THE MUSCULAR BASIS OF AQUATIC AND AERIAL VENTILATION IN THE AIR-BREATHING TELEOST FISH *CHANNA*

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SUMMARY

The mechanisms of aquatic and aerial ventilation in four bimodally breathing species of teleosts of the genus *Channa* were studied by high-speed light and X-ray ciné films, pressure transducer recordings and electromyography.

Neither inter- nor intraspecific differences were found in the pattern of aquatic and aerial ventilation. Aquatic ventilation proceeds with a partially air-filled buccopharyngeal cavity. The electromyographic patterns, pressure waveforms in the buccal and opercular cavities, and kinematics of jaw and opercula resemble those found for aquatically breathing teleosts.

The pattern of aerial ventilation differs significantly from that of aquatic ventilation. Aerial ventilation is initiated by a reversed flow of water brought about by a low pressure in the buccal cavity. In this way the air in the suprabranchial cavity is moved forward and expelled from the mouth. The buccal, suprabranchial and opercular pressures are maintained at below ambient pressures throughout the aerial respiratory cycle. The kinematic pattern of bone movements, pressure waveforms and electromyographic profiles during air ventilation are identical to those of the cough and do not differ interspecifically. The complex aerial ventilation mechanism in *Channa* may be homologous with the primitive cough mechanism. Fishes employing the cough mechanism for air ventilation remain totally dependent on water and are therefore poorly adapted to explore terrestrial habitats.

INTRODUCTION

The mechanical basis of air ventilation in the lungfish *Protopterus aethiopicus* is well understood by the application of modern techniques (McMahon, 1969). In Dipnoi, the air-breathing mechanism is derived from the aquatic mechanism: inspiration occurs by a buccal force-pump and expiration proceeds by the release of compressed pulmonary gas, aided by the elasticity of the lung wall. Air-breathing has evolved independently in many unrelated phyletic teleostean lineages (Gans, 1970; Johansen, 1970; Hughes, 1976; Lauder & Liem, 1983). The mechanism of air ventilation in bimodally breathing teleosts is still poorly known. Most studies on air

ventilation in teleosts are based on dissection, light ciné film, and X-ray ciné film
(Peters, 1978; Kramer, 1978; Liem, 1980; Hellin & Chardon, 1981; Ishimatsu &
Itazawa, 1981). Preliminary information on the electromyography of some respirat-
ory muscles in the Anabantoid fishes *Anabas* and *Helostoma* suggests that two modes
of air-ventilation exist (Liem, 1980). One mode seems to be homologous with the
biomechanical and neuromuscular patterns underlying prey capture, while the second
mode resembles the cough reflex. However, a comprehensive analysis integrating data
obtained from anatomy, high-speed light and X-ray ciné films with pressure record-
ings and electromyography has not been presented for any air breathing teleost fish.
This paucity of experimental information has hindered our attempts to understand
the physiological basis and evolutionary derivation of air ventilation in the different
phyletic lineages of teleosts.

    This study attempts to fill an important gap in our knowledge of the development
of respiratory mechanisms in air-breathing teleosts. Four main lines of approach were
used on representative species of *Channa*, bimodally breathing representatives of the
teleostean order Channiformes, inhabiting waters of the Old World tropics (Lauder
& Liem, 1983): high-speed X-ray ciné films, high-speed light ciné films, simultaneous
pressure recordings of the buccal, opercular and suprabranchial cavities, and electro-
myography of 11 muscles active in the aquatic and aerial respiratory cycles.

MATERIALS AND METHODS

    The fishes used in this study were imported from Thailand and Malaysia, and
maintained in well-aerated water at a temperature of $28 \pm 4\,°C$. They were fed on live
goldfish (*Carassius auratus*) and frozen smelt (*Osmerus*), but were starved for 2 days
before experimental procedures were carried out.

    The following species have been studied:
    Channa punctatus, 8 specimens, 12–31 cm in total length,
    Channa striatus, 11 specimens, 8–27 cm in total length,
    Channa gachua, 2 specimens, 15 and 17 cm in total length,
    Channa marulius, 2 specimens, 13 and 18 cm in total length.

    High-speed X-ray ciné films were produced by a Siemens radiographic unit with
a Sirecon image intensifier and an Eclair GV-16 camera at 150 frames s^{-1}. Kodak Plus-
X Reversal film was exposed at 300 mA and 46 kV. The fish was filmed under water
in a chamber constructed from thin Plexiglas. The chamber was made just large
enough to hold the fish and to minimize the loss of contrast caused by the Plexiglas
and water. Films were taken from lateral and dorsoventral views and were analysed
with a Lafayette variable speed projector.

    High-speed light ciné films were produced with the fish in a 40-l aquarium. The fish
were illuminated by three 600 W tungsten lamps located approximately 30 cm in front
of the aquarium. Fishes were filmed with Kodak 4X Reversal film and a Photosonics
16 mm 1 PL camera at 200 frames s^{-1}, f 5·6 lens aperture and 1/1000 s exposure for
each frame.

    The pressures developed in buccal, opercular and suprabranchial chambers were
recorded by the introduction of flexible polyethylene cannulae (i.d., 0·86 mm; o.d.,
1·52 mm). Each cannula was introduced *via* a hole drilled through a skeletal element

▶sing a dental drill. The size of the burr was chosen to produce a hole that fitted a 13 gauge needle. The tubing with one flared end was passed through the bore of the needle, which was withdrawn and the flared end secured against the roof or wall of the cavity under study. The buccal cavity was reached by a hole drilled in the ethmoid and parasphenoid from a point mid-way between the snout and eyes, and passed through the roof of the mouth cavity just posterior to the vomerine teeth. The suprabranchial chamber was introduced through the pterotic bone, posterior to the eye and medial to the hyomandibula. This method of cannulation was often problematic due to the extremely vascular nature of the wall of the suprabranchial chamber. However, satisfactory placement was accomplished in six specimens out of 15. The opercular cavity was readily reached by a hole through the anterior surface of the cleithrum dorsal to the origin of the pectoral fin. Cannulae inserted in this way were well tolerated by the fishes and remained patent for at least 7 days.

Electromyograms were obtained with bipolar electrodes (Evenohm S) using the method of Basmajian & Stecko (1962). Pairs of wires, cleaned and hooked at the tip were threaded through a 25 gauge hypodermic needle. This needle was inserted through the skin to the desired site and was then withdrawn leaving the electrodes hooked into the muscles. The electrodes, which were colour-coded with acrylic paint, were glued together using Testor's Cement for Plastic Models and fixed to a small plastic clamp attached to an artificial spine of surgical steel placed through the epaxial muscles midway between skull and dorsal fin. The cannulae for the pressure recordings were also attached to this spine with mini-plastic clamps in order to stabilize the tubes.

