THE FUNCTIONAL MORPHOLOGY OF GILL VENTILATION IN THREE SPECIES OF ANURAN TADPOLES

By

Norman Gradwell

Summary. The water pumping apparatus of Rana catesbeiana is described and a numerical terminology which is proposed for its anterior visceral muscles, is also extended to Ascaphus truei and Xenopus laevis. Muscle activity, mechanical displacement, hydrostatic pressures, and water flow during water pumping, are investigated in the three species by electrophysiology, photography, and direct observation. In catesbeiana, phasic ventilation and hyperventilation are effected by two structurally different types of striated muscle fiber. A muscle in the operculum of catesbeiana alone, operates an auxiliary pump behind the gill clefts. A passive oral valve in truei dispenses with muscular activity to maintain sucker adhesion and to move the jaws during gill ventilation with the sucker disengaged. Frequency and amplitude of ventilation in truei are independent of ambient water velocity. A valvular ventral velum facilitates dual water pumps in catesbeiana and truei, but laevis lacks a valvular ventral velum and therefore has only a single water pump.

Department of Biology,
McGill University,
Montreal, Quebec.

PhD thesis
July 1970

Short title:

FUNCTIONAL MORPHOLOGY OF GILL VENTILATION IN ANURANS

THE FUNCTIONAL MORPHOLOGY OF GILL VENTILATION IN THREE SPECIES OF ANURAN TADPOLES

By

Norman Gradwell

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Doctoral committee:

R. Carroll

P.S.B. Digby

V.M. Pasztor (Chairman)

Department of Biology

McGill University

Montreal, Quebec

July, 1970

Preface

Considering the growing literature on fish ventilation mechanisms (mainly from the laboratory of Dr. G.M. Hughes, University of Bristol), anuran tadpoles, which are the only amphibians with internal gills, have been neglected. The present thesis attempts to lay a broad foundation for later detailed studies of gill ventilation mechanisms in the Anura. For this reason, three species showing marked differences in their gill ventilation mechanisms were chosen for investigation: Rana catesbeiana represents a phylogenetically advanced tadpole which is essentially a bottom-dweller in sluggish water such as that found in small lakes; Ascaphus truei is generally believed to be phylogenetically the most primitive living anuran, and its tadpole clings by an oral sucker to rocks in mountain torrents; Xenopus laevis represents a phylogenetically primitive tadpole, which is nektonic in ponds, and which is aberrant in several interesting aspects of its water pumping mechanism. Although these three types of tadpole by no means show all the possible variation of the gill ventilation mechanisms, they exhibit functional and morphological differences which reveal close adaptations to their respective habitats.

The present thesis is arranged in autonomous chapters, each covering a specific aspect of the subject. I believe that the greater clarity afforded by this arrangement outweighs the very small extent of duplication. The final chapter is devoted to an overall discussion of the research, in which the chief points in the comparative functional morphology of gill ventilation are emphasized, and hypothetical models are presented as a working basis for further investigations.

I appreciate Dr. D.M. Steven's welcoming me back to McGill University to elaborate on my earlier studies of anuran tadpoles, and I am grateful for Dr. V.M. Pasztor's interest to see this research progress to its present state of development.

N.G.

CONTENTS

CHAPTER 1

THE MORPHOLOGY OF GILL VENTILATION IN *RANA CATESBEIANA*

ABSTRACT. The gross and histological anatomy of the gill ventilation apparatus are discussed. Two histologically different types of muscle fiber are identified in certain muscles by virtue of the variation in the proportion of fibrillar to sarcoplasmic material in the muscle fibers. An accessory jaw tendon, the valve of the first gill cleft, and several ligaments are described for the first time in an anuran tadpole. An opercular muscle and a fascia lateralis are also described for the first time in a ranid tadpole. The rugulose lining of the pharynx is well vascularized; attention is also drawn to conical epithelial cells whose points project into the lumen of the pharynx. Several discrepancies in the literature on tadpole anatomy are discussed and a numerical terminology is proposed for the muscles of the gill ventilation apparatus.

C O N T E N T S

DISCUSSION CONT.

Legend

A	auricle
A.BR	afferent branchial artery
AR	arteriole
AC	auditory capsule
AH	ligamentum anterohyoideum
A.PH	afferent pharyngeal artery
ARY	arytenoid cartilage
BC	buccal cavity
BV	blood vessel
B1-B14	branchial muscles 1 to 14 (Table I)
CA	ceratohyalian articular surface
CB1-4	ceratobranchialia 1 to 4
CB.C	cuboidal cell
CC	chondrocranium
CG	ciliated groove
CH	ceratohyale
CHL	" lateralis
CHM	" medialis
C.HY	cornu hyalis
CM	cartilago Meckeli
CN.T	connective tissue
CP.B	capillary bed
CQL	ligamentum cornu quadratum laterale
CR.H	crista hyoidea (46)
CRI	cartilago rostrale inferior
CRS	" " superior

CS	ligamentum cornu suprarostralis
CT	cornu trabeculae
C1,2	copulae 1, 2
D	dorsal velum
DVP	" velar pad
E	esophagus
E.BR	efferent branchial artery
EC	epithelial cushion
EJ	external jugular vein (32)
FC	filter canal
FF	" fold
FLS	fascia lateralis, Schulze
FN	filter niche
FR	" ridge
FRP	fascia rostrale posterior
FS	fenestra subocularis (35)
GC	gill cavity
GT	" tuft
G1-4	" clefts 1 to 4
HP	hypobranchial plate
H1-H7	hyoidean muscles 1 to 7 (see Table I)
LG	lymph gland (1)
LS	stem of lung
MS	ligamentum mandibulo-suprarostrale (46)
MVO	membrana vasculosa opercularis
M1-M10	mandibular muscles 1 to 10 (see Table I)
NV	nasal valve
OC	opercular canal

P	cartilago pterygoquadratum
PAH	processus anterior hyalis
PAQ	" articularis quadrati
PB	planum basale
PH	pharynx
PH.M	pharyngeal mucosa
PMQ	processus muscularis quadrati
PO	" oticus
PP	" pseudopterygoideus
PPH	" posterior hyalis
PR	pars reuniens
PRA	processus retroarticularis
PS	parasphenoid
PT.C	pointed cell
Q	cartilago quadratum
QE	ligamentum quadrato-ethmoidale
RA	musculus rectus abdominis (34)
RSQ	ligamentum rostrale superior quadrati
SC	" supraorbitalis cranii
SE	" " ethmoidale
SP1-4	spicula 1 to 4
SV	subclavian vein
S1,2	spinal muscles 1 and 2 (see Table I)
TA	tendo accessorius
TC	trabeculae cranii
V	ventral velum
VN	ventricle

Introduction

Complementary but independent reports discuss the structure and functioning of the jaw apparatus of the larvae of Rana temporaria (5) and R. catesbeiana (18). The findings (5) on the pumping apparatus of temporaria do not include the branchial muscles but otherwise compare closely with the situation in catesbeiana. Measurements of hydrostatic pressures in the ventilation system of normally breathing bullfrog tadpoles (23) are consistent with earlier evidence (29) for two regularly alternating force pumps in front of the gill clefts. However, a better understanding of the bullfrog tadpole's water pumping mechanism requires familiarity with its structure. Hence, the present report describes the gross and histological anatomy of the gill ventilation apparatus of the bullfrog tadpole preparatory to a more detailed functional study of the system (21).

Incidental references have been made by many authors to structures in the gill ventilation apparatus of anurans, but only for the following species is there enough detail to permit functional deductions and useful comparisons with Rana catesbeiana: R. temporaria (35, 37, 13, 5); Pelobates fuscus (45, 46); Xenopus laevis (8, 54, 33, 49); Rana dalmatina (= R. agilis) (29); Ascaphus truei (38, 53, 22); Bufo regularis (47, 48); and Phyllomedusa trinitatus (26).

The most important early research on the visceral skeleton of anuran tadpoles concerns Rana temporaria (35, 36, 37; 13, 14). Other findings were based on dissections of Pelodytes punctatus (39), Alytes obstetricans (40), Xenopus and Pipa (41). There is also a comparison of the hyobranchial skeleton of Alytes with that of several genera from other anuran families (42). More recently,

the visceral skeleton has been described in Bufo regularis (48, 50), Xenopus laevis (33, 49), Rana tigrina (3), and R. temporaria (5). Concerning R. catesbeiana, the only published information on the larval visceral skeleton is contained in brief references (55, 18, 20), which indicates the need for further data on this component of the gill ventilation apparatus.

It has been pointed out (49) that very little published research exists on the visceral musculature of larval anurans. There is great diversity in the feeding and water pumping mechanisms of anuran larvae and so little is known of the concomitant specializations of musculature, that further studies along these lines are called for. It is therefore also the object of the present paper to help fill this hiatus, thus completing the structural basis needed for further functional studies of the bullfrog tadpole (21).

Materials and Methods

Bullfrog tadpoles (Rana catesbeiana Shaw) were collected at Lake Hertel, St. Hilaire, Quebec, and thirty large specimens were selected for study. These tadpoles were at stage 35 of Gosner (17).

Canadian populations of bullfrog tadpoles attain a snout-to-vent length of over 4 cm before metamorphosis. It was therefore possible to substitute gross dissection for reconstructions based on serial histological sections. The results were checked on the basis of seven sets of histological sections.

Dissections were made on tadpoles which had been deeply anesthetized in 1 % urethane and then killed by immersion in 40° C tap water. The animals were bathed for 5 min in 10 % glacial acetic acid. This procedure softened the skin, permitting its easy removal with watchmaker's forceps. The normally transparent hyaline cartilages became translucent when contacted by the acid, and easier to see under a dissecting microscope; this procedure was found preferable to staining.

Tissues destined for histological study were fixed in aqueous Bouin's solution, dehydrated in a graded series of aqueous ethanol solutions and cleared in xylene before being embedded in paraffin. Serial sections were cut at 9 μm thickness, stained with Ehrlich's haematoxylin, counter-stained with erythrosin, and mounted in balsam.

The dissections were photographed with a single lens reflex camera and the enlarged image (X10) was printed on mat paper. India ink was used to outline the relevant structures on the print, which was then dried, and the photographic emulsion bleached with 5 % potassium ferricyanide. Frosted acetate film was used as an overlay to trace the inked photograph and the details were then added by freehand drawing from dissections. The same photographic and drawing procedure was used for illustrating histological sections photographed through a photomicroscope.

Results

As it is necessary to reiterate certain somewhat cumbersome terms in the text, the following abbreviations will be used: ceratobranchiale = CB, ceratohyale = CH, ceratohyale lateralis = CHL, ceratohyale medialis = CHM, hypobranchial plate = HP.

EPITHELIAL AND VASCULAR LININGS

Aspects of the mucosa of the gill ventilation apparatus of anurans have been investigated in Pelobates fuscus (45, 46); Rana dalmatina (29); R. temporaria (5, 27); Phyllomedusa trinitatus (26, 27); and several other species (27). In this respect, there are close structural similarities between Rana catesbeiana and these other species. Therefore, in the present section of this report, appropriate references to these other investigations has permitted the avoidance of needless duplication of research. Moreover, in deference to Mr. Van Kuijen, University of Leiden, who is preparing a detailed study on the vascularization of larval R. temporaria (de Jongh, in litt.), this subject has been only briefly considered in the present report on R. catesbeiana.

Buccal cavity

At the entrance to the buccal cavity, keratinized skin covers the single cartilago rostrale superior and also the bilateral cartilagines rostrale inferiores to form the crescentic upper and lower beaks respectively (Figs. 1-4). Rows of horny denticles on the upper and lower lips form the other component of the dental apparatus.

Immediately within the oral entrance a pad of epithelium covers the cornua trabeculae and consists of squamous cells which are partly stratified (EC, Fig. 3). The cushion-like structure so formed is prominent anteriorly where its surface is studded with hillocks of slight epithelial elevations, but its thickness

diminishes more posteriorly. The rest of the buccal roof is formed by the ventral cartilages and nascent parasphenoid of the skull and is lined by mucosa which projects papillae into the buccal cavity (Fig. 3). The epithelium of the buccal roof is similar to that of Pelobates (45) and Phyllomedusa trinitatus (26).

Papillae also project into the buccal cavity from the mucosa covering the cartilages of the buccal floor (Fig. 4). Figure 4 also shows that the adult tongue, which develops above copula 1, is not yet present at stage 35 of Gosner (17). Medial to the CHL, at gill cleft 1, the mucosa is continuous with the lining of the gill cavity (Figs. 4, 8). The epithelium of the buccal floor consists of one or two layers of squamous cells.

Relative to the pharynx and gill cavity, the buccal lining is poorly vascularized and it is probably of little significance for blood ventilation by the buccal water current.

The vela

The ventral velum has been found to act in a valve-like fashion during normal gill ventilation; both dorsal and ventral vela serve as hydrofoils in deflecting the ventilation current (20). A consideration of the structure of the vela may permit a better understanding of these functions.

The flexible cartilaginous spicula on both sides of the middle line, are spanned and covered ventrally and dorsally by epithelium between which there is a highly vascular plexus, thus forming the single, non-muscular ventral velum (V, Figs. 4, 6AB, 8). It extends bilaterally and then turns upward and backward to become the paired dorsal vela (D, Figs. 4, 6B, 8). These are mucosal folds attached along their length to the pharyngeal roof and they pass toward the entrance of the esophagus (see also 46,

26, 29). Approaching the middle line, the dorsal vela become reduced in size until, at 1 to 2 mm in front of the esophageal entrance, they are mutually joined by a low, transverse fold of mucosa. Whereas the dorsal velum protrudes mainly anteroventrad, its medial aspects are folded toward the esophagus. The dorsal velum has neither skeletal nor muscular support. However, there are a pair of small swellings, the dorsal velar pads (DVP), on both left and right sides of the pharynx. They hang immediately behind the dorsal vela, near the lateral junctions of the dorsal and ventral vela with each other. These pads consist of squamous epithelium which covers a resilient connective tissue. In vivo, the convex surface of these bilateral pads is oval, the more medial ones appearing posteriorly more bulbous, but their shapes are distorted in fixed material (DVP, Fig. 6B). Between the pads there is a groove which is 1 mm wide and 2.5 mm long. Similar pad-like structures have been called "pressure cushions" in Rana dalmatina (29) and Phyllomedusa (26).

The dorsal epithelium of the ventral velum consists of non-glandular simple squamous cells at its margin, and stratified taller squamous cells farther back, over small posterior processes of the velar edge. There are five bilaterally paired processes and a single median process (Fig. 4).

Like Phyllomedusa (26), the ventral velum of Rana catesbeiana also has secretory columnar cells on its underside. These cells are orientated in continuous rows parallel to the velar edge, but the rows between the spicula are somewhat crescentic, being slightly convex to the anterior. The secretory rows are separated from one another by squamous cells. The nuclei of adjacent secretory cells are sometimes at different levels, which gives them a stratified appearance. Filter traps under the ventral velum, similar to those of Phyllomedusa (26), are also present in Rana catesbeiana.

Pharynx

In the middle line, the pharynx is partially divided by the dorsal bulge of the heart (Fig. 6A), but latex casts showed that an interchange of water across this partial division is possible at least during expiration, when the pharynx is expanded. The left and right sides of the pharynx may therefore be regarded as a single chamber.

Ventrally, the pharynx is perforated by three gill clefts (G2-4, Figs. 1, 4, 6B, 8). These clefts correspond to endodermal pouches 3, 4, and 5, as was deduced from the position of the aortic arches, using the method of Millard (32; see also Table II). In all amphibian larvae except those of the Gymnophiona, the endodermal pouch 1 (the spiracle of elasmobranchs) does not perforate (34). Rana catesbeiana is no exception in this respect, but it differs from Ascaphus, Xenopus, Scaphiopus, Phyllomedusa, and many other genera in that the endodermal pouch 2 perforates to become the membranous gill cleft 1 of the tadpole. It has been proposed that the ventral velum be used to demarcate the buccal cavity from the pharynx (23). Therefore, unlike the three clefts between the pharynx and gill cavity, the first cleft opens directly into the gill cavity from the buccal cavity (G1, Figs. 1,4,8).

Valvular activity at the first gill cleft is facilitated by the rostrad projection of CB 1, which is covered by mucosa of the buccal floor (Fig. 4). The connective tissue sandwiched between this cartilaginous support and its epithelium has a resilience which probably adds to the efficiency of the valve.

The pharyngeal roof is lined by mucosa covering the planum basale, the nascent parasphenoid, the floor of the orbit (fenestra subocularis,35), and the pterygoquadrate (Figs. 3, 6B). The mucosa contains a glandular zone in the same position as that of

Pelobates (45) and Rana dalmatina (29), but unlike Kenny's Fig. 1 (27) of Hyla geographica, the zone extends across the middle line in Rana catesbeiana, where it is V-shaped, pointing toward the esophagus. Nevertheless, the histological character of this zone in R. catesbeiana is similar to Kenny's descriptions (26, 27). Injected latex revealed that a dense capillary bed in the pharyngeal roof, like that of the glandular ventral velum, coincides precisely with the position of the glandular zone in catesbeiana.

Simple columnar and pseudostratified columnar cells form a ciliated groove immediately behind the dorsal velum, running from the bilateral junctions between the dorsal and ventral vela toward the esophagus (CG, Fig. 8). The groove is narrow at first, but, as it passes obliquely backward, it broadens. In the middle line the grooves of both sides meet and together they form a continuous ciliated funnel near the esophageal entrance.

The dorsal aspect of the CBs are covered by a rugulose mucosa in gross structure like that of Pelobates (Fig. 9). As in Phyllomedusa (26), this mucosa is arranged in single filter plates on CBs 1 and 4, and double filter plates on CBs 2 and 3. Superficial ridges, supported by an arteriole system, form an intricate latticework (Fig. 10A,B,C). The crests of these ridges are sharp, because they are formed by pointed cells (PT.C, Fig. 10C). A dichotomously branching arteriole system, exactly corresponding to the lattice of epithelial ridges was demonstrated by the injection of latex via the truncus arteriosus. These arterioles run along the bases of the ridges and they ramify into a superficial capillary bed (mesh diameter, 20 to 60μm) in the vertical sides of the filter folds (Fig. 10B). In vivo staining with aniline blue revealed that there are no "filter shelves" or "filter crevices" (of Kenny, 26) in Rana catesbeiana.

Gill cavity

Unlike the other gill clefts, the first cleft is not completely surrounded by cartilage, has no gill tufts along its anterior border, and it does not interconnect the gill cavity and the pharynx; it lies between the CHL and a forward projection of CB 1 and it connects the gill cavity directly with the buccal cavity (G1, Figs. 1, 4, 8). The absence of this gill cleft in several other genera is an important difference in respect to water flow.

The fold of the integument forming the operculum, which has grown back from the hyoidean arch (2, 34), is fused at the level of the diaphragm to the skin covering the abdomen, except for a left, lateral branchial outlet. This outlet is neither analogous nor homologous to the spiracle of certain elasmobranchs. It is free from membranous or muscular valves and projects into a short spout whose orifice faces backward and slightly upward.

As the left and right sides of the gill cavity are joined by a fairly broad opercular canal across the ventral midline (OC, Figs. 1, 6A), the gill cavity may be considered as a single chamber. Anteriorly, the opercular canal is, however, slightly constricted by the meeting in the middle line of the dorsal and ventral bundles of the H7 muscle (Figs. 1, 6A,B).

There are four pairs of gills, and they are structurally similar to those of Pelobates (Fig. 9). The gills consist of soft tufts attached linearly by connective tissue and by the arteries at their bases, to the respective muscles B2, B6, and B9, along the CBs. As there is no branchial muscle along CB 4, its few gill tufts are attached directly to the cartilage by connective tissue. The gill tufts form flexible, arborescent villosities which are highly vascular and they are continuously bathed in water within the sac-like gill cavity. There is occasional

variation in the number of gill tufts per arch (see Table II). Simple squamous epithelium covers the arborescent parts of the tufts and the stems of the tufts which, as in Pelobates (46, Fig. 34), are traversed by an afferent and an efferent blood vessel. The gill tufts gain some turgidity from the blood pressure transmitted to them from the heart.

In addition to gill tufts, the bullfrog tadpole has a fine network of capillaries in the mucous lining which connects the bases of the gill tufts with one another and which is continuous with the capillary bed of the pharyngeal mucosa. Strawinski (52, for Rana esculenta) was the first to point out the respiratory importance of this vascular lining, the membrana vasculosa operculari s. It lines the gill cavity and it is posteriorly in contact with the diaphragm and ventrally with the opercular skin (MVO, Figs. 1, 8). Anteriorly, the membrane is separated from the stratum compactum to form the anterior border of the gill cavity (Fig. 8). The membrane is reflected on to CB 1 and it lines gill cleft 1; at a point just medial to the cleft, connective tissue attaches the membrane to the dorsal surface of the H6 muscle just before the latter inserts on the CHL. This lateral attachment of the opercular lining enables venous and arterial vessels to augment the rich blood circulation of the membrana vasculosa operculari s (19). In the ventral midline, the opercular lining is attached by connective tissue to the posterior, fast fibers of the H6 muscle (24; Fig. 1). In addition to serving as a bridge for nerves, this connection also permits unilateral or bilateral blood supply to the membrana vasculosa operculari s (19).

CARTILAGES

De Beer's terminology (4) will be used in the present study except where otherwise indicated.

The movable cartilages of the gill ventilation apparatus are essentially those of the visceral skeleton. This structure has been so well investigated in Rana (e.g. 35, 37, 13, 14, 15, 42, 29, 3, 5) that only a summary of its anatomy in R. catesbeiana is necessary in the present study.

In the middle line, transverse, parallel collagen fibers interconnect the bilateral cartilagines rostrale inferiores (CRI, Figs. 4, 5, 7). These short fibers are attached peripherally around the joint, forming a strong sheath and enclosing what appears to be a small median copula as in R. temporaria (cf. 5). However, in R. catesbeiana this median structure has small, closely packed chondrocytes in a sparse matrix, and it resembles a chondrified ligament. Further account of the mandibular skeleton of catesbeiana is unnecessary as it closely resembles that of temporaria (cf. 5).

The structure of the cartilages of the buccal floor is a compromise between the requirements of an expansive area with firm support, and flexibility and articulation at the joints between the elements of this complex (Figs. 1, 4, 5, 6A, 11). The medial union of the CHMs by the pars reuniens and by the copulae, and the median connection between the HPs, facilitates bending along the axis of the tadpole, while the bilateral junctions of the HPs with the CHMs and with the unpaired copulae 2, also permit transverse bending. Each CHM has two forward protrusions, and a processus posterior hyalis (14) (PPH, Fig. 4) which slightly overlaps the HP dorsally. The processus anterior hyalis (14) (PAH, Fig. 4), which is the more medial of the two forward

protrusions, is larger and more robust than the second protrusion, the cornu hyalis (C.HY, Fig. 4), which has apparently not been hitherto described.

The CHL is more robust than the dorsoventrally flattened CHM. A firm prominence on the ventral CHL, oblique to the axis of the tadpole, subserves the insertion of an important pumping muscle (H6, Figs. 1, 7, 11). A surface for articulation of the CH with the quadrate is afforded by a condyl on the dorsal aspect of the CHL (CA, Fig. 5). The outer face of the CHL is relatively flat and provides support for the attachment of three hyoidean muscles (CHL, Fig. 7).

Copula 1 is somewhat cylindrical and bluntly pointed at its ends. It lies in the posterior part of the ligamentum interhyoideum, transversely spanning the hyoglossal sinus between the anterior processes of the CHMs (C1, Figs. 4, 6A). The pars reuniens lies immediately behind copula 1, and it is cup-shaped in transverse section, being convex toward the ventral aspect. This shape, combined with the resilient material of the pars reuniens, render the structure well suited to withstand such tensile and compressive forces that seem to occur due to dorsoventral oscillations of the buccal floor (Fig. 11). Histological inspection of the pars reuniens revealed that, unlike the hyaline elements of the rest of the visceral skeleton, its smaller chondrocytes are clustered in a sparse matrix.

The coupling of the CHMs by the pars reuniens (PR, Figs. 1, 4, 5, 6A) and the additional strong but flexible tissue between the other cartilages of the buccal floor, ensure the efficient transfer of movement, enabling the CHs, copulae and HPs to function as a concerted whole during operation of the buccal pump.

Unlike the rest of the visceral skeleton, copula 2 is not derived from cells of the neural crest (4, pp. 406, 407, 474). The hyaline cartilage of copula 2 merges anteriorly with the pars reuniens. A short posteroventral projection of copula 2, the crista hyoidea (46) (CR.H, Fig. 6A), provides a firm site for the origin of the B7 muscles (Fig. 1) which couple the bilateral CBs to the buccal cartilages.

There is controversy regarding the ontogenetic origin of the pars reuniens and copula 2 (4, pp. 406, 407, 474). These cartilages, like the HPs, are more closely associated with the branchial complex, in an ontogenetic sense, than they are with the hyoidean arch. However, in a functional sense, the pars reuniens, copula 2, and HPs are active elements of the buccal pump, because they form part of the buccal floor (Figs. 1, 5).

Posterolaterally, the CBs are directly fused with one another by the commissurae terminales. CB 1 is directly fused with the HP (Figs. 4, 5). Lateral to its junction with the HP, CB 1 is anteriorly expanded and dorsoventrally flattened into a blunt process (Figs. 4, 5) which supports the valve of the gill cleft 1. The CBs 2, 3, and 4 are not directly fused with the HP by hyaline cartilage but the junctions between these CBs and the HPs are flexible and of a similar histological character to the pars reuniens.

The CBs 2, 3, and 4 are fused with one another where they meet the HP. A ventromedial prominence of CB 2 (the processus branchialis) near the medial junction of CBs 1, 2, and 3, forms a buttress for the attachment of seven branchial muscles (Table I). Posteriorly directed spicula (SP, Fig. 5) project freely from the dorsal aspect of the junctions between CBs 2, 3, and 4, and the HP. Spiculum 1 (SP^1, Fig. 5) is not attached to the HP, but it extends posterolaterally from CB 1.

Unlike the rest of the visceral skeleton, copula 2 is not derived from cells of the neural crest (4, pp. 406, 407, 474). The hyaline cartilage of copula 2 merges anteriorly with the pars reuniens. A short posteroventral projection of copula 2, the crista hyoidea (46) (CR.H, Fig. 6A), provides a firm site for the origin of the B7 muscles (Fig. 1) which couple the bilateral CBs to the buccal cartilages.

There is controversy regarding the ontogenetic origin of the pars reuniens and copula 2 (4, pp. 406, 407, 474). These cartilages, like the HPs, are more closely associated with the branchial complex, in an ontogenetic sense, than they are with the hyoidean arch. However, in a functional sense, the pars reuniens, copula 2, and HPs are active elements of the buccal pump, because they form part of the buccal floor (Figs. 1, 5).

Posterolaterally, the CBs are directly fused with one another by the commissurae terminales. CB 1 is directly fused with the HP (Figs. 4, 5). Lateral to its junction with the HP, CB 1 is anteriorly expanded and dorsoventrally flattened into a blunt process (Figs. 4, 5) which supports the valve of the gill cleft 1. The CBs 2, 3, and 4 are not directly fused with the HP by hyaline cartilage but the junctions between these CBs and the HPs are flexible and of a similar histological character to the pars reuniens.

The CBs 2, 3, and 4 are fused with one another where they meet the HP. A ventromedial prominence of CB 2 (the processus branchialis) near the medial junction of CBs 1, 2, and 3, forms a buttress for the attachment of seven branchial muscles (Table I). Posteriorly directed spicula (SP, Fig. 5) project freely from the dorsal aspect of the junctions between CBs 2, 3, and 4, and the HP. Spiculum 1 (SP^1, Fig. 5) is not attached to the HP, but it extends posterolaterally from CB 1.

CONNECTIVE TISSUE

Mandibular arch

The ligamentum rostrale superior cartilago Meckeli has been illustrated in Rana catesbeiana (18). It has since been realized that Schulze (46, p. 8) called this ligament the l. mandibulo-suprarostrale in Pelobates, a term which is adopted in the present study by virtue of its priority. However, in Rana catesbeiana there are two such ligaments interconnecting the suprarostral and Meckel's cartilages. They have the same general attachments, and they run parallel and close to each other. The one ligament (MS, Fig. 7) is more superficial than the other and its fibers are attached to the lateral edge of the cartilago rostrale superior, whence they run to the dorsal aspect of the medial epiphysis of the cartilago Meckeli and diverge slightly as they attach themselves to it. The second ligament (not shown in Fig. 7) is slightly deeper than the first and its fibers have a wider attachment on the inner surface of the cartilago rostrale superior, at its lateral edge and just behind the attachment of the first ligament. The fibers then run toward the cartilago Meckeli and are attached to it immediately within the attachment of the first ligament.

A short, chondrified ligament, the l. cornu suprarostrale (CS, Fig. 2), joins the cornu trabeculae with the cartilago rostrale superior, while the median notch in the cartilago rostrale superior (CRS, Fig. 2) is spanned by a fascia rostralis anterior.

The H3 muscle in Fig. 7 covers a short l. quadrato Meckeli, which joins the most lateral aspect of the cartilago Meckeli to the outer surface of the pars articularis quadrati. The other jaw ligaments have already been discussed (18) and are shown in Figs. 2, 3, and 7.

Hyoidean arch

Such tensile forces that are imposed on the copulae during the extreme dorsoventral excursions of the CHMs are probably relieved to some extent by a strong axial ligament, which has been called the l. interhyoideum (5). It interconnects the anterior processes of the CHMs by bridging the hyoglossal sinus immediately rostral to the pars reuniens (Figs. 1, 4). Anteriorly, this ligament is partly chondrified to form the first copula. A thin l. anterohyoideum, not hitherto described, interconnects the processus anterior hyalis and the cornu hyalis (AH, Fig. 4).

The triangular space bounded by copula 2, the CHM, and the HP (Figs. 1, 4) is occupied by a l. intrahyoideum which is attached tangentially to the lateral aspect of copula 2 near the posterior limit of the pars reuniens. It then diverges as it passes caudad to form a wide attachment to the anterior edge of the HP.

In the posterolateral region of both sides of the buccal cavity there is a small pad of connective tissue. The anterior edge of the pad is attached to the posterior aspect of the CHL. The flat, inner face of the pad is lined with non-glandular squamous epithelium, while its outer face is convex and is covered by some ten muscle fibers of the H2 muscle (Fig. 7).

A bilateral ligament which has not been previously reported, arises from the ventral surface of the CH, medial to the base of the cornu hyalis. The ligament is flattened anteroposteriorly and it diverges slightly as it passes into the stratum compactum of the skin below this area of the CH, just medial to the fascia lateralis Schulze (FLS, Fig. 1).

The median raphe of each of the M10 and H6 muscles is joined by weak connective tissue to the underlying buccal skin.

Branchial arches

The medial fusions between the CBs are reinforced by short interconnecting ligaments (Fig. 1) which have not been mentioned in earlier publications on tadpole morphology. Gill clefts 3 and 4 are each medially spanned by such a ligament, while the second gill cleft is spanned by two such ligaments, which converge on a point of mutual attachment to CB 2. There is no ligamentous connection between the CH and CB 1.

The fascia lateralis, Schulze (FLS, Fig. 1), occurring symmetrically on both sides of the ventral midline, is a pigmented sheet of strong connective tissue. Along the line FS, the fascia is attached to the ventrolateral edges of the cartilagines quadratum and pterygoquadratum, and along LS, it is attached to the anterior border of the lateroventral bundle of the H7 muscle. The side FL is entirely free from attachment.

Cranial elements

Aside from the visceral apparatus, there are other ligaments which are also important in the gill ventilation system. For example, the most dorsal part of the processus muscularis quadrati is bound to the chondrocranium and to the ethmoid region of the quadrate cartilage by a strong connective tissue containing two main ligaments. A l. supraorbitalis cranii (SC, Fig. 11) joins the processus muscularis quadrati to the roof of the brain case, while the l. supraorbitalis ethmoidale (SE, Fig. 11) connects the processus muscularis quadrati to the dorsal surface of the quadrate which lies at the base of the processus pseudopterygoideus (PP, Fig. 3). In addition, a l. intertrabeculare across the midline, spans the rostral ends of the cornua trabeculae (CT, Fig. 2). Immediately behind the l. intertrabeculare, a sheet of connective tissue, the fascia rostrale posterior (FRP, Fig. 2), interconnects the medial edges of the cornua trabeculae.

MUSCLES

Nomenclature

Of the various nomenclatures used by earlier authors (6, 16, 46, 31, 29, 9, 30), Edgeworth's (9) is finding the most popular usage in recent works (e.g. 38, 47, 5). It was also used in Rana catesbeiana (18, 20, 24) but more detailed studies of this species (21), and general studies of other species (22; Gradwell in preparation) have revealed inadequacies in Edgeworth's terminology. They stem mainly from his variable reliance on function, based on cadavers and histological sections, as a nomenclatorial criterion.

The value of function as a standard for naming muscles is questionable when unsupported by detailed physiological evidence. There are many instances where electromyography has failed to confirm conventional functional anatomy as an infallible criterion of muscle function. Moreover, muscles often have more than one function, and in the absence of detailed research, it may be difficult to assess the relative importance of the functions. For example, Edgeworth's "Levatores arcuum branchialium" in larval anurans are believed by Kratochwill (29) to raise the CBs as well as open the gill clefts. It is also difficult to grasp from Edgeworth's "Constrictores branchialium" which components of the branchial apparatus are constricted. Kratochwill has suggested that these muscles might constrict the afferent branchial arteries, while in Xenopus tadpoles where these muscles are clearly visible through the transparent operculum, they appear to constrict the gill clefts (Gradwell, unpublished). The naming of muscles according to functions deduced from non-living anatomy may therefore lead to controversy.

Edgeworth's system is not consistent, as it names certain muscles from their supposed functions and others from their locations (e.g. Constrictor branchialis; Intermandibularis). To overcome the problem of naming muscles which perform the same function, for instance the elevation of the mandible, Edgeworth used combinations of functional and locational criteria, but this yielded cumbersome terms like "Levator mandibulae posterior superficialis".

In some cases, Edgeworth's terms are anatomically misleading. For example, the "Geniohyoideus" of tadpoles does not originate on the hyoidean arch. In addition, Pusey (38) has presented a cogent argument opposing Edgeworth's use of the term "Transversus ventralis II" (see discussion).

Notwithstanding the impartial precepts of the investigator, the naming of muscles from their supposed functions deduced from classical anatomy, tends to prejudice enquiry. This is perhaps why recent studies by some physiologists designate muscles by numbers, aside from the helpful brevity of such a system.

In preserved material, which is usually first to be documented, it is far more reliable to name muscles according to their topography than to name them according to functions deduced from such material. However, for accurate description of the origins and insertions of the muscles, their names would become inordinately long. In the bullfrog tadpole (Table I) and in the tadpole of Ascaphus (22), this problem has been solved by dividing the visceral muscles of the gill ventilation apparatus into groups according to their innervations, numbering the muscles in each group, and tabulating their origins and insertions for a particular stage of development. The system is particularly suitable for larval anurans since during metamorphosis these

visceral muscles change their positions by migration (if they do not atrophy). Their functions may also alter owing to changes in muscle fiber composition or changes in attachments, or by the combined effect of both these conditions. The advantage of the proposed numerical system over names incorporating topography or function, is that the new positions and functions which individual muscles acquire during and after metamorphosis, cannot conflict with their numerical names. In other words, the numerical system stabilizes the nomenclature and permits the tabulation of topographical and functional properties of the muscles before, during, and after the metamorphic stages of the species.

Conventions

Myogenetic data for Rana catesbeiana are not available. The following conventions for the descriptions of the muscles have therefore been adopted in the present study. "The place of attachment which in any particular movement remains fixed when the muscle contracts is called the origin; that which is caused to move is the insertion ..." (25, p. 200). Where the attachments of the two ends of a muscle are equally movable or inert, the central end is the origin and the peripheral end is the insertion.

In view of the conspicuous difference in natural coloration of the two types of muscle fiber in the H6 muscle, it has been convenient to refer to them as pink and white fibers (24). It has also been shown (24) that these fibers have functional and structural properties in common with conventional slow and fast muscle fibers respectively. However, in other ventilation muscles of the tadpole the two types of muscle fiber are not so well segregated and they do not show their pinkish or whitish color as when they are viewed en masse. Therefore it is not generally practicable to use color for distinguishing between these two types of muscle fiber. Instead, the following terms will be used as histological criteria in the present study:

(i) "plasmic" for muscle fibers with a large amount of sarcoplasm relative to their myofibrils

(ii) "fibrillic" for muscle fibers in which the myofibrils are so abundant that the sarcoplasm forms a thin peripheral sheath around them.

These terms are most suitable for such muscles as the H6, where the muscle fibers are reasonably well segregated (Fig. 12A). However, in certain muscles these criteria can only be applied to extremes of the two fiber types because there seems to be a

mergence of the one type of fiber into the other, for example the H1 muscle (Fig. 12B,C). Motor innervation or relative speed of muscle fiber contraction might be better criteria, but they were outside the scope of the present study.

Non-phasic skeletal muscles of the bullfrog tadpole, when examined by light microscopy, usually have only fibrillic fibers. On the other hand, phasic skeletal muscles show both plasmic and fibrillic fibers, but in variable proportions and distribution. Conspicuous cases of such variation will be mentioned in the text.

The attachments of most of the muscles to the visceral skeleton are ventral, musculous and tangential. Departures from these conditions will also be specified in the text.

The M8 muscle has no median raphe and it may therefore be regarded as a single muscle in an anatomical sense. However, it is bilaterally innervated and ontogeny might reveal that it has a dichotomous origin. The other visceral muscles of the tadpole are symmetrically paired about the middle line; each muscle of a pair is unilaterally innervated.

During gill ventilation, the visceral skeleton undergoes considerable mechanical displacement. Therefore in the present study, all the muscles with attachments to the visceral skeleton will be described, although some evidence has been given (18) that certain of these muscles do not participate in normal gill ventilation.

With the exception of the H7 muscle, the jaw and hyoidean muscles of the bullfrog tadpole have already been cursorily described (18). However, the exact position of muscles in relation to the surrounding structures, and the orientation of their muscle fibers are important considerations for the understanding of their functions (7, 11). Therefore these jaw and hyoidean muscles will

be reconsidered in the present report, in addition to the branchial and hypoglossal muscles.

Mandibular group

M1 (MP-ML)(Figs. 7, 8, 11)

The M1 is the deepest of the three muscles on the floor of the optic orbit. Its fibers arise on the anterodorsal surface of the processus ascendens pterygoquadrati. They run forward, laterad, and somewhat diagonally as a dorsoventrally flattened strap. The fibers diminish until, at the anterior edge of the processus muscularis quadrati, only a tendon remains. This dorsoventrally flattened tendon continues rostrad to the anterodorsal face of the cartilago Meckeli, where it is inserted on the cartilage just medial to its articulation with the pars articularis quadrati. Figure 5 shows only the tendon at the level of the processus muscularis quadrati.

M2 (MM-RS)(Figs. 7, 8)

The M2 originates low on the medial face of the processus muscularis quadrati, near its anterior edge. From their dorsoventrally broad origin, the muscle fibers converge on their way rostrad and slightly mesiad, to insert by a short tendon on the outer surface of the cartilago rostrale superior, near its lateral edge.

M3 Appears at stage 40 of Gosner (17)

M4 " " " 39 " " "

M5 (MM-MD)(Figs. 7, 8)

The few muscle fibers of the somewhat cylindrical M5 arise low on the medial face of the processus muscularis quadrati, from where they pass rostrad and slightly mesiad. They insert on the anterodorsal cartilago Meckeli just medial to the insertion of the M1.

M6 (MP-MD)(Figs. 7, 8, 11)

The M6 arises at the base of the processus ascendens of the pterygoquadrate. The muscle begins as a wide crescentic group of fibers on the dorsal surface of this cartilage and above the M7. The origin of the M6 is immediately lateral and slightly rostral to the origin of the M1. The fibers of the M6 form a dorsoventrally flattened bundle at first, but they converge as they run forward on the floor of the optic orbit. Near the anterior edge of the processus muscularis quadrati, the muscle is circular in cross-section just before ending in a robust tendon. The insertion of this tendon is on the anterodorsal surface of the cartilago Meckeli, near the cartilago rostrale inferior.

M7 (MP-RS)(Figs. 7, 8, 11)

This muscle's fibers originate just anterior and ventral to the origin of the M6, partly on the anterior dorsal face of the processus ascendens pterygoquadratum. The medial fibers arise just lateral and anterior to the origin of the M1. The other fibers follow the curvature of the orbit as they arise more laterally and anteriorly on the pterygoquadratum to form a dorsoventrally flattened muscle. It passes forward and becomes somewhat cylindrical but still more flattened above, as it leaves the orbit. It continues forward as a main tendon and then runs parallel and ventral to the shorter smaller tendon of the M2. The main tendon of the M7 inserts on the dorsal surface of the cartilago rostrale superior, close to the lateral edge of this cartilage and usually just dorsal to the insertion of the tendon of the M2. The main tendon is dorsoventrally flattened and passes forward to the diapophysis of the cartilago Meckeli. A few collagen fibers, forming the tendo accessorius (TA, Fig. 7), diverge from the ventral surface of the main tendon at about the level of the cartilago Meckeli and pass into a larger mass of collagen fibers at the bases of the dermal papillae near the medial end of the cartilago Meckeli. Along its entire length, the M7 is separated from the M6.

M8 (MRI)(Figs. 1, 7)

This is a small muscle at stage 35 of Gosner (17); it becomes prominent during metamorphosis. Its muscle fibers are attached bilaterally to the cartilagines rostrale inferiores and they straddle the joint between these cartilages. The muscle does not have a median raphe of connective tissue separating its left and right moieties.

M9 (MM-L)(Figs. 1, 7)

The medial epiphysis of the cartilago Meckeli, near the cartilago rostrale inferior, is the site of origin of the M9. Its few muscle fibers arise on the posteroventral face of the cartilage from where they diverge and end in the dermis of the lower lip.

M10 (MR-M)(Figs. 1, 6A, 7)

This muscle is bib-like, behind and below the mouth. Its origin is in a broad sagittal raphe from which its muscle fibers curve laterad and slightly rostrad. They also converge, and end in a short tendon which inserts on the ventral face of the paired cartilago Meckeli, about midway along its length.

Cross-sections of this muscle clearly show that it is composed of both plasmic and fibrillic fibers.

Hyoidean group

H1 (HM-CL)(Figs. 1, 4, 7, 11)

The H1 arises chiefly on the peripheral aspects of the lateral face of the processus muscularis quadrati. About 12 of the most medial of its fibers originate on the concave central area of the process. The muscle runs as a wide, thick, but somewhat flattened band ventrad and slightly caudad, passing over the origins of the H2, H3, and H4 to insert on the dorsolateral surface of the CHL.

Cross-sections of the H1 show that, like the H6 (Fig. 12A), it consists of plasmic and fibrillic fibers (Fig. 12B,C). Except peripherally and somewhat anteriorly, where the plasmic fibers predominate, there is much intermingling and gradation of structure among the muscle fibers of the H1.

H2 (HMP-CL)(Figs. 4, 7, 11)

The H2 originates along the lower two-thirds of the posterolateral edge of the processus muscularis quadrati. Its muscle fibers are covered by the posterior region of the H1. Part of the H2 also originates on the lateral edge of the cartilago pterygoquadratum that is confluent with the base of the processus muscularis quadrati. The H2 is laterally flattened into a thin sheet. Those fibers originating on the processus muscularis pass downward to cross over the origin of the H3, and together with those H2 fibers originating on the pterygoquadratum, all these fibers insert posteriorly, on the outer surface of the CHL.

Cross-sections of the H2 showed that it is composed entirely of plasmic fibers.

H3 (HMS-M)(Figs. 4, 7, 8)

A course parallel to the shorter H4 is taken by the converging fibers of the H3 from its origin on the posterolateral processus muscularis quadrati. At this point its fibers are covered by the H1 and to a lesser extent by the H2. The H3 inserts by its own aponeurosis on the processus retroarticularis of the cartilago Meckeli, medial to the aponeurosis of the larger H5.

There is a slight predominance of fibrillic fibers over the plasmic type, as seen in cross-sections of the H3.

H4 (HMI-M)(Figs. 1, 4, 7, 8)

From its origin on the ventral face of the processus muscularis quadrati, the H4 passes forward and inward to insert on the processus retroarticularis of the cartilago Meckeli by its own aponeurosis ventral to the aponeuroses of the H3 and H5. The point of origin of the H4 is anterior to the CHL-quadratum joint and anterior to the origin of the larger H5.

Plasmic and fibrillic fibers are about equally well represented in this muscle.

H5 (HCL-M)(Figs. 1, 7, 8)

The H5 originates on the outer face of the CHL, where its muscle fibers are covered by the H1. In profile, the H5 narrows as it emerges from under the H1 and it then runs forward and inward. The H5's insertion is by an aponeurosis which somewhat envelops the lateral aspect of the processus retroarticularis (PRA, Fig. 4) of the cartilago Meckeli. The muscle partly covers the H3 and H4 laterally and ventrally.

The fibers of the H5 are of the plasmic and fibrillic type in approximately equal proportions.

H6 (HR-CL)(Figs. 1, 6A, 7, 8, 11, 12A)

The fibers of the H6 originate at a raphe of connective tissue in the middle line; they then pass laterad to insert on the CHL as shown in Fig. 1. The absence of nervous connections across this raphe explains the need for bilateral innervation of the contralateral moieties of the H6, which is provided by the rami hyoidei jugularis (VII). The larger, sensory, glossopharyngeal component of the ramus jugularis is shown in Fig. 1, running caudad, inferior to the H6. The nerve has been truncated at the hind edge of this muscle.

Near the middle line, some of the whitish posterior muscle fibers (fibrillic type) of the H6 run obliquely backward and are attached by connective tissue to the membranous lining of the operculum. The other fibrillic fibers and the pinkish anterior fibers (plasmic type) are aligned parallel to one another in a transverse direction. The plasmic fibers partly disappear after the onset of metamorphic climax, at about stage 41 of Gosner (17), but a few plasmic fibers are still present in the newly metamorphosed frog; in older frogs the plasmic fibers disappear.

H7 (HR-Q)(Figs. 1; 6A,B; 8)

The membranous lining of the operculum is reinforced by this muscle. Its fibers, like those of the H6, originate in a median raphe of connective tissue, from either side of which, two bundles of muscle fibers immediately diverge. By virtue of the fascia lateralis ending on the quadrate and along the lateral edge of the pterygoquadrate as well as the auditory capsule, the H7 has a wide region of insertion. Some of its fibers also insert in the diaphragm.

A lateroventral bundle spreads laterally from the median raphe and adheres to the ventral aspect of the superficial opercular lining. The fibers of this bundle terminate near the lymph gland (l) at the level of the auditory capsule while still in contact with the opercular lining and without themselves inserting on the cartilage. Furthermore, the medial part of this muscle bundle is crossed obliquely and ventrally by single muscle fibers (Fig. 1) which have not been previously described in anuran tadpoles. These single fibers, although usually separated from one another, are closely applied to the lateroventral muscle fibers of the H7 and together with them, form the superficial component of this muscle.

A laterodorsal bundle (H7', Fig. 1) passes laterocaudad along the dorsal surface of the deep opercular lining and then crosses under the origins of the S2 and B11 in the diaphragm, and itself then ends in a wide contact with the anterior aspect of the diaphragm. In the present study, the bundle will be called the deep component of the H7. Both muscle components are dorsoventrally flattened, being only one to three fibers thick, and they are intimately applied to the superficial and deep opercular linings respectively.

The medial connective tissue between the H6 and H7 affords a bridge for the spread of innervation from the rami hyoidei jugularis to the H7 (24).

All the fibers of the H7 are of the fibrillic type.

Branchial group

B1 (BP-IL); B5 (BA-IL); B8 (BA-2LA); B10 (BA-2LP)(Figs. 1, 4, 6B)

The B1 originates on the posterolateral edge of the pterygoquadrate, while B5 and B8 originate on the lateral edge of the processus oticus. Immediately behind the base of the processus oticus and dorsally on the auditory capsules, the B10 has its origin. B1 and B5 insert ventrolaterally on CB 1 while B8 and B10 insert ventrolaterally on CB 2 near its junction with CB 3. B10 inserts on CB 2, immediately ventral and somewhat posterior to the insertion of B8. B10 is elliptical, almost circular in cross-section but the other members of this subgroup are only one or two muscle fibers thick, each muscle forming a sheet which is outwardly convex when relaxed.

The fibers of these muscles are almost entirely of the plasmic type.

B2 (B2M-1.2); B6 (B2M-2.3); B9 (B3M-2.3)(Figs. 1, 8, 9)

The ventral face of the processus branchialis of CB 2 is the site of origin of the B2 and B6, while B9 originates close to them, but on CB 3. These three muscles then curve backward along the ventral aspects of the branchial arches. They insert on the commissura terminalis joining CB 1 and CB 2 (B2), and on the commissure of CB 2 and CB 3 (B6 and B9). Each muscle is apposed to its CB, half encircling the gill cleft and running parallel to the respective branchial arteries, as in Pelobates (Fig. 9).

Each of the muscles of this subgroup have 10-15 muscle fibers. Half of the fibers are clearly of the fibrillic type, but the others are somewhat like the plasmic type, although their sarcoplasm is not exactly copious relative to the myofibrils.

B3 (BCM-1M), B4 (BCM-2M), B12 (B2M-4M) (Figs. 1, 4, 6B, 11)

B3 and B4 originate on the laterocaudal edge of the processus posterior hyalis of the CHM. The muscle fibers run backward to insert ventromedially near the base of the anterior process of CB 1 (B3) and on the ventral face of the processus branchialis of CB 2 (B4). The third muscle of this subgroup (B12) arises on the ventral face of the processus branchialis next to and in contact with the insertion of B4. B12 ends on the ventral aspect of CB 4 immediately medial to the short ligament joining CB 4 to CB 3.

B7 (BH-2M)(Fig. 1)

Unlike H6, the ventral median raphe of connective tissue in which the B7 originates, is attached to a skeletal support, the crista hyoidea of copula 2. The muscle, which is nearly cylindrical, then crosses the S1 ventrally and inserts on the ventral face of the processus branchialis of CB 2. Not all the B7 fibers have a tangential insertion; a few of the more dorsal fibers insert perpendicularly on the medial, vertical face of the processus branchialis.

Cross-sections of the B7 showed that it is composed of intermingled plasmic and fibrillic fibers with a slight tendency for the plasmic fibers to concentrate themselves in the deeper region of the muscle.

B11 (BD-2.3)(Figs. 1, 8)

A transverse course is taken by the B11 from its origin in the diaphragm laterocaudal to the heart, to its insertion on the commissure of CB 2 and CB 3. Its fibers originate in the anterior and lateral aspect of the diaphragm; the more ventral fibers originate dorsal to the deep component of the H7. B11 runs ventral to B13 and B14, but slightly dorsal to B9. B11 is somewhat fan-shaped in an almost vertical plane, its fibers converging toward their insertion.

B12 (B2M-4M)(Figs. 1, 6B)

This muscle originates on the processus branchialis in close association with B4 and B7. The B12 is a flattened, short muscle which runs straight back and is inserted near the front, medial edge of CB 4.

B13 (BA-4L)(Fig. 1)

Arising on the auditory capsule adjacent and medial to B10, the fibers of B13 pass mesiad and cross B11 dorsally, before ending on the ventral face of CB 4 near the end of gill cleft 4.

B14 (B4L-S)(Fig. 1)

B14 originates ventrally on the CB 4 close to the distal fusion of CB 4 with CB 3. The muscle passes posterolaterad and ends on the anterior face of the nascent scapula.

Immediately behind the B 14, a muscle bundle (not shown, Fig. 1) arises in the connective tissue between the glottis and the medial CB 4 and it is separated from the origin of its contralateral moiety by a space in the middle line. The bundle runs posterolaterad and converges on B14 to also become inserted on the anterior face of the nascent scapula. The muscle bundle is not easily accessible and as it is not inserted on the branchial skeleton, its role (if any) in gill ventilation is probably minor. The muscle bundle runs alongside and anterior to the dilator laryngeus muscle but does not fuse with it.

Spinal group

S1 (SH-RI)(Figs. 1, 7, 11)

S1 arises posteroventrally on the HP, near the CBs. The muscle crosses B7 dorsally and almost at right angles and the left and right moieties tend to converge on each other as they pass forward. Anteriorly, the S1 lies immediately ventral to the CHM and therefore dorsal to the M10. The S1 inserts ventrally on the cartilago rostrale inferior, just lateral to the M8.

There are 25 to 35 fibers in this muscle, of which usually more than half are of the plasmic type while the others are of the fibrillic type.

S2 (SD-2M)(Figs. 1, 6B)

S2 arises in the diaphragm lateral and caudal to the heart. Its fibers are widely spread in the diaphragm but they soon converge into a compact bundle which is elliptical in cross-section, being dorsoventrally wider. The muscle runs rostrad and slightly laterad, to end on the ventral face of the processus branchialis of CB 2, alongside and medial to the origin of B12. Some of the fibers of the S2 are also inserted along the medial edge of the ventral side of CB 3.

Fibrillic fibers are present in this muscle to a slightly greater degree than the plasmic type.

Discussion

The aim of the present research is to establish a structural basis for experimental studies of the gill ventilation mechanism in the bullfrog tadpole (see 21). For this reason, the more dynamic components, such as cartilages and muscles, are emphasized, although attention is also given to connective tissues and epithelial and vascular linings wherever they are considered important from a mechanical point of view.

Certain discrepancies in the literature on tadpole morphology are pointed out in the following discussion.

EPITHELIAL AND VASCULAR LININGS

Vela

Rows of secretory columnar cells along the undersurface of the ventral velum of Rana temporaria were mistaken for muscle fibers during dissections (43). Microtomy of the ventral velum has revealed that there are no muscle fibers present in this structure in R. temporaria, Phyllomedusa trinitatus, "... several Hylidae, Bufonidae and Leptodactylidae..." (26, p. 243), or in Rana catesbeiana (20), Pseudis paradoxa, Pelobates syriacus, Scaphiopus bombifrons and Ascaphus truei (Gradwell, unpublished).

Pharynx

Although epithelial cells with points which project into the lumen of the pharynx, were drawn for Pelobates (46, Figs. 26, 32), Rana dalmatina (29, Fig. 14), Xenopus (32, Fig. 1), this type of cell has not been discussed in recent publications which concern anuran larvae in general and Phyllomedusa trinitatus in particular (26, 27). The pointed cells are present on the crowns of the rugulose pharyngeal epithelium in Rana catesbeiana which in this respect closely resembles Pelobates (see 46, Figs. 26, 32).

Gill cavity

The respiratory importance of the membrana vasculosa opercularis of Rana esculenta was first pointed out by Strawinski (52), whose findings were based on measurements of capillary density. In R. catesbeiana kept under specific conditions, the main opercular blood vessels have been mapped and identified and the density of the capillaries has also been measured (19) and found to support Strawinski's postulate. However, there are many anuran genera in which the blood system of the opercular lining is poorly developed and is therefore apparently of little value for blood ventilation (Gradwell, unpublished).

In Rana catesbeiana water passing through the first pair of gill clefts enters the gill cavity directly from the buccal cavity, thus by-passing the pharynx and gill clefts 2 to 4 (20, 23). In the present study, attention has been drawn to the well vascularized rugulose lining of the pharynx. Although the flow through the first gill cleft bathes the first gill, much of this water impinges directly on the membrana vasculosa opercularis, affording better utilization of it for ventilation of the blood in this membrane than the water reaching the membrane via the gill clefts 2 to 4. The coincidence of first gill clefts and a well vascularized opercular lining would seem to be consistent with the view that this association is an adaptation for the increased ventilation of the blood. In contrast, a first gill cleft is generally absent in those anurans having a poorly vascularized operculum (Gradwell, unpublished).

It is well known that the position of the branchial outlet varies among the genera of anuran tadpoles. This variation has been explained by Brock (2, p. 339): "Since the branchial opening is at one time a single wide sweep from side to side across the

ventral surface of the body, it is easy to understand that the definitive position may vary in different forms."

CARTILAGES

Without implying homogeneity of the cartilages, Gaupp (13, 14, 15) described the larval hyobranchial skeleton of Rana temporaria (= R. fusca) as a cartilaginous continuum ("Homokontinuität"). The homogeneity of the hyobranchial skeleton is interrupted by a youthful type of cartilage (5) between certain of its cartilaginous elements. This condition is especially evident in the large bullfrog tadpole, in which the inherent flexibility of hyaline cartilage tends to be reduced and there is a greater need for zones of bending to facilitate the movements of the hyobranchial components. However, as Gans (12) has pointed out, anatomical components need not necessarily represent functional units; several anatomical components may act together to express one or more functions.

De Beer (4) has expressed doubt regarding the ontogeny of the pars reuniens and he considered it to originate independently of copulae 1 and 2. Ridewood (42) referred to the "fibrous" nature of the pars reuniens in dissected material. A histological difference between the pars reuniens and the other visceral cartilages was reported by Chacko (3). According to de Jongh (5), the anterior region of the pars reuniens has a more "youthful" appearance than its posterior. However, the precise nature of the intercellular substance has not been histologically identified and may hold the answer to the particular resilience of the pars reuniens.

Parker (35, p. 155) introduced the term "hypobranchial plate" for the relatively flat, broad cartilage lying between the hyoidean and branchial elements in Rana temporaria. This term has become

so well established in the literature that it is also used in the present study, but with the reservation that, when Rana tadpoles are in a natural prostrate orientation, the hypobranchial plate does not lie below the level of any component of the branchial apparatus (HP, Figs. 1, 4, 6A).

MUSCLES

Concerning the terminology of the muscles, the present study is a departure from earlier publications on larval anurans. The application of the nomenclature of Edgeworth (9) was attempted in an earlier functional study of Rana catesbeiana (18), but more detailed research has led to the proposal in the present report of a numerical terminology for the visceral muscles associated with the gill ventilation apparatus of anurans. A numerical system has the advantage of avoiding controversies that have stemmed partly from attempts to homologize tadpole muscles with those of other vertebrates.

Notwithstanding Edgeworth's monograph (9), some misunderstandings of tadpole visceral musculature persist, the most relevant of which are considered in the following discussion.

