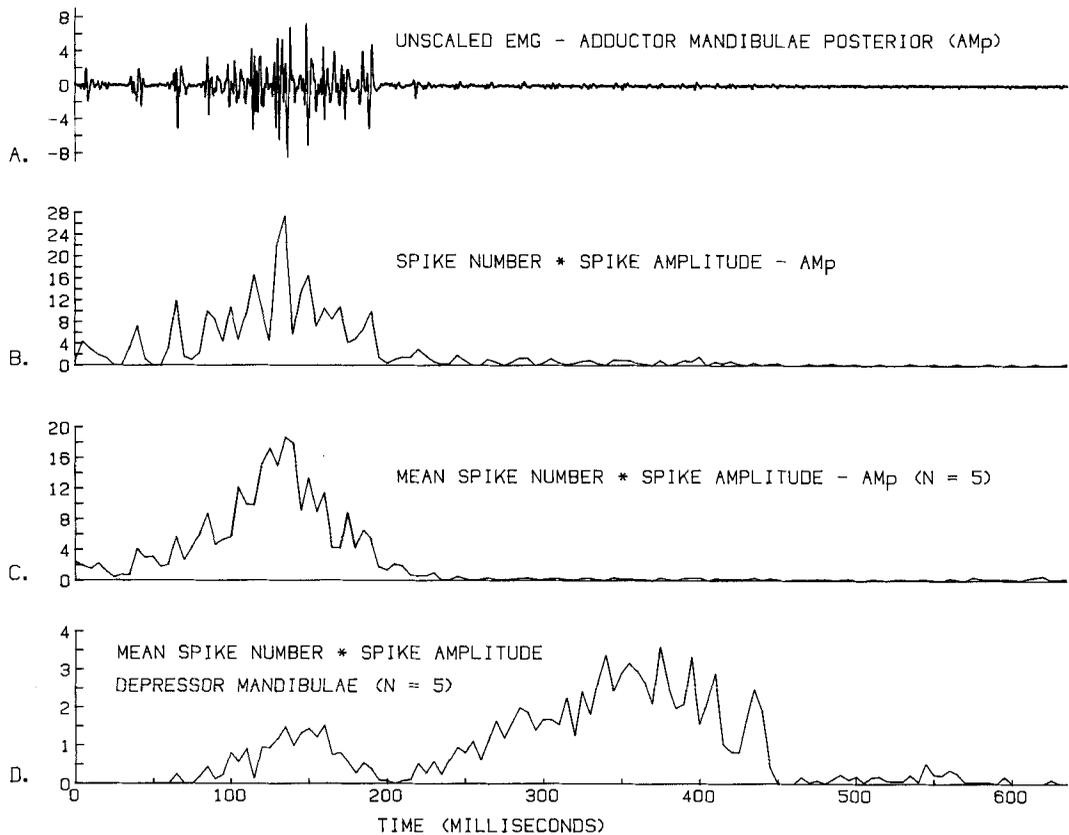


Fig. 14. Patterns of spike frequency and amplitude in the adductor mandibulae posterior and depressor mandibulae muscles during chewing. A) Raw, unsealed EMG of an adductor mandibulae burst as stored digitally; B) plot of spike number times amplitude for the myogram shown in A (see text for further information); C) mean spike number times amplitude for five bursts of the

adductor mandibulae muscle; D) a similar analysis for the depressor mandibulae muscle, simultaneously recorded. Note the rhythmic pulses in the adductor muscle, and that the first burst in the depressor is of lower amplitude than the second. These electromyographic patterns were observed consistently.

prey capture. The interhyoideus in particular, but also the intermandibularis, tend to elevate the hyoid region, forcing the prey item forward to position it between the tooth plates.

Figure 14 presents an analysis of the activities of the adductor and depressor muscles averaged over five successive chewing cycles. A sample electromyogram for the adductor mandibulae posterior muscle from one of the five cycles is presented at the top of the figure. A transformed version of this same myogram, in which the number of spikes in a given bin width has been multiplied times the mean amplitude of the spikes in that bin (Beach et al., '82), is presented in the second trace. Like most of the adductor bursts, activity in this muscle appears rhythmic. Thus,

three small trains of spikes precede the start of major activity. Once the major period of activity does start, a degree of rhythmicity is retained, as is particularly obvious from the transformed EMG. The average time between these pulses of activity seen in the transformed EMGs is 19 milliseconds ($N = 65$; $SD = 8.92$), indicating an average pulse frequency of 53 Hz. This pulse frequency is considerably lower than the mean ($N = 5$ bursts) power peak of 297 Hz determined by FFT analysis of the entire EMG. Note also that the myogram builds up to a peak in activity, and then declines before the end of the period of contraction. The trace below the transformed myogram is the average of five successive transformed adductor bursts. This trace shows that there is a central peak in

TABLE 2. Three level nested ANOVA on the duration of electrical activity in the adductor mandibulae posterior muscle*

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	Significance
Among individuals	4	330,023	82,506	1.6	NS
Among implants within indiv.	3	209,349	69,783	3.5	NS
Among days within implants	7	136,871	19,553	6.0	P < .001
Among bursts within days	74	240,800	3,254		
Total	88	917,045			

*Variance component (%): Among individuals = 17.5; among implants = 30.8; among days = 24.1; and among bursts = 27.6.

activity for all five of these myograms. Finally, the bottom trace in Figure 14 shows the corresponding pattern of activity in the depressor mandibulae for these five chewing cycles. There is no evidence of crosstalk in raw myograms of these two muscles, and we regularly recorded strong alternating activity in both muscles (e.g., Fig. 5B). Note that the synchronous depressor bursts are not symmetrically located with respect to the adductor bursts. Note also that the second period of activity in the depressor mandibulae is much larger than the synchronous burst. This is interpreted as the major burst of activity responsible for mouth opening. There is a one to one correlation between the timing of the rhythmic pulses of activity in the two muscles ($r = .99$; $N = 37$).