The insertion of all cannulae and electrodes was performed under anaesthesia induced by immersion of the fish in varying concentrations (1·0–5 %) of solutions of tricaine methane-sulphonate (Crescent Research Chemicals) and sodium hydroxide (Lauder, 1980*a*). During the entire surgical procedure, the subject's respiration was monitored carefully and the anaesthesia was adjusted with fresh water when necessary.

Pressures were recorded with Statham P23 Gb and P23 Db pressure transducers, which were damped (Lauder, 1980*a*) by filling them with a mixture of 45 % water and 55 % glycerine. Such a procedure optimizes the trade-off between frequency response and rise time. Great care was taken to ensure that no air was present in the fluid-filled cannulae, syringes and pressure transducers. After each air breath, the cannula was flushed. Signals from the pressure transducers were amplified by Gould Carrier Amplifiers, and recorded on a Bell & Howell 4020 A FM 14 channel tape recorder and a Gould-Brush 260 pen recorder. Electromyographic signals were amplified with Grass 5 11J preamplifiers, recorded on the Bell & Howell 4020 tape recorder and displayed on the Gould-Brush 260 pen recorder. Because fluid-filled pressure transducers have low frequency responses, some recordings were made (on *Channa striatus*) with Millar catheter-tip transducers (Model PR-249; diameter of the catheter, 0·7 mm), which were passed through polyethylene cannulae (i.d., 1·4 mm; o.d., 1·9 mm). These recordings were essentially the same as those obtained with the fluid-filled Statham pressure transducers.

During the pressure and electromyographic recordings the fish was given sufficient room in a small aquarium to manoeuvre its way to the surface to breathe and to turn. ▶he electrodes and polyethylene cannulae were left implanted for up to 5 days, during

which time multiple sets of experiments were conducted on different but overlapping
sets of muscles. Over 50 air ventilation sequences were recorded in this fashion.

<div style="text-align:center">RESULTS</div>

Detailed anatomical descriptions have been made of the cranial and branchial
muscles (Munshi, 1962, 1976; Liem, 1980; Lauder & Liem, 1983), air-breathing
organs (Munshi, 1962; Hughes & Munshi, 1973; Hakim, Munshi & Hughes, 1978),
circulatory system (Ishimatsu, Itazawa & Takeda, 1979) and osteology (Lauder &
Liem, 1983). A brief description of the anatomical position of the cranial muscles and
their actions is given here (Fig. 1).

The levator arcus palatini (*lap*) arises from the sphenotic, runs posteroventrad and
inserts on the large hyomandibula. Action of the levator arcus palatini is abduction
of the hyomandibula, and because of the connections and joints, it also abducts the
entire palatal complex and operculum.

The adductor arcus palatini (*aap*) is the antagonist of the levator arcus palatini and
is a continuous strip of muscle arising from the parasphenoid and inserted along the
dorsal rim of the palatal complex. It adducts the entire palatal complex or suspensory
apparatus and the operculum.

The dilatator operculi (*do*) arises from the pterotic just posterior to the hyoman-
dibula and inserts on a lever-like process on the lateral aspect of the operculum, dorsal
to its articulation with the hyomandibula. Action of the dilatator operculi is strong
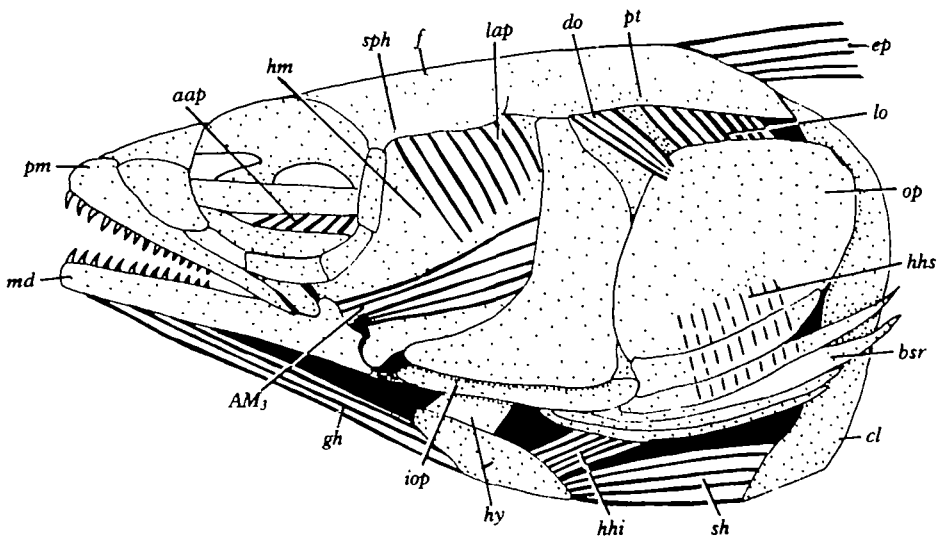opercular abduction.

Fig. 1. Diagrammatic representation of the major skeletal elements and muscles of the head of
Channa. Abbreviations: *aap*, adductor arcus palatini; *AM$_J$*, part A_J of adductor mandibulae muscle
complex; *bsr*, branchiostegal rays; *cl*, cleithrum; *do*, dilatator operculi; *ep*, epaxial muscle; *f*, frontal;
gh, geniohyoideus (protractor hyoidei); *hhi*, hyohyoideus inferior; *hm*, hyomandibula; *hhs*,
hyohyoideus superior; *hy*, hyoid; *iop*, interoperculum; *lap*, levator arcus palatini; *lo*; levator operculi;
md, mandible; *op*, operculum; *pm*, premaxilla; *pt*, pterotic; *sh*, sternohyoideus; *sph*, sphenotic.

The adductor operculi originates from the underside of the pterotic. It is a long, cylindrical muscle running laterad to insert on the medial surface of the operculum, just behind the articulation of the operculum with the hyomandibula. It closes the operculum and reduces the size of the opercular cavity.

The levator operculi (*lo*) arises from the lateral ridge of the pterotic behind the dilatator operculi. It inserts on the medial aspect of the operculum covering some distance of the bone. It lifts the operculum and thereby lowers the mandible (Osse, 1969; Liem, 1970).

Functionally the branchiostegal apparatus is an integral component of the operculum.

The hyohyoideus superior (*hhs*) runs between the operculum and suboperculum to the upper branchiostegal rays. It adducts the branchiostegal apparatus and thereby opens the ventral opercular cleft. The hyohyoideus inferior (*hhi*) runs between the hyoid arch and the ventral branchiostegal rays. Its action fans out the branchiostegals and closes the opercular cleft.

The large sternohyoideus (*sh*) runs between the cleithrum and the hyoid arch especially the urohyal. Its primary effect is to lower the floor of the buccal cavity and also to lower the mandible.

The geniohyoideus (*gh*) or protractor hyoidei muscles run between the mandibular symphysis and the hyoid arch. Its primary action is to pull the hyoid arch anterodorsad, thus raising the floor of the buccal cavity.

