THE MECHANISM OF GILL VENTILATION IN THREE FRESHWATER TELEOSTS

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(With Plate 13)

INTRODUCTION

The general nature of the respiratory movements of teleost fishes has been known for a long time, but several significant details of the mechanism are only now becoming clear as a result of modern experimental methods. Reviews of the extensive literature have been written by Babak (1921), Leiner (1938), and Fry (1957). Du Verney (1701) is generally credited with the first description of breathing movements in fish, and his view that mouth and opercula expand and contract together was later supported by Paul Bert (1870). However, Bert's method, using ampoules in the buccal and opercular cavities, interfered considerably with the movements. This was pointed out by François-Franck (1906 a, b), who was one of several workers to make use of smoked drum techniques. It became clear from this work that movement of the mouth preceded that of the opercula by a brief interval of time in all phases of the breathing cycle. The importance of both the buccal and the opercular valves in producing a unidirectional flow of water was clearly appreciated by Bert. Baglioni (1907, 1910) made a comparative study of different marine fishes and drew up a classification based on the degree of development of the branchiostegal apparatus. Despite some variations between different fishes, his account agrees substantially with that of François-Franck and forms the basis of the descriptions given in most elementary text-books.

These experimental investigations tended to draw attention to the intermittent nature of the water flow into the buccal cavity and out of the opercular cavities. The breathing mechanism was regarded as a single pump because the gill arches were thought to hang quite separately in the buccal cavity, there being no contact between the filaments of adjacent arches. The direction of water flow in such a system would have been parallel to the long axis of the gill filaments, with little movement of water between the secondary lamellae, which are the plates running across the upper and lower surfaces of the filaments.

A completely different view was expounded by Woskoboinikoff (1932), who maintained that the gills were not such passive features of the system but that, by virtue of their arrangement in the living animal, they functionally separated the buccal from the opercular cavities. The gill filaments of adjacent gill arches were

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thought by him to interdigitate so that the whole gill structure formed a continuous sieve. The only pathway for the water through this sieve would therefore be at right angles to the long axis of the gill filaments through the small spaces between the secondary lamellae. Van Dam (1938) subscribed to a very similar view of the gill arrangement, and this was finally confirmed by Hofdijk Enklaar (in Bijtel, 1949), who looked at gills directly through a celluloid window inserted in the operculum and saw that the tips of the filaments remained in contact throughout the normal breathing cycle. In Woskoboinikoff's scheme it was proposed that the gills also acted as a valve. He stressed the importance of the phase of opercular abduction during which the pressure was lowered in the opercular cavity, water being drawn through the gills by the action of this so-called opercular suction pump. During opercular adduction the gills in some way prevented reflux of water, the opercular valve opened and the water was forced out of the cavity. Woskoboinikoff ascribed little function to the buccal apparatus in this paper.

In subsequent papers, however, Woskoboinikoff & Balabai (1936, 1937) corrected this over-emphasis on the suction pump and suggested that the water current was maintained by the action of a buccal force pump before the gills and of a suction pump behind them. These important papers have not been widely known and indeed were unknown to the authors when the major part of the work in this paper was carried out. Nor were they mentioned by van Dam (1938) who was the first worker to consider in any detail the nature of the flow of water across the respiratory epithelium. He drew attention to two important functional adaptations which are involved in the high utilization (50–80 %) of oxygen that he measured. The first of these is that the direction of water flow is opposite to that of the blood circulation in the secondary lamellae. Secondly, he suggested that the flow of water across the respiratory surfaces is continuous throughout the whole breathing cycle. He substantiated this suggestion by connecting the two cavities externally by means of a glass tube and showing that water in this tube always moved in the direction mouth-to-operculum. From this experiment van Dam concluded that the pressure in the buccal cavity was always greater than that in the opercular cavity. These views were incorporated in a generalized scheme of teleostean breathing mechanisms by Henschel (1939).

Measurements of the pressures in the two cavities had in fact been made by Woskoboinikoff & Balabai at about the same time as van Dam was doing his work, and in general their observations confirmed the hypothesis outlined above. However, the pressure in both cavities fluctuated and they found that, while both the maximum and minimum values in the buccal cavity were generally higher than the corresponding pressures in the opercular cavity, there was an overlap in the two ranges such that the maximum pressure in the opercular cavity was often greater than the minimum pressure in the buccal cavity. In order to obtain further proof of van Dam's suggestion of a continuous flow of water across the gills it is therefore important to know the exact time course of these pressure fluctuations. Observations of this kind have been reported briefly (Hughes & Shelton, 1957) and more detailed descriptions are given in the present paper.

METHODS

Measurement of pressures, such as those produced by the respiratory movements of fish, presents considerable practical difficulties, particularly if a study is to be made of their time course. The manometer should have a high natural period in order to record rapid changes of pressure. In addition, the connexions between the respiratory chambers and the manometer must be made with fine tubing so that the breathing movements are not restricted in any way. In all manometers these two features are in some degree mutually exclusive and in water manometers, such as those used by Woskoboinikoff & Balabai in their quantitative measurements and by van Dam in his qualitative demonstration, an adequate compromise cannot be obtained.
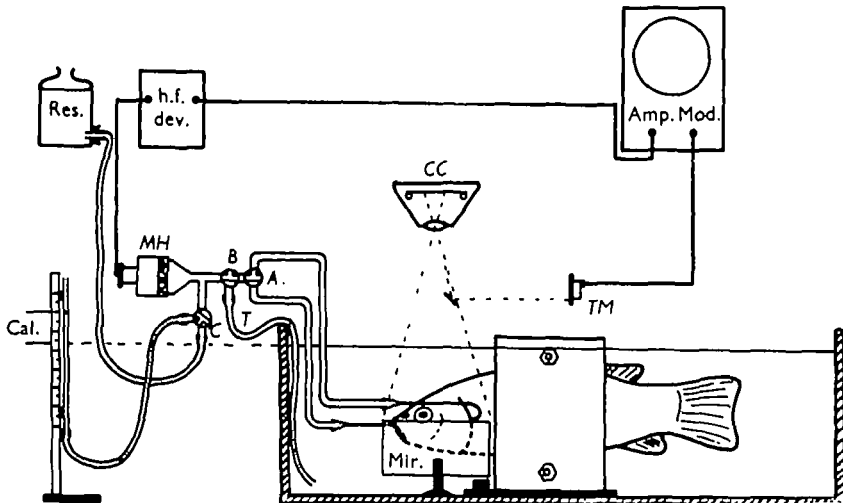
Text-fig. 1. Diagram of the apparatus used for the simultaneous recording of movements and pressures. A detailed description will be found in the text.