Activity in the adductor mandibulae muscle shows no overall pattern during a complete feeding sequence, but there is a tendency for later bursts within a bout to occur more frequently (Fig. 15). These data were measured from electromyographic records of a long chewing sequence, consisting of a total of 46 chewing cycles in six separate chewing bouts. The burst period (length of time that the adductor mandibulae was active) and interburst period (length of time between successive bursts of adductor mandibulae activity, unless this was the end of a chewing bout, in which case no measurement is reported) were measured in these 46 chewing cycles. The two period measurements are plotted against chewing cycle number (Fig. 15A,B), and against each other (Fig. 15C). These graphs suggest that there is neither a consistent change in the pattern of activity within a total feeding event (Fig. 15A,B), nor is there an obvious relationship between the length of time that the adductor muscle is active and the length of time that it is inactive (Fig. 15C). However, within each of the six chewing bouts, there is a significant relationship between the chewing cycle num-

ber within the bout and the length of time that the muscle is inactive. This is investigated further in Figure 15D, which shows the burst and interburst periods plotted against cycle number within each of the six chewing bouts. The regression line through the points indicating the burst period is not significantly different from 0 ($r = -.26$; $t = 1.59$, NS). However, the slope of the line through the interburst periods is significantly different from 0 and is negative ($r = -.71$; $t = 5.83$, $p < .001$). Thus, during an average chewing bout, the later chewing cycles tend to be more rapid, due to the shorter interburst period.

We performed three-level nested analyses of variance on the periods of activity for several muscles to determine the extent of variation and to evaluate the repeatability of muscle activity patterns. In Table 2, we report the results for the adductor mandibulae posterior, the key muscle used in chewing: the analyses of other muscles (e.g., depressor mandibulae) show comparable results. Activity periods for 89 bursts of activity in the adductor mandibulae posterior were measured. These bursts were grouped by experimental day within electrode implant and by implant within individual lungfishes. Variation within days accounted for nearly 28% of the total variance. Variation among days accounted for about one quarter of the total variance and was significant. Although the greatest percentage of variation (30.8%) was present among implants, this variation is not significant, and the relatively low variation (17.5%) among individuals was not significant. This analysis suggests that: 1) there was a good deal of repeatability from individual to individual, and 2) that our electrode placements recorded consistent patterns of activity. The only significant variation is at the level of days, which presumably reflects small differences in the details of a particular feeding event, such as the prey size.