The adductor mandibulae complex consists of several parts (Lauder & Liem, 1983, p. 180) of which the deepest head, the adductor mandibulae pars A_3 (AM_3) plays an important role during aquatic as well as air ventilation. It arises from the palatal complex, and runs anteroventrally to insert on the angular of the mandible. Its primary action is to raise the mandible.

The epaxial muscle (*em*) is the dorsal continuation of the body musculature, which inserts on the posterior aspect of the neurocranium.

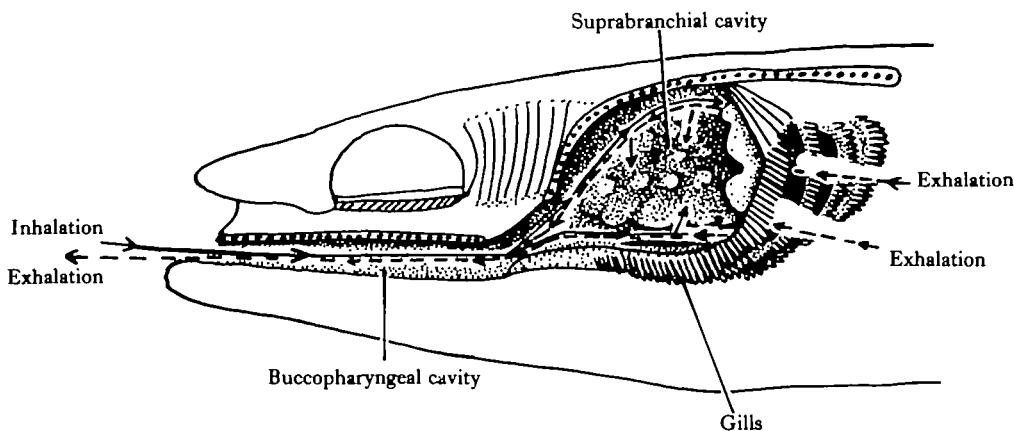The suprabranchial cavity (Munshi, 1962) is located above the gills (Fig. 2) and is

Fig. 2. Diagrammatic representation of the buccal cavity, suprabranchial cavity, and gills of *Channa* after removal of the entire opercular apparatus, suspensory apparatus and jaws. Path of water flow during exhalation is indicated by broken line, and during inhalation by solid line.

surrounded by the otic components of the neurocranium dorsally and medially, and by the hyomandibula laterally. Thus the cavity is surrounded mainly by bone. The suprabranchial cavity is in open communication with the buccal cavity, whereas the entrance to the opercular cavity can be regulated by changing the position of the gill arches (Fig. 2).

### High-speed X-ray ciné films

High-speed X-ray ciné films at 150 frames s^{-1} showed clearly the outlines of the air-filled suprabranchial and buccal chambers, and the principal skeletal elements. The movements of air and of the skeletal elements were clearly discernible from the films (Fig. 3).

Eleven sequences were filmed of C. punctatus, C. striatus and C. gachua. Neither interspecific nor intraspecific differences in air ventilation were found. Air ventilation begins when the fish approaches the water surface at angles of 30–40°. (The same angle was recorded for C. argus by Ishimatsu & Itazawa, 1981.) At this stage air in the suprabranchial and buccopharyngeal cavities remains stationary. The jaws are adducted and the hyoid is in a resting position (Fig. 3a). When the fish reaches the surface (Fig. 3b), the hyoid moves posteroventrally, the jaws remain adducted, but the operculum is abducted (as seen from a dorsoventral film). Thus, the buccopharyngeal cavity is expanded by the lowering of the hyoid and therefore the floor of the cavity, while the gill cover is opened. When the snout breaks the surface, the jaws are opened and air moves out of the posteroventral compartments of the suprabranchial and buccopharyngeal cavities and out of the opened mouth. Exhalation is completed when all air has left the suprabranchial and buccopharyngeal cavities (Fig. 3e). In the next stage, inhalation begins with a posteroventral movement of the hyoid and co-incident filling of the anteroventral compartments of the buccopharyngeal and supra-branchial cavities (Fig. 3f). Inhalation is completed when the entire suprabranchial and buccal cavities are filled with air, the hyoid apparatus is raised and the jaws adducted (Fig. 3g). Finally the fish sinks below the surface (Fig. 3h).

High-speed light ciné films

Ishimatsu & Itazawa (1981) gave a description of ventilation of the air-breathing organ in Channa argus on the basis of 8 mm ciné films at 24 frames s^{-1}. Because my findings differ from those of Ishimatsu & Itazawa, I present a composite graphical representation on the basis of 49 air ventilation sequences obtained with a graphics tablet and an Apple II microcomputer (Fig. 7). Air ventilation begins with a strong abduction of the palatal complex or suspensory apparatus, followed by depression of the hyoid and a lowering of the mandible (Fig. 7). When the mouth is open the

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Fig. 3. Representative prints from an X-ray ciné film at 150 frames s^{-1} during air ventilation in Channa striatus (standard length, 39 cm). (a) Fish approaches the surface with suprabranchial cavity (sb) and buccopharyngeal cavity (bc) filled with air. Note parasphenoid (ps) and hyoid (hy); (b) air ventilation begins, air moves forward; (c) air is moved from the suprabranchial cavity into the buccopharyngeal cavity and out of the opened mouth, the hyoid is depressed; (d) all air has been moved out, the mouth is closed and the hyoid is raised, but the fish remains with the snout protruded out of water; (e) mouth is opened and new air taken in; (f) and (g) filling of buccopharyngeal and suprabranchial cavities; (h) inhalation is completed, mouth is closed and fish sinks below the surface.

