

PATTERNS OF VENTILATION IN DRAGONFLY LARVAE

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INTRODUCTION

Respiration in the aquatic larvae of anisopteran dragonflies is of interest from a comparative point of view in that it utilizes a tidal ventilation mechanism in an aquatic medium. In most animals where water is the respiratory medium the flow over the respiratory surfaces is generally unidirectional and this probably requires less energy for the extraction of a given amount of oxygen. Tidal or reciprocating pumping mechanisms in water are only truly found here and in some sea-cucumbers. The respiratory surface in dragonfly larvae is also of interest since it is made up of tracheal gills so that the oxygen is transferred from an aquatic medium to a gaseous phase. The general nature of ventilation is well known but accounts of the detailed patterns are largely dependent upon direct observation. There are some excellent accounts, but inevitably they are based upon observation over a relatively short period and small number of individuals. Tonner (1936) divided the respiratory patterns into three main types. He and many other authors also observed the way in which the respiratory mechanisms for ventilating the rectal chamber had been made use of in the locomotion of these insects. Methods of more continuous recording previously used, mainly for the study of swimming were applied to the study of ventilation in this animal by recording pressures in the respiratory chambers using a condenser manometer (Hughes, 1958).

The purpose of the present investigation was to give a more complete account of the patterns of respiration using electrophysiological recording methods which would also give information concerning the detailed mechanisms which produce the required flow of water over the respiratory surface. The respiratory patterns of different individuals of the same species were compared and also those found in the three genera *Anax*, *Aeshna* and *Libellula*. In general the results have served to confirm the major types of ventilation but have shown that the patterns of a given individual can vary from time to time and that there are variations between individuals. While there are certainly differences between the three genera, the overall patterns are similar and presumably the mechanisms producing them are essentially the same. Because of differences of opinion regarding the morphological relationships and functioning of the muscles and cavities concerned in ventilation it has also been necessary to investigate these aspects.

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MATERIALS AND METHODS

The dragonfly larvae used were *Anax imperator*, *Aeshna cyanea*, *Libellula* sp. They were usually of either the last or the penultimate instar. The methods used for recording pressures and the propulsive force on the insect were substantially the same as described previously (Hughes, 1958), and made use of a Hansen condenser manometer. All pressures are given in cm. H₂O with respect to a zero pressure which is the pressure in the water surrounding the insect. The experimental set-up is shown in Fig. 1. Sternal

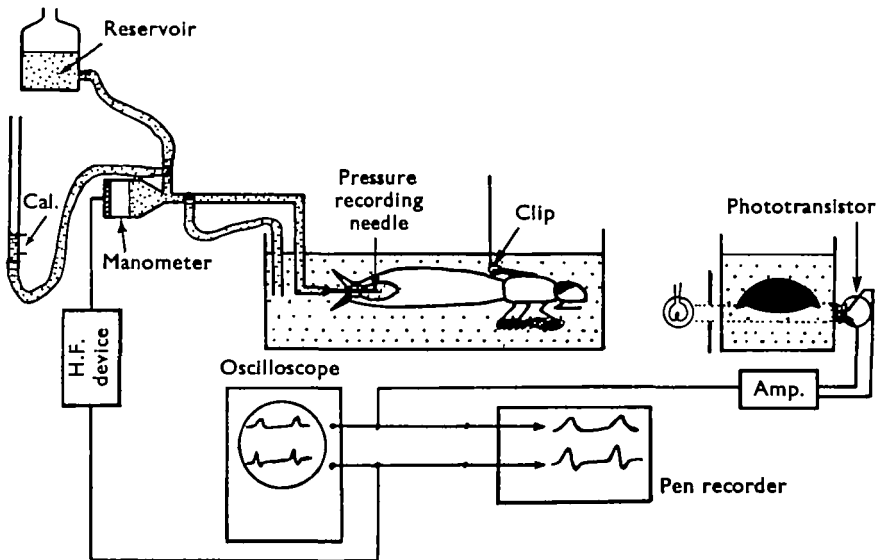


Fig. 1. Diagram of the apparatus used to record the pressure changes in the respiratory cavities and the dorso-ventral abdominal movements of a dragonfly larva. The method of pressure recording is shown by reference to a longitudinal view of the insect and that of movement recording by transverse section. For details, see text.

movements were recorded by means of a phototransistor and a horizontal beam of light which was interrupted by the profile of the abdomen. Such a technique was found satisfactory so long as the abdomen remained in a constant orientation, and this was aided by the support provided by the pressure needle inserted into the rectal chamber. The needles used had an outside diameter of 0.46 mm. and even though they were small they must have interfered to some extent with the normal movements, but this was inevitable. In order to reduce this effect, the insect was allowed to remain with the needle *in situ* for about $\frac{1}{2}$ hr. before recordings were made, and little change was noted during the habituation period. Recordings of the movements and pressures were displayed on an oscilloscope and Ediswan pen-recorder.

RESULTS

1. The respiratory system

Gaseous exchange takes place across the tracheal gills which are found in the branchial chamber which fills most of the posterior part of the abdominal cavity. These gills are arranged in six rows and receive their tracheal supply principally from four of the six main longitudinal tracheal trunks. There is considerable variation in the precise nature of the gills, but in the three genera studied here they are of the