In the present experiments a condenser manometer (Hansen, 1949) consisting of a manometer head (*MH*, Text-fig. 1) and 'electrical aggregate' (the high-frequency device (h.f. dev.) and d.c. amplifier (Amp.)) was used. The connexions between the buccal and opercular cavities and the manometer were made with short lengths of hypodermic tubing of 0·5 mm. outside diameter joined to stainless steel tubing of much larger diameter and bore. In the first series of experiments only one such tube was connected to the manometer, and to change the connexion from one cavity to the other involved exchanging the hypodermic tubing and readjusting its position. Subsequently a three-way tap (*A*) was obtained to which two large diameter connecting tubes, made of lead, were attached (Text-fig. 1). This made it possible to insert the fine hypodermic tubes into both cavities at the beginning of an experiment. Because the hypodermic tubes entered the cavities through the mouth and opercular opening they were of necessity arranged along the line of water flow. To avoid dynamic complications in the pressures recorded, a small hole was

made in the side of the tubing near its tip and the hole at the tip was sealed. Thus the connexion between the pressure source and the recording apparatus was at right angles to the main direction of water flow. Connected to the manometer head by another tap (*C*) was the reservoir (Res.) and the calibrator (Cal.), the latter providing a head of water of known height above the level of water in the fish tank. The zero pressure level on the oscilloscope screen was set when the manometer head was connected directly to the water in the tank by tube *T*. When the manometer was working under these conditions a full-scale deflexion on the oscilloscope screen could be produced when a pressure of 1 cm. of water was applied to the manometer head, the noise level being equivalent to 0·5 mm. of water. The natural frequency of the instrument when a pressure was applied suddenly at the open end of the hypodermic tubing was about 100 cycles/sec.

Cinematography was used to record the breathing movements, the photographs being taken at speeds of 16–30 frames/sec. with either a Sinclair 35 mm. or a Zeiss 16 mm. ciné camera. The camera (*CC*, Text-fig. 1) was arranged to photograph the dorsal view of the fish's head directly and the side view reflected in a mirror (Mir.) inclined at 45°. Illumination was provided by two photofloods and two spotlights. Subsequent analysis of the ciné films enabled accurate plots to be made of respiratory movements which had not been hampered by mechanical restraints. The two points from which plots of the mouth and opercular movements were obtained are shown in Pl. 13, which is a single frame from one of the ciné films. The positions of the buccal and opercular valves could also be plotted. A time marker (*TM*, Text-fig. 1; Pl. 13) with a rotating contact was included in the camera field, and this made it possible to match exactly the pressure records and movement plots (Hughes, 1958).

When these experiments were done only one Hansen manometer was available, and so successive recordings of the pressures within the buccal and opercular cavities were taken. The two recordings were made within a minute of each other. In the regularly breathing fish these records were readily superimposed by using the movement plots obtained at the same time as the pressure records. Another method used in superimposing successive pressure traces, particularly when no ciné films were taken, was to display the opercular movements as deflexions of the second beam on the cathode-ray tube. An RCA 5734 transducer valve was used for this, the movements being transmitted to the anode pin of the valve by a long lever arm resting lightly on the operculum.

Three species of fish were examined by these methods, the tench (*Tinca tinca*), the roach (*Leuciscus rutilus*) and the trout (*Salmo trutta* f. *fario*). Some recordings were also made of the breathing movements and pressures in the pike (*Esox lucius*), but this fish was not examined in detail as the results were similar to those obtained from the trout and roach. The tench and roach used were between 45 and 70 g. in weight and the trout were a little larger, the heaviest weighing approximately 80 g.

The fish were deeply anaesthetized in a 0·5 % urethane solution and placed in a clamp, which firmly held the trunk and head (Shelton, 1958). The clamp was then

secured in the experimental tank which was of 10 l. capacity. After the fish had been arranged in the apparatus, the urethane concentration was reduced to approximately 0·2 %, which allowed the fish to recover to a lightly anaesthetized level sufficient to keep it quiet during the experiment. This solution was constantly aerated and its temperature maintained at 17°–20° C. The difficulties involved in working on respiratory processes in fish are well known (Fry, 1957). Activity or excitement cause wide fluctuations in metabolic rate and in the general breathing activity. Anaesthesia overcomes some of these difficulties, particularly when it is intended to experiment on the breathing mechanisms or metabolic rate of the inactive fish. However, it must be recognized that anaesthesia may introduce its own complications, and largely because of this possibility it has not found favour as a means of suppressing excitement. Urethane in high concentrations produces breathing which is slower and deeper than normal. In still higher concentrations the respiratory movements get smaller and less frequent until eventually they cease. At low levels of anaesthesia such as those used here, however, there seems to be little effect on the respiratory patterns or responses, though the animal remains quiet. These therefore were the conditions under which the experimental results were obtained.