Fig. 3
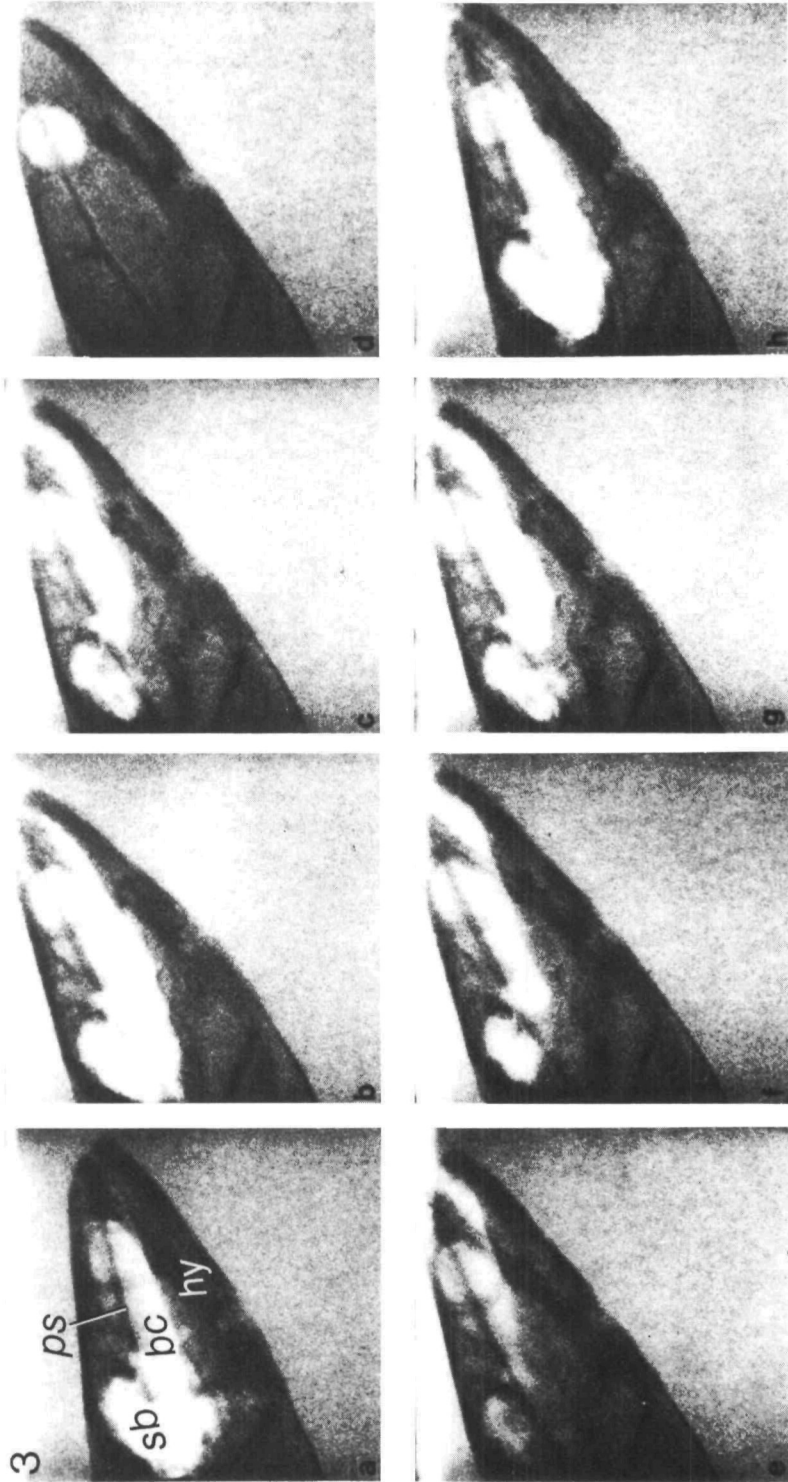
opercula are abducted. This is followed by a rapid adduction of the suspensory apparatus while the mouth is still fully abducted. Then the hyoid is raised, and the mouth and opercula are closed. In the next stage the mandible and hyoid are lowered abruptly, followed by a gradual and partial abduction of the suspensory apparatus. Finally, the jaws, hyoid, the partially abducted suspensory apparatus and the opercula are adducted. Thus the mouth is opened twice (see also Fig. 3) during each ventilation cycle and not once as suggested by Ishimatsu & Itazawa (1981).

Pressure studies

Pressures developed in buccal, opercular and suprabranchial cavities were recorded during both aquatic and aerial respiratory cycles from free-swimming fishes. Great differences were found between the pressure waveforms during air- and water-breathing.

During aquatic ventilation, pressure changes occur in the buccal and opercular cavities (Fig. 4) but never in the suprabranchial cavity. Few differences were seen in the basic form of the pressure profiles recorded from seven individuals of *C. striatus*, three individuals of *C. punctatus* and two individuals of *C. gachua*. However, variations in amplitude and frequency of the waveforms occur according to variation of physiological activity and degree of acclimatization. An air breath is always followed by an increase in both frequency and amplitude of the waveforms of the aquatic cycle. The pressure waveforms (Fig. 4) in the partially air-filled buccal cavity and water-filled opercular cavity closely resemble those recorded for water-breathing teleosts by Hughes (1960b) and Ballintijn (1969). Thus aquatic ventilation in *Channa* proceeds by interacting buccal and opercular pressure changes (Fig. 2) which combine to produce a differential pressure across the gill resistance, forcing water over the gills throughout most of the cycle. As in most teleosts (e.g. Ballintijn, 1969) three phases can be distinguished in the respiratory cycle. The first phase, the respiratory pause, is short and coincides with maximal abduction of the opercula and inactivity of the muscles. Buccal and opercular cavities have negative pressures. The second phase is compressive, during which the pressures in both the buccal and opercular cavities become positive. During the third phase, which is expansive, the negative pressure in the buccal cavity draws water through the wide open mouth, while a considerable negative pressure in the opercular cavities sucks water through the gills from the mouth cavity. From X-ray ciné film (Fig. 3) one can see that during aquatic ventilation water flow is confined to the ventral part of the buccal cavity, the dorsal half being occupied by air. Accordingly the gills are located primarily on the ventral, horizontal gill bars while the roof of the mouth is highly vascularized and provided with respiratory nodules (Liem, 1980; Ishimatsu & Itazawa, 1981). *Channa* continues branchial irrigation while the dorsal half of the buccopharynx is filled with air.