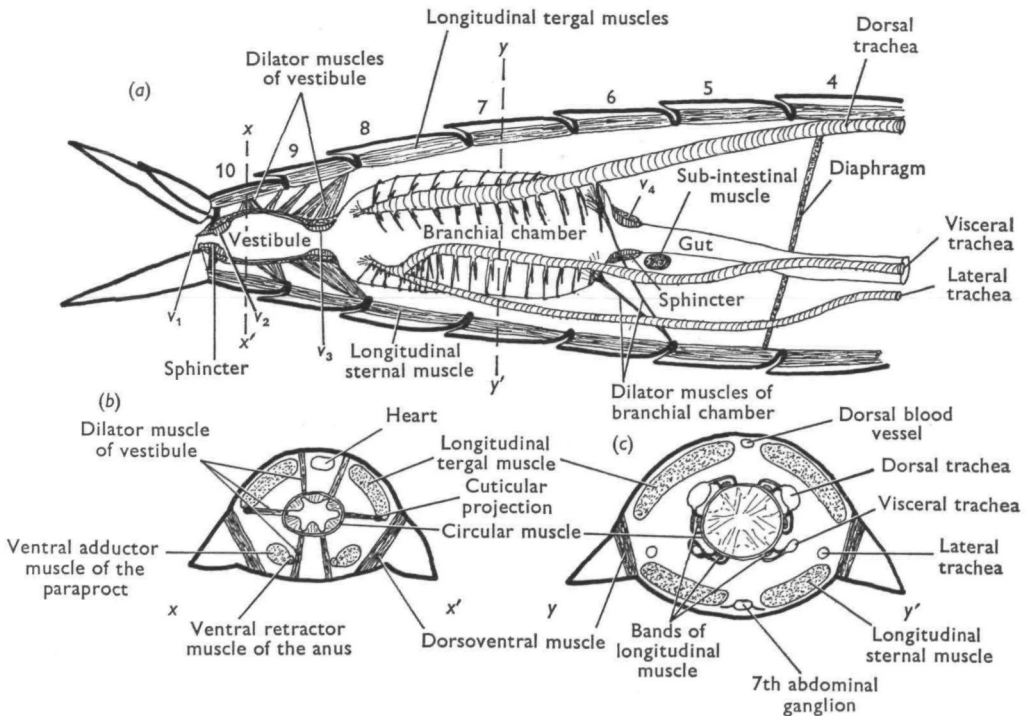


Fig. 2. Diagrams to show the morphology of the respiratory system in a dragonfly larva. (a) Longitudinal section, (b, c) transverse sections through the regions indicated.

duplex type, i.e. each row of gills is double (Fig. 2). In *Aeshna* the gills are foliate while those of *Anax* are of the papillo-foliate subtype which are not so large and are swollen up into oval humps on short stalks. The surface of the humps is covered with a number of minute papillae each of which contains five or six capillary loops of the tracheal system. The total number of capillaries in the gill basket of *Anax* reaches 100,000 or more (Tillyard, 1917). The gill type found in *Libellula* is considered to be a separate evolution in which the gills are formed of very flat lamellae.

In all three genera the gills are contained within a cavity, the branchial chamber, which is sometimes considered to be part of the rectum, but Snodgrass (1954) homologizes it with the colon. This cavity can be closed by muscular valves at both ends. The anterior one (v_4) is the ileal valve and the posterior one (v_3) separates the branchial chamber from the vestibule. The vestibule, which is probably derived from the

rectum, is very muscular and has both longitudinal and circular layers of muscle. It is also provided with six rows of extrinsic dilator muscles which have their origin on the exoskeleton. Other muscles are inserted at the bases of the paraprocts and are important in controlling movements of the valves which guard the anal opening. In contrast to the vestibule, the branchial chamber has few extrinsic muscles and its intrinsic musculature is relatively weak. Ventilation of the gills contained in this cavity is achieved by mechanisms which move water in and out of the anal opening. The mechanisms involve the structure of the exoskeleton, the hydrostatic function of the body cavity, the action of the extrinsic and intrinsic musculature of the branchial chamber and vestibule, and the muscles which control the different valves in the system. Of considerable importance are those muscles which change the volume of the whole abdominal cavity. During respiration these are principally the respiratory dorso-ventral muscles but in addition there are two transverse muscles. The nature and role of these structures are discussed below.

The exoskeleton. The abdomen comprises ten segments each of which is strongly arched dorsally (tergite) and flattened ventrally. The sterna are convex and in the first nine segments each sternum is divided into a broad median plate (sternite) and two small lateral plates (laterosternites). The latter are movably hinged on the tergum and on the medial sternal plate. These lateral plates are often called pleurites. The main action of the respiratory muscles is to draw the pleurites upwards towards the tergites and thus distort the shape of the segments and reduce the volume of the abdominal cavity. It is clear that the elasticity of the exoskeleton can play an important role in the respiratory mechanism. Snodgrass (1954) noted that if the sternum of a single segment is pressed inwards, the lateral margins of the tergum are drawn closer to one another. On release of the pressure the more convex shape of the tergum acts as a spring which restores the sternum to its original position. The normal respiratory movements of the resting larva are comparable, and it is clear that these elastic forces play an important role in the inspiratory action of the abdomen. In addition to this type of movement the abdominal segments may be telescoped longitudinally, but this action plays little part in normal respiration although it is very important during swimming (Hughes, 1958). The abdomen terminates in five appendages, two lateral paraprocts and a median dorsal epiproct and a small cercus on each side. The paraprocts and epiproct each have a valve (v_1) at the base which closes the vestibule or rectal chamber as a result of the contraction of a well-developed sphincter muscle.

The abdominal musculature. The important muscles from the point of view of ventilation may be divided into those intrinsic to the branchial chamber and vestibule and the extrinsic muscles which either insert on them from the exoskeleton or are muscles of the abdominal wall itself. The intrinsic musculature is found mainly in the vestibule and comprises some fairly strong longitudinal muscles together with several circular strands. Also of this type are the strong muscles which close the anal valves and other sphincter muscles which operate the valves between the branchial chamber and vestibule, and between the branchial chamber and the next anterior region of the gut. The branchial chamber itself has few muscles, mainly bands of longitudinal fibres running between the rows of tracheal gills. There are also numerous fine circular fibres, but no dilator muscles. The main respiratory muscles, however, belong to the dorso-ventral group of muscles. In abdominal segments 4-9 these muscles are divided

on each side into anterior and posterior parts and a respiratory dorso-ventral muscle. The latter are the principal expiratory muscles of the body wall and their contraction causes the pleurites to be lifted with a consequent arching of the tergum. The volume of the abdominal cavity tends to be reduced and the increase in the pressure within it will be transmitted to the contents of the respiratory chamber.

In addition to these muscles there is a pair of transverse muscles. One of these is the sub-intestinal transverse muscle which lies at the anterior end of the sixth segment and is entirely ventral to the gut. It is attached laterally to the tergum on both sides. In addition there is a muscle which almost separates the abdominal cavity into two compartments. This, the so-called diaphragm or supra-intestinal muscle, has dorsal and ventral portions lying above and below the gut. There has been considerable debate regarding not only its function but also its morphological position since it was first mentioned by Amans (1881). He described it as an elliptical membrane stretched across the body cavity between the fourth and fifth segments in front of the Malpighian tubules. Several other authors have given a variety of descriptions of its position (see Mill, 1965) and the degree to which it separates the abdominal cavity into two parts.