<div align="center">RESULTS</div>

A. *The respiratory cycle in the trout*

The respiratory rhythm is extremely regular and in the trout used in these experiments there were usually 100 cyc./min. A typical result obtained by superimposing pressure curves and movement plots of the mouth and operculum can be seen in Text-fig. 2. In all the figures and descriptions the pressure at the water-level in the experimental tank will be called zero pressure, so that the pressures higher and lower than this become positive and negative respectively. All the determinations were made relative to this zero level, and descriptions are greatly facilitated if this convention is retained. As Fig. 2 shows, the durations of the opening and closing phases were approximately equal for both the mouth and the opercular movements but movements of the mouth preceded those of the operculum by one-fifth to one-quarter of a cycle. As changes in volume of the buccal and opercular cavities accompanied these movements it was to be expected that similar relationships would also apply to the pressure curves. Closing of the mouth and operculum is associated with an increase in the pressure within the buccal and opercular cavities respectively. The pressure in both cavities exceeds that of the surrounding water during this part of the cycle; this positive pressure is greater in the buccal cavity. Correspondingly, as the mouth and operculum open the cavities increase in volume and the pressures within them become negative with respect to the surrounding water, but in this case the pressure change in the opercular cavity is the greater. Thus during these two main phases of the cycle the pressure in the buccal cavity is positive with respect to that in the opercular cavity, as is shown by the differential pressure curve. This generalization is not true for the whole cycle, however, because there is a brief period during which the pressure in the buccal cavity is

negative with respect to that in the opercular cavity. But in general the pressure curves confirm earlier suggestions (Woskoboinikoff & Balabai, 1936, 1937; van Dam, 1938; Henschel, 1939) that the flow of water across the gills is maintained by
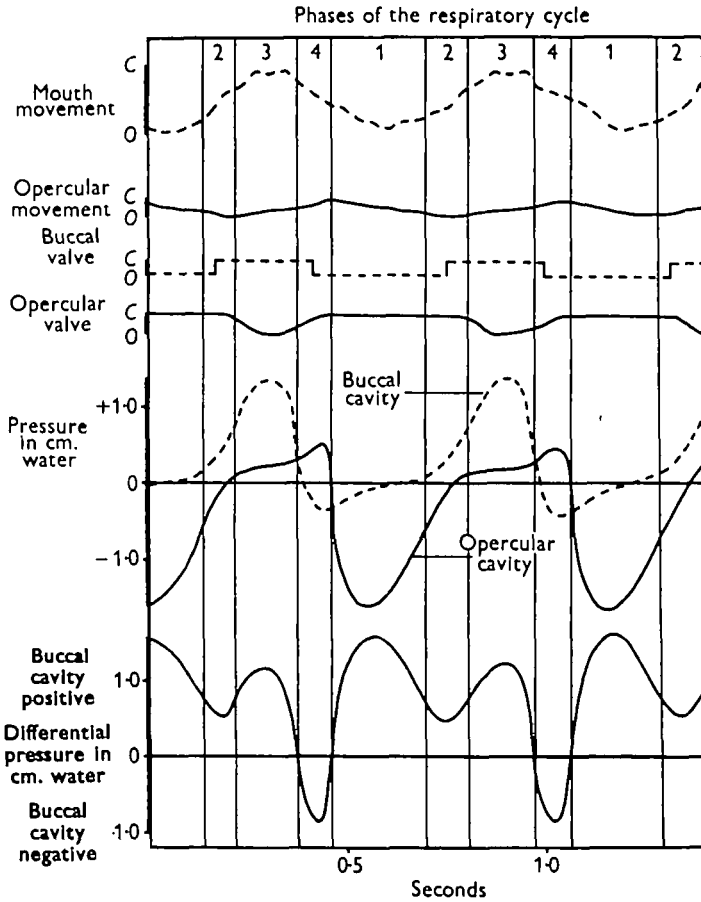
Text-fig. 2. *Trout* (70 g.). The breathing movements of the mouth and operculum, together with associated pressure changes in the buccal and opercular cavities. The differential pressure between these cavities is shown below. *O* and *C* indicate the opened and closed positions of the mouth, operculum and their associated valves. Temp. 17° C.

a dual mechanism. The first component of this involves a buccal pressure pump which forces water through the gills and the second component, the opercular suction pump, draws water through the gills into the opercular cavity. These two main phases are separated by two periods of transition, so that a complete breathing cycle may be artificially divided into the following four phases.

(1) *The opercular suction pump predominant*

The negative pressure produced in the buccal cavity, as this increases in volume, gradually falls as the mouth reaches the fully open position and then begins to

close. At this time the operculum is abducting and as the opercular cavity is closed externally by the opercular valve a considerable negative pressure is produced and so water flows through the gills from the buccal cavity.

(2) *Transition with a fall in the differential pressure between the buccal and opercular cavities*

The mouth continues to close and the buccal cavity decreases in volume. At first water leaves the buccal cavity through the still open mouth and the increase in pressure within the cavity is small. By the time the closing movement is about one-third of the way to completion, closure of the buccal valves diminishes this reflux and the pressure within the buccal cavity begins to rise more steeply. Meanwhile, the pressure in the opercular cavity is becoming less negative because water is still flowing through the gills into the maximally expanded opercular cavity, the operculum having almost reached the end of its abduction stroke. The opercular valve opens just as the pressure in the opercular cavity becomes equal to that in the external medium.

(3) *The buccal pressure pump predominant*

The operculum now begins to adduct, and during this initial period of adduction there is a considerable gap between the operculum and the flank of the animal. There is little resistance to the movement of water from the cavity to the exterior and so the positive pressure produced in the cavity is small at first. The positive pressure in the buccal cavity, on the other hand, rises steeply to its maximum value as the mouth closes and then begins to fall off as the final stages of the closing movement are reached. Water is therefore forced over the gills during this phase and there is little reflux through the mouth as the upper and lower lips are quite close together, making the buccal valves much more effective.