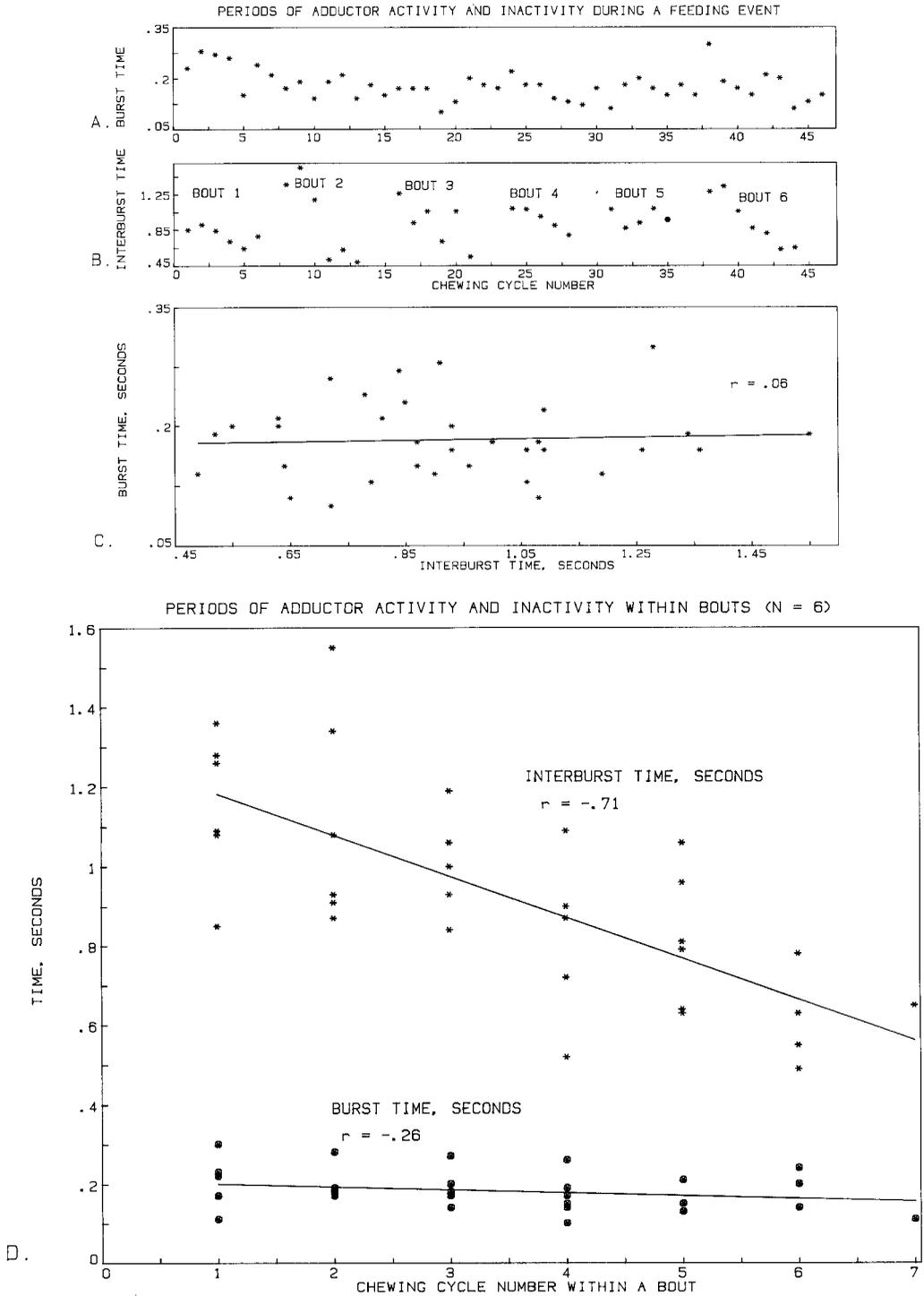


Fig. 15. Periods of adductor activity and inactivity. A) Burst duration (time in seconds of adductor activity) in the adductor mandibulae posterior muscle, plotted against chewing cycle number within an entire feeding sequence consisting of 46 chews; B) interburst durations (time in seconds between successive adductor bursts) for these same 46 chews; C) burst duration as a function of

interburst duration, showing no relationship; D) burst and interburst durations within single chewing bouts, showing a significant relationship between the length of the interburst and the number of the chewing cycle. Thus, later chews within a chewing bout tend to occur closer together in time.

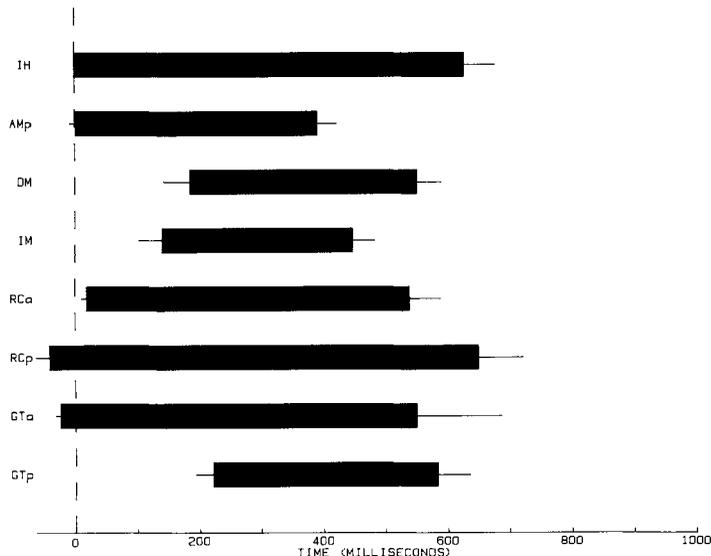


Fig. 16. Summary diagram of the pattern of muscle activity during constriction (buccal compression) following chewing bouts. Conventions for this figure follow those of Figure 10. This figure summarizes data from 45 constrictions. All muscles recorded are active during

constrictions, although at different amplitudes. Note the overlapping but not synchronous bursts in the adductor and depressor mandibulae, and the extended activity in the interhyoideus (IH), rectus cervicis (RCp), and geniothoracicus (GTP).

Figure 16 diagrams patterns of electrical activity during constriction. The most important muscle during constriction movements is the interhyoideus and it is used as the reference muscle for this diagram. These eight muscles all showed significantly overlapping activity during constriction. Three of the muscles (the depressor mandibulae, intermandibularis, and geniothoracicus posterior, Fig. 16: DM, IM, GTP), are active significantly later than the other muscles examined. The last half of depressor mandibulae activity does not overlap activity in the adductor mandibulae.

DISCUSSION

Behavioral patterns and functional morphology

We distinguish three basic behavioral patterns in the feeding system of *Lepidosiren*. These are: 1) the strike or prey capture by suction; 2) chewing cycles, each consisting of an adduction phase and a transport phase; and 3) constriction or buccal compression. A given feeding event includes an initial strike, followed by a variable number of chewing cycles arranged into chewing bouts. Usually, each chewing bout is terminated by one or

more constriction events. When chewing is complete, a final series of constrictions forces the prey item into the esophagus, and swallowing ensues.

