# ANAL VALVE MOVEMENT AND NORMAL VENTILATION IN AESHNID DRAGONFLY LARVAE

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## INTRODUCTION

In anisopteran dragonfly larvae respiratory gases are exchanged over the surface of tracheal gills, which are contained in a modified portion of the hind gut, the branchial chamber. This is separated from the anus by a smaller, muscular chamber called the vestibule. The sternal movements and the associated pressure changes in
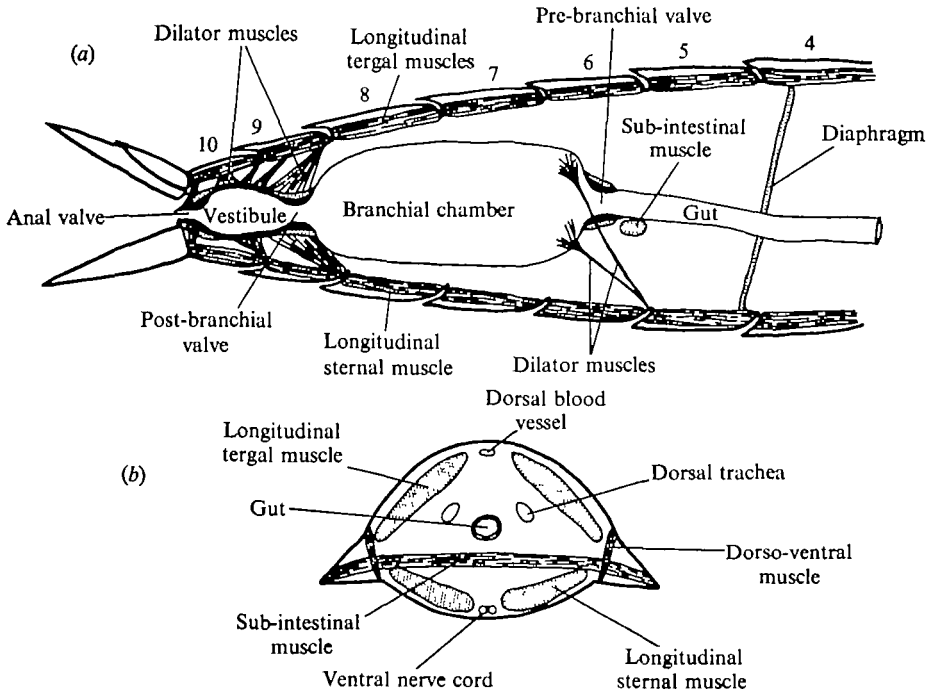


Text-fig. 1. Diagrams of the larval abdomen showing the principal structures involved in ventilatory activity. (*a*) Longitudinal, (*b*) transverse; in the anterior region of segment 6. (After Hughes & Mill (1966). *J. exp. Biol.*)

the branchial chamber which occur during ventilation have been described by Hughes & Mill (1966). Normal ventilation ($V_n$) consists of a rhythmic raising and lowering of the posterior abdominal sterna, with commensurate increasing and decreasing of pressure in the branchial chamber. Occasionally, two other types of ventilation occur: 'gulping' ventilation ($V_g$) and 'chewing' ventilation ($V_c$) (Tonner, 1936; Hughes &

Mill, 1966). Ventilation also occurs during jet propulsive swimming ($V_s$) (Hughes, 1958). The basic anatomy of the ventilatory system is shown in Text-fig. 1.

Expiration is the initial phase in a normal ventilatory cycle ($V_n$). The sterna are raised by contraction of the respiratory dorso-ventral segmental muscles. This action increases the pressure within the branchial chamber, the anal valve opens and water is forced out through the anus. The anal valve remains open while the sterna are lowered to their resting position and during this phase a small negative pressure arises in the branchial chamber, drawing water in again. The extent to which the intrinsic musculature of the branchial chamber and vestibule is involved is not known, neither has the importance of cuticular elasticity in restoring the sterna to their resting position been assessed. However, the increase in pressure during expiration is probably enhanced by contraction of the muscles in the wall of the branchial chamber and vestibule. Similarly, the vestibule is presumably expanded during inspiration by contraction of its dilator muscles. The timing of valve movements within the ventilatory cycle is not known. This paper is concerned with the relationship between anal valve movement and other events in the normal ventilatory cycle.