(4) *Transition with a reversal of differential pressure*

At this point, however, the mouth begins to open, the volume of the buccal cavity increases and the buccal valves open. As the aperture produced by the opening mouth is small at first, the pressure within the buccal cavity falls rapidly, reaching a maximum negative value when the mouth is half open. The operculum, on the other hand, continues to adduct and the pressure within the opercular cavity becomes increasingly positive, no doubt because the space through which water from the cavity can escape to the outside is becoming smaller. Finally, a point is reached when the gap between operculum and flank becomes vanishingly small so that water flow from the cavity must be negligible. Though the pressure is still positive within the opercular cavity, this is the point at which the opercular valve appears to close on many of our records (see below). As the pressure within the buccal cavity is negative with respect to that in the opercular cavity, there will be a tendency for the flow of water across the gills to be reversed during this phase of the movement cycle.

### (5) *The opercular suction pump predominant*

The operculum begins to abduct and as the valve is closed the pressure within the cavity falls extremely rapidly to its maximum negative value. Any reversal of water flow will no doubt contribute initially to the sudden nature of this pressure change. The process then continues with the next cycle.

By dividing the respiratory cycle into these four phases stress is laid upon the flow of water across the gills rather than on its flow into the buccal cavity and out of the opercular cavity. Such a division has much to recommend it in a system whose main characteristic is a unidirectional flow of the respiratory medium in contrast to the tidal flow characterizing those systems which are usually divided into expiratory and inspiratory phases.

This can be regarded as the typical respiratory cycle. In the trout it is relatively constant in its many components, but even in this species we have observed differences between individuals and, at different times, in the same individual. For example, the differential pressure may be reduced because the positive pressure in the opercular cavity and/or the negative pressure in the buccal cavity are relatively greater than those shown in Text-fig. 2. The presence of a transition phase during which the opercular pressure is greater than the buccal pressure has invariably been found. One of the most difficult parts of the analysis has been the timing of the buccal and opercular valves. The general picture, obtained from analyses of both trout and tench breathing, suggests that these act as passive valves, since they tend to open and close according to the direction of the pressure gradient.

The action of the buccal valve was studied from films taken with specially arranged illumination. In addition, it was found that if the pressure was recorded just inside the buccal cavity near the maxillary valve a characteristic small negative pressure occurred at the instant when the valve closed, as confirmed by comparison with the films. The nature of this pressure is obscure, but it made it possible to decide when the valve closed without the necessity of filming. The result of superimposing pressure records obtained from the buccal and opercular cavities with such a record is shown in Text-fig. 3. It was possible to study the action of the opercular valve in films taken with the standard illumination, since its posterior edge was clearly silhouetted against the floor of the tank. Text-fig. 4 shows the outline of the operculum and its valve throughout the cycle. The degree of expansion of the operculum is indicated, together with the position of the valve. The point at which the valve opens is easily established and can be seen just to precede the position of maximum abduction of the operculum. The instant at which the valve is truly closed is more difficult to decide, as the tracings show. Although the valve appears to be closed at frame 17–18 it is quite possible that it is not really closed until frame 19. This is of particular interest, as a number of our analyses suggest that the valve closes when the pressure within the opercular cavity is positive with respect to the surrounding water. Though this appears improbable if the valve is a passive one, it is possible if, during the later stages of its adduction, the operculum is actively

pressed against the side of the body, as it were short-circuiting the valve. The appearance of the valve on the ciné records would agree with this interpretation.
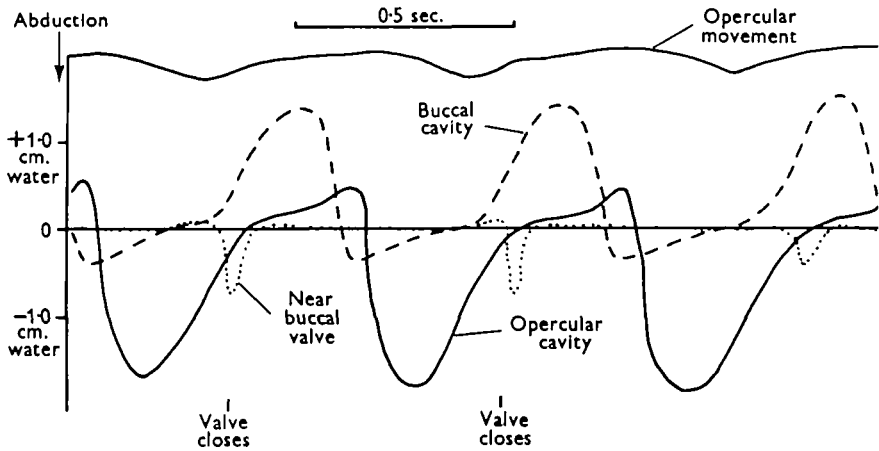

Text-fig. 3. *Trout.* Superimposed pressure records from the buccal and opercular cavities, and in the neighbourhood of the buccal valve. The instant at which the valve was observed to close on a ciné film taken simultaneously is indicated.
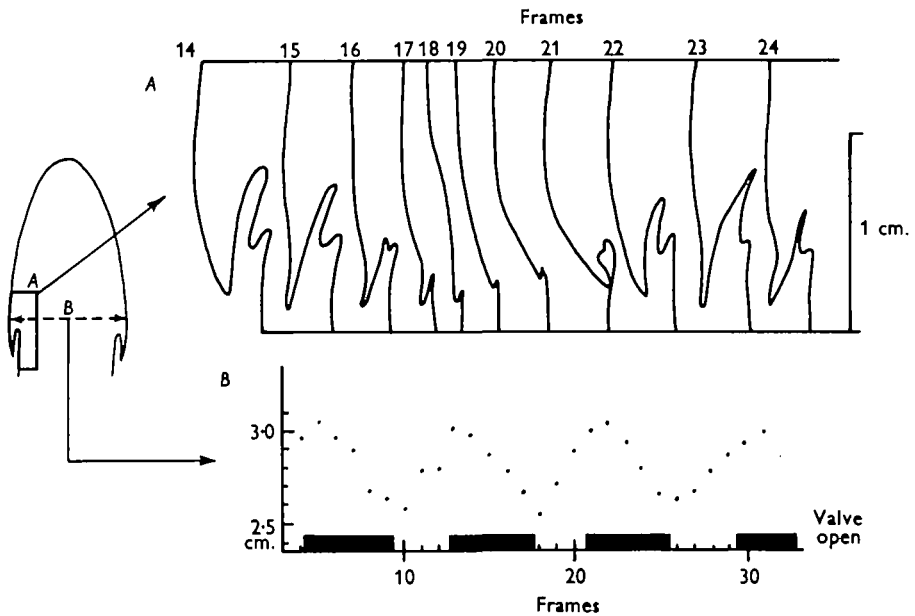
Text-fig. 4. *Trout.* (A) The outline of the left operculum and its valve seen in dorsal view traced from successive frames of a ciné film (20 frames/sec.). (B) The opercular movements obtained by measuring the distance across the fish at this level are plotted below, together with the time during which the valve appeared to be open.