The pressure waveforms during air ventilation are more complex and different from those of aquatic ventilation (Fig. 5). The pressure in the suprabranchial cavity changes according to a pattern which resembles that of the buccal cavity (Fig. 6). The pressures in the buccopharyngeal and suprabranchial cavities remain lower than those of the opercular cavities throughout the air ventilation cycle (Fig. 6). In the first phase he pressure in the buccal cavity begins to drop, followed (after 120–180 ms) by a
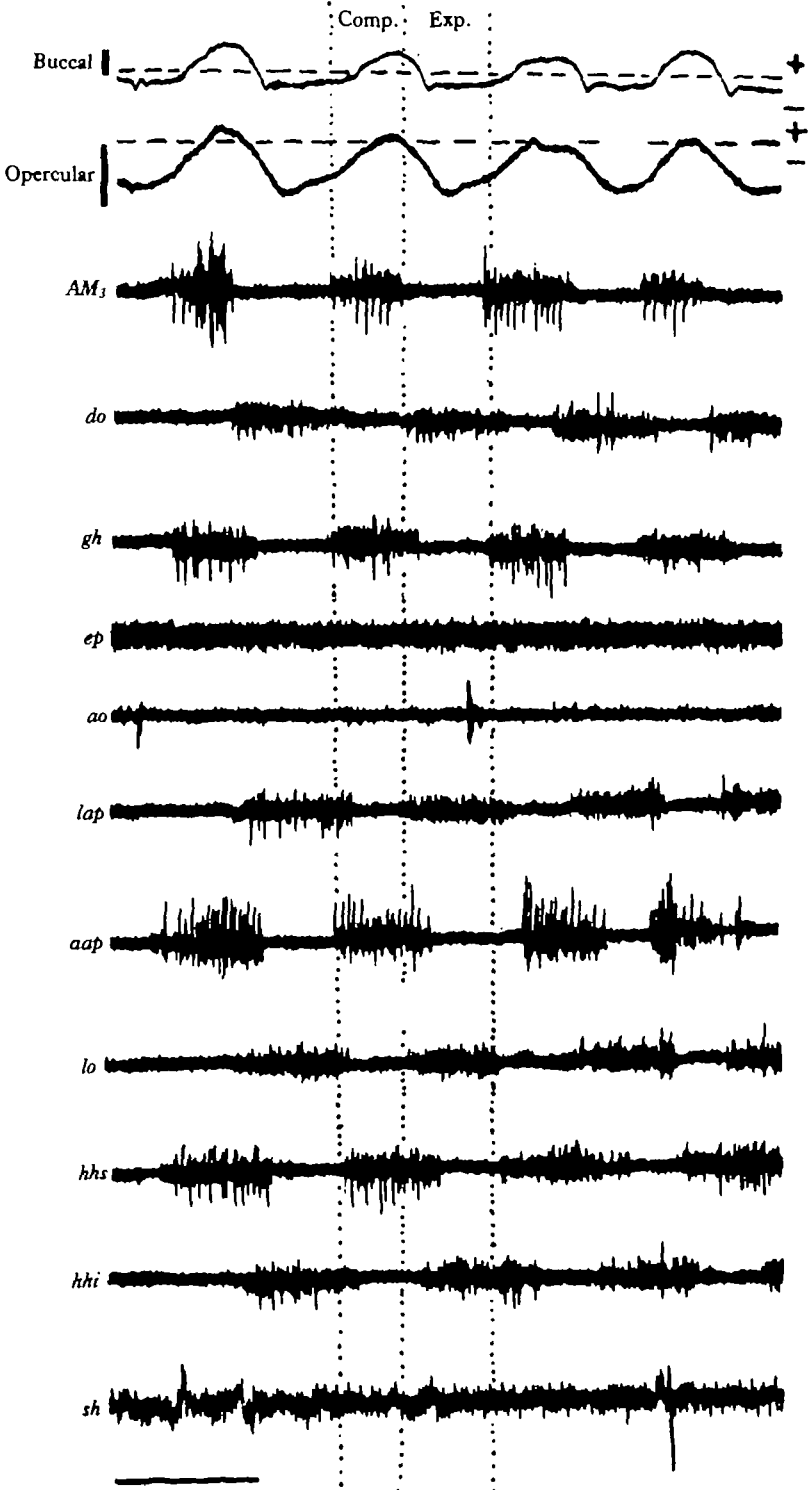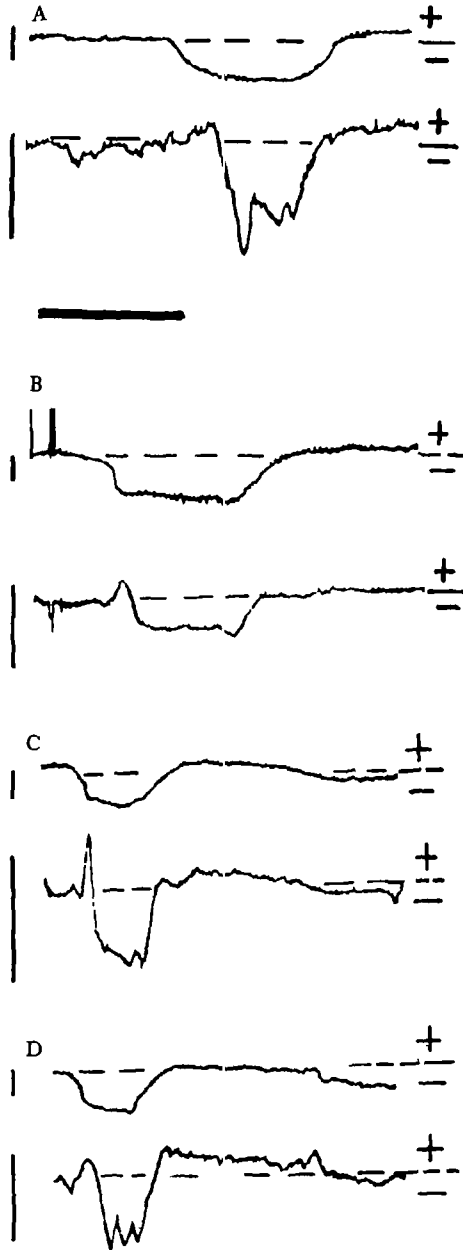
Fig. 5. Representative pressure waveforms during air ventilation of the buccal cavity (top of each pair) and opercular cavity (bottom of each pair) in *Channa punctatus* (A and B), and *Channa gachua* (C and D). Time scale is 250 ms; pressure scale is 50 cmH₂O.

Fig. 4. Pressure waveforms of the buccal and opercular cavities together with electromyograms of the head muscles during aquatic hyperventilation in *Channa striatus*. The electromyographic and pressure profiles are composites of recordings selected from 45 sequences. Pressure scale is 5 cmH₂O; time scale is 1 s, Comp., compressive phase; Exp., expansive phase. (For explanation of other abbreviations, see Fig. 1.)
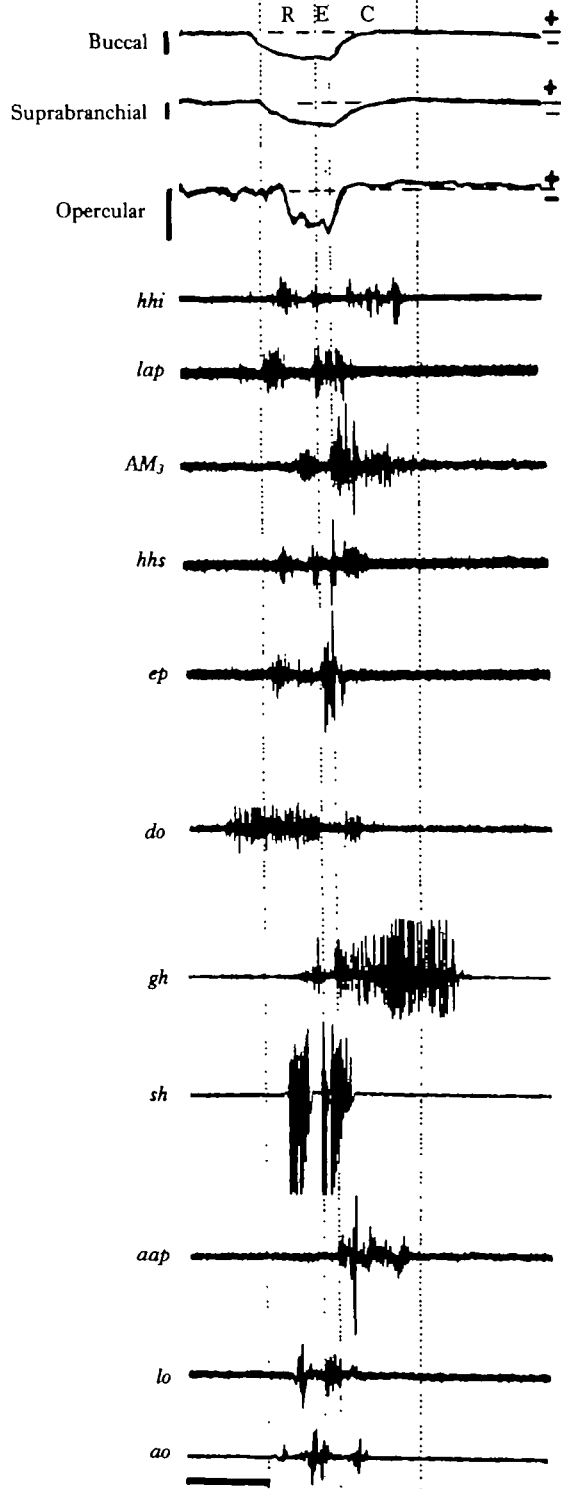
similar drop in the suprabranchial cavity, while the pressures in the opercular cavities show an initial rise in pressure before dropping. As the first phase proceeds the pressures in the buccal, suprabranchial and opercular cavities continue to decline very gently. In the second phase the low pressures in the buccal, suprabranchial and opercular cavities are maintained, and the mouth is opened. In the third phase, the opercular pressure rises steeply to the ambient level, while the pressures in the buccal and suprabranchial cavities show a slower rise to ambient levels. No significant intraspecific and interspecific differences were found in the pressure waveforms of the buccal and suprabranchial cavities (Fig. 5).