## MATERIALS AND METHODS

In all experiments large larvae of the Emperor dragonfly, *Anax imperator* were used.

*Simultaneous recording of valve position and sternal movement.* A test-tube was partially filled with wax to create a chamber at the top of the tube equal in length of that of a larva. The larva was inserted into the tube head-first, and the chamber was filled with water. A coverslip was placed on top of the tube, taking care to exclude any air bubbles. The water was renewed at frequent intervals. The tube was placed vertically in a holder and a Bolex ciné camera was arranged so that the anal valve and the 'sternal horizon' were in focus. Films of normal ventilation were taken at speeds of 18–64 frames/second. The films were analysed with the aid of a Watson microscope with a projector attachment. Drawings were made of successive frames, and from these drawings changes in open valve area and sternal movement were measured.

*Simultaneous recording of water flow and sternal movement.* The experimental bath used is described in the previous paper (Pickard & Mill, 1972). To record tidal movements of water passing in and out of the anus a water-vane was pivoted, parallel to the partition, in the ventilatory current, as close to the anus as possible. In some experiments the paraprocts and epiproct were removed close to their bases so that the vane could be positioned nearer to the anus. Such animals were allowed at least 7 days recuperation before being used in the apparatus. After being secured in the experimental bath, all of the larvae were left for half-an-hour before any measurements were taken. A square of light foil was attached to the axis of the vane. This interrupted a light beam falling on an OCP 71 photo-transistor.

Sternal movements were simultaneously monitored by placing a small straw vertically on the sixth sternum. This was maintained in its vertical position by a thread running transversely across the bath. (The thread also supported most of the weight of the straw.) A square of foil was attached to the straw and this interrupted a light beam falling on a second photo-transistor. The outputs were displayed on a Telequipment D 43 oscilloscope and photographed with a Cossor 1428 camera.

Controls were made on the water-vane to check its sensitivity and range. These were effected by using a teat pipette to produce currents of water.