The mechanics of each of these phases are summarized in Figure 17. The white arrows represent the movements seen externally. The black arrows represent the approximate lines of action of the muscles involved, as well as an estimate of the relative amplitude of electromyographic activity. During suction, the lower jaw is opened by jaw-depressing muscles, the depressor mandibulae and rectus cervicis, and the hyoid is depressed by the large recti cervicis. During the adduction phase of chewing, the jaw-closing muscles are strongly active, while most other components are weakly active. During the transport phase, the adductor muscles are inactive. The lower jaw is abducted by the depressor mandibulae, while the hyoid region is moved up or down through the action of the recti cervicis or interhyoideus muscles. During constriction (buccal compression), the muscles elevating the hyoid region, principally the interhyoideus muscle, are strongly active. Other muscles, notably the adductor mandibulae, are active to prevent food from escaping through the front of the mouth.

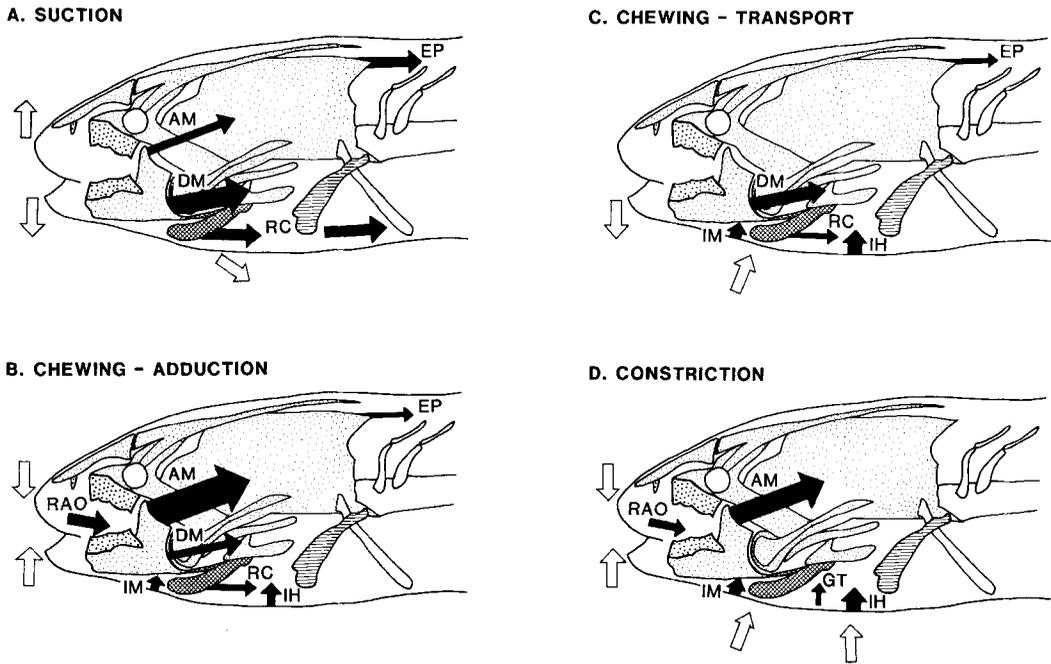


Fig. 17. Summary diagram showing the dominant features of the kinematic and electromyographic patterns during four stages of feeding. White arrows indicate head movements visible externally, while black arrows indicate EMG activity. Width of the black arrows approximates the relative amplitude of EMG activity.

An especially noteworthy feature of muscle activity during chewing is the pattern of spike activity within adductor muscle bursts. During the adduction phase of chewing, the adductor mandibulae muscles consistently show rhythmic pulses of electrical activity so that each overall burst is composed of shorter, relatively high amplitude and high frequency activity followed by lower level activity (Fig. 14A,B). The result is a "pulsed" pattern with an average frequency of 53 Hz, which stands out sharply in contrast to the average peak power frequency of the myograms, 297 Hz. This pulsed pattern is sufficiently consistent that averaging several transformed bursts does not decrease its clarity (Fig. 14C). During these adductor muscle bursts, the antagonistic depressor mandibulae muscle is also active. It also exhibits a rhythmic pattern of pulses, with a one to one correspondence to the adductor pulses.

A pulsed pattern of EMG activity during crushing may reflect biomechanical or physiological properties advantageous in crushing (Irish, '83). Gans et al. ('85) documented a pulsed pattern of adductor activity in

skinks feeding on snails. They suggest that because such a pattern produces graded force development at the tooth row, it may reduce shock impacts between opposing teeth. Irish ('83) reported that pulsed adductor activity occurred less frequently in the fish, *Colosoma*, when fed on soft rather than hard food items. Lungfishes are widely regarded as durophagic, yet the earthworm prey used in this study scarcely qualify as hard. Nevertheless, we did find pulsed activity during feeding on earthworms. It will be interesting to study the pulsed adductor burst patterns for lungfish feeding on hard prey items.

There are few relevant data on the physiological properties of fish muscles (e.g., Bone, '78; Johnston, '83), and the comparative data that do exist suggest that there can be important differences among taxa. Nevertheless, based on the data of Johnston ('80) for skate and cod fin muscle fibers, our observed pulse frequency of 53 Hz indicates that the muscle probably is being stimulated at a rate sufficient to produce at least unfused tetanus (see also Gans and de Vree, '84). By stimulating the muscle at about 50 Hz, the tension devel-

oped probably rises and falls, as in unfused tetanus. These and other ideas need further study before the functional significance of the pulsed EMG pattern in lungfish adductor muscles is fully understood.