According to de Jongh (5, for Rana temporaria), larval muscle fibers become replaced during metamorphosis be new muscle fibers which differentiate into those characteristic of adults. In R. catesbeiana, there are two histologically different types of muscle fiber in several muscles (particularly the H1 and H6) of the gill ventilation apparatus before the major events of metamorphosis begin at about stage 41 of Gosner (17).

Mandibular group

M2

This muscle was regarded as the levator mandibulae externus pars anterior to distinguish it from the l.m.e. pars posterior (18), but in the general absence of a l.m.e. pars posterior at stage 35 of Gosner (17) in Rana catesbeiana, the M2 is probably homologous to the l.m. externus (Table I).

M6, M7

The M6 is regarded as being fused posteriorly with the M7 in Rana temporaria (l.m. posterior superficialis and l.m.p. profundus respectively, 5). In R. catesbeiana, the M6 and M7 are distinctly separate from each other at stage 35 of Gosner (17). Moreover, the tendon of M7 in catesbeiana is not fused with that of M2, which also contrasts with temporaria (5).

Occasionally the fibers of the M7 are segregated into dual, parallel bundles. On this basis a l.m.e. pars posterior of Sedra (47) was shown in Rana catesbeiana (18). However, further research has shown that this muscle is generally absent at stage 35 of Gosner (17) in R. catesbeiana.

Hyoidean group

H3, H4, H5

In Rana temporaria (5), the quadratoangularis has a similar topography to the H4 of R. catesbeiana, except that this muscle does not insert with the hyoangularis (H5) by a common aponeurosis on the cartilago Meckeli. Whereas the insertions of the H3 and H5 are lateral on this cartilage, the H4 is inserted more ventrally and medially (Figs. 1, 4, 7). This distinction has functional implications (see 21).

H6

Earlier reports on the H6 have not furnished details on its histological composition (6, 46, 9, 30, 38, 47, 49). Recently, it has been found (5) to consist entirely of "larval fibers" at stage 25 of Kopsch (28) (= stage 40 of Gosner, 17). In the bullfrog tadpole the H6 is composed of two structurally different types of muscle fiber, each specialized for a particular function (24). This difference has also been found in several other species of Rana tadpoles (Gradwell, unpublished).

H7

The H7 has been reported (9, 38) as being absent in Rana (particularly R. temporaria). More recent work has shown that it occurs generally in Rana (including R. temporaria), Pseudis, and Pelobates, but not in pipids, Bufo, and Scaphiopus (Gradwell, unpublished).

The H7 has been described (46) in Pelobates as two muscles, the diaphragmatopraecordialis and the subbranchialis in approximately the same positions as the deep and superficial components respectively of the H7 in Rana catesbeiana. Further research is needed to decide whether this is one or two muscles.

Branchial group

B1, B5, B8, B10

Kratochwill (29, for Rana dalmatina) described four branchial levator muscles ("Kiemenbogenheber") as originating on the underside of the pterygoquadrate ("Lamina pterygo-temporalis"). Edgeworth (9, for Pelobates, Rana, and Bufo) did not recognize a levator arcus branchialis 4. Pusey (38) reported that in Ascaphus there is no separate constrictor branchialis 4, but that the combined constrictor branchialis 4 and levator branchialis 4 are

inserted farther ventrally on the branchial skeleton than are the l.a. branchialium 1 to 3. If the muscle B10 (Figs. 1, 4) really is a combination of Edgeworth's l.a. branchialis 4 and c. branchialis 4, then Pusey's description of the condition in Ascaphus also applies to Rana catesbeiana.

Sedra and Michael (33, 49, for Xenopus) have assigned the names "constrictores branchialium i-iv" to the muscles which Edgeworth (9, for Rana temporaria), Pusey (38, for Ascaphus), and earlier researchers called the "levatores arcuum branchialium i-iv".

B2, B6, B9

Having given the name "constrictores branchialium i-iv" to the conventional "levatores arcuum branchialium i-iv", Sedra and Michael (33, 49) identified the customary "constrictores branchialium i-iii" as their "subarcuales recti ii-iv" (Weisz's "subarcual muscle strands", 54; see also Hymenochirus, Sokol 51).

B3, B4, B12

Edgeworth (9, p. 157, for Rana temporaria) reported that the subarcuales recti 1 and 2 have fused to form a single muscle in 8 mm larvae, and later (11 mm larvae), two muscles reappear: "A fasciculus subsequently separates from the anterior part of the fused subarcuales recti i and ii". However, no data in this regard are available for R. catesbeiana.

Edgeworth's subarcuales recti i and ii (9) were described as a single "ceratobranchialis" by Schulze (46) in the fully developed tadpole of Pelobates. He also described an "interbranchialis", apparently the subarcuales recti iii and iv of Edgeworth (9). Kratochwill (29, for Rana dalmatina) referred to the subarcuales recti i and ii by the term "hyo-keratobranchiales"

and adopted Schulze's "interbranchialis" as the term for Edgeworth's subarcuales recti iii and iv (9).

B7

Edgeworth (9, pp. 156, 162) regarded the primitive amphibian branchial segments as each containing a transversus ventralis, and a subarcualis which was in the rectus or obliquus form, but not in both forms at once. Pusey (38, p. 144) disagreed and mainly on the basis of his Ascaphus research, he suggested that "... a primitive branchial segment could contain simultaneously a S. rectus, a S. obliquus, and a Transversus ventralis muscle pair", and, "In future the term 'Transversus ventralis II' must be abandoned in favour of 'S. obliquus II' in the Anura".

Fox (10) reported on the ontogeny of the lungfish, Neoceratodus, and showed that the adult has a transversus ventralis and a subarcualis rectus but it has no S. obliquus in each of its five branchial segments. For this reason, Fox (in litt. 1969) favors the retention of Edgeworth's transversus ventralis ii in the Anura.

In the present study and in agreement with Sokol (in litt. 1969), the cogent reasoning of Pusey is regarded as sufficient grounds for favoring the term "subarcualis obliquus" for the muscle which Edgeworth has called the "transversus ventralis ii". However, the subject of muscle ontogeny and homology is outside the scope of the present report.

B11

Edgeworth (9, p. 133) states for Pelobates: "... a downgrowth from Constrictor iv forms the Diaphragmatico-branchialis ...". There is no "Constrictor branchialis iv" in Rana catesbeiana, but as a "diaphragmatobranchialis iv" (B11) is present and is separate from the muscle labelled B10 in Figs. 1 and 3, its ontogenetic

origin is a problem in embryology.

B13

The tympanopharyngeus of Pelobates (Schulze 46) was not discussed by Edgeworth (9) although he reproduced Schulze's Fig. 16 which shows this muscle. By virtue of a close similarity of position, this muscle is probably homologous to the B13 of Rana catesbeiana.

Spinal group

S2

In view of almost identical origins and insertions, the S2 (which Edgeworth, 9, calls the "rectus cervicis") may be homologous to the muscle described by Schulze (46) and Kratochwill as the "diaphragmato-branchialis medialis."

Acknowledgments

Versions of this report, in its various stages of preparation, have been critically read by the following gentlemen, to whom I am most grateful: H.J. de Jongh, C. Gans, and O.M. Sokol. There are some points of contention, however, and I am therefore alone responsible for the final version.

References

1. Baculi, B.S., and E.L. Cooper. 1967. Lymphomyeloid organs of Amphibia. II. Vasculature in larval and adult Rana catesbeiana. J. Morphol. 123: 463-480.

2. Brock, G.T. 1929. The formation and fate of the operculum and gill chambers in the tadpole of Rana temporaria. Quart. J. Microscop. Sci. 73: 335-343.

3. Chacko, T. 1965. The development and metamorphosis of the hyobranchial skeleton in Rana tigrina, the Indian bull frog. Acta Zoologica, Stockholm 46: 311-328.

4. De Beer, G.R. 1937. The development of the vertebrate skull. Oxford Univ. Press. London and New York.

5. De Jongh, H.J. 1968. Functional morphology of the jaw apparatus of larval and metamorphosing Rana temporaria. Neth. J. Zool. 18: 1-103.

6. Dugès, A. 1834. Recherches sur l'osteologie et la myologie des Batraciens à l'Académie royale des Sciences. Paris. Sci. math. et phys. Vol. 6: 1-216. Cited from Edgeworth (1935).

7. Dullemeijer, P. 1958. The mutual structural influence of the elements in a pattern. Archs. neérl. Zool. 13 suppl. 1: 74-88.

8. Edgeworth, F.H. 1930. On the masticatory and hyoid muscles of Xenopus laevis. J. Anat. 64: 184-188.

9. ---- 1935. The cranial muscles of vertebrates. Cambridge Univ.

10. Fox, H. 1965. Early development of the head and pharynx of Neoceratodus with a consideration of its phylogeny. J. Zool. Lond. 146: 470-554.

11. Gans, C. 1966. Some limitations and approaches to problems in functional anatomy. Folia Biotheoretica, 6: 41-50.

12. ---- 1968. Functional components or mechanical units. Am. Zool. 8 (4): 808.

13. Gaupp, E. 1893. Beiträge zur Morphologie des Schädels. I. Primordial-Cranium und Kieferbogen von Rana fusca. Morphologische Arbeiten 2: 275-481.

14. ---- 1894. Idem. II. Das Hyo-Branchial-Skelet der Anuren und seine Umwandlung. Ibid. 3: 399-438.

15. ---- 1904. Das Hyobranchialskelet der Wirbeltiere. Ergeb. Anat. EntwGesch. Abt. 3, 14 Band: 965-1008.

16. Götte, A. 1875. Die Entwicklungsgeschichte der Unke (Bombinator igneus) als Grundlage einer vergleichenden Morphologie der Wirbeltiere. Leipzig.

17. Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16: 183-190.

18. Gradwell, N. 1968. The jaw and hyoidean mechanism of the bullfrog tadpole during aqueous ventilation. Can. J. Zool. 46: 1041-1052.

19. ---- 1969. The respiratory importance of the vascularization of the tadpole operculum in Rana catesbeiana Shaw. Ibid. 47: 1239-1243.

20. ---- 1970. The function of the ventral velum during gill ventilation in Rana catesbeiana. Ibid. In the press. See Ch. 2.

21. ---- 1970. The musculoskeletal mechanism of gill ventilation in Rana catesbeiana (see Ch 3).

22. ---- 1970. The tadpole of Ascaphus: experiments on the suction and gill ventilation mechanisms. (See Ch 4).

23. Gradwell, N., and V.H. Pasztor. 1968. Hydrostatic pressures during normal ventilation in the bullfrog tadpole. Can. J. Zool. 46: 1169-1174.

24. Gradwell, N., and B. Walcott. 1970. Dual functional and structural properties of the interhyoideus muscle of the bullfrog (Rana catesbeiana). J. Exp. Zool. In the press.

25. Hyman, L.H. 1922. Comparative vertebrate anatomy. Univ. Chic. Press. Second edition 1942.

26. Kenny, J.S. 1969a. Feeding mechanisms in anuran larvae. J. Zool. Lond. 157: 225-246.

27. ---- 1969b. Pharyngeal mucous secreting epithelia of anuran larvae. Acta Zool. 50: 143-153.

28. Kopsch, F. 1952. Die Entwicklung des braunen Grasfrosches, Rana fusca Roesel. Darstellt in der Art der Normentafeln zur Entwicklungsgeschichte der Wirbeltiere: 1-70.

29. Kratochwill, K. 1933. Zur Morphologie und Physiologie der Nahrungsaufnahme der Froschlarven. Z. Wiss. Zool. 164: 421-468.

30. Lubosch, W. 1938. Muskeln des Kopfes: Visceralemuskulature. C. Amphibien und Sauropsiden. In Bolk, et al.(eds.), Handbuch der Vergleichenden Anatomie der Wirbeltiere. 5: 1025-1064. Urban and Schwartzenberg. Berlin and Vienna.

31. Luther, A. 1914. Über die vom N. trigeminus versorgte Muskulatur der Amphibien, mit einem vergleichenden Ausblick über den Adductor mandibulae der Gnathostomen, und einem Beiträg zum Verständnis der Organisation der Anurenlarven. Acta Soc. Sci. Fennica 44 (7): 1-151.

32. Millard, N. 1945. The development of the arterial system of Xenopus laevis, including experiments on the destruction of the larval aortic arches. Trans. Roy. Soc. S. Africa. 30: 217-234.

33. Nieuwkoop, P.D., and J. Faber (editors). 1956. Normal table of Xenopus laevis, Daudin. North-Holland Publ. Co., Amsterdam.

34. Noble, G.K. 1931. The biology of the Amphibia. McGraw-Hill, N.Y. Reprinted 1954, Dover Publ., N.Y.

35. Parker, W.K. 1871. On the structure and development of the skull of the common frog (Rana temporaria L). Phil. Trans. Roy. Soc. 161: 137-211.

36. ---- 1876. On the structure and development of the skull in Batrachia. Part II. Ibid. 166: 601-669.

37. ---- 1881. Idem. Part III. Ibid. 172:1-266.

38. Pusey, H.K. 1943. On the head of the liopelmid frog, Ascaphus truei. I. The chondrocranium, jaws, arches, and muscles of a partly-grown larva. Quart. J. Microscop. Sci. 84: 105-185.

39. Ridewood, W.G. 1897. On the structure and development of the hyobranchial skeleton of the parsely frog (Pelodytes punctatus). Proc. Zool. Soc. Lond. 26: 577-595.

40. ---- 1898a. On the development of the hyobranchial skeleton of the midwife toad (Alytes obstetricans). Ibid. 27: 4-12.

41. ---- 1898b. On the structure and development of the hyobranchial skeleton and larynx in Xenopus and Pipa, with remarks on the affinities of the aglossa. J. Linn. Soc. Zool. 26: 53-128.

42. ---- 1898c. On the larval hyobranchial skeleton of the anurous batrachians, with special reference to the axial parts. J. Linn. Soc. Lond. Zool. 26: 474-486.

43. Savage, R.M. 1952. Ecological, physiological and anatomical observations on some species of anuran tadpoles. Proc. Zool. Soc. Lond. 122: 467-514.

44. ---- 1955. The ingestive, digestive and respiratory systems of the microhylid tadpole, Hypopachus aguae. Copeia 2: 120-127.

45. Schulze, F.E. 1888. Über die inneren Kiemen der Batrachierlarven. I. Mitteilung: Über das Epithel der Lippen, der Mund-, Rachen- und Kiemenhöhle erwachsener Larven von Pelobates fuscus. Abhandl. Königlichen Akad. Wiss. Berlin. 32: 715-768.

46. ---- 1892. Idem. Skelet, Muskulatur, Blutgefässe, Filterapparat, respiratorische Anhange und Atmungsbewegungen erwachsener Larven von Pelobates fuscus. Ibid. 13: 1-66.

47. Sedra, S.N. 1950. The metamorphosis of the jaws and their muscles in the toad, Bufo regularis Reuss correlated with changes in the animal's feeding habits. Proc. Zool. Soc. Lond. 120: 405-449.

48. Sedra, S.N., and M.I. Michael. 1956. The structure of the hyobranchial apparatus of the fully developed tadpole larva of Bufo regularis Reuss. Proc. Egyp. Acad. Sci. 12: 38-46.

49. ---- 1957. The development of the skull, visceral arches, larynx and visceral muscles of the South African clawed toad, Xenopus during metamorphosis (stages 55-66). Verhand. Koninkl. Akad. Wetenschap. Natuurk. 51 (4): 1-80.

50. ---- 1958. The metamorphosis and growth of the hyobranchial apparatus of the Egyptian toad, Bufo regularis Reuss. J. Morphol. 103: 1-30.

51. Sokol, O.M. 1962. The tadpole of Hymenochirus boettgeri. Copeia 2: 272-284.

52. Strawinski, S. 1956. Vascularization of respiratory surfaces in ontogeny of the edible frog, Rana esculenta. Zool. Polon. 7 (3): 327-365.

53. Van Eeden, J.A. 1951. The development of the chondrocranium of Ascaphus truei Stejneger with special reference to the relations of the palatoquadrate to the neurocranium. Acta Zool. 32: 41-176.

54. Weisz, P.B. 1945. The development and morphology of the larva of the South African toad, Xenopus laevis. J. Morphol. 77: 163-217.

55. Witschi, E. 1956. Development of vertebrates. W.B. Saunders Co. Philadelphia and London.

TABLE I

The positions of the anterior visceral muscles of Rana catesbeiana, stage 35 of Gosner (17). Motor innervations are parenthesized. Key letters designate the group to which each muscle belongs and refer to the origin-insertion relationship of individual muscles. For use in the text and illustrations, each muscle is also identified by a number, preceded by the initial letter of the group to which the muscle belongs.

Edgeworth (9), Rana tadpole	Origin-insertion[1], Rana catesbeiana	No.	Key letters
MANDIBULAR GROUP			
Levator mandibulae anterior	Pterygoquadratum ascendens, dorsal - Meckeli, lateral dorsal	M1	MP-ML
L.m. externus	Musculoquadratum, anterior - rostrale superior, lateral	M2	MM-RS
L.m.a. subexternus	Appears at ca. stage 40 of Gosner (17)	M3	
L.m.a. lateralis	" " " " 39 " " "	M4	
L.m.a. articularis	Musculoquadratum, medial - Meckeli, dorsal, lateral	M5	MM-MD
L.m. posterior superficialis	Pterygoquadratum, dorsal- Meckeli, dorsal, medial	M6	MP-MD
L.m.p. profundus	Pterygoquadratum, dorsal - rostrale superior, lateral	M7	MP-RS
Intermandibularis anterior	Interconnects left and right rostrale inferior; no median raphe	M8	MRI
Mandibulo-labialis	Meckeli, anterior - lower lip	M9	MM-L
Intermandibularis posterior	Median raphe - Meckeli, medial	M10	MR-M
(n. trigeminus)			

HYOIDEAN GROUP

Orbitohyoideus	Musculoquadratum, lateral - ceratohyale lateralis	H1 HM-CL
Suspensoriohyoideus	Musculoquadratum, lateral, posterior - ceratohyale lateralis	H2 HMP-CL
Suspensorioangularis	Musculoquadratum, lateral, superior - Meckeli retroarticularis	H3 HMS-M
Quadratoangularis	Musculoquadratum, lateral, inferior - Meckeli retroarticularis	H4 HMI-M
Hyoangularis	Ceratohyale lateralis - Meckeli retroarticularis	H5 HCL-M
Interhyoideus	Median raphe - ceratohyale lateralis	H6 HR-CL
Interhyoideus posterior[2] (n. facialis)	" " - quadratum + otic process + diaphragm, lateral	H7 HR-Q

BRANCHIAL GROUP

Levator arcus branchialis I	Pterygoquadratum, lateral - 1, lateral	B1	BP-1L
Constrictor branchialis I	2, medial - commissure 1, 2	B2	B2M-1.2
Subarcualis rectus I	Ceratohyale medialis, posterior - 1, medial	B3	BCM-1M
S.r. II	Ceratohyale medialis, posterior - 2, medial	B4	BCM-2M
Lev. arc. br. II	Otic process - 1, lateral	B5	BO-1L
Constric. br. II	2, medial - commissure 2, 3	B6	B2M-2.3
Transversus ventralis II[3]	Crista hyoidea - 2, medial	B7	BH-2M
Lev. arc. br. III	Auditory capsule - 2, lateral, anterior	B8	BA-2LA
Constric. br. III	3, medial - commissure 2, 3	B9	B3M-2.3
Lev. arc. br. IV[4]	Auditory capsule - 2, lateral, posterior	B10	BA-2LP
Diaphragmato-branchialis IV	Diaphragm, medial - commissure 2, 3	B11	BD-2.3
Subarc. rec. III+IV	2, medial - 4, medial	B12	B2M-4M
Tympanopharyngeus[5]	Auditory capsule - 4, lateral	B13	BA-4L
? Transversus ventralis IV	4, lateral - scapula	B14	B4L-S

(nn. glossopharyngeus + vagus)

SPINAL GROUP		
Geniohyoideus	Hypobranchial plate - rostrale inferior	S1 SH-RI
Rectus cervicis	Diaphragm, medial - 2, medial	S2 SD-2M
(n. hypoglossus)		

[1] Attachments are assumed to be ventral except where otherwise indicated. Arabic numerals refer to the ceratobranchialia.

[2] According to Edgeworth (9), this muscle is absent in Rana, but present in Bufo and Pelobates. Recent evidence shows this muscle to be generally present in Rana; it is also found in Pseudis, Phyllomedusa, and Hyla, but it is absent in Ascaphus, Scaphiopus, Bufo, pipids and microhylids (Gradwell, unpublished).

[3] Pusey (38) calls this muscle the Subarcualis obliquus II

[4] Edgeworth (9) states that this muscle is absent in Rana.

[5] From Schulze (46).

TABLE II. *Rana catesbeiana*. The ontogenetic relationship between the arches, endodermal pouches, visceral clefts and gills. Modified from a schema suggested by Dr. N. Millard (1967, in litt.).

Arch		Endodermal	Tadpole Condition	
Visceral	Aortic	Pouch	Visceral Clefts	Gill Tufts
Mandibular	1 Premetamorphic atrophy			0
Hyoidean	2 Vestigial	1	Does not perforate	0
Branchial 1	3 Carotid	2	Gill cleft 1 (ca. 1 mm)	11-13
" 2	4 Systemic	3	" " 2 (ca. 6 mm)	13-15
" 3	5 Metamorphic atrophy	4	" " 3 (ca. 7 mm)	13-15
" 4	6 Pulmonary	5	" " 4 (ca. 4 mm)	7-8
		6	Does not perforate	

ILLUSTRATIONS

FIG. 1. Ventral view of the cartilages and muscles of the gill ventilation apparatus. The gills and opercular lining have been removed on the right side of the animal. FLS demarcates the triangular area of the fascia lateralis, Schulze. PP', QQ', and RR' indicate the levels of microtomy of the sections drawn in Figs. 6 and 8.

FIG. 2. Anterior view of the upper jaw system. The beak covering the distal edge of the cartilago rostrale superior has been removed on the right side of the animal.

FIG. 3. Ventral aspect of the buccal roof. Papillae project into the buccal cavity from the epithelium shown on the left side of the animal.

FIG. 4. Dorsal aspect, buccal floor and pharynx after removal of overlying structures. However, a small part of the pterygoquadrate and certain otic cartilages have been left intact, showing the origins of the branchial muscles 1, 5, 8, and 10. The dorsal velum (D) is shown in its downward hanging orientation as during expiration, thus widening the space between the dorsal and ventral vela.

FIG. 5. The visceral skeleton shown from the dorsal aspect.

Fig. 6. A. Sagittal section at the level PP', Fig. 1. B. Cross-section at the level QQ', Fig. 1.

FIG. 7. The muscles, cartilages and ligaments of the jaw and hyoidean apparatus drawn from a lateral and slightly anterior aspect.

FIG. 8. An oblique vertical section at the level RR', Fig. 1.

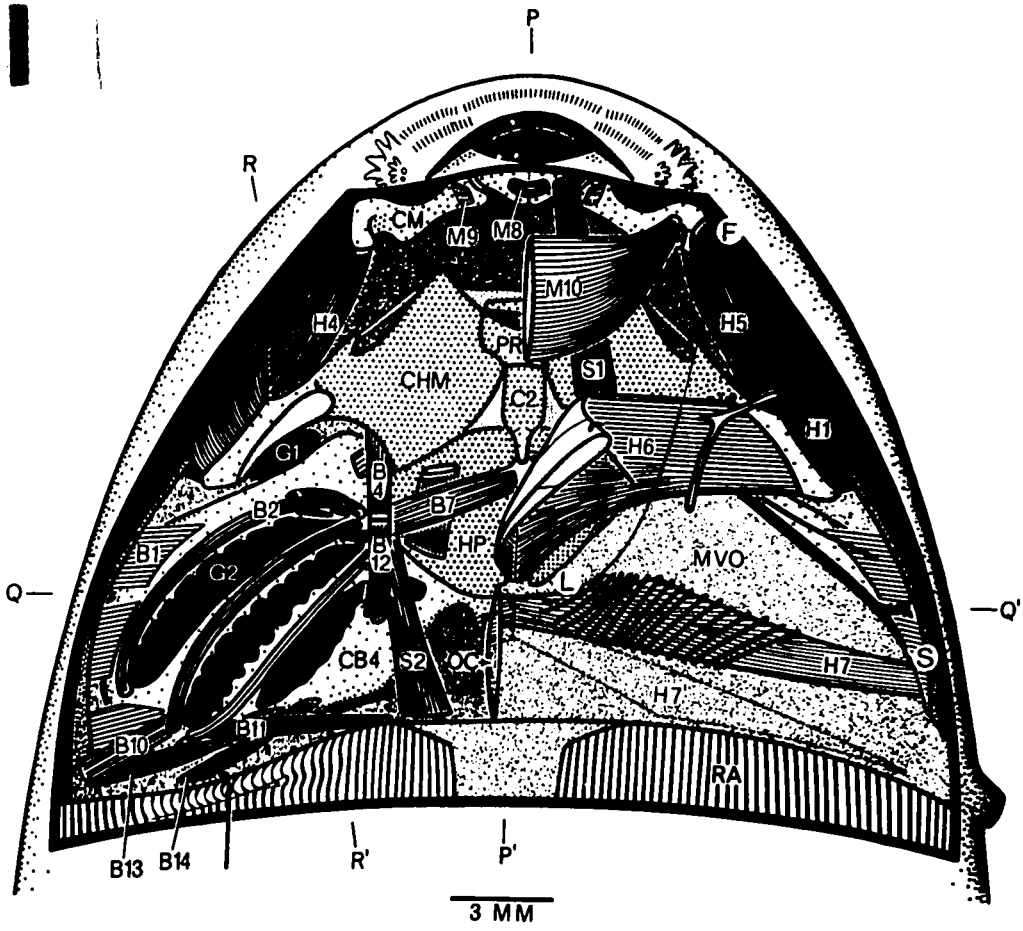
FIG. 9. A gill arch of Pelobates fuscus redrawn from Schulze (46).

FIG. 10. A. Two filter rows of a pharyngeal filter plate. B. Enlarged distal aspect of the filter folds. C. Vertical section through a filter ridge (XX', Fig. 10B). Scale: 0.5 mm in A.; 10 μm in C.

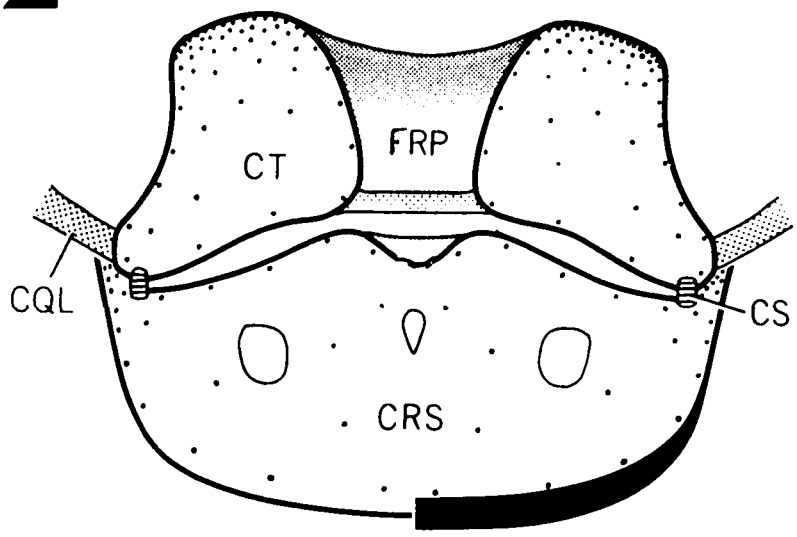
FIG. 11. The muscles, cartilages and ligaments seen in a cross-section of a tadpole's head at the level of copula 2 (Figs. 1, 4).

FIG. 12. Photomicrographs of cross-sections of parts of the two main pumping muscles. The arrows point anteriorly. BV, blood vessel. A. Muscle fibers of the H6 at its dorsal periphery. A clear separation is shown between the fibrillic (white) and plasmic (pink) fibers. B. Anterior muscle fibers of the H1 showing a predominance of plasmic over fibrillic fibers at the periphery of the muscle. C. Muscle fibers in the center of the H1 showing an intermingling between the plasmic and fibrillic fibers.

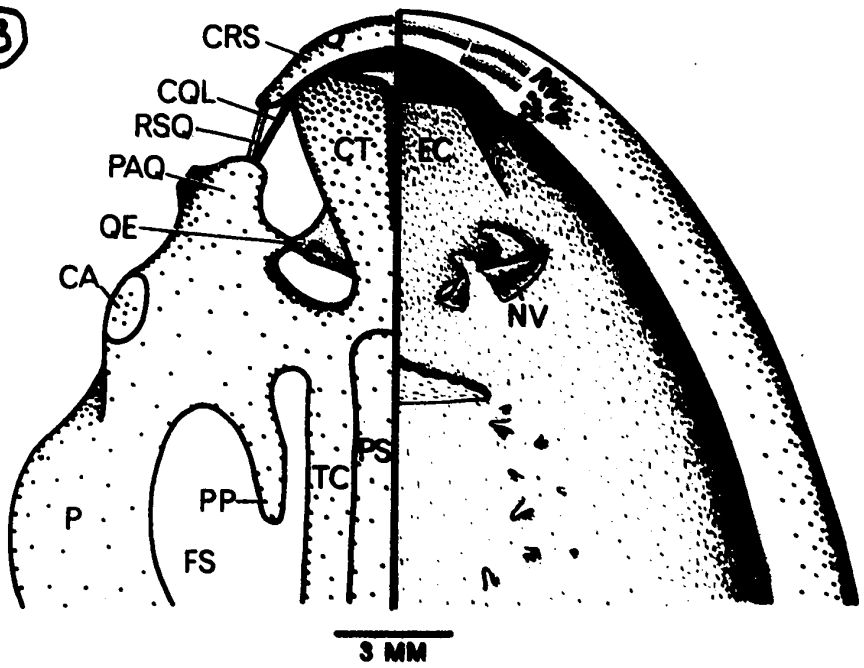
1


P
R
CM
M9
M8
F
M10
H4
H5
PR
S1
CHM
C2
H1
H6
G1
B4
B2
B7
B1
B12
HP
MVO
Q
L
Q'
G2
CB4
S2
OC
H7
S
H7
B11
B10
RA
B13
B14
R'
P'
3 MM

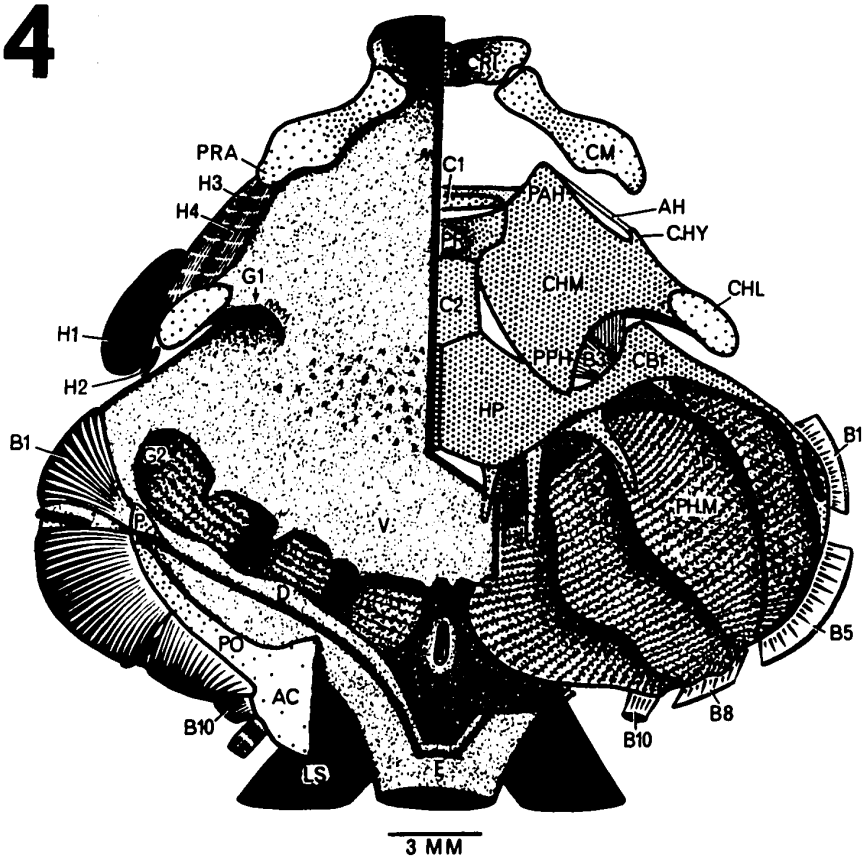
2


CT
FRP
CQL
CS
CRS
3 MM

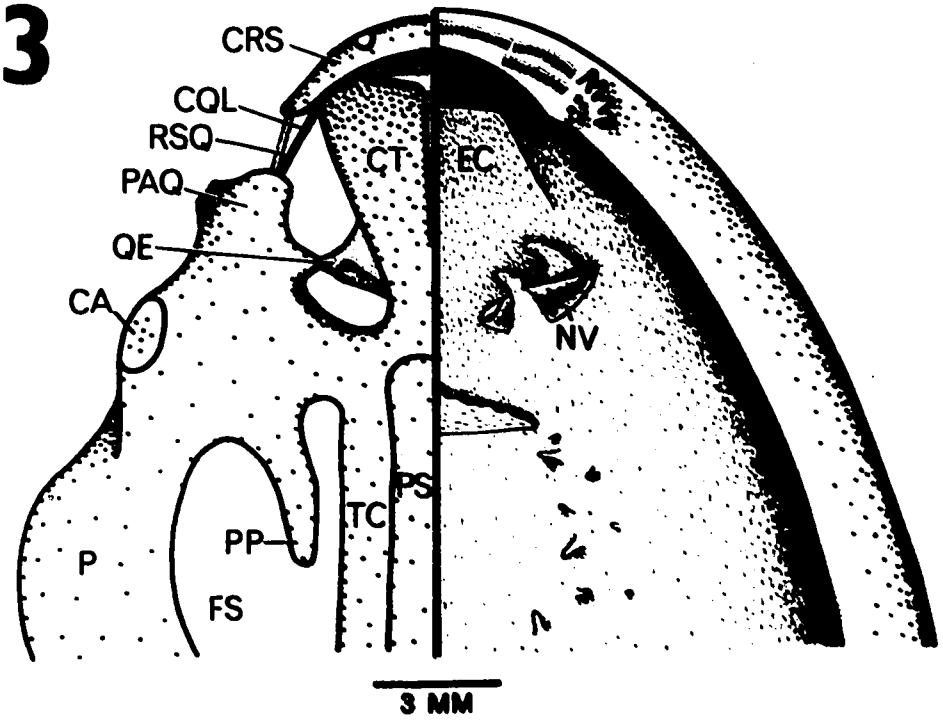
3


CRS
CQL
RSQ
PAQ
QE
CA
CT
EC
NV
PS
TC
PP
P
FS
3 MM

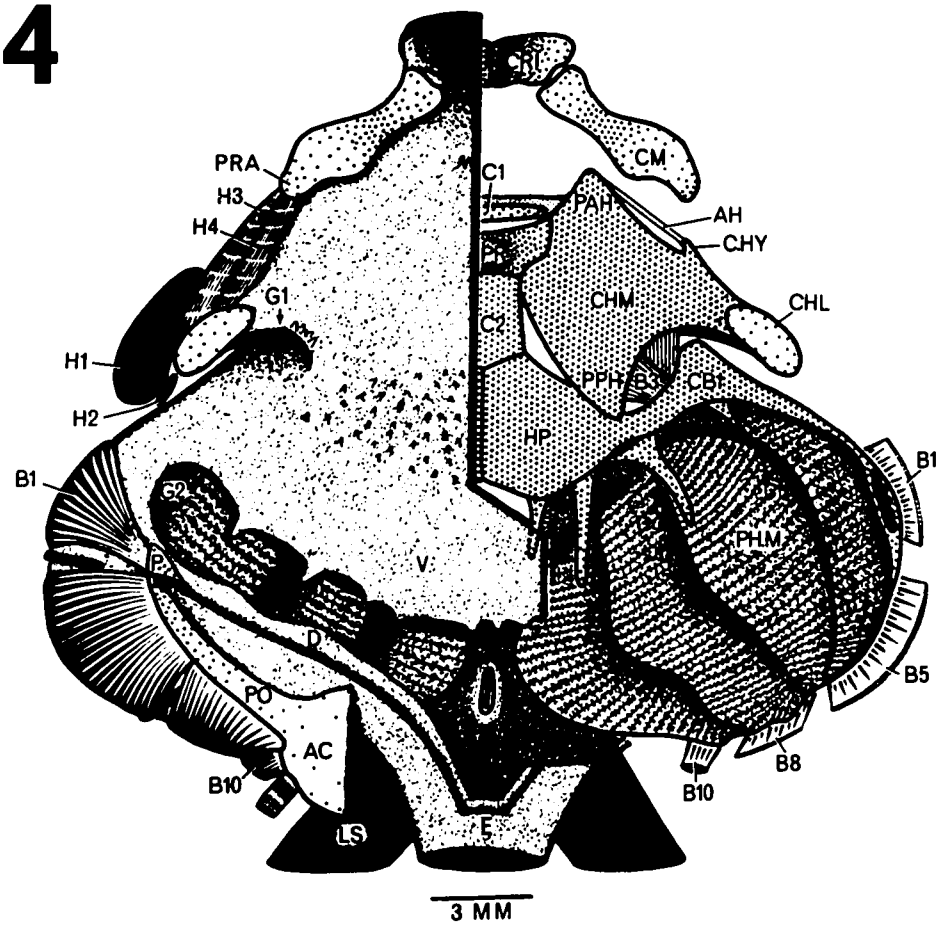
4


PRA
H3
H4
G1
H1
H2
B1
G2
PO
AC
B10
LS
CM
C1
PAH
AH
CHY
CHM
CHL
C2
PPH
CB1
HP
PH-LM
B5
B8
B10
3 MM

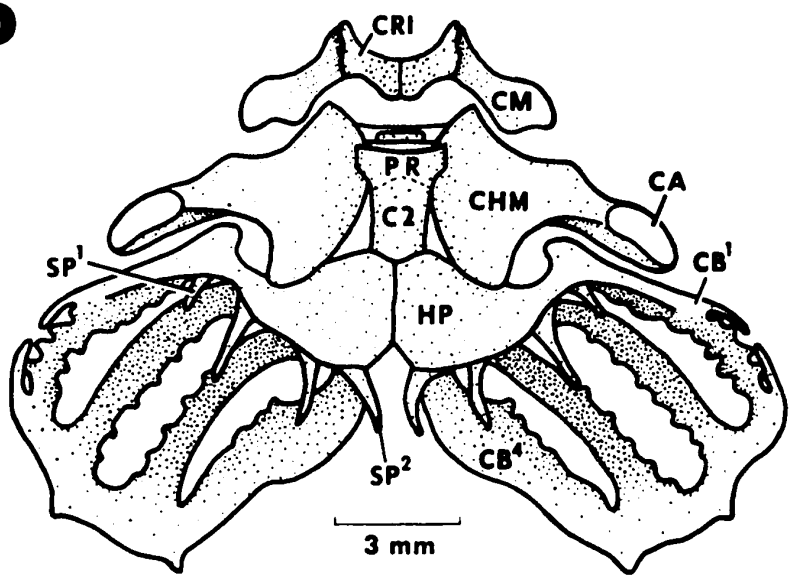
3

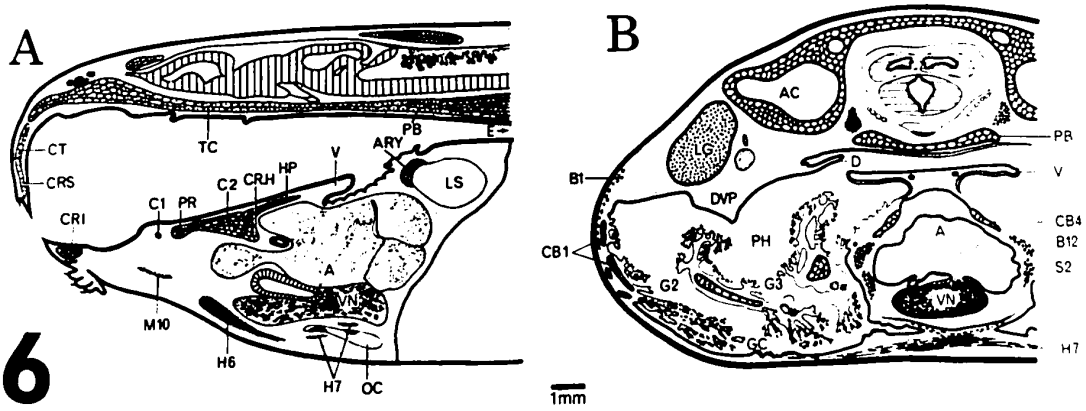

CRS
CQL
RSQ
PAQ
QE
CA
CT
EC
NV
PS
TC
PP
P
FS
3 MM

4


PRA
H3
H4
G1
H1
H2
B1
CRL
CM
C1
PAH
AH
CHY
C2
CHM
CHL
PPH
CBT
HP
PHM
V
D
PO
AC
B10
LS
E
B5
B8
3 MM

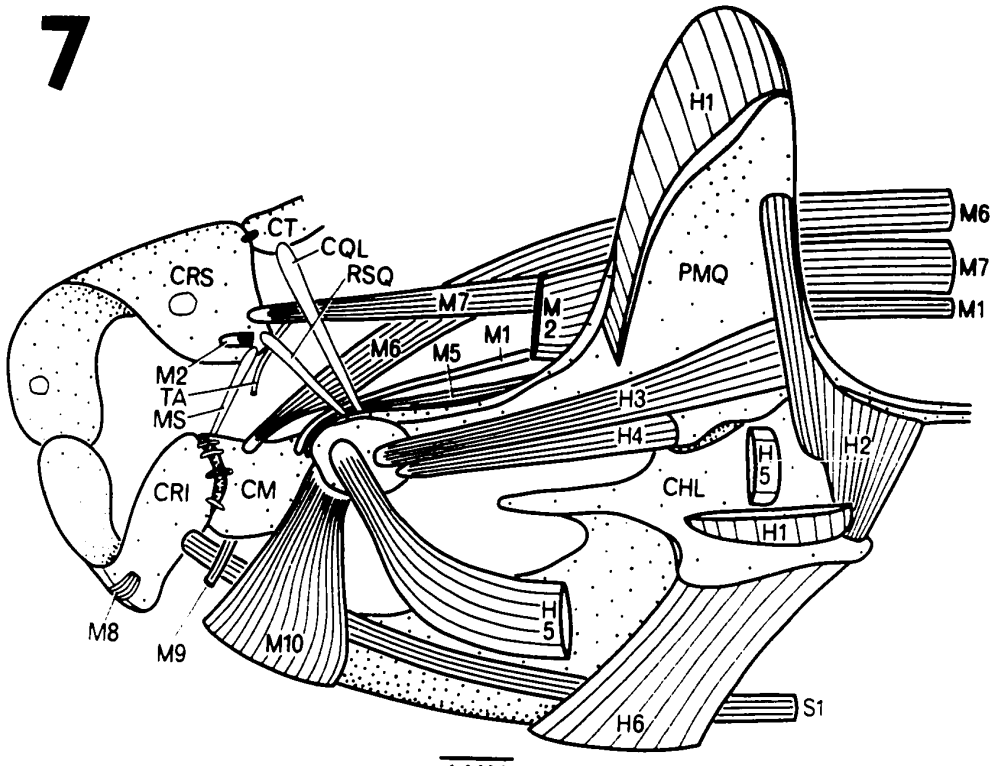
5


CRI
CM
PR
C2
CHM
CA
SP1
CB1
HP
SP2
CB4
3 mm

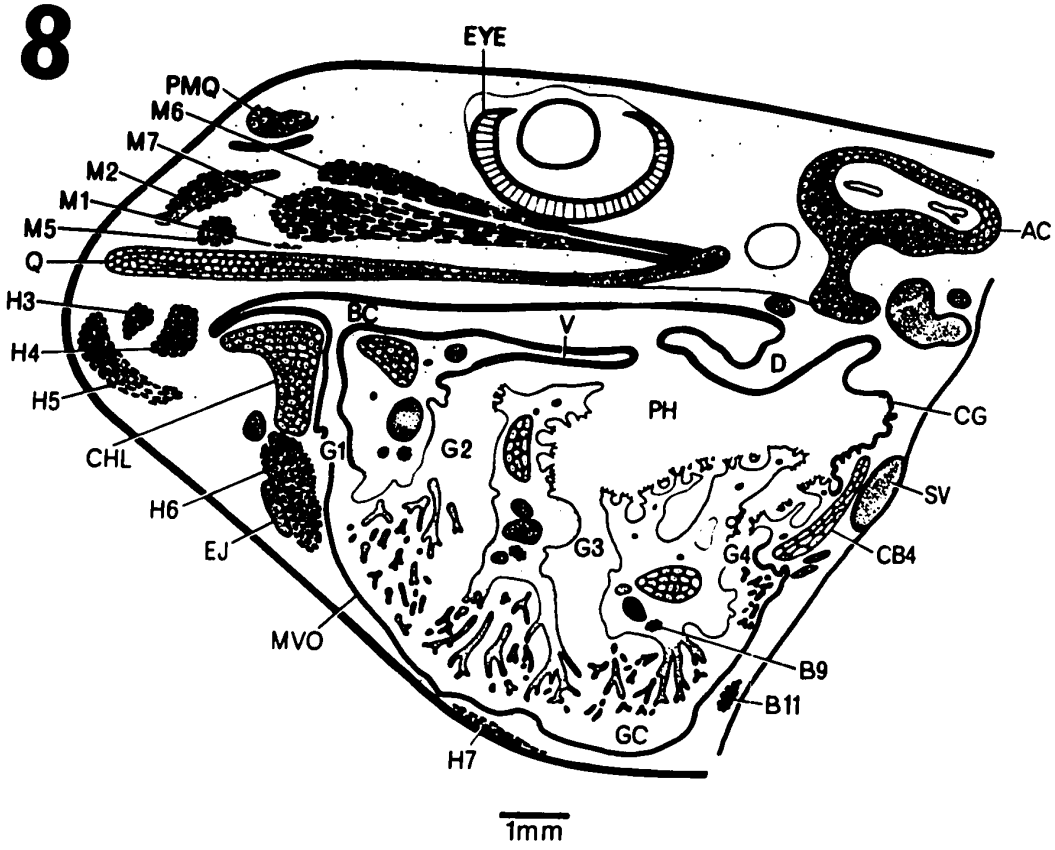

A
CT
CRS
CRI
TC
C1
PR
C2
CRH
HP
V
ARY
PB
LS
A
VN
M10
H6
H7
OC
B
AC
LG
PB
D
V
B1
DVP
CB4
B12
S2
CB1
PH
G2
G3
A
VN
H7
1mm

6

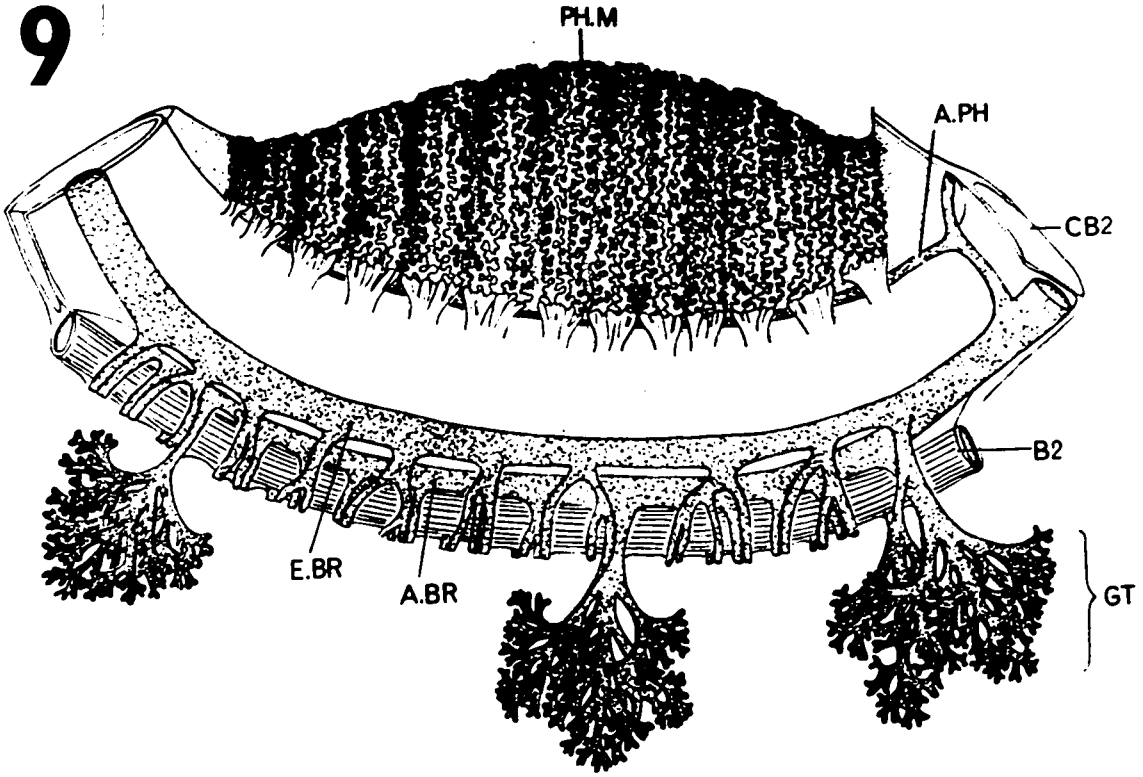
7


H1
M6
M7
M1
PMQ
CT
CQL
CRS
RSQ
M7
M2
M6
M5
M1
M2
TA
MS
H3
H4
H2
H5
CRI
CM
CHL
H1
H5
M8
M9
M10
S1
H6
1 MM

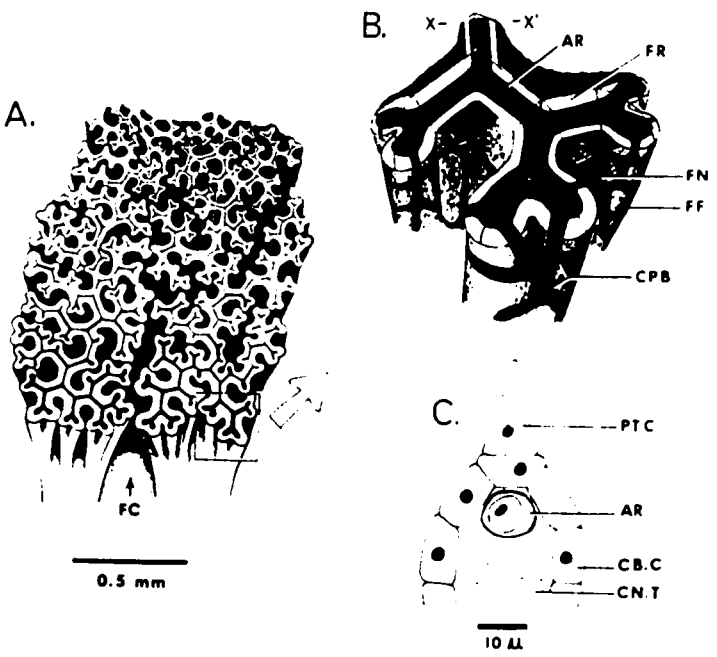
8


EYE
PMQ
M6
M7
M2
M1
M5
Q
AC
H3
BC
V
H4
D
H5
PH
CG
CHL
G1
G2
SV
H6
G3
G4
CB4
EJ
MVO
B9
B11
GC
H7
1mm

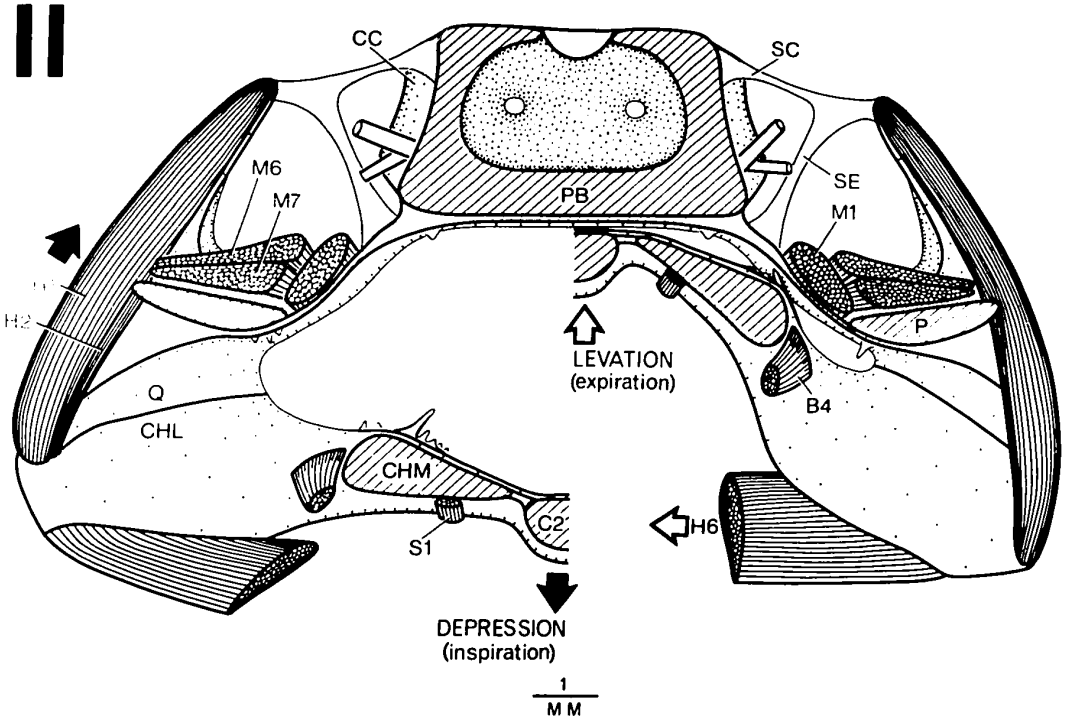
9


PH.M
A.PH
CB2
B2
E.BR
A.BR
GT
1mm

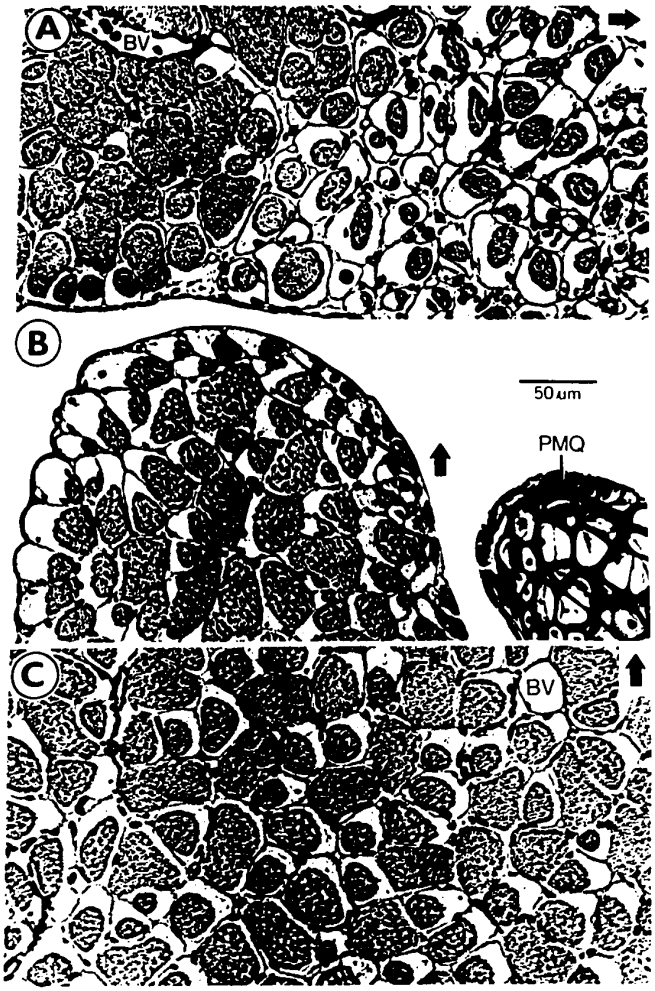
10


A.
FC
0.5 mm
B.
X-
-X'
AR
FR
FN
FF
CPB
C.
PTC
AR
CB.C
CN.T
10 µ

11


CC
SC
M6
M7
PB
SE
M1
H2
P
LEVATION
(expiration)
B4
Q
CHL
CHM
C2
S1
H6
DEPRESSION
(inspiration)
1
M M

12


A
BV
B
50 µm
PMQ
C
BV

CHAPTER 2

THE VELAR MECHANISM OF *RANA CATESBEIANA*

ABSTRACT. The direct observation of the ventral velum in normally breathing tadpoles confirms earlier evidence for a valvular function of this structure during inspiration. The ventral velum also acts as a hydrofoil during expiration and as a mucus secreting surface for the entrapment of suspended food particles.

As the volume of water pumped per ventilation cycle is normally less than the maximum volume of the buccal cavity, it is unnecessary for the pharynx also to become filled with inspired water during the sinking of the buccal floor. Therefore the ventral velum's cyclic occlusion of the buccal cavity from the pharynx is no handicap to normal ventilation; on the contrary, it is a prerequisite for the efficiency of the pharyngeal pump.

The dorsal velum does not participate in valvular activity, but deflects the respiratory current downward and inward to the gill cavity via gill clefts 2, 3, and 4. Therefore the strong flow pumped into the pharynx by buccal compression is prevented from impinging directly on the ciliated groove behind the dorsal velum and thereby interfering with the transport of food into the esophagus.

CONTENTS

Introduction

The concealed location of the ventral velum has prevented direct observation of it during gill ventilation and controversy has been generated in the literature by speculations regarding its function.

Schulze (1888, 1892) regarded this structure as a non-valvular "Kiemendeckplatte" in Pelobates fuscus. Kratochwill (1933) believed that in Rana dalmatina (= R. agilis) it was a valvular "Filterklappe", because he recognized that in addition to the buccal pump, there is a pharyngeal force pump which, when active, must be occluded from the buccal cavity to ensure that water flows into the gill cavity and not back into the buccal cavity. On the other hand, Savage (1952), in describing R. temporaria, used the term "ventral velum" (of Götte 1875) and did not see the need for a valvular hypothesis, as he recognized only one pump, the buccal cavity (p. 494): "... it is not necessary that there should be a valve, for a pump can act if there is a resistance of any kind in the outlet channel."