## RESULTS

### (1) *The relationship between anal valve and sternal movements*

The area of the anal orifice is controlled by the position of the anal valve, which has been observed to move rhythmically during normal ventilation (Hughes & Mill, 1966). The area of the anal orifice and the position of the sterna are plotted in Text-fig. 2 for several ventilatory cycles, and it can be seen that they are indeed synchronized.
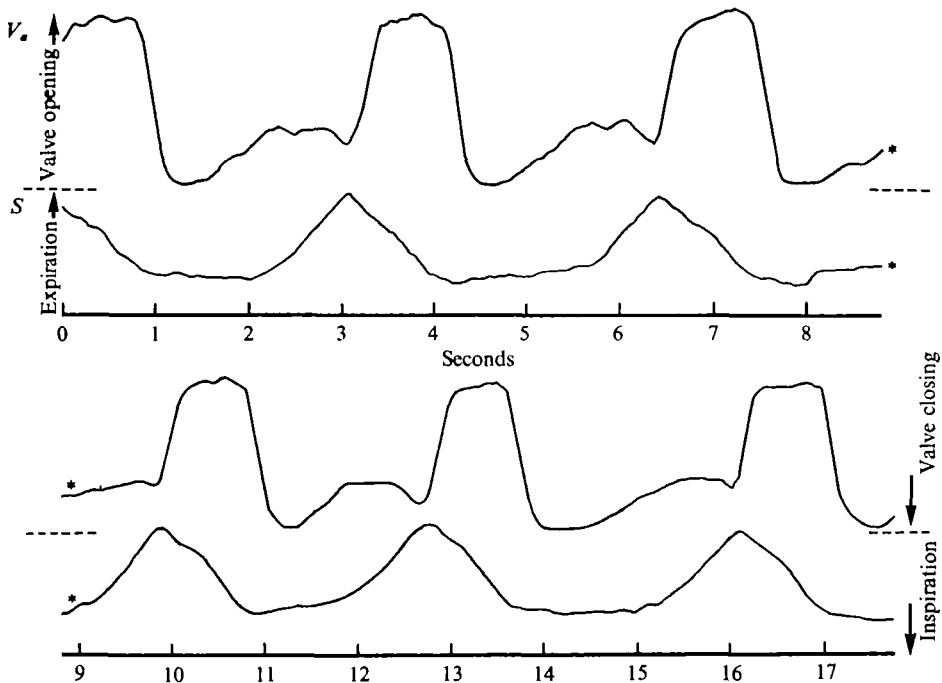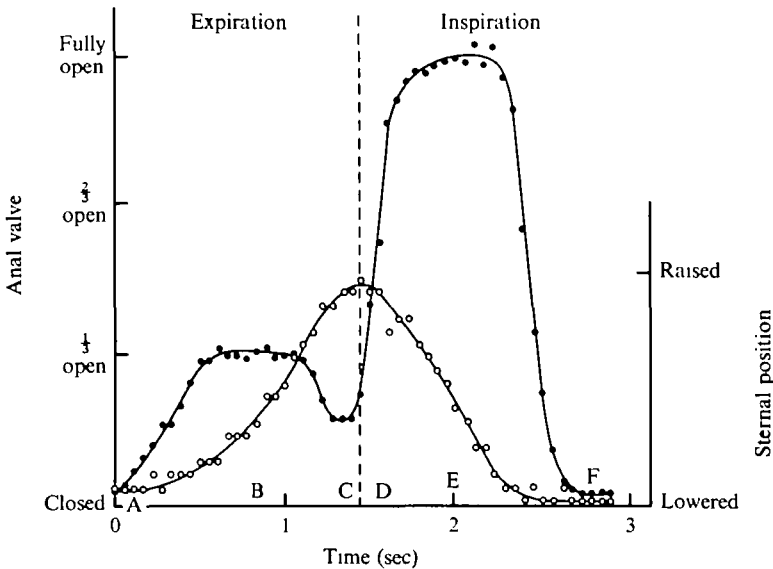


Text-fig. 2. Open valve area ($V_a$), and sternal movement ($S$), measured from successive ciné frames during normal ventilation ($V_n$).

For convenience in describing the observations, and to obtain a measure of consistency, expiration will be defined as commencing with the onset of sternal lifting and finishing when the sterna have reached their maximum lift; inspiration starts immediately after expiration and ends when the sterna have returned to their rest position.

During expiration the anal valve opens so that the area of the anal orifice is about one-third of its maximum, and this position is maintained until expiration is complete. At the onset of inspiration the valve rapidly opens to its fullest extent. This opening is generally preceded by a slight closing of the valve towards the end of the expiratory phase. The valve is rapidly closed at the end of inspiration. The occurrence of peak sternal movement coincident with the onset of full valve opening was particularly

precise.. Between cycles the valves do not fully close and an anal orifice of up to 5%
maximum opening is maintained. Text-fig. 3 shows a single cycle of sternal and anal
valve movement in detail and photographs taken at the labelled points in the cycle are
shown in Pl. 1.



Text-fig. 3. Open valve area and sternal movement in a single cycle of $V_n$. Measurements from
projected ciné-frames. A–F, selected points in the cycle exemplified in Pl. 1.