Activity in the respiratory muscles during aquatic ventilation

As in the carp (Ballintijn, 1969), the aquatic respiratory cycle of *Channa* can be divided into three phases: (1) a very short respiratory pause, coinciding with the peak abduction of the lower jaw and opercula, during which muscles are not active during quiet respiration; but when respiratory activity increases, the respiratory pause decreases in duration or disappears. (2) The compressive or contraction phase; and (3) the expansive phase (Fig. 4).

In the compressive phase, the A_3 part of the adductor mandibulae, the adductor arcus palatini, geniohyoideus anterior and hyohyoideus superior muscles are active (Fig. 4). Action of the adductor arcus palatini adducts the palatal arch, while action of the geniohyoideus raises the floor of the mouth. As a result, the volume of the buccal cavity is decreased and a positive pressure builds up forcing water through the gills into the opercular cavities. This influx of water raises the pressure in the opercular cavities, and water flows out through the opercular slit, which is opened by action of the hyohyoideus superior muscle (Fig. 4). During the compressive phase the pressure in the buccal cavity is always higher than that in the opercular cavity.

The expansive phase starts with activity in the levator arcus palatini, immediately followed by activity in the dilatator operculi and hyohyoideus inferior muscles. The levator operculi becomes active and the mouth opens. The results of the activity of these muscles are: pronounced abductions of the palatal complex, the jaws and the opercular apparatus, while the opercular slit is sealed by the hyohyoideus inferior muscle. Negative pressure in the buccal cavity creates an inflow of water through the opened mouth. An even lower pressure in the enlarged opercular cavities draws the water from the buccal cavity through the gills.

The pattern of activity of the muscles during aquatic ventilation resembles that of the perch *Perca fluviatilis* (Osse, 1969). The sternohyoideus muscle does not contract, and the adductor operculi remains inactive during the aquatic cycle. During increased aquatic ventilation all the muscles that are active during the expansive phase are indirectly coupled to the hyoid apparatus (Ballintijn & Hughes, 1965; Ballintijn, 1969; Osse, 1969; Elshoud, 1978; Vandewalle, 1979). Activity in the levator arcus

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Fig. 6. Pressure waveforms of the buccal, suprabranchial and opercular cavities together with electromyograms of the head muscles of *Channa striatus* during air ventilation. Electromyographic and pressure profiles are composites of recordings selected from 51 sequences. Pressure scale is 50 cmH₂O; time scale is 250 ms. C, compressive phase; E, expansive phase; R, reversal phase. (For explanations of other abbreviations, see Fig. 1.)

palatini, hyohyoideus inferior and dilatator operculi has an overall effect on the side-walls and floor of both the buccal and opercular cavities. Thus, *Channa* exhibits the same muscle activity pattern, pressure patterns and kinematics of jaws and opercula as those encountered in less specialized, purely aquatic breathing teleosts.

### Activity in the respiratory muscles during air ventilation.

Based on the pattern of muscle activity, three phases can be distinguished during an air ventilation cycle (Fig. 6).

The first is a reversal phase during which there is early activity in the dilatator operculi and levator arcus palatini muscles. It is immediately followed by activity in the sternohyoideus, epaxial, levator operculi and hyohyoideus superior muscles. In the final stages of the first phase, the adductor mandibulae complex, adductor arcus palatini and adductor operculi muscles become active. Early abduction of the palatal complex by action of the levator arcus palatini, lowering of the buccal floor by action of the sternohyoideus and head lifting as a result of epaxial muscle activity, will greatly enlarge the buccal cavity and thus lower the buccal pressure drastically. At the same time action of the dilatator operculi and hyohyoideus superior will open the opercular slit. As a result water is drawn through the opened opercular slit into the opercular and suprabranchial cavities and into the buccal cavity. This reversal flow created by the enlarged buccal cavity will draw the air out of the suprabranchial cavity which becomes rapidly filled with water from the opercular cavity. The mouth is then opened by a strong burst of activity of the levator operculi, to expel the air bubble from the opened mouth. The opercular slit is closed by combined action of the dilatator and hyohyoideus inferior muscles. Once the air bubble has been exhaled, the adductor mandibulae closes the mouth, the adductor arcus palatini adducts the palatal complex, and the adductor operculi adducts the opercular apparatus, in preparation for the second expansive phase.

At the onset of the second phase, which is of very short duration, the levator arcus palatini, epaxial muscles and the sternohyoideus muscles become active, thereby enlarging the buccal cavity and lowering buccal pressure. Because the mouth is opened by a strong burst in the levator operculi, a fresh air bubble is sucked into the buccal cavity. The second phase is clearly an expansive one and serves to draw new air into the buccal cavity.