That the diaphragm does separate the abdominal cavity into two parts has been demonstrated by injecting methylene blue into the anterior region, when the dye remains restricted to the part of the body in front of the diaphragm. If the injection is into the hind segments it stays in this part except for dye pumped forwards by the heart. Correspondingly, X-ray photographs taken after radio-opaque material had been injected into the posterior region showed that the injected material was restricted to that part of the abdomen. The precise position of the diaphragm seems to be that described by Wallengren (1914), namely, it is attached ventrally to the anterior border of the fifth abdominal segment and it remains attached round the lateral borders of that segment for about two-thirds of its length. Dorsally it is not attached and allows the tracheal trunks to pass above it. During life this dorsal opening will largely be occluded and hence fluid will not be able to pass readily from one compartment to the other. Regarding the functions of the diaphragm some authors have maintained that it is important as an inspiratory muscle (e.g. Matula, 1911; Tonner, 1936), but in his more recent monograph Snodgrass (1954) claims that the elasticity of the exoskeleton would be sufficient to produce inspiration and that the diaphragm is not therefore required for this purpose. He suggests that it is more important during expulsion of the labial mask. However, as such a muscle is absent in the Zygoptera which also have a 'mask' but a different respiratory mechanism, it seems more probable that it is linked in some way to ventilation. A respiratory function is clearly indicated by neurophysiological experiments (Mill & Hughes, 1966) in which rhythmic bursts of electrical activity were recorded in this muscle at the same frequency as in the respiratory dorso-ventral muscles.

Many experiments show that activation of both the diaphragm and the sub-intestinal muscle is simultaneous and alternates with that of the respiratory dorso-ventral muscles. Tonner (1936) maintained that the two transverse muscles were inspiratory in action so long as they contracted simultaneously. Contraction of the diaphragm alone was thought to result only in expiration. Tonner does not show the anatomical arrangement of these muscles correctly and although his conclusion regarding their

function is supported here, it seems that he laid insufficient stress on the elastic properties of the exoskeleton. In considering the electromyographic experiments mentioned above it must be remembered that they were made with the abdomen dissected and hence the mechanical arrangements were disturbed. If, as now seems reasonable, the diaphragm is an inspiratory muscle it is difficult to be certain of the nature of its action. Apart from Tonner's suggestion perhaps it also functions by producing a fall in pressure within the posterior abdominal cavity which is transmitted to the branchial chamber. Such an action would be analogous to the mammalian diaphragm but there is little evidence for such a function.

2. Types of ventilation

In addition to the normal regular ventilation of the respiratory system (V_n), two other main types can be recognized (Fig. 3). In normal ventilation there is a rhythmic expulsion of water from the anus followed by the entry of an equivalent volume during inspiration. Sometimes, however, the abdomen maintains its expiratory position for

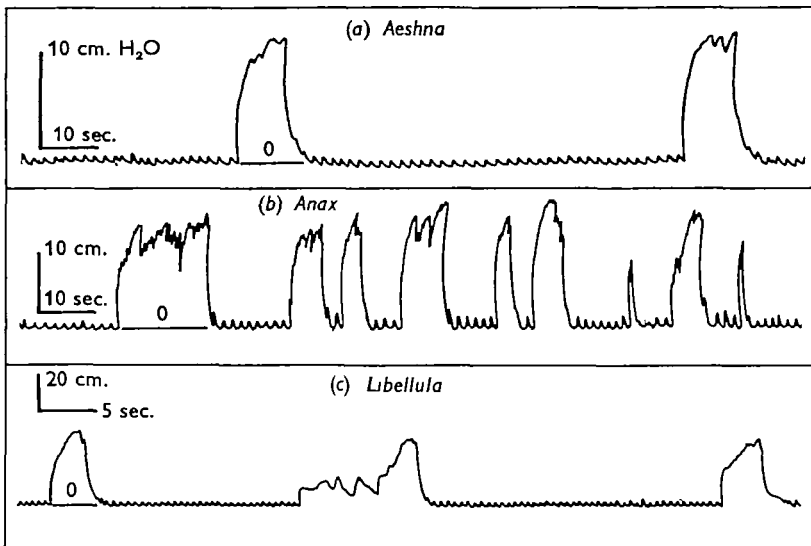


Fig. 3. Pen recordings of pressure changes in the respiratory chambers of *Aeshna*, *Anax* and *Libellula* to show the general pattern in the three species. In each case the normal respiratory rhythm is interrupted by periodic gulping movements. The zero pressure and calibration are indicated in each case.

a longer period. This is the so-called *Schluckatmung* (Tonner, 1936) or 'gulping breathing' (V_g). In certain cases the abdomen is maintained in a fairly expanded condition during each 'gulp'. Each 'gulp' is frequently accompanied by active mixing movements of the respiratory chambers produced by the intrinsic musculature and this has been described as *Kauatmung* or 'chewing breathing' (V_c). There are many variations in the detailed nature of these types and their relation to one another will be described below.

3. Normal ventilation (V_n) (Figs. 3-7)

During this activity there is a regular renewal of about 85% of the water contained in the respiratory chambers. This respiratory current is mainly produced by the action of the dorso-ventral muscles which increases the pressure in the body and branchial

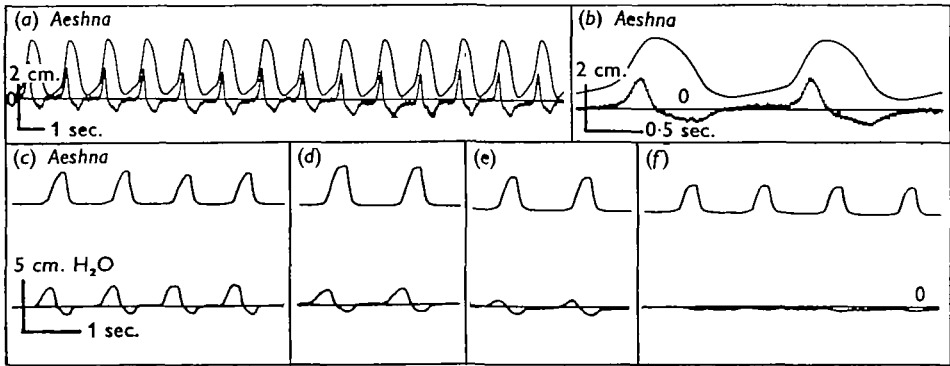


Fig. 4. *Aeshna*: recordings of normal respiration. The upper trace shows the dorso-ventral movement of the sternum (upwards indicating an upward movement). The lower trace is the pressure recorded in the respiratory chambers with respect to the outside zero pressure. In (a) the regularity of the rhythm can be seen, and in (b) more detail of the wave-form and the relationship between the mechanical and pressure recordings can be distinguished. (c-f) Separate recordings from an experiment in which the pressure needle was progressively withdrawn from the respiratory chamber. A gradual decrease in the positive phase of the pressure curve is noticeable and the negative phase only becomes lost when the needle is completely outside the insect. (c, d) Recordings from the branchial chamber, (e) from the vestibule and (f) from the region of the anal valve. (a, b) Oscilloscope recordings, (c-f) pen recordings.

