AN INVESTIGATION OF THE PHYSIOLOGICAL ADAPTATIONS FITTING CHIROCEPHALUS DIAPHANUS PRÉVOST FOR LIFE IN TEMPORARY PONDS

A THESIS

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N.B. Two uncommon abbreviations are used in the text.

Pc. is an abbreviation of critical oxygen concentration.

A.P.B.R. is an abbreviation of frequency of appendage movements.

Both terms are described and defined in the text before they are used in the abbreviated form.

The relevant figures are inserted at the end of each section.

Most results obtained from the closed chamber respirometer are plotted as curves from one experiment, the general properties of the curve being supported by the results of similar experiments. The number of experiments carried out has in each case been inserted at the foot of the relevant figure.

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INTRODUCTION.

Chirocephalus diaphanus Prévost is a member of the crustacean order Anostraca. All members of this order, with the exception of *Artemia salina* the brine-shrimp, are limited to temporary freshwater habitats. It was the known occurrence of *C. diaphanus* in temporary ponds in the New Forest, Hampshire which led to a study of its relationship with its environment.

Much of the previous ecological work on the freshwater Anostraca was purely observational, consisting of records of the occurrence of the different species, e.g. Moore, 1951 and 1955(a), Dexter, 1953. Experimental work following upon these observations was concentrated on the factors controlling egg hatching, e.g. Mathias, 1926, Hall, 1951 and 1961 and Nourisson, 1960; though Moore, 1955(b) did some work on the upper lethal temperature limits of *Streptocephalus seali*. Cannon, 1928 described the feeding mechanism of *C. diaphanus* and in some recent work on bile pigments Gilchrist, 1962 investigated haem synthesis in *C. diaphanus*.

The brine-shrimp, *Artemia* has received relatively more attention than the freshwater Anostraca, work having been done on its osmotic regulation (Croghan, 1958), response to sudden temperature change (Grainger, 1956) and the function of its haemoglobin (Gilchrist, 1954) ; plus numerous papers on its genetics and development.

The present investigation was designed primarily to test the effects of environmental conditions and changes in these conditions on the metabolism of the animals living in temporary ponds. In order to reach some general conclusions on the adaptations necessary for life in this habitat most experiments carried out on <u>C. diaphanus</u> were repeated on the larvae of <u>Chironomus riparius</u> Meigen, which were found in large numbers in the bottom fauna of the New Forest ponds. A summary of observations on the conditions in the temporary ponds of the New Forest forms the first section of the thesis. By simulating these conditions experimentally and measuring the~~ir~~ oxygen consumption of the two species it was intended that any common adaptations they ~~two species~~ may possess for life in temporary ponds would be detected.

From the observations in the New Forest ponds it appeared that the two species live in separate niches within their habitat, experiencing often quite different sets of environmental conditions. The experimental results were, therefore, examined to detect any adaptations the animals may possess which fitted them specifically for life in their immediate surroundings.

A very relevant criticism of measurements of metabolic activity, of any kind, on whole organisms is that their reactions are too complex for simple analysis. This is because any changes in ambient conditions will not only result in metabolic adjustment but will produce a behavioural response in the healthy animal. In a study which was initially concerned with the relationships between animals and their environment the possible disadvantages of a complex response to environmental change are outweighed by the importance of measurements on whole animals. If the animals were allowed sufficient time to become acclimated to each set of experimental conditions and these conditions were carefully controlled and monitored, it was possible to obtain repeatable measurements of their oxygen uptake. Whole and comparatively normal individual animals were, therefore, used for most of this study.

The investigation was extended by designing experiments which not only tested the effects of environmental factors but also gave information on the extrinsic, environmental and intrinsic, physiological factors determining the relative respiratory dependence of both species (this term is defined in a later section) . This was done in two ways. Firstly the ambient conditions were varied experimentally and the animal's rate of oxygen consumption in response to the changing conditions was measured. Secondly the animal's physiological state was changed experimentally so as to expose the separate intrinsic factors controlling its response to low oxygen concentrations. This was achieved by changes in external physical conditions of water movement, light intensity and temperature and by chemical poisoning with carbon dioxide, carbon monoxide and drugs.

The work on C. diaphanus was further extended by a study of the functioning of its appendage movements in swimming and ventilation. An addition to the thesis, included in a separate section at the end, is a note on the discovery in one of the New Forest ponds, of the uncommon crustacean species Triops cancriformis Breb.

THE ENVIRONMENTAL CONDITIONS AND CHARACTERISTIC FAUNA IN TEMPORARY PONDS.

INTRODUCTION.

All members of the crustacean order Anostraca, with the exception of *Artemia salina* which is found in brine-pools, are limited to temporary freshwater habitats (Moore 1951 , 1955(a) ; Dexter, 1953 ; Hall 1961) . *Chirocephalus diaphanus* Prevost, occurs sporadically in temporary ponds in the New Forest, Hampshire (Hall, 1953 , 1961) . The present study started as an experimental investigation into the physiological adaptations of *C. diaphanus* for life in temporary habitats. Some knowledge of the physico-chemical conditions in the ponds was required, in order that the animals' reactions to these conditions could be tested experimentally. This section deals with the few environmental measurements made, and considers the characteristic fauna found associated with *C. diaphanus*, in the temporary ponds.

FIELD OBSERVATIONS.

Great Britain, because of its temperate climate, is poorly supplied with temporary ponds compared to the countries which have hotter and more variable climates. The occurrence of several species of Anostraca has been studied by workers in North America (Moore, 1959 Dexter, 1953) . Moore, 1955(a) recorded seven successive occurrences of Streptocephalus seali Ryder, separated by periods of drying of the pool, during a one year period, in Louisiana. He described (Moore, 1951) two general types of habitat for S. seali: "road-side ditches ..... less than twelve inches deep, of small area, devoid of vegetation and with very turbid water" ; also "piney-woods pools ..... up to three feet deep, of considerable area, algae or other vegetation present and highly coloured water." Hall, 1953 described the conditions in two ponds in the New Forest, from which he collected C. diaphanus. Both were roadside ponds approximately 20 yards across, one was muddy the other contained clear water over a grassy depression. He later (Hall, 1961) described a third habitat as a shallow depression at the side of a gravel road, frequently trampled by cattle, and containing opaque, sometimes thickly muddy water. A superficial simularity between the habitats described by Moore and Hall is apparent.

The animals used in the present study were collected from two other localities in the New Forest. Both were searched on the advice of Mr. R. E. Hall. One was a roadside pond near Ringwood (Plate 1), from which *C. diaphanus* was first collected in the Spring of 1962. That Summer the water level decreased and the population of *C. diaphanus* disappeared, newts and some large dytiscid larvae being observed in the water. The pond dried out completely in August and after some heavy rainfall mature male and female *C. diaphanus* were collected on the 21st September, 1962. On this occasion the water was approximately two feet deep near the centre of the pond and was clear but highly coloured. The bottom was of soft, black mud and contained fairly dense patches of submerged and emergent aquatic plants. This single hatching of *C. diaphanus* was observed again in October and finally in December, when a few males and females were collected from under a thin covering of ice. They appeared to be larger in size but fewer in number than the September collection. This pond was filled with water throughout the Winter of 1962 - 3 and was frozen to a depth of 11 inches for over 3 weeks in January and

February 1963. It became permanent from then on, and the C. diaphanus population never reappeared. A net sample, taken on the 14th November, 1963, showed that a typical permanent pond fauna was established, including many predatory forms such as dytiscids, gyrinids and notonectids. This pond was eventually filled in, during a road widening scheme, early in 1964.

The other locality was a roadside pond near Fordingbridge, Hampshire (Plate 2) . It consisted of a very shallow grassy depression approximately 40 yards long and 20 yards wide. Hall collected C. diaphanus from this pond approximately 5 years previous to the present observations.

The pond was non-existent through the Summer of 1962, consisting of a grassy depression with a small patch of marsh grass in the centre. The first collection from this site was made on the 2nd April 1963. The water was very clear and shallow over a substratum of turf and mud. A small number of immature, half grown C. diaphanus were collected, plus some Daphnia sp., Cyclops sp., ostracods, very small dytiscid and ephemerid larvae, tadpoles and large amounts of frog spawn. The water contained patches of submerged aquatic plants and filamentous algae.

On the 5th June, 1963 the pond had shrunk to a very shallow pool approximately 8 feet across. The water was deep brown in colour and the bottom was soft, black mud. The only animal observed was a single dytiscid beetle.

On the 14th November, 1963 the pond had refilled. It contained thick patches of submerged vegetation and filamentous algae. Four large, mature, female Chirocephalus were collected, plus many large predatory notonectid bugs and dytiscid beetle larvae. This fauna probably represented the remnants of a C. diaphanus population, depleted by predators which had invaded the pond after it had been full for some time.

The pond contained water throughout the Winter of 1963 - 4, and was sampled again on the 5th February, 1964. It contained quite a large volume of water but very sparse animal and plant populations. No C. diaphanus were found. On the 8th March, 1964 the pond had shrunk to a small, muddy patch of water at one side of the depression. It contained several gravid frogs and masses of spawn. Net samples included Daphnia sp., and ephemerid larvae; numerous chironomid larvae and tubificid worms were present in the mud.