The third phase is compressive: the adductor arcus palatini, adductor mandibulae and geniohyoideus muscles show considerable activity, indicating that the buccal pressure is increased to fill the suprabranchial cavity with new air even though hydrostatic pressure must play an important role. Activity in the hyohyoideus superior muscle will open the opercular slit to allow excess water and air to be expelled. The intensity and duration of activity of the adductor muscles during the compressive phase is variable. Activity in the adductor mandibulae complex can be very low, while

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Fig. 7. Summary diagram of the time relationship of respiratory muscle activity, pressures in buccal and opercular cavities, and movements of the mandible, hyoid and operculum. Black bars indicate regular activity in over 66 % of the recordings, open bars indicate activity in less than 34 % of the recordings. C, compressive phase; E, expansive phase; R, reversal phase. For explanation of other abbreviations, see Fig. 1.
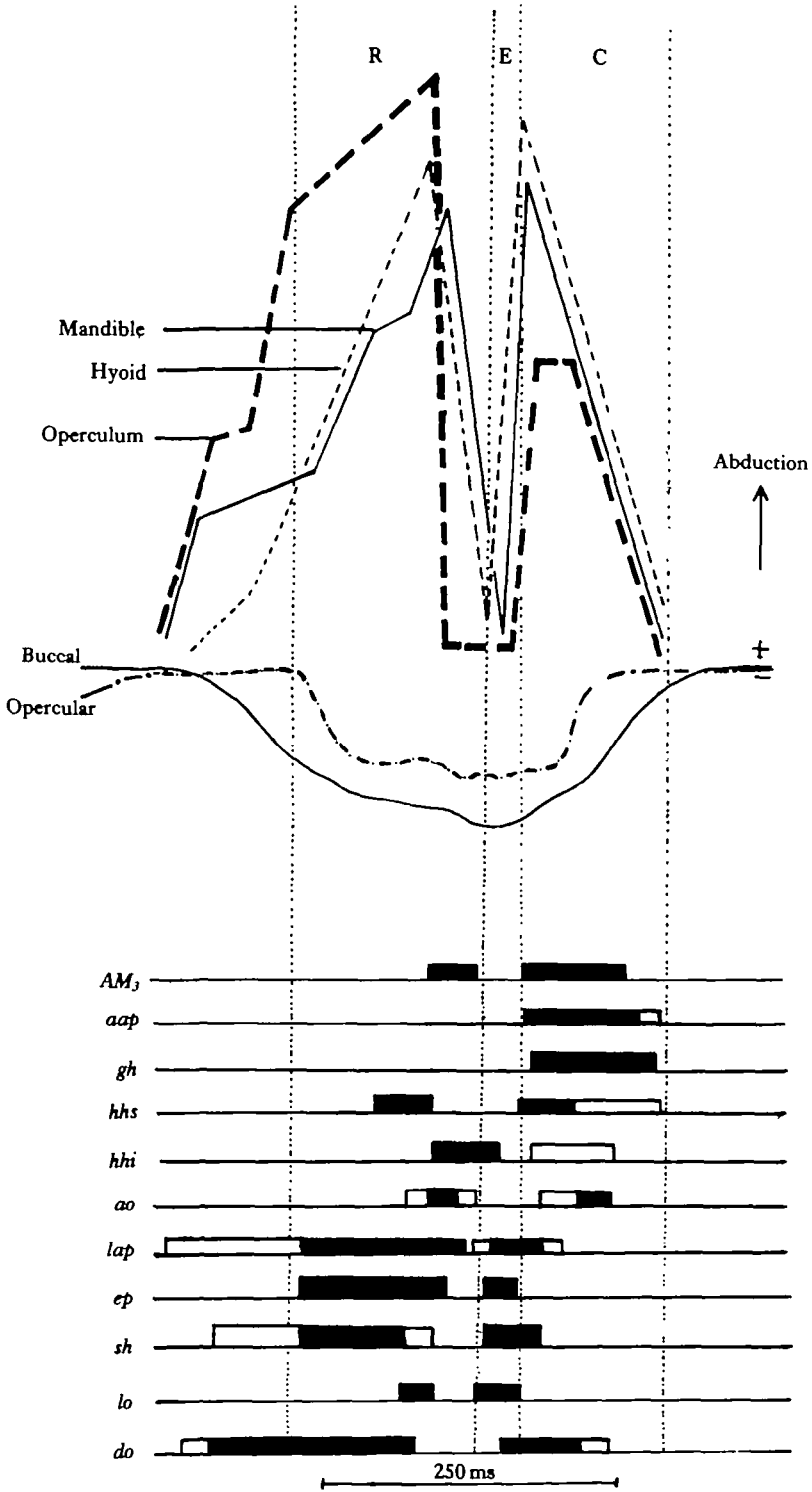
the geniohyoideus can exhibit firing patterns of very short low amplitude to prolonged high amplitude. In less than 25 % of the recordings the usually silent dilatator operculi is active together with the hyohyoideus superior muscle during the compressive phase.

DISCUSSION

It is now possible to furnish a functional interpretation of the mechanisms underlying the aerial and aquatic cycles of *Channa* on the basis of anatomical data, EMG and pressure recordings, and the high-speed light and X-ray ciné films.

The aquatic respiratory cycle

Even though *Channa* is an evolutionarily very specialized air-breathing teleost (Munshi, 1962; Ishimatsu & Itazawa, 1981; Lauder & Liem, 1983), the gross sequencing of the movements, muscle actions and pressure changes during aquatic ventilation in *Channa* is very similar to that described for more generalized teleosts (e.g. Hughes & Shelton, 1958; Hughes, 1960b; Saunders, 1961; Ballintijn & Hughes, 1965; Hughes & Umezawa, 1968; Ballintijn, 1969; Elshoud, 1978; Vandewalle, 1979), sturgeons (Burggren, 1978), elasmobranchs (Teichman, 1959; Hughes, 1960a; Hughes & Ballintijn, 1965) and lungfish (McMahon, 1979). Gill ventilation is achieved by synchronous movements of both the buccal cavity sidewalls and floor, and the operculae. Large positive pressures are produced anterior to the gill sieve by a buccal force pump, which is activated by action of the A_3 part of the adductor mandibulae, the adductor arcus palatini and geniohyoideus anterior muscles. Alternating pressures below ambient hydrostatic pressure posterior to the gills are produced by the opercular suction pump, which is activated by contractions of the dilator operculi and hyohyoideus inferior muscles. It is postulated that water flow will follow the resulting pressure gradient across the gills (Holeton & Jones, 1975; Lauder, 1984). While pressure-flow relationships have not been analysed in detail in *Channa*, the tracing of movements of radio-opaque dye carefully added to the inhalant water indicates that a continuous flow of water from the buccal to the opercular cavities occurs throughout the respiratory cycle. Because the vascularized dorsal compartment of the buccal cavity is filled with air, the water flow within the buccal cavity is confined to the ventral compartment.

The mechanism of aerial ventilation

The results of electromyograms, pressure changes and kinematic measurements from high speed X-ray and light movies recorded during a large number of air-breathing cycles are summarized diagrammatically in Fig. 7. The aerial respiratory cycle differs significantly from the aquatic cycle and can be subdivided into three phases.