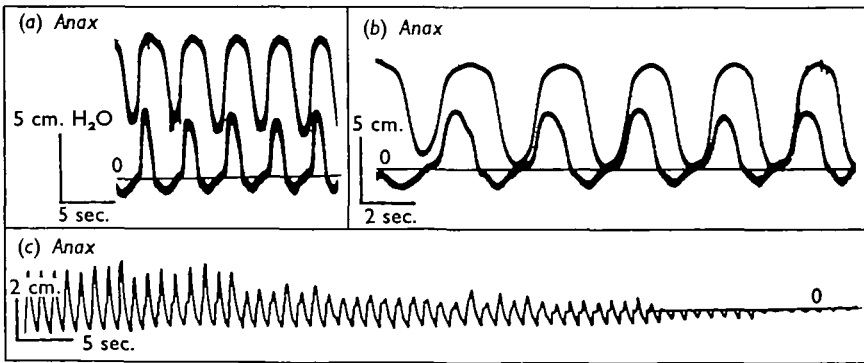


Fig. 5. *Anax*: normal respiration. (a, b) Simultaneous oscilloscope recordings of the mechanical movement of the sternum (upwards being raising the sternum) and the pressure changes in the branchial chamber. (c) Pen recording of change in the pressure waveform as the needle is withdrawn from the insect.

cavities and so forces water through the open anal valves leading to a decrease in volume of the branchial cavity. These muscles relax and the elasticity of the exoskeleton tends to restore the normal volume and so produces a fall in pressure and a consequent inflow of water. It is probable that the transverse muscles assist in this

activity. There is some activity of the intrinsic musculature but its exact timing relative to the body musculature is difficult to establish. There is scarcely any change in length of the abdomen during these movements. In *Aeshna* the frequency normally ranges between 25 and 50/min. It is about the same in *Anax* but usually higher in

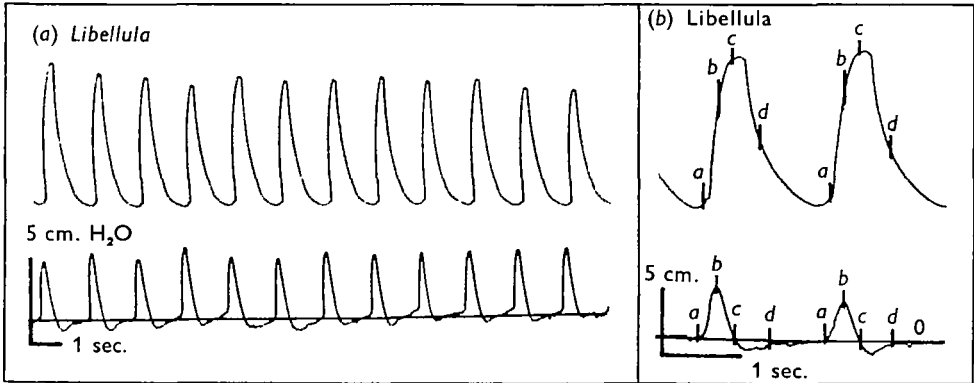


Fig. 6. *Libellula*: normal respiration. (a) Simultaneous pen recordings of the dorso-ventral movements of the sternum and the pressure changes within the branchial chamber. (b) Details of these recordings to show the relationships between the pressure and mechanical records. Corresponding points on the two curves are indicated.

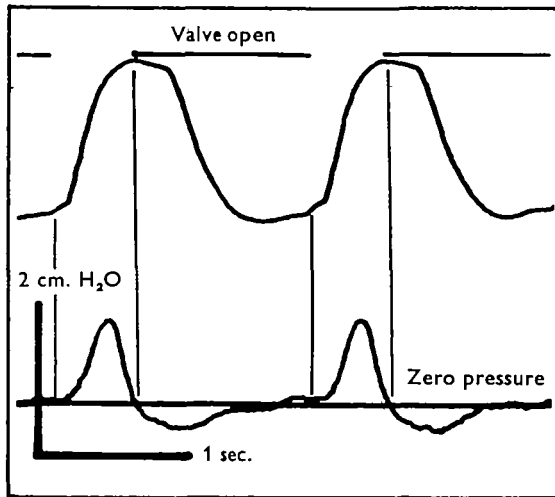


Fig. 7. Two cycles of the dorso-ventral movements of the sternum and of the pressure changes within the branchial chamber.

Libellula (55–90/min., Table 1). In all three larvae the pressure changes include a brief but marked increase in pressure relative to the outside water, usually between 2 and 5 cm. water, within the branchial chamber. This phase occurs as the sterna are raised during expiration. The pressure returns to zero at the end of this movement and then becomes negative as the sterna return to their resting position. The pressure during this inspiratory movement is about 0.5–1 cm. H₂O (0.2–0.5 cm. in *Libellula*)

below the zero level and this phase is usually of longer duration than the positive phase (Figs. 3-7). It can be seen that the rising phase of the pressure curve takes place more rapidly than its falling phase which occurs as water leaves the system, the volume of the respiratory cavities decreases and the pressure falls to that of the outside water. The overall rate of pressure increase in the positive phase is in the range 6-12 cm. H₂O/sec. The fact that the negative phase of the pressure curve is not so great as the positive may be because of its more passive nature and also because of the relatively large aperture of the anal valve during this stage. Changes in rhythm take place by alterations in the interval between the inspiratory phase and the next active expiration.