On 12th April, 1964 the pond had refilled to a high level. Net samples revealed large numbers of

C. diaphanus , both mature and immature, and many other species: including Cladocera, Ostracoda, *Planorbis* sp. and small corixid, ephemerid and dytiscid larvae. This represented a population only recently established, in the period after the 8th March, during which time the pond probably dried out completely and was then refilled. The pond was permanently filled with water for many weeks after this observation. On the 18th May, 1964 it was found to have shrunk to approximately two-thirds of its maximum area and had a soft, muddy bottom and highly coloured water. Some large, mature *C. diaphanus* were observed, also some large dytiscid beetle larvae. Predatory species were, therefore, beginning to become established. In a net sample on the 12th June, 1964 only 7 mature *C. diaphanus* were caught. They were all bright red in colour indicating the presence of a high concentration of haemoglobin in their blood. Also in the net collection were: many corixid bugs, dytiscid beetle larvae of two species, a dragon-fly nymph, numerous ostracods, *Planorbis*, and two species of chironomid larvae. The *C. diaphanus* population had apparently been extensively depleted by the invasion of predatory species, after the pond had become semi-permanent.

On the 3rd September 1964, after a prolonged dry spell, the site was visited again. It consisted of a grassy depression without water or visible signs of a recent pond; the earth was hard and cracking with dryness.

Near the completion of this study a collection of mud from this pond yielded a single hatching of Triops cancriformis (Breb) about which an appendix is included at the back of this thesis (see pages 190 - 199). It was later discovered that this was the same locality as that described by Fox, 1949. This connection allows earlier observations, recorded by Fox, on the occurrence of C. diaphanus in this locality, to be described. Hobson and Omer - Cooper, 1935 mentioned that C. diaphanus was known to occur there in 1934 and Fox records a collection of 'abundant' C. diaphanus from the same locality in February 1948. It is apparent from these earlier records that there is a permanent settlement of C. diaphanus in this locality in contrast to its somewhat sporadic occurrence in other New Forest ponds (Hall, 1953). This may be explained by the more truly temporary nature of this pond, which was less deep and more often dry than all other New Forest ponds observed by the author.

These random field observations indicate that there is a cycle of events in these temporary ponds which determine the appearance and survival of C. diaphanus. This cycle is summarised in FIG. 1.

The three basic problems facing animals such as C. diaphanus, which are characteristic of temporary ponds, are:

(a) DESICCATION.

the pond may be dry for prolonged periods during the Summer months.

(b) STAGNATION.

when the pond contains only a small volume of water there may be extensive long and short-term fluctuations in temperature and the concentration of the respiratory gases.

(c) PREDATION.

should the pond become semi-permanent i.e. filled for more than a few weeks, it is colonised by predatory animals which feed on the defenceless population associated with C. diaphanus.

DESICCATION.

Many of the inhabitants of temporary ponds are only able to survive in this habitat by virtue of a drought resistant stage (see Table 3) . The Anostraca produce eggs which are able to resist prolonged desiccation (for a review of the observations and work on this phenomenon see Moore, 1957) . These eggs remain in the dried mud at the site of a pond and give rise to a new generation of animals as soon as it is refilled.

Hall, 1953 detected an apparent gradual development in the eggs of C. diaphanus during prolonged desiccation. This may account for the animals rapid appearance in a refilled pond after a long period of dryness. There are indications that the eggs of various species of Anostraca require a complex of conditions to promote hatching. Hall, 1959 found that the period required for development and hatching of the eggs of C. diaphanus varies with the depth of water. Nourisson, 1960 believes the marked change in ambient temperature associated with desiccation may end diapause in the eggs of C. stagnalis. In these ways the hatching of the eggs may be timed so that the animals appear when the habitat will be favourable for their growth and reproduction. Moore, 1955(a) thought it possible that the successive generations of S. seali recorded in a pond throughout a year may all have originated from a successively hatching store of eggs from the previous year.

An additional advantage of a drought resistant stage is its possible role in the dispersal of the species. Dried up in the mud a small egg can be carried to a new locality by the wind or on the feet of a semi-aquatic animal. Without this fortuitous means of dispersal each pond would support an isolated community. The relative isolation in small freshwater habitats and its effect on the fauna were discussed by Macan, 1961.

The temporary nature of their habitat will often result in the animal population having only a very short time in which to grow to maturity and reproduce. Moore, 1955(a) reported the extremely fast Winter and Summer growth rate and onset of maturity in S. seali from the flooded ditch habitats of Louisiana. Further discussion of this point is included, in an appendix at the end of the thesis, with the author's observations on the growth rate of Triops cancriformis. Also contributing to a fast rate of replacement and multiplication in the temporary pond fauna is the widespread occurrence of asexual reproduction and hermaphroditism (see Table 3). This allows all individuals in a population to produce offspring, and in the case of self-fertile or asexually reproducing forms a single surviving individual can re-establish a population. The Anostraca do not have this particular adaptation, the sexes are separate and occur in approximately equal numbers.

STAGNATION.

This section deals with the extremes of temperature and concentration of the respiratory gases occurring in temporary ponds during the periods when their animal population is rapidly multiplying whilst their volume is being decreased by evaporation. It was the effects of these environmental conditions upon *C. diaphanus* which formed the basis of the present investigation.

Moore, 1955(a) described the extreme variability of conditions in the pools inhabited by *S. seali*. The p.H. varied between 4·9 and 6·2 . Forest pools had a high free carbon dioxide tension of 17 - 20 p.p.m. (mg./l.) and a low oxygen tension of 1 - 4·5 p.p.m. (approx. 0·8 - 3·5 ml./l.) The minimum water temperature he recorded was 8°C, though he reported a collection of *S. seali* from beneath a thin covering of ice when the air temperature was -3°C . He collected animals from shallow ditches exposed to the full heat of the sun in which the temperature rose to 42°C . This is an example of exceptional temperature resistance as later experiments by Moore, 1955(b) indicated that the lethal temperature threshold for *S. seali* was at approximately $44{\cdot}5^{\circ}$C .

Moore also reported collecting *S. seali* from a beach pool in which the water was mildly brackish (315 p.p.m. chloride) . The author found that *C. diaphanus* could survive for a period of days in 10 – 12·5% S.W. (approximate salinity of 3·5°/oo) and one male was found apparently healthy after thirteen days in water which had risen by evaporation from 10% to 15% seawater (approx. 5°/oo total salinity). This salinity tolerance seems part of a general physiological resistance to unfavourable environmental conditions in the Anostraca.

The author's measurements of temperature, p.H. and oxygen concentration in the temporary ponds of the New Forest are summarised in Table 1. These random samples show that the temperature varies considerably through the year and that the oxygen concentration may fall to very low levels on occasions. This is a very limited picture of environmental conditions in the ponds, particularly as diurnal changes were not considered.

Bamforth, 1962 measured the changes in the environmental conditions of a shallow pond in Louisiana. Between sunrise and mid-afternoon of a day in May the temperature rose from 24° to 30°C, the oxygen concentration from 1 mg./l. to 9 mg./l. and the carbon dioxide concentration fell from 12 mg./l. to 2 mg./l.

A record of the temperature changes in the Ringwood pond was obtained over prolonged periods during 1963 using a 'Thermograph' temperature recorder. One probe was suspended in the water and the other placed just under the surface of the mud at the bottom of the pond. FIG. 2. shows the diurnal temperature fluctuation measured during a hot spell in June 1963. The fluctuation in the water mass is very large, changes of 12°C occurring in under 6 hours. This exceptional short-term variability is demonstrated in FIG. 3. which traces the maximum and minimum temperatures at the two positions in the pond during July, 1963. The limits of the temperature variation in the water mass during this period were 10·5°C and 28°C .

These very variable temperature conditions will affect the activity and in consequence the respiratory rate of the poikilothermic invertebrate, bacterial and plant populations, which will in turn affect the oxygen levels in the water. Light intensity is another important ecological factor which fluctuates diurnally. This affects the rate of photosynthesis of the plant population and will again result in marked fluctuations in oxygen levels in the water. This was likely to have been particularly important in the pond near Fordinbridge which contained dense clumps of submerged vegetation and filamentous algae.

The possible effects of diurnal variations in temperature and light intensity upon the concentrations of the respiratory gases in the water was studied by measurements over a single 24 hour period. An artificial pond was set up in a large polythene tank sunk into the ground outside the laboratory and allowed to fill naturally with rain water. ·A layer of mud from the pond near Fordingbridge was added, and most of the fauna and flora characteristic of the primary population in temporary ponds (see Table 2) , was introduced. This pond remained full of water and maintained an apparently typical temporary pond community for some weeks. Over a 24 hour period from the 12th to the 13th June 1964, water samples were taken in a stoppered bottle midway between the substratum and the water surface, close to a clump of vegetation. The temperature of each sample was taken, its' oxygen content was measured polarographically and its p.H. taken with a p.H. meter. The results are summarised in FIG. 4.

The first sample was taken at 3 p.m. when bright sunlight was falling directly on the patch of submerged vegetation. The water was supersaturated with oxygen. As the light faded the oxygen concentration dropped, until at 5 a.m. the water was less than 50% saturated. This oxygen depletion results from respiratory uptake by the plants and animals in the absence of the replacement by photosynthesis. The potential depletion was probably not realised during this night as a heavy rainstorm around midnight added some oxygenated water to the pond and may have masked the actual rate of depletion. Nevertheless the fluctuation in oxygen concentration was very large. An equivalent depletion under natural conditions could result in periods of anoxia during the night, especially as the oxygen concentration recorded in the ponds, was never very high, even in daylight.