Opposite points of view concerning the ventral velum are taken by de Jongh (1968) and Kenny (1969a; "anterior filter valve"), but like earlier researches, their findings are based on functional anatomy and visual observations of surrounding structures. In addition, de Jongh used cinematography on R. temporaria and his support for the valvular hypothesis agrees with evidence based on hydrostatic pressures monitored simultaneously in the buccal cavity and pharynx of R. catesbeiana (Gradwell and Pasztor 1968). However, de Jongh's support for a valvular ventral velum is not based on the recognition of Kratochwill's pharyngeal pump (1933), for de Jongh contends that there is only a single pump in tadpoles,

and that it lies in the oral (buccal) cavity. Kenny worked mainly on Phyllomedusa trinitatus but from his examinations of the anatomy of Rana temporaria and Bufo bufo he concludes (p. 244) that the water pumping mechanisms of these species are the same: "... the primary feeding mechanism must function in exactly the same way in these forms." Elsewhere (pp. 242, 243), he makes close comparisons between his description of the pumping mechanism of Phyllomedusa and Kratochwill's work (1933) on Rana dalmatina, and rejects Kratochwill's valvular hypothesis of the ventral velum on the grounds that: "While this action is theoretically possible, it is inconsistent with the sequence of movements of the pumping mechanism (of Phyllomedusa ?)[1]. The valve would have to be maintained in this position on the intake stroke of the pump, when the entire buccal floor, including the anterior filter valve (ventral velum)[1], is lowered." However, in Rana dalmatina (Kratochwill 1933) the hind part of the buccal floor is not lowered, but is somewhat raised during the intake stroke of the buccal pump and the ventral velum is closed against the buccal roof. Kenny (1969a) was chiefly concerned with the feeding mechanism and proposed that large particles are filtered from the water flowing through the slit between the ventral velum and the dorsal velum.

It will be realized that if valvular closure of the ventral velum occurs during inspiration, it must preclude inhaled water from entering the pharynx. In the bullfrog tadpole the water flow is almost completely unidirectional. During each ventilation cycle the water volume inhaled should therefore equal the volume

---

1 Parentheses mine

exhaled. It follows that if the exhaled volume is greater than the inhaled volume of the buccal cavity, the pharynx must also become filled to some extent by inhaled water during inspiration and the ventral velum could then not act as a valve. It is therefore important to establish whether the buccal volume alone is adequate to account for the spout outflow per ventilation cycle. The present research was undertaken with this object in view as there is no published information on the volume of water pumped by tadpoles per ventilation cycle nor on the volume of the buccal cavity.

An attempt is also made in the present study to settle the velum controversy, at least in Rana catesbeiana, by direct observation and by determining the effect on hydrostatic pressures of experimental interference with normal velar movements.

By virtue of their close association with the ventral velum, some notes are included on the function of the paired dorsal vela.

Materials and Methods

Ten bullfrog tadpoles (stage 30 of Gosner 1960), having an entire length of 10.2 cm and a snout-to-vent length of 4.0 cm, were used for the volumetric measurements. The animals were acclimated to 22°C for two weeks in dechlorinated tap water and fed on algae and cooked spinach.

Individual tadpoles were placed in a 500 ml Perspex trough (Fig. 1) and deeply anesthetized with 1 % urethane for ca. 10 min. A cannula of 2 cm polyethylene tubing (PE 160, Clay-Adams Inc., N.Y.) with an inner diameter of 1.14 mm and an outer diameter of 1.57 mm was flared at one end by means of a hot iron. Heat was also applied along the length of the cannula to bend it in a smooth curve through 90°. The spout (diameter, 1.1 mm) of the tadpole was slightly enlarged by a 0.5 mm cut at right angles to its anterior edge to accommodate the flared end of the cannula. This end was placed in the spout and fixed with a fine copper wire (diameter, 80μm) tied in a moderately tight knot around the skin of the orifice. The cannula was withdrawn slightly until the flared end was stopped by the tied circumference of the spout. Tightening of the knot then made all the exhaled water flow through the cannula without leaks at its junction with the spout. The branchial outflow via the cannula would encounter more frictional resistance than through the normal spout, were it not for the greater inner diameter of the cannula than the spout. Therefore the resistance to the branchial outflow was probably negligible in the experiments.

After the cannula was passed through a hole in the side of the trough, a sealant (Dow Corning Silicones Ltd) was used to stop leaks and to fix the cannula with its distal end vertical. A stream of aerated 0.5 % urethane (prepared with dechlorinated tap

water) was passed through the trough. Displacement of the 1 % urethane occurred, and a constant volume was maintained in the trough by adjusting the inflow until the liquid surface in the trough was kept level with the cannula's aperture. When the tadpole was breathing with a frequency (ca. 75 cycle/min) and amplitude (judged from the vertical oscillations of the buccal floor) identical with that of conscious, unrestrained tadpoles, the water issuing from the cannula's aperture was collected during 100 cycles of ventilation.

The tadpoles were killed by deep anesthesia after the experiments and the ventral velum was exposed by dissecting the pharynx away. Latex was injected through the mouth of each tadpole to fill the buccal cavity completely while the ventral velum was held closed against the buccal roof.

Some 20 tadpoles of the same size and developmental stage as those used for the volumetric measurements, were also acclimated and anesthetized by the same procedures. These tadpoles were used under a binocular dissecting microscope for anatomical and experimental studies of the vela. Hydrostatic pressures were monitored by the method of Gradwell and Pasztor (1968).

Results and Discussion

The anatomy of the dorsal and ventral vela has already been discussed (see Chapter 1). In the present study, following the lead of Savage (1952), the term "velum" is retained both by reason of priority and for its suggested accessory role of deflecting water (Gradwell and Pasztor 1968), although it will be shown that it also functions as a respiratory valve and as a secretory surface.

One objection to the acceptance of the ventral velum as a valve, is that such a function would restrict inspired water to the buccal cavity and this volume would therefore be insufficient for normal ventilation. The present study is arranged so as first to overrule this objection with evidence based on measurements of the buccal volume and of the total volume pumped per ventilation cycle. The the results of functional anatomical studies, direct observation, and hydrostatic pressure measurements will be presented as further evidence for a valvular function of the ventral velum.

Buccal and ventilation volumes

Anuran tadpoles (except pipids, Rhinophrynus and certain microhylids) are the only amphibians with internal gills. In Rana catesbeiana, the gills are irrigated by means of a complex water pumping mechanism that ensures an intermittent oral and nasal inflow and a continuous branchial outflow (Gradwell and Pasztor 1968). The single exhalent aperture of the gill cavity is projected into a short spout, which facilitates the experimental collection of the branchial outflow (Fig. 1). In addition, these tadpoles grow to a large size and as they then have a surface-to-volume ratio unfavorable for cutaneous gas exchange, gill ventilation tends to be increased. It is therefore convenient to measure the ventilation volume with the simple apparatus shown in Fig. 1.

Five determinations of the ventilation volume were made on each of the ten tadpoles, and ranged from 4.16 to 4.77 ml per 100 cycles. The mean value was 4.51 ml per 100 cycles. Therefore the mean volume pumped per ventilation cycle was 0.0451 ml.

The volume of latex injected into the buccal cavity ranged from 0.045 to 0.060 ml for the ten tadpoles and had a mean value of 0.055 ml.

If valvular action of the ventral velum occludes the buccal cavity from the pharynx, the same volume of water should leave the spout during each ventilation cycle as enters the buccal cavity during inspiration. However, the spout outflow (0.0451 ml) during moderate breathing is less than the direct measurement of maximum buccal volume (0.055 ml). The difference in volumes would be explained if the buccal cavity was not exerting its full capacity, as seems likely from the observation that during the collection of the spout outflow in moderate breathing, the tadpoles did not depress the buccal floor maximally and were presumably not expanding the buccal cavity completely. This observation therefore emphasizes the adequacy of the buccal cavity to account for all the spout outflow per ventilation cycle.

The significance of the above results is best judged in the light of what is known at present of water flow through the bullfrog tadpole (Gradwell 1968, Gradwell and Pasztor 1968) (Fig. 2). Inspiration begins with a slight opening of the mouth and a passive sinking of the buccal floor by its inherent elasticity. Simultaneously, pharyngeal constriction occurs, and the consequent rise in pharyngeal hydrostatic pressure (second peak, Fig. 3A) probably seals the ventral velum against the buccal roof and prevents backflow of pharyngeal water into the buccal cavity. In this respect, graphic results were previously misinterpreted

(Gradwell and Pasztor 1968), for pharyngeal constriction occurs early and not late in the inspiration phase. Water, rendered visible with dyes, emerges through gill clefts 2, 3, and 4, and leaves the gill cavity via the spout. During the second part of inspiration there is a wider opening of the mouth simultaneously with further depression of the buccal floor, but this time by the abductor muscles of the hyoidean arch.

During expiration, buccal levation by the pink fibers of the H6 muscle (see Chapter 1) causes a rise in the buccal hydrostatic pressure and this probably opens the ventral velum, allowing buccal water to enter the pharynx. The pharynx fills rapidly until its hydrostatic pressure is sufficient to drive water through gill clefts 2, 3, and 4 into the gill cavity and out through the spout. A small volume also leaves the buccal cavity by gill cleft 1 and flows into the gill cavity, thus by-passing the pharynx.

The measured buccal and ventilation volumes show that it is unnecessary for both the buccal cavity and pharynx to become filled with water during inspiration; the buccal volume is more than adequate to account for all the water leaving the spout during the ventilation cycle. These findings therefore invalidate the argument that valvular action of the ventral velum cannot occur because the inspired water would be restricted to the buccal cavity and therefore would be insufficient for normal ventilation.

Functional anatomy

After partial removal of the pigmented skin covering the ventral aspect of the ventilation apparatus, bullfrog tadpoles under light anesthesia continue to breathe normally. The movements of the water pumps can then be easily seen, but the movements of the ventral velum are difficult to determine with certainty

because this structure is concealed from view, lying between the buccal cavity and pharynx (Fig. 2).

In pithed, bisected tadpoles, manipulation of the spicular supports of the ventral velum revealed its possible movements. The simultaneous displacements of the adjoining cartilages were also noted and compared with their displacements in normally breathing, partly dissected animals. The experiments showed that the dorsoventral movements of the medial aspects of the ceratohyalia caused obligatory but much smaller dorsoventral excursions of the hypobranchial plates. During normal buccal depression, the sinking of the hypobranchial plates was hardly enough to pull the edge of the ventral velum free from the buccal roof. It was also found that depression of the medial aspects of the ceratobranchialia simultaneous with elevation of the lateral aspects, as occurs during pharyngeal constriction, caused a slight depression of the hypobranchial plates. Manual displacements of this kind in bisected tadpoles tended to bend the distal ends of the spicula toward the buccal roof, suggesting that this mechanism may be a factor in maintaining the edge of the ventral velum against the buccal roof during at least the early part of normal inspiration.

Direct observation and hydrostatic pressures

It was previously thought that exposure of the ventral velum in breathing tadpoles by cutting into the buccal cavity or pharynx would influence the hydrostatic pressures of these chambers and probably disturb normal velar movements. This discouraged earlier attempts at direct observation of the ventral velum. However, in the present study, parts of the ventral velum were exposed in lightly anesthetized, regularly breathing tadpoles, while hydrostatic pressures in the buccal cavity and pharynx were simultaneously monitored. The effect of this surgery on the buccal and

pharyngeal hydrostatic pressures could therefore be determined.

Ablation of the second gill arch of one side after removal of the overlying operculum and ligation of the relative branchial arteries, caused a reduction of the buccal and pharyngeal pressures. It was therefore possible to observe movements of the ventral velum from the posterior aspect. The velum closed in a valve-like fashion against the buccal roof during pharyngeal constriction and buccal expansion, and deflected water against the dorsal velum during buccal constriction and pharyngeal expansion.

In other bullfrog tadpoles, the ventral velum was exposed to view during lightly anesthetized breathing by less extensive dissection. After implantation and connection of the buccal and pharyngeal cannulae to the pressure transducers, a small patch of skin (<1 mm^2) was removed from the region covering the H2 muscle (see Chapter 1) just below the eye and on the same side as the pharyngeal cannula. A small slit cut between the posterior fibers of this muscle permitted observation of the functioning of the lateral part of the ventral velum from an anterior aspect. The reduction in hydrostatic pressures caused by this light surgery was insignificant, and the visual observations of the ventral velum's movements generally agreed with those obtained by ablations of a second gill arch. Study of the ventral velum through the slit in the H2 muscle was so successful that with a strong spotlight even the medial part of the velum was visible. The lateral part of the velum showed greater flap-like dorsoventral movements than the medial part during opening and closing of the valve. It was also seen that a pad of resilient connective tissue covered by a few of the posterior fibers of the H2 muscle was forced inward by cyclic contraction of this muscle. Therefore

the contiguous buccal lining was also pressed inward and against the most lateral part of the ventral velum's edge, ensuring a flush contact and efficient valvular action.

Direct observation through the slit in the H2 muscle revealed that the dorsal velum did not participate in the valvular activity of the ventral velum. There are two pads of resilient connective tissue covered by squamous epithelium behind the most lateral aspect of the dorsal velum. These pads helped to support this region of the dorsal velum so that it was not unduly deformed by water impinging on it from the buccal cavity. On the contrary, the impact of this water spread the dorsal velum out like an unfolding sail, enabling it to deflect the water downward and toward the more medial aspects of the gill clefts 2, 3, and 4. By this means, water from the buccal cavity was prevented from displacing the mucous cord containing entrapped food particles behind the dorsal velum. The ciliated tract of this region could therefore continue moving the cord of food particles into the esophagus unhampered by the periodic flow of buccal water into the pharynx.

As final evidence for a valvular ventral velum, an experiment was conducted to determine the effect on hydrostatic pressures of manually preventing closure of the ventral velum. A loop of fine wire was passed through the small slit in the H2 muscle while hydrostatic pressures were monitored in the normally breathing tadpole. Figure 3B,C shows the depression of the second pharyngeal pressure peak when the lateral edge of the ventral velum was prevented from making contact with the buccal roof during pharyngeal constriction. The buccal pressure and its transmitted effect in the pharynx (first peak) were scarcely influenced by the operation. This is not surprising, as the

efficiency of the buccal pump is negligibly dependent on the closing of the ventral velum. Withdrawal of the wire allowed the velum to resume its valvular action and the second pharyngeal pressure peak was promptly restored. Consistent with the view of separate left and right respiratory streams entering the pharynx from the buccal cavity (Kratochwill 1933, Kenny 1969a), the velar interference affected the pharyngeal pressure significantly only if the implanted cannula was on the same side as the experimental abduction of the ventral velum.

The regularity of normal gill ventilation is occasionally disturbed by irritants, such as suspended debris, in the oral intake. In nature, tadpoles respond to such stimuli with greater depression of the buccal floor. Sometimes this behavior also occurs spontaneously in lightly anesthetized tadpoles. Furthermore, this behavior was induced by adding dyes or debris to the oral intakes of unrestrained conscious tadpoles, and alternatively, by mechanically stimulating the opercular lining with a fine wire inserted into the gill cavity through the spout. The phenomenon has been called "coughing" (Gradwell 1968) but the term "hyperinspiration" is more descriptive. Hyperinspirations are caused by ventrad jerks of the buccal floor of variable amplitude during the inspiration phase of the ventilation cycle, while the mouth is open (Fig. 4A). In consequence, abnormally large negative hydrostatic pressures are generated in the buccal cavity and also in the pharynx (Fig. 4B), but the comparison of Fig. 3A with Fig. 4A reveals that the hyperinspirations occur after pharyngeal constriction (second pressure peak). Hyperinspiration may occur early or late during inspiration but no evidence was found of its occurrence so early that it might completely abolish valvular activity of the ventral velum during the power stroke of

the pharyngeal pump.

Taken together, the above results permits the conclusion that the ventral velum of Rana catesbeiana acts as a valve during pharyngeal constriction and as a hydrofoil to deflect water against the dorsal velum during expiration. The function of the dorsal velum is to deflect the respiratory current downward, preventing it from displacing the mucous cord of entrapped food particles which lies behind the dorsal velum. The secretion of mucus by the underside of the ventral velum is indicated by its glandular columnar epithelium and by the high density of its vascular bed. However, the detailed investigation of Kenny's proposal (1969a) that the ventral velum participates mechanically in food filtration, was outside the scope of the present research.

Acknowledgments

My thanks are extended to Dr. B.R. McMahon, Department of Biology, University of Calgary, for a critical reading of the manuscript.

I am indebted to Dr. J.A. Peters, Smithsonian Institution, Washington D.C., for the loan of tadpoles of Phyllomedusa guttata (96643). I also gratefully acknowledge a gift of Rana temporaria tadpoles from Dr. R. Maxwell Savage.

References

De Jongh, H.J. 1968. Functional morphology of the jaw apparatus of larval and metamorphosing Rana temporaria. Neth. J. Zool. 18: 1-103.

Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16: 183-190.

Götte, A. 1875. Die Entwicklungsgeschichte der Unke (Bombinator igneus) als Grundlage einer vergleichenden Morphologie der Wirbelthiere. Leipzig. Cited from Savage (1952).

Gradwell, N. 1968. The jaw and hyoidean mechanism of the bullfrog tadpole during aqueous ventilation. Can. J. Zool. 46: 1041-1052.

Gradwell, N., and V.M. Pasztor. 1968. Hydrostatic pressures during normal ventilation in the bullfrog tadpole. Ibid. 46: 1169-1174.

Kenny, J.S. 1969a. Feeding mechanisms in anuran larvae. J. Zool. Lond. 157: 225-246.

---- 1969b. Pharyngeal mucous secreting epithelia of anuran larvae. Acta Zool. 50: 143-153.

Kratochwill, K. 1933. Zur Morphologie und Physiologie der Nahrungsaufnahme der Froschlarven. Z. Wiss. Zool. 164: 421-468.

Savage, R.M. 1952. Ecological, physiological and anatomical observations on some species of anuran tadpoles. Proc. Zool. Soc. Lond. 122: 467-514.

Schulze, F.E. 1888. Über die inneren Kiemen der Batrachierlarven. I. Mitteilung: Über das Epithel der Lippen, der Mund-, Rachen und Kiemenhöhle erwachsener Larven von Pelobates fuscus. Abhandl. Königlichen Akad. Wiss. Berlin. 32: 715-768.

---- 1892. Idem. II. Skelet, Muskulatur, Blutgefässe, Filterapparat, respiratorische Anhänge und Atmungsbewegungen erwachsener Larven von Pelobates fuscus. Ibid. 13: 1-66.

Illustrations

FIG. 1. Stereogram of the simple method used to determine the ventilation volume of the bullfrog tadpole. The cannula's outflow was collected in a beaker while the ventilation cycles were counted by observing dorsoventral oscillations of the buccal floor. C, cannula; S, sealant; SP, spout; W, copper wire.

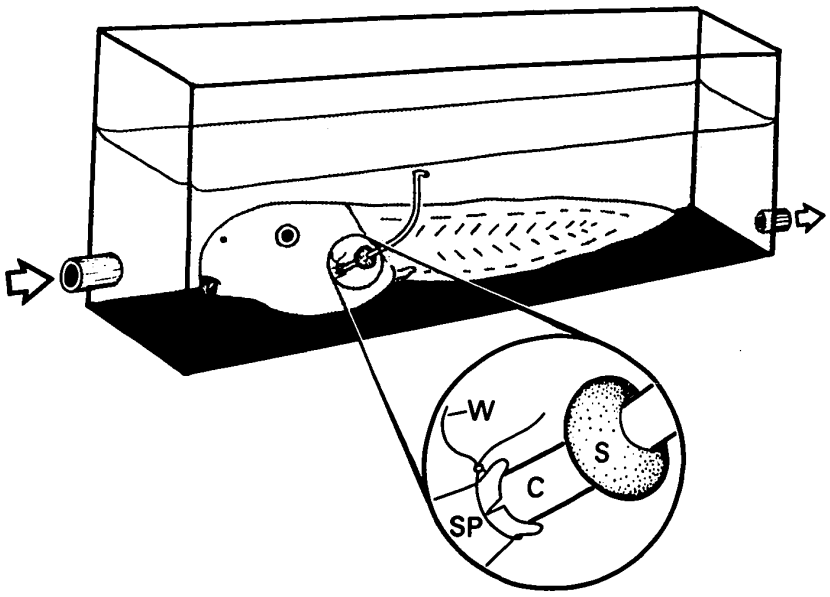
FIG. 2. Composite diagram of sagittal and vertical oblique sections through the head of a bullfrog tadpole. Orientations of the anatomy are shown at the onset of expiration, when the mouth and nares are closing and the buccal floor has begun to rise. The ventral velum has just been pulled away from the buccal roof, presumably by an increase in buccal hydrostatic pressure. The flow of buccal water over the ventral velum and into the pharynx is beginning (large arrow). Gill cleft 1 has opened in preparation for the flow from the buccal cavity into the gill cavity. BC, buccal cavity; D, dorsal velum; GC, gill cavity; PH, pharynx; V, ventral velum; 1-4, gill clefts 1 to 4.

FIG. 3. The effect on buccal and pharyngeal hydrostatic pressures of experimentally preventing valvular closure of the ventral velum in lightly anesthetized tadpoles. Positive pressures appear above the horizontal lines. Upper traces: pharyngeal pressures; lower traces: buccal pressures. A. Regular breathing before surgery. B,C. Experimental abduction (AB) of the edge of the ventral velum caused depression of the second pharyngeal pressure peak. After the period of experimental velar abduction (shown by the arrow in B.), withdrawal of the wire loop allowed gradual recovery and reappearance of the second pharyngeal pressure peak. In C., midway along the arrow AB, the wire was manually released and was left <u>in situ</u> during subsequent deep anesthesia and dissection to

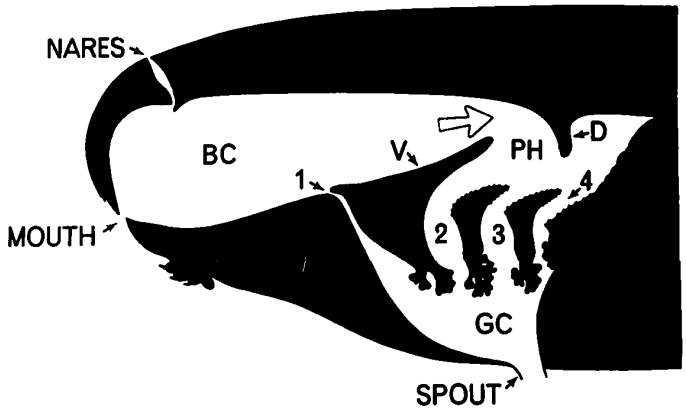
confirm that it was mechanically obstructing velar adduction. There was no reappearance of the second peak in pharyngeal pressure. AR, artifact caused by insertion of the wire. Calibrations: 1 cm water; 1 sec.

FIG. 4. Simultaneous records of the buccal floor movements and hydrostatic pressures in a lightly anesthetized bullfrog tadpole during gill ventilation. Hyperinspirations are indicated by the arrows. A. Movements of the medial ceratohyalia (CH) correlated with buccal pressures. B. Buccal and pharyngeal pressures recorded simultaneously. Pressure calibration: 1 cm water.

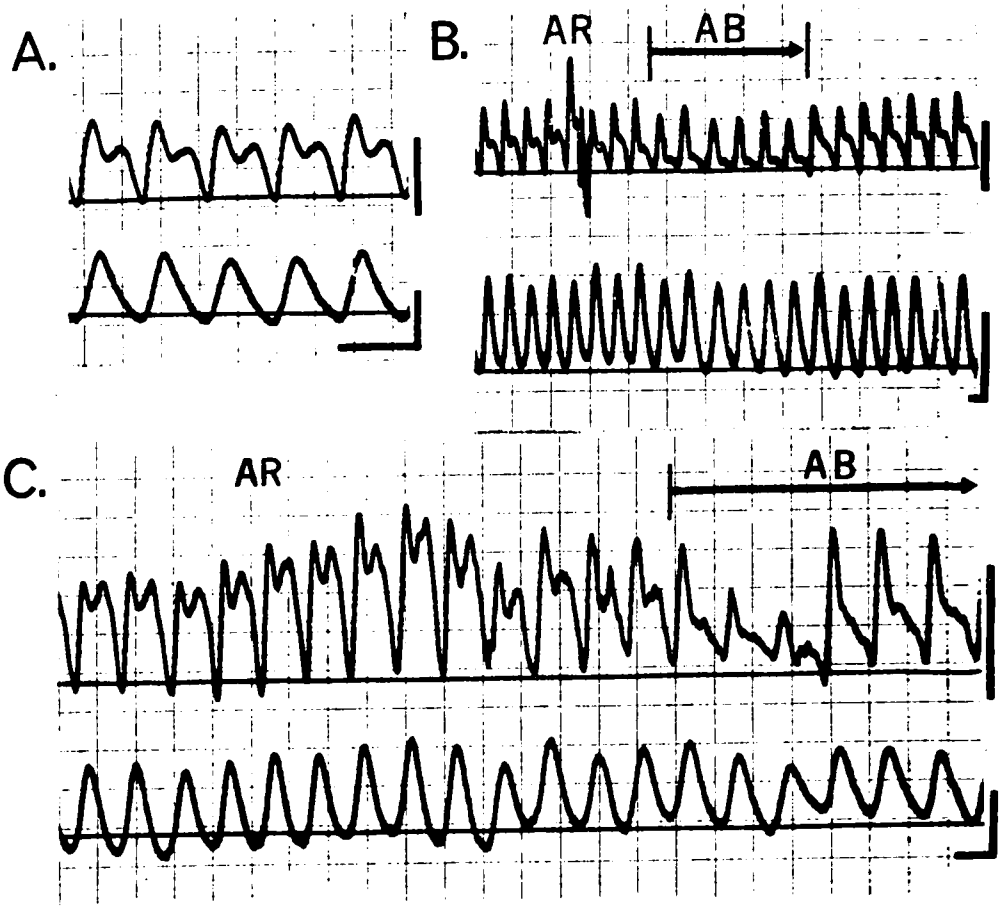
1



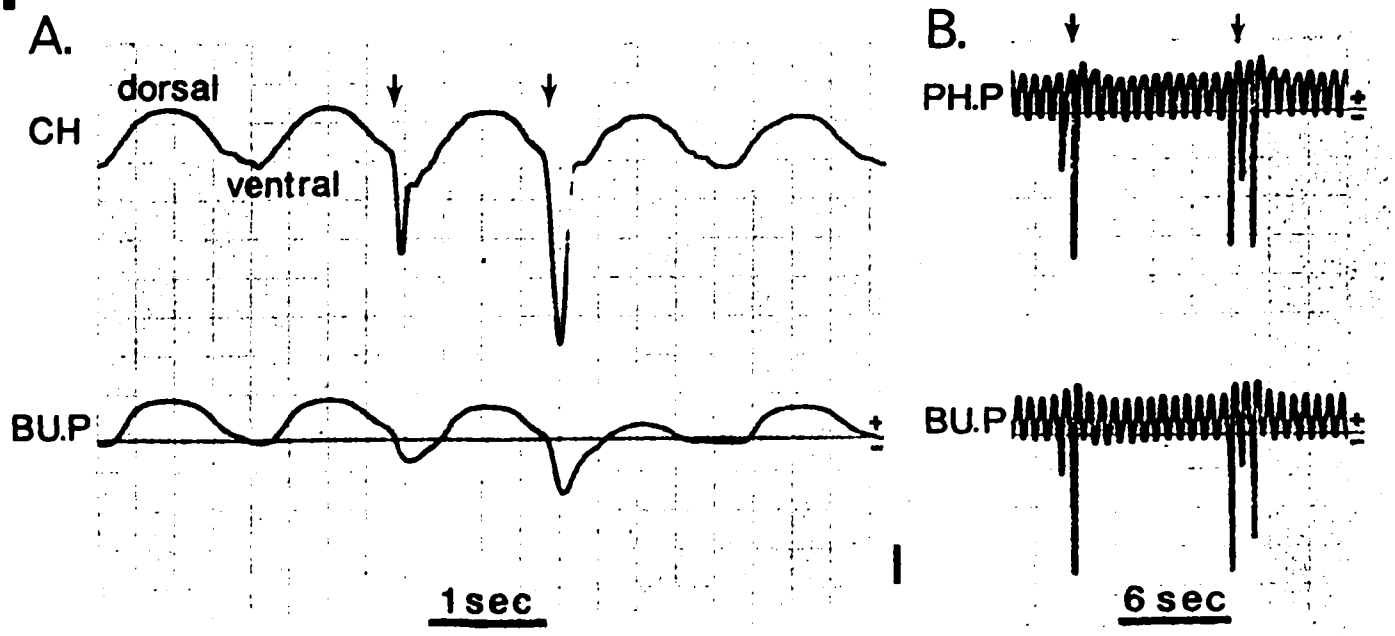
2



3



4



CHAPTER 3

THE MUSCULOSKELETAL MECHANISM OF GILL VENTILATION IN *RANA CATESBEIANA*

ABSTRACT. The jaw and hyoidean movements have been correlated with hydrostatic pressures in the ventilation system by video recording. The time dependence of hydrostatic pressures in the ventilation system and of five ventilatory muscles has been established. Intermittent variations in the amplitude of ventilation have been found to result from natural and experimental irritation of the gill cavity. These variations, or hyperventilations, have been correlated with the activity of special muscle fibers, called fibrillic fibers, in the H1, H3, H6, and B7 muscles. Motor denervations of the H6 muscle during phasic ventilation have revealed its importance for the power stroke of the buccal water pump. The respective contributions of the alternating buccal and pharyngeal pumps to phasic ventilation has been shown to depend on ambient temperature. An auxiliary branchial pressure pump behind the gill clefts is powered by the H7 muscle which lies in the soft opercular skin of the gill cavity.

C O N T E N T S

Introduction

Anuran tadpoles are the only amphibians with internal gills, although true gills are lacking in some genera. As in the case of other animals with enclosed gills, a means of ventilating these structures with the ambient medium is usually necessary for efficient respiration in tadpoles, and has caused the evolution of suitable pumping mechanisms. However, to varying degrees, tadpoles also practice microphagous ingestion by filtering suspended organic particles from water pumped through the pharynx. This water enters the gill cavity and if gills are present, the water flows over them and is then exhaled through one or two apertures in the operculum of the gill cavity. The problem of how water is pumped through tadpoles has therefore received the attention of researchers interested in gill ventilation as well as those interested in microphagous feeding.

It was inadvertently overlooked (Gradwell 1968) that Schulze (1892) first discovered the mechanism of upper jaw opening in the tadpole of Pelobates fuscus. Indeed, Schulze (1888, 1892) published the first and only existing detailed account of anuran gill ventilation, and described the relevant anatomy, skeletal movements, muscle activity and water flow of normal ventilation. Schulze's conclusions on function were based on observations of uninjured or partly dissected tadpoles while they pumped stained water through their ventilation systems. His emphasis was on a rhythmic buccal force pump, but he also mentioned a subsidiary water pump, for he occasionally observed a "not inconsiderable" spout outflow during oral and narial inspiration. He seems to have correctly correlated this outflow with branchial movements

but he overlooked pharyngeal constriction and valvular activity of the ventral velum. These are two prerequisites for the operation of this pharyngeal pump, and are undoubtedly satisfied by Schulze's description of the elevation of the lateral aspects of the gill arches. It is therefore clear that Schulze did not properly understand the mechanism of this spout outflow during inspiration. He also described an occasional branchial or oral expulsion of suspended foreign particles by an auxilliary pressure pump behind the gill clefts.

Willem's description (1920) of water flow through tadpoles agrees essentially with that of Schulze (1892).

Except for de Jongh (1968), all other published information on anuran water pumping is concerned with filter feeding. Similar research methods as those of Schulze (1888, 1892) were used by Kratochwill (1933) whose study of water flow through tadpoles of Rana dalmatina (= R. agilis) was also aided by their poorer pigmentation than tadpoles of Pelobates fuscus. Kratochwill described a buccal pumping mechanism like that of Pelobates except that in Rana dalmatina the hind part of the buccal floor, supported by the hypobranchial plates, was somewhat raised and not lowered like the rest of the buccal floor during inspiration. This new observation enabled Kratochwill to realize the valvular nature of the ventral velum and to interpret the simultaneous elevation of the lateral aspects of the gill arches as causing constriction of the pharynx. The raising of the hind part of the buccal floor during inspiration is important for this mechanism because it permits the ventral velum to be pressed against the buccal roof during pharyngeal constriction. This valvular action of the ventral velum prevents a reflux of pharyngeal water into the buccal cavity and pharyngeal water is consequently forced to

the outside through the gill clefts, gill cavity, and the spout. Kratochwill had therefore added more detail to Schulze's original concept (1892) of a pumping mechanism to explain this flow of water from the spout during inspiration. In other respects, Kratochwill's findings are generally consistent with those of Schulze for both the normal ventilation mechanism and the auxilliary branchial constriction by the somewhat muscular lining of the operculum. However, the more recent publications of Savage (1952, 1961), de Jongh (1968), and Kenny (1969) do not corroborate a branchial constriction mechanism and a pharyngeal pumping mechanism of the type described by Schulze (1892) and Kratochwill (1933) respectively.

According to Savage (1952), the buccal floor is the only pump for transporting water through tadpoles of Rana temporaria and Bufo bufo. He does not refer to the earlier reports of a pharyngeal pump and an auxilliary branchial pump. Savage's error in reporting muscles in the ventral velum has been pointed out (Chapter 2). Responding to Kratochwill's views on the function of the ventral velum, Savage believed that valvular action of the ventral velum was unnecessary because of the high resistance of the pharyngeal filters to branchial backflow during the intake stroke of the buccal pump. He therefore seems to have missed Kratochwill's point that a valvular ventral velum is related to the pharyngeal pump and is not just a device for preventing branchial backflow during the intake stroke of the buccal pump.

In his report on Rana temporaria, de Jongh (1968, p. 77) states: "In tadpoles there is only one pump chamber, the oral (buccal)[1] cavity, and the water current is discontinuous." These

[1] Parenthesis mine

views and a few others conflict with Schulze (1892) and Kratochwill (1933), and with electrophysiological findings on Rana catesbeiana (Gradwell and Pasztor 1968). De Jongh also does not mention the first gill cleft of R. temporaria, which connects the buccal cavity directly with the gill cavity, and he therefore fails to take account of the ability of buccal water to by-pass the pharynx. On the problem of velar function, de Jongh supports the valvular hypothesis (see Chapter 2).

The water pumping mechanism which Kenny (1969) describes in Phyllomedusa trinitatus and extends to include Rana and Bufo on account of their structural affinities with Phyllomedusa, conflicts with the situation in Rana catesbeiana (Gradwell and Pasztor 1968). Although Phyllomedusa has all the necessary apparatus of Kratochwill's pharyngeal pump, Kenny proposes a sequence of pumping movements discordant with Kratochwill's pharyngeal pump. Moreover, Kenny's rejection (p. 242) of Kratochwill's valvular hypothesis of the ventral velum adds to existing controversy because it disagrees with the findings of de Jongh (1968) and Gradwell and Pasztor (1968). Finally, Kenny proposes a branchial pump powered by a muscle in the opercular lining, but in disagreement with Schulze (1892) and Kratochwill (1933), he regards this pump as active during normal ventilation and not during occasional flushing of the ventilation system to expell debris. Kenny's extension of his scheme to include Bufo, provides further evidence that his views on the pumping mechanism are questionable, as muscle fibers in the operculum of Bufo are few and sparsely scattered, if at all present, and can hardly be regarded as a rhythmically active ventilatory muscle.

Earlier reports on Rana catesbeiana (Gradwell 1968, 1969a, 1969b; Gradwell and Pasztor 1968) have covered specific aspects

of the complex functional morphology of branchial ventilation. These findings and the further detail on the relevant morphology given in Chapter 1 of the present thesis, now permit an account of the musculoskeletal mechanism of water transport through the bullfrog tadpole. Such an account, with a detailed discussion of the pertinent literature, is called for in view of the existing controversies on the subject of water transport through anuran tadpoles.

Materials and Methods

Some 80 bullfrog tadpoles (Rana catesbeiana Shaw) were selected from collections made at Lake Hertel, St. Hilaire, Quebec. The developmental stage of these tadpoles was 35 of Gosner 1960, and the snout-to-vent length of them was 3.8 ± 0.5 cm. The animals were kept at 14°C for three weeks before commencement of experimentation, and they were fed on boiled spinach and algae.

Hydrostatic pressures, functional anatomy, and direct extracellular electrical stimulation were studied in some tadpoles by methods already described (Gradwell 1968, Gradwell and Pasztor 1968). The methods included rectilinear pen recordings on a Gilson Polygraph (CH-CBPP), which were monitored with Statham P23BB pressure transducers. In addition, hydrostatic pressures and electromyograms (recorded with a bipolar electrode of varnished and twined 70 μ copper wire) were monitored in upright tadpoles allowed to recover consciousness after anesthesia (ca. 25 min in 1% urethane at 14°C). A polaroid camera was used in some cases to record data from the oscilloscope screen to permit the precise correlation of breathing events. Blood circulation during experimentation was maintained by the animal's normal heart beat.

Mouth and hyoidean movements were correlated with buccal pressures by dual, synchronized television cameras, thus freeing the moving anatomy from constraint of any kind. One camera photographed the moving Polygraph record of hydrostatic pressures while the other camera monitored the mouth and hyoidean movements. The two television signals were displayed as juxtaposed pictures on a television screen. These data were simultaneously recorded at 32 frame/sec on video tape for subsequent analysis at slow replay speeds.

Results

Bullfrog tadpoles (Rana catesbeiana) in nature and in simulated natural conditions in the laboratory, usually rest lightly on the substratum in an upright position (Fig. 1). Sufficient buoyancy is afforded by the partially inflated lungs, to reduce the mechanical pressure between the soft abdomen and the substratum, and to maintain the tadpole's upright posture. Neither are the movements of the visceral apparatus, which also does not touch the substratum, encumbered by mechanical constraints imposed by gravity. Moreover, in an aqueous medium the effect of gravity on mechanical displacements of the visceral apparatus is probably of little importance.

The oral and narial intakes are elevated above the level of the substratum, thus decreasing the tendency for debris to enter the ventilation system by these channels during inspiration.

Against a natural background of benthic detritus, the disruptive camouflage provided by the chromatophores of the integument render motionless tadpoles difficult to see, even in clear, shallow water. The small orifice (1 mm diameter) of the sinistral spout tends to cause a strong exhalent flow which would wash away the protective camouflage of the detritus from its immediate vicinity and so assist detection by predators, were it not for the posterodorsally pointing spout (Fig. 1).

In the visceral skeleton of the large bullfrog tadpole and probably in many other species of tadpoles, there are specialized zones of bending where a different type of cartilage affords greater flexibility than that which is normally inherent in hyaline cartilage (Fig. 2). As in fishes (Tchernavin 1948), the variable mobility of articulation is also an important factor governing mechanical displacements in R. catesbeiana. Furthermore,

the articulation of elements at a joint is governed by:

(a) motive forces acting on the elements

(b) degrees of freedom of articulation

(c) friction between the elements

(d) flexibility of the elements themselves

(e) viscosity of the medium through which the elements are moved

(f) weight of the elements

The visceral arthrology is therefore in itself a deep subject. The present study of it considers only the motive forces (or muscles) and the degrees of freedom (or bending) of the cartilages; these two aspects and their behavioral effect will together be termed the musculoskeletal mechanism. To facilitate description of this mechanism, the abbreviations used in Chapter 1 for the relevant morphology, will be retained in the present study.

1. Jaw mechanism

The mandibular anatomy will be considered together with the cornua trabeculae (which are apparently part of the premandibular arch; see de Beer 1937, p. 476), as forming the upper and lower jaw complex. Included in this system are also three hyoidean muscles which open the jaws.

In the present research, the jaw mechanism was studied in partly dissected tadpoles allowed to gradually recover from deep anesthesia (20 min in 1% urethane at 20°C) after the implantation of electrodes in the jaw muscles. The complete range of jaw movements were analyzed by this method.

Opening

During deep anesthesia the lower beak is folded out of sight behind the upper beak (Fig. 3A) and no movements of the jaws occur. When these tadpoles are bathed in fresh water, the first sign of recovery from anesthesia is a feeble, rhythmic dorsoventral movement of the lower jaw although the opening of the mouth does

not yet occur. The friction of the lower beak against the upper beak causes a small degree of upper jaw movement, but this disappears as the amplitude of the lower jaw movements increases, and a gap appears between the beaks, representing the opening of the mouth (Fig. 3B). The H4 muscle is responsible for this abduction, by causing rotation of the cartilago Meckeli at its transverse and horizontal joint with the processus articularis quadrati. This rotation moves the medial epiphysis of the cartilago Meckeli and the cartilago rostrale inferior away (outward) from the buccal cavity. As recovery proceeds, a stage is reached when part of the H5 muscle begins contracting at about the same time, or immediately after the beginning of the H4 activity (Fig. 3B). As the aponeurosis of the H5 muscle is inserted partly ventrally on the processus retroarticularis of the cartilago Meckeli, this muscle is able, if necessary, to cause the same effect as the H4 muscle. The H5 muscle normally augments the action of the H4 muscle, and causes a greater outward rotation of the lower jaw. The narrow opening phase of jaw abduction ends when the cartilago Meckeli is maximally rotated outward while it lies in a transverse horizontal position.

The onset of the wider opening phase of jaw abduction is smoothly continuous with the end of the narrow opening phase because the activity of the H5 muscle is spread over both phases. Immediately after the ventral muscle fibers of this muscle have begun contracting, the laterally inserted fibers which form the greater bulk of the muscle, contract. A slight forward swing of the medial epiphysis of the cartilago Meckeli results (Fig. 3C) and carries the cartilago rostrale inferior into a wider state of abduction, which effect is also relayed to the upper jaw by

the mandibulo-suprarostrale ligaments (MS, Fig. 3C). At 20°C the mouth does not open more than this during normal gill ventilation. But higher temperatures and polluted water promote an even wider cyclic opening of the mouth by greater activity of the same muscle system and by interaction with hyoidean abduction.

The greatest opening of the mouth is effected by protrusion of the jaws during hyperinspiration (Fig. 3D). During recovery from anesthesia this event is occasional at first, but it becomes more frequent until bouts of 4 to 5 consecutive hyperinspirations occur, each characterized by jaw protrusion (Fig. 4). The insertion of the H3 muscle on the most lateral aspect of the processus retroarticularis Meckeli (Fig. 3D) permits a strong backward movement of this process. The resulting leverage at the quadrate joint causes a large forward protrusion of the medial epiphysis of the cartilago Meckeli and the cartilago rostrale inferior; the effect is also again relayed by the mandibulo-suprarostrale ligaments to the upper jaw. The coupling provided by these ligaments thus permits both jaws to protrude simultaneously.

The above description reveals that the opening of the mouth may be regarded as a superimposition of wider jaw opening on narrow opening, and during hyperinspiration, of the superimposition of jaw protrusion on wide opening.

The H4 and H5 muscles are phasically active and give larger bursts during hyperinspiration, but the H3 muscle is active only during hyperinspiration (Fig. 5). Bilateral denervation of these muscles and of the S1 muscles reduced, but did not abolish rhythmic jaw movements. The jaws opened by their natural elasticity alternating with normal jaw adduction by the relevant muscles (p.114). The mandibulo-suprarostrale ligaments were not active, and

protrusion of the jaws did not occur. Nevertheless, a wide opening of the jaws was possible through hyomandibular interaction (p.113). It therefore seems likely that jaw elasticity and hyomandibular interaction complement the normal muscular opening of the jaws.

Certain ligaments (RSQ, CQL, Fig. 3D) are stretched taut during jaw protrusion and therefore restrain the jaws from excessive abduction. These constraining ligaments are assisted by the ligamentum intratrabeculare, fascia interrostralis, l. cornu suprarostralis, and tendo accessorius (Chapter 1, Figs. 1, 7).

Closing

The closing phase of the mouth occurs more rapidly than the opening phase (Gradwell 1968, Fig. 5) and involves both a transverse horizontal and a vertical rotation of the cartilago Meckeli at its processus articularis quadrati joint. These rotations move the medial epiphysis of the cartilago Meckeli and the cartilago rostrale inferior toward the buccal cavity. Irrespective of their degree of opening, adduction of the upper and lower jaws occurs together. Toward the end of this movement, the lower beak closes within the crescent of the upper beak without the cutting engagement between the beaks that is typical of purposive feeding on large organic material (Gradwell unpublished).

The lower jaw is apparently pulled closed by the simultaneous contraction of the M1, M5, and M6 muscles acting on the cartilago Meckeli, but no electromyographic confirmation of this observation was obtained, nor for the apparently simultaneous contraction of the M2 and M7 muscles which seem to close the upper jaw. However, some closing is also caused by the elastic return of the upper jaw to its resting position, especially from a widely open or protruded state. Part of the closing effect of the M7 muscle is

conveyed to the lower jaw via the tendo accessorius but this seems negligible in comparison with the tendon's possibly more important role during feeding.

Jaw interaction

Interaction between the upper and lower jaw is important, especially via the dichotomous, paired mandibulo-suprarostrale ligaments. If the upper jaw is manually held closed, the lower jaw cannot open. Conversely, the lower jaw cannot close if the upper jaw is held in a wide open or in a protruded position. The same effects are caused on the upper jaw if the lower jaw is similarly displaced.

When the ambient water was lowered to ca. 10°C, the jaw movements became slower and it was possible to observe them more precisely. Their amplitude of movements also decreased but before this occurred to an appreciable extent, it was possible to see that the rhythmic cycle of mandibular movements that begins with the opening, and ends with the closing of the mouth, consists of a scoop-like maneuver of the lower jaw because the closing movement is not an exact reversal of the opening movements. Instead, the closing movement is a simultaneous combination of the transverse horizontal and the vertical rotations at the cartilago Meckeli-quadrate joint. The lower jaw therefore follows a direct path upward and inward until it comes to rest in a closed position behind the cartilago rostrale superior.

2. Hyoidean mechanism[1]

Lightly anesthetized, partly dissected tadpoles were used for direct observation of the ventilation apparatus while simultaneous records were made of hydrostatic pressures and electromyograms in these animals.

A. Direct observation

At its articulation with the quadrate, the CHL is capable of longitudinal horizontal rotation which moves the CHM alternately dorsad and ventrad. The CHL is also capable of slight transverse horizontal rotation at its quadrate joint, which permits anteroposterior tilting of the CHL. Like the cartilago Meckeli, the CHL therefore has two degrees of freedom at its articulation with the quadrate.

Hl muscle

The intermingling between phasic and fibrillic fibers of the Hl muscle increases the difficulty of direct observation of the effects of these two types of fiber of this muscle. However, anteriorly and peripherally there is a predominance of plasmic fibers over fibrillic fibers (Chapter 1, Fig. 12B,C), and these plasmic fibers were seen contracting phasically during buccal depression. It is the anterior aspects of the buccal floor that are more depressed than the posterior aspects and the concomitant forward tilting of the hind parts of the buccal floor facilitates the valvular closure of the ventral velum against the buccal roof. The force of buccal depression leaves little doubt that there is simultaneous complementary contraction of the plasmic fibers that are intermingled with the fibrillic fibers.

---

[1] including the pars reuniens, copula 2 and HPs

Although there is non-uniformity in the proportion of contractile material to sarcoplasm in the plasmic fibers, the usually copious nutritive sarcoplasm per unit cross-sectional area in these fibers would seem to correlate with their continuous phasic activity. On the other hand, the large amount of contractile material per unit cross-sectional area of the fibrillic fibers suggests a greater capability than the plasmic fibers for the generation of tension and it may be conjectured at this stage, that the fibrillic fibers are responsible for the enhanced depression of the buccal floor during hyperinspiration (Chapter 2, Fig. 4A).

H2 muscle

The H2 muscle may help to preserve the integrity of the CHL-quadrate joint (Gradwell 1968), especially during hyper-inspiration. However, observation of the exposed H2 muscle in lightly anesthetized tadpoles showed that it contracts rhythmically and assists the closing of gill cleft 1 by pulling the CHL toward the anterior horn of the CB 1. Simultaneously, those fibers of the H2 muscle which have their origin on the pterygoquadrate, serve to brace the anterior, lateral parts of the pharynx against the lateral edge of the ventral velum, thus assisting its valvular action (see Chapter 2).

The exclusive presence of plasmic fibers in this muscle correlates with the energy demands of its continuous cyclic contraction.

H6 muscle

The plasmic (pink) fibers of the H6 muscle were seen to contract rhythmically during regular gill ventilation. It has been demonstrated that these contractions raise the CHMs of the buccal floor and alternate with contractions of the H1 muscle which

depresses the CHMs (Gradwell 1968). Chapter 2, Fig. 4A shows that the normal vertical oscillations of the CHMs are correlated with buccal hydrostatic pressures. The fibrillic (white) fibers of the H6 muscle do not contract during this activity nor during hyperinspiration.

During buccal levation, the medial buccal floor is at first lower anteriorly than posteriorly, but near the end of levation the medial parts of the buccal floor are nearly horizontal and almost in contact with the buccal roof. As only the anteriorly located plasmic fibers of the H6 muscle contract rhythmically, it is the anterior part of the buccal floor, supported by the CHMs and pars reuniens, that is preferentially raised, allowing displaced buccal water to flow caudad over the posterior buccal floor (supported by the HPs) and into the pharynx.

In the middle line, the posterior border of the H6 muscle is attached to the opecular lining by connective tissue. This attachment apparently has no mechanical value for normal buccal levation by the plasmic fibers since L1, Fig. 6 shows that buccal pressures are unaffected by complete cutting of this medial connective tissue. However, it is an important nervous and vascular route from the H6 muscle to the opercular lining (Chapter 1).

The fibrillic fibers are inactive during regular breathing but they twitch intermittently during natural or artificial irritation in the gill cavity. These twitches are correlated with powerful elevations of the buccal floor, and cause the large pressures of hyperexpiration (Fig. 7B to E). The contractions of the fibrillic fibers occur so rapidly that it is difficult to see their other effects, but they seem to assist in the closure of gill cleft 1 and also to pull forward the raphe of the H7 muscle, thus helping to keep open the opercular canal during simultaneous H7 contraction.

The continuous cyclic activity of the plasmic fibers is cogent reason for their rich vascular bed. The fibrillic fibers, on the other hand, are dormant for relatively long periods between twitches and therefore do not require such a rich blood supply. In addition, relative to the myofibrils, the greater amount of sarcoplasm in the plasmic fibers than in the fibrillic fibers, correlates with the continuous phasic activity of the plasmic fibers. The fibrillic fibers are structurally better adapted for occasional powerful twitches.

H7 muscle

The H7 muscle contracts even more intermittently than the fibrillic fibers of the H6 muscle. Accordingly, the H7 muscle itself consists entirely of fibrillic fibers. Therefore its occasional contractions are nevertheless powerful enough to cause vigorous constriction of the gill cavity and the consequent expulsion of branchial water through the spout. When these contractions occur, they are always synchronized with activity of the H6 fibrillic fibers (see below).

Hyoidean interaction

As the H6 fibrillic fibers do not contract rhythmically, it was necessary to excite them experimentally, but this also evoked responses from other muscles and calls for study of the interactions within the hyoidean mechanism.

Aside from the less reliable stimulation provided by addition of suspended carmine to the oral inflow, it was possible to evoke greater ventilation amplitudes by direct mechanical stimulation of the opercular lining. The one end of a fine wire (diameter, 0.18 mm) was inserted into the gill cavity through the continuously open spout. This end was touched against the opercular lining by gentle manual manipulations of the other end.

The response to this stimulation was variable and complex and apparently not unlike the behavior of conscious tadpoles while flushing debris from the ventilation system:

(a) Consecutive hyperinspirations occurred without participation of the H6 fibrillic fibers (Fig. 7A). This response was effected by greater than normal activity of the H1 muscle.

(b) Hyperexpirations occurred and were caused mainly by the H6 fibrillic fibers and were without accompanying or intermittent hyperinspirations. This response was often preceded by a brief pause of the entire ventilation apparatus, during which hydrostatic pressures in the system were brought to the reference level of the baseline (Fig. 7B).

(c) Hyperinspirations occurred, interspersed with hyperexpirations (H6 fibrillic fibers active). When a hyperinspiration was followed by a hyperexpiration, the buccal floor, formed largely by the CHMs, underwent maximal dorsoventral excursion, causing the greatest positive and negative pressures in the buccal cavity (Fig. 7C), and indirectly in the pharynx and gill cavity.

(d) When stimulation was intense, consecutive hyperexpirations were caused by the H6 fibrillic fibers (Fig. 7D), but H7 contractions were sometimes superimposed on the H6 activity. Consecutive hyperexpirations were also sometimes markedly different in amplitude and noticeably different in timing (Fig. 7E).

(e) Less frequently, intense stimulation caused the mouth to open during the time that it usually closes. Water under pressure of the rising buccal floor (H6 fibrillic fibers active) was therefore expelled through the mouth and not via the more resistant pharynx and gill cavity.

During hyperexpirations the effect of the H6 fibrillic fibers was enhanced by more vigorous contractions of the H6 plasmic fibers. Facultative contraction of the H7 muscle occurred during opercular stimulation, and like the H6 fibrillic fibers, the H7 muscle was easily fatigued by repeated activity. Determination of the precise timing of the H7 muscle was difficult by direct observation. The branchial constriction that it caused, appeared to be superimposed on hyperexpiration, but some evidence was found of partial branchial constriction asynchronous with hyperexpiration. H6 fibrillic activity independent of H7 contraction, or combined with it, seems to reinforce the closure of the first gill cleft, thereby preventing reflux of branchial water directly into the buccal cavity.

Further information on the functioning of the hyoidean mechanism was obtained by mechanically stimulating the operculum after the H6 fibrillic fibers were cut close to their bilateral insertions on the CHLs. Hyperexpirations still occurred but were less vigorous than in the intact system (Fig. 8). Branchial constriction by the H7 muscle was also still possible. The failure of these lesions to abolish the typically greater amplitude of buccal levation during hyperexpiration demonstrates the capacity of the H6 plasmic fibers for particularly strong contractions, thus compensating for the loss of H6 fibrillic fiber function. Several such contractions resulted from a stimulation of the operculum, and they were superimposed on the phasic contractions of regular breathing. The trains of activity were followed by ventilation pauses of 5 to 10 sec when the mechanism had been repeatedly activated. The pauses may therefore have been due to the fatigue produced by such muscular exertion.

After the medial connective tissue joining the H6 and H7 muscles was severed, mechanical opercular stimulation never caused H7 contraction. Neither did contraction of the H6 fibrillic fibers then occur. The lack of response from the H7 muscle may be explained by the lesion to its motor nerve supply in the medial connective tissue, but as the motor innervation of the H6 muscle was still intact, the absence of response of its fibrillic fibers to opercular stimulation requires further investigation. It seems that although the medial connective tissue contains motor nerves to the H7 muscle, it may not be the route (or the only route) of sensory nerves from the opercular lining, as the H6 plasmic fibers still responded to mechanical opercular stimulation.

The bullfrog tadpole has an exhalent aperture from the gill cavity only on the left side. A transverse opercular canal is important for the flow of water from the right side of the gill cavity into the left side and then out via the spout. The H6 fibrillic fibers are partly concerned with helping to keep the transverse opercular canal open when the H7 muscle contracts. The forward pull on the medial connective tissue probably also provides support for the H7 muscle (especially its deep component) when it contracts. In the breathing tadpole, no occlusion of the opercular canal was seen during branchial expulsions caused by the combined contraction of the H6 fibrillic fibers and the H7 muscle. However, as the gill cavity became empty at the end of branchial constriction, there was a transient loss of opercular distension, including the area covering the opercular canal. One or two ventilation cycles later, the operculum regained its normal, inflated appearance.

Sporadic branchial constrictions would seem to be a useful mechanism in nature for the expulsion of suspended debris and perhaps parasites in the oral intake that escape the pharyngeal filters and enter the gill cavity. The usually strong, continuous branchial outflow from the permanently open, non-valvular spout, probably hinders the entrance of foreign material into the gill cavity via the spout.

Motor denervation

Bilateral denervation of the H1 and H2 muscles in breathing tadpoles greatly reduced but did not eliminate rhythmic buccal depression. The S2 muscles as well as the elastic recoil of the buccal floor after levation, were responsible for the persisting cyclic depressions of the buccal floor.

Cutting the ramus jugularis facialis on the right side of the H6 muscle paralyzed the plasmic fibers on this side of the muscle and reduced the amplitude of the buccal and pharyngeal pressures, but the lesion did not paralyze rhythmic contractions of the contralateral plasmic fibers (L2, Fig. 6). Contraction of these plasmic fibers deflected the median raphe toward the side of active innervation because the lesion had eliminated the synchronized, rhythmic tensions produced in both sides of the H6 muscle. Bilateral denervation (L3, Fig. 6) paralyzed both moieties of the H6 muscle and almost abolished buccal and pharyngeal pressures; the persisting small pressures were caused by certain other visceral muscles (p.118). Cutting these nerves sometimes evoked convulsive sinking of the buccal floor (hyperinspiration), depending on the depth of anesthesia.

The absence of participation of the H6 fibrillic fibers in normal buccal levation (produced by the H6 plasmic fibers) was demonstrated by severing them at their bilateral insertions on the

CHLs: normal buccal pressures were unaffected. In tadpoles whose muscle fibers were uninjured, bilateral denervation of the H6 muscle abolished responses of the fibrillic fibers of this muscle to opercular stimulation.

Electrical stimulation (direct, intercellular)

Bilateral electrical stimulation of the H1 muscles caused maximal depression of the buccal floor if the mouth was allowed to open. However, bilateral stimulation applied independently to the H2 muscles caused only a slight depression of the buccal floor irrespective of the functional condition of the mouth. Such stimulation applied after the H1 muscle of one side was removed to expose the CHL-quadrate joint, was seen to tilt the CHL backward.