Table 1. Comparison of the ventilation patterns in *Aeshna*, *Anax* and *Libellula*

	Aeshna		Anax		Libellula	
Normal ventilation (<i>V_n</i>)						
	Usual range	Observed range	Usual range	Observed range	Usual range	Observed range
Duration of active cycle (sternal movement) (sec.)	0.8-1.1	0.7-1.4		1.0-1.7*		0.5-1.5*
Frequency/min.	23-48	12-57	32-48	26-55	53-90	40-114
Positive phase (cm. H ₂ O)	2.4-4.9	1.3-6.9	2.1-3.2	1.6-8.2	2.2-4.5	1.4-5.0
Negative phase (cm. H ₂ O)	0.3-0.9	0.2-1.5	0.5-0.9	0.2-0.9	0.2-0.5	0.2-0.7
Overall rate of increase (cm. H ₂ O/sec.)	8.1†		6.2†		11.5†	
Gulping ventilation (<i>V_g</i>)						
	Maximum		Maximum		Maximum	
Approximate frequency/min	0.5-5		2-10		1-6	
Duration (sec.)	7-16.5		15-16		4-10.5	
Positive pressure (cm. H ₂ O)	10-35		11.3-14		26.3-30	
Chewing ventilation (<i>V_c</i>)						
Pressure change (cm. H ₂ O)	2.5-6		1.6-3		8.3-9.1	
Swimming (<i>S</i>)						
Frequency/min.	126-210		180‡		100	
Positive pressure (cm. H ₂ O)	32.7-40.5§		30‡		23.1	
Time to reach peak of positive pressure (sec.)	—		0.03‡		—	
Overall rate of increase (cm./sec.)	—		1000‡		—	

* Based on limited data. † Approximations. ‡ Hughes (1958). § 52 cm. H₂O on one occasion.

Clearly the respiratory cycle is made up of expiration followed by inspiration, and not vice versa as in the case of ventilation of the mammalian lung. In both cases, however, the change in volume of the cavity containing the respiratory chambers is transmitted to them and they act relatively passively. The exact role of the intrinsic muscles during normal respiration is difficult to estimate because it has not been possible to obtain good pressure recordings from the abdominal cavity itself (i.e. analogous to the intra-thoracic pressure of the mammal).

It has been shown that both the positive and negative phases are present, not only throughout the branchial chamber but also in the vestibule. As the tip of the needle is slowly withdrawn from the gut, the positive phase steadily decreases but the negative

phase is hardly affected. In the vestibule, close to the anal valve, the positive phase ultimately disappears and the negative phase becomes considerably reduced.

4. 'Gulping' ventilation (V_g) (Figs. 3, 8, 9)

This type of ventilation has mainly been recognized here as a periodic increase of between 10 and 30 cm. H_2O in the pressure within the branchial chamber which is maintained for about 10 sec. Externally it is usually accompanied by the closing together of the three terminal appendages and presumably of the anal valves. The abdomen is usually in an expanded condition but sometimes 'gulping' follows the

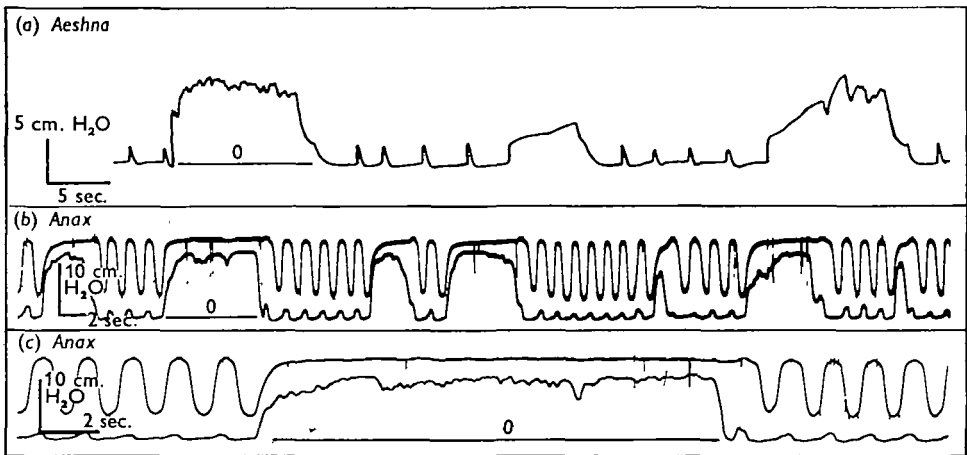


Fig. 8. Gulping and chewing ventilation in *Aeshna* and *Anax*. (a) Pen recording of pressures in the branchial chamber of *Aeshna* (notice particularly the absence of chewing in the second gulping cycle). (b) Simultaneous oscilloscope recording of the sternal movement and pressures in the branchial chamber of *Anax*. (c) The same experiment, only at faster film speed. In both (b) and (c) the fast transients on the upper trace are artifacts produced by leg movements in front of the phototransistor.

typical expiratory movement. 'Gulping' periodically interrupts the normal respiratory rhythm and its onset usually occurs about the time when an expiratory movement normally takes place. Its occurrence is variable in frequency and may be regular or irregular. In addition the duration and the form of the 'gulp' are also variable (Fig. 3*b*). 'Gulping' seems to be less common in *Anax* than in the other two genera. However, when it does occur in *Anax* the interval between successive gulps is often small. It is infrequent in *Libellula* but seems to occur quite frequently and normally in *Aeshna* and often with a rhythm which regularly interrupts that of normal ventilation.

'Gulping' is sometimes preceded by a small jet and is usually associated with 'chewing' movements. In fact it is relatively rare to find pure 'gulping', but it can be recognized by the smooth time-course of its pressure recordings as, for example, the second 'gulp' in Fig. 8*a*. Notice how the third cycle in this recording begins with the same time-course, the initial rising phase being identical with a typical expiration, but later it shows the rhythmic fluctuations associated with 'chewing'. The frequency of the 'chewing' pressures is sometimes close to that of V_n .