The first phase occurs as the fish approaches the surface and activity is recorded in the levator arcus palatini, dilatator operculi and sternohyoideus muscles. This activity is maintained as the epaxial and hyohyoideus superior muscles become active. The buccal floor is lowered, and the buccal sidewalls and operculae are abducted. Pressures in the buccal and opercular cavities decrease rapidly, with the pressure drop

in the opercular cavities lagging behind that of the buccal cavity. In this way a reversed flow of water is produced. Water is drawn from underneath the gill cover, which is opened by the combined action of the dilatator operculi and hyohyoideus superior muscles. It is then drawn from the gill cavity, into the suprabranchial cavity and buccal cavity, thereby forcing the air forward (Figs 2, 3). Once the air has been moved forward in the buccal cavity, the fish opens its mouth by action of the levator operculi muscle, and all air is released from the mouth. Release of air is immediately followed by action of the adductor mandibulae closing the mouth and adduction and closure of the operculae by contractions of the adductor operculi and hyohyoideus inferior muscles. Adduction of the jaws and the operculae in the terminal stage of the first phase has no effects on the pressures in the buccal and opercular cavities. The buccal and opercular pressures are maintained at below ambient levels. Low buccal pressure is maintained by keeping the sidewalls abducted, which in turn may contribute to the continued low pressure in the opercular cavities by means of the couplings described for other teleosts by Ballintijn & Hughes (1965) and Ballintijn (1969).

The second phase closely resembles the expansive phase so typical during prey capture in teleosts (Osse, 1969; Liem, 1978; Lauder, 1980b). The mandible is lowered by the combined actions of the levator operculi and sternohyoideus muscles, while the fish is at the surface. Activity in the levator arcus palatini, sternohyoideus and epaxial muscles maintains the enlarged buccal volume and reduced pressure with which new air is drawn into the buccopharyngeal cavity. The opercular cavity is abducted by the dilatator operculi muscle. Once the buccopharyngeal cavity is filled with air, the third phase begins.

The third phase is moderately compressive and characterized by a rapid closure of the mouth and a rise in buccal and opercular pressures. The floor of the buccal cavity is raised by contraction of the geniohyoideus and the sidewalls adducted by the adductor arcus palatini. Adduction of the operculum is correlated with an increase in opercular pressure and activity in the adductor operculi, hyohyoideus superior and hyohyoideus inferior muscles. Hyohyoideus superior activity brings about the opening of the branchiostegal apparatus to allow water to escape from the pressurized opercular cavity. During this phase, the water in the suprabranchial cavity is forced out and replaced by air. At the end of the compressive phase, the suprabranchial cavity is completely filled with air, and air is retained in the dorsal half of the buccopharyngeal cavity as well. The ventral half of the buccopharyngeal cavity becomes water-filled allowing aquatic ventilation to proceed independently of aerial respiration.

The nature and origin of the aerial respiratory mechanism

The pressure waveforms, patterns of muscle actions, and movements of the major skeletal elements recorded during the aquatic and aerial cycles differ both quantitatively and qualitatively. The aerial cycle (0·11–0·2 s) is at least four times faster than the aquatic cycle (0·8–2·1 s). *Channa* minimizes the time spent at the surface, during which it releases and captures air. During aquatic ventilation, the sternohyoideus, epaxial and adductor operculi remain inactive whereas during aerial ventilation the three muscles exhibit high amplitude and extended activity. In sharp contrast to the pattern in the aquatic cycle, buccal pressure remains lower than opercular

pressure throughout the aerial ventilation cycle. Thus the mechanism of suprabran⁺ chial cavity ventilation in *Channa* is fundamentally different from the basic aquatic cycle.

Early and prolonged activity in the levator arcus palatini, dilatator operculi, sterno-hyoideus and hyohyoideus inferior muscles and the creation of a low pressure in the buccal cavity are strong indications that the air ventilation cycle is actually homologous to the basic cough mechanism (Ballintijn, 1969; Osse, 1969; Hughes, 1975). As during the cough, the buccal pressure is first lowered by the expansive muscles while the opercular slit is opened to create a reversed flow of water from the outside into the opercular cavity, thence the buccal cavity. In *Channa*, the suprabran-chial cavity is interposed between the opercular and buccal cavity (Fig. 2). The reversed flow of water is first drawn into the suprabranchial cavity before entering the buccal cavity. No special modification of the basic cough mechanism has taken place in the pattern of muscle actions in *Channa*. The pressure waveforms and EMG pattern of *Channa* during air ventilation closely resemble those of the cough mechan-ism in the perch (Osse, 1969) and carp (Ballintijn, 1969). Whenever *Channa* is induced to cough in the aquarium by introducing sand in the respiratory current, it invariably expels air from its mouth and the fish immediately surfaces to take in a new air bubble. The electromyographic pattern and pressure waveforms during such a cough are identical to those during air ventilation. Thus the mechanism of exhalation in *Channa* is identical to the basic cough mechanism. Instead of clearing debris from its gills, the fish simply uses the reversed flow of water to move air from the suprabran-chial cavity out of the mouth.

Inhalation is accomplished by a rapid expansion, during which all expansive muscles including the epaxial and sternohyoideus muscles are recruited, followed by a compressive phase. The pressure waveforms, EMG pattern and high velocity move-ments of skeletal components during inhalation resemble those of the feeding cycle except that there is less overlap in activity periods of the expansive and compressive muscles. Essentially the mechanism of inhalation in *Channa* is based on, and easily derived from, the feeding cycle. In sharp contrast with the condition in the lungfish (McMahon, 1969), the air chamber in *Channa* is filled by a buccal pressure that does not rise above ambient pressure. The aerial ventilation cycle in *Channa* is therefore a characteristically low pressure system, which requires little energy.

On the basis of anatomy, Munshi (1962) has made the assumption that exhalation takes place through the opercular clefts and is brought about by contractions of numerous specialized branchial muscles, and that inhalation is brought about by elastic recoil. Based on experimental data, it has been shown that Munshi's assump-tions must be rejected (Liem, 1980; Ishimatsu & Itazawa, 1981). The pressure waveforms recorded from the suprabranchial cavity are identical to those of the buccal cavity indicating that the former cavity plays a passive role and is not equipped to alter its pressure. It is unnecessary to postulate the evolutionary development of a new and complex aerial ventilation mechanism in *Channa* when the existing cough mechanism can be so simply modified to serve the new function. It is suggested that several phylogenetically unrelated teleosts (Anabantoidei, Peters, 1978; Clariidae, Hellin & Chardon, 1981), in response to fluctuating hypoxic and hypercarbic environments, have come to use their coughing mechanism, previously used only to power the

reversed flow of water to clear debris from their gills, to move their respiratory air. Because air ventilation by means of a modified cough mechanism is a water-dependent system, it cannot function outside the aquatic habitat (Ishimatsu & Itazawa, 1981). It can be predicted that fishes employing the cough mechanism for air ventilation are unable to invade terrestrial habitats for long periods unless they possess an additional mode of air ventilation, which is independent of water.

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