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B. *The respiratory cycle in the roach*

The frequency of the movements was again about 100 cyc./min. This rhythm was occasionally interrupted by 'coughs' which were rarely found in the trout. This behaviour has been described by several authors (Bijtel, 1949; François-Franck, 1906a) and the function usually ascribed to it is that of cleaning the gills. The pressure changes produced in the course of one type of 'cough' can be seen in Text-fig. 5. During one of these interruptions the operculum, though making
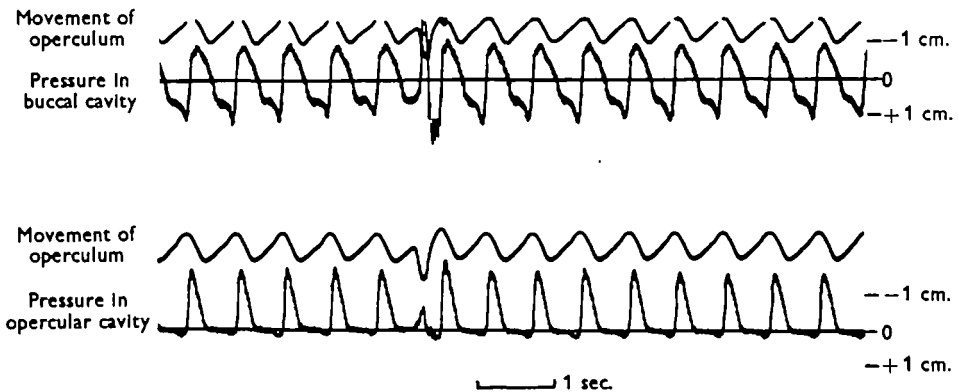

Text-fig. 5. Pressure curves recorded from the buccal and opercular cavities in the roach. A single 'cough' is included on each record. Transducer records of the opercular movements are shown on the second beam of the oscilloscope, abduction of the operculum being downwards in all records.

some small movements, does not close sufficiently to obstruct the opening into the opercular cavity and the mouth opens and closes rapidly. This means that there is relatively little change in pressure in the opercular cavity whilst there are two large changes, first negative and then positive, in the buccal cavity. These fluctuations must cause the water flow through the gills to be reversed and then to be re-established in the original direction. Sudden changes in the direction of water flow of this sort, together with movements of the gill filaments themselves (Bijtel, 1949), are effective in cleaning the respiratory surfaces.

The pattern of normal breathing is extremely similar to that described in detail for the trout, and the respiratory cycle can be divided into the same four phases. Superimposed pressure curves and movement plots are shown in Text-fig. 6. The mouth begins to open about one-eighth, and to close about one-fifth, of a cycle before the corresponding movements of the operculum. The period during which the mouth opens and the operculum abducts occupies less than half a cycle so that the wave-form of the movement plots is not so regular as that in the figure of trout breathing. In the roach, after the mouth has closed completely, rotation of the mandible and maxillae causes the lips to move backwards relative to the rest of the head. The volume of the buccal cavity continues to decrease during this time and a positive pressure is still recorded therein. Unfortunately this backward move-

ment causes those points on the upper and lower jaws, which are used in plotting movements, to move apart. Thus the mouth appears to be opening gradually in Text-fig. 6 when it is in fact closed. The instant when the mouth begins to open is indicated by an asterisk. The upper and lower lips then move forward and a negative pressure is produced within the buccal cavity by the lateral and dorso-ventral expansion of its walls. After a delay period, during which the pressure in
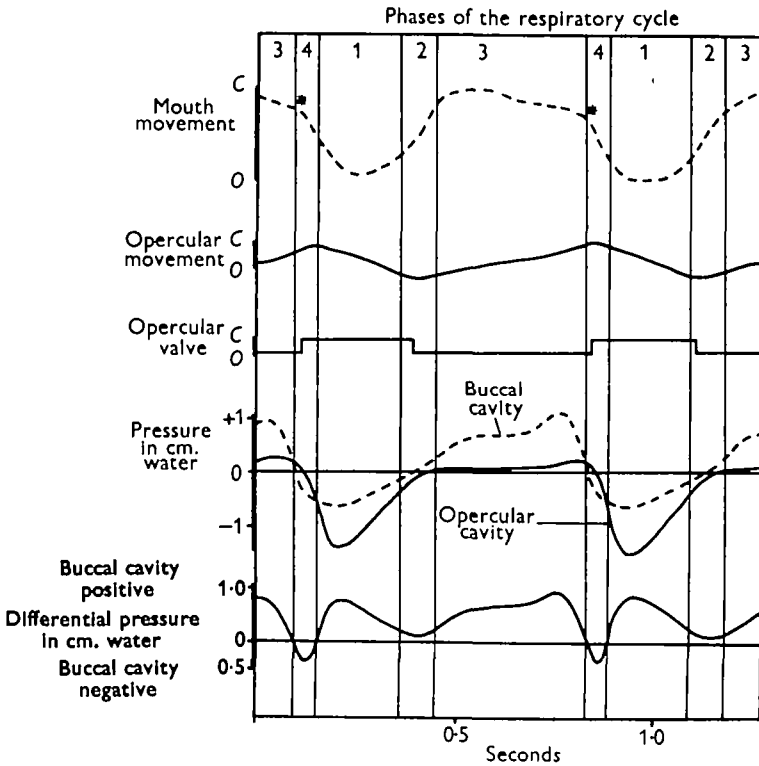