Evidently 'gulping' is a distinct pattern of ventilation, although it also involves the

respiratory dorso-ventral muscles and probably some of the longitudinal ones. It is characterized by the maintained closure of the anal valves and hence the prolonged increase in pressure. Presumably it serves to evacuate the whole of the respiratory cavities so that they can become refilled with oxygenated water. In addition it is possible that it allows some rearrangement of the gill lamellae. In a way it seems analogous to the 'coughing' movement of a fish during which there is a temporary

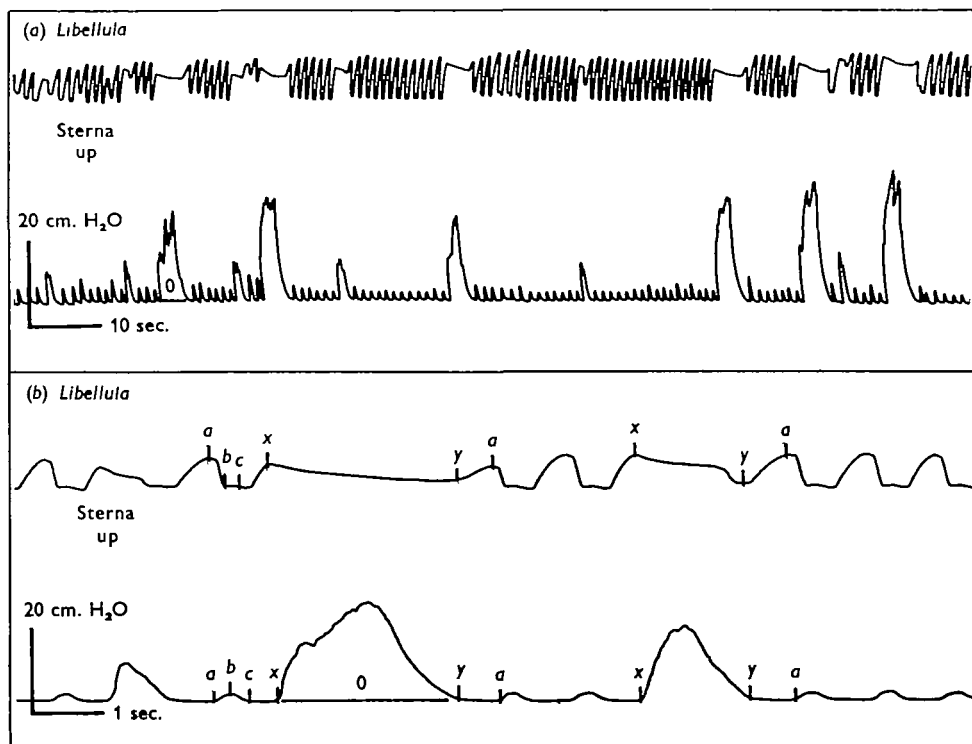


Fig. 9. *Libellula*: gulping and chewing ventilation (pen recordings). Simultaneous recordings of the sternal movements and pressure changes in the branchial chamber. Notice that in these recordings the upward movement of the sterna is *downwards* on the tracings. The recording speed in (b) is faster than in (a) and shows the relationship between the mechanical and pressure recordings.

reversal of the respiratory current and a rearrangement of the complex gill filaments. Whether or not the frequency with which 'gulping' occurs is closely related to some physical feature of the water such as the oxygen or carbon dioxide tensions, or temperature does not seem to be established. It could also be related to the presence or absence of some foreign bodies in the respiratory medium. It is certainly the type of respiratory pattern found in these larvae which is most distinct from normal ventilation.

5. 'Chewing' ventilation (V_c) (Figs. 3, 8, 9)

It is difficult to distinguish this as an entirely separate form of ventilation because it always seems to be accompanied by gulping ventilation (but see § 6 below). Its essential feature is the change in pressure which occurs within the respiratory cavities

during the maintained increase of pressure during V_g . The V_c pressure changes may appear as further increases or decreases in pressure but are often biphasic and sometimes rhythmical (Figs. 3*a, b*; 8*a*). These pressures result from contractions of the intrinsic and extrinsic musculature of the respiratory cavities and pass along the branchial chamber and vestibule as peristaltic waves. The intrinsic muscles are the main ones involved, particularly as possible dilators of the branchial chamber are only weakly developed (Mill, 1965). Because of the hydrostatic relationships of the posterior chamber of the abdomen it is clear that a contraction of one region of the gut will tend to reduce the pressure within the body cavity and that this will assist the expansion of other regions where the intrinsic muscles are more relaxed. A great deal of expansion movement of the branchial chamber can therefore take place in the absence of dilators, and such a mechanism is comparable to that which operates in the dogfish heart within the rigid pericardial cavity. The dilator muscles play an important role in drawing water into the system when the anal valve is open.

No consistent differences have been observed between the three genera in respect of V_c although it seems to be less rhythmic and more pronounced in *Libellula*. The function of this type of ventilation is presumably to ensure a more complete removal of oxygen from a given volume of water by disturbing any stagnant layers. It gives a longer time for diffusion to take place and it may be more economic from the energy point of view because the movement of liquid into and out of such a cavity will certainly use up a great deal of the resting oxygen consumption.

6. Other patterns of ventilation (Figs. 10, 11)

The most important of the other ways in which the contents of the respiratory cavities are renewed is during swimming (V_s) when water is actively 'jettted' out of the anus. These movements involve a reduction in the volume of the abdomen as a result of dorso-ventral and longitudinal contractions (Hughes, 1958). The frequency of the jets is far greater than normal ventilation and may be as high as 200/min. in *Aeshna* and *Anax* but about 100/min. is the upper limit for *Libellula*. The peak pressures measured were between 25 and 40 cm. water which is in the same range as the maximum recorded during 'gulping' ventilation. During normal hyperventilation water leaves the anus at quite a high velocity and there is a distinct tendency for the body to move forwards. Clearly normal ventilation must involve some rhythmic activity in the leg muscles to counteract this tendency.

The overall rate of increase in pressure is about 1000 cm. H_2O /sec. (Hughes, 1958) which is around 100 times that of normal ventilation. Swimming may be initiated by removal of a piece of paper or cotton wool that is being grasped by the suspended insect. Alternatively it is readily elicited by sharply tapping the bench or the metal support to which the insect is attached. The normal respiratory rhythm is usually inhibited for a period of about 10 sec. following a bout of swimming (Fig. 10*a, b*). In each of the bursts of swimming activity there are up to thirty movements. In certain cases repeated taps on the bench increase the amount of activity in each burst (Fig. 10*c*). However, following later taps in the series, the response becomes completely absent, suggesting some sort of adaptation or habituation. In this preparation (Fig. 10*b, c*) notice the unusual displacement of the base-line by about 5 cm. of water. These particular recordings were made using a needle with the opening in its tip and were not found

normally, that is with the opening in the side of the needle. The inhibition of V_n following a burst of V_s suggests that this type of ventilation has resulted in an increase in the oxygen and a decrease in the carbon dioxide tensions in the tracheal supply to the respiratory centre which may be responsible for the inhibition. It would be of interest to ventilate the branchial chamber artificially and to see the effect on V_n .