The problem of survival in these stagnant conditions leads to the establishment of a fauna characterised not only by their ability to survive desiccation, but also by physiological adaptations to the inclement temperatures and gas concentrations. The detection of these latter adaptations formed the starting point for the present study.

THE FAUNA.

Moore, 1951 listed the characteristic animal communities associated with S. seali populations in Louisiana. "In late fall and early winter (September through November) the typical associated pond fauna included crawfish, midge and mosquito larvae, water boatmen and backswimmers, a few aquatic beetles and their larvae, and frog tadpoles. From January through March, with the ponds refilled and a new generation of fairy shrimp present, the associated fauna included enormous populations of copepods and cladocera, crawfish, mosquito larvae, aquatic isopods and tadpoles." With few exceptions the fauna in the New Forest ponds was found to be very similar to that described by Moore. It appeared to be made up of three waves of colonisation which naturally graded into one another.

(a) The first animals to appear hatched from the mud a very short time after the pond was refilled. This initial (emergent) fauna included C. diaphanus and several other species which shared common characteristics. They all possessed a drought resistant stage and many had a blood pigment which possibly aided their survival in poor oxygen conditions.

(b) If the pond contained water for as long as 3 weeks a secondary wave (the primary invading fauna) of colonisation was observed. This was comprised of species which invaded the pond from outside, either as mobile individuals (e.g. Limnaea truncatula) or as eggs laid by flying adults (e.g. insect larvae) . This population contained a few small predatory forms; most species were without a drought resistant stage, and a few were air breathers. C. diaphanus was by this time sexually mature and found associated with this expanded fauna, often in quite large numbers.

(c) Whenever a pond lasted for over eight weeks the population of C. diaphanus rapidly diminished in numbers. This may be partially due to the onset of senescence in the population, as suggested by Moore, 1955(a) . It is also due, however, to an increase in predatory forms making up the third wave (the secondary invading fauna) of colonisation, which consists solely of larger predators, both arthropod and vertebrate.

The fauna list covering this ecological succession in the New Forest ponds is given in Table 2. The animals and plants were classified as far as was possible using the simple keys given by Mellanby, 1951 , Ward and Whipple, 1959 and the keys published by the Freshwater Biological Association. The specific accuracy of identification, particularly of some of the arthropods, may be a little suspect.

From these observations on the conditions and fauna of the New Forest ponds it can be seen that they do not represent simple temporary habitats. The fauna specifically adapted to life in temporary ponds is the initial emergent one, consisting of species lying dormant in the mud at the site of the pond and emerging directly it fills with water. These species are characterised by a set of features all of which have been mentioned earlier, and are summarised in Table 3. (the information in this table was gleaned from numerous sources including the keys mentioned above) .

A drought resistant stage is of course a fundamental requirement for these species. The possession of asexual means of reproduction and hermaphroditism, often linked with a fast growth rate, enables the population to build up quickly in the short-time available before desiccation or predation begin to limit its growth. The presence of a respiratory pigment may enable the animals to survive periods of stagnation. This is a remarkable example of the convergent evolution of adaptations to a common habitat by a number of unrelated animals. It was noticed that with the exception of T. cancriformis, which has a phenomenally high growth rate (see appendix) , all these species are relatively small.

The second and third waves of animals are not characteristic of temporary habitats, most being cosmopolitan species, and do not possess the necessary adaptations. They are, therefore, killed when the pond does eventually dry up, or they may escape to another body of water. They continually invade the pond from outside, and their presence, because many of them are predatory forms, is often correlated with the disappearance of C. diaphanus, though many of the other initial species survive alongside this invading fauna.

PREDATION.

Lowndes, 1933 suggested that the occurrence of *C. diaphanus* in temporary ponds only is entirely a matter of animal association. Moore, 1951 pointed out: "fairy shrimp are quite defenceless and wherever the pond fauna included fishes or fairly heavy populations of such predacious invertebrates as damsel and dragon-fly, naiads, amphipods or beetles, the fairy shrimp population was either very scanty or non-existent". Dexter and Sheary, 1943 observed salamanders and leeches to prey upon fairy shrimps. Pearce, 1913 and Mathias, 1937 have listed the principle enemies of fairy shrimps to be: tadpoles, frogs, salamanders, dytiscid, hydrophyllid and gyrinid beetles, phryganeid caddis larvae and water birds. Reference to Table 2 shows that almost without exception the same species appear in the New Forest ponds when they become semi-permanent. Perhaps the most destructive predators in the New Forest ponds were the dytiscid beetle larvae, which were often numerous and large whenever a pond was more than two months old. A single larva of *Dytiscus marginalis*, accidentally included in a field collection and kept in an aquarium tank in the laboratory, killed the entire population of approximately 20 adult *C. diaphanus* within 4 days.

TEMPERATURE AND OXYGEN STRATIFICATION.

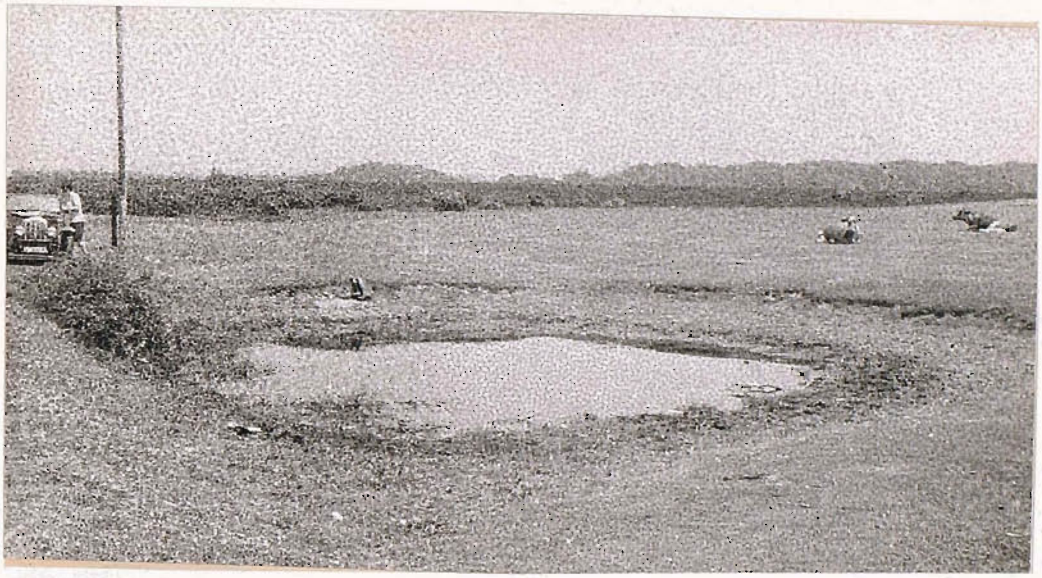
The measurements of diurnal temperature fluctuation (see FIGS. 2 & 3) indicated that the temperature range near the water surface was entirely different from that in the mud at the bottom of the pond. The high specific heat and low thermal conductivity of the water insulated the deeper parts of the pond from the wide fluctuations in air temperature.

The dense populations of bacteria and protozoa in stagnant temporary ponds give the water a measurable respiration, and the mud appeared to consume oxygen at a high rate. Most oxygen depletion is localised at the mud surface and may result in a vertical oxygen gradient through the water. The tube-building macro-invertebrate population of chironomid larvae and tubificid worms was often very dense in the mud. Edwards, 1958 described how the presence of a dense population of tube-dwelling animals may increase the total oxygen demand of organic deposits, by increasing the amount of oxidative decomposition taking place. The oxygen microstratification at the mud surface in the profundal zone of a lake, and its effects on the fauna, were described by Brundin, 1951. Brafield, 1964 measured the oxygen content of the interstitial water in sandy shores. He found exceptionally low oxygen concentrations of

0 to 8% air saturation in the sand of flat, poorly drained beaches. Jones, 1955 recorded values of 1·48 and 0·76 ml. O_2/litre in Arenicola burrows, directly after they were exposed by the tide. Cole, 1932 working in freshwater habitats with an oxygen content of 4 - 5 ml. O_2/litre in the surface layers of the water, found the oxygen concentration to be 2·36 ml./litre just above the mud surface, and between 0·13 - 0·6 ml./litre 4 inches below the mud surface. Buscemi, 1958 measured the oxygen concentration in a lake at a series of depths, through a covering of Elodea canadensis. At midday the water in the upper levels of the vegetation was supersaturated with oxygen due to photosynthesis; below the level of light penetration, however, the oxygen concentration decreased rapidly down to the mud surface where it was below 10% air saturation. The gradient is unlikely to be as steep in the temporary ponds, as the sparser vegetation would not cut down light penetration to the same extent. Similar conditions may, however, have been present in the Fordingbridge pond when the masses of filamentous algae and floating duckweed may have prevented sunlight from reaching the deeper vegetation. When the Elodea broke away from the substratum and freer vertical mixing was possible, the 24 hour cycle of oxygen values given by Buscemi was very

similar to the picture obtained from the temporary pond (see FIG. 4.) .

All this work indicates the existence of two "microgeographic habitats" within the temporary ponds, possibly with a graded physico-chemical gradient from the surface down to the substratum. Briefly, the animals living within the substratum will experience a more stable temperature regime, but potentially greater oxygen depletion, than the free-swimming species able to live near the water surface. In order that the adaptations to both sets of environmental conditions could be studied much of the work carried out on C. diaphanus has been repeated on the larvae of Chironomus riparius Meigen which were very numerous in the bottom living fauna in both ponds. These larvae had the advantage of being good experimental animals, could be collected in very large numbers, and were related to species which formed the subject of earlier work by various authors which served for comparison and eliminated unnecessary duplication.