Stimulation of the H6 plasmic fibers evoked a twitch to single shocks and rapid twitches to repetitive stimulation below 30/sec. Buccal floor levation resulted from the twitches thus produced. Repetitive stimulation exceeding 30/sec (the fusion frequency) caused tetanus of the plasmic fibers, which could be sustained for over 1 min. It was not possible to produce tetanus in the H6 fibrillic fibers by direct electrical stimulation. Instead, twitches occurred as a rapid vibration at high frequencies of stimulation (30-60/sec). The vibration did not cease suddenly but faded toward the end of the response to repetitive stimulation of longer than ca. 1 min (identical voltage and interelectrode distance were used as for the plasmic fibers). Therefore the fibrillic fibers of the H6 muscle fatigue much sooner than its plasmic fibers, as was also shown by Kuffler and Williams (1953) for fast versus slow muscle in adult frogs, and similarly by Andersen et al. (1963) in the hagfish.

Stimulation of the H7 muscle after the medial connective tissue between the H6 and H7 muscles had been cut, caused partial occlusion of the transverse opercular canal, whereas this effect was far less apparent when the medial connective tissue was left intact, and direct shocks applied simultaneously to the H6 fibrillic fibers and the H7 muscle.

B. Electromyography

The small size and difficult accessibility of the H2 and H7 muscles prevented electromyographic recording from them in the present study.

When the apparent functional difference between the plasmic and fibrillic fibers of the H6 muscle was investigated by electromyography, it was evident that while rhythmic electrical discharges occurred in the plasmic fibers, the fibrillic fibers were electrically inactive during normal gill ventilation (Fig. 9). This result was repeatedly confirmed in different tadpoles.

The timing and effect of the H6 plasmic fibers on buccal hydrostatic pressure is shown in Fig. 10A. Buccal pressure begins to rise 10-20 msec after the onset of electrical activity in the plasmic fibers (at 70-90 ventilation cycle/min). There is a small further increase in buccal pressure after electrical activity in the muscle has apparently ceased. However, in some tadpoles the electrical discharge was sustained throughout the period of increasing buccal pressure (Fig. 10B). It seems that this discrepancy is related to the particular location of the electrode among the plasmic fibers, for it is possible that, within the active period of the muscle, some muscle fibers contract at different times than others.

Figure 7C shows the synchrony between the contralateral moieties of the H6 muscle which, it has been noted (Chapter 1), is bilaterally innervated, and consists of two muscles mutually joined by connective tissue in the middle line.

The timing of the H6 plasmic fibers relative to the antagonistic H1 plasmic fibers, is shown in Fig. 10B, while Fig. 10D shows that the timing relationship between these muscles is the same in conscious tadpoles as in anesthetized animals. The phasic relationship of these two muscles (from Fig. 10B) is 25 mm/33 mm or 0.76 (scale = 0 to 1.0, where 0 and 1.0 are in phase and 0.76 is antiphase).

3. Hyomandibular interaction

The efficiency of the buccal pump depends on a close coordination of it with mouth movements. The interaction between the mandibular and hyoidean arches has been adequately described in anesthetized tadpoles (Gradwell 1968). In addition, the timing that has been advanced for the jaw and hyoidean movements relative to buccal pressures (Gradwell and Pasztor 1968) has been confirmed in the present study by dual, synchronized video recording while buccal pressures were simultaneously monitored from the same tadpoles. It now requires only a few observations to be commented on for conscious tadpoles.

During inspiration, the anterior parts of the CHMs are much more depressed than the hind parts, which remain near the buccal roof. Permitting the anterior CHMs this greater freedom is the opening of the mouth, for if the mouth is held closed in breathing tadpoles, it constrains to a large extent, the sinking of the buccal floor, even though the H1 muscles are still rhythmically active.

Tadpoles whose CHMs were manually held against the buccal roof, could not protrude their jaws but were able to open them only by a downward rotation of the cartilagines Meckeli. During this type of mouth opening the H4 muscle was normally active but the H5 muscle quivered under the strain of trying to effect the normal slight jaw protrusion which characterizes the wider opening of the mouth. Under these conditions the mandibulo-suprarostrale ligaments were scarcely active in opening the upper jaw. Instead, the slight opening of the upper jaw seemed to be caused by its elastic recoil upon relaxation of the jaw adductor muscles.

In tadpoles whose CHMs were manually held in a fully depressed position, the jaws could close rhythmically, but with difficulty. When the mouth was held in a wide open condition, the CHMs retained a large amplitude of rhythmic movement but they could not quite reach the buccal roof.

These manual operations constrained the mandibular and hyoidean cartilages from their normal displacements during regular breathing by causing excessive tension in the general connective tissue interconnecting the mandibular and hyoidean arches.

The insertion of the S1 muscle on the cartilago rostrale inferior and the position of this muscle immediately beneath the CHMs warrants its consideration in the context of hyomandibular interaction. No electromyographic evidence is available for the S1 muscle, but it would seem that a slight contraction of it at the onset of expiration would help buccal levation and also assist in pulling the ventral velum free from the buccal roof, but a strong contraction at this stage would probably hinder lower jaw adduction.

It is difficult to see contraction of the S1 muscle during dorsoventral oscillations of the buccal floor and it is therefore still questionable if the S1 muscle contributes to the rhythmic opening of the lower jaw, but contraction of this muscle was clearly seen during the hyperinspirations which sometimes interrupt apnoeia in tadpoles allowed to recover consciousness. On these occasions, the effect of the S1 muscle was to open the lower jaw (the jaw abductor muscles H3, H4, and H5 had been denervated) and to pull the HPs anteroventrad. Such contraction of the S1 muscle during normal hyperinspiration, when the CHMs sink suddenly, would help to distribute the force of the CHMs to the HPs, thus ensuring concerted sinking of the elements forming the buccal floor. Bilateral S1 denervation showed that it is not indispensable for jaw opening, but jaw protrusion during hyperinspiration then appeared less efficient.

4. Branchial mechanism

The branchial skeleton (Chapter 1, Fig. 5) consists of the HPs and the CBs. Bilaterally, the CBs 2, 3, and 4 have more flexible junctions with the HPs than that afforded by the continuity of the hyaline cartilage between the CB 1 and the HP (Fig. 2). These flexible junctions are important regions of articulation, while the HP-CB 1 junction provides an elastic recoil to assist the posterolaterad displacement of the CBs during the normal ventilation cycle. Bending also occurs along the median symphysis between the HPs and at the junctions between the HPs and CHMs. The least amount of bending occurs between the HPs and copula 2 (Fig. 2).

The pharynx and gill cavity lie on the dorsal and ventral sides respectively of the CBs. The pharynx certainly undergoes changes of volume during the movements of the CBs which constitute

the pharyngeal force pump. However, appreciable changes in volume of the sac-like gill cavity occur only during occasional branchial constriction by the H7 muscle in the lining of the operculum.

The cyclic displacements of the CBs during normal breathing consist primarily of an anteromesiad stroke which alternates with a returning posterolaterad stroke. Both these displacements are parallel to the B7 muscle. However, there are subsidiary movements of the CBs that also influence water flow in the ventilation system.

Anteromesiad stroke

A forward bending of the CBs toward the middle line is powered chiefly by the B7 muscle whose contraction is synchronized with that of the H6 muscle (Figs. 10A, 11). The medial aspects of the CBs are bent somewhat dorsad, but the lateral aspects undergo an excursion slightly ventrad.

There are two subsidiary displacements of the CBs that occur simultaneously with the anteromesiad movement of the CBs. One displacement is the forward bending of the medial CBs 1 and 2 by contraction of the B3 and B4 muscles. This movement tends to open gill cleft 1 connecting the buccal cavity directly to the gill cavity. The other subsidiary displacement is the mesiad movement of the lateral aspects of the CBs by the B11 muscle which is therefore antagonistic to the B1, B5, B8, and B10 muscles.

These three displacements of the anteromesiad stroke are bilaterally synchronized with one another and with buccal levation (Fig. 12). They result in the gills, which are borne on the ventral sides of the CBs, being swept anteromesiad through the water in the gill cavity. An important consequence of the anteromesiad stroke is its preparatory orientation of the branchial

apparatus for the action of the pharyngeal pump during the following (return) stroke of the CBs.

Posterolaterad stroke

Immediately after the CBs have reached their most forward position, a backward and laterad return begins, and this overall displacement is also bilaterally synchronized (Fig. 12). The gills are again swept through the water in the gill cavity, but this time in a posterolateral direction.

The elastic recoil of CB 1 and 2 after release of the forces drawing them forward, is one agent of the posterolaterad stroke. The backward component is effected by contraction of the S2 muscles (Fig. 13), while the somewhat subsidiary laterad component is produced by the activity of the B1, B5, B8, and B10 muscles (antagonists of B11). The B1 muscle also simultaneously helps in the closure of gill cleft 1 and in the opening of gill cleft 2. The activity of these branchial muscles, which are slightly convex when relaxed, was apparent from the shortening and flattening of their muscle fibers during contraction.

5. Hyobranchial interaction

In lightly anesthetized, breathing tadpoles, the movements of the branchial apparatus are largely inhibited if the crista hyoidea of copula 2 is manually held in a fully elevated or fully depressed position.

In pithed tadpoles, manual levation of the CHMs caused a slight anteromesiad bending of the CBs, especially when the displacement of the CHMs caused the copula 2, HPs, and the spicula to contact the buccal roof. This passive anteromesiad movement of the CBs was greatly reduced if the B7 muscles were severed. Apparently, the B7 muscle is able to simulate a

ligament by coupling the crista hyoidea to the processus branchialis, which permits the crista hyoidea to pull the CBs anteromesiad during buccal levation. In breathing tadpoles, this effect is accentuated by the superimposed contraction of the B7 muscle. Simultaneously, the B3 and B4 muscles contract and draw the processus posterior hyalis and the medial CBs 1 and 2 closer together, which tends to open gill cleft 1. By these contractions the slight, passive anteromesiad stroke of the CBs that accompanies buccal levation, is accentuated.

Alternatively, the manual depression of the CHMs and crista hyoidea (with the B7 muscles intact) caused a slight posterolateral movement of the CBs in pithed tadpoles. This movement of the CBs was hardly affected if the B7 muscles were severed before the manual depression of the CHMs and crista hyoidea. Electromyography and motor denervation in breathing tadpoles, and direct electrical stimulation of muscles in pithed tadpoles, disclosed that this hyobranchial interaction is promoted by other muscles. These are the S2, B1, B5, B8, and B10, which all contract together.

The anteromesiad stroke contributes to the compression of the buccal cavity because it causes slight elevation of the HPs, through contraction of the B3, B4, and B7 muscles, which explains the persistence of buccal pressure oscillations after bilateral denervation of the H6 muscle (Fig. 6).

6. Sequence and integration of muscle activity

While the terminology proposed in Chapter 1 for the ventilatory muscles is retained in the present study, these muscles will also be divided into functional groups to facilitate the description of the sequence and integration of muscular activity. Inspiration and expiration, the two regularly alternating phases of the normal ventilation cycle, are each driven by a complement of muscles whose makeup overrides anatomical groupings.

During inspiration there occurs a simultaneous decompression of the buccal cavity and a compression of the pharynx, and vice versa during expiration. This mechanism is operated by antagonistic muscles which drive oscillatory pumping movements and cause water to flow continuously over the gills.

(a) Inspiratory muscles

Individual, rhythmically active muscles of normal inspiration have different effects, which are coordinated to facilitate inspiration.

(i) Mouth opening. The action of the H3, H4, and H5 muscles in opening the mouth has been considered earlier in the present thesis (p.98). The precise timing of the H4 muscle has not been definitely established, but visual observation of it in breathing tadpoles, and studies of the functional anatomy of tadpoles, suggest the timing shown in Fig. 13. This muscle seems to initiate lower jaw abduction at the peak of the buccal pressure curve. The ventral fibers of the H5 muscle appear to enhance the initial opening of the lower jaw. The lateral fibers, forming the bulk of this muscle, definitely contract later in inspiration and are in phase with depression of the CHMs by the H1 and H2 muscles. Functional anatomy has shown (p.114) that the wider opening of the jaws is possible only if the

CHMs are simultaneously depressed. The synchrony between the H5 lateral fibers and fibers of the H1 and H2 muscles is therefore important for the unimpeded opening of the jaws. Electromyography has shown (Fig. 5) that fibrillic fibers of the H3 muscle are active during hyperinspirations, when there is a convulsive sinking of the buccal floor. As jaw protrusion is only mechanically possible when the CHMs are depressed, it is important that this muscle has a timing like that of the buccal floor depressors. However, the presence in the H3 muscle of some plasmic fibers suggests that part of this muscle is also phasically active, probably during the slight jaw protrusion that accompanies rhythmic jaw abduction (p.99). Twitches of the S1 muscle were seen during hyperinspiration, but the small girth of this muscle, the passive displacement of it by lower jaw movements, and the dorsoventral oscillations of the buccal floor, hampered the arrival at definite conclusions. However, the presence in this muscle of plasmic fibers, is circumstantial evidence supporting the proposal (Gradwell 1968) that it is phasically active.

(ii) Buccal depression. The comparison between Fig. 10B and Chapter 2, Fig. 4A, shows that the main buccal depressor (H1) is active only after buccal depression has begun. The initial buccal depression therefore seems to be a passive movement, probably caused by elasticity of the hyobranchial apparatus. The H2 muscle was seen contracting in phase with the H1 muscle. The synchrony between the H1 and H2 muscles which lower the buccal floor, and the H3 and H5 muscles of wide jaw abduction, is necessary because the normal amplitude of buccal floor depression (at ca. 20°C) and also of convulsive buccal depression during hyperinspiration, are not mechanically possible if the jaws are held closed (p.113). The sinking of the buccal floor during mouth opening also permits water

to be drawn into the buccal cavity through the mouth. However, a small volume of water enters the buccal cavity through the nares and this seems to be adequate for ventilation below ca. 8 °C; the jaws are then hardly active, if at all.

(iii) Pharyngeal constriction. The pharynx is constricted by the B1, B5, B8, B10, and S2 muscles which, probably together with the B13 and B14 muscles, contract simultaneously at the peak of buccal pressure and are therefore synchronized with the jaw abductor, H4 (Fig. 13). The contraction of the pharyngeal constrictors at this time allows the resultant hydrostatic pressure to be superimposed on that which is transmitted to the pharynx from the buccal cavity during expiration (Fig. 14B). The work of the pharyngeal constrictors is therefore less than that needed to increase the hydrostatic pressure of the pharynx from an ambient level to the level suitable to permit water to traverse the gill clefts 2, 3, and 4, and enter the gill cavity. It would seem that the backward pull exerted on the HPs by mainly the S2 muscles, would brace this element in preparation for possible contraction of the S1 muscle during regular breathing but especially during hyperinspiration.

(iv) Hyperinspiration. The plasmic fibers of the H1 muscle, which are cyclically active during normal inspiration, show greater electrical activity during hyperinspiration (Fig. 9). Stronger than normal contractions have also been observed in the H2 muscle during hyperinspiration, while this may possibly also be true of plasmic fibers in the H3 and S1 muscles. It is postulated here that the gill cleft constrictors, B2, B6, B9, B12, contract during hyperinspiration. Such action would prevent the transmission of large negative hydrostatic pressures from the pharynx and buccal cavity to the gill cavity. In this way the delicate gill cavity would be protected from a reflux of unfiltered ambient water through

the non-valvular spout. The simultaneous recording of hydrostatic pressures from the pharynx and gill cavity during hyperinspiration supports the postulated contraction of the gill cleft constrictors, because these recordings show that the large negative pharyngeal pressures do not reach the gill cavity (Fig. 16). However, electromyography and visual evidence of the contraction of these gill cleft constrictors is lacking. On the other hand, twitches of the S1 muscle were unmistakably seen during hyperinspirations, but their effect was too rapid for accurate interpretation.

(b) Expiratory muscles

As the expiration phase is shorter than the inspiration phase, visual interpretation of the coordination of the relevant individual muscles is difficult and electromyographic evidence is particularly important.

(i) Mouth closure. Judging from the shortness of the closing phase of the mouth relative to its opening phase (Gradwell 1968), it seems that the jaw adductors contract simultaneously, or nearly so. However, phasic electrical activity could not be detected in the M1, M6, and M7 muscles, which suggests that they are not active during rhythmic breathing. When the eye was lifted slightly above the floor of the orbit, small passive anteroposterior displacements of the M6 muscle were seen, but contractions of this muscle were not apparent during phasic ventilation. The almost complete lack of plasmic fibers in the muscles on the floor of the orbit (M1, M6, M7) suggests that these adductors may be more important for intermittent activities such as feeding and hyperventilation than for phasic ventilation. Contractions of the M2 muscle could also not be detected visually or electrically, although it was occasionally found to have some plasmic fibers. The M5 muscle, on the other hand, has the greatest proportion of plasmic to fibrillic fibers (usually 1:1) of all the jaw adductors. However, this muscle is also the deepest in position and it was not possible in the present study to establish its functional relation to phasic jaw adduction.

(ii) Buccal levation. Lesion experiments (Fig. 6) have shown that the most important agent of the power stroke of the buccal force pump is the H6 muscle. Only its plasmic fibers are phasically active, and they are responsible for the rise in the hydrostatic pressure in the buccal cavity during expiration

(Figs. 9, 10AB). After bilateral denervation of the H6 muscle, rhythmic buccal compression still occurs; it is of smaller amplitude than before H6 denervation, but it shows the same phase difference as before (Fig. 6). These persisting buccal pressures depend on the activity of the B7 muscle. The normal synchrony between the H6 and B7 muscles (Figs. 10AB, 11) is in accordance with the simultaneous elevation of the anterior and posterior parts of the buccal floor. However, even when both these muscles are bilaterally denervated, small rhythmic elevations of the buccal floor, of still smaller amplitude and with the same normal phase difference, persist. These persisting movements are then abolished by bilateral denervation of the B3 and B4 muscles. It therefore seems that the H6, B3, B4, and B7 muscles are all synchronized to promote an even power stroke of the buccal force pump.

(iii) Pharyngeal expansion. The B3, B4, and B7 muscles, which assist buccal floor levation, also effect the anteromesiad stroke of the CBs that affords pharyngeal expansion. The B11 muscle also contracts during expiration but it was not possible to establish whether it is exactly synchronized with the muscles of buccal levation. In anesthetized, breathing tadpoles, the rhythmic contractions of the B11 muscle were clearly seen to be antagonistic to the pharyngeal constrictors, B1, B5, B8, and B10 muscles, and possibly also to the B13 and B14 muscles.

(iv) Hyperexpiration. The muscles which have been recorded active (Fig. 9) and those which are proposed to be active during hyperexpiration, are schematized in Fig. 13. The fibrillic fibers of the H6 and B7 muscles, which are inactive during phasic ventilation, contract during hyperexpiration, while the plasmic fibers of these muscles show stronger contractions than during phasic ventilation. Simultaneously, contraction of the H7 muscle apparently occurs

and seems to be accompanied by unusually strong contractions of the B3, B4, and B11 muscles. The jaw adductors, M1, M2, M5, M6, and M7 muscles, may possibly also contract at this time. An unusually strong elevation of the buccal floor, and expansion of the pharynx, results from the integrated activity of the above-mentioned muscles. Gill cleft 1 is apparently closed, and the great compression of the ventilation chambers expells water from the system via the spout, or less frequently, via the spout and the mouth.

7. Hydrostatic pressures, functional anatomy, and water flow

General description

The anatomy shown in Fig. 14A and the records of Fig. 14B will be used as a basis for the present description of ventilation in the bullfrog tadpole. However, reference will also be made to an earlier study on bullfrog tadpoles from Carolina, USA (Gradwell and Pasztor 1968). The present investigation of larger tadpoles of the same species from Quebec, includes details that, together with earlier studies, now permits a better understanding of water flow during gill ventilation in the bullfrog tadpole.

Acclimated tadpoles were studied at 14°C, which is a close approximation to ambient water temperature in the natural habitat of Quebec tadpoles during most of the year. Experimental animals were anesthetized for 25 to 30 min in 1% urethane at 14°C and after the implantation and connection of the pressure cannulae, recordings were made from upright tadpoles allowed to recover consciousness in stream water of identical temperature. Figure 15B shows the condition of the ventilation pressures 3 to 5 min before the first sign of consciousness (feeble tail undulations).

At 14°C the graphs of buccal pressure (BU.P), pharyngeal pressure (PH.P), and branchial pressure (BR.P), are regular and slow enough to facilitate visual interpretation of the sequence of mechanical events during the typical ventilation cycle. In addition, dual video recording of mouth and buccal floor movements simultaneously with pressure recording has permitted the precise correlation of these breathing phenomena.

A variety of indicators (e.g. carmine, India ink, algae, and milk) have all shown that oral and nasal inflow is intermittent whereas the spout outflow is continuous. However, indicators pipetted into the inflows do not begin emerging from the spout

until several ventilation cycles later. It usually takes a few minutes for the pharyngeal filters to become saturated with suspended particles before particulate matter begins emerging from the spout in the respiratory outflow. As such loading of the filters was considered too great an interference with the natural ventilation current, the continuous spout outflow was also demonstrated in tadpoles without the use of indicators.

Streamers were made from freshly coagulated frog blood and were held at the inflow and outflow apertures of conscious, unrestrained tadpoles under normal ambient conditions. The intermittent character of the oral and nasal inflows was confirmed. The spout outflow was found to be continuous but less effusive during the expiration phase of ventilation.

Video recording showed that the mouth opens at the peak of the BU.P graph, and closes when BU.P rises from the horizontal baseline of zero (ambient) pressure (Fig. 14B). The buccal floor movements were found to confirm the recording in Chapter 2, Fig. 4A.

The dual peaks of the PH.P graph in Fig. 14B are evidence for the existence of two alternating force pumps in front of the gill clefts. At 14^{o}C the buccal pump is a more important driving force of the ventilation stream than is the pharyngeal pump, but at temperatures below about 10^{o}C, the pharyngeal pump assumes a larger share of ventilation (Fig. 15). The BR.P is kept positive by the alternate action of the buccal and pharyngeal pumps. Although the BR.P has the same phase difference as the BU.P and PH.P graphs, the BR.P lags behind the BU.P graph. Moreover, the BR.P peaks are much lower than the BU.P and PH.P peaks, which indicates that there is considerable resistance to the ventilation

current in the pharynx and gill cavity. On the other hand, there is negligible resistance to the flow from the buccal cavity into the pharynx (Fig. 14A), as the amplitude of the transmitted BU.P peak in the pharynx is not noticeably lower than the amplitude of the BU.P peak itself.

The ventilation cycle

A) Inspiration

There is a smooth continuation of the ventilation rhythm from the end of expiration to the beginning of the next phase, which is that of inspiration. Inspiration begins at the peak of the BU.P graph, which then shows a progressive decline throughout this phase. However, the PH.P in Fig. 14B shows that inspiration has two components.

1. The mouth opens but water does not enter through it nor through the nares at this time, when BU.P is greater than the ambient pressure. Water would be expected to flow out of these apertures were it not for the fact that at this stage, the buccal floor is elevated, the narial valves are closed, and the buccal water has already been pumped into the pharynx. There is a transverse fold of mucosa on the buccal roof, which may possibly also hinder reflux through the mouth. Moreover, the buccal cavity begins expanding passively at the onset of inspiration and causes a steep fall in the BU.P. A small reflux occurs from the gill cavity into the buccal cavity through gill clefts 1, just before they close.

During the first third of inspiration, the PH.P shows a slight fall before a second rise (Fig. 14B). In small tadpoles at 22°C, this slight fall in pressure is absent before the second rise, which immediately reinforces the BU.P that is transmitted to the pharynx (Gradwell and Pasztor 1968). In the larger Quebec tadpoles

at 14°C, the buccal pump is still more important than the pharyngeal pump, for producing a positive pressure in the ventilation system. This is shown by the greater height of the first peak than the second peak of PH.P in Fig. 14B. Figure 15 shows, at 8° and 10°C, the BU.P and PH.P of the same tadpole used for Fig. 14B. The second PH.P peak shows increasing prominence relative to BU.P, as the temperature falls. This confirms the postulate of Gradwell and Pasztor (1968) that such a temperature contingency exists.

At the onset of inspiration, the BR.P begins to rise and continues to do so throughout inspiration. This is therefore the phase of greatest flow from the spout and, together with the results of observations of streamers, contradicts all previous publications, which propose a stronger spout outflow during expiration.

2. The second component of inspiration occupies the next two thirds of this phase. The second third of inspiration is passive expansion of the buccal cavity, causing the inflow of water through the mouth and nares. Relaxation of the pharynx also occurs at this time and its pressure begins falling, but PH.P is still positive relative to BU.P and the ventral velum therefore remains closed while water continues to enter the gill cavity from the pharynx.

During the last third of inspiration, there is a simultaneous wider opening of the mouth and an active depression of the buccal floor. The wide mouth opening and the large buccal volume at this stage tend to dampen the fall of BU.P, and its value tends to level at equilibrium with the ambient pressure. PH.P continues to fall but as it is still positive relative to BU.P, the ventral velum remains shut. Water continues to flow from the gill cavity.

B)Expiration

With respect to water flow, the expiration phase may also be analyzed into two components which, like inspiration, are coordinated to effect changes in pressure.

1. Buccal pressure begins to rise at the instant of rapid mouth closure and simultaneous elevation of the buccal floor. The valves of the internal nares are closed by the increasing BU.P relative to ambient pressure. Opening of the valvular ventral velum occurs at the moment that the BU.P becomes positive in respect to the PH.P. The buccal cavity and pharynx therefore become one confluent chamber during expiration, but only the buccal cavity is compressed during this phase. The first buccal water flowing over the ventral velum (Fig. 14A) helps to expand the pharynx after its constriction during inspiration, and water does not flow through gill clefts 2, 3, and 4 at this early stage in expiration. Neither does a flow through gill cleft 1 occur at this stage. The absence of water flow into the gill cavity during early expiration causes a decline in BR.P and water flows from the spout less effusively than during inspiration.

2. Immediately after the entrance of buccal water into the pharynx, the gill clefts 2, 3, and 4 are opened widely by the greater PH.P relative to BR.P. Immediately thereafter, or at the same time, a strong flow gushes into the gill cavity directly from the buccal cavity through gill cleft 1. This water through cleft 1 therefore by-passes the pharynx; it impinges on the anterior surfaces of the first gill and also on the membrana vasculosa opercularis. Toward the end of expiration the gill clefts 2, 3, and 4 appear to close, but some pharyngeal water still passes through them. Moreover, the apparent closure of these clefts is so transient that this event does not stop

branchial outflow. The great flexibility of the operculum also tends to offset sudden changes in BR.P and branchial outflow.

Discussion

The present study has permitted a re-examination of earlier findings (Gradwell 1968, Gradwell and Pasztor 1968) and further experimentation has yielded details which have increased the understanding of the musculoskeletal mechanism of the bullfrog tadpole. Salient points of this contribution, and discrepancies in the relevant literature now require discussion.

Jaw mechanism

The jaws, with their two degrees of freedom, ligamentous interactions, 12 pairs of muscles, elasticity, and variable modes of function, are undoubtedly the most complicated mechanisms in the gill ventilation systems of anuran tadpoles.

The present study has definitively correlated the jaw movements of Rana catesbeiana with buccal pressures by means of video recording. Electromyography has shown that the H3 muscle has muscle fibers which are active during the jaw protrusion of hyperinspiration but not during phasic ventilation. It has not been possible to record electrical activity from several of the jaw muscles, either because they are inactive during phasic ventilation or because of the technical difficulties of smallness and inaccessibility. A muscle particularly calling for further study is the S1, whose function has been somewhat clarified by the observation that it contracts during hyperinspiration, but phasic activity and the true effect(s) of this muscle are remaining problems for investigation.

Sedra (1950) used dissection and microtomy on cadavers of Bufo regularis. He interpreted the jaws as possessing only one degree of freedom at the cartilago Meckeli-quadrate joint. Examination of the functional anatomy of this species in the present study showed that like Rana catesbeiana, Bufo regularis has two degrees of freedom at the cartilago Meckeli-quadrate joint.

It also has a ligament interconnecting the upper and lower jaws, but Sedra overlooked the ligamentous interaction between the jaws and therefore drew attention only to the elastic component of upper jaw opening. Furthermore, as Sedra regarded the jaw abductors, H3, H4, and H5, as participating in jaw closure (see Gradwell 1968, pp. 1046, 1047), he was left with only the S1 muscle to propose as an abductor of the lower jaw. Sedra's morphological data are a useful contribution but although the muscular anatomy of Bufo regularis differs slightly from that of Rana catesbeiana, the jaw mechanism of regularis seems to be like that of catesbeiana and therefore Sedra's functional account calls for re-examination.

According to de Jongh (1968, p. 76) for tadpoles of Rana temporaria: "The respiratory and feeding movements are essentially similar". This statement is true for R. catesbeiana only when the animal is not actively feeding by means of its dental apparatus, but is ventilating its gills phasically with water which has been filtered of particulate matter (e.g. algae) in the pharynx. A small volume of unfiltered water also enters the gill cavity directly from the buccal cavity through gill cleft 1. In this regular normal ventilation, involuntary microphagous feeding and gill ventilation occur simultaneously and the jaw mechanism described in the present study for phasic ventilation also serves for incidental and involuntary filter feeding in which the dental apparatus plays no masticatory role. However, R. catesbeiana and several species of American Rana tadpoles have been seen to feed intermittently by mastication of food (Gradwell, unpublished). This is a voluntary behavior where tadpoles swim in search of food and they utilize their crescentic beaks and labial denticles for masticating such food as algae growing on substrata, plant

leaves, and animal tissues. This food is then probably filtered in the pharynx by mechanisms similar to that of involuntary feeding. The jaw and respiratory mechanisms are more vigorous during this type of feeding than during phasic ventilation. In view of its close anatomical affinities with R. catesbeiana, R. temporaria probably also has an involuntary method of filter feeding during which the dental apparatus is not active in mastication.

In the laboratory, the masticatory feeding described by de Jongh, occurs frequently soon after cooked spinach is placed in aquaria. It is incidental that during masticatory feeding, water that enters the mouth and is probably needed for food conveyance to the pharynx, is also passed over the gills after filtration. Some postulates have been advanced concerning masticatory feeding (Gradwell, 1968), and enough observations of such feeding in conscious, uninjured tadpoles were made in the present study to support the notion that masticatory feeding and involuntary feeding involve distinctly different jaw mechanisms. The views of Savage (1952, 1961) and Kenny (1969) on R. temporaria tadpoles and examinations of the anatomy of this species in the present study tend to support the belief that temporaria has a jaw mechanism similar to that of catesbeiana.

Two of de Jongh's hypotheses concerning the jaw mechanism are questionable:

(i) Unless appropriate peristaltic musculature can be shown to exist in the esophagus, it is doubtful whether tadpoles are capable of "swallowing"; the movement of food down the esophagus occurs at least to some extent by ciliary action.

(ii) The protrusion of the jaws as shown in de Jongh's Fig. 15A, requires recognition of two degrees of freedom at the cartilago

Meckeli-quadrate joint; de Jongh refers only to downward and upward rotation of the quadrate articular surface of the cartilago Meckeli, but he also mentions a "translation" (p. 80) without associating it with jaw protrusion.

In the light of the present research, a few discrepancies in an earlier publication (Gradwell 1968) call for discussion. Further study of the geniohyoideus (S1) muscle has given rise to some doubt regarding its proposed phasic activity. Some doubt now also exists concerning the proposed phasic activity of the suspensoriohyoideus (H3) muscle. Wide opening of the mouth can be produced by strong electrical stimulation of the S1 muscle, but this was not considered typical of the normal condition because it did not involve the simultaneous contraction of the other jaw abductors. The present study suggests that the S1 muscle assists jaw protrusion during hyperinspiration by acting together with the other jaw abductors. In view of muscular interactions in the normal animal, the electrical stimulation of individual muscles for the study of their functions, has limitations, and should not be relied upon too greatly without supporting evidence.

Passive displacements of the jaw adductors have been misinterpreted (Gradwell 1968) as contractions of these small, generally deep-lying muscles. Although the action of these muscles is to close the jaws, electromyography is needed to show definitively when they are active.

Hyoidean mechanism

Schulze (1892) and Kratochwill (1933) considered the hyoidean mechanism as a simple dorsoventral oscillation, and they correctly described the muscles performing this action. However, Sedra (1950) has incorrectly named the H1 and H2 muscles as the elevators of the buccal floor, and he has omitted mention of the H6 muscle. De Jongh (1968) has rightly described the phasic function of the H1 and H2 muscles and given an interesting account of the CH-quadrate joint of Rana temporaria, but he has also omitted discussion of the function of the other hyoidean muscles, the H6 and H7. In trying to explain irregularities of branchial outflow, Kenny (1969) has speculated that the H7 muscle is normally active in gill ventilation.

In the present study, it has been found that the CHs of Rana catesbeiana are movable mainly dorsoventrally, but there is a slight degree of backward tilting, and there are also two types of functionally and structurally different muscle fibers in the two chief hyoidean pumping muscles, the H1 and H6. These newly discovered facts have helped to elucidate the intermittent variations of CH amplitude which are manifested as hyperinspiration or hyperexpiration.

Hyperinspiration is effected by a sudden sinking of the CHMs. The phasically inactive H1 fibrillic fibers become active at this time and are accompanied by stronger than normal contractions of the plasmic fibers. This produces a large negative buccal pressure. If the mouth opens simultaneously, water is drawn through it into the buccal cavity, but air is evacuated from the lungs if the mouth closes at this time. On the other hand, the CHMs are elevated abnormally vigorously during hyperexpiration, thus causing an unusually large positive buccal pressure. If the mouth

is simultaneously closed, water is driven through the gill clefts and gill cavity with great force; if the mouth opens at this time, water is expelled through it. The phasically inactive fibrillic fibers of the H6 muscle become active during hyperexpiration and they are augmented by abnormally vigorous contractions of the plasmic fibers (which are phasically active). The H7 muscle often also participates in hyperexpirations to cause a superimposed branchial constriction (first discovered by Schulze 1892). However, contrary to Kenny's proposal (1969), the H7 muscle is not phasically active; it shows rapid fatigue and it has no plasmic fibers.

In a correlation between the H1 muscle and CH movement (Gradwell 1968, Fig. 7B), this muscle is correctly shown as depressing the CHM. However, the recording equipment was apparently insufficiently sensitive to monitor the passive depression of the CHM by elasticity of the hyobranchial apparatus at the onset of inspiration. The present study has shown that the H1 muscle contracts late in the inspiration phase, just before the onset of expiration.

Branchial mechanism

The movements of the branchial apparatus proposed by Schulze (1892) for Pelobates, and by Kratochwill (1933) for Rana dalmatina (= R. agilis), are essentially similar to those of R. catesbeiana.

Kenny's description (1969) of the movements of the CBs concerns food filtration in the pharynx and the pumping of water through tadpoles in general. As the branchial apparatus lies on the ventral side of the CBs, Kenny's proposals have a bearing on the branchial mechanism and warrant consideration in the present discussion, particularly since he has extended his findings on Phyllomedusa to include Rana tadpoles.

(i) Kenny proposes that the "branchial levators III and IV" (B8, B10) contract during mouth adduction. Video recording has shown that this is certainly not the case in Rana catesbeiana, where the mouth is decidedly open during simultaneous contraction of the B1, B5, B8, and B10 muscles.

(ii) Kenny's "gill chamber musculature" is the H7 muscle, which he states is active cyclically (p. 237): "Superimposed on this cycle, is the asynchronous contraction of the gill chamber musculature ..." It seems likely that Kenny was observing occasional branchial constriction, elicited by the experimental introduction of "carmine or other particles" into the ventilation current. However, he has interpreted this response as a component of normal cyclic ventilation, which certainly it is not. This discrepancy points to the advantage of using alternative methods for following the respiratory stream, for example the colored streamers which have been used in the present study.

(iii) As the edge of the dorsal velum is deflected away from the ventral velum during the power stroke of the buccal pump, it is unlikely that the dorsal and ventral vela serve to filter food

by their juxtaposition in Rana. Kenny's findings in this regard may, however, be valid for Phyllomedusa, of which living tadpoles were unavailable during the present study.

The small muscles of the branchial apparatus, and the concealed location of most of them, present considerable technical difficulties for their electromyographic study. Yet this is at present the most desirable means of demonstrating the time dependence of the activity of these muscles and so elucidating the details of the branchial mechanism. The functioning of the gill cleft constrictors, B2, B6, B9, and B12, are particularly difficult to study because they are small and invested with delicate blood vessels. These muscles seem to contract during hyperinspiration, but it is also feasible that they may contract during hyperexpiration. Therefore this problem is another that awaits the sophistication of experimental technique.

Sequence and integration of muscle activity

The time dependence of muscles which have been proven or are believed to be active during normal gill ventilation and/or during hyperventilation, are summarized in Fig. 13. Several of the muscles still require electromyographic investigation, and the summary is therefore offered as a working hypothesis pending further research. It has been shown in the present study that certain muscles have two functionally and structurally different types of muscle fiber. It is therefore important in future studies, to take cognizance of the possibility that some parts of a muscle may contract independently of other parts of the muscle and that, in addition to the temporal difference, there may also be a difference in the effects produced by segregated components of a muscle.

The integration of muscle activity to produce specific behavioral effects is, in part, a consequence of their individual timing or sequence of contraction. However, the ability of single muscles to influence the effects of others is important in concerted behaviors such as feeding and gill ventilation. The understanding of interactions between individual muscles or groups of muscles inevitably requires a thorough knowledge of the relevant morphology. This concerns not only the muscles themselves, but also their skeletal relationships and their associations with soft tissues such as ligaments.

Hydrostatic pressures, functional anatomy, and water flow

Regarding hydrostatic pressures, the present research is consistent with an earlier study (Gradwell and Pasztor 1968). However, two misinterpretations have been found. Firstly, the occurrence of the second pharyngeal pressure peak immediately after the first peak, is clear evidence that constriction of the pharynx occurs early and not late in the inspiration phase of the ventilation cycle. Secondly, the rise in the branchial pressure graph during inspiration, corroborates evidence obtained with streamers held at the spout of slowly breathing tadpoles, that the branchial outflow is stronger during inspiration than during expiration.

Intermittent variations of hydrostatic pressure of abnormally large amplitudes have been recorded in the present study simultaneously in the three chambers of the ventilation system. These variations are termed "hyperinspiration" (negative buccal and pharyngeal pressures) and "hyperexpiration" (positive pressures throughout the system).

The functional anatomy and water flow of normal and hyperventilation have been broadly studied partly with the object of isolating problems for solution during continuing investigations. Many problems have been found whose solutions are required for a better understanding of gill ventilation in anuran tadpoles. The most important of these problems now appear to be:

(i) The time dependence of several muscles (see Fig. 14) needs to be established.

(ii) The functioning of all the muscles of gill ventilation needs further investigation, including the hyoidean group, which has so far been better studied than the others. Especially perplexing at present, are the B2, B6, and B9 muscles. Schulze (1892) believed

that when these muscles contract, they constrict the afferent branchial arteries and so help with the circulation of blood through the gills. As these muscles are so intimately invested with blood vessels, this proposal deserves close study.

(iii) Chapter 2 of the present thesis considers the subject of velar function, which is so important for an understanding of water flow. The precise route followed by water in the pharynx is a problem of greater concern to filtration feeding than to gill ventilation.

(iv) Numerous mucosal papillae that project from the lining of the buccal cavity into its lumen, call for a functional explanation. Preliminary experiments (Gradwell, unpublished) indicate that they are either flow receptors or mechanoreceptors for detection of large particles in the oral intake.

(v) A fine network of blood capillaries in the pharyngeal filter folds is reported in Chapter 1 of the present thesis. This vascular bed seems consistent with the view (Calori 1842, Götte 1875, and Weisz 1945) that the rugulose pharyngeal mucosa contributes to respiratory gas exchange. If this is so, the water from the pharynx reaching the gills and membrana vasculosa operculari through gill clefts 2, 3, and 4, may not be so efficient for ventilation in the gill cavity. In this case the importance of gill cleft 1, bringing fresh water to the gill cavity directly from the relatively poorly vascularized buccal cavity, is emphasized. Stained water flowing through gill cleft 1 was seen to impinge on the gill tufts of branchial arch I and was deflected by them against the membrana vasculosa operculari where the capillary density is relatively high (250-350 meshes/mm^2, Gradwell 1969b). Therefore the provision of a pharyngeal by-pass in the form of gill cleft 1 would seem to be an advantage for branchial

ventilation. Quantitative data are now needed to test this hypothesis.

(vi) The production of hyperventilation and branchial constriction by mechanical stimulation of the inner surface of the operculum is always successful in rested, normally breathing tadpoles, but mechanical stimulation of the gills themselves is less effective in producing these responses. However, there is great variation and adaptation of these responses to branchial stimulation. In all cases when the responses were elicited, the fibrillic fibers of some muscles were brought into action, whereas during phasic ventilation they are inactive. The further investigation of hyperventilation may yield important data on the functioning of the fibrillic fibers. The fibrillic fibers of the H6 muscle have several structural and functional properties in common with conventional fast contracting muscle fibers of vertebrates (Gradwell and Walcott 1970). In this regard, the fibrillic fibers of other muscles still need investigation. Intracellular recordings from fibrillic fibers and measurements of their tensions would seem to be the next stage of research pointed to by the functional studies of the present thesis.

(vii) In much of the present research, for easier observation of breathing movements, tadpoles have been studied at lower ambient temperatures than those of previous studies (Gradwell 1968, Gradwell and Pasztor 1968). The relative importance, one to the other, of the buccal and pharyngeal pumps has been shown to change with temperature. Further research is needed to establish whether other temperature dependent functions of gill ventilation exist.

The solution of the above-listed problems in the bullfrog tadpole will undoubtedly increase the understanding of gill ventilation in this species. However, useful and perhaps more important information relevant to this subject may also be gathered by comparative studies of gill ventilation in other species of anuran tadpoles (see Chapters 4, 5, and 6 of the present thesis).

Acknowledgment

I am exceedingly grateful to Dr. W.J. Karlé, McGill Instructional Communications Center, for his generous technical assistance during video recording.

References

Andersen, P., Jansen, J.K.S., and Løyning, Y. 1963. Slow and fast muscle fibres in the atlantic hagfish (Myxine glutinosa) Acta Physiol. Scand. 57: 167-179.

Calori, A. 1842. Descriptio anatom. branchiarum maxime internarum gyrini Ranae esculentae etc. Novi comment. Acad. Scient. Instituti Bononiensis. 5: 111. Cited from Kenny (1969).

De Beer, G.R. 1937. The development of the vertebrate skull. Oxford Univ. Press. Lond. and New York.

De Jongh, H.J. 1968. Functional morphology of the jaw apparatus of larval and metamorphosing Rana temporaria. Neth. J. Zool. 18: 1-103.

Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16: 183-190.

Götte, A. 1875. Die Entwicklungsgeschichte der Unke (Bombinator igneus) als Grundlage einer vergleichenden Morphologie der Wirbelthiere. Leipzig. Cited from Kenny (1969).

Gradwell, N. 1968. The jaw and hyoidean mechanism of the bullfrog tadpole during aqueous ventilation. Can. J. Zool. 46: 1041-1052.

---- 1969a. The function of the internal nares of the bullfrog tadpole. Herpetologica 25: 120-121.

---- 1969b. The respiratory importance of vascularization of the tadpole operculum in Rana catesbeiana Shaw. Can. J. Zool. 47: 1239-1243.

Gradwell, N., and V.M. Pasztor. 1968. Hydrostatic pressures during normal ventilation in the bullfrog tadpole. Ibid. 46: 1169-1174.

Gradwell, N., and B. Walcott. 1970. Dual functional and structural properties of the interhyoideus muscle of the bullfrog tadpole (Rana catesbeiana). J. Exp. Zool. In the press.

Kenny, J.S. 1969. Feeding mechanisms in anuran larvae. J. Zool. Lond. 157: 225-246.

Kratochwill, K. 1933. Zur Morphologie und Physiologie der Nahrungsaufnahme der Froschlarven. Z. Wiss. Zool. 164: 421-468.

Kuffler, S.W., and Vaughan Williams, E.M. 1953. Properties of the slow skeletal muscle fibers of the frog. J. Physiol. 121: 318-340.

Savage, R.M. 1952. Ecological, physiological and anatomical observations on some species of anuran tadpoles. Proc. Zool. Soc. Lond. 122: 467-514.

---- 1961. The ecology and life history of the common frog. Pitman and Sons, Ltd., Lond. 221 pp.

Schulze, F.E. 1888. Über die inneren Kiemen der Batrachierlarven. I. Mitteilung: Über das Epithel der Lippen, der Mund-, Rachen und Kiemenhöhle erwachsener Larven von Pelobates fuscus. Abhandl. Königlichen Akad. Wiss. Berlin. 32: 715-768.

---- 1892. Idem. II. Skelet, Muskulatur, Blutgefässe, Filterapparat, respiratorische Anhänge und Atmungsbewegungen erwachsener Larven von Pelobates fuscus. Ibid. 13: 1-66.

Sedra, S.N. 1950. The metamorphosis of the jaws and their muscles in the toad, Bufo regularis, Reuss correlated with the changes in the animal's feeding habits. Proc. Zool. Soc. Lond. 120: 405-449.

Tchernavin, V.V. 1948. On the mechanical working of the head of bony fishes. Proc. Zool. Soc. Lond. 118:129-143.

Weisz, P.B. 1945. The development and morphology of the larva of the South African toad, Xenopus laevis. J. Morphol. 77: 163-217.

Willem, V. 1920. Observations sur la respiration des Amphibiens. Deuxième note. Bull. Acad. r. Belg. Cl. Sci. Series 5. 6:339-347.

Illustrations

Fig. 1. Rana catesbeiana shown in profile, resting on the bottom of an aquarium. The only visible movements are those of the mouth, and the dorsoventral oscillations of the buccal floor. The inflows and outflow of ventilatory water are shown by the arrows.

Fig. 2. Semidiagramatic view of water flow relative to the larval visceral skeleton of Rana. Immediately on entering the mouth and nares, the inflows diverge into left and right streams, but intermixing of the outflowing water occurs just before it leaves through the sinistral spout. In addition to the three gill clefts between the ceratobranchialia (CB), there is a first gill cleft between the ceratohyale (CH) and CB 1. Broken lines indicate water flow dorsal to the skeleton. Zones of particular bending of the skeleton are between the : (i) CB and the hypobranchial plates (HP), (ii) bilateral moieties of the HP, (iii) copula 2 (C2) and CH, (iv) CH and HP, (v) CH and C2, and (vi) cartilagines Meckeli (CM) and infrarostral cartilages which lie between them, but there is little or no natural bending between the bilateral infrarostrals themselves.

Fig. 3. The jaw skeleton shown in profile during stages of opening and protrusion of the jaws. CQL, ligamentum cornu quadratum laterale; CRI, cartilago rostrale inferior; CRS, c.r. superior; MC, cartilago Meckeli; MS, ligamentum mandibulo-suprarostrale; PAQ, processus articularis quadrati; RSQ, ligamentum rostrale superior quadrati; TC, trabeculae cranii. See Ch 1, Table I for H3, H4, and H5 muscles.

Fig. 4. Pharyngeal pressures during increasing frequency of hyperinspiration on recovery from anesthesia. Each hyperinspiration is characterized by a large fall in pressure and by conspicuous protrusion of the jaws. Pressure calibration: + 1 cm water. Frequency: 70 cycle/min.

Fig. 5. Simultaneous recording of buccal pressures (top trace) and electrical activity in the H3 muscle. Only during the three hyperinspirations, shown by the greater troughs of the pressure graph, were electrical discharges monitored from the fibrillic fibers in the muscle. Pressure calibration: + 1 cm water. Frequency: 75 cycle/min.

Fig. 6. Simultaneous recording of buccal and pharyngeal pressures during experimental lesions. L1, medial connective tissue cut between the H6 muscle and the opercular lining; L2, dennervation on left side of H6 muscle; L3, bilateral dennervation of H6 muscle completed.

Fig. 7. Hydrostatic pressures during mechanical opercular stimulation (arrows) in semiconscious bullfrog tadpoles. A. Normal gill ventilation punctuated by hyperinspirations producing particularly large negative pressures. B. A single hyperexpiration (large pressure peaks) following a brief pause in the normal ventilation rhythm. C. Regular breathing is shown interrupted by hyperinspirations and hyperexpirations. D, E. Consecutive hyperexpirations caused by intense opercular stimulation. Pressures above the horizontal lines are positive. BR.P, branchial pressure; BU.P, buccal pressure; PH.P, pharyngeal pressure. Calibrations: 1 cm water; 1 sec.

Fig. 8. Hydrostatic pressures in the buccal cavity during hyperexpirations evoked by mechanical stimulation of the operculum (arrows). Only the plasmic fibers of the H6 muscle were active on account of bilateral lesion of the fibrillic fibers. Further details are given in the text. Calibrations: 1 cm water; 1 sec.

Fig. 9. Normal and hyperventilations recorded in the buccal cavity simultaneously with electromyography of (A) H6 plasmic fibers, (B) H6 fibrillic fibers, (C) H1 plasmic fibers, and (D) H1 fibrillic fibers. Pressure calibration: 1 cm water.

Fig. 10. A. Buccal pressure (BU.P) recorded simultaneously with the electromyography of the H6 plasmic fibers. B. Electromyography of the H6 and H1 plasmic fibers monitored simultaneously with buccal pressures C. Recording of electrical discharges from the left and right sides of the H6 plasmic fibers, showing that their contractions are exactly synchronized. D. Buccal pressures correlated with electromyography of the H6 and H1 plasmic fibers of a small, semiconscious tadpole (left), and of the same tadpole allowed to recover consciousness (right). Calibrations: time, 0.5 sec; pressure, 1 cm water; electromyography, 30 mv.

Fig. 11. Buccal pressures (BU.P) monitored simultaneously with electrical activity in the B7 and H5 plasmic fibers. Calibrations: pressure, 1 cm water; electromyograms, 35 mv; frequency, 75 cycle/min.

Fig. 12. Mechanography (force transducer, Gilson Polygraph) of the left and right branchial skeleton during light anesthesia. Upward deflection: anteromesiad stroke. Downward deflection: posterolaterad stroke. Frequency: 70 cycle/min.

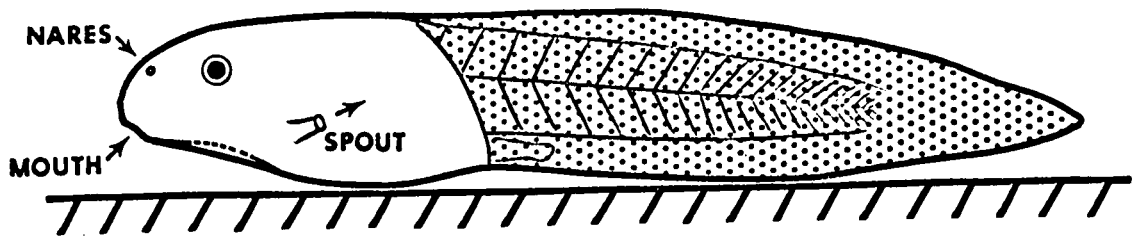
Fig. 13. Schematic summary of muscular activity during normal regular and hyperventilation. The blackened rectangles represent data acquired by electromyography; the blank rectangles represent data gathered in studies of functional anatomy and direct observations. HE, hyperexpiration; HI, hyperinspiration. Pressure calibration: 1 cm water.

Fig. 14. A. Composite diagram of sagittal and parasagittal sections through the head of a bullfrog tadpole. The anatomy is shown in its orientation at the onset of expiration. The large arrow indicates water which is beginning to flow over the ventral velum. BC, buccal cavity; D, dorsal velum; GC, gill cavity; PH, pharynx; V, ventral velum; 1 to 4, gill clefts 1 to 4.
B. Correlated hydrostatic pressures in the buccal cavity (BU.P), pharynx (PH.P), and gill cavity (BR.P) of a regularly breathing tadpole at 14°C.

Fig. 15. Simultaneous recording of buccal (BU.P) and pharyngeal (PH.P) pressures to show the greater importance of the pharyngeal pump at ambient temperatures below the acclimation temperature of 14°C. Calibrations: pressure, 1 cm water; time, 0.5 sec.

Fig. 16. Branchial (BR.P) and pharyngeal (PH.P) pressures monitored simultaneously during regular phasic ventilation which is intermittently interrupted by hyperinspirations. Although abnormally large negative pressures occur in the pharynx, the pressures in the gill cavity are simultaneously made abnormally positive. Frequency, 75 cycle/min. Pressure calibration: 1 cm water.