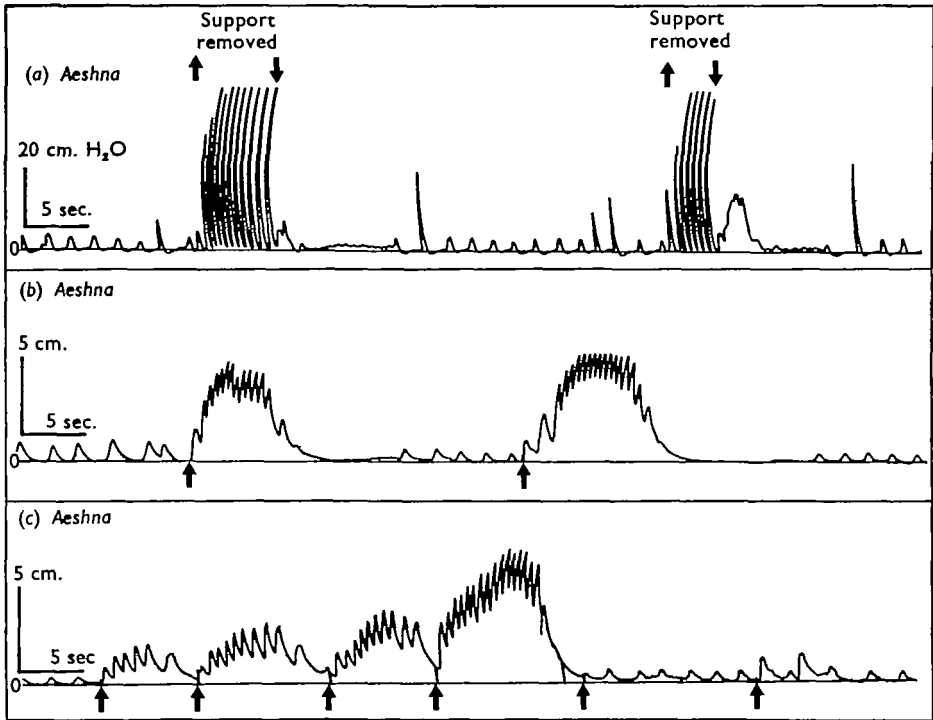


Fig. 10. *Aeshna*: initiation of swimming. Pen recordings of pressure changes within the branchial chamber. (a) Effect of removing support to the legs. (b) Effect of tapping the bench (arrows). Notice the production of swimming and subsequent inhibition of normal ventilation. (c) Effect of repeated tapping to show the gradual increase in response to consecutive stimuli, followed by habituation or fatigue.

A pattern of ventilation found in certain specimens of *Aeshna* is shown in Fig. 11. Here the normal ventilation has a positive pressure phase of 4 cm. H₂O and a negative phase of about $\frac{1}{2}$ cm. water. The V_n rhythm is periodically interrupted, however, by bursts of increased positive pressure within the respiratory chambers. These are not accompanied by decreases in volume of the abdominal cavity but rather by stepwise increases in the dorso-ventral dimension recorded. After about ten such cycles the pressure gradually subsides and the whole act is terminated by a normal respiratory cycle in which the decreased dorso-ventral movement produces a pressure wave which is superimposed on the steadily declining pressure resulting from the previous activity. Close analysis of the oscilloscope recordings (Fig. 11 b, c) shows how the rise of the V_n pressure curve takes place during the phase when the abdominal sternites are raised, as is usual. The negative phase accompanies the return of the sternites to their expanded condition. During the burst activity the positive waveform is very similar

to the normal one but it is not followed by a negative phase. Furthermore, the whole of the positive phase is accompanied by an increased depression of the sternites, which exhibits summation. It appears that the positive pressures during this activity are probably produced by the intrinsic muscles of the branchial region and that they serve gradually to 'pump up' these cavities and so lead to the expansion of the abdomen. This whole activity is suggestive of the types of mechanism which may be involved in chewing ventilation, only in this case they are not superimposed upon gulping ventila-

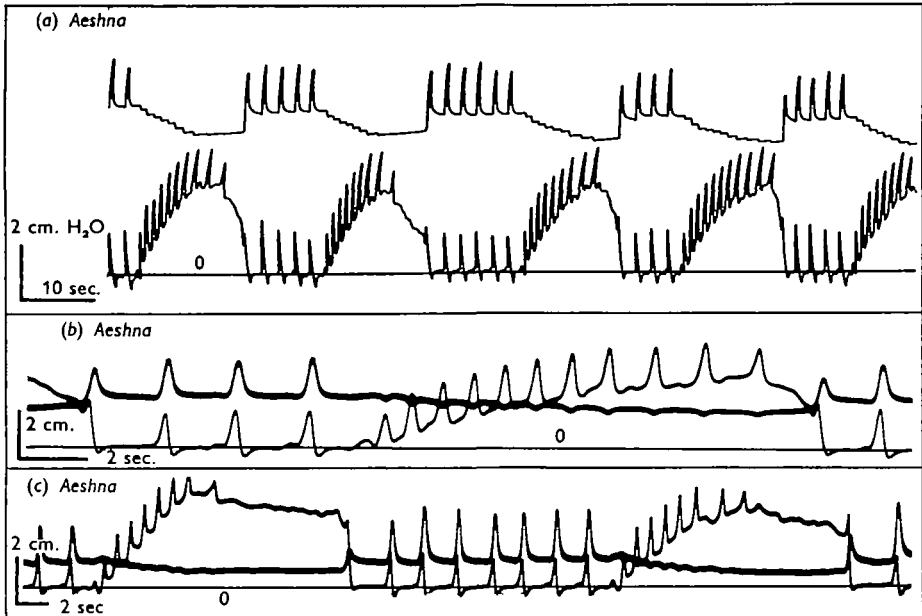


Fig. 11. *Aeshna*: simultaneous movement and pressure recordings in an experiment in which there is a gradual build-up in the pressure levels within the respiratory chamber. Notice the periodic increase in pressure during the build-up accompanied by stepwise depressions of the sterna. Upward movement of the sterna is indicated by upward movement of the top trace. (a) Pen recording. (b) Oscilloscope recording of part of the same preparation at faster recording speed. (c) Oscilloscope recording to show maintenance of pressure in the absence of normal ventilatory movements.

tion. It may be that they are not normally noticeable in recordings of gulping ventilation because the amplification to record chewing respiration is lower than in an experiment of the type now under consideration. It is of interest to notice that the frequency during the burst of activity is here about double that of the normal V_n rhythm.