### (2) The relationship between the ventilatory current and sternal movements

It has been possible to record the expiratory current fairly precisely by using a small
vane positioned in the current. There are obvious limitations to this method of
recording the time course of the ventilatory current. First, the positioning of the vane
outside the anus will introduce a lag which can account for all of the time observed
between the beginning of the cycle and detection of flow. Secondly, the mass of the
vane will cause some damping and so obscure the end-point of the expiratory current
and the timing of the weak inspiratory current. However, some useful information is
provided.
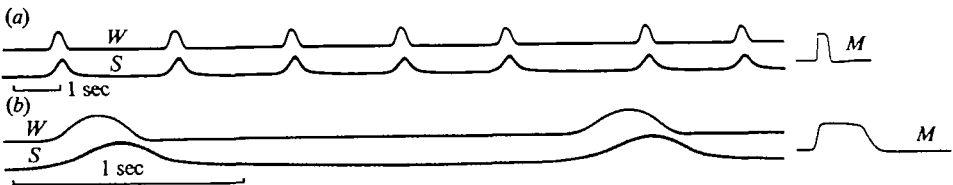


Text-fig. 4. Water flow (W), measured with a vane, and sternal movement (S) during $V_n$.
Upward deflexion of the sternal trace indicates lifting towards terga. Mechanical controls on
the water vane (M) are given at the end of each record.

During expiration the abdominal sterna of the posterior few segments are raised and
water is expelled from the branchial chamber through the vestibule and anus, the
reverse occurring during inspiration. It is noticeable from the records (Text-fig. 4)

that water begins to pass out of the anus almost immediately after the sterna start to lift and that peak water flow is recorded before maximum dorso-ventral contraction is achieved. By the time the latter state has been reached, the water-vane is already returning towards its rest position. This indicates that flow has slowed down rather than ceased at this point since test deflexions of the vane indicate that in the absence of water flow the vane returns to rest rather more rapidly.

## DISCUSSION

Text-fig. 5 is a summary diagram of the results described in this and previous papers (Hughes & Mill, 1966; Mill & Hughes, 1966; Mill, 1970; Pickard & Mill, 1972). The sequence of events is as follows. The expiratory bursts in the second seg-
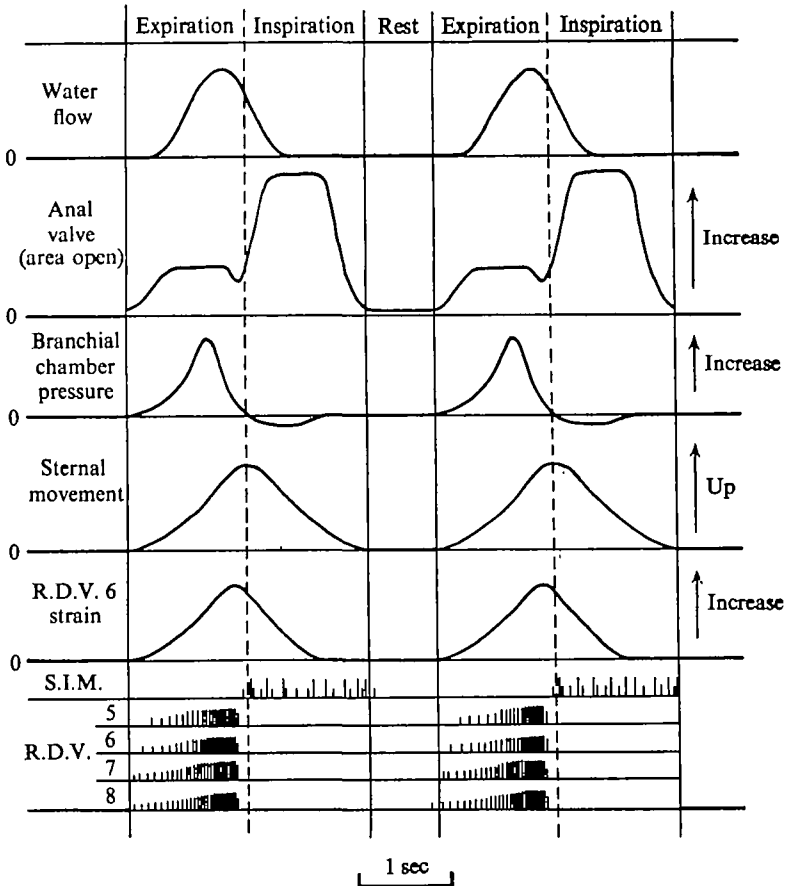


