ELECTROMYOGRAPHY
OF THE RESPIRATORY MUSCLES AND GILL WATER
FLOW IN THE DRAGONET

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INTRODUCTION

The electromyographic study of muscles involved in the respiratory movements of fishes (Hughes & Shelton, 1962) has already proved of value from at least three points of view. First, it has made it possible to decide which muscles are active during ventilation and has demonstrated that they are not always those suggested by purely anatomical studies (Hughes & Ballintijn, 1965). Secondly, electromyographic studies have shown how the pattern of muscular activity may vary according to the depth of ventilation and the type of respiratory manoeuvre. Thirdly, they have emphasized the need for studying the whole pattern of muscular activity and the considerable importance of couplings between different parts of the respiratory mechanism (Ballintijn & Hughes, 1965). In that study on trout, differences in the muscle activity were related to the depth of breathing qualitatively and no quantitative study was attempted either by measuring the electrical activity itself or the volume of water pumped across the gills. For such a study, it was desirable to measure the water flow directly as this makes it possible to follow changes over a shorter period than if measurement of ventilation volume is based upon the determinations of total oxygen consumption and the change in P_{O_2} of the water during its passage across the gills (Saunders, 1961). For this purpose, the preparation described in the previous paper (Hughes & Umezawa, 1968) seemed ideal as all the water leaving the opercular cavities is collected and can be measured while the fish remains at the bottom of the experimental tank in a resting condition.

The fish is specialized from several points of view in its respiration; it was first of all necessary to carry out an anatomical study and to determine electromyographically which muscles are most important in ventilation. Later the electrical activity in certain of these muscles was studied quantitatively in order to follow changes that occur when the fish was made to alter the volume pumped across the gills by either changing the hydrostatic pressure gradient (Δp) or by changing the P_{O_2} of the water and in some instances the P_{CO_2}. The effect of changing gas concentrations has not yet been studied in great detail and was used only as a means of effecting changes in pumping.

The results showed not only the usefulness of this fish for correlating the electrical activity of different muscles and stroke volume, but also provide information about

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the control mechanisms concerned in the response to alterations in the environmental P_{O_2}. In certain instances, this seems to involve peripheral receptors in the gills, but other observations suggest that the P_{O_2} of the blood affecting the respiratory centre is another mechanism.

MATERIALS AND METHODS

Specimens of the dragonet, *Callionymus lyra*, had been freshly caught and kept in the sea-water circulation at the Plymouth Laboratory. Males were generally used because of their larger size (80–120 g.) which not only facilitated the experiments but
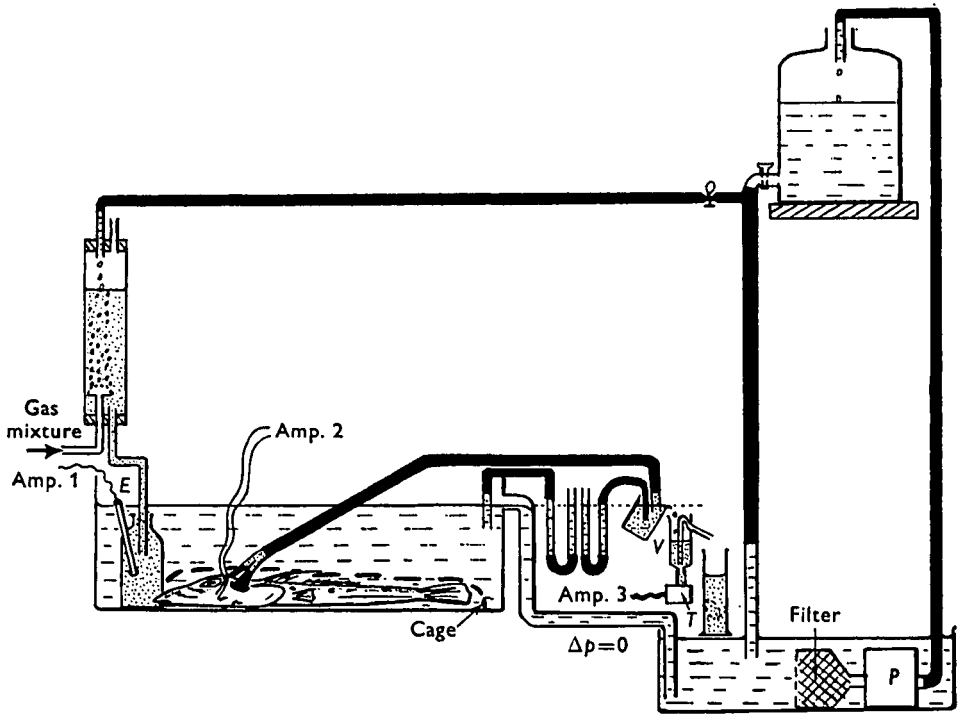

Fig. 1. Diagram to show the method used for measuring the volume of water pumped across the gills under different gradients of hydrostatic pressure (Δp), and following changes in the gas content of the water inspired by the fish. Amp. 1, Amplifier for oxygen electrode (E); Amp. 2, preamplifier for electromyogram; Amp. 3, carrier preamplifier for pressure transducer (T); P, water pump; V, volume recorder.

also decreased the dynamic loading effect of the experimental procedure (Hughes & Knights, 1968). The basic experimental set-up (Fig. 1) was similar to that used by Hughes & Umezawa (1968). The load imposed upon the fish by collecting tubes consists of two parts, (*a*) a static load due to frictional forces of the water against the tube wall, and (*b*) the dynamic load caused by the acceleration and deceleration of the water contained in the tube on account of the intermittent flow. To obtain an estimate of the dynamic load imposed upon the animal under different conditions, a model pump was constructed with which the dynamic load of different tubes could be

measured and compared with an equivalent hydrostatic load. From these experiments, it was possible to estimate that the dynamic load of the tubes used in this work was equivalent to about 1 mm. of water for the normally breathing fish, but was greater during very heavy ventilation.

The fish was first anaesthetized in MS 222 and the 'horns' were fixed around the two external opercular openings. The water issuing from the horns was collected into a single rubber tube of about ⅜ in. bore which led directly to the levelling beaker. The water level in the experimental tank was held constant by an overflow and the circulation was maintained by a submerged 'hippo' pump. The water overflowing from the levelling beaker could be collected and measured directly but for most experiments an electrical recording method was used in which a syringe containing a self-starting siphon was connected to a Sanborn 268B pressure transducer. As water collected in the syringe, the recorded pressure gradually increased to a maximum when the siphon operated and returned the pressure to a low level once more. This gave a useful tracing of volume on the pen recorder which was calibrated from time to time. Changes in gas content of the water were effected by bubbling through the exchange column either nitrogen or different gas mixtures made up in a 'Platen' gas mixing apparatus. The P_{O_2} of the water breathed by the fish was monitored using a Beckman macroelectrode placed in the plastic bottle which contained the snout of the fish. This arrangement assisted the rapid change of the P_{O_2} in the inspired water.

Electromyograms were recorded using fine stainless steel pins insulated except at their tip, and in other experiments pairs of hooked Diamel-coated stainless steel wires were thrust into the muscles by means of a hypodermic needle. The electrical activity was amplified by Tektronix 122 amplifiers and was displayed on a Devices 6-channel pen recorder and a Tektronix 565 oscilloscope. The electrical activity was integrated (Fig. 2) using integrators whose time constant could be varied.