①


NARES
MOUTH
SPOUT

②

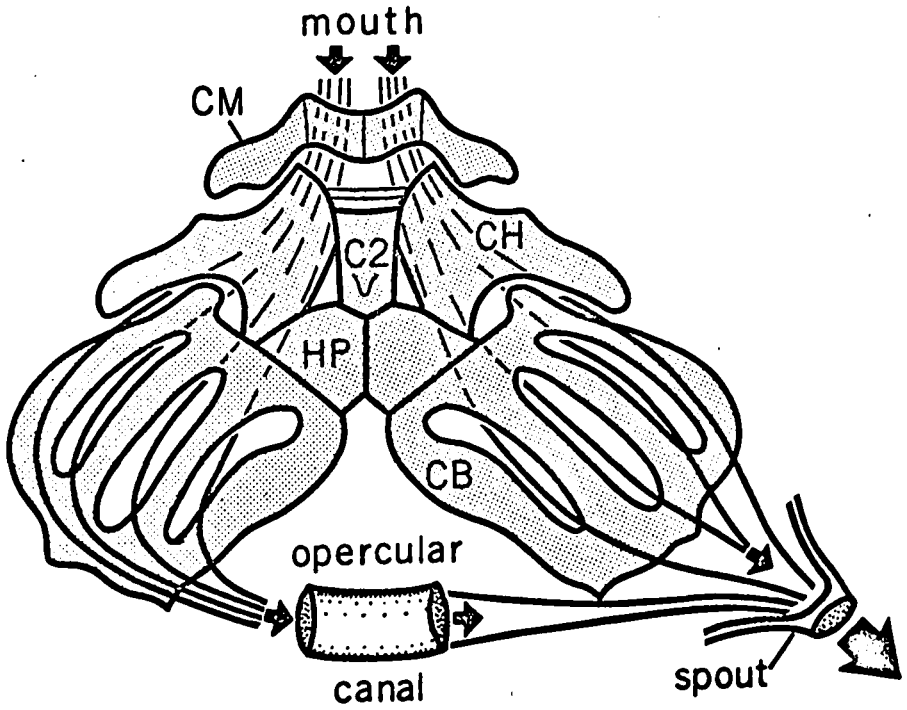
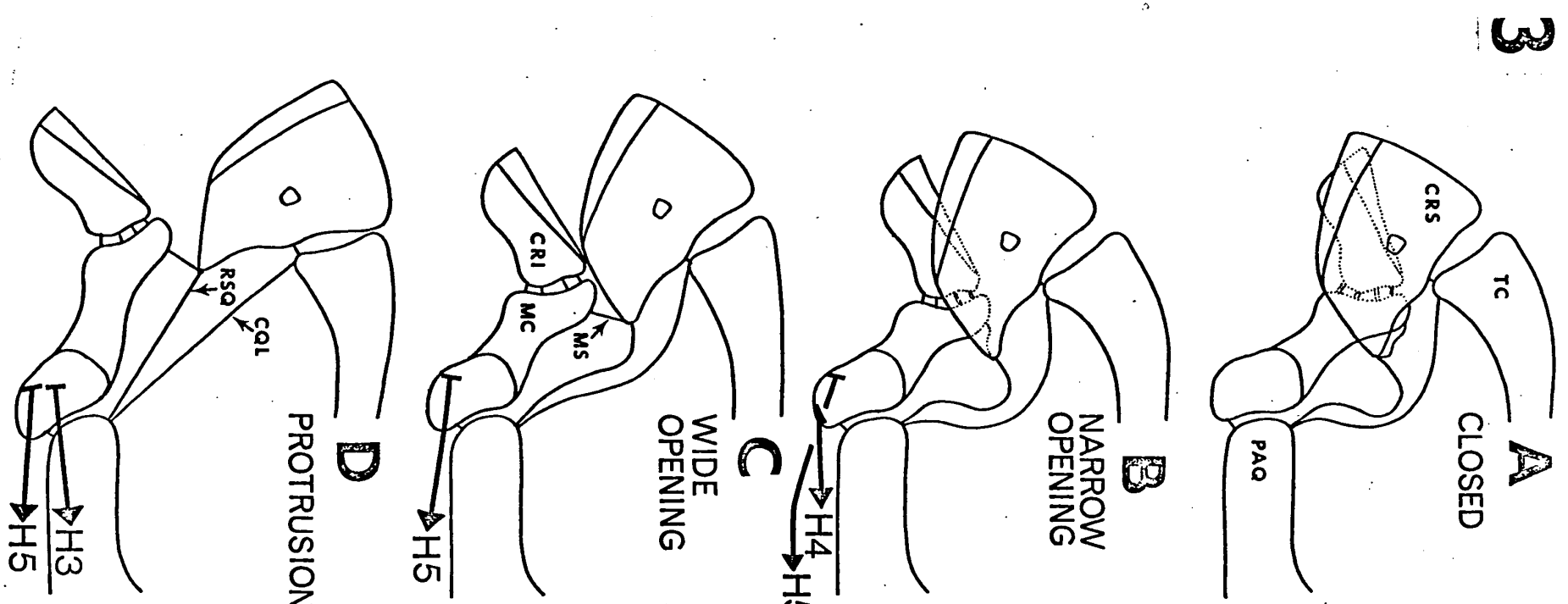
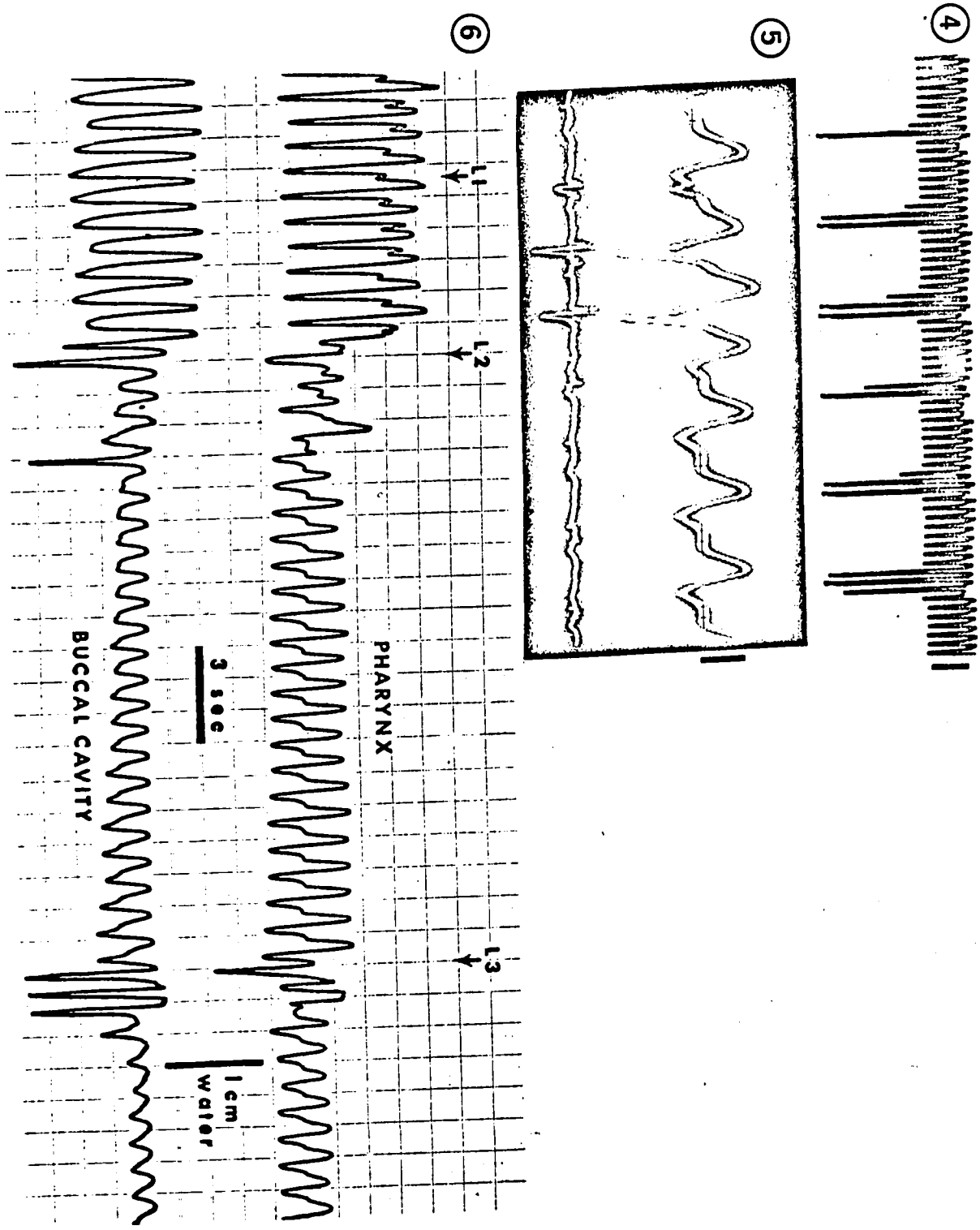
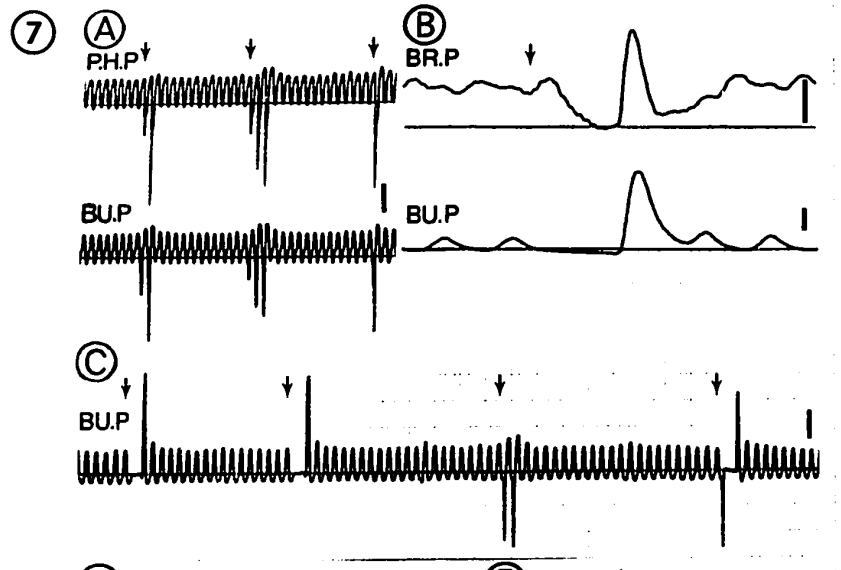

mouth
CM
C2
V
CH
HP
CB
opercular
canal
spout

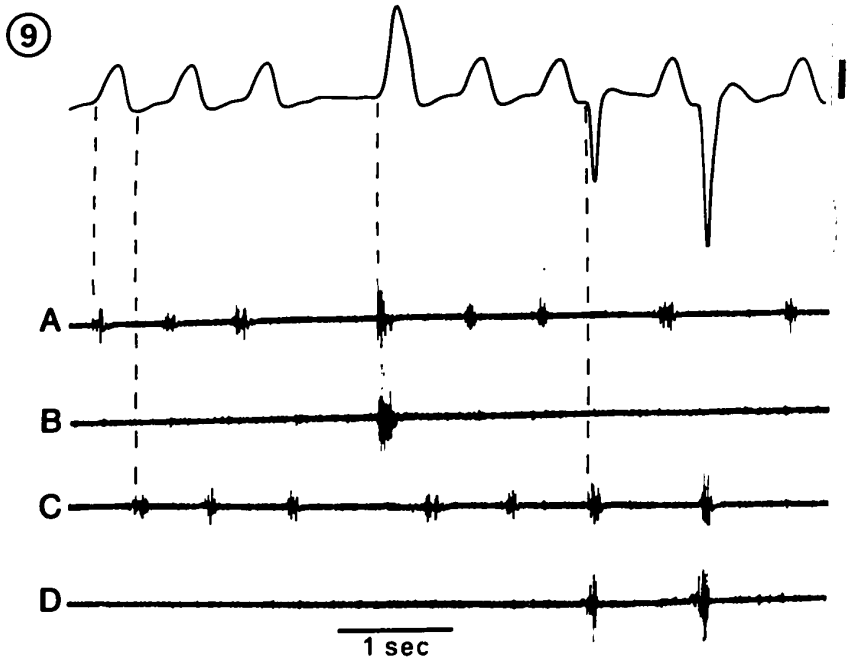
FIG. . N. Gradwell, 1102 Cottonwood Drive, Richland, Wash 99352. USA.
'Gill irrigation in Rana catesbeiana. II. Function.

Reduce this line to 19.7cm.



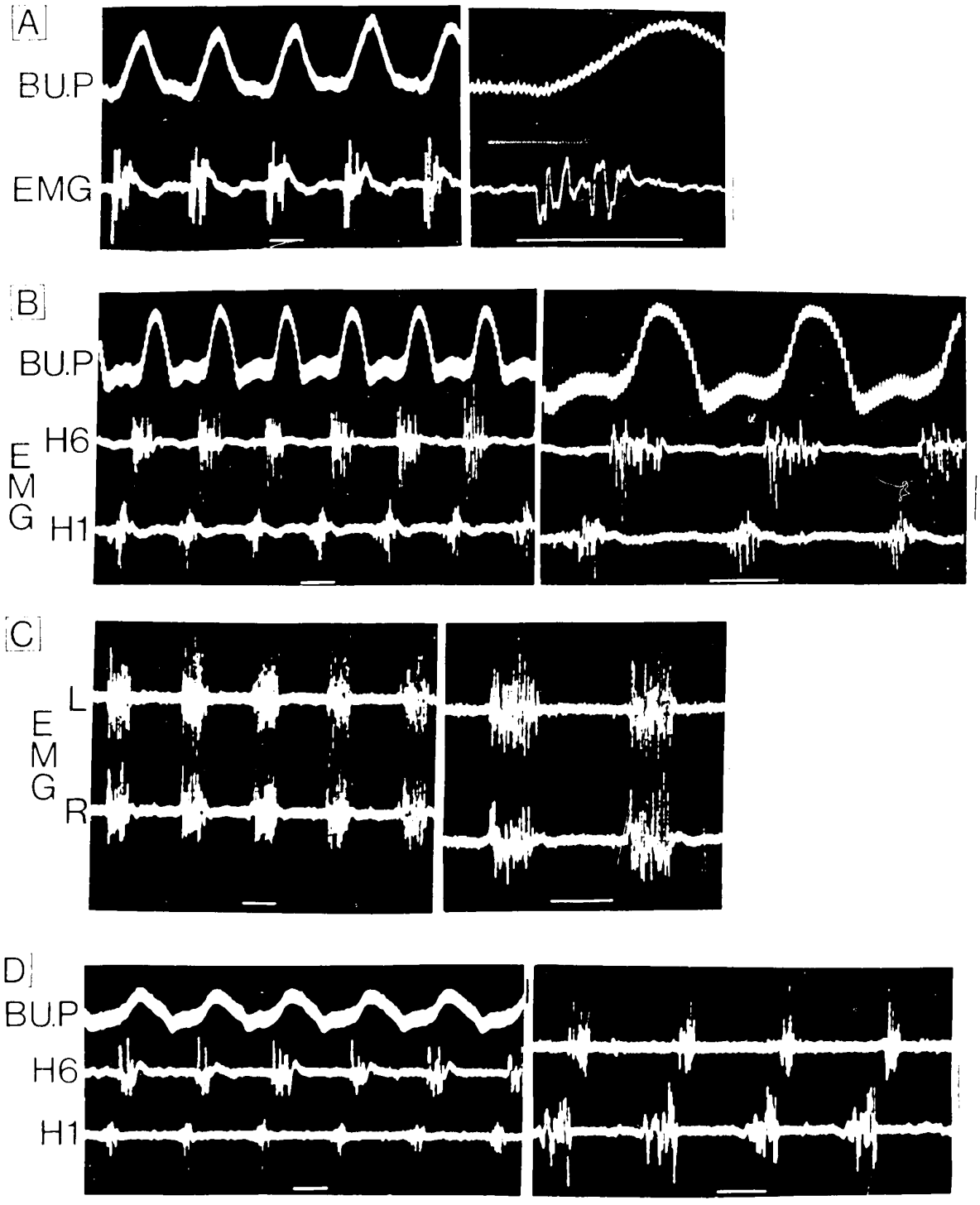

4
5
6
L1
L2
L3
PHARYNX
BUCCAL CAVITY
3 sec
1 cm water


⑦
Ⓐ
P.H.P
BU.P
Ⓑ
BR.P
BU.P
Ⓒ
BU.P
Ⓓ
BR.P
BU.P
Ⓔ
PH.P
BU.P

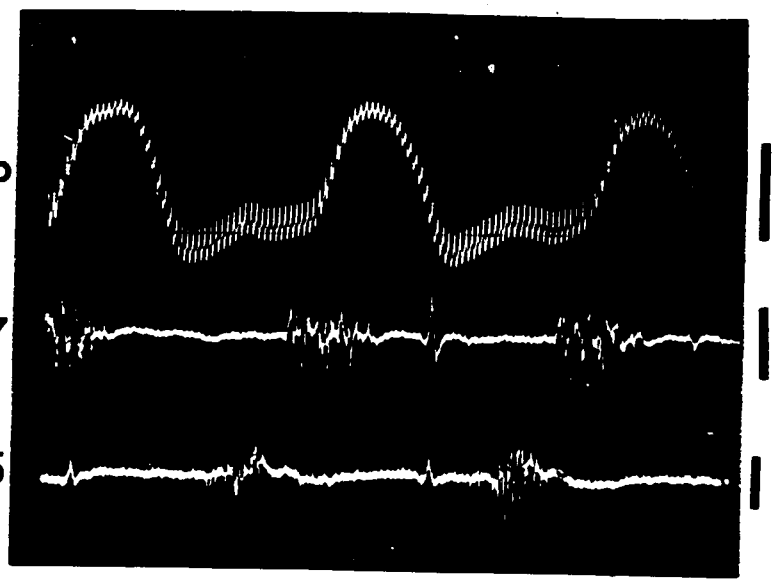

⑨
A
B
C
D
1 sec


+
-
⑧

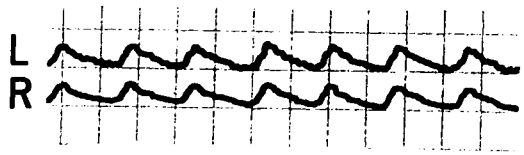
(10)



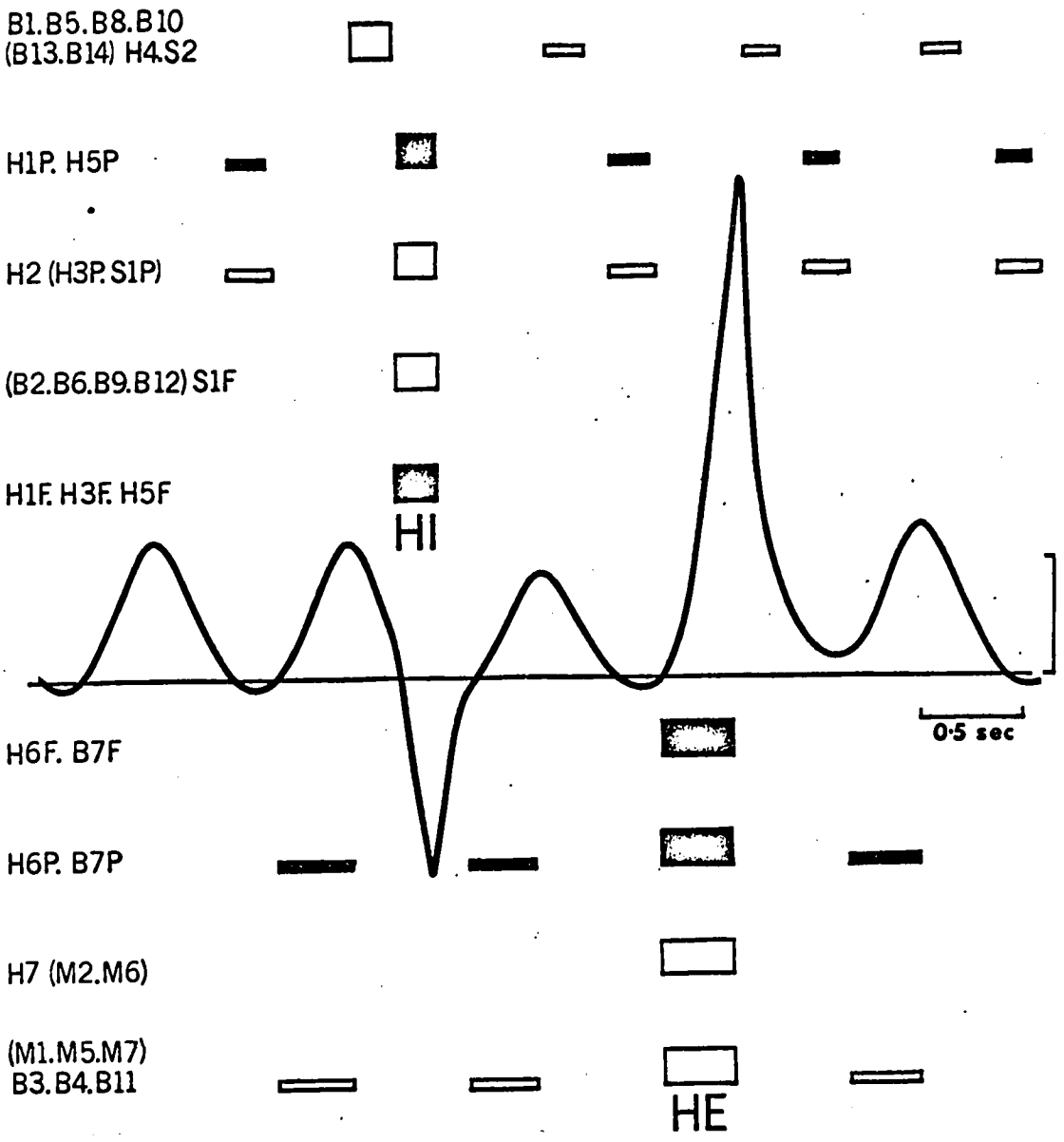
⑪



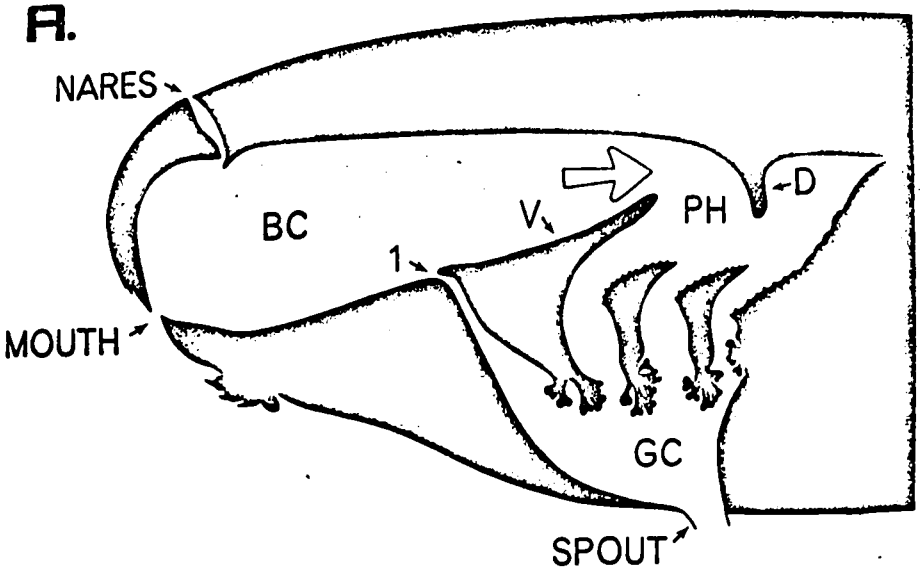
⑫

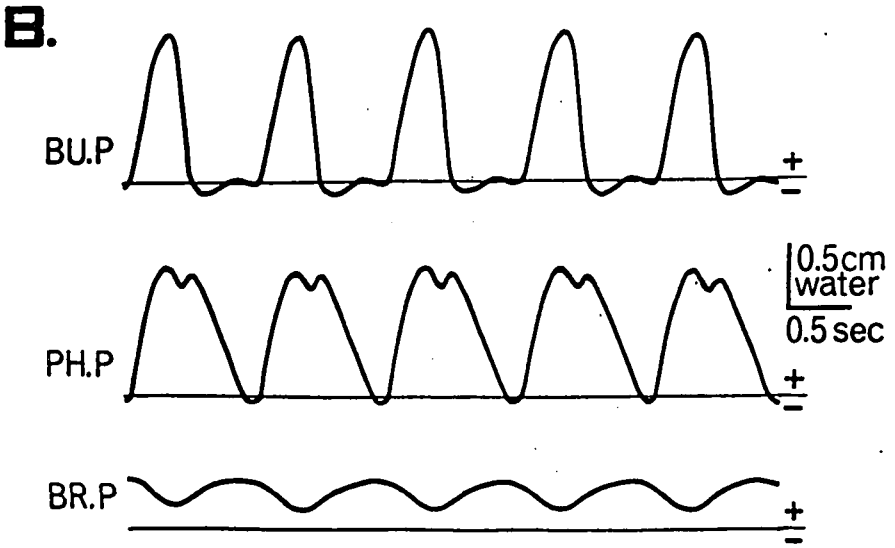


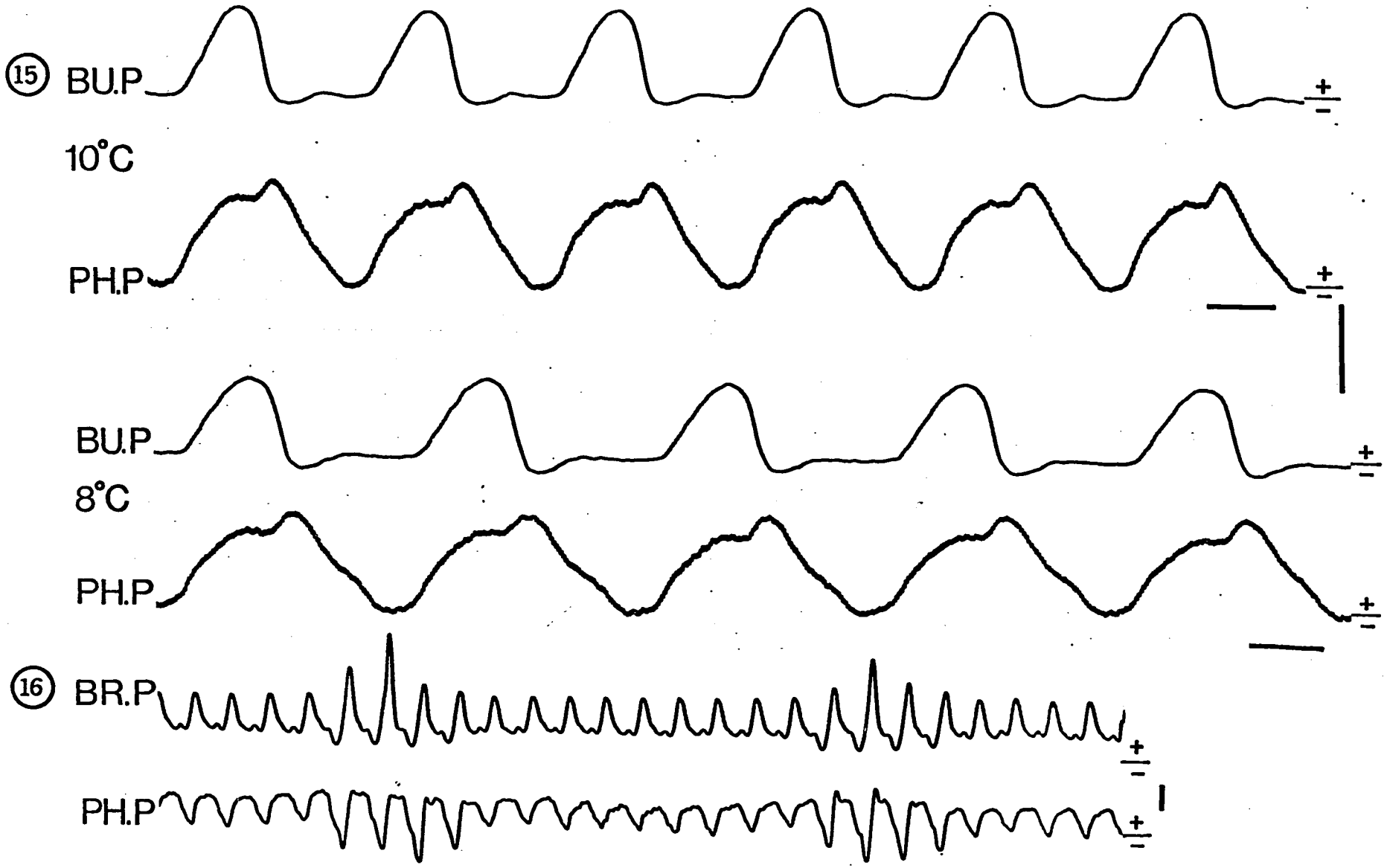
(13)


B1.B5.B8.B10
(B13.B14) H4.S2
H1P. H5P
H2 (H3P. S1P)
(B2.B6.B9.B12) S1F
H1F. H3F. H5F
HI
0·5 sec
H6F. B7F
H6P. B7P
H7 (M2.M6)
(M1.M5.M7)
B3.B4.B11
HE

(14)






⑮ BU.P
10°C
PH.P
BU.P
8°C
PH.P
⑯ BR.P
PH.P

CHAPTER 4

THE TADPOLE OF *ASCAPHUS*: EXPERIMENTS ON THE SUCTION AND GILL VENTILATION MECHANISMS

ABSTRACT. Sucker engagement is effected by application of the oral disc to a reasonably flat surface. Increased buccal pumping immediately begins, and the negative pressures of buccal inspiration are transmitted to the sucker through the open mouth. However, during expiration, the transmission of positive buccal pressures to the sucker is hindered by a membranous oral valve. Therefore a partial vacuum is progressively created between the sucker and the substratum. Buccal pumping and the oral valve enable ambient pressure to ensure and maintain sucker engagement. Adhesion of the sucker is immediately increased by greater buccal pumping when forces operate which tend to disengage the sucker.

There are two continuous, rhythmically alternating force pumps in front of the gill clefts. Habitual sucker attachment to the substratum restricts buccal inflow to the nares, and despite the intermittent nature of the narial inflow, the action of these pumps ensures a continuous flow of water over the gills. Tubular external nares facilitate their closure during spontaneous protrusion of the snout above water, but they do not completely close while submerged, nor does ventilation stop, even in the fastest tolerable current.

CONTENTS

Introduction

Ascaphus truei is America's most primitive anuran (35, 38). It occurs in the North-West United States and British Columbia (47). In addition to having an oral sucker armed with a distinctive dental apparatus, Ascaphus tadpoles have several anatomical features in common with urodeles (38). Notwithstanding the peculiarities of Ascaphus, except for scattered incidental remarks in the literature, no functional or experimental research has been published on the genus.

Several reports exist on the anatomical adaptations of tadpoles to a habitat in torrential mountain streams in North and Middle America, South Africa, and South-East Asia (18, 19; 1, 2; 9; 3; 20-23; 33-36; 4; 9; 46; 24-26; 44, 45; 43; 30, 31; 50; 7). Of these adaptations, ventral suctorial discs have been most often noted or described in the literature; it has been pointed out (3) that these discs should not be mistaken for the secretory adhesive organs which, when present in newly hatched anuran larvae, tend to disappear when a fold of the integument grows back from the hyoidean arch to form the tadpole's operculum. However, even in fishes, where there are such powerful suckers as that of Remora, there is a paucity of published findings on the functioning of the sucker. Reports on the suctorial mechanisms of anuran tadpoles have been chiefly observational or based on deductions of function from preserved material (e.g. 34, 4); they show the need for an experimental approach to the problem. Moreover, the little information that does exist, is confined to Staurois, an Asian genus, where the suctorial disc lies posterior to the mouth.

Most species of anuran tadpoles live in stagnant or sluggish water and need to ventilate their gills and sometimes a vascular

lining of the operculum as well, by continuous water pumping (42; 28; 39-41; 48; 6; 11-16; 27). However, Ascaphus tadpoles inhabit cold, well-oxygenated mountain streams and prefer location in a swift current, although they also occur in stagnant or semistagnant water (9, 30). The respiratory anatomy of Ascaphus nevertheless suggests that it ventilates its gills by pumping water through a pair of gill cavities; but Noble (33) reported that the external nares close in swift water. As these are the only inhalent apertures during sucker engagement, one might expect suspension of gill ventilation under such conditions. The present study was therefore undertaken to ascertain whether ventilation occurs in tadpoles anchored by their suckers in a torrential flow, or only when the animals are washed into quiet pools.

In many species of tadpoles inhabiting stagnant or sluggish water, lung ventilation is well developed. But there are nonfunctional lung rudiments in fully developed Ascaphus tadpoles (e.g. stage 39, of Gosner 1960). This is not surprising, as the mouth and nares of Ascaphus tadpoles are structurally incapable of air breathing and are adapted for other purposes. The tadpoles of torrential streams have greater need for the stability gained from compactness than for the buoyancy provided by lungs. The absence of lung breathing in Ascaphus tadpoles increases the importance of gill ventilation, but there exists no published information on the ventilation of its larval gills. Except for Hora (23), neither has this subject been reported on for anurans in Africa and Asia which also inhabit mountain streams.

In the present research, the publications of Pusey (38) and van Eeden (49) have been useful and should be referred to for details on the cranial anatomy of Ascaphus tadpoles.

Materials and Methods

Tadpoles of Ascaphus truei[1] (stage 32, of Gosner 1960) were placed in four Dewar flasks, each containing ten tadpoles in one liter of stream water. By occasional addition of ice during the flight to Montreal, the animals were kept at 8 to 11°C, which was the diurnal range at the collecting site, 1 to 3 Sept. 1969.

At McGill University, ca. 18 hr after the tadpoles had left the Snoqualmie River, they were placed in a 3 kiloliter spill-over aquarium with a fast inlet stream of dechlorinated tap water at 10°C. Immediately on liberation from the Dewar flasks, the tadpoles began swimming about and alighting and feeding on the algae growing on the walls of the glass and slate aquarium, by rasping movements of the horny beak and labial denticles; they grew ca. 1 mm in four weeks and relative to their counterparts in nature, they appeared normal in all obvious respects.

Hydrostatic pressures

Hydrostatic pressures associated with the suction and ventilation mechanisms were monitored in 20 tadpoles (Figs. 1, 2A). The snout-to-vent length of these animals was 10 ± 1 mm. Experiments were performed at 10°C except where otherwise indicated in the text and illustrations.

---

1 From Alpental Ski Resort, near Snoqualmie Pass, Washington. Collections were made along the Snoqualmie River from the start of Snow Lake Trail, near the foot of the ski lift of the Resort to a meadow ca. 1.5 km upstream.

Simultaneous pressure recordings from the buccal cavity, pharynx, and gill cavities were made with methods similar to those described earlier (15). However, for recording sucker pressures in moderately stagnant water, a hole of 1.5 mm diameter was made in the center of a Perspex disc of 5 mm thickness. The blunted point of a hypodermic needle was sealed into the hole to form a pressure monitor, and the other end of the needle was attached to a pressure transducer (Fig. 1). Concentric graduations on the Perspex disc permitted determination of the precise location of parts of the sucker relative to the pressure monitor.

Sucker pressures were monitored from tadpoles in a stream of water, with the apparatus shown in Fig. 2A. Smyth (43, p. 109) points out the need for data on the velocity of streams inhabited by such species as Ascaphus. A flowmeter was not available on the collecting trip of the present research but one was used in the laboratory to determine flow velocity. A water flow of 200 to 2,000 ml/min was passed through tubing of 1.5 mm internal radius (r). Therefore by calculation[1], the velocity of the water emerging from the tubing was 47 to 472 cm/sec. As Ascaphus tadpoles cannot attach themselves to submerged rubber foam, the sides of the container were lined with this material. Therefore when tadpoles became detached from the inclined Perspex plate (Fig. 2A), the only place where they could reattach themselves was the plate. They could then be easily returned to the recording position over the monitor of the pressure transducer by manual sliding, without disengaging the sucker.

---

[1] $\Pi(r)^2 = \Pi(0.15)^2 = 2.25\ \Pi \times 10^{-2} = 7.08 \times 10^{-2}\ cm^2$;

$$\frac{2 \times 10^2\ cm^3\ min^{-1}}{7.08 \times 10^{-2}\ cm^2} = 2830\ cm\ min^{-1} = 47.2\ cm\ sec^{-1}$$

The adhesive capability of the oral sucker, as well as the influence of this structure on buccal pressures, was investigated by gradually abducting a conscious tadpole from a pressure monitor embedded in Perspex (Fig. 1). The pressure monitor was orientated downward so that the tadpole was turned into a supine position, hanging from the horizontal Perspex. Loads were added to a scale pan attached to the post-cranial dorsum by a polyethylene loop through the dorsales trunci muscles. Sucker shapes were studied by allowing the animal to attach itself to the underside of horizontal Perspex, through which the sucker was photographed during progressive loading.

The signals from the pressure transducers were amplified and recorded graphically on a rectilinear Gilson CH-CBPP Polygraph. Pressures were calibrated manometrically (Fig. 1), and baseline reference pressures were determined after the experiments by anesthetizing the tadpoles until ventilation ceased.

Cannulation

Intramedic polyethylene tubing (PE 10, Clay-Adams Inc., N.Y.) was used for implantation in the buccal cavity and pharynx of tadpoles anesthetized ca. 30 min in 1% urethane at 10°C. The one end of the tubing was flared near a heated iron. The other end was fitted to a steel needle and shaved to form a tight, tapered sleeve. The needle with its attached tubing was passed through the tadpole's mouth and then through the buccal roof until the flange of the tubing formed a flush seal inside the buccal cavity. After detachment of the needle, the free end of the tubing was passed over the hypodermic needle of a pressure transducer (Fig. 1). Hydrostatic pressure curves from cannulae implanted posteriorly in the buccal cavity near the ventral velum, were of

identical shape and phase difference to those from cannulae farther forward. The region of the buccal cavity between the nares was therefore chosen for implantation in most cases on account of its easier accessibility and the absence in this region of muscles and large nerves and blood vessels.

Implantation in the pharynx of Ascaphus tadpoles was more difficult than in Rana catesbeiana (cf. 15) because of the smallness of the Ascaphus specimens and greater inaccessibility of the pharynx.

The small branchial outlet of Ascaphus prevented cannulation of the gill cavities with the available materials. Branchial pressures were therefore monitored by inserting the hypodermic needle (30-gauge, BD Yale) of a pressure transducer into the gill cavities through the valveless branchial outlet. The branchial orifice was widened with a small cut to compensate for the slight plugging effect of the needle.

Water velocity

A water tunnel (Fig. 2B) was used to determine the maximum velocity of turbulent water, as occurs in natural streams, which could be tolerated by the sucker without its slipping. The water velocity at which the flow changes from streamlined to turbulent in the tunnel is estimated by assuming this change to take place when Reynold's number has the value 2000 for a round pipe having the same cross-sectional area and carrying the same flow as the actual tunnel.

Let V_R = water velocity in circular pipe at which Reynolds' number has the value 2000.

D = diameter of round pipe having the same cross-sectional area as the tunnel. Its value is calculated to be 2.26 cm.

μ = viscosity of water at the experimental temperature of 10°C. Its value is 1.31×10^{-2} g cm^{-1} sec^{-1}.

ρ = density of water, taken to be 1 g cm^{-3}.

From the definition of Reynolds' number for a pipe of circular cross-section, it follows that

$$V_R = \frac{2000\,\mu}{D} \quad \frac{2000 \times 1.31 \times 10^{-2} \text{ g cm}^{-1} \text{ sec}^{-1}}{2.26 \text{ cm} \times 1 \text{ g cm}^{-3}} = 11.6 \text{ cm sec}^{-1}$$

Thus the flow through the water tunnel is turbulent at water velocities greater than about 12 cm sec^{-1}.

Flow indicators

The nature of the inhalent and exhalent flows in tadpoles was studied with a dissecting microscope while flow indicators such as carmine, milk, and algal suspensions were pipetted near the mouth, external nares and branchial outlet. The flow through the exposed gill clefts of anesthetized tadpoles was also studied by this method.

Dental inscriptions

Impressions made on the substratum by the horny beak, were recorded directly on photographic film. The emulsion of uniformly exposed and developed 35 mm film was softened by a potassium hydroxide solution. After thorough rinsing in water, the film was attached to a wall of an aquarium. When tadpoles clung to the film they were manually displaced backward, forward and sideways. The backward displacement caused contact between the upper beak and the emulsion and inscriptions were thus produced which could be printed on photographic paper by normal procedures.

Results

To avoid reiteration, "pressure" will be used in its hydrostatic sense in this report.

The small size of the available Ascaphus tadpoles was discouraging for the measurement of pressures. However, considering the difficulty of manually detaching tadpoles from a substratum, it was expected that measurement of the suction pressure would be simple. The first attempt was gratifying, for, contrary to the expected constant negative pressure, the graph showed an oscillating negative pressure. Inspection of the animal revealed that the undulations were synchronized with pulsations of the skin covering the tadpole's pharynx. The data suggest that internal pressures caused by pumping movements of the ventilation apparatus are transmitted to the sucker cavity (Fig. 3B). In agreement with this view is the simultaneous recording in the same animal (Fig. 4) of buccal and sucker pressures, of identical amplitude and frequency during regular breathing. Therefore, it seems that sucker function is influenced by the buccal pump of gill ventilation. On the other hand, as efficient gill ventilation still occurs in tadpoles with their suckers disengaged, the sucker is not indispensable for gill ventilation. However, it will be shown that the functional state of the sucker affects the course of the ambient flow into the ventilation system. On account of this interdependence between the suction and ventilation mechanisms, the separate treatment of them in the present study is somewhat artificial, and is intended to facilitate description.

The anatomy of the visceral muscles of Ascaphus tadpoles has been described in adequate detail (38) to guide functional studies. The positions of these muscles are summarized in Table I, which

also proposes a simplified nomenclature based on the innervation and origin-insertion relationships. Until verified by electromyography, the functions of certain relevant muscles are presented tentatively in Table II.

The Suction Mechanism

In their natural habitat at the Touchet and Snoqualmie Rivers, Washington, USA, Ascaphus tadpoles were seen clinging to rocks and stones by an oral sucker. They freed themselves from such adhesion only for swimming short distances from one place of attachment to another. They preferred the undersides of substrata and tended to face upstream in swiftly flowing water. However, at the Snoqualmie site, where isolated, stagnant pools occur, tadpoles were often seen in these pools, and wherever denied an undersurface for adhesion, they were seen clinging to the vertical or upper surfaces of substrata. When these tadpoles were placed in aquaria and allowed choice of orientation, they, like those caught on the undersides of rocks in a fast current, preferred fast water and the undersides of substrata. Ascaphus tadpoles are negatively phototropic, which is consistent with their preference for shaded undersurfaces.

1. Sucker engagement

In the disengaged sucker, the periphery of the upper and lower lips forms a rim around a shallow, central concavity (Figs. 3, 5). The periphery of the upper lip, and the lateral flaps (LF, Fig. 6) are supported by a hydrostatic skeleton of lymph canals which are turgid when the sucker is not engaged, but they become somewhat compressed during the achievement and maintenance of sucker engagement. The lower lip has no lymph canals, but flexible papillae (P, Fig. 6) instead of denticles at its peri-

phery probably assist sucker engagement on slightly irregular surfaces by filling the small spaces between the posterior rim of the sucker and the substratum. The ability of the rim of the sucker to mold itself to irregularities of the substratum, facilitates the creation of a water-tight seal when, at the onset of sucker engagement, the entire rim of the sucker is applied to the substratum (Fig. 3B).

The second stage of sucker engagement ensues when indirect muscle action, operating the buccal pump, causes substantial negative buccal pressures (Figs. 7, 8A). Water trapped between the sucker and the substratum is sucked into the buccal cavity through the mouth, thereby generating a negative sucker pressure. In consequence, the central area of the suctorial disc, like the periphery, becomes firmly pressed against the substratum (Fig. 3C). Therefore the sucker cavity (Fig. 3B) becomes restricted to the small area immediately outside the mouth (Fig. 3C; No. 3, Fig. 6). The passive, flap-like oral valve (OV, Figs. 3A, 5, 9) closes the mouth at the instant that the sucker pressure becomes negative relative to the buccal pressure. The flow of water into the buccal cavity through the mouth (but also through the nares), combined with a change to predominance of the compression phase over the decompression phase of the buccal pump, then tends to overcome the large negative buccal pressure. To account for the simultaneous partial removal of the negative sucker pressure, it is proposed that the oral valve bends toward the sucker cavity, thereby reducing its volume. It was not possible to verify this postulate by direct observation as the oral valve is obscured by the beak, especially in the engaged sucker. However, the elasticity of the central area of the sucker tends to cause it to

withdraw from the substratum and to form a concavity like that of the disengaged sucker (Figs. 3A, 5). This slight natural abduction probably helps to maintain a pressure between the sucker and the substratum that is negative with respect to the ambient water and to the buccal cavity. Once the oral valve has shut, it is probably maintained in this condition even during subsequent regular gill ventilation, provided that buccal pressure is kept positive relative to the sucker pressure.

The maximum sucker pressure generated by spontaneous buccal pumping during sucker engagement, is -18 to -25 torr.

2. The engaged sucker

The weight of Ascaphus tadpoles in water is very small (<0.01 g for those used in the present study). Therefore it is not surprising that they were able to hang upside-down by their suckers for periods exceeding an hour, without showing evidence of discomfort.

In anuran species which do not have suckers, conscious tadpoles exhibit vigorous struggling if they are turned upside-down. This contrasts with the indifference of Ascaphus tadpoles to all possible orientations in three dimensions when their suckers are engaged.

When the sucker of a tadpole is apposed to a surface, the tendency for water to be drawn into the mouth by decreasing buccal pressure during inspiration causes a general negative pressure between the sucker and the substratum. The oral valve (Figs. 3C, 5, 9) so restricts the rising phase of buccal pressure, that the residual negative pressure is not overcome to the degree that sucker adhesion is lost. Presumably, the fluctuations of this residual sucker pressure synchronous with buccal pressure (Fig. 4), are caused by bending of the oral valve during compres-

sion and decompression of the buccal cavity. Synchronized with breathing, there are also slight dorsoventral movements of the more medial parts of the lower jaw, but it is doubtful if these movements are caused by direct muscle action. It seems more likely that the lower jaw cartilages (e.g. CRI, Fig. 9) are pulled dorsad by the ceratohyale and that their return movement is by elasticity. Whatever the contribution of these lower jaw movements, it is essentially the oral valve that opens and closes the mouth during normal gill ventilation.

The pipetting of indicators around the periphery of the sucker disclosed no leakage of ambient water directly into the sucker cavity and thence into the buccal cavity via the mouth.

During prolonged sucker engagement in stagnant water, the sucker pressure was usually found to vary between -2 and -4 torr.

It was noticed that although the posterior areas of the sucker registered pressures of the same frequency, and locked to the same phase of buccal pressures, the shapes of these posterior sucker pressures were unlike those monitored in the sucker cavity nearer the mouth. To clarify this point, recordings were made along a midline transect of the sucker (Fig. 6). The graphs show that areas of the sucker demarcated by the lower lip yield curves with two peaks per ventilation cycle and there is a progressive fall in amplitude the farther away that pressures are monitored from the mouth. The changes in sucker pressure caused by manual repositioning in Fig. 6 agreed with those monitored during spontaneous sucker repositioning over the pressure monitor (e.g. Fig. 10).

Simultaneous recording of buccal pressures and posterior sucker pressures showed smooth single buccal pressure peaks, indicating that the dual-peaked curves are not an inherent proper-

ty of the ventilation mechanism. The general shape of the dual peaks remains the same for each ventilation cycle. Therefore whatever causes these dual peaks, acts synchronously with the ventilation cycle. The dual peaks persisted regardless of the orientation of the tadpole on the pressure monitor, provided that pressures from areas 4 to 6 (Fig. 6) were being recorded. The possibility that the heart, which lies near the posterior border of the sucker, is responsible for the notch in the sucker pressures, was disproven by the electrocardiogram, which does not have the same frequency as the ventilation rhythm (Fig. 11). Therefore further investigation is needed to determine the cause of these dual peaks.

Two shapes of pressure curve were recorded from area 6, Fig. 6. When recordings are first made from this area, the curves have a relatively shallow notch between their peaks and it is possible to distinguish one ventilation cycle from the next. However, the shape of the curves from this area usually changes before ca. 3 min after the location of the sucker over the pressure monitor and the beginning of recording. There is a deepening of the notch between the pressure peaks until it is possible to distinguish individual ventilation cycles only by assuming that one cycle is composed of two peaks.

3. Effects of lesions

As the Perspex substratum used in the experiments on the sucker was perfectly smooth like some rock surfaces found in the natural habitat of Ascaphus, the beak, (B, Fig. 6) and labial denticles may be assumed to have played little or no frictional role in the engagement of the sucker and in the maintenance of its adhesion. When the labial teeth and the edge of the beak were dissected away, sucker adhesion was still efficient and

sucker pressures did not differ significantly from those monitored in normal animals. Therefore these structures do not seem to be used for effecting and maintaining sucker adhesion; they may be concerned with rasping plant material off the substratum (cf. Fig. 12) rather than with providing a mechanical grip for the sucker.

Lesion such as a radial cut at any part of the periphery of the suctorial disc, reduced the amplitude of sucker pressures by causing a leak of ambient water into the sucker cavity. This effect was greatest when the lateral parts of the periphery were cut near the junction of the upper and lower jaws (LF, Fig. 6).

Surgical damage to the oral valve (OV, Figs. 3A, 5, 9), so as to destroy its valvular action, did not prevent sucker engagement, but to maintain sucker engagement, the tadpole had to keep breathing at the high frequency and amplitude characteristic of the onset of sucker engagement. As this could not usually be maintained for longer than ca. 30 sec, adhesion gradually weakened until sucker disengagement resulted. These effects were demonstrated by such a surgically injured tadpole which engaged its sucker to a vertical side of the aquarium. At first, the animal was nearly horizontal. Immediately buccal pumping became subdued, a gradual loosening and rotation of the sucker occurred, so that the animal assumed a perpendicular orientation with its tail pointing downward. The animal then slid slowly downward by gravitational pull and became disengaged from the glass. Figure 13 shows such a tadpole just before disengagement. The tadpole then slid on to the bottom of the aquarium, where it lay with its sucker disengaged like the normal but fatigued tadpole shown in Fig. 14A. Lesion to the oral valve therefore demonstrates the importance of this structure for the maintenance of sucker engagement.

4. Effects of water velocity

In a strong current, tadpoles are always aligned so that the stream washes over their bodies, from head to tail. Once sucker engagement has been effected, this alignment can, if necessary, be achieved through the passive rotation of the sucker and the streamlined body and tail into an orientation of least resistance to the ambient flow.

The fall in pressure concomitant with sucker engagement over the pressure monitor of a transducer is shown in A, Fig. 15. As a gentle flow of water (47 cm/sec) began over the tadpole's head (4 cm from the inlet, Fig. 2A), the slight further fall in pressure which occurred, is shown in B, Fig. 15. Simultaneously, the rhythmic pressures of the buccal cavity, registered in the sucker cavity, showed a small increase in amplitude. These effects were exaggerated when the ambient flow was substantially increased (236 cm/sec; C, Fig. 15). However, while the ambient flow was kept at this high level, the pressure curve tended to return to its earlier condition, monitored in stagnant water, except that its average value was more negative (D, Fig. 15), owing to the greater adhesion of the sucker. When the flow was further increased, to 472 cm/sec, tadpoles could not usually maintain their position at 4 cm from the inlet for longer than ca. 2 min. They then began to slip backward in the stream, gradually and passively, to ca. 5 cm from the inlet where, in the slower current, they remained for periods exceeding 10 min. Disengagement of the sucker was caused when the inlet was moved closer to the tadpole than 4 cm.

The use of a water tunnel (Fig. 2B) permitted the accurate determination of the maximum velocity of a turbulent stream which could be withstood before slipping of the sucker occurred. Of

five tadpoles tested, only one slipped backward (at a constant slow rate) when the ambient velocity reached 105 cm/sec. Slipping occurred in the other tadpoles at 140 to 185 cm/sec (163 cm/sec average).

Tadpoles resist sucker disengagement by a slightly greater frequency and much greater amplitude of buccal pumping when forces of abduction act on them. For example, Fig. 15 shows increased suction during an increase (C) in the velocity of the ambient flow. During the onset of such a vigorous pumping response, the inspiration phase or reduced pressure phase of ventilation, is greater than the expiration or increased pressure phase. When the net reduction of buccal pressure falls below the sucker pressure, the oral valve probably opens and further water is sucked from the sucker cavity (Fig. 3) into the buccal cavity. This generates an even greater negative sucker pressure, and it usually draws the first row of lower lip denticles slightly anterodorsad to the edge of the beak and therefore out of sight. The double tooth pattern of the first row of denticles distinguishes it from the other rows, which facilitates the recognition of the phenomenon (Fig. 16A,B). The increased adhesion is maintained by the resealing of the oral valve. By this means, the animal regulates the adhesion of its sucker to suit the velocity of ambient flow, and thereby resists abduction.

5. Properties of adhesion

Clinging tadpoles can be manually slid parallel to the plane of the substratum, but their slippery, streamlined bodies make it difficult to abduct them from the substratum. Therefore a hold was secured by passing a polyethylene loop through the dorsales trunci muscles of anesthetized animals, which were then returned to the aquarium for recovery. Two days later, when their

wounds had healed reasonably well, the suckers of tadpoles could be disengaged by manually pulling the polyethylene loop at right angles away from the substratum. The tadpoles then swam away and re-engaged their suckers elsewhere in the aquarium. Tadpoles could also be held by the loop and induced to engage their suckers over a pressure monitor embedded in a Perspex plate (Fig.1).

The suckers of dead tadpoles were engaged over the pressure monitor by manually pressing their heads ventrad (i.e. toward the Perspex) and then suddenly releasing the pressure. In this case, the adhesion mechanism is simpler than in the breathing tadpole. Immediately the periphery of the sucker contacts the substratum, a water-tight seal is formed (Fig. 3B). Consequently, a space (the sucker cavity) is created between the central, concave surface of the sucker and the substratum. For engagement of the sucker, the water in the sucker cavity must flow through the mouth and into the buccal cavity, thereby flattening the central area of the sucker and greatly reducing the sucker cavity. These effects can be achieved by manually pressing the dead tadpole's sucker against the substratum, simulating the effect of buccal pumping when breathing tadpoles engage their suckers. The elastic tendency of the central area to withdraw from the substratum then creates a negative pressure in the sucker relative to the buccal cavity because the passive oral valve is deflected closed and it prevents buccal water from re-entering the sucker cavity. The negative sucker pressure maintains adhesion while the oral valve remains closed and the peripheral seal of the sucker remains intact. Dead tadpoles, whose suckers were engaged in this way, remained clinging to the substratum for over an hour.

Forces of sucker abduction tend to increase the size of the sucker cavity, but as this is a closed chamber, an even greater suction is produced and the oral valve becomes more tightly sealed. The sucker pressures generated by manually pulling a conscious tadpole away from the pressure monitor are little different from the pressures generated by abducting the same, but killed, animal from the pressure monitor (Fig. 17A,B). Therefore it seems that adhesion is passive after sucker engagement has been achieved, and no further muscular expenditure is normally needed for maintaining the suction. However, when necessary, breathing tadpoles are able to vary the strength of adhesion by regulating the degree of buccal pumping.

Study of the sucker during abduction of it from a smooth Perspex plate was aided by a photomicroscope for direct observation through the Perspex, and for photography. The first noticeable effect of sucker abduction is an infolding of the first row of denticles (Fig. 16A,B; unlike the other rows, it is double, and there is also a space dividing it in the middle line). As gradual sucker abduction progresses, the bilateral horny plates of the upper jaw, forming the beak (B, Fig. 6), are slowly drawn away from the substratum by virtue of the flexible cleft between the plates (Fig. 16C to F). This flexibility of the beak is facilitated by the thinner keratin in the middle line than elsewhere on the beak. There is also a further withdrawal of the bilateral moieties of the lower jaw from the substratum, and the second to fifth rows of denticles fold toward the mouth and also away from the substratum. The slightly transparent lateral flaps (LF, Fig. 6) are drawn mesiad and wrinkles occur at the edge of the lower lip, especially at the moment of sucker

disengagement (Fig. 16F). Simultaneously, as ambient water floods the sucker cavity and destroys its negative pressure, the tooth rows of the lower lip become more visible again (Fig. 16E,F).

The oral valve is not visible during this abducting procedure but the great fall that abduction causes in sucker pressure (Fig. 17A,B), would probably pull the oral valve (OV, Figs. 3, 5, 9) into the sucker cavity were it not for bilateral ligaments which firmly bind the lateral aspects of the valve to Meckel's cartilages. On both sides, the ligament runs laterad, caudad, and slightly ventrad and is attached to the medial face of Meckel's cartilage immediately dorsal to the insertion of the M5 muscle (Table I) on this cartialge. Study of the partly exposed oral valve of a breathing, anesthetized tadpole disclosed that these ligaments do not interfere with the normal range of movements of the valve.

Sucker abduction by loads permitted an estimate of the force required to cause sucker disengagement under water. Figure 18A shows the stepwise increase in suction in a conscious tadpole by loads, until the climax of disengagement was reached (7.79g; 7.5 torr/g load). In five tadpoles tested (Table III), 5.79g was the greatest tolerable load borne under water for 8 sec by instantaneous application (Fig. 18B). A load of 6.79g was borne for 4 sec before it also disengaged the submerged sucker. On the other hand, after the sucker was attached to a submerged load, the tadpole was held by the polyethylene loop through its back muscles and lifted manually above the water surface. By this procedure, the mean maximum load which could be lifted into the air was 48.3g, about six times greater than the maximum submerged load borne for 4 sec. Further research is needed to explain this inconsistency, but it may be speculated that closure of the external nares

when exposed to air, would facilitate the generation of greater negative buccal pressures and these may help the sucker to resist disengagement.

Parker (37) defines adhesion efficiency as

$$\frac{\text{force required for disengagement}}{\text{area of sucker}^1 \times \text{atmospheric pressure}}$$

In conscious tadpoles, the mean maximum pressure in the sucker[1] is -125 torr (Table III). From elementary mechanics, it follows that force required for disengagement

= area of sucker[1] X (atmospheric pressure - pressure in sucker[1])

Thus adhesion efficiency

$$= \frac{\text{area of sucker}^1 \times (\text{atmospheric pressure} - \text{pressure in sucker}^1)}{\text{area of sucker}^1 \times \text{atmospheric pressure}}$$

$$= \frac{\text{atmospheric pressure} - \text{pressure in sucker}^1}{\text{atmospheric pressure}} = \frac{(760 - 635)\ \text{torr}}{760\ \text{torr}}$$

$$= \frac{125}{760} = \text{ca. } \frac{1}{6} \text{ or } 17\% \text{ (for the submerged sucker).}$$

---

[1] at the instant of disengagement.

6. Sucker locomotion and feeding

Like their counterparts in nature, laboratory tadpoles of Ascaphus crawled forward by alternate backward and then forward jerks of the lower lip. In a weak stream of aquarium water at 10°C, the crawling occurred sporadically. A crawl consisted of one to ca. 15 cyclic jerks of the lower lip. When transferred to a trough of fresh but stagnant water at 15°C, the tadpoles showed a greater crawling tendency during the first 5 min and they generally exhibited more cyclic hitches per crawl (up to 24 were counted). During the next 5 min, their crawling was interspersed with progressively more frequent swimming between places of sucker attachment to the substratum, possibly because, on finding no algae on it, their behavior became more searching than in the algae-laden aquarium. Both types of errant behavior became greatly reduced during the third 5 min period and the tadpoles then remained clinging to the sides of the trough for several minutes before further, but less vigorous searching. Generally consistent results were obtained in two further experiments of this kind.

As the backward movement of the lower lip encountered friction with the substratum, the tadpole's snout was thrust forward and simultaneously the trunk and tail were lifted from the substratum (Fig. 14C,D). During its alternate forward movement, the lower lip again encountered friction with the substratum and the snout was then pulled backward (Fig. 14D,E). However, as the snout's forward displacement (AD, Fig. 14D) was usually greater than its alternate backward displacement (AB, Fig. 14E), the net result (X) was a slight forward progression during each cycle (usually of duration 1 sec) of the crawl. Depending on the material nature of the substratum, its slope, and the density of

its covering vegetation, the net forward progression during each cycle varied, but was usually 1 mm on horizontal, smooth Perspex and glass. No backward or lateral progression by this means was seen,nor, from study of the functional anatomy would such progression seem to be possible in Ascaphus.

Described above are the conspicuous characteristics of sucker locomotion. Further information on the behavior was gathered in three ways. Firstly, a dissecting microscope was focussed through the aquarium glass and on the ventral surface of the sucker to study its movements under conditions close to those obtaining in nature. Secondly, pressures were recorded from the suckers of five tadpoles during their crawling on Perspex in which was embedded the monitor of a transducer. The Perspex was orientated vertically and the tadpoles were placed on it in a head-up position. This arrangement sometimes caused the forward movement of the animal to be cancelled by its alternate backward movement (probably helped by gravitational pull), so that the net result kept the sucker over the pressure monitor and yielded consistent recordings of sucker pressures associated with crawling (Figs. 19, 20). The rise and fall of sucker pressures correspond respectively with the forward and backward displacements of the snout during crawling. The recorded pressures therefore furnish data on the frequency and amplitude of crawling and on its periodicity. Thirdly, a tadpole was allowed to engage its sucker on a glass cover-slip. The tadpole was then gradually anesthetized until it could be turned supine without struggling, to permit study of the sucker through the glass with a dissecting microscope. The lower lip was gripped with forceps and slowly pulled caudad, imitating its natural,

though faster, movement during crawling, and the changes in the dispositions of the relevant anatomy were noted.

The data gained during these experiments permit general description of sucker locomotion. A cycle of this behavior may be considered as composed of two phases:

Abduction phase: the snout is thrust forward

Sucker locomotion begins with a backward (abduction) movement of the lower jaw and the entire lower lip (Fig. 16G,H) as a consequence of the muscular (cf. Table II) ventrocaudad rotation of the bilateral Meckel's cartilages. However, the peripheral seal of the sucker is maintained by a stretching apart of the overlapping junctions between the bilateral flaps (LF, Fig. 6) and the anterolateral edges of the lower lip. This backward displacement of the lower lip is facilitated by a transverse fold in the skin joining the upper and lower jaws at the corners of the mouth. The fold at no time contacts the substratum; it permits the free backward displacement of the lower jaw and it is pulled somewhat taut near the end of the phase. The displacement opens the mouth by pulling the oral valve (OV, Figs. 3, 5, 9) free from its contact with the upper jaw. Therefore buccal water enters the sucker cavity and tends to obliterate its negative pressure. Accordingly, Fig. 19 shows that there is usually a rise in the graph of sucker pressure. However, the inspiration phase of the ventilation cycle immediately preceding the onset of crawling, is sometimes accentuated, causing a sudden large fall in the graph of sucker pressure (Figs. 7, 20B). Consequently, the following rise in sucker pressure, as the snout is thrust forward, is of an amplitude generally greater than when crawling is not preceded by a vigorous inspiration.

Synchronous with the lower jaw's backward displacement, there is a bilateral indentation of the skin below the eyes that covers the H1 muscles (Table I). The relaxation and stretching of these muscles, which the sagging of the skin seems to indicate, would be in accord with the contraction of the H6 muscle which raises the buccal floor and increases the buccal pressure at this time. The transmission of this buccal pressure to the sucker cavity as the mouth opens, reduces suction and the contact which the broad lower lip makes with the substratum provides enough friction for it to thrust the snout forward. The small denticles and the beak of the upper jaw have their edges directed backward, and, as a result of the decrease in suction, they tend to be pulled away from the substratum by the snout's elasticity. Therefore their friction with the substratum during this phase of the locomotion cycle is probably too small to hamper the forward sliding of the snout. Figures 19, 20 show that the sucker pressure during this phase does not quite reach the baseline reference pressure. Therefore, in sluggish or stagnant water, where crawling seems to occur most readily, sucker engagement is not greatly endangered by this partial loss of suction.