Text-fig. 6. *Roach* (60 g.). The breathing movements of the mouth and operculum, together with associated pressure changes in the buccal and opercular cavities. The differential pressure between these cavities is shown below. *O* and *C* indicate the open and closed positions of the mouth, operculum and their associated valves. Temp. 18° C.

the opercular cavity is positive with respect to that in the buccal cavity, the oper-culum begins to abduct and the negative pressure associated with this movement is produced. The opercular valve is closed during this phase but it is very difficult to be certain of its precise movements. The valves in the individuals we examined were shorter than those in the tench and trout. They appeared to remain open longer along the dorsal border than on the mid-opercular border and so the move-ments were not so readily plotted from the films. However, the movements of the mid-opercular border of the valve have been plotted and appear in Text-fig. 6. They again follow the direction of the pressure gradient. Buccal valves are not obvious in this fish; no doubt the behaviour already described, in which a

considerable part of the buccal pressure pump phase is accomplished with the mouth closed, compensates in some degree for this lack.

C. *The respiratory cycle in the tench*

The exact superposition of pressure curves and movement plots was difficult because of irregularities in the breathing rhythm of the tench. There are usually about 60 breathing cycles per minute but they are regularly punctuated by 'coughs' which are more frequent than in the roach. The pressure changes produced during a 'cough' (Text-fig. 7) are often very like those seen in the roach and are caused by
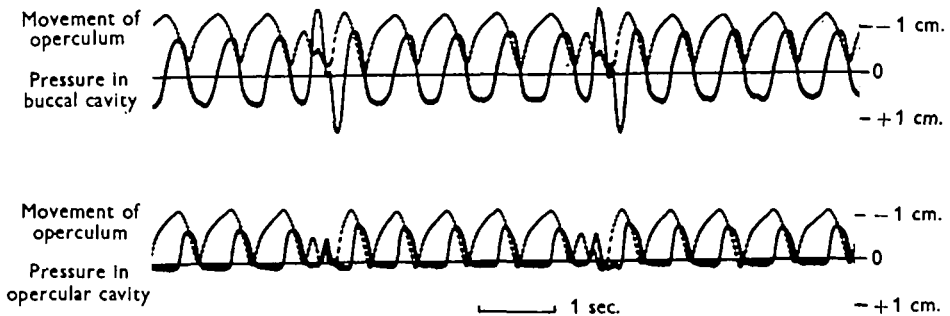

Text-fig. 7. Pressure curves recorded from the buccal and opercular cavities in the tench. Two 'coughs' are visible on each record. Transducer records of the opercular movements are shown on the second beam of the oscilloscope, abduction of the operculum being downwards in all records.

the same pattern of movement. The cycles both before and after a 'cough' often vary both in amplitude and frequency and so each 'cough' has a considerable effect on the rhythm. Because of this, and because of the greater range of individual variation, many more experiments were carried out on this species. When the records could be superimposed satisfactorily results such as those in Text-fig. 8 were obtained. During all phases the mouth movements precede those of the operculum by about one-eighth to one-fifth of a cycle. Once again the backward rotation of the upper and lower jaws when the mouth is closed forms a distinct part of the cycle. This can be seen in the mouth movement plots of Text-fig. 8 as a second hump during the closing movement. As in the roach this results in the final stages of the buccal pressure pump phase (phase 3) occurring when the mouth is closed. When the mouth begins to open, the pressure in the buccal cavity falls rapidly and there is the usual momentary reversal in pressure gradient between the two cavities. Abduction of the operculum then follows and the pressure within the opercular cavity becomes negative. The most striking feature of the pressure curves, however, is the relatively large negative pressure which is recorded in the buccal cavity during this phase. In some experiments it exceeds that in the opercular cavity (Text-fig. 7). In the majority of records the negative pressure in the buccal cavity, though large, is not so large as that in the opercular cavity. In these cases, as in the trout and roach, the pressure in the buccal cavity is positive with respect

to that in the opercular cavity, with the single exception of phase 4 of the breathing cycle.

The action of the opercular valve is readily visible in the film. Its movements, in general, follow the pressure gradient between the opercular cavity and the external environment, yet some analyses show it to be open when the pressure inside the opercular cavity is negative (Text-fig. 8). In the tench from which the
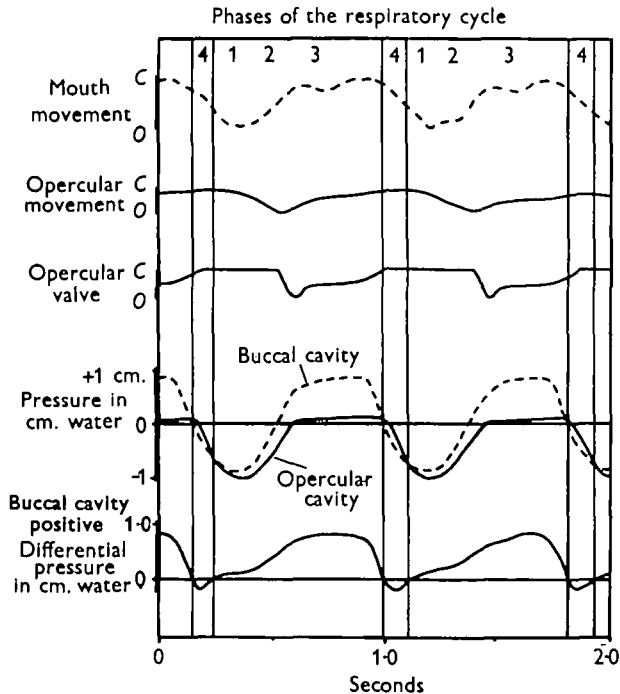
Text-fig. 8. *Tench* (60 g.). The breathing movements of the mouth and operculum, together with associated pressure changes in the buccal and opercular cavities. The differential pressure between these cavities is shown below. *O* and *C* indicate the opened and closed positions of the mouth, operculum and their associated valves. Temp. 18° C.