In these integrators the signal was fed into a capacitor via a rectifier circuit and a charging resistance. The rectifier circuit could be switched to full wave or half wave, and in the latter case the positive or the negative part of the signal could be chosen. The voltage across the capacitor, a measure for the integral of the signal, was displayed on the pen recorder. The capacitor was continuously discharged by one of a bank of resistances, the choice of which determines the time constant of discharge.

RESULTS

A. *Anatomical*

Skeleton.

Callionymus lyra has a curiously specialized skull in which the greatly enlarged ascending processes of the premaxillae are particularly prominent. These are received posteriorly into deep fossae formed chiefly in the lateral ethmoid. It is this arrangement which forms the basis of the protrusible upper jaw. The skull is flattened dorsoventrally and is generally triangular in shape, the posterior corners of the triangle being protected by groups of spines attached to the pre-opercular bones. The dorsal side of the opercular cavity is mainly supported by the subopercular which is enlarged more than the opercular bone. The latter articulates with the hyomandibula. The suborbitals are a pair of well-developed membranous bones which form the lateral

edge of the skull below the eyes. The lower jaw articulates posteriorly with an elongated quadrate but its relationship with the maxilla and premaxilla is more complex and has not been studied in detail. The ventral and lateral walls of the opercular cavity are relatively thin and supported by the branchiostegal rays. The exit from the opercular cavity lies in a notch of the opercular bone. Details of this skull are figured by Gregory (1959).
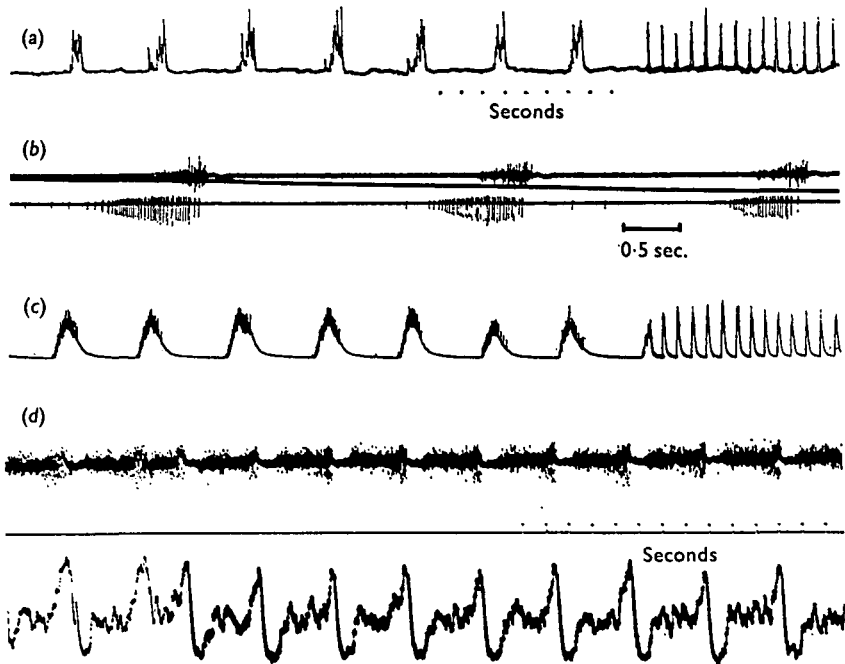

Fig. 2. Recordings to show use of integrated electromyogram to give pattern of electrical activity in respiratory muscles of *Callionymus*. (*a*) and (*c*) are simultaneous recordings of the integrated EMG of the protractor hyoideus and adductor mandibulae respectively. (*b*) is an oscilloscope recording taken shortly afterwards and shows the electrical activity in the same two muscles. Notice the gradual increase in height (facilitation of j.p's) of the adductor mandibulae and particularly the gradual decline in height and frequency which contrasts with the sudden reduction in the EMG of the protractor hyoideus (upper beam). The third beam is from the volume recorder. (*d*) Electrical activity in levator hyomandibulae and simultaneous recording of integrated EMG. Notice the marked increase in activity just before the muscle becomes inactive.

Musculature (Fig. 3)

There are four main pairs of muscles visible in a dorsal dissection.

(1) *The adductor mandibulae.* This is the largest muscle in the head and forms its latero-frontal border. It is formed of two main parts, one lateral and the other medial and more dorsal. The separation between the two is where the mandibular branch of the trigeminal nerve enters the muscle mass. The lateral portion has its origin on the preopercular and quadrate bones and runs from a more ventral position to a slightly more dorsal one, whereas the inner branch is initially more dorsal and becomes more ventral at its insertion. The outer branch inserts on the maxilla and ligaments at the angle of the lower jaw. The inner and larger muscle mass inserts on the mandible and partly on the maxilla. When pulled, the medial branch lifts up the mandible, pulls

back the maxilla and premaxilla and hence closes the mouth; the lateral branch pulls the maxilla down but it also lifts the mandible to a lesser extent. There are several ligamentous connexions in this region, notably between the maxilla and mandible and between the maxilla and premaxilla, so that movement of one of these bones affects the others.
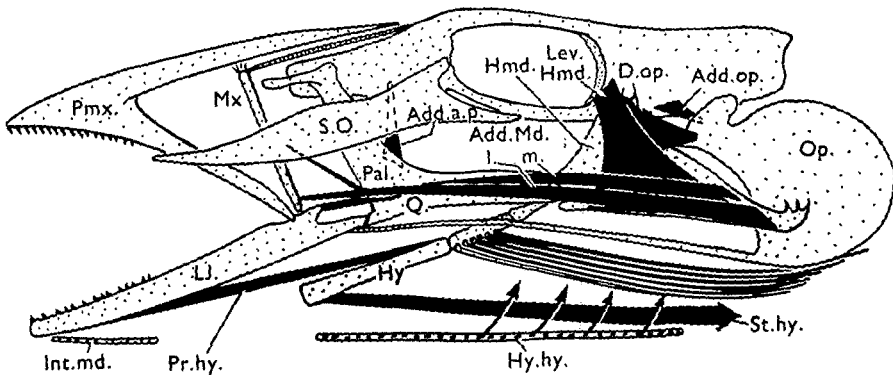

Fig. 3. *Callionymus lyra*. Diagram of the skeletal elements and muscles used in respiration. The muscles expanding the system are the levator hyomandibulae (Lev. Hmd), dilator operculi (D. op.), and sternohyoideus (St. hy.). Muscles contracting the system are: the adductor arcus palatini (Add. a.p.), adductor operculi (Add. op.), adductor mandibulae medialis and lateralis (Add. md. m. and l.), protractor hyoideus (Pr. hy.) and the hyohyoideus (Hy. hy.). Hmd, hyomandibula; Hy, hyoid arch; L.J., lower jaw; Mx, maxilla; Op, operculum; Pal., palatine; Pmx, premaxilla; Q, quadrate; S.O. suborbital. Skeletal elements are dotted; ligaments striped and muscles black.

(2) *The levator arcus palatini et hyomandibulae.* This is a well-defined muscle situated beneath the orbit. It has its origin on the cranium in the posterior orbital border and runs across the hyomandibula upon which it inserts towards the spine which forms the lateral edge of the head, above the opercular cavity. This muscle causes the hyomandibula to lift and indirectly abducts the operculum. Its action would also appear to be involved in the edge-raising of the lateral part of the head, exposing the spines, when the fish is attacked. Posterior to the levator hyomandibulae is a well defined ligament which runs just in front of the opercular opening from the cranium to these spines.