Text-fig. 5. Summary chart of events occurring in normal ventilation: from muscular activity, through mechanical effects, to resultant behaviour.

mental nerves start from the eighth ganglion with a delay of 50–150 msec for successively anterior ganglia, but all finish simultaneously and this is reflected in the timing of the contraction of the respiratory dorso-ventral muscles. Each pair of segmental respiratory dorso-ventral muscles exerts a force which lifts the pleura and sternum of that segment. The effect is to increase the pressure within the abdominal cavity and

hence in the branchial chamber. Simultaneously the anal valve starts to open and allows water to be ejected via the anus. Maximum respiratory muscle strain, branchial chamber pressure and water flow all occur before the end of expiration. It would appear that the increase in frequency of the expiratory bursts and the concomitant facilitation apparent in the muscles is necessary to offset the cuticular restoring force imposed by the movement of the sterna away from their rest position. It is interesting that these muscles cease activity before the sterna are fully lifted, and so the force on the sterna is already falling before the movement is complete. It may be that the sterna have been lifted past a fulcrum and that they then 'click' into a stable position (i.e. fully raised). The inertia of water moving out of the branchial chamber would also promote the continued rise of the sterna. At the peak of sternal movement, which marks the transition between expiration and inspiration, the pressure in the branchial chamber has fallen to zero and the outward flow of water ceases.

The subintestinal muscle starts to contract before the sterna are completely raised and thus presumably exerts a braking effect on their movement. Its role is to help in lowering the sterna and its main function may be to bring them to the point at which cuticular elasticity can act to restore the rest position. The inspiratory muscle continues to contract until the end of the cycle. The diaphragm probably serves a similar function (Mill & Hughes, 1966). As soon as the sterna start to fall, the pressure in the branchial chamber becomes negative and the anal valve immediately opens to its fullest extent. It is probable that the resultant intake of water is aided by active dilation of the vestibule. Inspiration is terminated when the sterna have attained their rest position, by which time the pressure in the branchial chamber has returned to zero and the anal valve has closed.

The constricted opening of the anal orifice during expiration combined with the positive pressure developed in the branchial chamber causes a 'jetting' of water away from the anus, and this will serve to clear external debris from the immediate vicinity of the anal valve. The inspiratory current is much weaker than the expiratory one, with a low negative pressure in the branchial chamber (Hughes & Mill, 1966) and with the anal orifice fully open. Thus water is drawn in over a fairly wide area from close to the anus, so avoiding the intake of previously expelled water. The risk of drawing debris into the branchial chamber is also reduced by use of the weaker inspiratory current. The valvular restriction during expiration also means that the turbulence in the branchial chamber will be increased so ensuring a good change-over of water.