A further noteworthy feature of the chewing phase is the consistent presence of electrical activity in antagonistic muscles. The best example of this is the antagonistic activity in the depressor and adductor mandibulae muscles when prey are crushed (Fig. 14D). The first, or synchronous, burst of depressor mandibulae activity is a regular feature of our data, although it is usually of lower amplitude than the second burst. Antagonistic muscle activity in vigorous movements is common in musculoskeletal systems (Basmajian, '74), and perhaps reflects the stabilization of joint articulations or increased control due to modulation of activity in antagonists.

The nested ANOVA performed on the burst duration in the adductor mandibulae provides considerable insight into the experimental procedure and into the variability present in the feeding mechanism. By partitioning the variation in one variable (such as burst duration of a muscle) into three levels, variation among individuals, among electrode implants on the same individual on different days, and among recording sessions on different days with the same electrode implants, it is possible to assess the effect of repeated experiments on the same individuals as well as to determine how much individuals differ from each other. Our results (Table 2) show that although there is variation in our data owing to different electrode implantations on the same individuals (many weeks apart), this variation is not statistically significant. There is also no evidence from this analysis that the individuals we studied differed significantly from each other. The analysis does show, however, that there is significant variation among days given that the same set of electrodes was used. This is consistent with the results of Shaffer and Lauder ('85a) who found a significant variation among days in many of the EMG variables they used in a study of suction feeding in aquatic ambystomatid salamanders. We recommend this type of analysis in studies of vertebrate functional morphology because of its heuristic value and the increased understanding of the sources of variation in the experimental data (also see Shaffer and Lauder, '85b).

A final important aspect of the functional morphology of feeding in lungfishes is the mechanism of chewing and the process of food transport within the oral cavity. During a chewing bout, the prey is alternately crushed and transported, in a fashion reminiscent of generalized mammalian masticatory systems (see examples in Gans et al., '78; Hiiemae, '78). Unlike mammals, however, the mechanism of prey movement is *hydraulic*, with water movement and its direction of flow determined by the hyoid apparatus. As the mouth is opened during the transport phase of a chew, the hyoid can be elevated to push water and the prey anteriorly. At other times in a chewing bout, the hyoid acts in a similar manner to its role in suction feeding and draws water and the prey posteriorly. Thus, it is the control of water movement by the hyoid apparatus that allows positioning of prey between the tooth plates and provides control of prey position in a manner analogous to the tetrapod tongue.

Our results on the functional morphology of mouth opening conflict with some of the limited literature available on the feeding systems of living lungfishes, particularly in the case of the jaw opening system. Thomson ('69) realized the correct function of the depressor mandibulae muscle, but in the absence of functional data, did not elaborate on this point. Edgeworth ('35) considered that jaw opening in lungfishes was the result of geniothoracicus activity. This idea was reiterated by Perkins ('72), who stated (pg. 69): "The m. geniothoracicus lowers the jaw during feeding; no other muscle appears to be active at this time." Perkins went on to suggest that the depressor muscle is not active during feeding. However, neither Edgeworth nor Perkins used electromyography to determine muscle activity patterns, and our results suggest that they misinterpreted function on the basis of static morphology. It is more difficult to resolve a difference between our study and McMahon's ('69) electromyographic study of respiration in the African lungfish, *Protopterus aethiopicus*. McMahon found that the depressor mandibulae muscle was only active during peak movements of the lower jaw. Thus, he stated ('69, pg. 417): "Activity in the retractor (= depressor) mandibulae was always associated with maximal depression of the lower jaw, as seen in the 'yawning movements' discussed below." This conflicts with our finding that the

depressor was active every time the lower jaw was opened.

Evolution of the feeding mechanism in lower vertebrates

A key component of our understanding of vertebrate evolution is the analysis of both morphological and functional patterns in the feeding mechanism. The definition of such patterns forms a necessary basis for discussions of the rate of evolution in the skull, the origin of terrestrial feeding, and scenarios about morphological changes and the environment. The recognition of such morphological and functional patterns is difficult because general patterns become clear only after extensive comparative analyses of the relevant clades. This study contributes to our understanding of the evolution of the vertebrate feeding mechanism by providing comparative data on a critical clade, the lungfishes, and allowing the definition of primitive characteristics of the tetrapod feeding mechanism. The present data must be considered in the light of other recent work on patterns of evolution in the feeding mechanism of aquatic vertebrates (salamanders, Shaffer and Lauder, '85a, '85b; coelacanth, Lauder, '80a; ray-finned fishes, Lauder, '80b, '82, '85).

The comparative data cited above on the major lower vertebrate clades indicate unequivocally that at least four aspects of the feeding mechanism are primitive for teleostome fishes (reviewed in Lauder, '80): 1) initial prey capture occurs by suction feeding; 2) the epaxial muscles produce cranial elevation during mouth opening; 3) the hyoid apparatus plays a major role in mediating expansion of the mouth cavity and is one biomechanical system involved in depressing the mandible; and 4) peak hyoid excursion occurs after maximum gape has been achieved. This study confirms that all of these features also characterize *Lepidosiren*, strongly suggesting that the four primitive characteristics of the teleostome feeding mechanism are also primitive for tetrapods. The fundamental biomechanical systems involved in initial prey capture exhibit remarkable conservatism. For example, abduction of the mandible during suction feeding in primitive ray-finned fishes, coelacanth, lungfishes, and aquatic salamanders occurs at least in part by posteroventral movement of the hyoid and the transmission of this movement to the retroarticular process of the mandible by the mandibulohyoid ligament. This mechanical system remains

intact after 400 million years of vertebrate evolution and extensive divergence in feeding form, function, and habit.