(3) *Dilator operculi.* This is a small muscle which lies just posterior and slightly dorsal to the levator hyomandibulae and at first sight seems to be a part of the latter as its origin is similarly placed on the anterior otic region just behind the eye. It operates across the hyomandibula-opercular hinge and expands the operculum.

(4) *Adductor operculi.* This muscle is more posterior and dorsal but it still lies anterior to the opercular opening. It has its origin in the posterior otic region and is inserted behind the hyomandibula-opercular joint. Its contraction serves to rotate and adduct the operculum. When the opercular cavity is opened up from the inside, a well-defined muscle is seen from the cranium to the inside of the hyomandibula. This presumably is a branch of the adductor arcus palatini et hyomandibulae. It does not run to the operculum itself.

These are the main superficial muscles of the dorsal and lateral aspect of the skull, but deeper and anterior to the orbital region there is a well-defined *adductor arcus*

palatini which has its origin on the cranium and is inserted on the medial aspect of the palatine. A part of this muscle seems to extend as a thin sheet beneath the eyeball.

On the ventral side there are a number of muscles lying just beneath the skin, the most posterior being a thin sheet of fibres running transversely which form the *hyo-hyoideus*. Anteriorly a similar sheet (*intermandibularis*) runs between the two rami of the lower jaw. The hyohyoideus spreads out over the branchiostegal rays, extends backwards and behind the spines of the lateral side of the head. Sometimes it is difficult to differentiate the hyohyoideus from the protractor hyoidei but the latter muscles run along either side of the lower jaws from their anterior origin on the lower jaw to the hyoid arch. Unlike this muscle in many teleosts, the two parts do not appear to meet so definitely in the mid-ventral line. The *sternohyoideus* is a well-defined large muscle having a broad origin on the pectoral girdle and is inserted medially on the hyoid arch. Its insertion on the hyoid is anterior to that of the protractor hyoidei. A well-defined ligament which is ossified seems to represent the combined hyoideo-mandibular and mandibulo-interopercular ligaments of other fishes. It remains ligamentous in the region where it joins the mandible. The opercular bones are very thin and membranous and the single opercular opening is slightly dorsal to them.

This is a brief summary of the main muscles used in the present study. No attempt has been made to work out the complex mechanical interactions between different parts of the skull nor the detailed musculature of the gill arches.

B. *Experimental*

(1) *Electromyographic analysis of the respiratory cycle*

As is known from studies of the respiratory movements and pressures (Hughes, 1960), the cycle in *Callionymus* is made up of two distinct phases. Water is sucked across the gills during the expansion phase and this is followed by a much briefer contraction of both buccal and opercular cavities which produces a large increase in pressure as water is ejected from the opercular slits. The period of ejection is clearly marked as an artifact on the electromyogram records and forms convenient markers with which to divide the cycle (Fig. 4). Most muscles recorded are active during the brief ejection phase but there are slight differences in their timing as mentioned below. The main muscle active during expansion appears to be the levator hyomandibulae, and to a lesser extent the sternohyoideus, as in the trout. The adductor mandibulae, protractor hyoideus and hyohyoideus are active during the contraction phase. The other muscles active during this phase in the trout form a continuous strip (adductor arcus palatini et operculi) but in *Callionymus* they are separate and only the adductor operculi could be reached. The general pattern of activity in the individual muscles is summarized in Fig. 5 and discussed below.

Levator hyomandibulae. This muscle already comes into action before the expulsion phase has been completed; it begins with activity of low amplitude and gradually increases until it ends just slightly before the operculum is opened. In some instances this latter part is quite marked as shown by the integrated myogram (Fig. 2d).

Sternohyoideus. Activity begins some time after the opercular slit is closed and with very low-amplitude signals barely discernible from the noise. It gradually increases its activity which ends at about the same time as, or usually slightly before, the levator
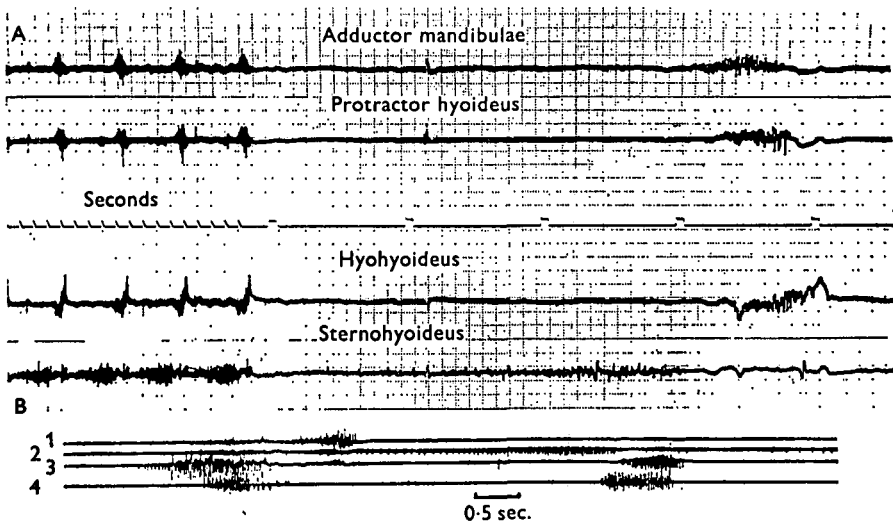
Fig. 4. Electromyograms from respiratory muscles. (A) Pen recording of activity in the adductor mandibulae, protractor hyoideus, hyohyoideus and sternohyoideus. Notice artifact on all recordings during opening of the opercular valve. (B) Oscilloscope record from 1, sternohyoideus; 2, levator hyomandibulae; 3, hyohyoideus; 4, adductor mandibulae. Notice that the sternohyoideus is only active at the end of the first respiratory manoeuvre, which was a sort of cough. This muscle was inactive during the typical respiratory pattern which begins when activity in the sternohyoideus has ended.
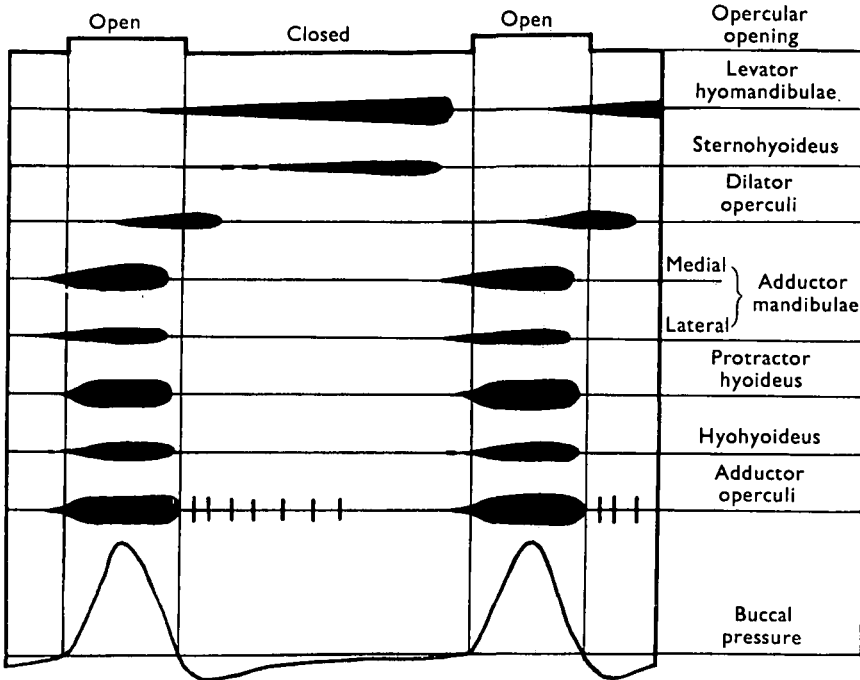

Fig. 5. Summary diagram to show the phase during which different muscles are active during the respiratory cycle. The phase of opening of the opercular slit has been deduced from the artifact on the electromyogram recordings and coincides with the increase in pressure recorded in the buccal and opercular cavities.