records of this figure were obtained, it was observed that methylene blue pipetted near the posterior border of the operculum entered the cavity momentarily, as the valve opened, and was then expelled. An active valve with its own musculature could produce this sort of effect, but this is not the only possible explanation. As the operculum abducts the valve has to bridge a gap, which is increasing in size, between the opercular flap itself and the side of the body. A point must be reached when the valve, because of its dimensions or stiffness, is pulled away from the flank. If the operculum continues to abduct after this has occurred then water will enter the opercular cavity from outside. It is difficult to be absolutely certain that this is the correct interpretation because the valve moves from the closed to the maximally open position in one twenty-fifth of a second and so the whole procedure is recorded on, at the most, two frames of the ciné film. This was by no means a constant feature of the breathing pattern of the tench, however, and in other

individuals there was no inflow of water to the opercular cavity as the valve opened. Analyses confirmed that the pressure in the cavity was positive when the valve opened in these fish.

In order to decide what sort of range existed in breathing behaviour a group of six fresh tench were treated in an identical way as regards depth of anaesthesia, time intervals before pressure records were made, etc. The mouth or the opercular movements were recorded by means of the transducer valve. The results confirmed that the pattern of breathing as shown in Text-fig. 8 is the most common. In all six fish, and indeed in every tench examined, a very distinct positive pressure was developed in the buccal cavity, always exceeding that produced in the opercular cavity. The main variable was the relative size of the negative pressures recorded in the two cavities. In three of the tench the negative pressure in the buccal cavity was distinctly less than that in the opercular cavity. In the other three fish there was very little difference between the pressures in the two cavities during phases 1 and 2. In occasional records from two of the fish the negative pressure in the buccal cavity during these phases was greater than that in the opercular cavity.

In view of these variations it was interesting to find later that Woskoboinikoff & Balabai (1937) also record similar variations from the basic pattern in this fish. They found, for instance, that in 22% of the cases the negative pressure in the opercular cavity was less than that in the buccal cavity and that in 9% they were approximately equal.

DISCUSSION

The use of cinematography has made it possible to record and analyse the breathing movements with as little interference with the fish as possible. This method has the added advantage that the action of the buccal and opercular valves could be observed at the same time. The recording of pressure changes simultaneously with the filming of movement provides a more complete picture of the functioning of this complex system than has previously been possible. The results obtained support those of François-Franck and others on the time relations of the movements and have confirmed the relationships expected between these movements and the pressure changes in the buccal and opercular cavities. Because of the differences between the pressure curves the system must be considered as consisting of three chambers, the opercular cavities being separated from the buccal cavity by a continuous gill sieve offering resistance to water flow. It is not a single cavity in which the gills are freely suspended.

The differential pressure curves (Text-figs. 2, 6 and 8), obtained by subtraction of the opercular from the buccal pressures, show two maxima in each cycle. The opercular suction pump and the buccal pressure pump alternately produce these pressure maxima and, during the transitions between their action, periods of very low-pressure differential occur. Indeed, as the suction pump takes over from the pressure pump, the differential becomes negative and the flow will tend to reverse. Division of the ventilating system into these two pumps is justifiable, provided it is realized that the terms refer only to the passage of water across the gills. The

opercular suction pump is not directly involved in drawing water into the system nor the buccal pressure pump in forcing it out.

The relationships of the pressure curves from the buccal and opercular cavities suggest that, except for the very brief period of phase 4, water will flow continuously across the gill filaments from buccal to opercular cavity. It is not possible, however, to make exact deductions about the rate of flow without some knowledge of the properties and numerical value of the gill resistance. The volume of water flowing over the gills at any time is, of course, directly related to the pressure difference which exists between the two cavities. If, to take the simplest case, the resistance were constant and the same for flow in both directions, then the flow would always be related to the pressure difference by the same factor and the form of a curve showing flow across the gills would be identical with that of the differential pressure curve. In order to get some idea of the relationship between differential pressure and flow, simultaneous determinations were made of the flow and of the pressures in the buccal and opercular cavities of the tench. The volume of water passing over the gills was measured by separating the mouth and opercular regions of the fish with a rubber membrane. Any water which came over a spillway in the 'opercular' part of the tank was pumped by the fish from the 'mouth' compartment. The water in the latter was maintained at a constant level which was the same as the level of the spillway in the 'opercular' compartment. A convenient way of varying the rate at which water was pumped through the system was by changing the tension of carbon dioxide in the water by arbitrary amounts. If the gill resistance remains constant the ratio mean pressure/minute volume should be the same for all rates of flow. As Table 1 shows, this is not the case, the ratio decreasing as the minute volume and mean pressure difference increase. It is clear, then, that under these conditions the gill resistance is variable, and that it probably decreases as the pressure difference across the gills increases. Some support for this conclusion comes from preliminary measurements made on fish which had stopped breathing under very deep anaesthesia. Water could be made to flow over the gills of such fish by raising the level of water in the 'mouth' compartment of a tank similar to that described above. Measurements of the flow, made as this pressure head was maintained at progressively greater values, showed that there was an accompanying decrease in the gill resistance.