One aspect of the feeding system of lepidosirenid lungfishes appears to be a specialization at least functionally similar to a feature found in the feeding mechanism of salamanders. *Lepidosiren* possesses a well-developed muscle (Fig. 2) that is consistently active during mouth opening. In recognition of this function, we term the muscle depressor mandibulae, in accordance with Thomson ('69) but different from a widely used name, retractor mandibulae (e.g., McMahon, '69; Fox, '65; Edgeworth, '35). Aquatic salamanders possess a depressor mandibulae muscle in a topographically comparable position that is also used in mouth opening (Shaffer and Lauder, '85a). Although the depressor mandibulae muscles of lungfishes and salamanders are both derived from the hyoid arch, they are not regarded as homologues by embryologists (e.g., Fox, '63, '65; Edgeworth, '35). The basic developmental difference between lungfishes and salamanders is that the medial portion of the constrictor hyoideus sheet gives rise to the depressor muscle of lungfishes (Edgeworth, '35: pg. 97), while the depressor muscle of salamanders is derived from the dorsal (= levator hyoideus) portion of the constrictor hyoideus sheet (Edgeworth, '35, pg. 102).

The depressor mandibulae muscle is similar in size and position in *Lepidosiren* and *Protopterus*. In the Australian lungfish, *Neoceratodus forsteri*, however, the muscle is apparently much less well developed, originating from the ceratohyal and passing to its insertion on the lower jaw deep to the opercular elements, instead of originating from their outer surface (Fox, '65; Perkins, '72). The size and position of the muscle in *Neoceratodus* is probably closer to the primitive condition for lungfishes, because there has been a general tendency during dipnoan evolution to reduce both the number and size of the opercular and subopercular elements (e.g., *Uranolophus*—*Scaumenacia*—*Neoceratodus*—*Lepidosiren*), and there is no indication of a muscle attachment on the outer face of the opercular and subopercular in the descriptions of phylogenetically primitive dipnoans (e.g., *Uranolophus*, Denison, '68; *Griphognathus*, Miles, '77). The enlarged depressor mandibulae of *Lepidosiren* and *Protopterus* is, therefore, a specialized condition within the Dipnoi, and is yet another synapomorphy indicative of the close relationship between these two genera. Even though the muscle is

unknown in outgroup osteichthyans, the embryological considerations discussed above render it unlikely that the depressor mandibulae is a synapomorphy of lungfishes and tetrapods. The convergent development of depressor mandibulae muscles in lungfishes as well as several lineages of tetrapods is striking evidence of the functional importance of specialized jaw opening systems.

The salient specialization in the feeding mechanism of *Lepidosiren* does not lie in initial prey capture, but rather in the extensive intra-oral processing of food after it has been captured. Movement of the prey in and out of the mouth accompanied by strong adduction of the jaws to crush prey between the massive tooth plates is an aspect of the feeding mechanism not found in most lower vertebrates. Associated with the tooth plates and crushing behavior is the hydraulic transport of food that allows positioning of food within the oral cavity. In many ways, this hydraulic food transport system in lungfishes performs analogous functions to those of the tongue in tetrapods (e.g., Hiiemae, et al., '78; Bramble and Wake, '85; Gorniak, et al., '82; Smith, '84). Water movement mediated by the hyoid apparatus positions prey between teeth, aids in swallowing (constriction or buccal compression in *Lepidosiren*), and can act to remove unwanted particles from the oral cavity. Although other fishes have been documented to use water flow through the oral cavity to position food (see, for example, Lauder, '81), ray-finned fishes have not yet been shown to do the extensive processing of food that *Lepidosiren* does with the oral teeth. Hiiemae et al. ('78: 206) stated that "Transport is the fundamental mechanism of feeding and the hyoid apparatus is a fundamental part of that mechanism," a conclusion as appropriate for *Lepidosiren* as it was for the mammalian feeding systems they discussed, despite the major anatomical differences between tongue and hydraulic transport systems.

This paper and the comparative data now available on other lower vertebrate clades set the stage for a new examination of the feeding mechanism in "rhipidistian" fishes. The presence of several features of the initial prey capture mechanism in all living lower vertebrate clades studied to date indicates that *Eusthenopteron*, *Rhizodus*, *Glyptolepis*, and their relatives (whatever their phylogenetic position) shared several key characteristics of the lower vertebrate feeding mechanism. Although there is no evidence for the presence of a depressor mandibulae muscle,

mouth opening is best interpreted as having been achieved by a combination of cranial elevation caused by the epaxial muscles, and mandibular depression mediated by posteroventral excursion of the hyoid. The mandibulohyoid ligament would transmit hyoid movement to the mandible as in other lower vertebrates. No evidence available from fossils contradicts these inferences, but neither does fossil evidence provide much critical information on the presence of fleshy lips that occlude the margins of the gape (as in *Protopterus* and *Lepidosiren*), or on the possible presence of a depressor mandibulae muscle. Further progress in interpreting the functional characteristics of extinct lower vertebrates awaits a more precise understanding of the interrelationships between form and function in living clades.