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hyomandibulae becomes active. In some preparations the sternohyoideus does not appear to be active during normal ventilation but may come into action during hyperventilation following a decrease in oxygen concentration.

Adductor mandibulae consists of inner and outer portions which may or may not differ slightly in their timing, sometimes the inner or medial part appears to become active before the lateral part and it is almost always found that the inner portion is more active than the outer. Activity of both parts of the adductor mandibulae begins slightly before opening and ends slightly before closure of the opercular slits.

Protractor hyoidei. Activity in this muscle again starts slightly before the opercular slit opens and with activity of fairly low amplitude. It builds up and sometimes gives the impression of a definite burst of higher amplitude. Its activity seems to begin slightly after that of the adductor mandibulae. After opercular opening, activity in this muscle is markedly increased and ends slightly before opercular closing.

Hyohyoideus may or may not start before opercular opening. If it does, its intensity is always low and gradually increases until after opercular opening when its activity diminishes and ends before the next opercular closing. The recordings suggest that this muscle becomes active after the protractor hyoideus, so we have the sequence adductor mandibulae, protractor hyoideus, hyohyoideus.

The *adductor operculi*, like the protractor hyoideus, starts before the opercular slit opens and with low-intensity activity. It increases considerably after opercular opening but ends later when the opercular slit closes. It was remarkable to find in one preparation that firing of a single unit persisted as a series of single spikes after the whole muscle ceased to be active. About seven action potentials were fired during the period from opercular closing till about half way to the next opercular opening phase.

The pattern of muscular activity described here has some similarities to that described for the trout (Ballintijn & Hughes, 1965). Thus, the three muscles, adductor mandibulae, protractor hyoideus and hyohyoideus contract almost simultaneously in both species and in the order given here. The levator hyomandibulae plays a relatively more important role during the expansion phase of the cycle of *Callionymus*, though during quiet and moderate ventilation of the trout the levator hyomandibulae is active whereas the sternohyoideus is not. In the present experiments relatively few cases were found where there was a difference in the pattern of muscular activity at different depths of ventilation, although the sternohyoideus is one example which did occur. Thus, in some preparations this muscle was initially inactive but became active when the stroke volume increased following a lowering of the P_{O_2}

(2) *The relationship between the electromyograms and the volume pumped through the gill system under varying conditions.*

The main purpose of this work was to determine quantitatively the modifications in electrical activity under varying depths of ventilation. The latter were produced either by altering the hydrostatic pressure across the system or by changing the concentration of the respiratory gases. The differences between the results obtained using these two methods are of interest and suggest different control mechanisms regulating ventilation.

(a) *Changes in hydrostatic pressure gradients* (Δp) *imposed across the gills.* (Figs. 6, 7). Studies on this effect have already been carried out in *Callionymus* by Hughes & Umezawa (1968) who showed that there was a more or less linearly increasing effect

on the flow when the pressure in the buccal cavity was increased relative to the opercular chamber. Where negative gradients were imposed, i.e. the pressure outside the opercular cavity made greater than that on the buccal cavity, the rate of change in minute volume per unit Δp was much less. It was suggested there, and is evident on general grounds, that changes in Δp will affect the output of the respiratory pump, partly passively because a change in the hydrostatic pressure head across the gill resistance will alter the flow of water. In addition to this passive influence, there may also be a change in the pump output produced by the fish actively trying to compensate for the imposed gradient.
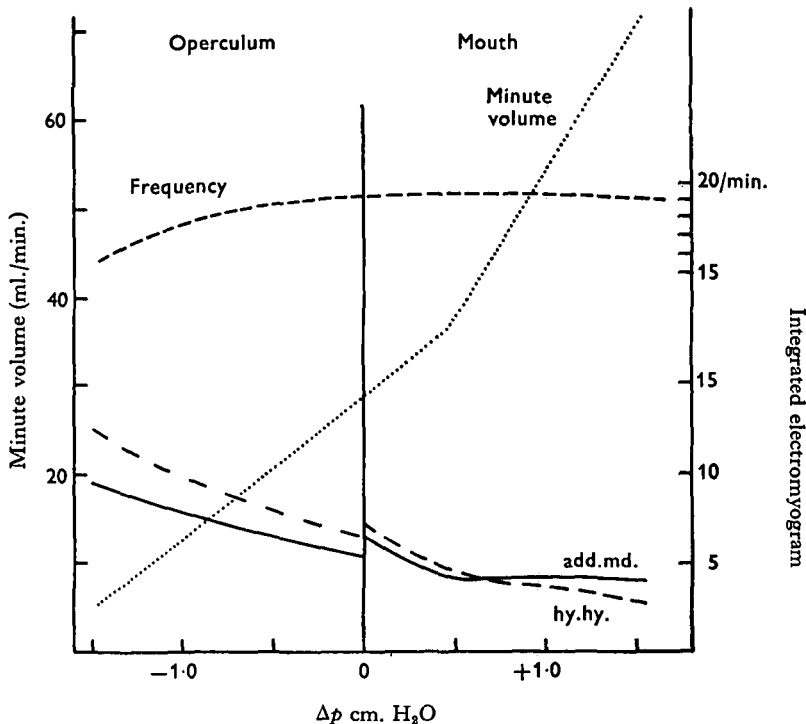

Fig. 6. Effect of changing the Δp on the minute volume, respiratory frequency and level of integrated electromyogram of the adductor mandibulae and hyohyoideus. Notice that in this preparation the gradient of the minute volume curve changes at a positive gradient of $+0.5$ cm. H_2O; in most preparations the inflection occurs nearer the zero Δp.

The frequency of respiration does not generally show any marked effect with changes in Δp, and consequently the curves for minute volume and stroke volume are very similar but variations occur between different individuals. Only as the flow becomes lower with negative pressure gradients, is there a fall in respiratory frequency (Fig. 6) which is almost certainly due to oxygen lack because a similar response occurs when the P_{O_2} breathed by the fish is reduced. The fact that the over-all frequency does not change during the Δp experiments is of significance because it suggests that changes in flow with different pressure gradients are related to changes in the gill resistance. There are, in fact, three series resistances involved, the mouth opening, the gills and thirdly the exit from the opercular cavities. Any changes in these resistances

will affect the flow at a given Δp. As the over-all frequency does not change, it tends to suggest that the respiratory movements are continuing more or less normally but it is still possible that the relative duration of the phase when the mouth and opercular slits are open may in fact alter at different Δp's. If this is true, then the constancy of frequency is not quite so significant. Observations on the duration of the burst from individual muscles suggests that the duration of the opening phases is more or less constant. Another possibility is that there is no change in frequency or change in any of the three resistances but that there is a higher intensity of activity of the muscles in a given phase. In fact this relationship has been observed with negative Δp's. Clearly these are important factors which will also affect the volume of water flowing through the system as a result of the imposed pressure gradient, and must be analysed before a more detailed interpretation of the Δp/volume curves can be given.