Table 1

Minute volume	Frequency/ minute	Stroke volume (ml.)	Mean pressure (cm. water)	Mean pressure / Minute volume
39	39	0·99	0·51	0·0131
55	35	1·56	0·76	0·0138
66	48	1·38	0·64	0·0097
67	49	1·37	0·75	0·0112
69	41	1·70	0·66	0·0096
82	43	1·90	0·65	0·0080
124	44	2·83	0·91	0·0074
154	57	2·72	1·12	0·0073
164	56	2·94	1·12	0·0068

The changes in volume of buccal and opercular cavities and in area of the mouth and opercular openings are clearly of fundamental importance in determining the form of the pressure curves recorded in the two cavities. Depending upon the relative size of the cavities and on the extent of their volume change, it is possible for either the buccal pressure pump or the opercular suction pump to become the dominant ventilating mechanism in different species. The pressure curves suggest that these two pumps are fairly well balanced in the trout and roach, whereas in the tench the buccal pump appears to be the more important. It is difficult to be quite certain that this is the case, however, because it must now be recognized that another factor, namely the resistance of the gills to water flow, also has a significant effect on the pressures measured in the two cavities. It has been shown that this resistance changes as the mean differential pressure and the ventilation volume are altered. It must be concluded that changes of gill resistance will also occur during a single respiratory cycle as the differential pressure fluctuates. It is probable that in different species differences will exist not only in the numerical value for the mean gill resistance but also in the range over which the resistance varies with changing pressure.

In spite of these variations the results so far obtained conform sufficiently to the same general plan that it seems reasonable to accept the breathing pattern of a fish such as a trout as representing a general, unspecialized mechanism. Variations on this plan have already been described and it is hoped that further investigations will disclose more fully the range of possible modifications.

SUMMARY

1. A study has been made of the respiratory movements of three species of freshwater fish. The time course of pressure changes in the buccal and opercular cavities was recorded and movements of the mouth and operculum plotted from ciné films taken simultaneously.

2. Opening and closing of the mouth precede respectively abduction and adduction of the operculum by about one-fifth of a cycle.

3. The most prominent part of the buccal pressure curve is a positive pressure associated with mouth closing. The size of a negative pressure as the mouth opens is small in the trout but may be relatively large in the tench.

4. Abduction of the operculum produces a marked negative pressure in the opercular cavity of all three species and there is a slight positive pressure during its adduction.

5. The respiratory system is divided into a buccal and two opercular cavities and the concept of gill resistances separating them is introduced.

6. The respiratory cycle is made up of four phases which succeed one another. These are: phase (1) opercular suction pump predominant; phase (2) transition with a reduction in differential pressure between the buccal and opercular cavities; phase (3) buccal pressure pump predominant; and phase (4) transition with reversal of differential pressure.

7. With the exception of phase (4), which occupies only about one-tenth of a
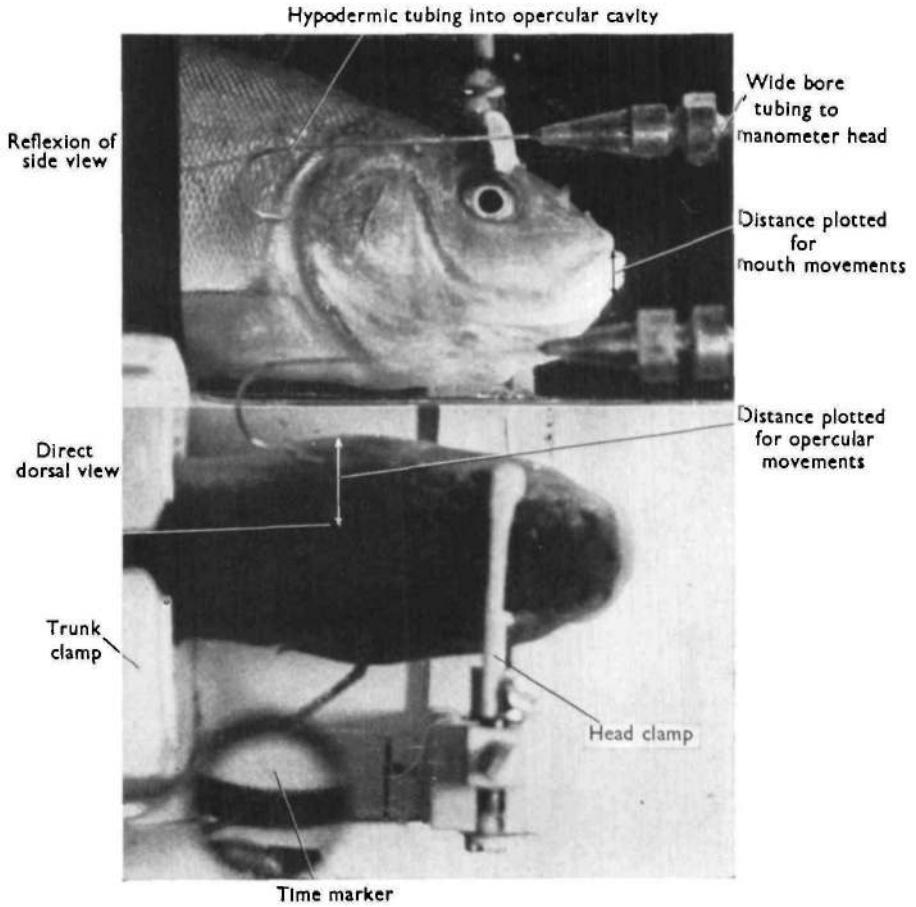
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cycle, the pressure in the buccal cavity exceeds that in the opercular cavity through-out the cycle. It is therefore concluded that water will flow across the gills for almost the entire cycle but may reverse for this brief period. The quantitative relationship between the pressures and the volume of water flowing across the gills during different parts of the cycle will depend upon the properties of the gill resistances.

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EXPLANATION OF PLATE

A single frame from a ciné film to show a tench set up in the apparatus. The measurements made in plotting the respiratory movements are indicated.