Abduction of the trunk and tail from the substratum by the (?) dorsales trunci muscles also occurs during this phase (Fig. 14C,D). As the tail is laterally flattened, it encounters very little resistance from the ambient water during this movement. Depending on the orientation of the tadpole, the abduction of the trunk and tail (usually 30° to 40° when prostrate on the bottom) is influenced by gravitational force, but in water the magnitude of its effect is probably small. During the periods of rest between crawling, the tail is held against the substratum (Fig. 14B). A space between the branchial outlet and the substratum

(Figs. 6, 14B, 16G) during sucker engagement, permits free branchial outflow. The substantial increase in buccal pressure during the abduction phase (Figs. 7, 20C) accentuates the expiration of ventilation water. The concomitant lifting of the abdomen from the substratum (Fig. 14D) may safeguard the branchial outlet from occlusion by occasionally more vigorous backward displacements of the lower lip.

Adduction phase: the snout is pulled backward

The lower jaw and lower lip are capable of returning to their normal position (Fig. 6) by their natural elasticity. However, like the backward displacement of these structures in the abduction phase, the return movement (adduction) is so vigorous that it is undoubtedly also powered by muscles attached to Meckel's cartilages (Table II). The greater intercalation of the lateral flaps with the lower lip is restored by this movement. Simultaneous increase in suction (Figs. 19, 20) is probably caused by depression of the buccal floor and the flow of sucker water into the buccal cavity. As there is also at this time an outward bulging of the skin covering the H1 muscles, it seems likely that these muscles are responsible for buccal depression.

The greater suction produced by buccal depression, and the forward pointing denticles (contrary to 49) of the broad lower lip probably increase its friction enough to permit the lip to rasp algae off the substratum. This friction of the lip may also help in pulling the snout backward to its resting position. During this backward movement the upper jaw denticles and beak are pressed against the substratum by the prevailing suction. These conditions favor the view that the keratinized parts of the

sucker, especially the beak, are concerned with scraping algae free from the substratum, to be sucked into the buccal cavity by its lowered pressure during this phase (Figs. 7, 20C). The simultaneous, slightly delayed fall in sucker pressure during the greater part of this phase (Figs. 19A,B; 20A) seems to reflect the scraping action, particularly of the upper beak, because a smooth fall in sucker pressure occurred after the edge of the beak was dissected away (Fig. 20B,C). However, sucker locomotion at about the same rate still occurred after the beak had been completely dissected away. Inscriptions of the scraping action of the dental apparatus were sometimes found over areas where algae grew on the aquarium walls. Moreover, Fig. 12 shows similar marks produced by manual backward displacement of a tadpole's sucker which was adhering to photographic film. The continuous buccal pumping between crawling and also during crawling (Figs. 7, 19C, 20C) probably facilitates transport of the loosened algae to the pharyngeal ciliated tract[1], whence the food is carried in a cord of mucus into the esophagus. The details of this process are, however, outside the scope of the present research. In support of the above evidence for a feeding utility of sucker crawling, it was observed that on all occasions when continual crawling occurred over areas of algae, a continuous column of algal feces emerged from the anus, even after the aquarium had been flushed of all planktonic algae.

After one or several cycles like the one described above, the oral valve closes at the end of the adduction phase (Fig. 16I), and seals a negative pressure in the sucker cavity. Gill ventilation occurs mainly by buccal pumping (Figs. 7, 19C, 20C) during the pause which extends to the next series of crawling movements. The graphs also show a coordination between crawling

[1] not hitherto described in Ascaphus.

and gill ventilation; indeed, in some respects crawling seems to be scarcely more than an enhancement of the amplitude of gill ventilation. The length of the pause between crawling is highly variable, but in feeding tadpoles it was often 20 to 30 sec.

Sucker disengagement sometimes occurs immediately after crawling movements (Fig. 19B) and the animal then swims to a new place of attachment where crawling may again occur.

Sucker locomotion may not always be associated with feeding, nor restricted to still water, for although it was most often seen under these conditions, one tadpole in the water tunnel (Fig. 2B) was seen crawling slowly against a turbulent flow (ca. 60 cm/sec), in which the animal's forward progress was slower than normal and trunk and tail elevating movements did not occur. Crawling against a stream of water may occur by the general lowering of the range of sucker pressures to resist abduction.

Functional anatomy disclosed that the lower jaw movements are caused by muscular displacements of Meckel's cartilages, whose movements are always bilaterally synchronized. As the left and right sets of the relevant muscles appear usually to exert equal forces on Meckel's cartilages, the lower jaw and lip are moved straight back (abduction; Fig. 16H) and then straight forward during the adduction phase of crawling. Consequently, the animal advances in a straight line. However, occasionally the left and right sets of muscles seem to exert unequal forces, for it was plainly visible that the lower jaw sometimes did not move straight backward, but either the left or the right side of the jaw moved more than the other. The muscles moving the lower jaw forward would then presumably also exert different tensions in returning the somewhat skewed lower jaw and lip to its resting

place against the upper jaw (Figs. 6, 16I). This unequal displacement of the lower jaw and lip causes the tadpole to change its direction slightly at each hitch of the sucker. Consecutive step-like changes in direction always supplement one another and tadpoles are able to turn through 90° by this means with 7 to 13 hitches of the sucker. In other words, once a turning to the left or right has begun, consecutive hitches are cumulative in promoting the turn.

Backward sucker locomotion independent of tail movements was never seen in Ascaphus tadpoles, but by coordinated action with tail flips to left and right, and by loosening the sucker at the appropriate moment and then tightening it when suitable, the animal can pull itself slightly backward (usually 2 to 6 mm).

7. Sucker disengagement

At the Snoqualmie site, tadpoles in small sunlit pools would disengage their suckers and swim downward to reattach themselves in dark recesses when a shadow fell on them. Sucker disengagement was investigated in the laboratory by allowing tadpoles to afix themselves by sucker over the monitor of a pressure transducer (Fig. 1). After adaptation of the tadpoles to normal lighting, the lights were switched off and the usual response was that of Fig. 4, where the sucker became slightly loosened due to a sudden slight loss of suction, but in the absence of further disturbance, the animals immediately restored their normal condition. An identical response was elicited from dark adapted tadpoles when the lights were switched on, which suggests that the response is triggered by a change in illumination rather than only by a decrease in illumination.

The sudden decrease in suction in the above experiments is probably an early behavioral stage in sucker disengagement. The increase in pressure always occurred during the beginning of inspiration, despite the stage in the ventilation cycle of the change in illumination. However, in constant lighting, spontaneous sucker disengagement also occurs by a sudden rise in sucker pressure at the onset of expiration. The rise in the pressure curve is associated with an opening of the mouth by the slight caudad displacement of the lower jaw, possibly by the S1 muscles (Fig. 9; Table I). Study of the mouth through the glass substratum with a dissecting microscope during sucker disengagement, showed that the lower jaw displacement caused a pulling away of the oral valve from its seal against the skin of the upper jaw. Compression of the buccal cavity at this time by elevation of the buccal floor would help to destroy the partial vacuum of the sucker and therefore would facilitate sucker disengagement. However, technical problems and the short supply of tadpoles precluded the gathering of functional data to support this conjecture.

At the time of sucker disengagement, a bilateral buckling of the anterior rim of the snout (Fig. 21) lifts the front of the sucker off the substratum. The paired M2 muscles are responsible for this buckling of the snout. The effect is achieved by the lifting of the bilateral cartilages of the suprarostral system. Simultaneously, the head and tail are abducted by contraction of the (?) dorsales trunci muscles. Tail undulations then cause the displacement of the sucker to another area of engagement. Alternatively, buckling of the snout and lifting of the sucker off the substratum does not occur after opening of the mouth. Instead, a single tail flip to left or right causes a swivelling of the

loosened sucker in the same direction as the tail flip, often through about 90°, without producing complete sucker disengagement. The broad profile of the tail (Fig. 14) and consequently the high resistance it must encounter from the ambient water during these flips, is no doubt an important factor in this maneuver. The swivelling could be experimentally induced by plugging the external nares with organic debris suspended at their entrances.

8. The disengaged sucker

During anesthesia preparatory to buccal cannulation, the sucker became loosened from its normally engaged condition. After ca. 30 min in 1% urethane (10°C), the tadpole no longer responded to skin pricks, and it was turned supine with its sucker disengaged. The suctorial disc became somewhat cup-shaped through its inherent elasticity, and the median symphysis between the rostral cartilages of the lower jaw became more visible. Indicators pipetted at the nares, mouth, and branchial outlet, showed that regular breathing continued. Figure 8A shows that in the disengaged sucker the amplitude of buccal ventilation is slightly smaller than when the sucker is engaged, but the frequency is approximately the same in both conditions.

Microscopic examination of the slightly displaced lower jaw revealed that the mouth was regularly opened and closed by movements of the oral valve. Lower jaw movements such as occur faintly in the engaged sucker, were absent. Possibly the ceratohyalia (Fig. 9) do not under these circumstances reach far enough upward to exert a pull on the lower jaw.

The Ventilation Mechanism

The flow of ambient water into the ventilation system depends on the functional state of the sucker. Indicators pipetted at the entrances to the mouth and nares showed that when the sucker is engaged, water enters the buccal cavity only through the nares, but both nares and mouth serve as water intakes when the sucker is not engaged. For understanding gill ventilation, it is therefore necessary to take the sucker into account. Moreover, the identical relation between the sucker and buccal pressure curves (Fig. 4) permits the amplitude and frequency of gill ventilation to be monitored in conscious, uninjured and unrestrained tadpoles. The recordings can also be made in all possible orientations in three dimensions and in variable velocities of ambient flow. The sucker therefore provides a convenient way of collecting respiratory data without anesthesia, cannulation, or other interference with the animal.

Tadpoles at 10° C ventilate their gills continuously (ca. 50 cycle/min) even in a swift current of water. In tadpoles clinging to a substratum, ventilation is also of regular amplitude, but mechanical or visual disturbance in the laboratory, as experiments earlier in the present study have shown, tends to cause irregular amplitudes. Identical pressures recorded from the sucker of conscious, unrestrained tadpoles in all possible orientations suggest that in still water they can ventilate their gills equally well, regardless of orientation.

1. Buccal cavity

The buccal pump subserves both the suction and gill ventilation mechanisms. Figure 7 shows a slight increase in the frequency but a marked increase in the amplitude of buccal pumping, which causes a general fall in buccal pressure during sucker

engagement. These effects were also produced by lightly touching the skin of a tadpole with a blunt probe, or by manually sliding a tadpole from an engaged position alongside the pressure monitor to an engaged position over the monitor (Fig. 8B). The increase in pumping frequency is not shown in Fig. 8A because the animal was already breathing at a high frequency (18°C) at the commencement of sucker engagement. Further experiments showed that spontaneous hyperexpirations occur in the buccal cavity during recovery from deep anesthesia (Fig. 22). Unusually large buccal pressures also occur during elevation of the external nares above water (Fig. 23A,B). Moreover, there is greater buccal pumping when the velocity of ambient flow is suddenly increased (Fig. 15). Taken together, the above results demonstrate that the buccal pump is capable of greater effort than during quiet ventilation (Fig. 4).

The following experiments are concerned with the water intakes of the buccal cavity during gill ventilation.

During inspiration, suspensions of indicators were drawn through the tubular external nares by the buccal pressure which was lower than the pressure of the ambient water. There was no reflux of indicators pipetted at the external nares, because non-muscular valves of the internal nares were pressed closed by the buccal pressure which was greater during expiration than the pressure of the ambient water. For a similar reason, there was no reflux at the mouths of tadpoles whose suckers were in a disengaged condition. When the mouth of a tadpole was gently opened by displacing the lower jaw with a probe, it was seen that immediately within the mouth, and normally obscured by the horny beak, is a flap-like fold of skin (OV, Figs. 3, 5, 9). The

action of this fold, the oral valve, under the influence of buccal pressure, closes the oral entrance at the onset of expiration (pressure rises). The anterior surface of the valve becomes apposed against the skin of the upper jaw. Conversely, during inspiration, the fold is pulled away from the upper jaw by decreasing buccal pressure, which draws water into the buccal cavity through the mouth.

Tadpoles were sometimes seen clinging by their suckers to the sides of dimly-lit aquaria, and with their snouts and nares just protruding above the water surface. Metter (30) has witnessed this behavior in nature at night. As the water intakes of the buccal cavity become sealed by this protrusion of the nares above water, the effect of this behavior on buccal pumping was considered worthy of investigation in the present study.

In the laboratory, a conscious tadpole adhering to a vertical Perspex plate was slid until its sucker was over the submerged monitor of a pressure transducer. As the snout was pointing upward, it could be easily raised above water level by gradually elevating the transducer and Perspex plate until the nares became exposed to air. Tadpoles tolerated this procedure, even when raised above water for over 1 min. Pressures monitored in all areas of the sucker during snout protrusion showed increased amplitude and reduced frequency (e.g. Fig. 23A,B). The elevation of the nares above water often caused greater ventilation amplitudes when the nares were resubmerged, than those monitored before the nares were raised above water.

Closure of the external nares is facilitated by their tubular nature, which prevents air from entering the buccal cavity when the snout protrudes above water. This finding agrees with Noble (33). Closure of the external nares occurs immediately

on exposure of them to air and it is caused by their mesiocaudad displacement. As tadpoles usually crawl spontaneously above water while they are in a vertical position, snout upward, the force of gravity assists the caudad component of this displacement.

Maintained narial closure during snout protrusion above water and while the mouth is sealed by the sucker's engagement, prevents ambient water from entering the buccal cavity. Simultaneously, the branchial outlet appears collapsed and indicators pipetted at its orifice disclosed no water flow from the gill cavities. Nevertheless Fig. 23A,B shows that buccal pumping continues while the snout and nares are raised above water. Therefore, water that may be retained in the closed ventilation system is probably circulated by these pumping movements. If snout protrusion is a means whereby tadpoles can feed on vegetation above the waterline, the mixing of water in the closed ventilation system (except during oral ingestion) would probably facilitate respiratory gas exchange by ensuring that the fullest advantage is taken of the water in the system. However, the transport of food in the buccal cavity to the esophagus would probably require the tadpole to resubmerge its external nares.

Tadpoles crawl out of the water by the mechanical action of their suckers against the substratum, similar to the manner of the submerged crawling described earlier in this report. Aquarium tadpoles return themselves to the water from their elevated positions by a loosening of the sucker and a passive sliding into the water by gravitational pull, or by assisting this return with one or more vertical flips of the tail. These tail flips do not usually alternate, left and right, but are repeated on the left or right sides of the body until the animal returns to the water.

The external nares "bend down and close" when a flow of water is directed against them (33, p. 65), but in the present study it was found that in a fast stream, buccal pumping (Fig. 15) and branchial outflow continue, indicating that the external nares are probably not closed by a fast current. Submerged Ascaphus tadpoles even in a swift stream, can control narial inflow simply by changing the amplitude and frequency of buccal depression. Perhaps the most significant finding of monitoring ventilation pressures from Ascaphus tadpoles in an ambient flow is that, unlike the suckerfish, Remora, where ventilation decreases and may even stop in a fast stream (32), the ventilation frequency of Ascaphus is not reduced by increased velocity of the ambient flow (Fig. 15).

In Ascaphus there is no first gill cleft connecting the buccal cavity to the gill cavity (cf. Rana catesbeiana, 14). Therefore, as the valves of the internal nares and mouth are closed by buccal compression during expiration, the only normal channel for the exit of water from the buccal cavity is over the ventral velum and into the pharynx.

2. Pharynx and gill cavities

The difficulty of monitoring pharyngeal pressures by cannula permitted only a single recording to be made (Fig. 24). The cannula's position was determined by dissection after the experiment and found to be lodged laterally in the pharynx, near the edges of the dorsal and ventral vela. It is possible that either or both the vela may have interfered with the recording of normal pharyngeal pressures. Therefore the pressure recording is presented tentatively until verified in larger tadpoles. However, the two

pressure peaks per cycle are consistent with the functional anatomy of breathing, anesthetized tadpoles. The first pharyngeal pressure peak occurs in phase with an increase in buccal pressure and is due to the transmitted effect of buccal pressure in the pharynx during expiration. Pharyngeal constriction probably causes the second peak in the phayngeal pressure (Fig. 24) during the latter part of buccal inspiration.

The presence in Ascaphus of a ventral velum which, like that of Rana catesbeiana (14), is structurally suited to function as a valve between the buccal cavity and pharynx, is circumstantial evidence favoring the existence of a pharyngeal pressure pump in Ascaphus. The absence of first gill clefts prevents reflux of water directly from the gill cavities into the buccal cavity when the buccal floor depresses, and so facilitates the generation of negative buccal pressures. By increasing the pressure difference between the buccal cavity and pharynx, the fall in buccal pressure probably closes the passive ventral velum during early inspiration. Therefore neither is there reflux of pharyngeal water into the buccal cavity during this phase and the suction generated by buccal decompression is rendered more effective at the mouth and nares. The occurrence of pharyngeal constriction later in inspiration, maintains the pressure in the pharynx at a positive value relative to the buccal cavity (Fig. 24) and therefore keeps the non-muscular ventral velum shut. Residual water in the pharynx can then only leave via the paired gill clefts 2, 3, and 4, and enter the gill cavities.

During expiration all the buccal water must flow over the ventral velum and into the pharynx. The anatomical relation between the dorsal and ventral vela of Ascaphus supports the view that, as in Rana catesbeiana (14), the ventral velum of Ascaphus

acts as a hydrofoil to deflect water against the dorsal velum during buccal expiration. From here, the water must flow through the gill clefts 2, 3, and 4 to reach the bilateral gill cavities (there is no transverse opercular canal in Ascaphus).

The recording (Fig. 25) of sustained, though fluctuating, positive pressures from the gill cavities, and the continuous, though variable, flow from the single branchial outlet (as shown by dye to be greatest during buccal inspiration), indicate that the dual pumping mechanism facilitates a continuous water flow over the gills. Dissections of anesthetized, regularly breathing tadpoles showed that the gills are also themselves moved through the water in the gill cavities, synchronously with the ventilation cycle. Inspection of the gill arches revealed that their movements are bilaterally synchronized; no evidence was found for unilateral pumping by the pharynx or gill cavities.

The foregoing results may now be summarized by a description of the sequence of events during a typical ventilation cycle.

3. The ventilation cycle

Inspiration

Depression of the buccal floor causes a fall in the buccal pressure relative to the ambient pressure[1]. This probably closes the ventral velum in a valve-like fashion that occludes the buccal cavity from the pharynx. If the oral sucker is not engaged, the only inhalent channels are the nares and mouth, through which ambient water can flow to equilibrate the negative buccal pressure. On the other hand, if the sucker is engaged, an inflow still occurs through the nares, but an oral inflow is sealed by the sucker.

---

[1] ambient pressure = atmospheric pressure plus hydrostatic pressure at depth of tadpole below water surface.

Pharyngeal constriction occurs toward the end of inspiration and causes a rise in pharyngeal pressure, which probably keeps the ventral velum shut. Therefore water in the pharynx passes through the gill clefts and over the gills in the gill cavities. A strong flow leaves the non-contractile gill cavities by the mid-ventral branchial outlet. The lack of reflux at the branchial outlet during large negative buccal pressures may perhaps be explained by valvular action of the ventral velum.

Expiration

Elevation of the buccal floor causes compression of the buccal cavity. When the buccal pressure exceeds the ambient pressure, the valvular internal nares close and so does the oral valve if it is not already sealing the mouth owing to engagement of the sucker; but the ventral velum probably opens at this time. Therefore no reflux of water occurs at the nares or mouth, and the buccal water is instead deflected by the ventral velum against the dorsal velum. Hence the water is further deflected until it leaves the pharynx by the gill clefts. After passing over the gills, a weaker flow leaves the gill cavities via the branchial outlet than during inspiration.

Discussion

The present study assumes that the gills provide the more important respiratory surface in the ventilation system, but it is possible that the lining of the buccal cavity and pharynx also participates as a respiratory surface.

There is a functional interaction between the suction and gill ventilation mechanisms. For convenience of description, they are considered separately in the present report.

The denticulated sucker of Ascaphus is immediately suggestive of a feeding adaptation, but according to Noble (33, p. 66): "Undoubtedly, some particles are scraped off the vegetation on the rocks as the tadpole moves along. But only the lower mandible would function in this process and this structure is exceedingly small (Fig. 9)"; and again (p. 69): "The larval teeth are modified in order to better grip the irregularities in the rocks to which the larva holds." However, the present investigation suggests that the dental apparatus does not participate in sucker adhesion, as the sucker is efficient on smooth glass and Perspex, even when the teeth have been dissected away. Furthermore, although Noble believed that Ascaphus tadpoles feed by taking in suspended food particles through their nares, he nevertheless pointed out (p. 67) that the entire horny edge of the beak of the upper jaw is in nature continually worn down by abrasion against the substratum. The present study shows that the beak is used for scraping algae off the substratum.

On the basis of his discovery of nasal cilia in Ascaphus and on "food materials in both the nasal sacs and preoral buccal cavity", van Eeden (49, p. 50) supports Noble's contention (33) that the nares serve as feeding channels. Van Eeden also suggests (p. 49) that immediately within the mouth, a patch of ciliated

epithelium (which Pusey, 38, p. 113, found to be innervated by "a branch of the olfactory nerve") may assist this type of feeding. But these small areas of cilia are separated from the pharynx by a relatively wide expanse of non-ciliated epithelium (Gradwell, unpublished). Moreover, in comparison with the strong water flow produced by buccal and pharyngeal pumping, the significance of the nasal and oral cilia for food transport, could scarcely be of much consequence.

The dual peaks recorded per ventilation cycle from the posterior areas of the engaged sucker may be the result of synchronous lower jaw movements, for it has been established in the sucker catfish, Plecostomus (Gradwell, in preparation), that the withdrawal of the lower jaw from the substratum, causes identical dual peaks in the sucker pressure of this fish. However, the elucidation of this point requires an understanding of the relevant functional anatomy, to be described in a later contribution.

The similarity between pressures recorded from the sucker during detachment of living as well as pithed tadpoles from a substratum, indicates the dispensability of direct muscle action for resisting sucker abduction. But in the living animal, the indirect muscle action of buccal pumping effects sucker engagement and resists sucker disengagement by reducing the sucker pressure when forces of abduction act on the animal.

Mucosal flaps, which form an oral valve[1], are an important component of the sucker apparatus of Ascaphus tadpoles. This feature is reminiscent of certain fishes which have oral valves (17). In dead Lampreta, the tongue acts as a passive oral valve

---

[1] Absent in non-suctorial tadpoles

(5). The toothed sucker of L. wilderi is an adaptation for adhesion and feeding (5), but unlike the sucker of Ascaphus, it is incapable of active crawling over a substratum (Gradwell, unpublished). When the sucker of Ascaphus is engaged, the occlusion of the mouth confines the respiratory inflow to the nares. Lampreta have no nares for water flow into the respiratory system. In these fishes gill ventilation during sucker engagement therefore occurs solely through the inflow and outflow of water through the branchiopores (5). There are other comparisons that could be attempted between Ascaphus tadpoles and sucker cyclostomes, but such a study must be left until the functional anatomy of both groups is better understood.

In the disengaged sucker the oral valve and lower jaw movements open and close the mouth rhythmically in coordination with the ventilation cycle, but ambient water cannot enter the buccal cavity by this route while the sucker is afixed to a substratum. The importance of the nares for intermittent buccal inflow is therefore evident. As in Rana catesbeiana (12), valvular flaps of the buccal mucosa prevent narial outflow during buccal compression. In the light of the present findings, Noble's contention (33) that the external nares close when exposed to air seems correct, but he also stated, in conflict with the present research, that the external nares close in a fast current of water. However, in a stream of ambient water, the external nares are the only inlets to the ventilation system to account for the continuous flow from the distended branchial outlet. It therefore seems that, even in a stream, water enters the external nares cyclically during the inspiration phase of breathing, just as suspensions of indicators have shown it does in tadpoles clinging in non-flowing water.

The teleost, Remora, has its dorsal fin modified into a sucker. The fish decreases ventilatory pumping and eventually stops active ventilation of its gills in favor of passive ventilation by adjusting the degree of oral abduction if ambient water is experimentally driven over the fish (32). There is no such ability in Ascaphus tadpoles to utilize the ambient stream for passive gill ventilation, despite the somewhat funnel-shaped external nares. The mouth of Ascaphus is sealed by the sucker when the tadpole clings in a fast current, and relative to the size of the ventilation system, the nares are too small to admit a flow adequate for passive ventilation.

Ventilation in Ascaphus is by two cyclic force pumps in front of the gill clefts, as in Rana catesbeiana (14, 15). The cycles are out of phase with each other, and this ensures a strong, unidirectional flow of water over the gills during pharyngeal compression and a weaker branchial flow during pharyngeal decompression and buccal compression. Therefore, the gills are continually bathed by moving water and, in addition, they are themselves moved rhythmically to and fro through the branchial water. The development of this efficient gill ventilation mechanism in tadpoles which generally live in cold, well-oxygenated water, seems surprising, but may perhaps be explained by the absence of functional lungs and by the tendency for tadpoles to be washed into and trapped in stagnant or semistagnant pools.

Unlike Rana, Hyla, Bufo, Pseudis, and probably some other genera, Ascaphus tadpoles have no first gill clefts connecting the buccal cavity to the paired gill cavities. Therefore the buccal pump can drive water only over the ventral velum, through the pharynx and gill clefts 2, 3, and 4, into the gill cavities.

Constriction of the pharynx then drives residual pharyngeal water directly through the same gill clefts. A valvular ventral velum and the absence of a first gill cleft probably facilitate the large negative buccal pressures necessary to increase sucker adhesion when forces of abduction operate.

The presence of one or two branchial outlets is probably a primitive feature which tends to persist in nektonic tadpoles (e.g. Xenopus). Ascaphus has a single midventral branchial outlet, but this feature is not a prerequisite for the life of tadpoles in swift streams, as Heleophryne (18) and also Staurois (35) and several other sucker-bearing genera (e.g. 24-26) have sinistral branchial outlets. The lateral or posterior transposition of this feature in benthic species, and the frequent extension of it into a spout, may have occurred to prevent detritus and parasites from entering the delicate gill cavity. The confinement of the branchial outflow to a single, small aperture probably assists in the generation of a constant positive pressure in the gill cavity. Therefore water exits continuously through the branchial outlet and this too, guards against entry into the gill cavity of extraneous material through the branchial orifice. It may be conjectured that the sinistral branchial outlet of many species of stream-dwelling sucker tadpoles was derived from ancestors living in sluggish or non-flowing water. The feature may have persisted when such forms adapted to life in torrents, because it became no handicap to gill ventilation.

The short supply of tadpoles precluded extensive experimentation on the several phenomena associated with suction and ventilation and the present study should therefore be regarded as preliminary. However, it provides a basis for an understanding of the suction and gill ventilation mechanisms in Ascaphus.

Aspects of the relevant functional anatomy have been briefly considered, but they need to be amplified before a detailed elucidation of the suction and gill ventilation mechanisms of Ascaphus can be attempted.

Acknowledgments

Dr. C. Carl, British Columbia Provincial Museum, Victoria, kindly sent preserved tadpoles for anatomical study.

Dr. L.A. du Plessis helped with the tadpole collecting and gave useful advice on the improvement of the manuscript and on problems of hydrodynamics.

I am also indebted to Dr. R.L. Saunders, Fisheries Research Board of Canada, St. Andrews, New Brunswick, for a critical reading of the manuscript.

References

1. Annandale, N. 1918. Some undescribed tadpoles from the hills of southern India. Rec. Ind. Mus. 15: 17-23.
2. ---- 1919. The fauna of certain small streams in the Bombay Presidency. Ibid. 16: 109-161.
3. Annandale, N., and S.L. Hora. 1922. Parallel evolution in the fish and tadpoles of mountain torrents. Ibid. 24: 505-509.
4. Bhaduri, J.L. 1935. The anatomy of the adhesive apparatus in the tadpoles of Rana afghana Günther, with special reference to the adaptive modifications. Trans. Roy. Soc. Edin. 58: 339-349.
5. Dawson, J. 1905. The breathing and feeding mechanism of the lampreys. Biol. Bull. 9: 1-21; 91-111.
6. De Jongh, H.J. 1968. Functional morphology of the jaw apparatus of larval and metamorphosing Rana temporaria. Neth. J. Zool. 18: 1-103.
7. Duellman, W.E., and J.D. Lynch. 1969. Descriptions of Atelopus tadpoles and their relevance to atelopodid classification. Herpetologica 25: 231-240.
8. Edgeworth, F.H. 1935. The cranial muscles of vertebrates. Cambridge Univ. Press. 493 pp.
9. Gaige, H.T. 1920. Observations upon the habits of Ascaphus truei Stejneger. Occ. Pap. Univ. Mich. Mus. Zool. 84: 1-9.
10. Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16: 183-190.
11. Gradwell, N. 1968. The jaw and hyoidean mechanism of the bullfrog tadpole during aqueous ventilation. Can. J. Zool. 46: 1041-1052.
12. ---- 1969a. The function of the internal nares of the bullfrog tadpole. Herpetologica 25: 120-121.
13. ---- 1969b. The respiratory importance of vascularization of the tadpole operculum in Rana catesbeiana Shaw. Can. J. Zool. 47: 1239-1243.

14. ---- 1970. The function of the ventral velum during gill ventilation in Rana catesbeiana. Ibid. In press.

15. Gradwell, N., and V.M. Pasztor. 1968. Hydrostatic pressures during normal ventilation in the bullfrog tadpole. Ibid. 46: 1169-1174.

16. Gradwell, N., and B. Walcott. 1970. Dual functional and structural properties of the interhyoideus muscle of the bullfrog tadpole (Rana catesbeiana). J. Exp. Zool. In press.

17. Gudger, E.W. 1946. Oral breathing valves in fishes. J. Morph. 79: 263-285.

18. Hewitt, J. 1913. Description of Heleophryne natalensis, a new batrachian from Natal. Ann. Natal Mus. II: 475-483.

19. ---- 1922. Notes on some South African tadpoles. South Afr. J. Nat. Hist. III: 60-65.

20. Hora, S.L. 1923. Certain points in the anatomy of hill-stream tadpoles. Rec. Ind. Mus. 25: 579-599.

21. ---- 1927. Animal life in torrential streams. J. Bombay Nat. Hist. Soc. 32: 111-126.

22. ---- 1932. Development and probable evolution of the suctorial disc in the tadpoles of Rana afghana Günther. Trans. Roy. Soc. Edin. 57: 469-472.

23. ---- 1934. Further observations of the bionomics of the tadpoles of Rana afghana Günther. Rec. Ind. Mus. 36: 321-325.

24. Inger, R.F. 1954. Systematics and zoogeography of Philippine Amphibia. Fieldiana Zool., Chic. Mus. 33: 183-531.

25. ---- 1960. A review of the oriental toads of the genus Ansonia Stoliczka. Ibid. 39: 473-503.

26. ---- 1966. The systematics and zoogeography of the Amphibia of Borneo. Ibid. 52: 1-402.

27. Kenny, J.S. 1969. Feeding mechanisms in anuran larvae. J. Zool. Lond. 157: 225-246.

28. Kratochwill, K. 1933. Zur Morphologie und Physiologie der Nahrungsaufnahme der Froschlarven. Z. Wiss. Zool. 164: 421-468.

29. Lui, C.C. 1950. Amphibians of Western China. Fieldiana, Zool. Mem, Vol. 2. Chic. Nat. Hist. Mus.

30. Metter, D.E. 1964. A morphological and ecological comparison of two populations of the tailed frog Ascaphus truei Stejneger. Copeia (1): 181-195.

31. ---- 1967. Variation in the ribbed frog Ascaphus truei Stejneger. Ibid. (3): 634-649.

32. Muir, B.S., and R.M. Buckley. 1967. Gill ventilation in Remora remora. Ibid. (3): 581-586.

33. Noble, G.K. 1927. The value of life history data in the study of the evolution of the Amphibia. Ann. N.Y. Acad. Sci. 30: 31-128.

34. ---- 1929. The adaptive modifications of the arboreal tadpoles of Hoplophryne and the torrent tadpoles of Staurois. Bull. Amer. Mus. Nat. Hist. 58, Art. VII: 291-334.

35. ---- 1931. The biology of the Amphibia. McGraw-Hill, N.Y. Reprinted 1954, Dover Publ., N.Y.

36. Noble, G.K., and P.G. Putnam. 1931. Observations on the life history of Ascaphus truei Stejneger. Copeia (3): 97-102.

37. Parker, G.H. 1921. The power of adhesion in the suckers of Octopus bimaculatus Verrill. J. Exp. Biol. 33: 391-394.

38. Pusey, H.K. 1943. On the head of the liopelmid frog, Ascaphus truei. I. The chondrocranium, jaws, arches, and muscles of a partly-grown larva. Quart. J. Micr. Sci. 84: 105-185.

39. Savage, R.M. 1952. Ecological, physiological and anatomical observations on some species of anuran tadpoles. Proc. Zool. Soc. Lond. 122: 467-514.

40. ---- 1955. The ingestive, digestive, and respiratory systems of the microhylid tadpole, Hypopachus aguae. Copeia (2): 120-127.

41. ---- 1961. Ecology and life history of the common frog. Pitman, Lond. 221 pp.

42. Schulze, F.E. 1892. Über die inneren Kiemen der Batrachierlarven. II. Mitteilung: Skelett, Muskulatur, Blutgefässe, Filterapparat, Respiratorische Anhänge und Atmungsbewegungen erwachsener Larven von Pelobates fuscus. Abhandl. Konigl. Akad. Wiss. Berlin, Sonderh. 13: 1-66.

43. Smyth, H.R. 1962. Amphibians and their ways. Macmillan Co., N.Y.

44. Starrett, P. 1960. Descriptions of tadpoles of Middle American frogs. Misc. Pubns. Mus. Zool. Univ. Mich. 110: 1-37.

45. ---- 1968. A phylogenetic comparison of the jaw musculature in the anuran families. Ph.D. thesis, Univ. Mich.

46. Stebbins, R.C. 1951. Amphibians of western N. America. Univ. Calif. Press. Berkeley and Los Angeles.

47. ---- 1966. A field guide to western reptiles and amphibians. Riverside Press, Cambridge, Mass. USA.

48. Stramiński, S. 1956. Vascularization of respiratory surfaces in ontogeny of the edible frog, Rana esculenta. Zoologica Poloniae 7 (3): 327-365.

49. Van Eeden, J.A. 1951. The development of the chondrocranium of Ascaphus truei Stejneger with special reference to the relations of the palatoquadrate to the neurocranium. Acta Zool. 32:41-176.

50. Wager, V.A. 1965. Frogs of South Africa. Purnell, Cape Town.

Table I. The positions of the anterior visceral muscles of Ascaphus, stage 32 of Gosner (10). Motor innervations are parenthesized. Each muscle is identified by a number, preceded by the initial letter of the group to which the muscle belongs.

Edgeworth (8), Rana tadpole	Pusey (38)	Origin-insertion,[1] Ascaphus	No.
MANDIBULAR GROUP			
Levator mandibulae anterior		Absent	
L.m. externus		Absent	
L.m.a. subexternus		Absent	
L.m.a. lateralis		Absent	
L.m.a. articularis	lmam	Pterygoquadrate, dorsal, lateral - Meckeli, dorsal, lateral	M1
L.m. posterior superficialis	lmsm	Auditory capsule, anterior, superficial - rostrale superior lateralis, dorsal	M2
L.m.p. profundus	lmpm	Auditory capsule, anterior, deep - Meckeli, dorsal, anterior	M3
Intermandibularis anterior		Interconnects left and right infrarostrals; no median raphe	M4
Mandibulo-labialis		Absent	
Intermandibularis posterior	ipm	Median raphe - Meckeli, medial	M5
(n. trigeminus)			

HYOIDEAN GROUP			
Orbitohyoideus	ohm	Musculoquadrate - ceratohyale lateralis, lateral	H1.2
Suspensoriohyoideus	shm	Fused with the H1 muscle	
Suspensorioangularis	sam	Pterygoquadrate - Meckeli	H3
Quadratoangularis	qdm	Quadrate anterior, medial - Meckeli, medial	H4
Hyoangularis		Absent	
Interhyoideus	ihm	Median raphe - ceratohyale lateralis	H6
I. posterior		Absent	
(n. facialis)			

BRANCHIAL GROUP

Levator arcus branchialis I	labm	Auditory capsule, lateral - 1, lateral	B1
Constrictor branchialis I	cbm	2, medial - commissure 1,2	B2
Subarcualis rectus I, ? +II	sarm	Ceratohyale medialis, posterior - 1, medial	B3.4
Lev. arc. br. II	labm	Auditory capsule, lateral - 2, lateral	B5
Constric. br. II	cbm	3, medial - commissure 2,3	B6
Subarc. obliquus II[2]	saom	Crista hyoidea - 2, medial	B7
Lev. arc. br. III	labm	Auditory capsule, posterior - 3, lateral	B8
Constric. br. III	cbm	3, medial - 3, lateral	B9
Lev. arc. br. IV	labm	Nucha, superficial - 4, lateral	B10
Diaphragmato-branchialis IV	dbm	Diaphragm, medial - 4, posterior	B11
Subarc. rec. IV, ? +III, ? +V	sarm	1, medial - 4, medial	B12
Transversus ventralis IV	tvm	Median raphe - 4, medial	B14
Subarc. obliq. III	saom	Crista hyoidea - 3, medial	B15
Tympanopharyngeus[3]		Absent	
Subarc. obliq. IV	saom	Crista hyoidea - 4, medial	B16
Branchiohyoideus[4]	bhem	Ceratohyale lateralis, postero-dorsal - 1, lateral	B17

(nn. glossopharyngeal + vagus)

SPINAL GROUP

Geniohyoideus	ghm	Planum hypobranchialis - rostrale inferior	S1
Rectus cervicis	rcm, rcsm	Diaphragm, medial - crista hyoidea	S2
(n. hypoglossus)			

1 Attachments are assumed to be ventral except where otherwise indicated. Arabic numerals refer to the ceratobranchialia.

2 From Pusey (38). Edgeworth (8) calls this muscle the Transversus ventralis II.

3 From Schulze (42).

4 Pusey (38) placed this muscle with the hyoidean group, but it is not innervated by the n. facialis (Gradwell, unpublished). This muscle is absent in Rana (8).

Table II. The function of muscles of the suction and gill ventilation mechanisms. Application of 0.45% KCl in frog Ringer solution was used to depolarize the relevant muscles of pithed tadpoles while the effects of contraction were observed with a dissecting microscope. These effects were also compared with those which occur spontaneously in conscious tadpoles.

Muscle (from Table I)	Function[1]
M1, M3	Adducts lower jaw during snout retraction phase of sucker locomotion
M2	Buckles the anterior rim of the snout during sucker disengagement
H1.2	Depresses the buccal floor during inspiration and during snout retraction phase of sucker locomotion
H3, H4	Abduct lower jaw during snout advancement phase of sucker locomotion
H6	Elevates buccal floor during expiration and during snout advancement phase of sucker locomotion
B1, B5, B8, B10	Elevate lateral aspects of branchial skeleton during pharyngeal constriction
B2, B6, B9, B12	(?) Constrict the branchial clefts and/or branchial arteries
B3.4, B7, B11, B14, B15, B16, B17	Assist pharyngeal expansion

S1	Assists lower jaw abduction during snout advancement phase of sucker locomotion
S2	Assists buccal floor depression during snout retraction phase of sucker locomotion
Dorsales trunci[2]	Abduct trunk and tail during snout advancement phase of sucker locomotion; (?) assist head and tail abduction during sucker disengagement
Rectus abdominis[2]	Adducts trunk and tail during snout retraction phase of sucker locomotion

[1] Pending electromyographic evidence.

[2] From Noble (35).

Table III. Mean values from five Ascaphus tadpoles (stage 32, of Gosner 10).

Size

3.2 cm entire length; 1.0 cm snout-to-vent length

0.280 g weight in air

0.006 g weight in water

64.4 mm^2 area of engaged sucker

Water velocity: turbulent flow needed to cause slipping of sucker

163 cm/sec (140 to 185 cm/sec)

Loading tolerances of sucker (g)

48.3 max. wt. lifted for 3 sec into air

7.79 " " borne underwater by progressive loading (Fig. 18A)

5.79 " " " " for 8 sec (Fig. 18B)

6.79 " " " " " 4 sec (Fig. 18C)

Suction (torr)

min. 0 to -4 (usually -2 to -4)

max. -18 to -25 (spontaneous pumping)

" -125 (manual sucker abduction, living animal)

" -131 (" " " , pithed ")

" -59 (abduction by progressive loading)

" -80 (" " instantaneous ")

Illustrations

Fig. 1. Diagram of the apparatus used for the simultaneous recording of hydrostatic pressures in the sucker and buccal cavities. CAL, calibration; MAN, manometer; P, Perspex disc; PG, connection to Polygraph; PM, pressure monitor; PT, Statham P23BB pressure transducer; S, sealant (Dow Corning Silicones Ltd).

Fig. 2. Diagram of the apparatus used to study the properties of suction and ventilation during exposure of tadpoles to flowing ambient water. A. Monitoring hydrostatic pressures of the oral sucker. B. Determination of the maximum velocity of a turbulent stream which can be withstood without the slipping or disengagement of the sucker. FR, foam rubber; MAN, connection to manometer; PG, connection to Polygraph; PT, Statham P23BB pressure transducer; S, sealant.

Fig. 3. Composite diagrams of cross-sections of the mouth and nares during sucker engagement. A. The valves of the internal nares (NV) and the oral valve (OV) are shown in a closed condition during the expiration phase of the ventilation cycle. B. During inspiration, a greater volume of water is drawn into the buccal cavity (BC) through the mouth than through the nares. As the periphery of the sucker is sealed against the substratum, the progressive pumping of water from the sucker cavity into the buccal cavity, causes the central area of the sucker to become pressed against the substratum. C. The sucker cavity is reduced to the small region outside the mouth. During inspiration, the reduction in buccal pressure opens the internal nares and draws ambient water into the buccal cavity; but the oral valve remains closed because once sucker engagement has been effected, the buccal pressure does not fall below the sucker pressure.

Fig. 4. Pressures recorded simultaneously in the buccal and sucker cavities of a conscious tadpole at 14°C. The graphs show the effect on breathing of switching the lights off (in the case of a light- adapted tadpole, left), and of switching the ligts on (in the case of a dark-adapted tadpole, right). In both cases, the stimulus caused a rise in the pressure curve, which reflects a reduction in the adhesion of the sucker. Calibrations: 1 cm water; 1 sec.

Fig. 5. A composite diagram of sagittal and parasagittal sections through the head of a tadpole with its sucker in a disengaged condition. The valves are shown in their orientations during the beginning of the expiration phase of the ventilation cycle. The large arrow indicates the commencement of flow of buccal water over the ventral velum and into the pharynx. BC, buccal cavity; D, dorsal velum; GC, gill cavity; OV, oral valve; PH, pharynx; V, ventral velum.

Fig. 6. Ventral aspect of the sucker, left, to show the recording positions of sucker pressures monitored along a midline transect, 1 to 7. Dual peaks per ventilation cycle are recorded from the more posterior areas of the sucker (4 to 6). Records first made from area 6 have a shallow notch between the peaks, but the notch usually deepens before ca. 3 min and it is then difficult to distinguish the exact beginning and end of each ventilation cycle. B, beak; LF, lateral flap; P, papillae.

Fig. 7. The effects of sucker engagement (E) and crawling (C) on buccal pressures monitored in a conscious tadpole. Artificial changes in the baseline and distortions of the pressures were avoided by bringing the substratum, a glass cover-slip, into contact with the sucker and not vice versa. Calibration: 1 cm water; 3 sec.

Fig. 8. Buccal and sucker pressures recorded simultaneously in a conscious tadpole. A. The effects of sucker engagement (E) and disengagement (D) are shown. The baseline of the buccal pressures was changed by the need to move the tadpole's sucker into contact with the pressure monitor during engagement, and by the withdrawal of the sucker from the pressure monitor during disengagement. However, the larger amplitudes of buccal pressures when the sucker is engaged than when it is disengaged, are clearly shown. B. A conscious, quiescent tadpole's response to the sudden manual sliding of it on to the pressure monitor and then off again. The sucker was in the engaged condition throughout the recording, but the disturbances still caused increased buccal pumping like that of sucker engagement and like that elicited by forces of abduction (e.g. Fig. 15). Calibrations: 1 cm water; 3 sec.

Fig. 9. Photomicrograph of a parasagittal paraffin section (12 µ thickness) through the oral entrance of an Ascaphus tadpole (stage 32, of Gosner 10). B, beak; BC, buccal cavity; BV, blood vessel; CH, ceratohyale; CRI, cartilago rostrale inferior; CRS, c. rostrale superior; OV, oral valve; S1, musculus spinalis 1; T, nascent tongue.

Fig. 10. Spontaneous repositioning of a conscious tadpole over a pressure monitor. The numbers refer to pressures monitored from areas of the sucker in Fig. 6. Calibrations: 1 cm water; 1 sec.

Fig. 11. Simultaneous recording of the electrocardiogram (top trace) and buccal pressures (bottom trace) from a lightly anesthetized tadpole with its sucker in an engaged condition. The heart beat is out of phase with the ventilation cycle. Calibrations: 1 cm water; 1 sec.

Fig. 12. Photograph of dental inscriptions made directly on photographic film by the manual backward displacement of the engaged sucker. The contact between the beak and the substratum is broken by the median cleft of the beak (cf. Figs. 6, 16).

Fig. 13. A tadpole adhering weakly to the glass of an aquarium just before disengagement of the sucker occurred. The oral valve of the animal had been surgically injured but this did not prevent sucker engagement and adhesion to the substratum for short periods (usually $<$30 sec).

Fig. 14. Profile silhouettes traced from photographs of a tadpole with its sucker disengaged due to repeated manual disturbance (A) and of the stages in crawling while the sucker is in an engaged condition (B to E). AB, abduction (snout advancement); AD, adduction (snout retraction); X, net forward progression during one cycle of locomotion.

Fig. 15. Sucker pressures recorded from a conscious tadpole to show the effect of water velocity on suction and gill ventilation. A, sucker engagement; B, beginning of gentle water flow (47 cm/sec); C, water flow increased to 236 cm/sec; D, plateau reached while the flow was kept at 236 cm/sec, but the average value of the graph is lower than that recorded in still water. Calibrations: 5 cm water; 3 sec.

Fig. 16. Photographs of sucker shapes during abduction from a glass substratum (A to F), and during sucker locomotion (G to I). A. Weak suction of the undisturbed tadpole is shown by the exposed first row of denticles, which is divided by a space in the middle line. B. Increased suction caused by the beginning of abduction is revealed by the first row of denticles which has folded out of sight, behind the beak. The medial areas of the beak have also begun their withdrawal from the glass. C. Further abduction of the tadpole causes further folding of the denticles away from the glass and in toward the mouth. The medial areas of the beak also continue to withdraw from the substratum. D,E. The effects shown in "C" continue during progressive abduction of the tadpole. F. The instant of sucker disengagement. Wrinkles which occur at the edge of the lower lip, break the peripheral seal of the sucker. As water floods the sucker cavity, the denticles reappear. The deformities of the somewhat transparent lateral flaps (LF, Fig. 6) are not visible in the photographs. Visual examination of the lateral flaps during abduction showed that they are also progressively withdrawn from the substratum, but their edges do not lose contact with the substratum before the edge of the lower lip. G. The appearance of the sucker during the rest period between crawling activity of the sucker. The branchial outlet is shown by the arrow. H. Spontaneous opening of the mouth by backward movement of the entire lower jaw and lower lip. I. Strong suction at the end of the adduction phase of sucker locomotion is evident from the first row of denticles which is folded out of sight behind the beak.

Fig. 17. Sucker pressures during disengagement of the sucker by manual abduction of the tadpole from the substratum. A. The arrows indicate negative pressures generated by sucker engagements prior to abduction of the conscious, breathing animal. B. Abductions of a pithed tadpole after engagements of the sucker were effected by manually pressing the suctorial disc against the substratum. Calibrations: 10 torr; 30 sec.

Fig. 18. Sucker pressures during disengagement of the sucker by loading. Abduction of the conscious breathing tadpole from the substratum. A. After attachment of the scale pan (SP), gradual abduction was effected by progressive increments of load (500 mg, 1 to 14). B. The arrow indicates the immediate increase in suction caused by application of 5.79 g which was then borne for 8 sec. C. A weight of 6.79 g (arrow) caused a large increase in suction and was borne for 4 sec. Oscillations of the graphs are pressures transmitted to the sucker from the gill ventilation system. E, sucker engagement; D, sucker disengagement. Calibrations: 10 torr; 3 sec.

Fig. 19. Sucker pressures recorded from three different tadpoles (A,B,C) during spontaneous crawling over the monitor of a pressure transducer. The typical slightly delayed fall of the curves from their peaks is shown by the arrows. This effect is not so well demonstrated in the record from tadpole C. Spontaneous sucker disengagement after crawling is shown in B (left). In A and B (from area 1, Fig. 6) the apparent suspension of ventilation between crawling is partly attributable to the low sensitivity of the recording apparatus in order to monitor the relatively large pressures of crawling. In C, the sudden appearance of small oscillations in the curve was caused by the spontaneous movement of the animal into a more sensitive recording position over the pressure monitor (area 3, Fig. 6). The oscillations demonstrate that rhythmic gill ventilation by the buccal pump occurs between periods of crawling. Gill ventilation and crawling are so well coordinated that during crawling the curves are reinforced without noticeably changing their phase difference. Calibrations: 10 torr; 2 sec.

Fig. 20. Sucker pressures recorded from an uninjured tadpole during spontaneous crawling. Artifacts in some of the troughs were caused by overshoots of the writing pen. B. Sucker pressures from the same tadpole as in graph A, but recorded after the beak and denticles were dissected away. The smooth fall of the pressures from their peaks demonstrates that the dental apparatus (especially the beak) is responsible for the retarding effect on pressures from uninjured tadpoles (arrow, graph A). D, sucker disengagement. C. Sucker pressures (top trace) and buccal pressures (bottom trace) monitored simultaneously in an uninjured conscious tadpole. The restraining effect of the buccal cannula increases the difficulty of simultaneous recording from the buccal cavity and sucker during crawling activity. Therefore these graphs were the only ones made during many hours of recording. Three incidents of crawling activity caused spontaneous movement of the animal to a less sensitive recording position, which is reflected in the smaller amplitudes of the graphs after the third crawl. The movement also displaced the buccal pressure baseline. Calibrations: A,B: 5 torr, 3 sec; C: 10 cm water, 1 sec.

Fig. 21. Front views of a tadpole showing spontaneous disengagement of the sucker by a buckling of the anterior rim of the snout during contraction of the M2 muscles (arrows).

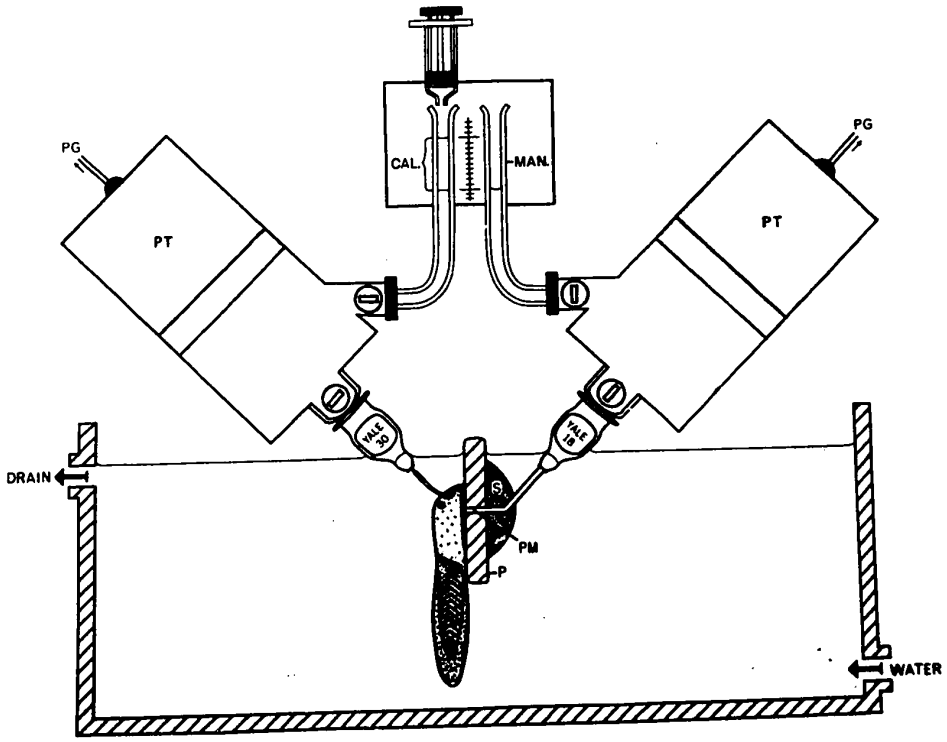
Fig. 22. Buccal pressures recorded during recovery from deep anesthesia of two tadpoles. Intermittent hyperexpirations show the capacity of the buccal pump for unusually large compression relative to the much smaller amplitudes of normal ventilation. Calibrations: 10 cm water; 20 sec.

Fig. 23. The effect on sucker pressures of protrusion of the external nares above water. Narial protrusion (P) causes a reduction in ventilation frequency and an increase in its amplitude. The protrusion also causes a progressive general increase in suction, but this may be a consequence, at least to some extent, of the change in depth of the tadpole during the experiment. Probably for the same reason, on submergence (S) of the nares, there is a gradual reduction in suction until the stable normal level is reached. A. Recording made from area 5, Fig. 6. B. Recording made from area 6, Fig. 6. Calibrations: 1 cm water; 1 sec.

Fig. 24. Pressures (PR) recorded simultaneously from the buccal cavity and pharynx of a lightly anesthetized tadpole. In each ventilation cycle, the first pharyngeal pressure peak is the transmitted effect of the buccal force pump. The second pharyngeal peak is caused by constriction of the pharynx during its occlusion from the buccal cavity by the ventral velum. Calibrations: 1 cm water; 1 sec.

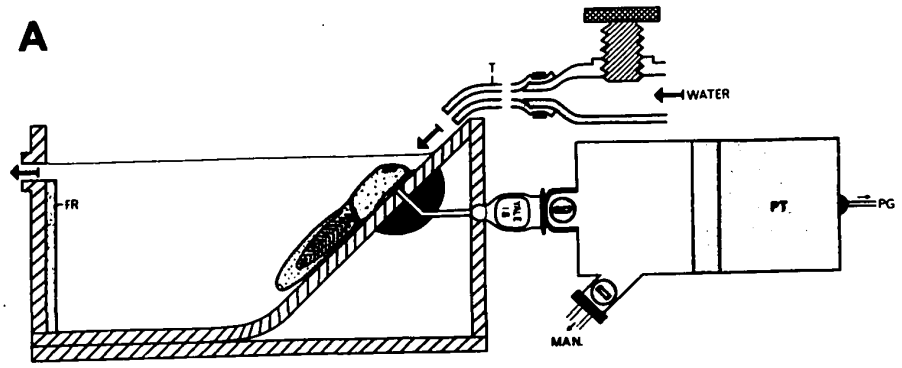
Fig. 25. Pressures (PR) monitored simultaneously from the buccal and gill cavities of an anesthetized tadpole. Branchial pressures remain positive throughout each ventilation cycle, ensuring a continuous branchial outflow. A. The arrows indicate slight evidence of the operation of two alternate force pumps. In lightly anesthetized and conscious tadpoles these pumps are usually so well coordinated that they produce single peaks in the branchial pressures. B. The recording of dual branchial pressure peaks during deep anesthesia, when the force pumps are not so well coordinated. Single branchial pressure peaks were gradually restored as the tadpole regained consciousness. Calibrations: 1 cm water; 1 sec.

1

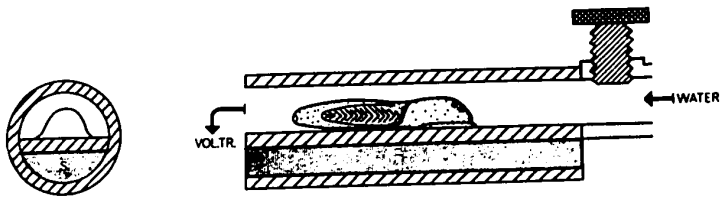

PG
PT
CAL.
MAN.
PG
PT
VALVE 2D
VALVE 1B
DRAIN
S
PM
P
WATER

2

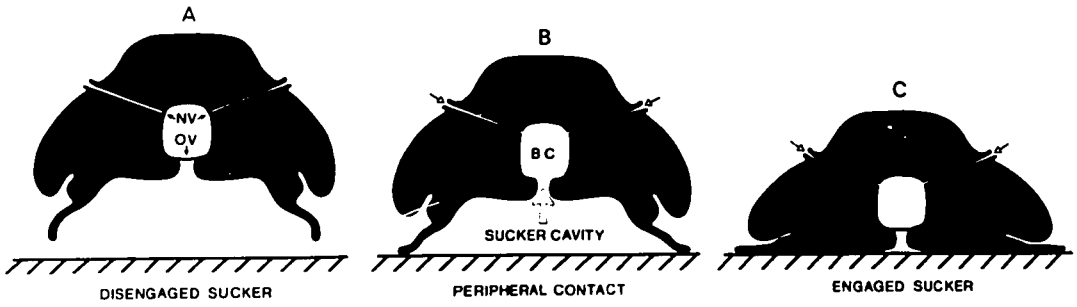
A


T
WATER
FR
PT
PG
MAN.