In the response to changes in Δp of the integrated electromyograms and consequently of the ventilation volume, there is often a more or less marked hysteresis. Such a hysteresis was also observed in the volume curves recorded by Hughes & Umezawa (1968). In all these cases it has been found that when Δp changes are made in a positive direction, the curve is higher than that obtained when the changes are in a negative direction. These differences have been taken into account in the plots shown in Fig. 6.

The effect of changing the Δp upon the intensity of the integrated myogram varies according to the state of the animal. Clearly where the fish is in very poor condition, increasing positive gradients will serve to flush water through the system and effectively it is being given artificial respiration which, as is to be expected, results in an increase in electrical activity with increasing flows. Since the fish cannot cope with negative gradients, the activity falls and may even cease altogether because of exhaustion. However, with fish in good condition there is relatively little change in the myogram with positive gradients, and it only increases for negative Δp's, thus indicating an active compensation which tends to maintain a good flow of water across the system. Where the respiratory reserve is less good there is still no influence of the Δp's upon the electromyogram at fairly positive gradients, but for less positive and negative Δp's there is a significant increase in the electromyogram with diminishing Δp. Where the condition of the animal is rather worse than this, the integrated EMG increases all over the range of diminishing Δp from positive to negative. The ventilation volume of course follows the change in muscular effort in the part of the graphs where the lowering of Δp is not compensated for by an increasing electromyogram, i.e. with higher Δp's. The ventilation volume decreases linearly with decreasing Δp because the passive component caused by the pressure head gradually diminishes. As mentioned earlier, in the region where the increasing EMG compensates for the decreasing Δp, the ventilation volume diminishes at a slower rate but it never seemed possible for the electromyograms to be able to compensate completely for the effect of the lower Δp upon the volume flow, i.e. the curve never becomes horizontal.

In Fig. 6 the two gradients of the volume curve are 30 and 17 ml./min./cm. H_2O. The steeper gradient is very typical of these preparations but in this particular fish the less steep gradient is not flattened out as much as in some other cases, presumably because it was not in perfect condition. This is also supported by the fact that the point of inflexion is not at zero Δp, but it is significant that the point of inflexion corresponds

to the flow at which the adductor mandibulae and hyohyoideus show their most marked increase in the integrated myogram. On the negative side of zero Δp the hyohyoideus seems to increase in activity more rapidly than the adductor mandibulae and is a general feature of many preparations. This may be because the adductor mandibulae is.more closely associated with the buccal pump, whereas the hyohyoideus
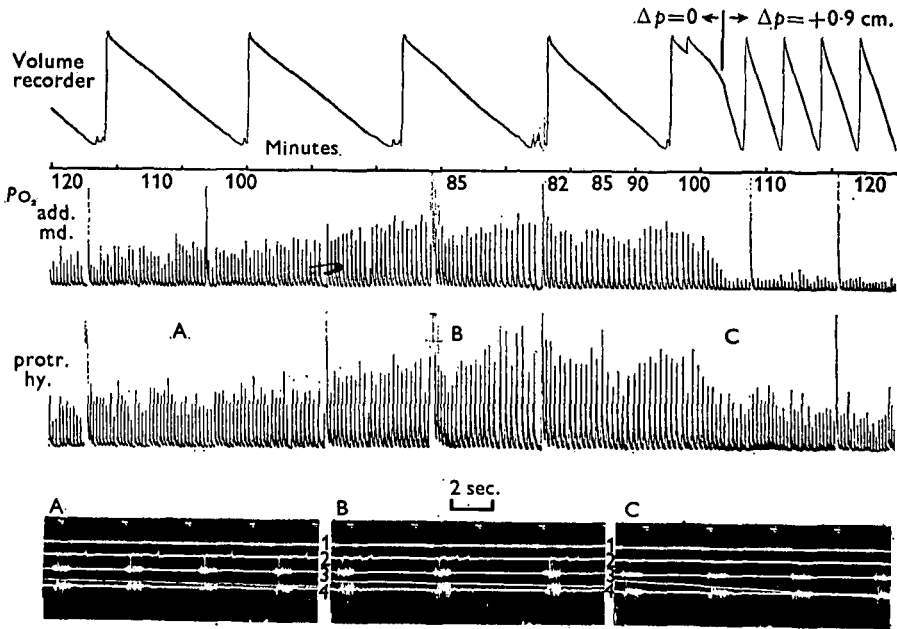

Fig. 7. Effect of low oxygen and change in Δp on respiration. The recordings are as follows: upper trace, volume recorder; time scale in minutes; P_{O_2} readings of inspired water; integrated electromyograms of the adductor mandibulae and protractor hyoideus. Sample records of the EMG's filmed directly from an oscilloscope are shown underneath; the letters corresponding with those between the integrated EMG traces. In these records the upper beam (1) is of the levator hyomandibulae, the middle two (2 and 3) from the adductor mandibulae, the lower beam (4) is the protractor hyoideus. The volume record is also present. .

	A	B	C
Minute volume (ml./min.)	10·9	11·5	35·8
Stroke volume (ml.)	0·61	0·82	2·1
Frequency (/min.)	18·0	14·0	17·0
Average height of EMG:			
add. md.	13·0	22·3	4·1
protr. hy.	19·1	35·0	18·0

Note the increase in height and decrease in frequency of the EMG during lowered oxygen. When Δp is increased the frequency rises to almost normal. The amplitude of the EMG of the adductor decreases to a very low level, that of the protractor hyoideus to a much lesser extent.

mainly changes the volume of the opercular cavities. The pressure change in the latter needs to be particularly forceful if the opercular valves are to open against the imposed gradient. In Fig. 8 the same data are plotted out and the lower two curves show the relationship between the integrated myograms and stroke volume under the influence of changing Δp. The other two show the influence of P_{O_2} changes upon the relationship of stroke volume and integrated EMG. It is clear that there is a very marked difference between the integrated myogram resulting from P_{O_2} changes and that from the Δp

change which in part may be explained by the fact that with lowering Δp the animal is not able to compensate fully for the passive decrease in water flow, hence the decrease in stroke volume with increasing EMG activity. With P_{O_2} changes, however, there is no such passive flow component and an increased muscular effort results in increased stroke volume. In both instances the hyohyoideus response seems to be greater than that of the adductor mandibulae.
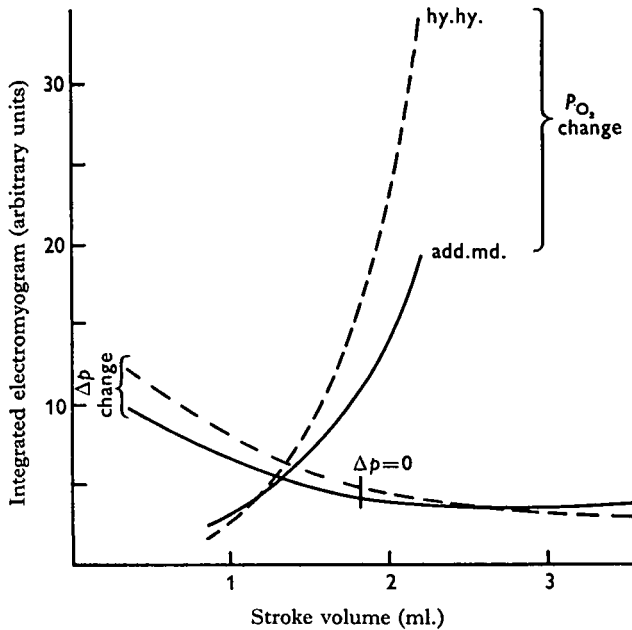

Fig. 8. Plot of the integrated electromyogram (height in arbitrary units) of the hyohyoideus and adductor mandibulae, at different stroke volumes which result from either a change in the P_{O_2} of the inspired water or by alterations in the static pressure gradient (Δp) across the system.