Plate, 1. The only available print of the temporary pond near Ringwood which has now been filled in.

Plate, 2. The temporary pond near Fordingbridge.

Table, 1. Measurements of environmental conditions in the temporary ponds of the New Forest.

DATE			CONDITIONS IN POND		
			TEMPERATURE	O_2 CONC.	p.H.
19th Feb.	1963		0.5°C. (ice)	3.2 ml./l.	
2nd April	"		9.0°C.	6.0 ml./l.	6.7
7th June	"	x	23°C.	1.5 ml./l.	
11th June	"	x	30°C.	1.8 ml./l.	6.6
16th July	"		20°C.		
30th July	"		25°C.		
3rd Nov.	"		13°C.		
15th Jan.	1964		8°C.	5.6 ml./l.	
8th March	"		5°C.		
18th May	"	o	19°C.	6.5 ml./l.	7.0
12th June	"	o	30°C.	3.9 ml./l.	

These measurements were taken from the two ponds in the late morning or early afternoon, and approximate to the conditions found in the ponds at midday.

The midsummer measurements marked, x were taken from the Ringwood pond where, at high temperatures, the oxygen concentration declined and the p.H. rose. This tendency was reversed in the Fordingbridge pond (marked, o), where the richer vegetation caused an increase in the oxygen content of the water at midday and kept the p.H. high by utilising carbon dioxide in photosynthesis.

Table, 2. A list of the fauna and flora collected from the temporary ponds of the New Forest.

The fauna are listed under three headings according to the chronological order of their occurrence after the pond is flooded.

PRIMARY POPULATION (EMERGENT SPECIES).

Crustacea		
	Anostraca	*Chirocephalus diaphanus* Prevost
	Notostraca	*Triops cancriformis* (Breb.).
	Cladocera	*Daphnia* spp.
	Ostracoda	*Cypris* spp.
Gastropoda		*Planorbis spirorbis*.
Platyhelminthes		
	Rhabdocoelida (probably *Stenostomum virginianum*).	

SECONDARY POPULATION (INVADING SPECIES)

Coelenterata		*Hydra oligactis* (possibly emergent)
Platyhelminthes		
	Tricladida	*Dendrocoelium lacteum*
Annelida		*Tubifex* spp.
Crustacea		*Cyclops* spp.
Arachnida		water mites.
Mollusca		

Gastropoda	*Limnea glabra*	All molluscs including Planorbis characteristic of drying habitats and stagnant conditions.
	L. truncatula	
Lamellibranchiata	*Pisidium obtusale*	

Insecta

Diptera	chironomid larvae
	Chironomus riparius Meigen
	Eukiefferiella sp.
	culicine larvae
	Corethra sp.
Ephemeroptera	*Leptophlebia* sp.
Hemiptera	*Sigara sahlbergi* (adults and nymphs).

TERTIARY POPULATION (INVADING, PREDOMINANTLY PREDACIOUS SPECIES).

INSECTA

Odonata	*Aeshna* sp. (nymphs) *Libellula* sp.
Coleoptera	
Dytiscidae	*Dytiscus marginalis* Linn.
	Hydaticus transversalis Leach.
Gyrinidae	*Gyrinus* sp.
Hemiptera	
Notonectidae	*Plea leachi*
Gerridae	*Gerris* sp.

Trichoptera	*Limnophilus* sp.

VERTEBRATA

Amphibia	*Rana temporaria* (adults, spawn and tadpoles) *Triturus vulgaris*

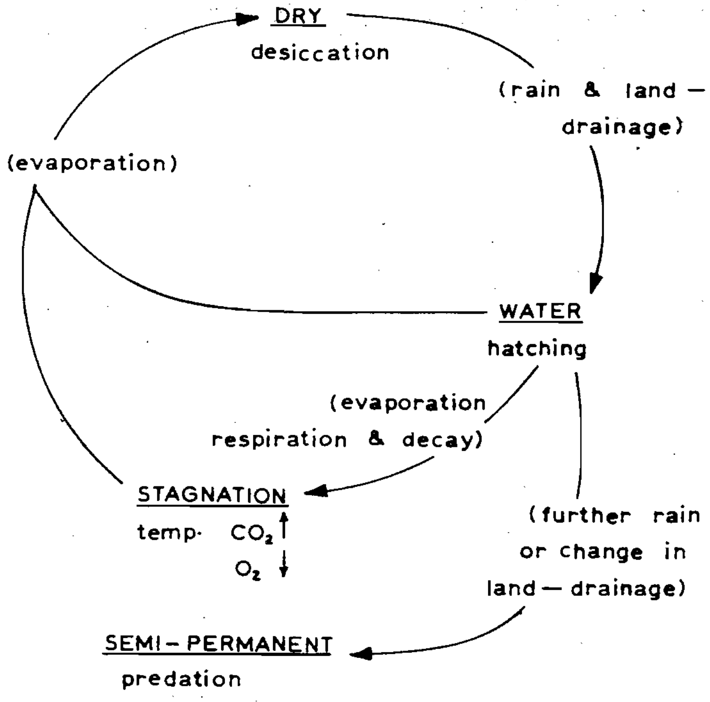
FLORA

Glyceria declinata (Breb.)	(large emergent grass).
Callitriche heterophylla	(in dense clumps).
Leptodictyum riparium	(long-stranded moss).
Lemna minor	(floating duckweed).

The Fordingbridge pond had a very rich flora, including the above species, with masses of filamentous algae interwoven with the other plants. When it was first flooded the water lay over a short turf of terrestrial grass species which lived for some time, contributing to the flora.

TABLE 3

ANIMAL	DROUGHT RESISTANT STAGE	BLOOD-PIGMENT	REPRODUCTION	CHARACTERISTIC HABITAT
Chirocephalus diaphanus	eggs	haemoglobin (synthesised in poor conditions)	separate sexes internal fertilization	limited to temporary ponds
Triops cancriformis	eggs	haemoglobin	hermaphrodite (self-fertile) rarely find males	limited to temporary ponds - very rare in this country
Daphnia sp.	ephippia (produced by sexual reproduction in bad conditions)	haemoglobin (synthesised in poor O_2 conditions)	parthenogenetic females. Males appear in poor conditions	shallow weedy pools among debris
Cypris sp.	eggs		parthenogenetic and bisexual	occur in immense numbers in weedy ponds
Stenostomum sp.	eggs		budding?	among water weed in small pools, particularly those liable to dry up in the Summer
Planorbis spirorbis	may bury itself in the mud	haemoglobin (+ air breathing)	hermaphrodite	marshes and ponds which dry up - also in lakes
Chironomus riparius (larvae)	NONE (adults mobile)	haemoglobin		still water with a deposit of sand, mud and organic debris
Sigara sp.	NONE (mobile)	NONE (air breathing)		ponds, lakes and tarns all over the country
Hydra sp.	eggs (sexual)	NONE low O_2 demand	budding + sexual	ponds, ditches & fens with covering of weed on surface



figure, 1. A summary of the cycle of environmental conditions in a small pond and the attendant problems facing the fauna.

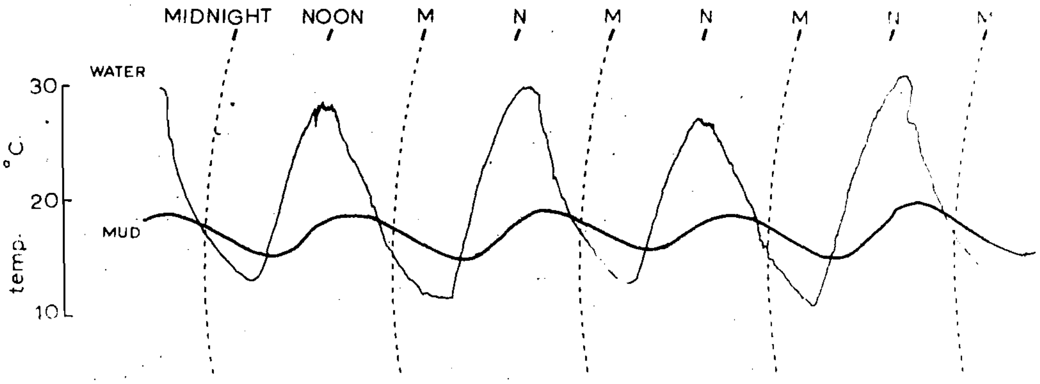


figure 2. Diurnal temperature fluctuation in the Ringwood pond during a hot spell in June 1963.

The water temperature varied between 12° and 29°C during this period, fluctuating at a rate of approximately 2° per hour. The mud temperature was relatively much more stable, varying by only 5° around a mean of 16°C.