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LITERATURE CITED

- Basmajian, J.V. (1974) Muscles Alive, Their Functions Revealed Through Electromyography, Ed. 3. Baltimore: Williams and Wilkins.
- Beach, J., G.C. Gorniak, and C. Gans (1982) A method for quantifying electromyograms. *J. Biomech.* 15:611-617.
- Bemis, W.E. (1984a) Morphology and growth of lepidosirenid lungfish tooth plates (Pisces: Dipnoi). *J. Morphol.* 179:73-93.
- Bemis, W.E. (1984b) Paedomorphosis and the evolution of the Dipnoi. *Paleobiology* 10:293-307.
- Beman, D.S. (1979) *Gnathorhiza bothrotreta* (Osteichthyes: Dipnoi) from the Lower Permian Abo Formation of New Mexico. *Ann. Carnegie Mus.* 48:211-230.
- Bertmar, G. (1966) The development of the skeleton, blood-vessels and nerves in the dipnoan snout, with a discussion on the homology of the dipnoan posterior nostrils. *Acta Zool. Stockh.* 47:82-150.
- Bishop, I.R. and G.E.H. Foxon (1968) The mechanism of breathing in the South American lungfish, *Lepidosiren paradoxa*; a radiological study. *J. Zool. Lond.* 154:263-271.
- Bone, Q. (1978) Locomotor muscle. In W.S. Hoar and D.J. Randall (eds): *Fish Physiology*, Vol. 7. New York: Academic Press, pp. 361-424.
- Bramble, D. and D.B. Wake (1985) Feeding mechanisms of lower tetrapods. In M. Hildebrand, D. Bramble, K. Liem, and D. Wake (eds): *Functional Vertebrate Mor-*