When the animal is in weak condition, breathing may stop altogether during low Δp's, but may be resumed again at more positive Δp's. Under these conditions the minute volume is the same despite the Δp being greater than before stoppage of breathing and in spite of an increased EMG. This strongly suggests an increase in gill resistance. Although there are not enough data available for the exact evaluation of the part the different muscles play, there is a strong suggestion that there may be differences in their reaction under certain circumstances.

(b) *Changes in ventilation volume resulting from changes in the O_2 and CO_2 content of the water.*

(i) *Changes in oxygen concentration and the intensity of the electromyogram* (Figs. 7, 10). The decrease in the oxygen content of the water is followed by an increased pumping effort in *Callionymus*; the significant effect is the increase in stroke volume of the pump which increases linearly with decreasing P_{O_2} of the inspired water (Fig. 9). This is not always accompanied by a corresponding increase in minute volume, however, because of changes in frequency as discussed below. This linear

increase in stroke volume is due to enhanced activity in the respiratory muscles, but the integrated myogram shows that there is more than a linear increase in recordings from the adductor mandibulae, protractor hyoideus and hyohyoideus. During recovery from the effect of low oxygen, the electromyogram returns very rapidly to the initial level although the change in frequency is much slower. There was one exception in which the stroke volume for an electromyogram of the protractor hyoideus was much lower before a period of hypoxia than the stroke volume for the same EMG
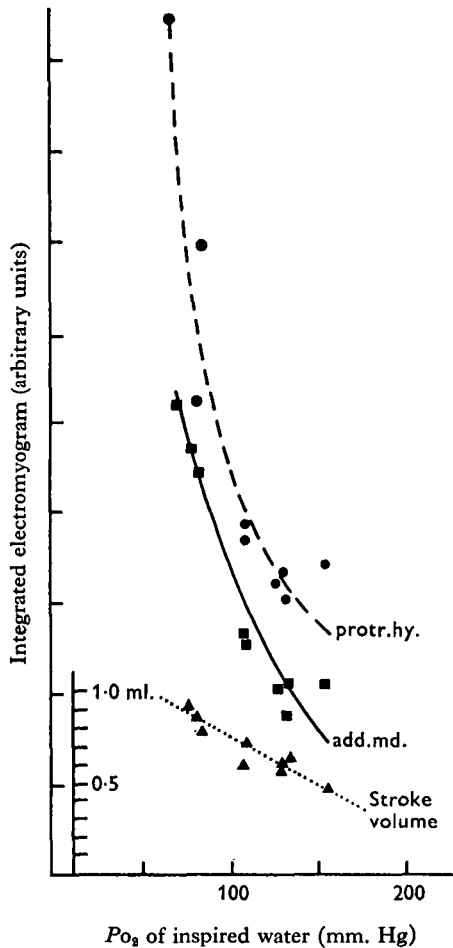

Fig. 9. The relationship between the integrated electromyogram of the protractor hyoideus and adductor mandibulae at different P_{O_2} of insp red water. The linear relationship between stroke volume measured at the same time and P_{O_2} of the inspired water is also illustrated.

after this period. There are two possible explanations of this phenomenon, first of all that the muscle was not so active before low oxygen and has now been recruited, and secondly that the resistance of the gills to the flow of water has increased as a result of the period of hypoxia.

The relationship between EMG and stroke volume is plotted in Fig. 8, where the levels of the integrated myogram can be compared with those obtained in a $\Delta p/$

volume experiment. This suggests that the change in electromyogram activity follow-
ing a reduction in the Δp of 1·5 cm. of water would normally increase the stroke volume
by approximately one-quarter of the resting level. Viewed in this light, it appears that
the muscles are not making anything near their maximal possible effort during the
Δp experiments even with the maximal adverse gradients. It would clearly be of
interest to know the state of saturation of the efferent blood during the Δp experiment
and also during a period of hypoxia.

(ii) *Influences on the respiratory frequency.* As was observed by Hughes & Umezawa
(1968), changes in the P_{O_2} of the inspired water very markedly affect the respiratory
frequency. With a lowering of the P_{O_2} there is a reduction in the respiratory frequency
(Fig. 10 & 11) and correspondingly where the oxygen level of the water is raised above
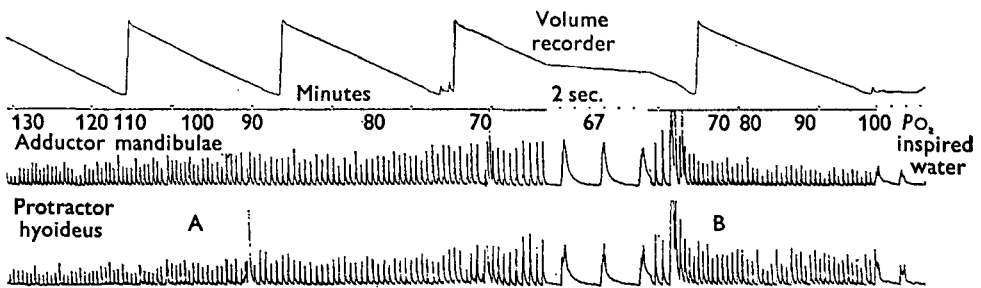

Fig. 10. Continuous recording at two different speeds to show the influence of oxygen
tension on respiratory activity. The upper trace shows the water flow which at time A was
10·6 ml./min. and at time B, 10·1 ml./min. The partial pressure of oxygen in the inspired
water is shown by readings taken at the time indicated. The two lower tracings are of the
integrated EMG from the adductor mandibulae and protractor hyoideus. Notice the marked
increase in activity as the P_{O_2} falls and the stroke volume rises. Following the replacement of
nitrogen by air in the exchange column there is a marked fall in the integrated EMG which is
more striking for the adductor mandibulae. During the decrease in P_{O_2} the amplitude of the
integrated EMG increases and the frequency decreases. During the increase the amplitude soon
gets back to normal, the frequency at a lower rate.

normal the respiratory frequency also increases. The relationship between electromyo-
gram and frequency of respiration is, however, more complicated than might be
expected. The reason being that when the P_{O_2} is lowered, both the EMG and the
frequency of respiration immediately respond, the EMG increasing and the respiratory
frequency decreasing. When the oxygen pressure is restored, however, the electro-
myogram nearly always reacts immediately and falls to its original intensity, but the
respiratory frequency remains low for some time after hypoxia before rising once
more to the normal value. This effect is shown in Fig. 11 where the intensity of the
integrated electromyogram immediately following a period of low P_{O_2} is indicated and
is clearly at the same average level as is found in normal aerated water. In other pre-
parations the situation is more complicated because following a stage corresponding
to that illustrated in Fig. 11 when the respiratory frequency has returned once more to
its original level, there is, however, an *increase* in the EMG. As has been suggested
earlier, this might be due to the change in resistance of the gills resulting from the
hypoxia. There may well be some modification which increases the contact between
the water flow and gill surfaces that would raise the resistance to water flow.