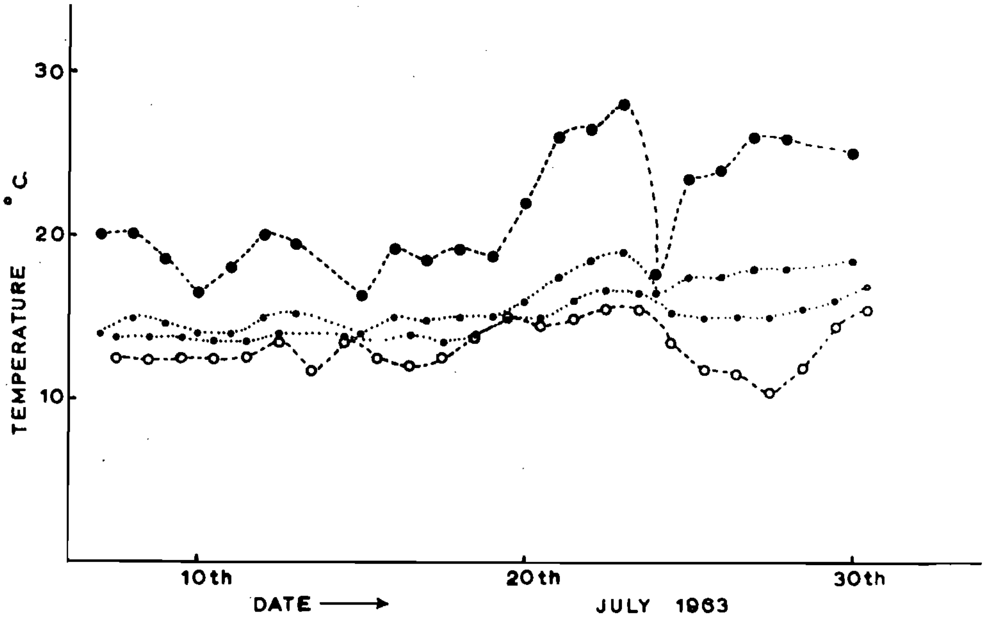


figure 3. The variations in maximum and minimum temperatures recorded in the Ringwood pond during July 1963.

● maximum temperature at each 24 hour period.

o minimum " " " " " "

The maxima and minima recorded at the surface of the substratum (small points) varied over a narrower range than at the water surface (larger points), but tended to follow the same pattern of variation, with a lag of approximately 6 hours.

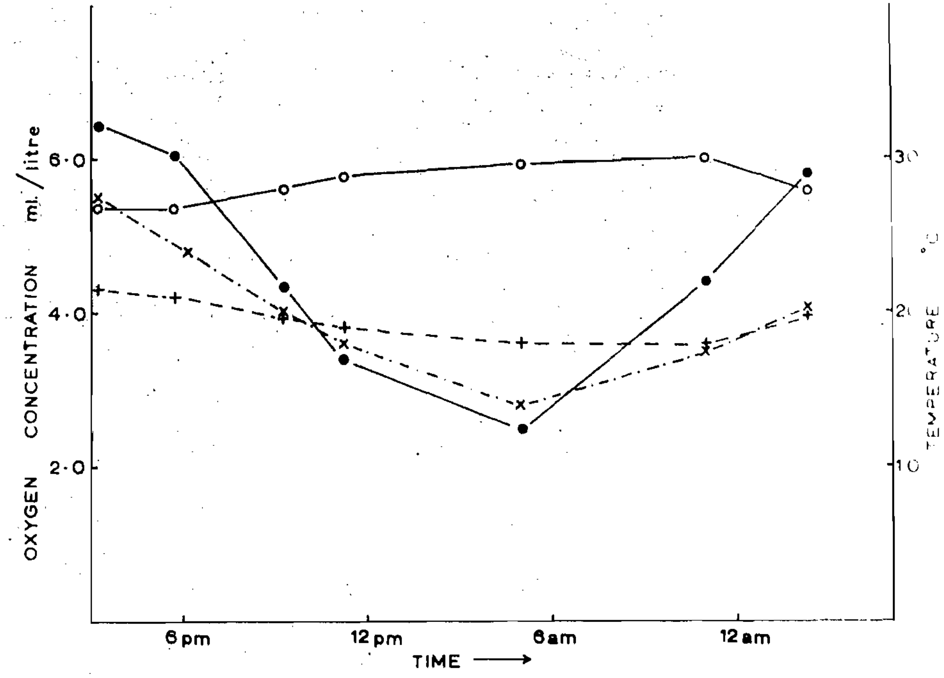


figure 4. The variation in oxygen content and temperature of an artificial pond during a single 24 hour cycle.

●, oxygen content of pond water.

o, " " " " " saturated with air.

x, air temperature; + , water temperature.

The pond water was supersaturated with oxygen in the afternoon when the sun was on the water, and less than 50% saturated in the early hours of the morning. The water temperature, taken at the bottom of the pond, did not fluctuate as much as the air temperature.

THE COMPARATIVE PHYSIOLOGY OF RESPIRATORY ADAPTATIONS IN C. DIAPHANUS AND THE LARVAE OF C. RIPARIUS.

INTRODUCTION.

This was fundamentally a comparative physiological study concerned with the functional adaptation of two species to a common habitat. Experiments were devised which measured the adaptive responses of their metabolisms to environmental stress. The most direct indication of metabolic rate is heat production which is difficult to measure on small aquatic invertebrates. Respiration rate, measured as oxygen consumption per gramme wet weight, was, therefore, chosen as the most convenient and accurate guide to metabolic activity for the purposes of the present study.

Results obtained from determinations of oxygen consumption are often difficult to interpret. This is due to variations in oxygen consumption with size and activity and also to the problem of attaining "normal" experimental conditions. These problems can only be overcome by ensuring a constant set of experimental conditions and a predictable response from the animal. The latter is the most difficult to attain and could only be attempted by choosing animals in the same stage of their life cycle, i.e. a certain instar of *C. riparius* and sexually mature *C. diaphanus* of a similar size. Careful observation of the results failed to detect any significant diurnal or seasonal fluctuations in activity.

When designing the experimental chamber a choice had to be made between aiming to reproduce "normal" environmental conditions or arranging conditions which would produce a standard response from the animals which although it could be considered "abnormal" from the ecological point of view would represent a definable physiological state and give a repeatable result. The latter design was adopted in the present study as the physiological significance of the results was considered to take priority.

It was possible to measure the oxygen consumption either of resting animals, which would represent their standard rate of metabolism, or of active animals, which would represent their active metabolic rate and have the additional advantage of an ecological significance. This point, to be considered more fully below, was discussed by Fry, 1947 who stated, "the ecological point of view recognises that much of the measure of success an animal may enjoy is due to its ability to be active." Animals in the field show variable levels of random activity, environmental stress will, however, normally result in an active response rising to a maximum level which will be constant for any individual, definable physiologically and useful in an ecological study. The experimental conditions were, therefore, designed to produce a high level of activity in the animals.

The aspects of the animals' oxygen consumption chosen for study and the terminology employed in expressing the results are summarised in the section entitled "Respiratory dependence."

Previous workers measuring the oxygen consumption of animals respiring inside experimental chambers have noted the depressant effects of overcrowding on the pattern of consumption they obtained (Wells, I935 and Grainger, I955). In exploratory experiments the author recorded a lower rate of oxygen consumption from C.diaphanus when more than 3 individuals were enclosed in the respiration chamber. Accordingly a maximum of 3 individuals was used throughout this study. The larvae of C.riparius were, however, apparently unaffected by crowding even when between 20 and 30 individuals were used in one experiment, possibly due to their tube living habit. Their oxygen consumption was, therefore, measured on 20 larvae. This large number gave a conveniently high rate of consumption and avoided the effects of individual variation.

METHODS.

Previous workers in the field of invertebrate respiration have used a few standard chemical and physical techniques to measure oxygen consumption. These methods, because they involve periodic sampling of the medium, limit the amount and type of data that can be taken. Recently, however, polarographic methods of oxygen determination have been widely adopted, and were used in this study, as they allow the oxygen content of the experimental medium to be monitored continuously.

The author's methods were based upon an oxygen electrode similar to that described by Kanwisher, 1959. See figure 7(a). It consisted of a platinum cathode made from four strands of 24. S.W.G. wire, cast in epoxy-resin inside a silver/silver oxide anode, in the form of a cylinder of 5 mm. external diameter. The cathode was held at - 0·8 volts to the anode, and operated in an electrolyte of 0·5 N potassium hydroxide solution, enclosed as a thin film beneath a polythene membrane, held in place by an "O" ring. This arrangement gave a current of approximately 5 microamps for air saturated water at 20°C.

This version of the Kanwisher electrode was adopted in this study for several reasons.

(a) Its small size and relative ease of manufacture made it possible for several to be put into use, in various small respiration chambers.

(b) A high degree of sensitivity and fast reaction to changes in oxygen concentration, (Kanwisher claimed 90% full response in 20 seconds) made the electrode useful for studying small, short-term changes in oxygen consumption.

(c) The very slight permeability of the polythene membrane to molecular oxygen and the small size of the platinum cathode minimises the oxygen consumption of the electrode which was almost indetectable by the methods of measurement employed. This electrode is, therefore, particularly suitable for use in a closed chamber respirometer.

The polarographic recording circuit is shown in figure 5 . A voltage was applied to the electrodes E_1, and E_2 from a 3 volt dry battery placed across a 10,000 ohm potential divider. A rotary changeover switch with two levels X and Y was used to connect the electrodes to the recording instrument. It was designed so that when a recording was being taken from one electrode the other continued to function via an alternative circuit. This arrangement is represented in figure 5 as a ganged changeover switch, drawn as a double-action lever rather than a rotary type, merely to simplify the circuit diagram. When the switch is in the A, A_1 position a reading is being taken from the electrode E_1 with a recording potentiometer in the X circuit. Meanwhile E_2 is operating via the alternative 50,000 ohm resistance included in the Y circuit. When the switch is turned to the B, B_1 position a reading is taken from E_2 while E_1 operates through the Y circuit. The rotary changeover switch makes before it breaks so that both electrodes operate the whole time, even during the change from circuit X to circuit Y . This arrangement avoids the sudden large surges in current produced when electrodes are switched on and off, and ensures that they give stable readings.