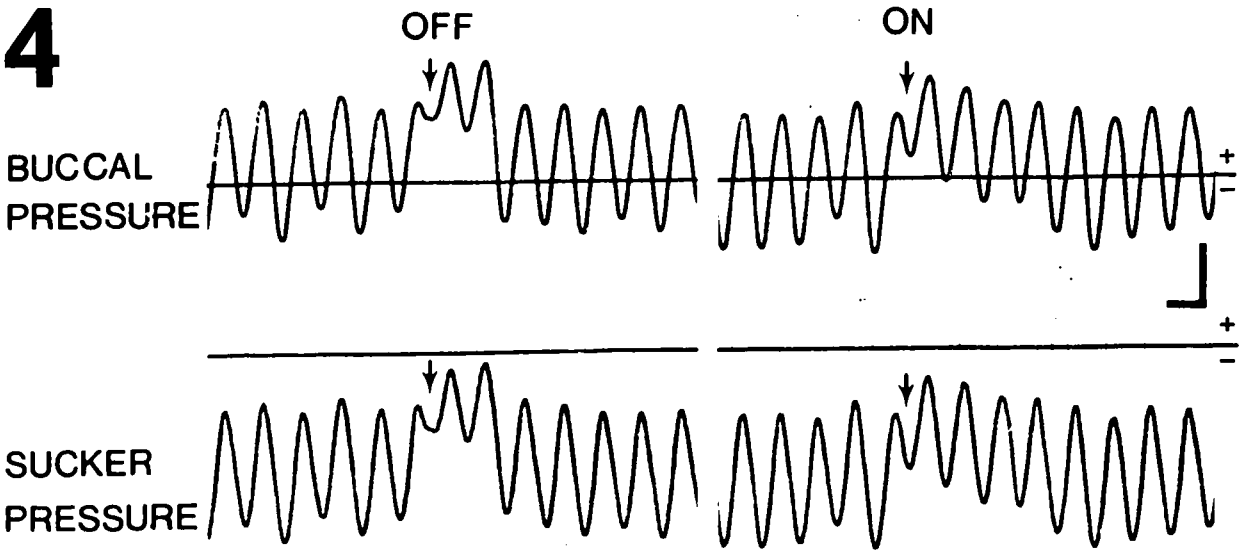
B


WATER
VOLTR.

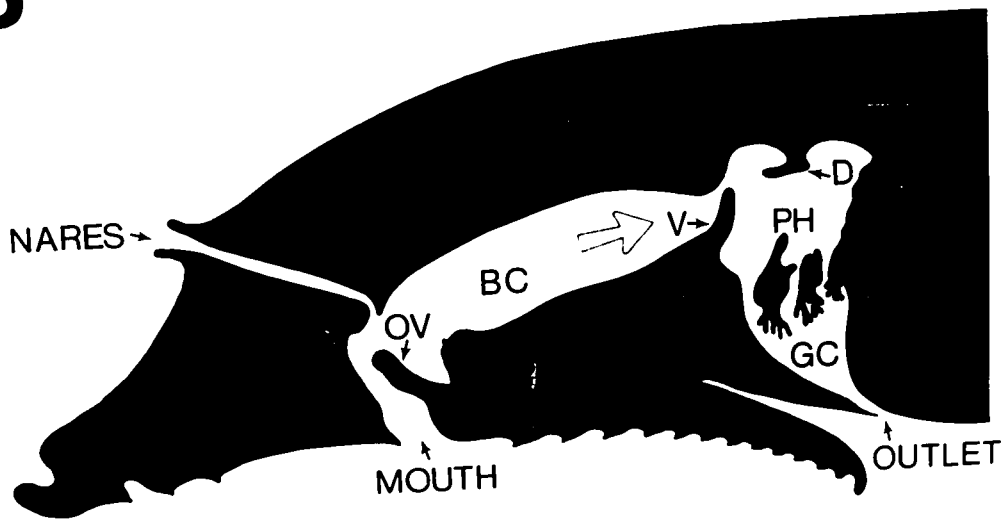
3


A
NV
OV
B
BC
SUCKER CAVITY
C
DISENGAGED SUCKER
PERIPHERAL CONTACT
ENGAGED SUCKER

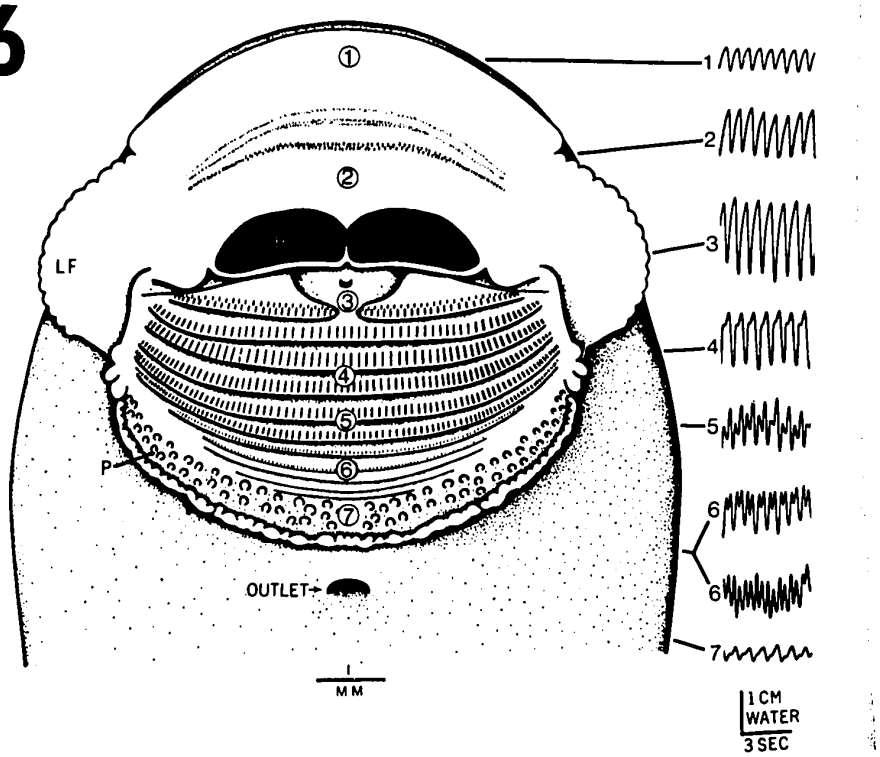
4


OFF
ON
BUCCAL PRESSURE
+
−
+
−
SUCKER PRESSURE

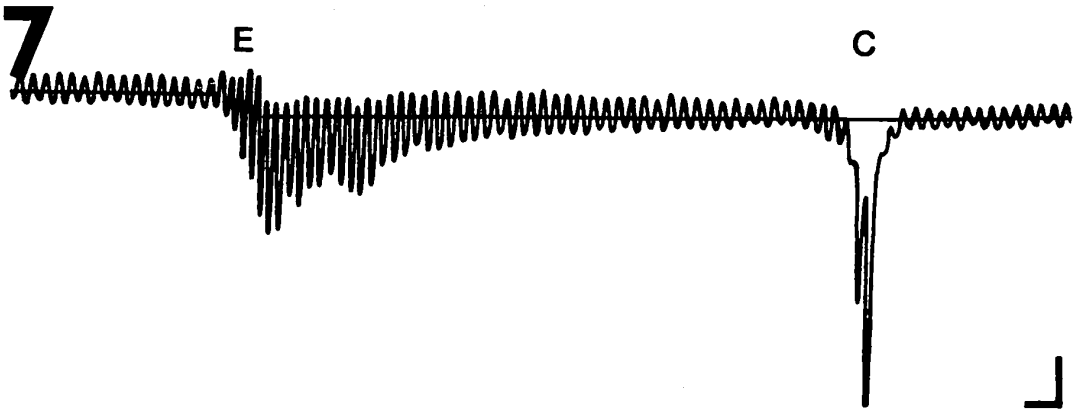
5


NARES
BC
V
D
PH
OV
GC
MOUTH
OUTLET

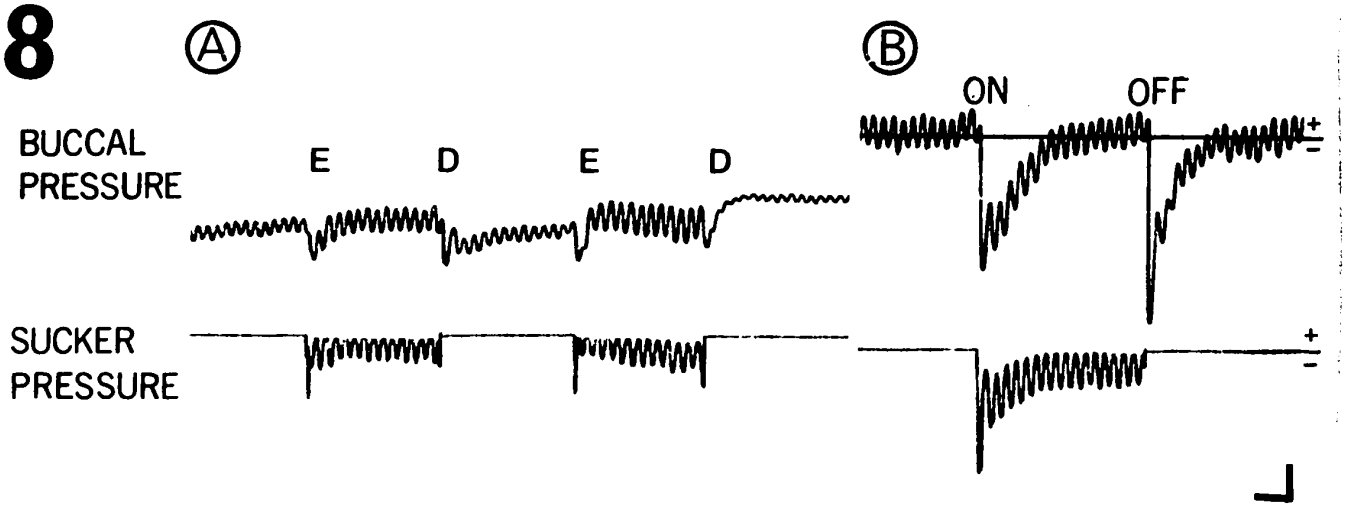
6

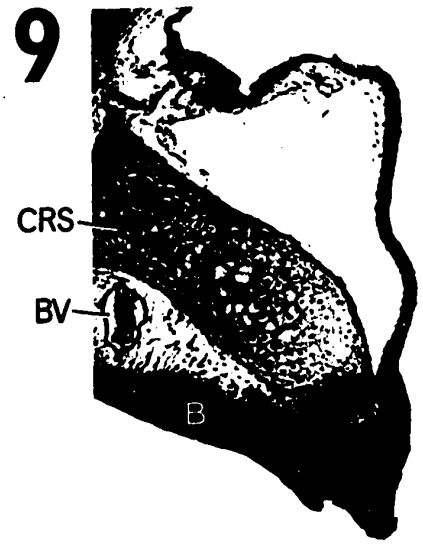

①
②
③
④
⑤
⑥
⑦
LF
P
OUTLET
1
2
3
4
5
6
6
7
1 MM
1 CM
WATER
3 SEC

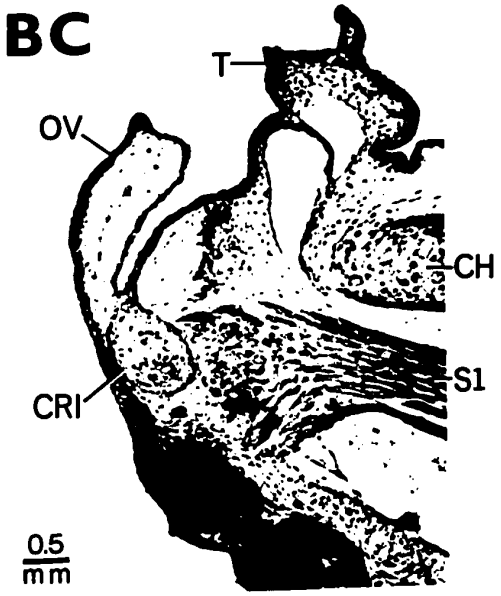
7

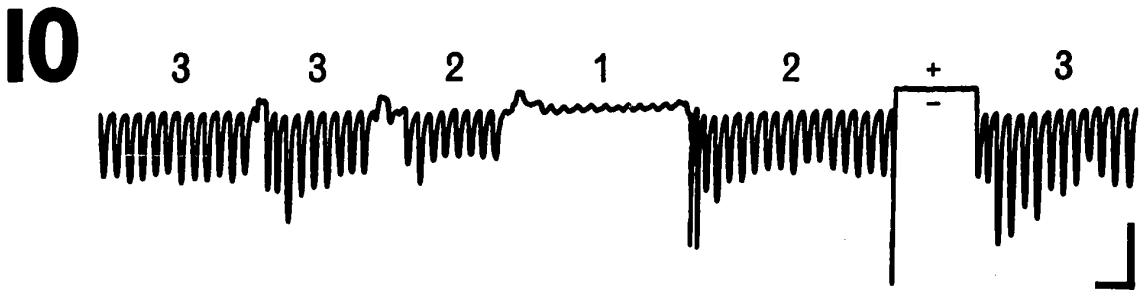

E
C

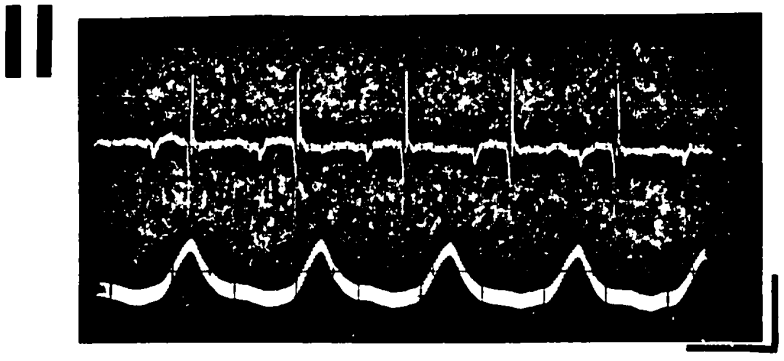
8


Ⓐ
Ⓑ
BUCCAL PRESSURE
SUCKER PRESSURE
E
D
E
D
ON
OFF
+
−
+
−


9
CRS
BV
B

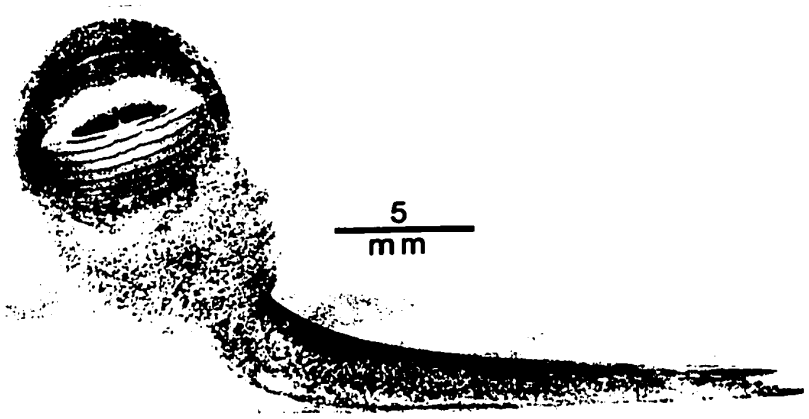

BC
T
OV
CH
S1
CRI
0.5
mm


10
3
3
2
1
2
+
−
3

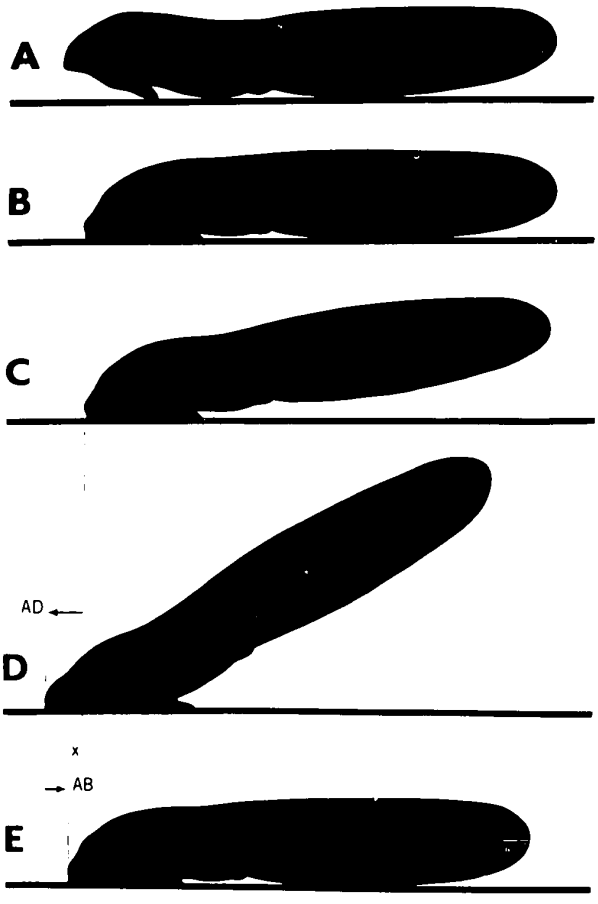

11

12

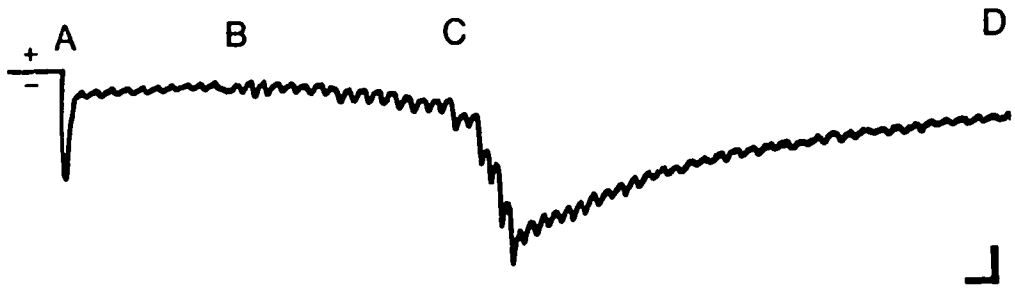
13



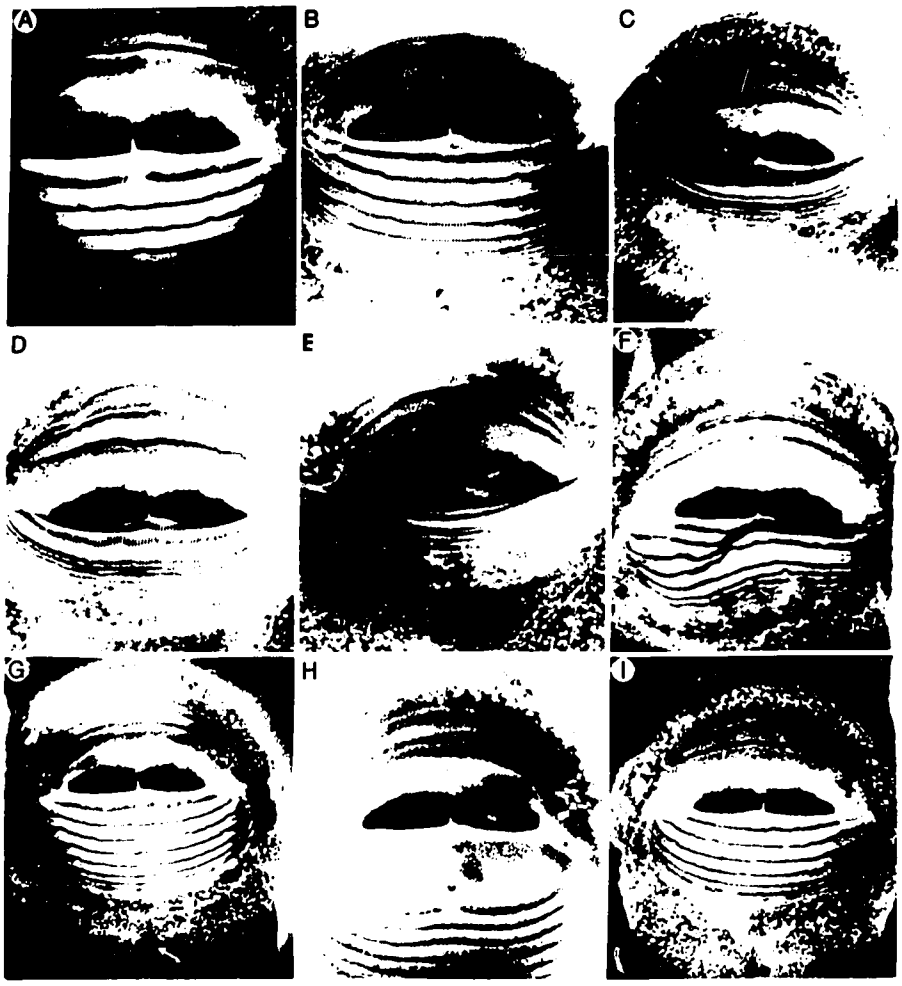
14



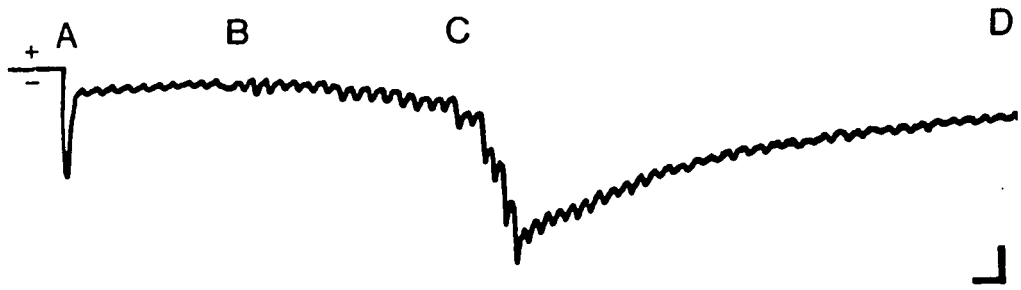
15



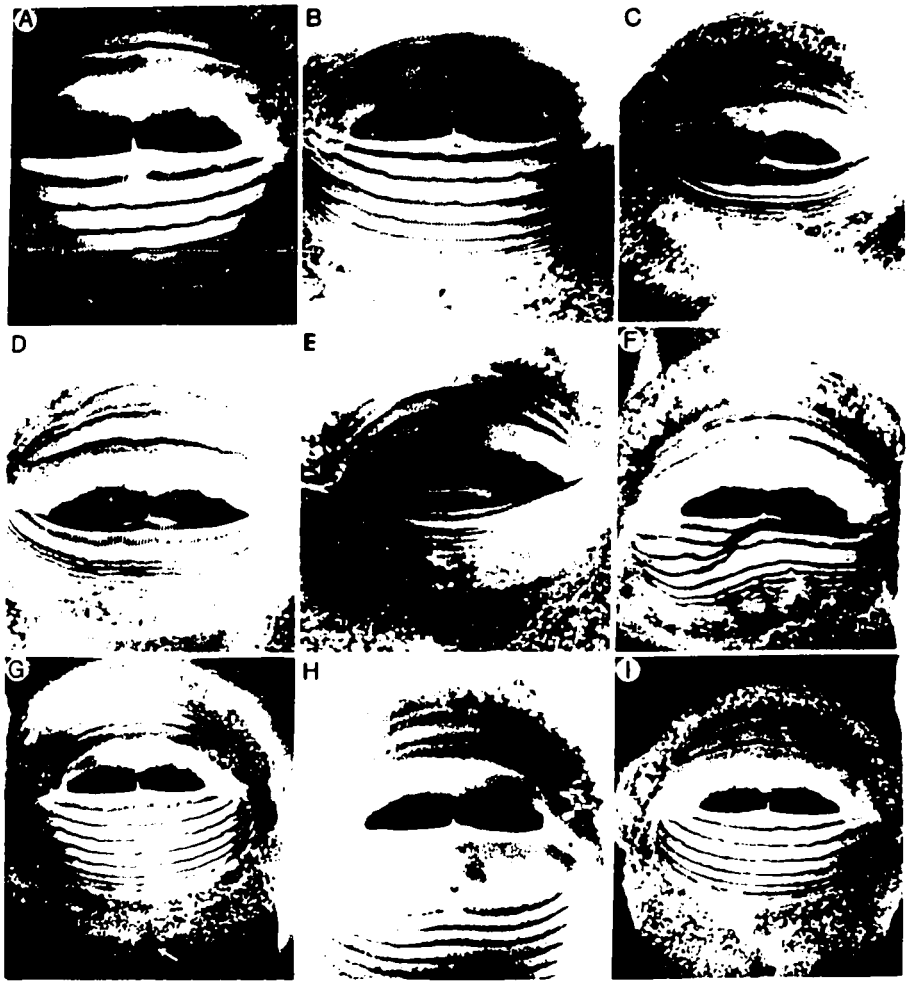
16



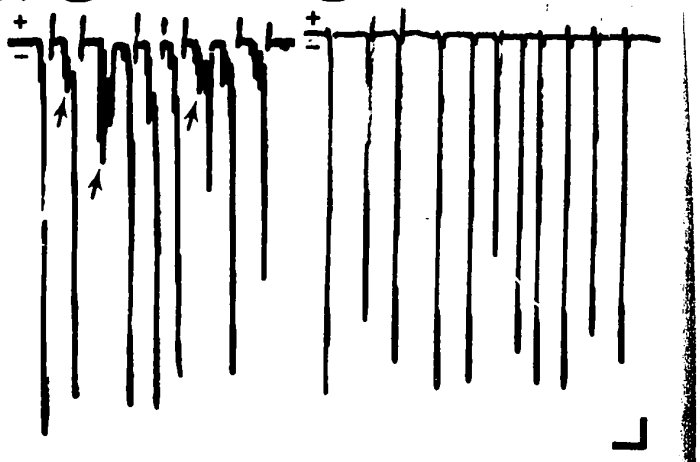
15



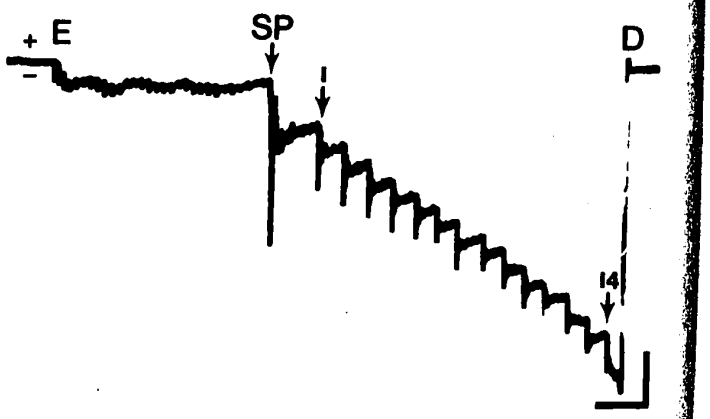
16



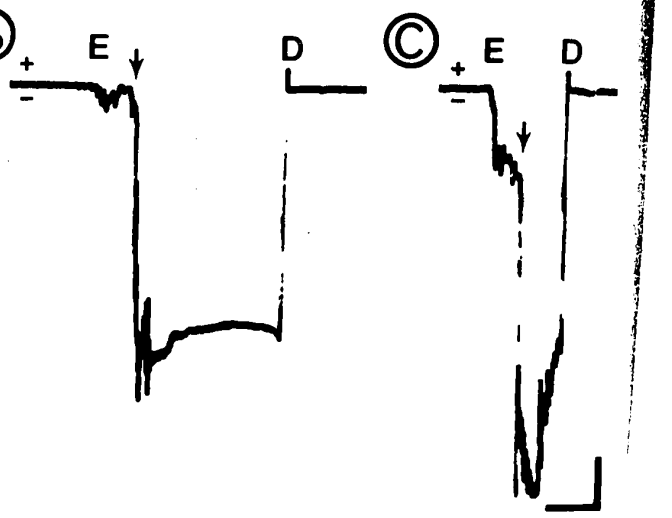
17Ⓐ Ⓑ



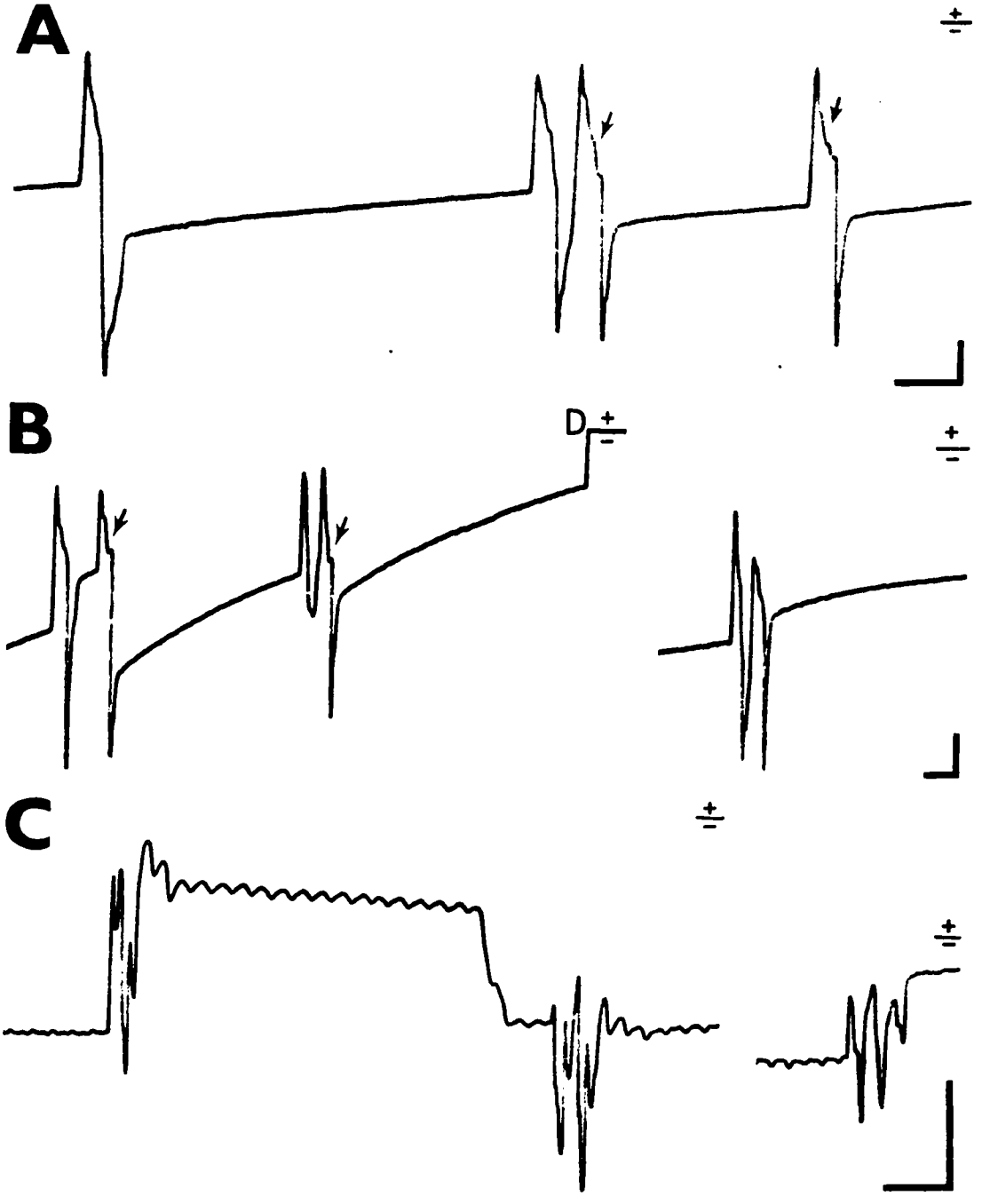
18Ⓐ



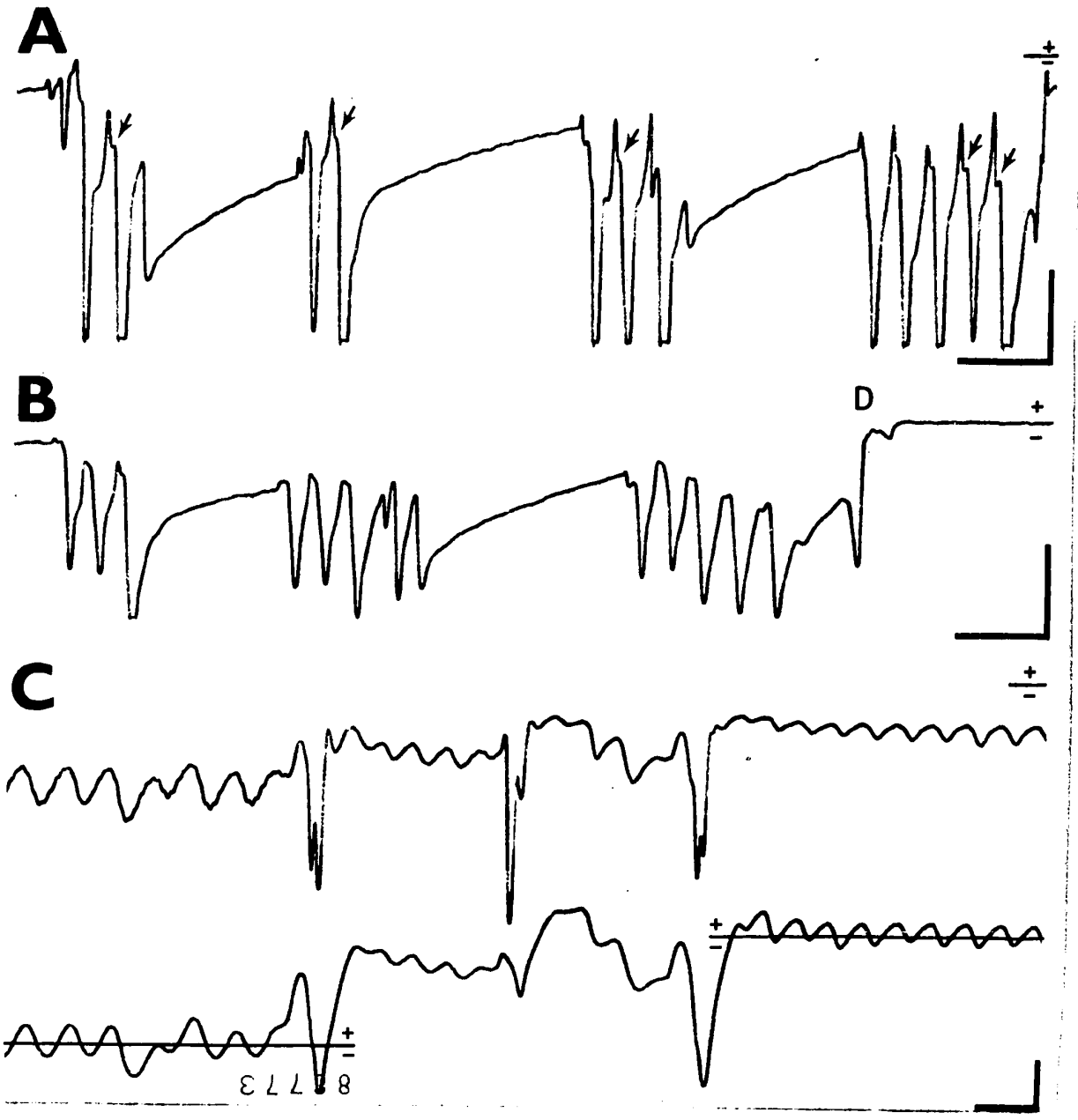
Ⓑ Ⓒ



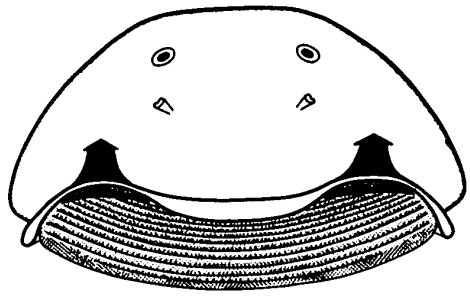
19 A



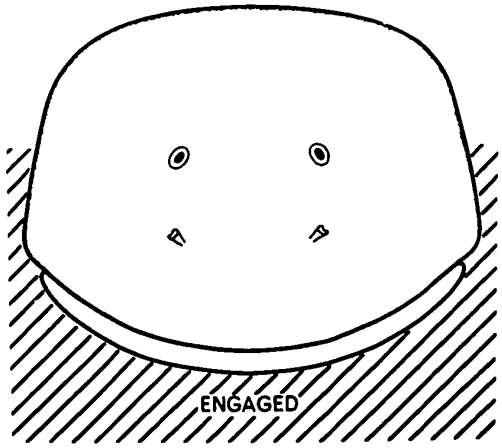
20



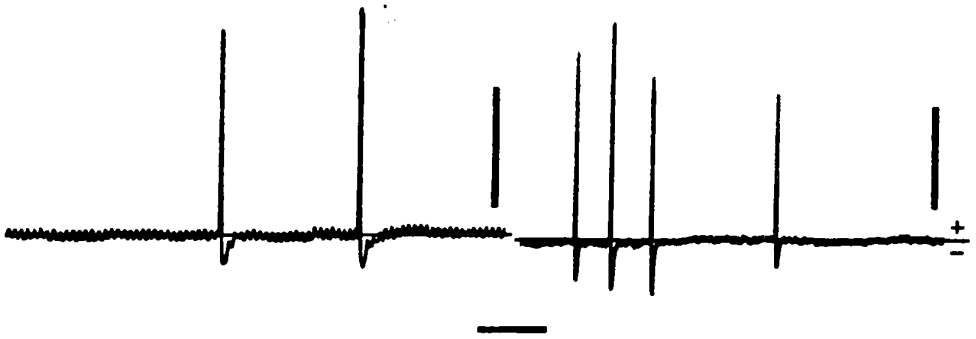
21



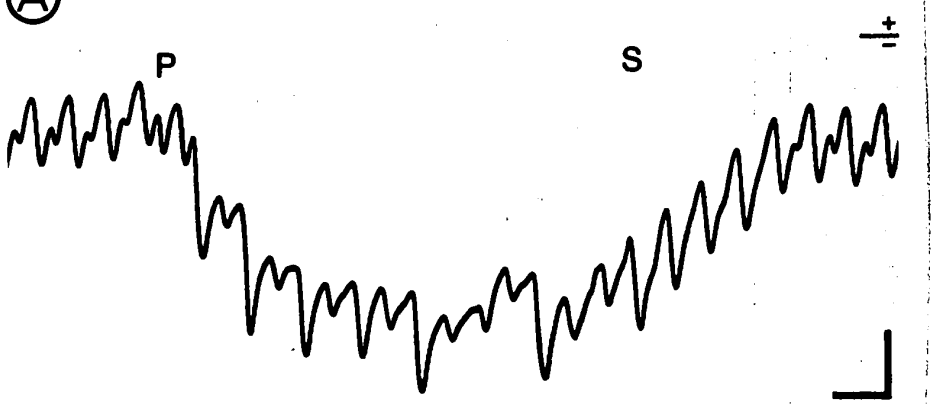
LIFT-OFF



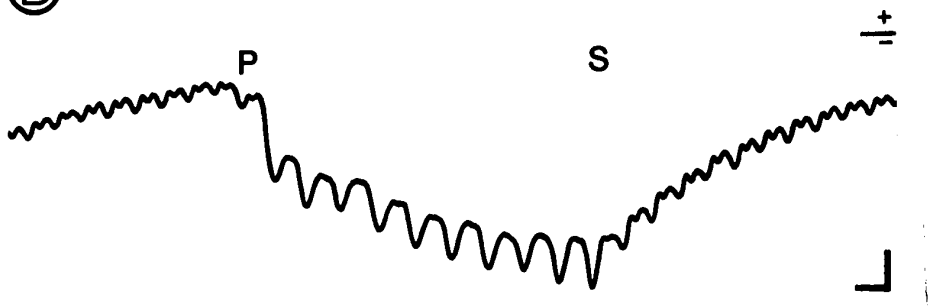
22



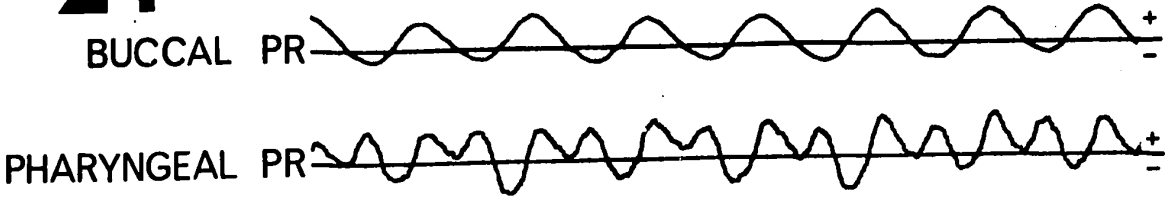
23 Ⓐ


P
S

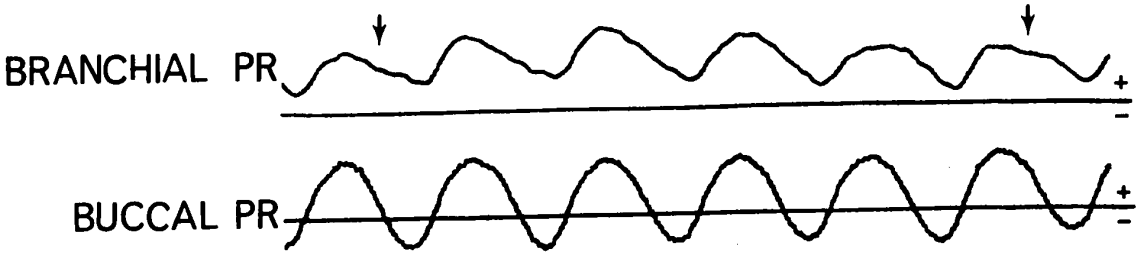
Ⓑ


P
S

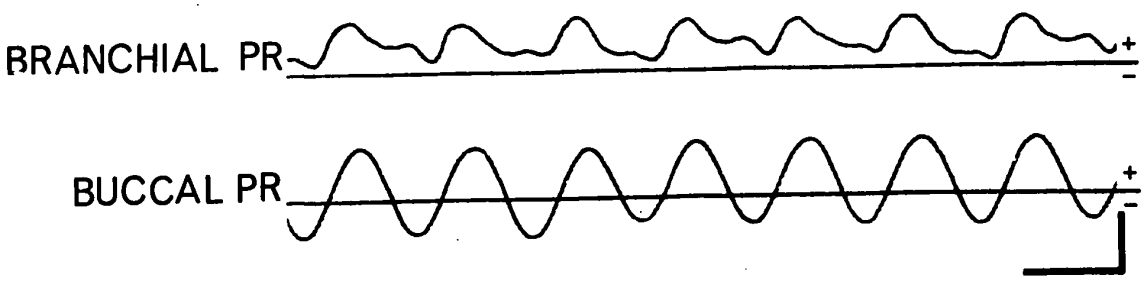
24


BUCCAL PR
PHARYNGEAL PR

25 Ⓐ


BRANCHIAL PR
BUCCAL PR

Ⓑ


BRANCHIAL PR
BUCCAL PR

CHAPTER 5

THE WATER PUMPING MECHANISM OF *XENOPUS*

ABSTRACT. Regular phasic water pumping normally occurs only during feeding in *Xenopus* tadpoles, but it can be induced by light anesthesia and sometimes also by restraining conscious tadpoles from swimming, when they are not feeding.

The peculiar simplicity of the water pumping apparatus is associated with an equally peculiar simplicity of the mechanism whereby water is rhythmically pumped through the animal. The jaws have neither two phases of opening nor the ability to protrude. No evidence was found to indicate the segregation of muscle fibers in the pumping muscles into different structural and functional groups. Nevertheless, tadpoles are capable of hyperexpiration when they are deeply anesthetized or when India ink is pipetted into the oral intake; no evidence of hyperinspiration was found.

The absence of a valvular ventral velum makes *Xenopus* unique among the Anura and it necessitates the presence of only a single force pump in front of the pharyngeal clefts. *Xenopus* also does not have an auxiliary pressure pump behind the pharyngeal clefts, in the opercular cavities. The cyclic functioning of the passive opercular valves is controlled by hydrostatic pressure alone. Closure of the pharyngeal clefts is effected by special muscles during intermittent hyperexpirations, when contaminated water is expelled, first through the mouth and then sometimes also through the opercular outlets, as the cleft constrictors relax.

C O N T E N T S

Introduction

The peculiarities of the tadpole of Xenopus laevis, the common South African clawed frog, were emphasized in a doctoral thesis submitted to McGill University by Paul Weisz. His views were also expressed in a published version of the thesis (1945, p. 163): "So unique are the peculiarities of the Xenopus tadpole that the latter, from an academic viewpoint, becomes one of the most interesting vertebrate forms."

Aside from Rana, the cranial morphology of Xenopus is better known than that of any other species of anuran tadpole (Parker 1876; Beddard 1894; Ridewood 1898; Bles 1905; Dreyer 1915; de Villiers 1932; Kotthaus 1933; Edgeworth 1930, 1935; Paterson 1939a, 1939b; Millard 1945; Weisz 1945; Smit 1953; Nieuwkoop and Faber 1956; and Sedra and Michael 1957. In these researches, the general anatomy of the water pumping apparatus is described sufficiently well to permit an examination of the pumping mechanism of this genus.

Young Xenopus larvae have external gills, but these gills degenerate when the operculum grows back from the hyoidean arch, and a set of true internal gills in gill cavities does not occur in Xenopus tadpoles (Beddard 1894, Bles 1904, Dreyer 1915, and Nieuwkoop and Faber 1956). This absence of gills in Xenopus tadpoles caused Beddard (1894) to ascribe a ventilatory function to the rugulose lining of the pharynx. Bles (1905) pointed out that Beddard had overlooked the respiratory function of the lungs and that if some oxygenation of the blood does occur in the pharyngeal lining, it is apparently subsidiary to the function of the lungs. Nevertheless, Xenopus tadpoles rhythmically pump water through the buccopharynx and opercular cavities and although this

activity seems more important for filter feeding, it is still possible that the blood of tadpoles is simultaneously oxygenated to some extent by this pumping. In support of this view is the observation by Dreyer (1915, p. 243): "The lungs are developed and supplied with pulmonary arteries and veins at a very early stage, but they remain sacs with such delicate walls that it is not probable that they are the only organs active in respiration. Also, the tadpoles do come to the surface to swallow air occasionally, but they can remain under water for such long periods that the air cannot be their only source of oxygen." Dreyer also showed that the rugulose lining of the pharynx is richly vascularized.

For want of functional data, the state of knowledge until 1945, on the oxygenation of blood in Xenopus tadpoles, was based largely on anatomy and it is best stated by Millard (1945, p. 226): "Blood from the filter apparatus is returned to the venous system and not to the aortic arches. In spite of this fact Beddard (1894) and Dreyer (1915) both ascribed a respiratory function to the filter apparatus in addition to its obvious one of filtering food material from the outgoing water current. This theory is favoured by the author on the grounds of the intense vascularisation of the filter apparatus. It is supported by the following facts: (a) internal gills are absent; (b) the arteria cutanea magna does not appear until metamorphosis; and (c) the lungs, though present from an early stage, are simple, undivided sacs and do not appear capable of playing any important part in respiration." Moreover, Weisz (1945) contributed some functional data (p. 188): "Tadpoles 7 days or older, when prevented for even an indefinite period to come to the surface of the water to use their lungs, stay perfectly normal and alive; since there are no external gills at this

advanced stage, the internal gill system[1] alone fulfills all respiratory requirements."

These views tend to favor a respiratory role of the rugulose lining of the pharynx. The definitive approach to this problem would seem to be through an analysis of blood gas content, but the smallness of Xenopus tadpoles suggests that there are technical difficulties in this approach. Analysis of water gas content before and after it is pumped through tadpoles may be technically easier. Perhaps the most feasible approach is to monitor the effects on pumping, of ambient water of known gas content. Light may be shed on the bearing of the phasic water pumping mechanism on respiration, if it can be shown that at constant temperature, the frequency or amplitude of water pumping is related to the oxygen content of the ambient water. Experiments of this kind may be assisted by the present broad study of the water pumping mechanism of Xenopus tadpoles. The present research also calls attention to several anatomical and functional peculiarities of Xenopus tadpoles for comparison with Rana and Ascaphus (see Chapter 6).

---

[1] refers to the rugulose lining of the pharynx, which Weisz also calls "pseudo-internal-gills".

Materials and Methods

The normal developmental table of Nieuwkoop and Faber (1956) will be used in the present study for staging the larvae of Xenopus laevis Daudin.

Adults of X. laevis were induced to ovulate by injections of frog pituitary extract into the dorsal lymph sac. Larvae were raised from eggs fertilized with sperm from extirpated testes, and hatched in stream water at 22°C. On the fifth day after hatching, when the mouth and operculum had developed (stage 46) and water pumping had begun, filter feeding was encouraged by transferring the tadpoles to stream water made slightly turbid with suspensions of dried baker's yeast. Feces were seen emerging from the cloaca ca. 30 min after commencement of feeding. The tadpoles were fed daily by this method and their behavior during laboratory rearing was like that of normal tadpoles observed in their natural habitat.

Experiments were performed on 26 larvae reared to stage 52 (snout-to-vent length, 14 mm). Tadpoles were anesthetized in 0.5% urethane (10 min at 22°C), which rendered them insensitive to light surgery. After surgery, individual tadpoles were transferred into 0.25% urethane for study of their functional anatomy during phasic water pumping. Indicators and streamers (see Chapter 3) were used to observe water flow during normal pumping and after lesions to ventilatory muscles were made. Microscopic observations on uninjured, anesthetized tadpoles and on unanesthetized, unrestrained tadpoles were also made. The smallness of the animals frustrated attempts at electromyography, but the lack of skin pigmentation was an aid to the study of the actions of the main pumping muscles in uninjured tadpoles.

For hydrostatic pressure recordings, cannulae of polyethylene tubing (PE10, Clay-Adams Inc. N.Y.) were constructed and implanted after anesthesia of individual tadpoles, by the method of Gradwell and Pasztor (1968). Implantation sites were mid-dorsal, between the nares, and posterolateral, behind and below the eye, where the cannula was passed through part of a thin sheet of muscle, the "constrictores branchiales" of Sedra and Michael (1957). The cannulae were connected to P23BB venous pressure transducers, the outputs of which were fed into a Gilson Polygraph (CH-CBPP) for amplification and recording by rectilinear pens. Calibration was performed as described by Gradwell and Pasztor (1968).

Results

Nomenclature

The morphology of the water pumping apparatus of the tadpole of Xenopus has been documented well enough to provide a basis for a functional interpretation of it in the present study. In particular, the publications of Weisz (1945), Nieuwkoop and Faber (1956), and Sedra and Michael (1957), should be consulted for morphological details, as frequent reference will be made in the present study to the morphology of tadpoles. To prevent the description from becoming clouded by lengthy Latin terminology, the same abbreviations will be used for the cartilages of Xenopus as those in Chapter 1 of the present thesis.

A numerical terminology has been proposed for the muscles of Rana and Ascaphus (Chapters 1 and 4), and it has been found to greatly facilitate the expression of functional themes in Chapters 3 and 4. For the same reasons given in Chapter 1, the muscles of the Xenopus tadpole are also numbered in the present study, on the basis of motor innervation. However, this practice is not intended to imply homology between the muscles of Rana, Ascaphus, and Xenopus, except to the extent of the primordial muscle plates, which are innervated by the nervi trigeminus, facialis, glossopharyngeus and vagus, and hypoglossus. On the other hand, the sequence of muscles in each major group of these genera, has been so arranged as to indicate analogies between the genera, as far as present knowledge permits. The numerical terminology proposed for Xenopus is shown in Table I.

The M1, M2, and M3 muscles are fused together at their origins, but they become separate fasciculi anterior to the processus muscularis quadrati and they have independent insertions.

Moreover, the M3 muscle is functionally independent of the M1 and M2 muscles (p.252). On these grounds, the three fasciculi are here regarded as individual muscles. These muscles arise on the posterior pterygoquadrate, while the M4 muscle arises on the anterior pterygoquadrate. However, Sedra and Michael (1957) have called the M1, M2, and M3, the "Levator mandibulae anterior", and they have called the M4, the"l.m. posterior". This confusion between anterior and posterior, might have resulted from the attempt by these authors to adapt Edgeworth's terms (1935) to the somewhat aberrant Xenopus. The absence of the M6 and M7 muscles in Xenopus may be associated with its simple jaw mechanism (p.252) relative to Rana, in which the M6 and M7 muscles are well-developed.

Sedra and Michael (1957) have proposed that their "Quadrato-hyoangularis" represents a fusion of the Suspensorio-, Quadrato-, and Hyoangulares of Rana. Until this proposal is investigated in detail, the Quadratohyoangularis of Sedra and Michael is assigned the numbers 3, 4, and 5 of the hyoidean group in Table I. The H7 muscle of Rana is not present in Xenopus at any stage of development.

The need for a new nomenclature for the visceral muscles of Xenopus is shown by the confusion generated by the use in Xenopus (Sedra and Michael) of the terms "Constrictor branchialis" and "Subarcualis rectus" for Edgeworth's terms (1935) "Levator arcus branchialis" and "Constrictor branchialis" respectively. In addition, the "Levator arcus branchialis IV" of Sedra and Michael would seem to be the "Tympanopharyngeus" of Pelobates (Schulze 1892) and of Rana (Chapter 1). The use of these two terms for

what is probably the same muscle, is a consequence of the double standards that have existed in the naming of muscles. The reasons for abandoning both these standards in favor of a numerical system, are presented in Chapter 1.

The water pumping mechanism

In the absence of suspended food, Xenopus tadpoles swim about restlessly, opening their mouths periodically to sample the nature of the water. During this searching behavior, the water pumping mechanism is not rhythmically active, but as soon as tadpoles encounter food, such as clouds of yeast suspension, they assume a head-down, tail-up orientation, and begin phasic water pumping. Filter feeding continues incessantly except for short dashes of the animal to and from the water surface for lung ventilation. The greater musculature of the tail is utilized for these dashes, which occur at 3 to 7 min intervals. However, during feeding, only the tip of the tail vibrates rapidly, and if it is amputated, the animal is unable to resume its normal feeding posture. Similarly, this posture could not be adopted by tadpoles which were prevented from inflating their lungs. It appears that the typical feeding posture of Xenopus depends on a delicate balance between the buoyancy of the inflated lungs and the downward propulsion afforded by the vibrating tip of the tail. The animal is thus able to vary its depth gradually, simply by varying the intensity of tail vibration. Amputation of the tail tip caused the animal to immediately rise passively to the water surface and float there, which proves the buoyancy of the lungs, for if they were then punctured, the tadpole sank passively to the bottom of the aquarium.

1. The jaw mechanism

The dental apparatus of anuran tadpoles has long been used as a reliable taxonomic criterion. As pipids, Xenopus tadpoles are characterized by the complete absence of labial denticles and keratinized beaks. In addition, the mouth is wider (3.8 mm) relative to the size of the head, than that of all non-pipid tadpoles. The head of Xenopus tadpoles is itself wider than the trunk of the animal. The gape is therefore considerable for a tadpole of this size, and like that of manta rays and baleen whales, it is probably an adaptation to filter feeding.

Opening

The opening of the mouth and the depression of the buccopharyngeal floor occur together. Protrusion of the jaws is not possible in Xenopus because the joint between the Meckelian and infrarostral cartilages is not flexible like it is in Rana. In addition, the cartilago Meckeli-quadrate joint has only one degree of freedom, which permits the rotation of Meckel's cartilage solely in the parasagittal plane. The jaw abductor, H3.4.5, is inserted ventrolaterally on Meckel's cartilage, medial to the base of the tentacle but lateral to the insertion of the M10 muscle. Contraction of the H3.4.5 muscle, therefore, can produce only a downward swing of the lower jaw.

Lesions to the bilateral moieties of the H3.4.5 muscle were made to test the hypothesis that it is the only jaw abductor during phasic pumping: normal lower jaw abduction was abolished. However, a wide opening of the lower jaw was intermittently caused during gasping movements. It seems that the S1 muscle is responsible for this intermittent abduction, for it no longer occurred after the S1 muscles were also severed. The buccopharyngeal floor was still rhythmically depressed, and as the

lesions prevented the simultaneous opening of the mouth, the only water inlets were the nares. Rapid elevation of the buccopharyngeal floor then caused such a great increase in the hydrostatic pressure of the buccopharynx that the mouth was forced open and some water was expelled through it. During this abnormal behavior, the jaw adductors might have relaxed, whereas in phasic pumping they (or some of them) contract during buccopharyngeal compression and thus close the mouth tightly. Therefore water is forced caudad and through the gill clefts because valves of the internal nares close at this time. The lesions to the H3.4.5 muscle showed that contraction of this muscle is the only normal way that tadpoles have of jaw abduction during buccopharyngeal depression. They are, however, still able to abduct their jaws by first drawing water into the buccopharynx through the nares and then vigorously compressing the buccopharynx. The resultant positive hydrostatic pressure then forces the mouth passively open, possibly because the jaw adductors do not actively oppose this opening movement.

Closure

The closing of the mouth occurs more rapidly than its opening. All or some of the jaw adductors, M1, M2, M4, and M5, cause phasic closure of the lower jaw. It was not possible to detect contractions of individual muscles, but it was definitely seen on repeated occasions that the mouth normally closes before the end of the elevation stroke of the buccopharyngeal floor. There is sometimes a slight oral reflux just before the mouth closes, but this was the exception rather than the rule. It is therefore likely that mouth adduction can occur even before compression of the buccopharynx begins with elevation of its floor.

During none of the movements of the lower jaw is there any independent or participatory movement of the upper jaw; opening and closing of the mouth is possible only through movements of the lower jaw.

The oral tentacles play no part in the normal phasic movements of the mouth. When the turbidity of the water begins to clear through the filtering behavior of tadpoles, they gradually increase the depth of their filtering stations. Eventually, when the ambient water has been cleared of suspended yeast or other food material, tadpoles begin to stir up such organic particles that are present on the substratum. The agitation of the substratum on these occasions was seen to be performed by the caudad flicking of the oral tentacles when they contacted the substratum. The M3 muscle, which is inserted near the base of the tentacle, is relatively superficial and it was therefore possible to see its spontaneous contraction through the transparent overlying skin during these flicking movements. There is no antagonistic muscle for the return of the tentacle to its resting position. However, manipulation of the tentacle after the M3 muscle was severed, disclosed that the elasticity of the tentacular cartilage was able to return the tentacle to its resting position after the force causing caudad displacement of it was released. The spontaneous flicking of the tentacles occurs so intermittently and rapidly that it was not possible to correlate this phenomenon with mouth movements.

2. The buccopharyngeal mechanism

When the hyobranchial apparatus of Xenopus is examined from the dorsal aspect, one is immediately struck by the absence in these tadpoles of the flap-like ventral velum that is present in all non-pipid tadpoles. However, the histological studies of Kratochwill (1933) and of Kenny (1969a, 1969b) have shown that the underside of the ventral velum of anuran tadpoles is glandular. On either side of the mid-dorsal line of the hyobranchial apparatus of Xenopus there is a structure (the "pharyngo-branchial tract" of Weisz 1945) whose position in relation to the branchial skeleton, and whose glandular nature, justify calling it the ventral velum.