(iii) *The influence of high CO_2 on respiration* (Fig. 12). The effect of increasing the P_{CO_2} of the water breathed by the fish are very similar to those obtained by lowering the P_{O_2}. It should be noted, however, that in experiments with extremely high P_{CO_2}'s, the P_{O_2} will consequently be lowered and some of the effects observed may be due to this factor in addition to the P_{CO_2} effect. Simultaneous monitoring of the P_{O_2} of the inspired water showed that in experiments where this remained almost unchanged there was nevertheless a very significant effect of the high CO_2 on the electromyograms. When the fish is made to breathe water containing high CO_2 concentrations,
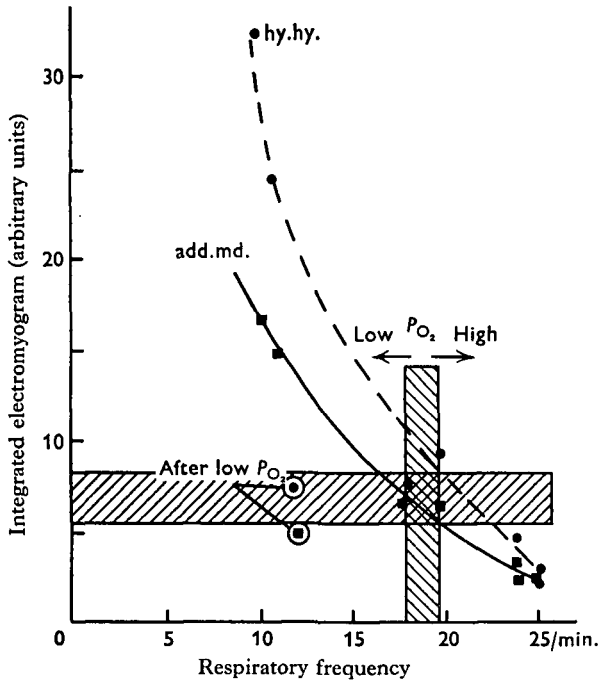

Fig. 11. Relationship between integrated electromyograms of the hyohyoideus and adductor mandibulae and respiratory frequency during a change of P_{O_2} below and above the normal level. When the P_{O_2} is restored to its normal level following a period of breathing water containing a low concentration of oxygen, the electromyogram drops almost immediately, although the frequency continues at a low rate, as shown by the two circled points.

this is followed by a marked increase in muscular effort which can be observed as an increase in the integrated electromyogram (Fig. 13). Contrary to the similar effects obtained by lowering the P_{O_2}, high CO_2 gives rise to an increase in stroke volume which is *proportional* to the increase in electromyogram (this may also be associated with some change in the gill resistance which increases less than when the fish is subjected to a low P_{O_2}). This more or less direct proportionality is well illustrated in Fig. 13 where two sets of data are included from experiments with the same fish at different times. It is of interest that the two curves for each muscle are parallel. This illustrates the difficulty of making any direct measure of stroke volume based entirely upon the integrated myogram, because there seem to be some shifts in base-line during the course of an experiment. Their precise cause is difficult to know and may largely reflect differences in the electrical conditions for picking-up the potentials.
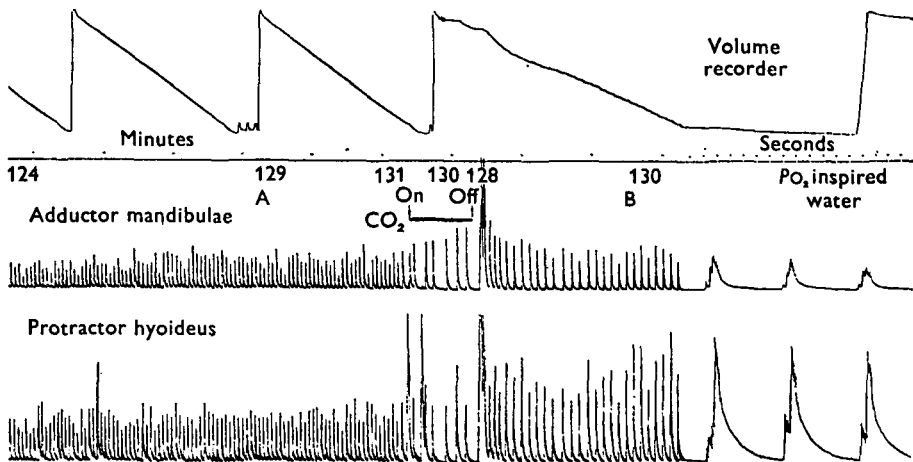
Fig. 12. Continuous pen recording to show the influence of high CO_2 on respiration. Channels are as follows: volume recorder, time marker, Po_2 of inspired water, integrated EMG's of the adductor mandibulae and protractor hyoideus. Note that the Po_2 during high CO_2 (bubbling air containing 0·57 % CO_2) remained almost unchanged. The amplitude of the integrated EMG's increased during high CO_2 and the frequency was considerably reduced.

	A	B
Minute volume (ml./min.)	9·0	5·8
Stroke volume (ml.)	0·63	0·64
Frequency (/min.)	16·0	9·0
Average height of integrated EMG		
add. mand.	10·3	13·6
protr. hy.	16·0	31·5

The frequency of respiration is lowered by high concentrations of CO_2 as illustrated in Figs. 12 and 14. This plot was obtained from measurements made during a period of high CO_2 followed by return to the normal level. It shows very clearly how there is a reduction in frequency during the period of increased CO_2 which is associated with an increase in the EMG, but when the CO_2 is returned to normal levels there is a sudden fall in the EMG whereas the return to the normal frequency of respiration is a much slower process, as has already been described for changes in P_{O_2} Correlated with the change in EMG there is an immediate fall in the stroke volume following a period of high CO_2. The frequency of ventilation remains low for some time, however, and as a result of this combination the minute volume remains lower than normal for quite a long time and even drops further. There are, then, some differences between the responses to low O_2 and high CO_2.

DISCUSSION

Although *Callionymus* is a specialized fish from many points of view, and its particular features have proved invaluable in the present type of work, nevertheless the pattern of some of its muscle activity is very similar to that recorded in the trout (Ballintijn & Hughes, 1965). This is particularly true, for example, of the muscles which reduce the volume of the respiratory cavities. However, the changes which occur during

alterations in gas content of the inspired water are not typical of all fishes and need to be investigated further in other species. It is clear that, as was the original aim, a general relationship can be established between the electromyograms and the amount of pumping being done by the fish. It is not, however, always a linear one but more often the integrated myogram increases more rapidly than in direct proportion to the increase in stroke volume. It appears from the literature on integrated electromyography (Inman *et al.* 1952; Bergstrom, 1959; Person & Kushnarev, 1963; Bigland &
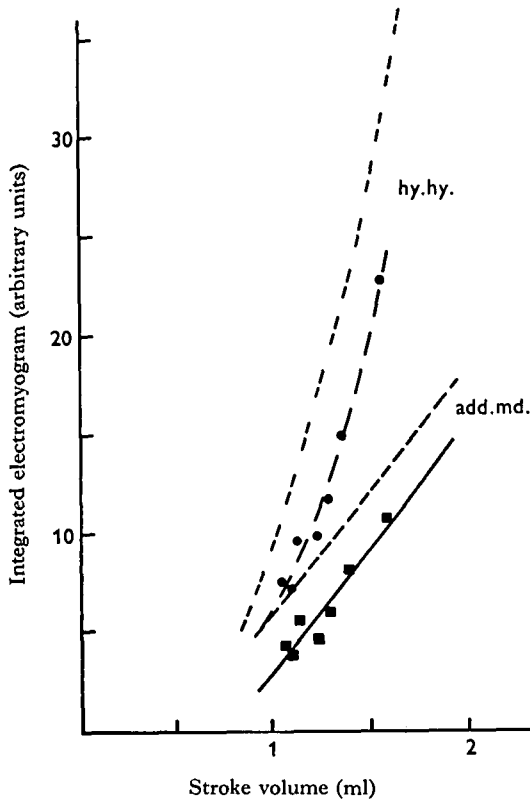