The oxygen electrode is a diffusion dependent device and, therefore, measures oxygen tension rather than content. To express the results as oxygen concentration, the electrodes were calibrated by the chemical method of oxygen estimation described by Fox and Wingfield, 1938. A graph of current or voltage readings from the electrode, plotted against chemical determinations of oxygen content, gave a straight line calibration curve for each experimental temperature. These curves were used to convert the experimental readings to changes in oxygen content. The accuracy of the values obtained was limited to 2% by the sensitivity of the chemical method.

As the sensitivity of the electrodes was found to vary slowly with time, due perhaps to deterioration of the electrolyte, they were recalibrated before and after each experiment. Only those experiments in which both sets of calibration figures fell on the same straight line were accepted, as this was taken to indicate a complete stability of the electrode throughout the experiment. The electrodes also respond instantly to any change in external temperature, all experiments were, therefore, conducted in accurately controlled constant temperature water-baths.

Two sets of apparatus, both involving oxygen electrodes, were used to measure oxygen consumption.

One was a continuous flow apparatus, based on a design described by Grainger, 1955 . This consisted of a gravity feed of water from a constant head, passing through a capillary which limited the rate of flow, and then over the animals. Grainger used chemical techniques to measure the oxygen content of the water before and after the animal chamber. In an open system, such as a continuous flow respirometer, sampling by a chemical method usually involves a change in flow rate. This temporarily effects the oxygen supply to the animals, and may also influence their behaviour enough to cause inaccuracies in later readings. In fact the stability of the system is limited by the sampling technique.

Wells, 1935 stated that the criteria of an efficient method of measuring the respiratory exchange of aquatic animals are:-

(a) that the physical and mechanical conditions remain constant,

(b) that duplicate water samples have the same oxygen content,

(c) that the rate of flow of the water passing through the respiratory chambers be maintained invariable over long periods of time.

To perfect this type of system it is desirable, therefore, to use a method of oxygen analysis which does not involve a change in flow rate. This was achieved by the insertion of oxygen electrodes before and after the animal chamber, both in more recent work by Grainger (personal communication) , and by the author.

Calibration of the electrodes at the beginning and end of an experiment which often lasted for ten hours was insufficient and wasteful, as whole runs were discarded because of electrode drift. In order that the electrodes could be calibrated during an experiment, and animals removed or introduced, without disturbing the continuous and constant water flow, a byepass system was introduced, see figure 6(a).

During an experiment the water from the gravity feed flowed over the animals in the experimental chamber. The oxygen concentration in the water before and after passing over the animals was measured by two electrodes inserted in front of and behind the respiration chamber. When a calibration sample was required, at the end of an experimental run, the three-way taps were altered to divert the water flow through a byepass chamber. When conditions were stable within the byepass tubes, and the electrodes gave steady readings, a water sample was collected under liquid paraffin at the outflow, and then taken up in a Fox and Wingfield pipette. Fresh animals could then be introduced into the experimental chamber when necessary, and the experiment continued.

To enable short-term changes in oxygen consumption to be followed, the time-lag for water flowing between the electrodes was reduced to a minimum. This was achieved by restricting the volume between them by the use of narrow bore glass tubing and small electrode chambers (see figure 6(b)). Also, because of the sensitivity of the electrodes small changes in oxygen concentration could be measured, and a relatively fast flow rate of approximately 1 ml. per minute could be used. This water flow was directed onto the face of the electrode through a narrow tube, to give the maximum possible stirring effect and ensure stable readings of oxygen concentration.

The advantages of using this continuous flow respirometer were found to be twofold.

(a) Apart from enabling the system to remain undisturbed during measurements, the electrodes gave a continuous, very accurate picture of any short-term changes in the animals' oxygen consumption. This allowed the effects of sudden changes in environmental conditions to be studied during the actual change. For example measurements were made of the instantaneous effects, upon the animals oxygen consumption, of sudden changes in temperature and oxygen concentration.

(b) It was suitable for measuring the animal's response to a constant oxygen concentration and temperature for a period of time limited only by the onset of the effects of starvation. The particular advantage of continuous flow system, in this respect, was that the accumulation of toxic waste products, particularly carbon dioxide, was avoided. Using this technique, rates of oxygen consumption were obtained from animals completely acclimated to the experimental conditions.

The other set of apparatus was adapted from that used by Teal and Halcrow, 1962 to measure the respiration rates of single small marine organisms (see figure 7(b)). It consisted of an ordinary 3 inch specimen tube with an electrode inserted through the top, and passing down to the bottom on a glass rod. This brought the electrode face into close proximity to a magnetic stirring bar. It also allowed the animals to be inserted in the animal chamber above, without disturbing the electrode, and avoided any effects of their movements upon the stirring rate about the electrode face. The lower electrode chamber connected with the animal chamber via a perforated 'Perspex' disc. The electrode was calibrated before each experiment, then the animals were introduced, and the top closed by a further 'Perspex' disc, sealed on with vaseline.

The tube stood in a bridge-shaped water-bath, see figure 8 and plate 3 ,through which water of a controlled temperature was circulated by a combined pump, stirring motor and heater. Temperature control was achieved as a balance between the heating of this machine, and the cooling effect of the ambient temperature and a glass coil through which tap-water was circulated. All experiments at low temperatures were carried out in a refrigerated room.

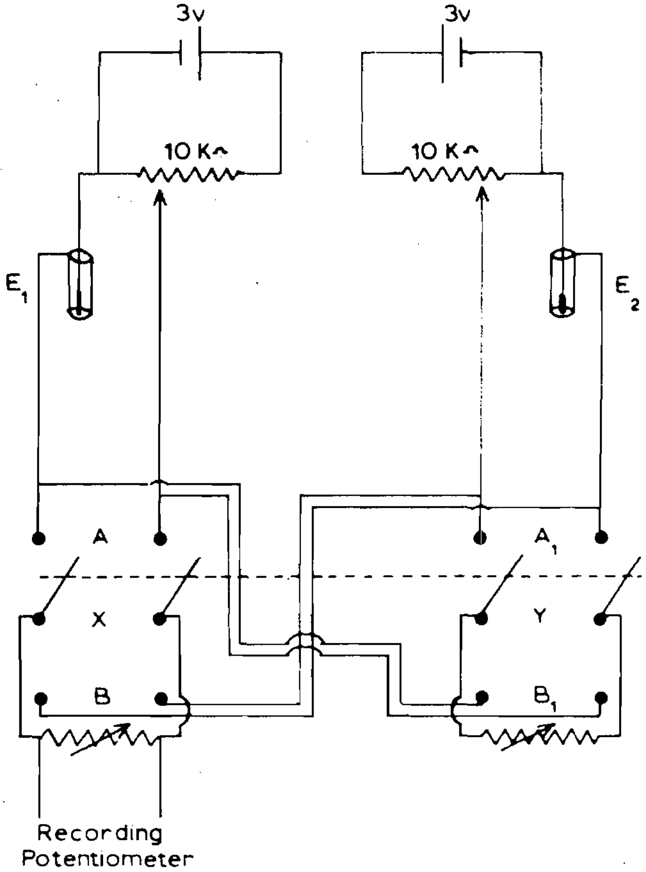
In this apparatus oxygen consumption was measured by the common method of allowing the animals to exhaust the oxygen in a closed container. The result obtained was a trace of decrease in oxygen concentration with time. By drawing tangents to this trace and plotting their gradients against the concentration at which they were taken, a curve relating oxygen consumption to concentration was obtained.

The advantage of this method is that the effects of varying oxygen concentration and any other environmental factor, such as temperature, can be assessed simultaneously. There is, however, a possible disadvantage, as the animals are not given time to adapt to the continuously changing oxygen conditions. Similar conditions may, however, be experienced in the environment, where bacterial, plant and animal respiration on the one hand, and plant photosynthesis on the other, will produce the quite extensive short-term fluctuations in oxygen concentration measured in the ponds. Experiments to be described below showed that the animals require little adaptation time for even quite large changes in external oxygen concentration. There seems, therefore, to be no objection on these grounds, to the use of a closed chamber to measure the oxygen consumption of these two species.

Most of the animals used in experiments were previously kept in air saturated water for several days, and every experiment was started at this level.

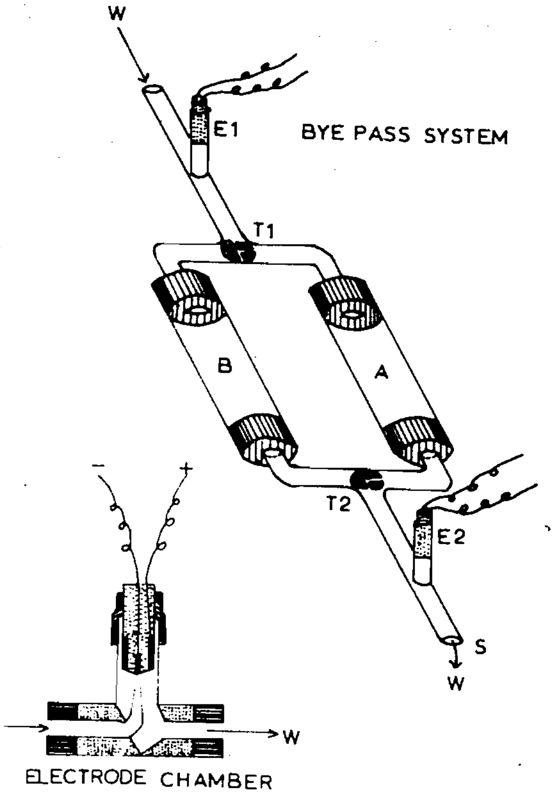
Another disadvantage which is inherent in this method is the possible effect, on the animal's rate of oxygen consumption, of the build up of carbon dioxide during experiments. As they were, however, of only short duration it was thought probable that the small increase in carbon dioxide would have no appreciable depressant effect. Later experiments proved that this was the case.