The function of the pre-branchial and post-branchial valves is uncertain at present. Presumably the former remains closed throughout the ventilatory cycle to prevent flow of water back into the gut. The post-branchial valve may serve to occlude the branchial chamber if debris is sucked into the vestibule during inspiration. More likely, however, a functional separation of the branchial chamber and vestibule may be important in 'gulping' and 'chewing' ventilation (Tonner, 1936; Hughes & Mill, 1966; Pickard & Mill, 1972) to adequately circulate the inspired water.
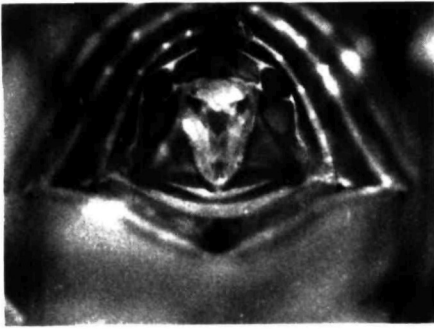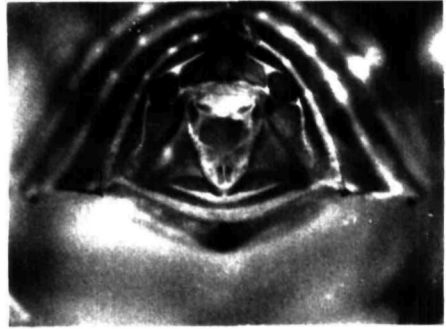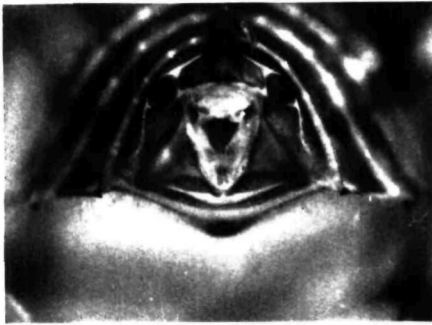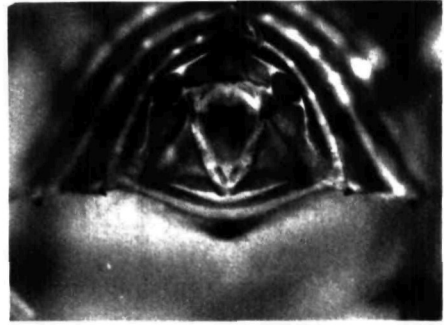
## SUMMARY
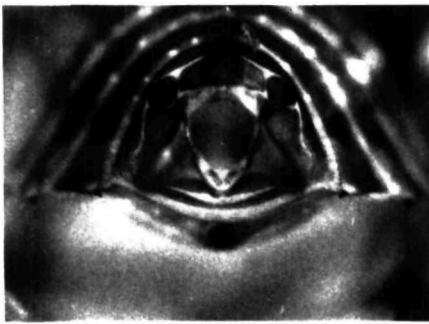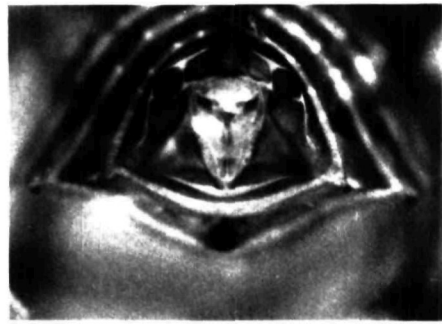
1. Anal valve movement and abdominal sternal movement were photographed with a ciné camera during normal ventilation to obtain a correlation between the two parameters.

2. As the sterna are raised (expiration) the anal valve opens to about one third of its maximum. At peak sternal movement the anal valve opens fully and these events mark the transition between expiration and inspiration. The anal valve finally closes towards the end of inspiration.

3. The narrow aperture of the anal valve during expiration, coupled with the increase in pressure in the branchial chamber, causes the expired water to be forced well clear of the anus. Conversely, the wide aperture during inspiration and the small negative pressure in the branchial chamber ensure that water is drawn in from close to the animal, and so there is minimal mixing of expired water with inspired.

4. The relationships between water flow, anal valve movement, branchial chamber pressure, sternal movement, pleural strain, and activity in certain expiratory and inspiratory muscles are discussed.

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## EXPLANATION OF PLATE

Fig. 1. A to F are ciné frames of the anal valve and abdominal sterna, filmed as described in text. They illustrate different points in the ventilatory cycle (Text-fig. 3). Silhouettes of the open valve areas are drawn below each frame as a guide, and frame numbers are also given. Camera speed, 18 frames/sec.