- phology. Cambridge: Harvard University Press, pp. 230-261.
- Bridge, T.W. (1898) On the morphology of the skull in the Paraguayan *Lepidosiren* and in other dipnoids. *Trans. Zool. Soc. Lond.* 14:325-376.
- Campbell, K.S.W. and R.E. Barwick. (1983) Early evolution of dipnoan dentitions and a new genus *Speonesdrion*. *Mem. Ass. Australas. Palaeontols.* 1:17-49.
- Carter, G.S. and L.C. Beadle (1930) Notes on the habits and development of *Lepidosiren paradoxa*. *J. Linn. Soc. Lond.*, Zool. 37:197-203.
- Corbet, P.S. (1961) The food of non-cichlid fishes in the Lake Victoria Basin, with remarks on their evolution and adaptations to lacustrine conditions. *Proc. Zool. Soc. Lond.* 136:1-101.
- Denison, R.H. (1968) Early Devonian lungfishes from Wyoming, Utah, and Idaho. *Fieldiana, Geol.* 17:353-413.
- Dingerkus, G. and L.D. Uhler (1977) Enzyme clearing of alcian blue stained whole small vertebrates for demonstration of cartilage. *Stain Technol.* 52:229-232.
- Edgeworth, F.H. (1926) On the development of the cranial muscles in *Protopterus* and *Lepidosiren*. *Trans. Roy. Soc. Edinburgh.* 54:719-734.
- Edgeworth, F.H. (1935) *The Cranial Muscles of Vertebrates*. Cambridge: Cambridge Univ. Press.
- Fox, H. (1963) The hyoid of *Neoceratodus* and a consideration of its homology in urodele amphibia. *Proc. Zool. Soc. Lond.* 141:803-810.
- Fox, H. (1965) Early development of the head and pharynx of *Neoceratodus* with a consideration of its phylogeny. *J. Zool. Lond.* 146:470-554.
- Gans, C., F. de Vree, and G.C. Gorniak (1978) Analysis of mammalian masticatory mechanisms: Progress and problems. *Zbl. Vet. Med. C. Anat. Histol. Embryol.* 7:226-244.
- Gans, C. and F. de Vree (1984) Temporal summation in the adductor musculature of the lizard *Trachydosaurus*: Treppe and tetanus in the crushing of snails. *Am. Zool.* 24:108A (abstract).
- Gans, C., F. de Vree, and D. Carrier (1985) Usage pattern of the complex masticatory muscles in the shingleback lizard, *Trachydosaurus rugosus*: A model for muscle placement. *Am. J. Anat.* 173:219-240.
- Gorniak, G.C., and C. Gans (1980) Quantitative assay of electromyograms during mastication in domestic cats (*Felis catus*). *J. Morphol.* 163:253-281.
- Gorniak, G.C., H.I. Rosenberg, and C. Gans (1982) Mastication in the tuatara, *Sphenodon punctatus* (Reptilia: Rhynchocephalia): Structure and activity of the motor system. *J. Morphol.* 171:321-353.
- Hiimae, K.M. (1978) Mammalian mastication: A review of the activity of the jaw muscles and the movements they produce in chewing. In P.M. Butler and K.A. Joysey (eds): *Studies on the Development and Function of Teeth*. New York: Academic Press, pp. 359-398.
- Hiimae, K.M., A.J. Thexton, and A.W. Crompton (1978) Intra-oral food transport: The fundamental mechanism of feeding. In D. Carlson and J. McNamara, eds. *Muscle Adaptation in the Craniofacial Region*. Ann Arbor: Univ. of Michigan Press, pp. 181-208.
- Humason, G.L. (1972) *Animal Tissue Techniques*, Ed. 3. San Francisco: W.H. Freeman.
- Irish, F. (1983) The dynamics of seed-eating in characiform fish, *Colossoma*. *Am. Zool.* 23:1027 (abstract).
- Jollie, M. (1982) Ventral branchial musculature and synapomorphies questioned. *Zool. J. Linn. Soc. Lond.* 74:35-47.
- Johansen, K. and D. Hanson (1968) Functional anatomy of the hearts of lungfishes and amphibians. *Am. Zool.* 8:191-210.
- Johnston, I.A. (1980) Contractile properties of fish fast muscle fibers. *Marine Biol. Letters.* 1:323-328.
- Johnston, I.A. (1983) Dynamic properties of fish muscle. In P.W. Webb and D. Weihs (eds): *Fish Biomechanics*. New York: Praeger, pp. 36-67.
- Lankester, E.R. (1896) On the *Lepidosiren* of Paraguay, and on the external characters of *Lepidosiren* and *Protopterus*. *Trans. Zool. Soc. Lond.* 14:12-24.
- Lauder, G.V. (1980a) The role of the hyoid apparatus in the feeding mechanism of the coelacanth *Latimeria chalumnae*. *Copeia* 1980:1-9.
- Lauder, G.V. (1980b) Evolution of the feeding mechanism in primitive actinopterygian fishes: A functional anatomical analysis of *Polypterus*, *Lepisosteus*, and *Amia*. *J. Morphol.* 163:283-317.
- Lauder, G.V. (1981) Intraspecific functional repertoires in the feeding mechanism of the characid fishes *Lebiasina*, *Hoplias*, and *Chalceus*. *Copeia* 1981:154-168.
- Lauder, G.V. (1982) Patterns of evolution in the feeding mechanism of actinopterygian fishes. *Am. Zool.* 22:275-285.
- Lauder, G.V. (1983a) Functional design and evolution of the pharyngeal jaw apparatus in euteleostean fishes. *Zool. J. Linn. Soc. Lond.* 77:1-38.
- Lauder, G.V. (1983b) Functional and morphological bases of trophic specialization in sunfishes (Teleostei, Centrarchidae). *J. Morphol.* 178:1-21.
- Lauder, G.V. (1985) Functional morphology of the feeding mechanism in lower vertebrates. In H.-R. Duncker and G. Fleischer (eds): *Functional Morphology of Vertebrates*. New York: Springer-Verlag, (in press).
- Luther, A. (1913) Über die von N. Trigeminus versorgte Muskulatur der Ganoiden und Dipneusten. *Acta Soc. Sci. Fennicae* 41:3-72.
- McMahon, B.R. (1969) A functional analysis of the aquatic and aerial respiratory movements of an African lungfish, *Protopterus aethiopicus*, with reference to the evolution of the lung ventilation mechanism in vertebrates. *J. Exp. Biol.* 51:407-430.
- Miles, R. (1977) Dipnoan (lungfish) skulls and the relationships of the group: A study based on new species from the Devonian of Australia. *Zool. J. Linn. Soc. Lond.* 61:1-328.
- Owen, R. (1839) Description of the *Lepidosiren annectens*. *Trans. Linn. Soc.* 18:327-361.
- Parker, W.K. (1892) On the anatomy and physiology of *Protopterus annectens*. *Trans. Roy. Irish Acad.* 30:109-230.
- Perkins, P.L. (1972) Mandibular mechanics and feeding groups in the Dipnoi. Unpublished doctoral dissertation, Yale University, New Haven, Connecticut.
- Rosen, D., P.L. Forey, B.G. Gardiner, and C. Patterson (1981) Lungfishes, tetrapods, paleontology, and pleiomorphy. *Bull. Am. Mus. Nat. Hist.* 167:159-276.
- Schultze, H.-P., and L. Trueb (1981) *Basic Structure and Evolution of Vertebrates*, Vols. I, II. E. Jarvik. 1980. London: Academic Press. (review). *J. Vert. Paleontol.* 1:389-397.
- Shaffer, H.B., and G.V. Lauder (1985a) Aquatic prey capture in ambystomatid salamanders: patterns of variation in muscle activity. *J. Morphol.* 183:273-284.
- Shaffer, H.B., and G.V. Lauder (1985b) Patterns of variation in aquatic ambystomatid salamanders: kinematics of the feeding mechanism. *Evolution.* 39:83-92.
- Smith, K.K. (1984) The use of the tongue and hyoid apparatus during feeding in lizards (*Ctenosaura similis* and *Tupinambis nigropunctatus*). *J. Zool. Lond.* 202:115-143.
- Sokal, R.R., and F.J. Rohlf (1981) *Biometry*. San Francisco: W.H. Freeman.
- Thomson, K.S. (1969) The biology of the lobe-finned fishes. *Biol. Rev. Camb. Phil. Soc.* 44:91-154.
- Wassersug, R.J. (1976) A procedure for differential staining of cartilage and bone in whole formalin fixed vertebrates. *Stain Technol.* 51:131-134.
- Wiley, E.O. (1979) Ventral gill arch muscles and the interrelationships of gnathostomes, with a new classification of the Vertebrata. *Zool. J. Linn. Soc. Lond.* 67:149-179.