Apart from electrode failure, the two most frequent causes of inaccuracy during experiments in both sets of apparatus were the "bubbling-out" of gas from solution and bacterial respiration. The former was prevented by preheating the water to slightly above the experimental temperature, then stirring and allowing it to stand. In the continuous flow apparatus this was carried out in the head-tank. Bacterial growth was prevented by washing the apparatus out after each experiment, and flushing it with 95% alcohol.



figure, 5. A diagram of the circuit used to measure oxygen concentration continuously with two oxygen electrodes.

The current from the electrodes was recorded on a Leeds and Northrup, single-channel recording potentiometer. (Speedo Max. type H model S.)



figure, 6. The oxygen electrode chamber and byepass system included in the continuous flow respirometer.

E_1 and E_2, oxygen electrodes; W, water stream;

T_1 and T_2, three-way taps; S, sampling point;

A, animal chamber; B, byepass chamber.

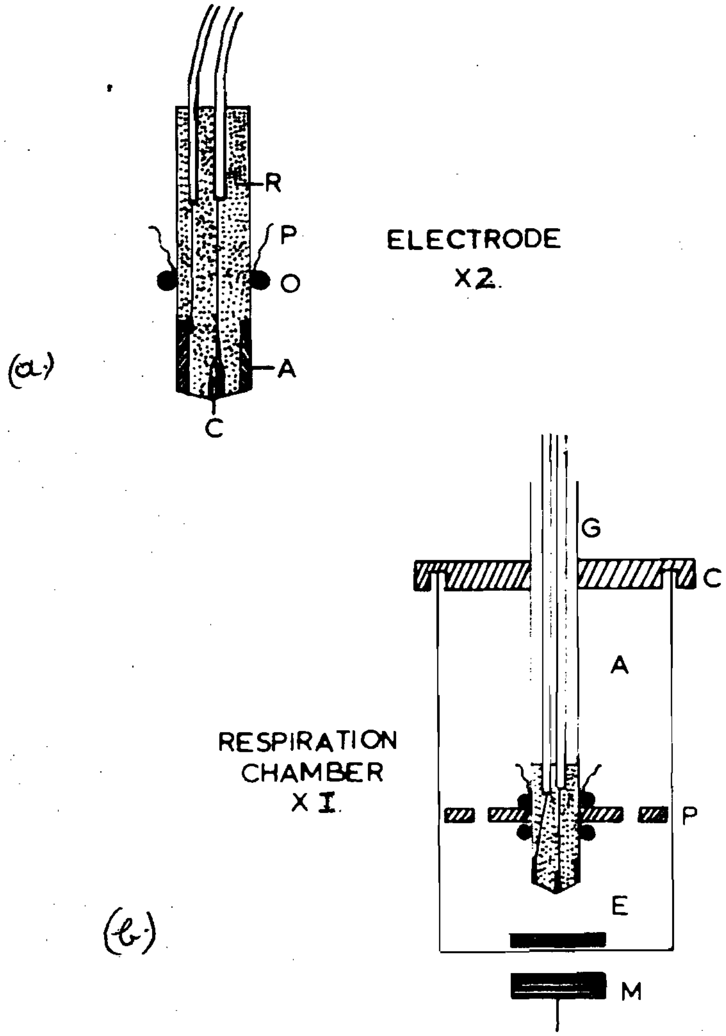
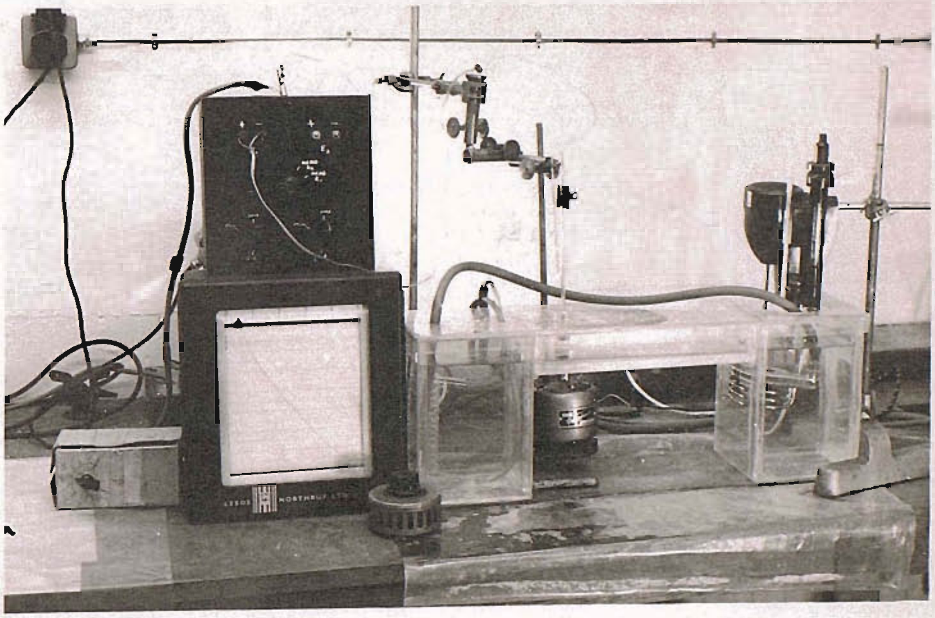


figure 7(a). Section of the oxygen electrode.

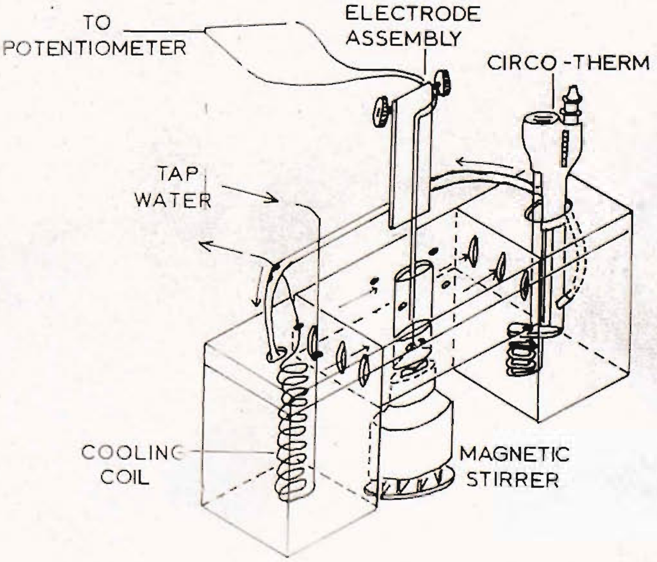
A, silver/silver oxide anode; O, 'O' ring;
C, platinum cathode; P, 'Polythene' membrane;
R, epoxy-resin mould.

(b). Section of the closed chamber respirometer.

A, animal chamber; C, 'Perspex' cap;
E, electrode chamber; G, glass tube;
M, magnetic stirrer; P, perforated disc.



Plate, 3. The water bath for the closed chamber respirometer and the recording apparatus.



figure, 8. The water bath for the closed chamber respirometer.

The effect of size variation on oxygen consumption.

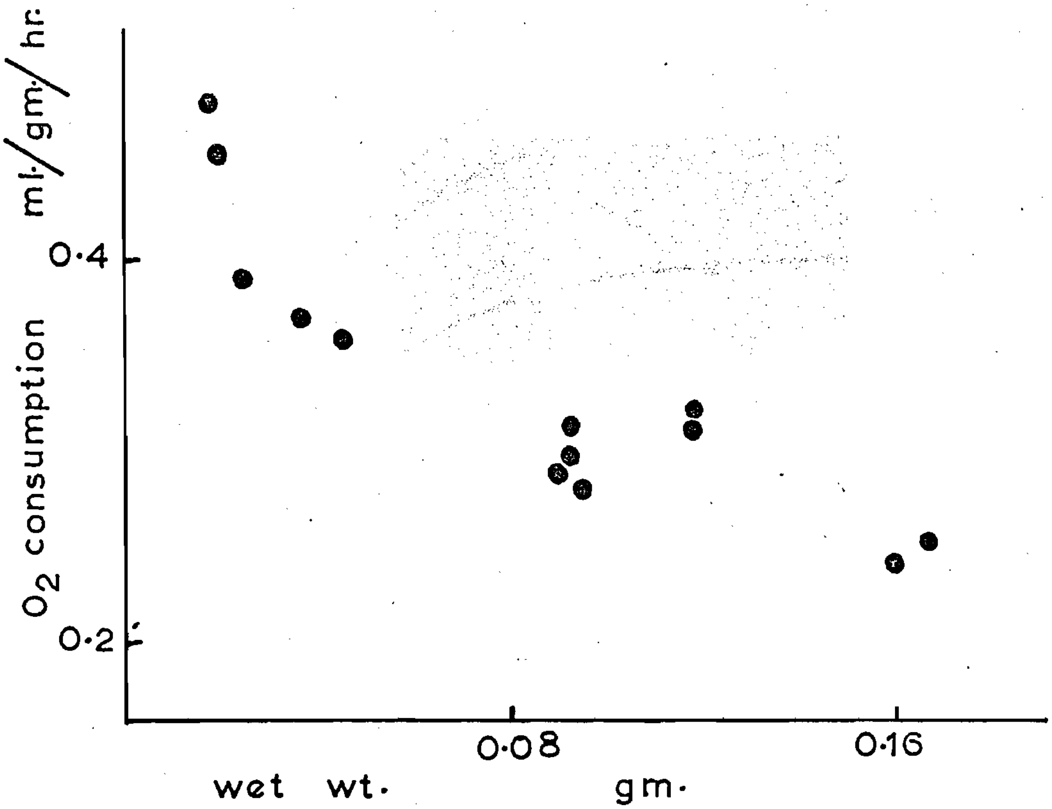
It is a general rule that small organisms have higher rates of oxygen consumption than larger organisms when determined both for animals within a given species and for those of closely related phylogenetic groups. Weymouth et al, 1944 obtained the same general correlation between body surface and oxygen consumption in a number of different species of Crustacea. This variation with size complicates the interpretation of measurements of oxygen consumption and must be catered for in a comparative study.