From extensive observations of uninjured, conscious tadpoles, and of partly dissected, anesthetized tadpoles which were cyclically pumping water, it was postulated early in the present research, that the ventral velum of Xenopus is non-valvular. This postulate was then tested by recording hydrostatic pressures simultaneously from the fore and hind parts of the pumping system. The sites of cannula implantation were chosen to ensure that if a straight line was passed between them, it would cross over the ventral velum. Therefore, the pressures recorded by the cannulae of the transducer apparatus, may be regarded as those of the water before and after it had passed over the ventral velum.

Figure 1 shows that the hydrostatic pressures monitored anteriorly and posteriorly in the tadpole are in phase with each other. Both graphs have a single peak to each cycle of pumping; the absence of dual peaks in the graph monitored posteriorly, immediately discloses the absence of a valvular ventral velum in Xenopus, which is consistent with the functional anatomy of these tadpoles. The amplitude of the pressures monitored posteriorly is about 30% lower than that of the pressures monitored anterior

to the ventral velum, which is to be expected for the unidirectional flow of water, anterior-to-posterior, through the animal. This fall in amplitude may be attributed to the greater volume of the posterior parts of the system than of the region nearer to the anterior cannula. In addition, some frictional resistance is probably encountered by the water in its course backward. The above results reveal that although there is a phasically active musculature which constricts the posterior parts of the pumping system, and although it is apparently active just after compression of the anterior parts of the system, it cannot be regarded as an independent pump because the ventral velum does not act as a valve.

The confirmation of Kratochwill's view (1933) that there is a valvular ventral velum between a buccal pump and a pharyngeal pump in Rana tadpoles, gave rise to the proposal (Gradwell and Pasztor 1968) that the buccopharynx of this genus be regarded as two functionally distinct chambers, the buccal cavity and pharynx. From a functional point of view, perhaps the most important peculiarity of Xenopus is its lack of a valvular ventral velum. Therefore, it is suggested that the classical term of "buccopharynx" be retained for Xenopus tadpoles.

Buccopharyngeal compression

During cyclic water pumping under the influence of light anesthesia, compression of the buccopharynx by elevation of its floor, begins simultaneously with the onset of mouth closure. A slight oral reflux was seen in these tadpoles. However, in semiconscious and in conscious tadpoles, the floor of the buccopharynx begins its elevation immediately after the mouth has closed, and no oral reflux of water occurs.

The elevation of the buccopharyngeal floor causes a positive increase in the buccopharyngeal pressure (Fig. 2). The H6 muscle is chiefly responsible for these effects, because they are greatly reduced if this muscle is denervated. However, small cyclic oscillations of the buccopharyngeal pressure of the same phase difference persist after H6 denervation, which reveals that there are also other muscles which participate in buccopharyngeal pumping. Contrary to Sedra and Michael (1957), it was found in the present study that the M10 muscle does not contract during phasic pumping in the tadpole. On the other hand, the B7 muscle contracts synchronously with the H6 muscle, and in addition, contractions of the B1, B5, B8, and B10 muscles were easily seen through the transparent skin. The B1, B5, B8, and B10 muscles are active toward the end of elevation of the anterior buccopharyngeal floor, or just after its elevation. In consequence, they enhance the compression effect of the H6 and B7 muscles.

All the buccopharyngeal compressors are well co-ordinated with one another and they cause a smooth compression of the buccopharynx, the movement beginning anteriorly and then passing caudad.

The effect of buccopharyngeal compression on water flow may now be considered. Experiments such as those performed on Rana (Gradwell 1969a) showed that the valves of the internal nares of Xenopus are closed during buccopharyngeal compression. As the mouth is also closed at this time, buccopharyngeal water has only one course to follow: backward, over the ventral velum and through the pharyngeal clefts. As gills are absent in Xenopus tadpoles, the space which the water then enters is better called the opercular cavity than the gill cavity. The bilateral opercular cavities have their exits to the outside via the flap-like

structures which have hitherto been called spiracles, but which will be called opercular outlets in the present study. Water which has entered the opercular cavities during buccopharyngeal compression, flows immediately to the outside via the opercular outlets (Fig. 3). As there is no transverse opercular canal in Xenopus, the water from opposite sides of the buccopharynx does not intermix before it flows to the outside.

Buccopharyngeal decompression

Water pumping does not occur in pithed tadpoles nor in deeply anesthetized tadpoles. In these animals the buccopharyngeal floor assumes by its inherent elasticity, a depressed position. If the buccopharyngeal floor is manually elevated, it returns to its depressed position soon after release of the force pressing it against the buccopharyngeal roof.

The passive depression of the buccopharyngeal floor by its natural elasticity is greatly enhanced by the simultaneous contractions of the bilateral Hl muscles. These are relatively large muscles but their effect (Fig. 3) in drawing water into the buccopharynx through the mouth and nares is not usually noticeable in the buccopharyngeal pressure graphs (Fig. 1). However, the effect of the Hl muscle (arrows) on buccopharyngeal pressures is demonstrated in Fig. 4, where the pressures were recorded from a tadpole while it was pumping water very much like tadpoles do while filter feeding. The contraction of the Hl muscles appears to be simultaneous with the opening of the mouth. As mouth closure and buccopharyngeal compression soon follow these events of decompression, the intake (decompression) phase may be seen in Fig. 4 to be much shorter than the outflow (compression) phase of water pumping.

From its topography, it would seem that the small H2 muscle is suited to contraction during decompression of the buccopharynx, but it was not possible to observe such contractions in the present study.

Water enters the buccopharynx mainly through the mouth, but a small volume also enters via the nares. As the ventral velum is non-valvular, it does not prevent water from filling the entire buccopharynx during decompression (Fig. 3). Another consequence of a non-valvular ventral velum is that the negative buccopharyngeal pressure would cause an opercular reflux were it not for the flap-like opercular valves which are passively closed when the hydrostatic pressure in the opercular cavities falls below the ambient pressure[1].

The natural, slight elastic tendency of the opercular valves to revert to a resting open condition, was studied in a pithed tadpole. As the tadpole was not pumping water, the opercular valves were immobile and were orientated in an open condition. Either or both the valves could be manually pressed closed, but on release of the weak force that was displacing them, they immediately sprang wide open again. Closure of these valves was also experimentally caused by manual depression of the buccopharyngeal floor of the tadpole, to thus produce a hydrostatic pressure which was lower in the opercular cavities than the ambient pressure. Conversely, the valves immediately opened when the buccopharyngeal floor was manually elevated.

---

[1] ambient pressure = atmospheric pressure plus hydrostatic pressure at the particular depth of the tadpole in the surrounding water.

Subsidiary muscles

The function of several muscles which are associated with the water pumping apparatus of Xenopus, but which are inactive during normal pumping, have not been considered in the foregoing description of the typical cycle of pumping.

Contractions of the B2, B6, and B9 muscles were never seen during regular phasic pumping, but these muscles become intermittently active during deepening anesthesia. The function of these muscles is to close the three pharyngeal clefts, and as they contract synchronously with the H6 and B7 muscles, an abnormally large positive pressure results in the buccopharynx (Fig. 5). These effects were also elicited by India ink which was pipetted into the oral intake of conscious, but restrained tadpoles (Fig. 6A,B). During the onset of the abnormally large positive buccopharyngeal pressure, the mouth, contrary to its normal timing, opened and there was a vigorous discharge of water through it from the buccopharynx. Careful observation of the pharyngeal clefts through the opercular skin during these experiments with India ink, revealed that immediately after the oral discharge, an opercular discharge of stained water occurred simultaneously with the relaxation of the B2, B6, and B9 muscles. This result may be the effect of mouth closure just before the buccopharyngeal pressure reaches equilibrium with the ambient pressure. Therefore the only remaining outlets through which the persisting positive buccopharyngeal pressure could drive water, would be the pharyngeal clefts.

In the present research, no information was gathered on the functioning of the remaining minute and inaccessible muscles of the water pumping apparatus (namely B3.4, B13, B14, B15, and S1).

Discussion

Most of the investigators of Xenopus who were interested in the mode of respiration of its tadpole, have favored the view that the rugulose lining of the buccopharynx is a respiratory surface. Indeed, Weisz (1945, p. 173) was unequivocal on this point and went so far as to call the rugulose lining, the "pseudo-internal-gill system", while Sedra and Michael (1957, p. 19) have assigned the name "branchial chamber" to the rugulose part of the buccopharynx.

The present investigation offers no evidence to refute or to substantiate the view that the rugulose lining is respiratory. Instead, the investigation has been devoted to the nature of the water pumping mechanism, with the supposition that, in view of the rich vascularization of the rugulose lining, at least some degree of respiratory gas exchange must occur here. On the other hand, relative to Rana catesbeiana (Gradwell 1969b), the operculum of Xenopus is poorly vascularized, and also considering the absence of gills, very little (if any) respiratory gas exchange would seem to be possible in the opercular cavities.

Electromyography has not been attempted in the small tadpoles of Xenopus. Therefore, the muscle activity that has been described earlier in this Chapter, is based solely on visual observations, and the timing suggested for the muscles is particularly in need of electromyographic evidence. The absence of such data and also the simplicity of the water pumping mechanism of Xenopus, has permitted the joint treatment of muscle activity, movement, hydrostatic pressures, and water flow in the foregoing description of the pumping mechanism.

The simplicity of the water pumping cycle of Xenopus is a consequence of the general and peculiar simplicity of the pumping apparatus:

(a) an immovable upper jaw

(b) a single plane of articulation at the cartilago Meckeli-quadrate joint

(c) an inflexible joint between the Meckelian and infrarostral cartilages

(d) absence of the M6 and M7 muscles

(e) structurally homogeneous muscle fibers in the hyoidean muscles

(f) absence of the H7 muscle and of a pars reuniens

(g) apparent fusion of the H3, H4, and H5 muscles into a single muscle

(h) a non-valvular ventral velum

(i) absence of a pharyngeal cleft between the ceratohyale and the first ceratobranchiale; also, the absence of gills, a membrana vasculosa opercularis, opercular canal, and the B11 and B12 muscles.

Apart from the need for electromyographic data for the pumping muscles of Xenopus, the detailed nature of the water flow through the tadpole has still to be established. In addition, further morphological information is needed on the intricate, small cavities and water channels in the buccopharynx and opercular cavities. An elucidation of the filtration mechanism would also do much to help in the understanding of water flow during phasic pumping.

With regard to its water pumping mechanism, Xenopus is certainly peculiar among tadpoles in having only a single force pump in front of the pharyngeal clefts. Other functional peculiarities of Xenopus are discussed in Chapter 6.

Acknowledgments

I am indebted to my former instructors, Drs. N. Millard, N. Paterson, and A.L. Smit, whose enthusiasm and research on Xenopus laevis first generated my interest in tadpoles. I am also grateful to Mr. Wontaik Han who helped me with the experimental induction of ovulation.

References

Beddard, F.E. 1894. Notes on the tadpole of Xenopus laevis. Proc. Zool. Soc. Lond. 1894: 101-107.

Bles, E.J. 1905. The life history of Xenopus laevis Daud. Trans. Roy. Soc. Edin. 41: 789-822.

De Villiers, C.G.S. 1932. Über das Gehorskelett der aglossen Anuren. Anat. Anz. 33-55.

Dreyer, T.F. 1915. The morphology of the tadpole of Xenopus laevis. Trans. Roy. Soc. S. Africa, 4: 241-258.

Edgeworth, F.H. 1930. On the masticatory and hyoid muscles of Xenopus laevis. J. Anat. 64: 184-188.

---- 1935. The cranial muscles of vertebrates. Cambridge Univ. Press.

Gradwell, N. 1969a. The function of the internal nares of the bullfrog tadpole. Herpetologica, 25: 120-121.

---- 1969b. The respiratory importance of vascularization of the tadpole operculum in Rana catesbeiana Shaw. Can. J. Zool. 47: 1239-1243.

Gradwell, N, and V.M. Pasztor. 1968. Hydrostatic pressures during normal ventilation in the bullfrog tadpole. Ibid. 46: 1169-1174.

Kenny, J.S. 1969a. Feeding mechanisms in anuran larvae. J. Zool. Lond. 157: 225-246.

---- 1969b. Pharyngeal mucous secreting epithelia of anuran larvae. Acta Zool. 50: 143-153.

Kotthaus, A. 1933. Die Entwicklung des Primordial-craniums von Xenopus laevis bis zur Metamorphose. Z. Wiss. Zool. 144: 510-572.

Kratochwill, K. 1933. Zur Morphologie und Physiologie der Nahrungsaufnahme der Froschlarven. Ibid. 144: 421-468.

Millard, N. 1945. The development of the arterial system of Xenopus laevis, including experiments on the larval arches. Trans. Roy. Soc. S. Africa, 30: 217-234.

Nieuwkoop, P.D., and J. Faber. 1956. Normal table of Xenopus laevis, Daudin. North-Holland Pub. Co., Amsterdam.

Parker, W.K. 1876. On the structure and development of the skull in the Batrachia. Part II. Philos. Trans. Roy. Soc. 166: 601-669.

Paterson, N.F. 1939a. The head of Xenopus laevis. Quart. J. Microscop. Sci. 81: 161-234.

---- 1939b. The olfactory organ and tentacles of Xenopus laevis. South African J. Sci. 36: 390-404.

Ridewood, W.G. 1898. On the structure and development of the hyobranchial skeleton and larynx in Xenopus and Pipa; with remarks on the affinities of the Aglossa. J. Linn. Soc. Lond. Zool. 26: 53-128.

Schulze, F.E. 1892. Über die inneren Kiemen der Batrachierlarven. II. Skelet, Muskulatur, Blutgefässe, Filterapparat, respiratorische Anhänge und Atmungsbewegungen erwachsener Larven von Pelobates fuscus. Abhandl. Königlichen Akad. Wiss. Berlin, 13: 1-66.

Sedra, S.N., and M.I. Michael. 1957. The development of the skull, visceral arches and visceral muscles of the South African clawed toad, Xenopus laevis, Daudin, during metamorphosis (stages 55-66). Verhandel. Koninkl. Akad. Wetenschap. Natuurk. 51 (4): 1-80.

Smit, A.L. 1953. The ontogenesis of the vertebral column of Xenopus laevis Daud. with special reference to the segmentation of the metotic region of the skull. Ann. Univ. Stellenbosch, 29A (3): 79-136.

Weisz, P.B. 1945. The development and morphology of the larva of the South African clawed toad Xenopus laevis. J. Morphol. 77: 163-217.

Illustrations

Fig. 1. Hydrostatic pressures recorded simultaneously from the anterior and posterior buccopharynx in a lightly anesthetized tadpole. Horizontal lines represent ambient pressure. Frequency: 50 cycle/min; calibration: 4 mm water.

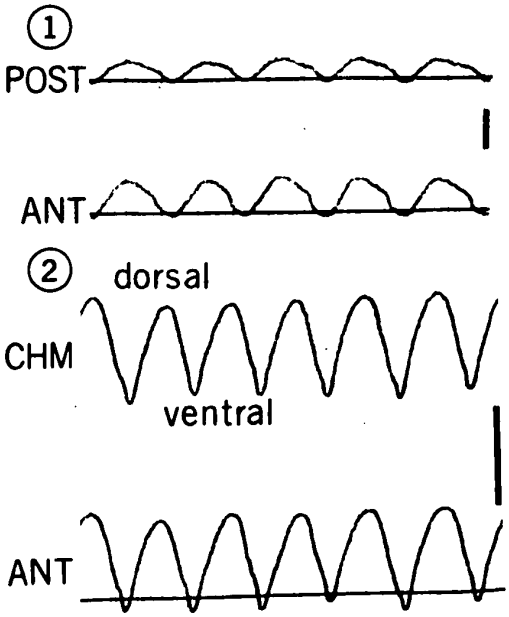
Fig. 2. Records from a conscious, restrained tadpole. Mechanogram (force transducer, Gilson Polygraph) of the medial ceratohyale (CHM) monitored simultaneously with hydrostatic pressure in the anterior part of the buccopharynx. Frequency: 60 cycle/min; pressure calibration: 6 mm water.

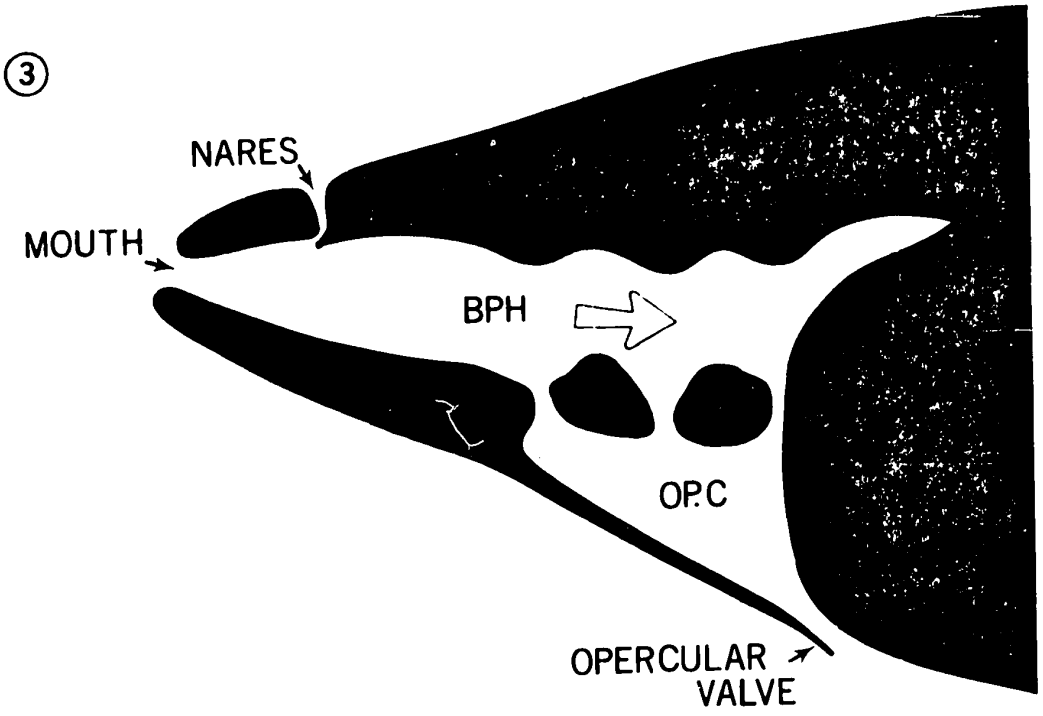
Fig. 3. Composite diagram of sagittal and parasagittal sections through the head of a tadpole. The anatomy is shown in its orientation at the onset of buccopharyngeal compression. The mouth and nares are closing while the opercular valves are opening. The flow of water past the ventral velum (not seen in profile) and into the entire buccopharynx is indicated by the large arrow. BPH, buccopharynx; OP.C, opercular cavity.

Fig. 4. Buccopharyngeal pressures monitored anteriorly in a conscious, unrestrained tadpole. The arrows indicate the onset of active depression of the buccopharyngeal floor by the Hl muscles during opening of the mouth. Mouth closure and buccopharyngeal compression begin so soon afterward, that the intake phase (decompression) is extremely short relative to the outflow phase (compression). Frequency: 60 cycle/min; calibration 7 mm water.

Fig. 5. Hydrostatic pressures monitored simultaneously from the anterior and posterior buccopharynx in a deeply anesthetized tadpole in which phasic pumping had ceased. Hyperexpirations are shown by the peaks of the graphs. Calibration: time, 6 sec; pressure, 1 cm water.

Fig. 6. Hydrostatic pressures of a lightly anesthetized tadpole during injection of India ink (arrows) into the oral intake. A. Anterior and posterior buccopharyngeal pressures monitored simultaneously. B. Anterior buccopharyngeal pressures. Frequency: 55 cycle/min; calibration: 5 mm water.


①
POST
ANT
②
dorsal
CHM
ventral
ANT


③
NARES
MOUTH
BPH
OPC
OPERCULAR
VALVE

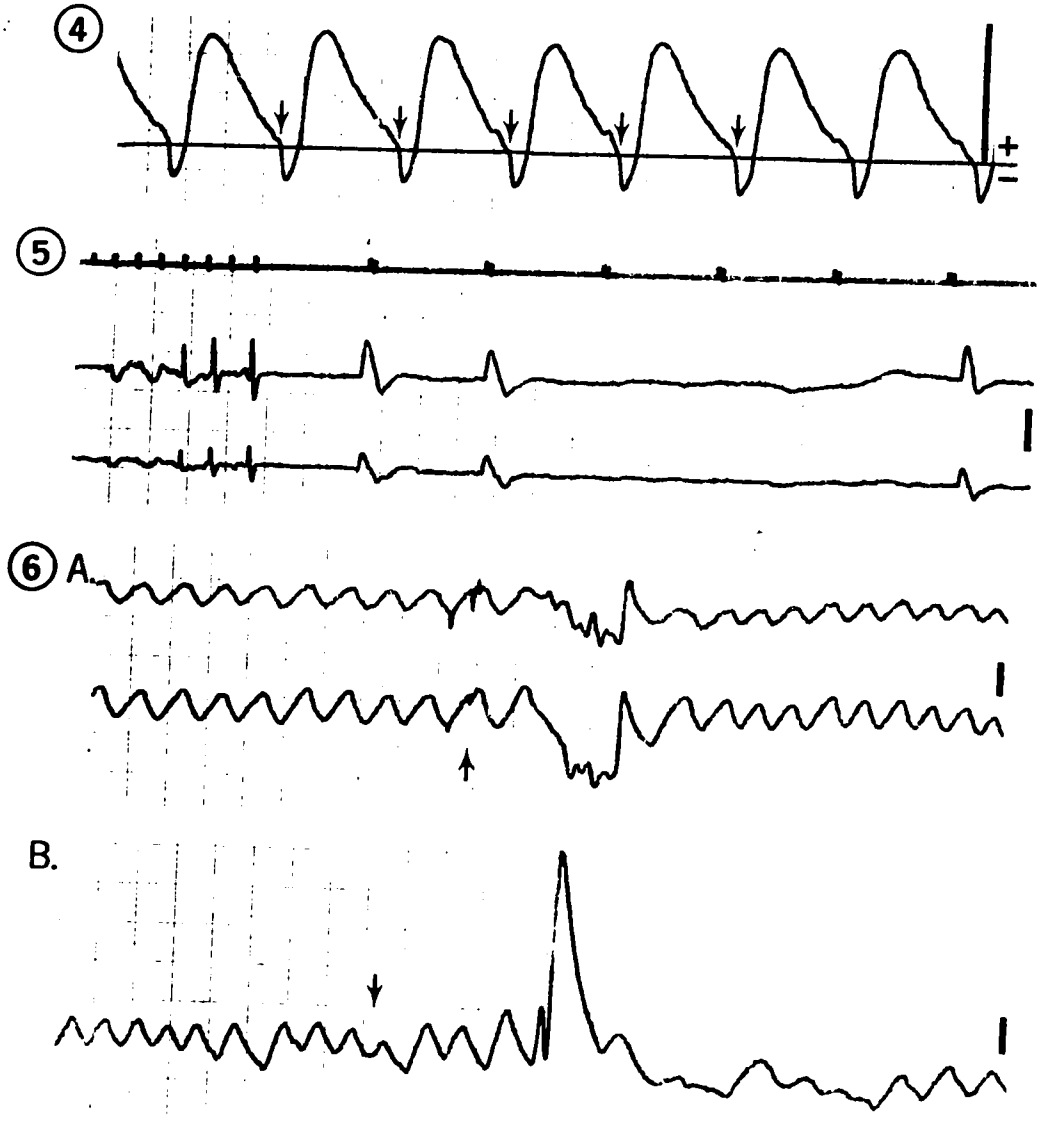

④
±
⑤
⑥ A.
B.

TABLE I

The positions of the anterior visceral muscles of Xenopus laevis, stage 52 of Nieuwkoop and Faber, 1956 (= stage 31 of Gosner 1960). Motor innervations are parenthesized. Key letters designate the group to which each muscle belongs and refer to the origin-insertion relationship of individual muscles. For use in the text, each muscle is also identified by a number, preceded by the initial letter of the group to which the muscle belongs.

Terms used by Sedra & Michael (1957)	Origin-insertion[1]	No.	Key letters
MANDIBULAR GROUP			
Levator mandibulae anterior	Pterygoquadrate, dorsal, posterior -		
pars medialis	(a) Meckeli, dorsal, medial	M1	MP-MDM
" intermedius	(b) " " intermedial	M2	MP-MDI
" lateralis	(c) Tentacle, posterior	M3	MP-TP
L.m. externus	Musculoquadrate, anterior - Meckeli, lateral	M4	MM-ML
L.m. posterior	Pterygoquadrate, dorsal, anterior - Meckeli, dorsal, posterior	M5	MP-MDP
Intermandibularis anterior	Appears at stage 56: interconnects medial aspects of the single rostrale inferior; no median raphe	M8	MRI
I. posterior (n. trigeminus)	Median raphe - Meckeli, medial	M10	MR-M

[1] Attachments are assumed to be ventral except where otherwise indicated. Arabic numerals refer to the ceratobranchialia.

HYOIDEAN GROUP			
Orbitohyoideus	Musculoquadrate, lateral - ceratohyale lateralis, lateral	H1	HM-CL
Suspensoriohyoideus	Pterygoquadrate, ventrolateral - ceratohyale lateralis, dorsal	H2	HP-CL
Quadratohyoangularis	Pterygoquadrate, lateral - Meckeli, ventrolateral	H3. 4.5	HP-MV
Hyo- and suspensorio-angulares	Absent as individual muscles		
Interhyoideus (n. facialis)	Median raphe - ceratohyale lateralis	H6	HR-CL

BRANCHIAL GROUP			
Constrictor branchialis I	Pterygoquadrate, ascendens - 1, lateral	B1	BP-1L
Subarcualis rectus II	2, medial - 2, lateral	B2	B2M-2L
S.r. I	Ceratohyale medialis, posterior - 1, medial	B3.4	BCM-1M
Constric. br. II	Pterygoquadrate, ascendens - 2, lateral	B5	BP-2L
Subarc. rec. III	3, medial - 3, lateral	B6	B3M-3L
Transversus ventralis II	Median raphe - 2, medial	B7	BM-2M
Constric. br. III	Crista parotica - 3, lateral	B8	BCP-3L
Subarc rec. IV	3, medial, posterior - 3, lateral, posterior	B9	B3M-3L
Constric. br. IV	Auditory capsule - 4, lateral	B10	BAC-4L
Levator arcus branchialis IV	Auditory capsule - 4, medial	B13	BAC-4M
Cucullaris	4, lateral - scapula	B14	B4L-S
Transversus ventralis IV	Median raphe - 4, medial	B15	BM-4M
(nn. glossopharyngeus + vagus)			
SPINAL GROUP			
Geniohyoideus	1, medial - rostrale inferior	S1	S1M-RI
(n. hypoglossus)			

CHAPTER 6

DISCUSSION OF COMPARATIVE AND CONTRASTING ASPECTS OF WATER FLOW IN THREE SPECIES OF ANURAN TADPOLES

The important points of the information furnished in the earlier Chapters of this thesis, may now be considered in a comparison between the water pumping mechanisms of Rana catesbeiana, Ascaphus truei, and Xenopus laevis. As an aid to this discussion, diagrams of hypothetical models are presented for each of these species (Figs. 1, 2, and 3).

The tadpole of Rana catesbeiana is generally a bottom-dweller in water having a wide annual range of temperature. As the tadpole grows to a large size (reaching ca. 4.5 cm, snout-to-vent), and as it is sometimes subjected to poorly oxygenated water, its respiratory system is well-developed, and both the lungs and gills are functional throughout the life of the tadpole. However, in Canada, the respiratory demands of this tadpole cannot be great in winter and as open bodies of water freeze over in winter, lung ventilation is suppressed during this season, but gill ventilation, although reduced in frequency and amplitude, continues.

The suctorial tadpole of Ascaphus truei habitually clings to substrata in cold, cascading mountain streams. It is a small tadpole, reaching ca. 2.0 cm, snout-to-vent. Although it can swim against the strong current in short dashes between feeding stations on the algae-covered rocks, powerful dashes to and from the water surface for air do not occur, and the tadpole is totally dependent on aqueous ventilation for its respiratory gas exchange.

Xenopus laevis has a small nektonic tadpole (reaching ca. 1.5 cm, snout-to-vent) found naturally in warm, stagnant water. Lung ventilation begins immediately the larva loses its external gills and becomes a tadpole. However, there is controversy in the literature regarding the respiratory or hydrostatic function of the lungs, as well as of the rugulose lining of the buccopharynx. As rhythmic water pumping begins only when the tadpole is feeding, it would seem that the animal is negligibly dependent on the feeding current for respiratory gas exchange. In such a small tadpole, ventilation of skin capillaries may be relatively more important for respiration than it is in the larger Rana and Ascaphus tadpoles.

1. The jaws

Anatomical and functional adaptations of anuran tadpoles to their habitats are particularly evident for the activities of feeding and respiration (Gradwell 1968). The specializations of the jaws of the three species studied in the present research are apt examples of the effects of selective pressures of habitat on anatomy and on function.

The jaws of Rana are protruded during hyperinspiration and during voluntary feeding on plant and animal tissues. Both jaws are movable, but the upper jaw is normally active together with the lower jaw only above ca. 10°C. The synchrony between the upper and lower jaws is ensured by the efficient co-ordination of their muscles, and secondarily, by ligaments interconnecting the upper and lower jaws. At temperatures below ca. 5°C both jaws are inactive and the nares are the only water intakes.

An oral valve in Ascaphus seems to be a unique feature among tadpoles, and the presence of it eliminates the need for rhythmic muscular movements of the jaws during gill ventilation at all tolerable ambient temperatures. When the sucker is engaged, the mouth is sealed closed by the oral valve and the nares are the only water intakes. When it is impossible for the sucker to become engaged to a substratum, the tadpole still pumps water rhythmically. The oral valve then opens and closes cyclically and both the nares and mouth serve as intakes. Oral suckers are found in many species of tadpoles inhabiting mountain streams, but oral valves have not hitherto been described in them. The highly denticulated jaws of Ascaphus are conspicuously active during cyclic hitches of the sucker while feeding. Both the upper and lower jaws are active during these movements, but in contrast with Rana, the jaws are not protrusible. Those mandibular muscles that are present in Ascaphus, are well-developed, but five others that occur in Rana, are absent in Ascaphus.

In Xenopus, the horizontal slit-like mouth is, relative to the width of the head, wider than that of all non-pipid tadpoles. This permits a large intake to occur rapidly during the very short decompression phase of water pumping. The wide mouth also dispenses with the need for two planes of articulation at the cartilago Meckeli-quadrate joint, and with the need for a flexible joint between the Meckelian and infrarostral cartilages, which are two conditions essential for the protrusion of the jaws in Rana. Moreover, there is no need for a movable upper jaw in Xenopus. The simplification of the jaws of the Xenopus tadpole

is also reflected in the total fusion of the jaw abductors, and in the partial fusion of the jaw adductors. Also, three mandibular muscles that occur in Rana, are absent in Xenopus.

Frazzetta (1966) has shown that in snakes, the jaw muscles of the primitive boas are simpler and fewer than those of more modern species. Such a phylogenetic trend seems to occur also in anuran tadpoles, because both Ascaphus and Xenopus, which show a reduction in jaw musculature, are universally regarded as more primitive than Rana.

2. Buccopharynx

On the basis of a valvular ventral velum, the classical buccopharynx of Rana has been divided into an anterior buccal cavity and a posterior pharynx (Gradwell and Pasztor 1968). The cyclic alternation of the buccal and pharyngeal force pumps facilitates the occlusion of the buccal cavity from the pharynx during inspiration, but the buccal cavity becomes confluent with the pharynx during expiration.

All the evidence that has so far been gained, points to the existence of the same type of pumping mechanism in Ascaphus, except that the small nares and the habitual sealing of the mouth while the sucker is engaged, adapts Ascaphus to the easy production of negative pressures in the buccal cavity. By light microscopy, the pumping muscles of Ascaphus appear to be composed of structurally homogeneous muscle fibers. Nevertheless, they are capable of producing both the hyperinspiration and hyperexpiration effects which, in Rana, are mainly caused by muscle fibers which appear to be analogous to conventional fast contracting or twitch muscle fibers of vertebrates (Gradwell and Walcott 1970).

Contrasting with Rana and Ascaphus, the large mouth of Xenopus hampers the production of large negative pressures in the pumping system. The absence of a valvular ventral velum is probably also a hindrance to the production of negative buccopharyngeal pressures in Xenopus. Rana, Ascaphus and Xenopus are all capable of hyperexpiration, but the phenomenon is best developed in Rana.

There is apparently little difference between the phasic buccal and pharyngeal pumping mechanisms of Rana and Ascaphus, but the absence of a valvular ventral velum in Xenopus seems to be

the chief handicap to a dual pumping mechanism in this tadpole. In Xenopus, the pharyngeal constrictors are active just after buccal compression and a peristaltic wave of buccopharyngeal constriction therefore forces water caudad, and through the pharyngeal clefts. Rana and Ascaphus have taken advantage of the slight contractile delay of the pharyngeal constrictors behind the buccal compressors, by evolving the valvular ventral velum between the buccal cavity and pharynx, to produce two alternating pumps. They appear to have then co-ordinated the mouth movements with these pumps, to facilitate an efficient dual pumping mechanism.

In Rana, buccal water is deflected by the ventral velum against the dorsal velum, which then deflects this water down toward the gill clefts in the rugulose floor of the pharynx. The same route is supposedly also followed by the buccal water in Ascaphus, whose anatomical relationships between the vela and the pharyngeal gill clefts are similar to those in Rana. Xenopus, on the other hand, has a complicated system of mucosal folds above its pharyngeal clefts. Therefore, except for the obvious fact that water leaves the buccopharynx by the three pairs of pharyngeal clefts, it has not been possible in the present research, to postulate the detailed course followed by the water. The nature of water flow in the pharyngeal region of Xenopus is probably important in its filtration mechanism, although mucus secreted by the ventral velum is also involved in this process.

A cleft between the buccal and gill cavities is present in Rana but not in Ascaphus and Xenopus. During buccal compression in Rana, buccal water reaches the gill cavity mainly via the pharyngeal gill clefts, but some buccal water reaches the gill cavity directly, through the first gill cleft, thus by-passing the pharynx. In Ascaphus, the absence of a first gill cleft between

the buccal and gill cavities causes buccal compression to pump water into the gill cavity only via the pharyngeal gill clefts. In both Rana and Ascaphus, some buccal water remains in the pharynx at the end of buccal compression and this water is then pumped into the gill cavity via the pharyngeal gill clefts. The force of this second pump is provided by constriction of the pharynx immediately after buccal compression. During buccopharyngeal compression in Xenopus (which, like Ascaphus, has only the three pharyngeal "gill" clefts), all the buccopharyngeal water is pumped through the pharyngeal clefts and directly into the opercular cavity, and there is no separate pharyngeal pumping phase.

3. The gill (or opercular) cavities

A transverse opercular canal in Rana, interconnects the right and left sides of the gill cavity, with the result that all the branchial water leaves the gill cavity through the single sinistral spout. Xenopus does not have gills and the water leaving the pharyngeal clefts enters an opercular cavity instead of a gill cavity as in Rana and Ascaphus. A transverse opercular canal is absent in both Ascaphus and Xenopus; the left and right gill cavities of Ascaphus and opercular cavities of Xenopus, have independent outflows. However, the single median branchial outlet of Ascaphus serves as a common orifice of discharge for the left and right gill cavities. In Xenopus, the opercular cavities each have an outlet which, unlike the spout of Rana and the branchial outlet of Ascaphus, is distinctly valvular. The outflow of Rana and Ascaphus is continuous but it is intermittent in Xenopus.

It has not been possible to establish the function of the B2, B6, and B9 muscles of Rana and Ascaphus, but their action is apparently that of closing the gill clefts. In Xenopus, the absence of gills and the transparency of the operculum has facilitated the study of these muscles of the pharyngeal clefts. They are not phasically active during regular water pumping, but they contract during hyperexpiration: they close the pharyngeal clefts and thus facilitate the oral expulsion of contaminated water.

In Rana, hyperexpiration may or may not be enhanced by branchial expulsion through the facultative contraction of an hyoidean muscle in the opercular lining. Such a muscle (the H7) is absent in Ascaphus and Xenopus.

The opercular lining is highly vascular in Rana, but not in Ascaphus and Xenopus. For Ascaphus, living in well oxygenated mountain streams, gills alone seem to be adequate for respiration. In the smaller Xenopus tadpole, lung ventilation and possibly also ventilation of the pharyngeal blood capillaries may explain the absence of gills and of a poorly vascular membrana vasculosa opercularis.

Diagrams of hypothetical models of water pumping in three species of anuran tadpoles

The diagramatic models (Figs. 1, 2, and 3) represent an overall summary of probable muscle activity, mechanical displacement, hydrostatic pressure, and water flow in the tadpoles compared with one another in the present Chapter. Functional anatomical studies of several other species of Rana have revealed no important differences in their water pumping mechanisms from that of R. catesbeiana. Ascaphus has only one species, namely truei. Dissections of cadavers of other species of Xenopus have shown that their tadpoles are almost identical to that of X. laevis, in regard to the water pumping apparatus. It therefore seems likely that the models presented here may serve as a useful guide to species other than those which have been investigated in the present research.

Chapter 3, Fig. 13 shows that there is little electromyographic basis for the muscle activity postulated in the model for Rana. For Ascaphus and Xenopus, no electromyography has been attempted in the present study. The muscle activity and the mechanical displacements shown in the models are chiefly based on visual observations of breathing, anesthetized tadpoles, dissected under a microscope. The H7 muscle is parenthesized in

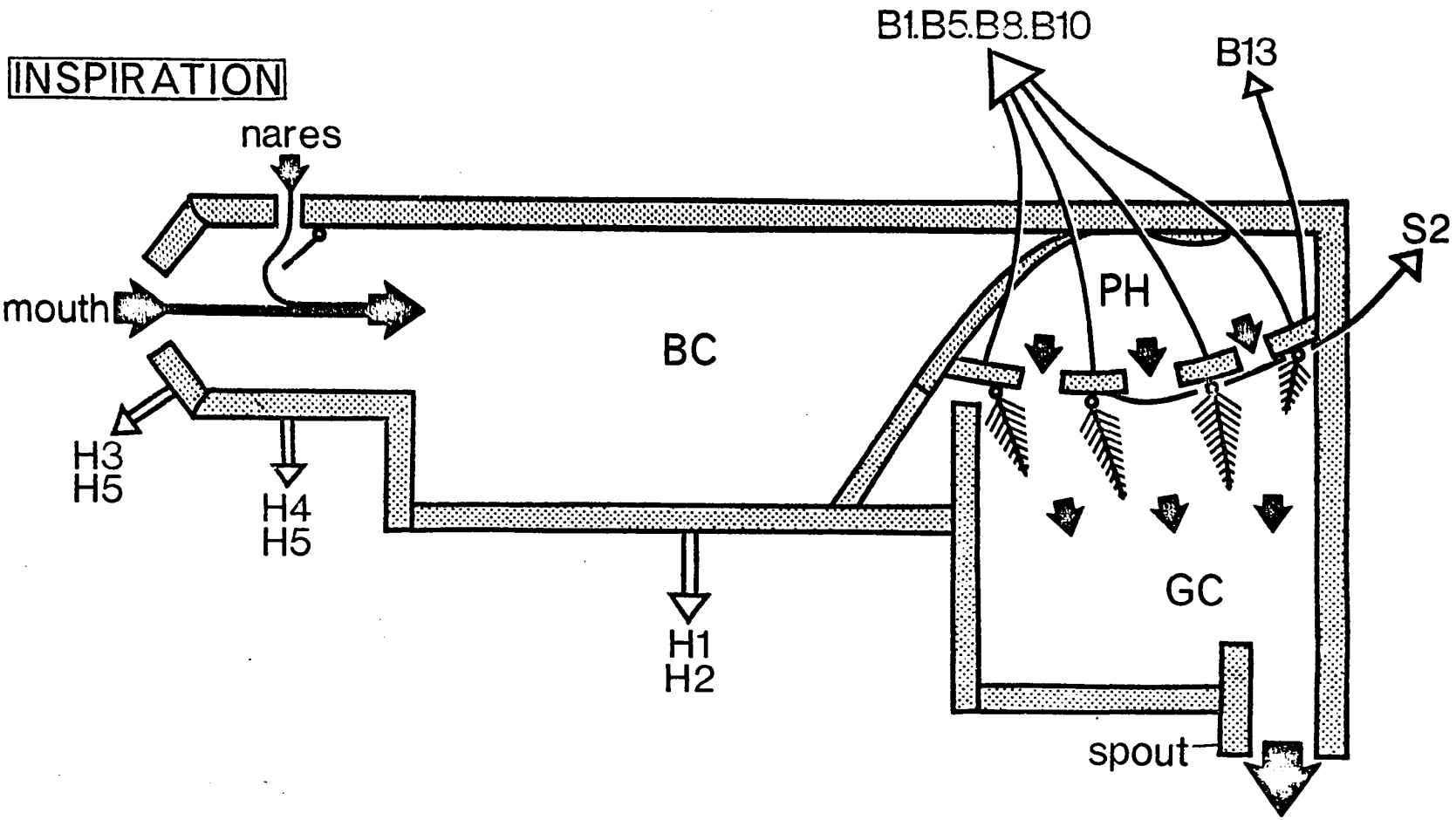
Fig. 1 because this muscle is not active during regular phasic breathing. It may or may not contract during hyperexpiration (see text above). Water flow has been deduced from the hydrostatic pressures shown graphically in the earlier Chapters, and has been found consistent with the results of studies with streamers and dyes.

Abbreviations

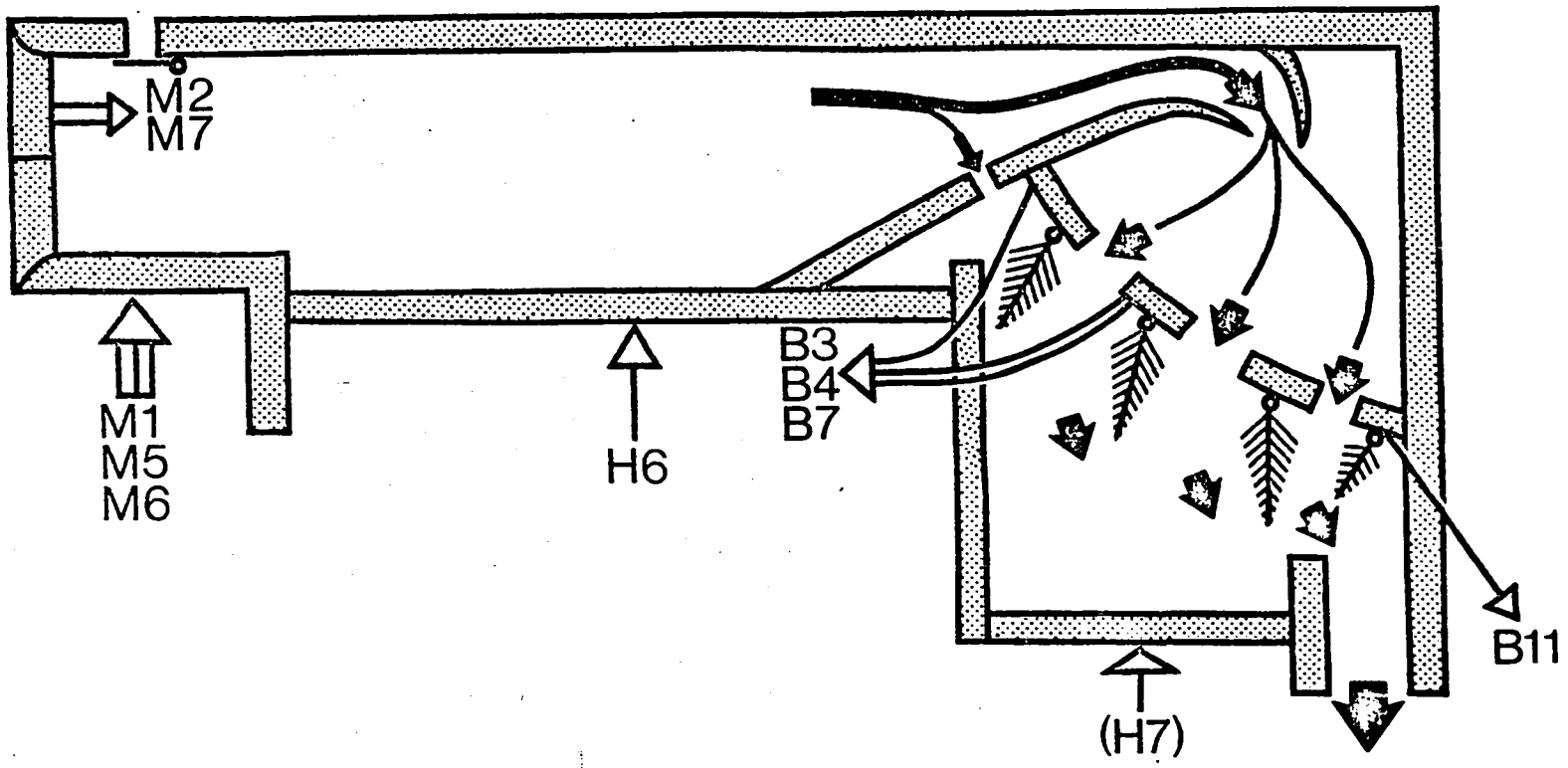
The origins and insertions of all the muscles referred to in the models have been tabulated in the Chapters describing the respective species. The lengthy conventional terminology for the muscles is also tabulated in these earlier Chapters (Table I, Ch 1; Table I, Ch 4; and Table I, Ch 5).

BC, buccal cavity; BPH, buccopharynx; br., branchial; GC, gill cavity; op., opercular; OP.C, opercular cavity.

RANA

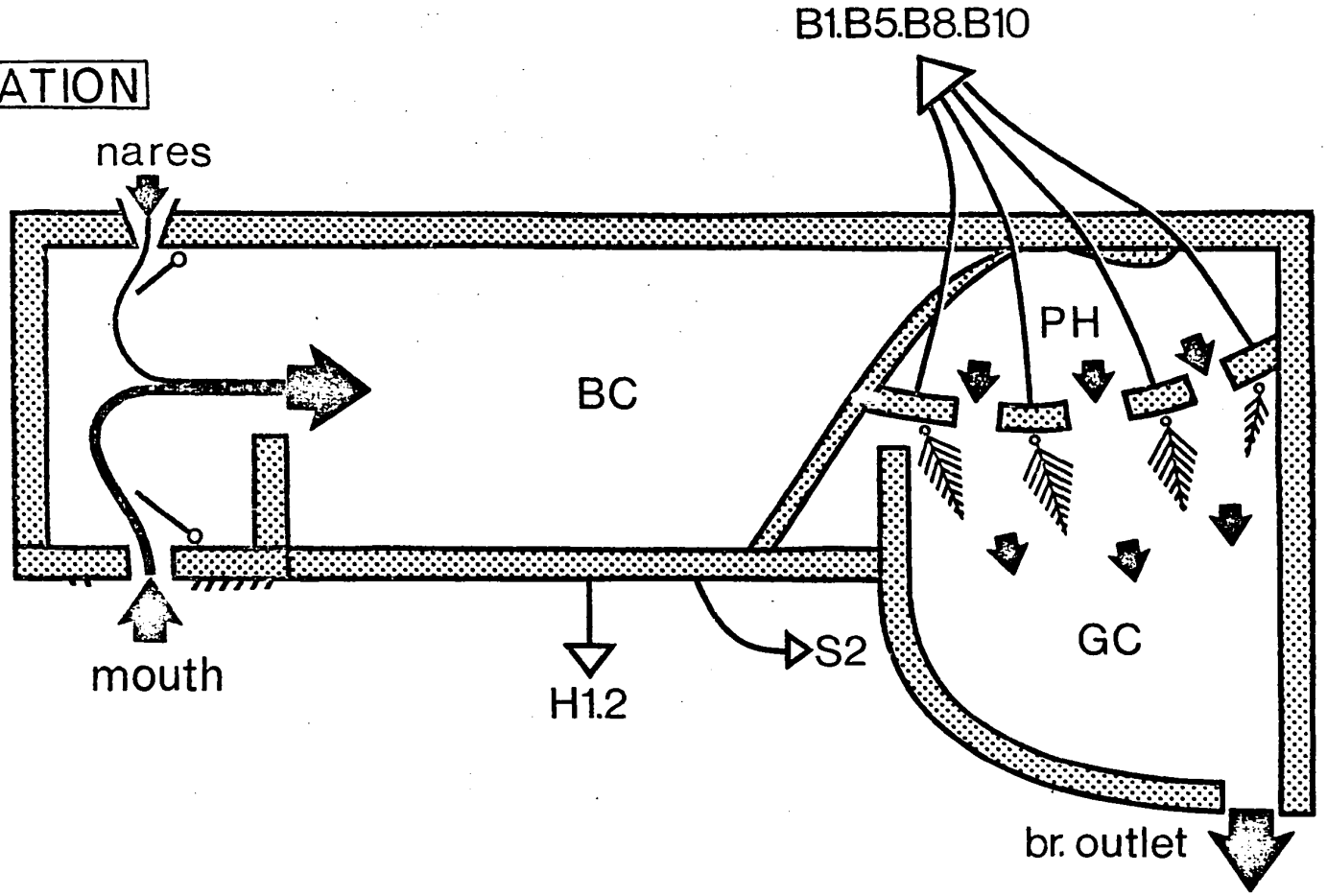

INSPIRATION
nares
mouth
H3
H5
H4
H5
BC
H1
H2
B1.B5.B8.B10
B13
S2
PH
GC
spout

EXPIRATION

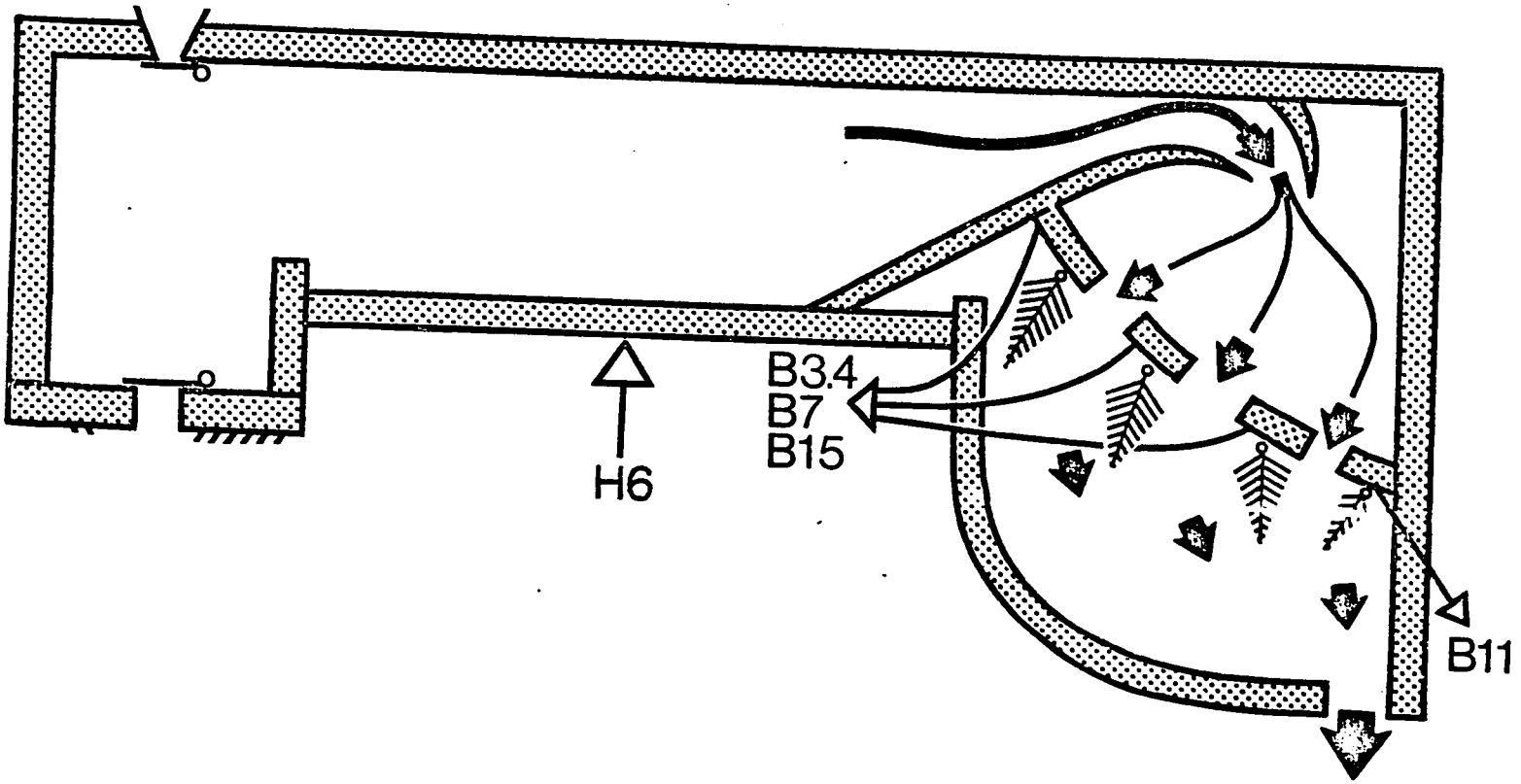


2 ASCAPHUS

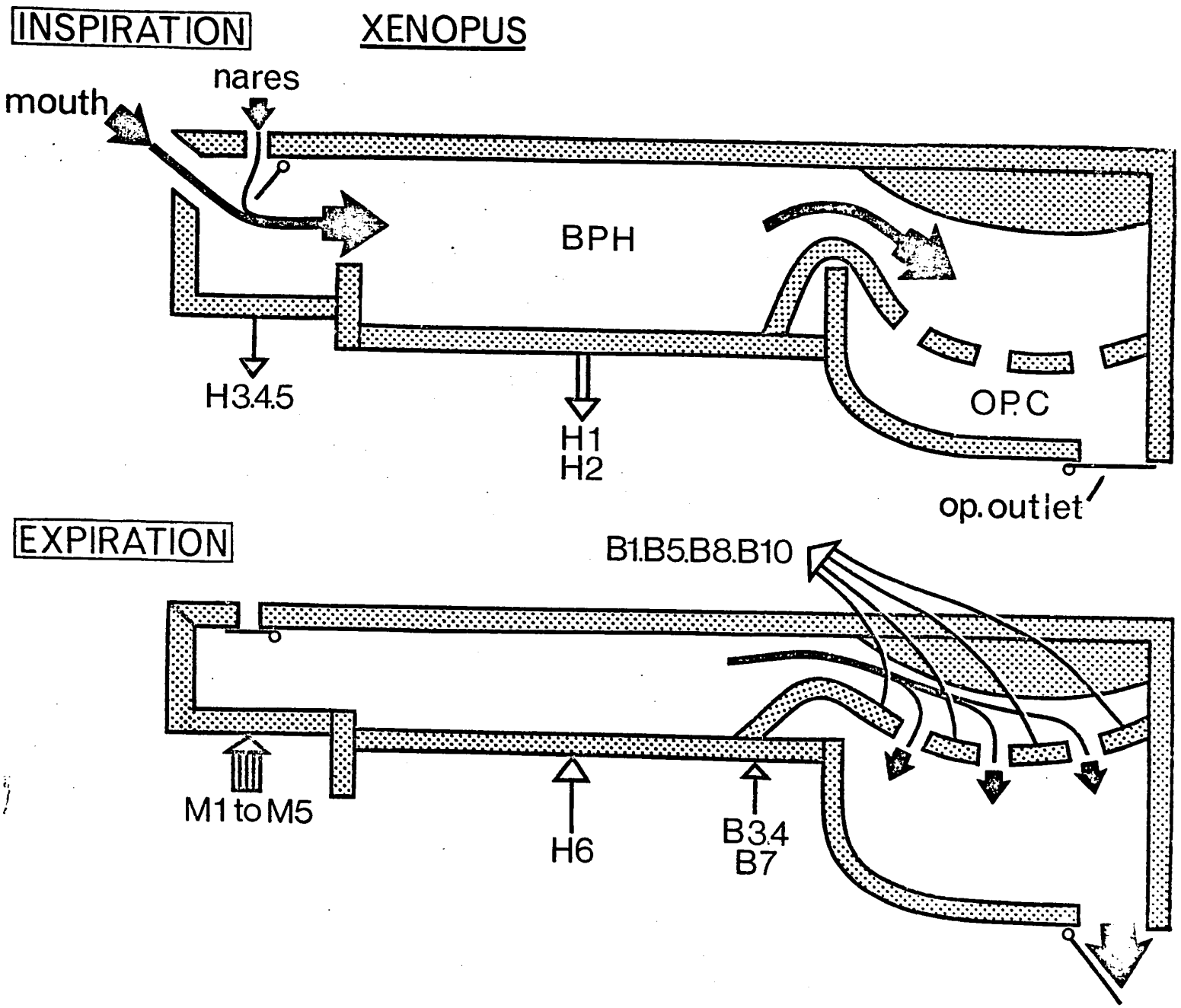
INSPIRATION



2 EXPIRATION



3


INSPIRATION
XENOPUS
nares
mouth
BPH
H3.4.5
H1
H2
OP.C
op. outlet
EXPIRATION
B1.B5.B8.B10
M1 to M5
H6
B3.4
B7

References

Frazzetta, T.H. 1966. Studies on the morphology and function of the skull in the Boidae (Serpentes). Part II. Morphology and function of the jaw apparatus in Python sebae and P. molurus. J. Morphol. 118: 217-296.

Gradwell, N. 1968. The jaw and hyoidean mechanism of the bullfrog tadpole during aqueous ventilation. Can. J. Zool. 46: 1041-1052.

Gradwell, N., and V.M. Pasztor. 1968. Hydrostatic pressures during normal ventilation in the bullfrog tadpole. Ibid. 46: 1169-1174.

Gradwell, N., and B. Walcott. 1970. Dual structural and functional properties of the interhyoideus muscle of the bullfrog tadpole (Rana catesbeiana). J. Exp. Zool. In the press.