Fig. 13. Relationship between the integrated electromyogram of the hyohyoideus and the adductor mandibulae muscles during a period when the inspired water contained a high concentration of CO_2 (0·57 % CO_2). The relationship between stroke volume and integrated electromyogram some time later in the same preparation is indicated by the dashed lines.

Lippold, 1954) that the relation between the integrated EMG and power in a contracting muscle is not a simple linear one except in certain circumstances. In the present paper the integrated EMG is used as a qualitative measure of muscular power which seems permissible as the same part of a given muscle's movement is compared under different conditions. Thus during changes in the depth of ventilation the respiratory muscles seem both to contract further and to be extended to a greater extent and hence the middle part is common at all depths.

The method of integration used here is particularly suited to this situation because it reduces the influence of the early parts of a burst of electrical activity and by measur-

ing the peak value the last part of a burst is neglected. In addition to having demonstrated a correlation between electrical activity in the muscles and the volume pumped across the gills by the fish, these investigations have provided interesting information concerning the sensory systems involved. Thus, the changes which occur when Δp is altered do not seem to be sensed by the same mechanisms as those which detect the P_{O_2} or P_{CO_2} because the former influences the EMG, whereas the changes in gas
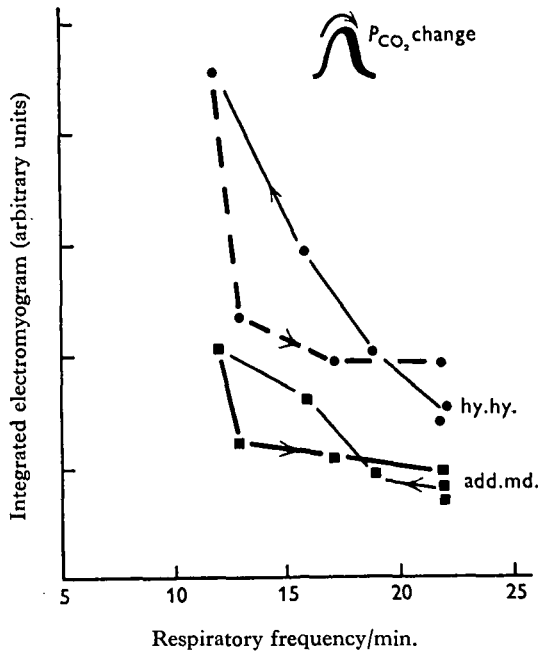

Fig. 14. Relationship between respiratory frequency and integrated electromyograms of the hyohyoideus and the adductor mandibulae muscles. The plots show how, following the return of the P_{CO_2} to normal level, there is a sudden fall in electromyogram activity at the same respiratory frequency, the latter gradually returning to its normal level.

tensions also affect the frequency of respiration. In some individuals, although the respiratory frequency is fairly constant over the whole range of Δp's, sometimes a slight decrease is present about the zero position (Hughes & Knights, 1968).

The influence of decreasing P_{O_2} of the respired medium and of increasing the concentration of CO_2 seems to work upon the same mechanism because their effects are very similar. Thus low O_2 and high CO_2 produce a decrease in frequency and an increase in electromyograms. Further analysis of the recordings suggests, however, that there is some difference because in low oxygen the stroke volume increases less than linearly with the electromyogram, whereas for high CO_2 the relationship between stroke volume and integrated electromyogram is more nearly linear. The difference may well be caused by differences in the effect on gill resistance which might be expected under the two conditions. With lowering of the P_{O_2} a tighter sieve with less dead space (Hughes, 1966) might be expected, whereas an increase in CO_2 concentration might be expected to encourage expansion of the sieve and facilitate the washing-out of CO_2. It is, of course, possible that a part of the response to increased CO_2 is in

fact a response of the respiratory centre to a lowered P_{O_2} brought about as a result of the Bohr and Root effects. When the P_{O_2} is reduced, any increase in gill resistance will require additional muscular effort for the same stroke volume.

There are, however, sufficient similarities between the responses to lowered oxygen and increased carbon dioxide to suggest similar receptor sites. This is particularly true in relation to differences in the time course of the recovery of the respiratory frequency and amplitude of the integrated EMG and indicates the involvement of different receptor mechanisms for these two responses. It may be suggested that the slower recovery of the frequency is governed by changes in the blood gas composition affecting receptors in the brain, whereas the faster responses of changes in EMG are detected by receptors of a more peripheral nature which are probably located on the gills. It may well be that these receptors are the same as those involved in the bradycardia which results from a lowering of the inspired P_{O_2} in this fish (Hughes & Umezawa, 1968).

SUMMARY

1. An account is given of the main skeletal elements and muscles involved in the respiratory movements of the dragonet, *Callionymus lyra*.

2. Using electromyographic techniques it has been shown that the muscles chiefly involved in rapid ejection of water out of the opercular slit are the adductor mandibulae, protractor hyoideus, and hyohyoideus. During the expansion phase of the cycle, which is about six times the duration of the contraction phase, the levator hyomandibulae and sternohyoideus are active, though in some cases the latter only comes in at higher levels of pumping.

3. Changes in volume flow across the gills have been produced by either (*a*) altering the hydrostatic pressure gradient (Δp) across the system, or (*b*) altering the oxygen or carbon dioxide content of the water inspired by the fish. With (*a*), the volume flow decreases linearly at a rate of about 30 ml./min./cm. H_2O static pressure head until an inflexion is reached in the curve at which rate of flow decreases and is normally when Δp is zero. That the relative increase in flow rate with negative Δp's is due to the activity of the fish pumping against the adverse pressure gradient has been confirmed by electromyogram recordings during such experiments. With (*b*), it was possible to demonstrate a clear relationship between stroke volume and the level of electrical activity as measured by the height of the integrated electromyogram. The integrated EMG increases more than linearly with increasing stroke volume during P_{O_2} changes, but this relationship seems to be more nearly linear during changes in CO_2 concentration.

4. The respiratory frequency is scarcely affected by changes in flow produced by altering the hydrostatic pressure gradient, but following a decrease in P_{O_2} or an increase in CO_2 there is a significant fall in frequency which accompanies the increased electromyogram. The time course of these changes during recovery from a decrease in P_{O_2} or an increase in P_{CO_2} suggests that the gas tensions of the inspired water are detected by receptors on the gills and thus influence the electromyogram activity, but the frequency change observed is due to a change in the blood affecting receptors in the brain.

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