DISCUSSION

It is clear that the basic morphological plan of the abdomen of the anisopteran larva provides an extremely efficient mechanism for renewing the water within the enlarged cavity of the colon containing the tracheal gills. The part of the abdomen behind the fourth segment is a more or less isolated hydrostatic compartment so that changes of pressure within it are transmitted to the gut contents in that region but probably not to the rest of the body. Changes in pressure within the anterior regions of the insect

are more concerned with protrusion of the mask. If there were not this isolation of the posterior respiratory part of the abdomen the mask would tend to be protruded each time the abdomen contracted strongly during jet propulsion. The special development of the dorso-ventral muscles in the fourth to the ninth segments also emphasizes the specialization of function in this region.

Tidal ventilation is not common in aquatic organisms. It is found, however, in the respiratory trees of holothurians and in the gill pouches of the lamprey when it is attached to its prey. In the latter case the path of water across the respiratory surface is probably unidirectional because of the nature of the valves which guard the external opening. As far as is known the flow is simply tidal in the holothurians. The problems of tidal respiration in a liquid medium are considerable because of the relatively low rate of diffusion of the respiratory gases in liquid as compared with air. It has the disadvantages of aquatic respiration such as low oxygen content, high viscosity and density of the water and also the difficulty of a large dead space as found in tidal air breathers. The work of ventilation must be relatively high and the mechanism is probably finely adapted to the conditions.

One of the main advantages of the mechanism of ventilation found in the anisopteran larva is that it circulates the water being respired, which is ideal for a relatively immobile animal which spends a lot of its time at the bottom of fairly stationary waters. It would, as Bidder (1923) pointed out in connexion with the feeding of sponges, serve to increase the 'diameter of supply' and so not foul its own water. The evolution of a diaphragm would be adaptive for a larva with a prehensile mask. The relationship between respiration and locomotion is apparent in many organisms and in this case one can see how the mechanism could become adapted for locomotion when conditions for respiration were not ideal. The jet propulsion mechanism is basically an enhancement of the respiratory pump and potentially serves the important respiratory function of removing the insect from a region low in oxygen.

A tidal mechanism has several disadvantages for liquid breathing of small mammals (Hughes & Kylstra, 1964). Thus, because of the lack of adequate mixing in the alveoli and the low oxygen capacity of the saline, it was necessary for the saline to be saturated with oxygen at about 5 atmospheres pressure. This serves to emphasize the design problem which has been overcome by the dragonfly larva.

In this context it is clear how important are the intrinsic muscles of the respiratory cavities, and especially the chewing movements, in reducing the stagnant layers adjacent to the respiratory epithelium. Once the gas has crossed the respiratory interface its rate of diffusion will be much greater within the tracheal trunks. In addition to their effect on ventilation of the branchial chamber the pumping movements may also play some part in encouraging circulation of the air within the tracheal system where the gas must be under a slight negative pressure.

The pressure recordings and also the neurophysiological experiments (Mill & Hughes, 1966) have shown that the respiratory cycle consists of an expiratory phase followed by inspiration, the latter mainly produced by elasticity of the exoskeleton. This is similar to what is found in lampreys, where the respiratory muscles are expiratory (Roberts, 1950). A similar important role of expiratory muscles combined with skeletal restoring forces has also been shown in the dogfish (Hughes & Ballintijn, 1965). It is of interest to note that the type of pressures recorded from the dogfish

parabranchial cavities and the associated movement record are almost identical in form to those recorded from the dragonfly larva.

On the whole this work has served to confirm the classification of Tonner (1936). The time-course of these pressure changes and movement recordings also fit in with his description of the action of the different valves. There are considerable similarities between the three genera and this method of recording ventilation has given a more complete description of the range of activities, particularly in respect of gulping and chewing. There is certainly a great variation in these activities, which are usually combined, but there is some suggestion that chewing may occur in the absence of gulping. The role of chewing in reducing stagnant layers has been referred to above, but the significance of gulping unaccompanied by chewing is not so apparent. It may be a sort of defence mechanism against a possible assailant or some noxious material within the water. The similarity between all three genera suggests that the adaptive value of these mechanisms is relatively independent of the type of gill organization within the branchial chamber. The functional significance of the different gill types may be related to the nature of the water which the insect normally inhabits, the utilization and minute volume and consequently the metabolic rate. The large plate-like lamellae of *Libellula* suggest adaptation to conditions of lower oxygen tension than those of, say, *Anax*. The latter, however, might be expected to be a more efficient swimming insect as the flow of water into and out of its branchial chamber will not be hindered so much by the gills. These differences are supported by the experimental data (Table 1).

In the present experiments no clear relationship was found between the pattern of ventilation and the gas content, but the latter was not adequately controlled. There are marked individual variations; some larvae showed very regular gulping and chewing movements whereas another larva placed immediately in the same environment showed a perfectly regular V_n rhythm.

These features pose considerable problems from the point of view of the nervous co-ordination of the movements and it would be of interest to know whether individual differences in respiratory activity of the intact insect are correlated with different types of intrinsic activity in the central nervous system.

SUMMARY

1. A study has been made of the patterns of ventilation in three genera of anisopteran dragonfly larvae by recording simultaneously the pressure changes in the branchial chamber and the dorso-ventral movements of the abdomen.

2. The three main types of ventilation described by Tonner have been confirmed and the characteristic pressure changes during normal, gulping, and chewing ventilation are described.

3. During normal ventilation there is a positive pressure phase of about 4 cm. water followed by a brief negative phase of $\frac{1}{2}$ cm. water. The main expiratory phase is produced by the respiratory dorso-ventral muscles which are found in abdominal segments 4-9. Inspiration, which closely follows expiration, results from the elastic properties of the exoskeleton together with the activity of the two transverse muscles. One of these, lying at the anterior end of the fifth abdominal segment, forms the so-called diaphragm and divides the abdomen into two functional cavities.

4. Gulping ventilation is characterized by a maintained pressure of 20–40 cm. water during which there may be fluctuations associated with the chewing movements, which are mainly due to the intrinsic musculature of the colon and rectum.

5. Despite some differences in detail the main patterns of ventilation are very similar in *Aeshna*, *Anax* and *Libellula*.

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