Size variation was reduced to a minimum in the present study by working only upon the last larval instar of *C. riparius* and on sexually mature *C. diaphanus*.

Given an adequate food supply and stable temperature conditions it was found that the individuals in each larval instar of *C. riparius* had an almost identical wet weight. Thus by confining experiments to the final instar the effects of size variation could be ignored in measurements of its oxygen consumption.

C. diaphanus moults throughout its life so that the wet weight of sexually mature individuals varied between 0·01 and 0·15 gm. The rate of oxygen consumption of mature *C. diaphanus* was therefore very variable, and is plotted against wet weight in figure 9 . To allow comparison of results from similar experiments on different individuals, all measurements of oxygen consumption in C. diaphanus were converted to the approximate rate expected from an animal of 0·05 gm., obtained by inspection of curves similar to figure 9 . This does not represent a thorough investigation of the size effects on oxygen consumption, such as is being carried out on a number of species of Anostraca by Dr. B. M. Gilchrist (personal communication) . Size variation had no measurable effect on the respiratory dependence of C. diaphanus and was ignored in all considerations of this aspect of its oxygen consumption.

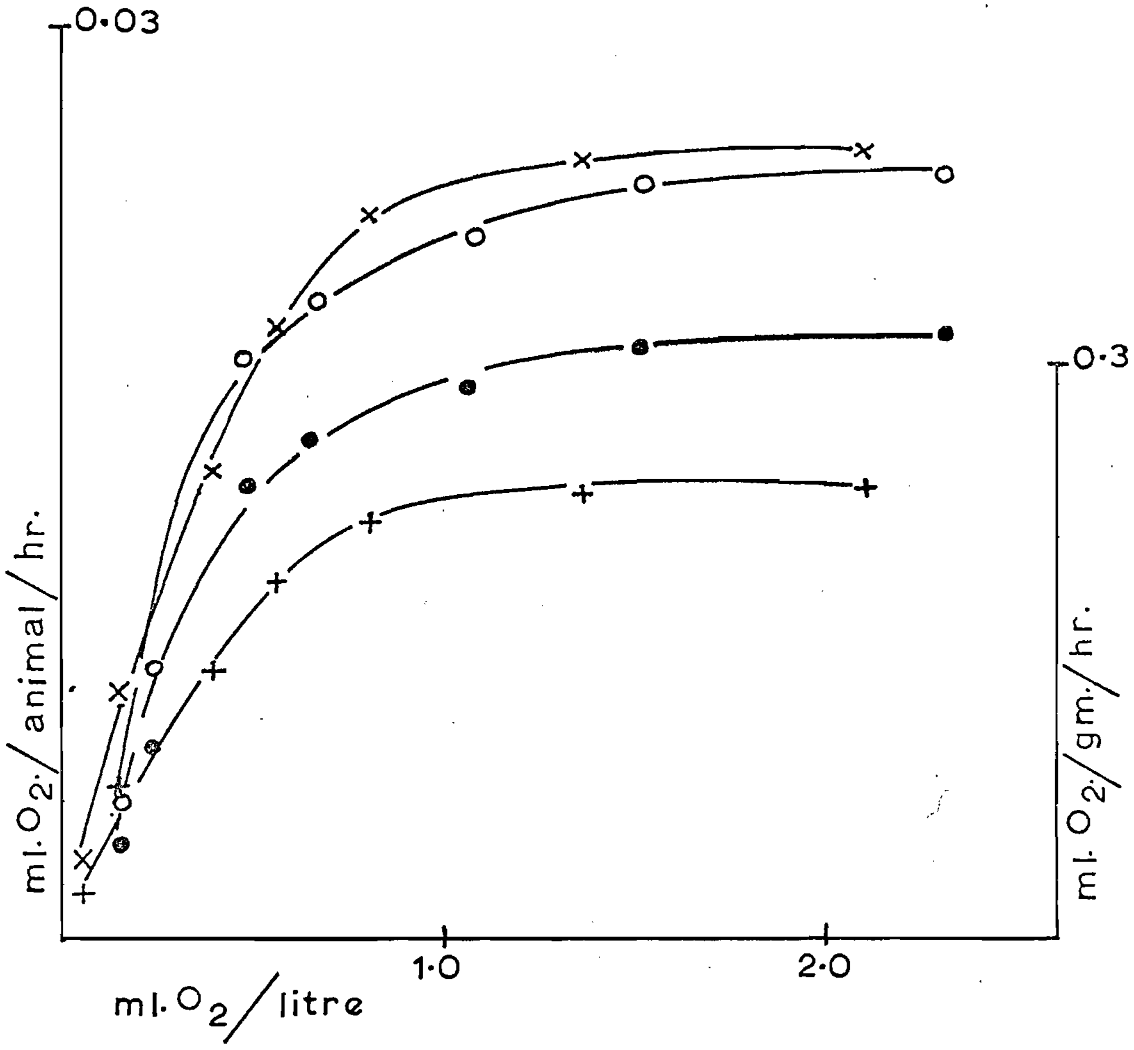
Experiments on earlier instars of C. riparius showed that both their rate of oxygen consumption and their level of respiratory dependence was affected by size variation (see figure 10) . This may be due to structural changes between instars. An interesting observation was that the oxygen consumption per individual larva, regardless of its instar, (see figure 10) was almost identical. This indicates a possible relationship between their rates of oxygen consumption and their dry weights or total nitrogen content which will not vary as much between instars as would their wet weights.



figure, 9. The effect of size variation on the rate of oxygen consumption of sexually mature C. diaphanus at 20°C.

Their rate of oxygen consumption, per gramme of tissue, decreased with increasing size.

Points taken from I3 experiments on unpoisoned animals in the closed chamber respirometer.



figure, 10. The oxygen consumption of the 3rd. and 4th. larval instars of *C. riparius*, in decreasing oxygen concentrations.

o, ● 3rd instar; x, + 4th instar.

The 3rd. instar larvae consumed oxygen at a higher rate, per gramme of tissue, than the 4th. (lower two traces). The consumption per individual larva was, however, very similar (upper traces). The 4th. instar larvae had a slightly lower Pc. than the 3rd.

2 experiments on 3rd instar and 4 on the 4th.

THE EFFECTS OF CHANGES IN OXYGEN CONCENTRATION ON THE OXYGEN CONSUMPTION OF C. DIAPHANUS AND THE LARVAE OF C. RIPARIUS .

RESULTS.

The measurement of oxygen consumption in the continuous flow and closed chamber respirometers.

The effects of a decrease in oxygen concentration on the oxygen consumption of C. diaphanus and the larvae of C. riparius was measured using both the open continuous flow and the closed chamber respirometers.

In the continuous flow apparatus the oxygen content of the water was changed by bubbling various mixtures of air and nitrogen through the head-tank. The animals were first exposed to each oxygen concentration for at least one hour. Readings were then taken from both electrodes, until a steady level of oxygen consumption was obtained. This value was then plotted against oxygen concentration, taken as the mean of the two values recorded from the electrodes.

The animals were allowed to exhaust all the available oxygen in the closed chamber. The pattern of removal of oxygen from the water in the chamber was followed polarographically as a trace on a recording potentiometer. The curve for decreasing voltage with time (see figure 11), fell gradually down to a level just above zero, which represented the animal's residual oxygen concentration. The animal's rate of oxygen consumption, related to oxygen concentration, was derived from these potentiometer traces by drawing tangents to the voltage curve at a series of points along its length. The gradient of each tangent was proportional to the rate at which the animals were withdrawing the oxygen from the chamber. The oxygen electrode was calibrated before each experiment, so that these measurements of the potentiometer traces could be converted to oxygen concentration, and the gradients expressed as the oxygen consumption of the weighed animals. By this method the animal's oxygen consumption was measured over a continuous range of oxygen concentrations, from air saturation down to their residual level.

The measurements of oxygen consumption in *C. diaphanus* using the two sets of apparatus are compared in figure 12 . There is a marked similarity in the pattern of oxygen consumption with decreasing oxygen concentration as measured by the different techniques. This similarity implies that the animals do not require the time to adapt to a rapidly changing oxygen concentration, which they were allowed in the open system. To test the validity of this implication further experiments were carried out in the continuous flow respirometer.

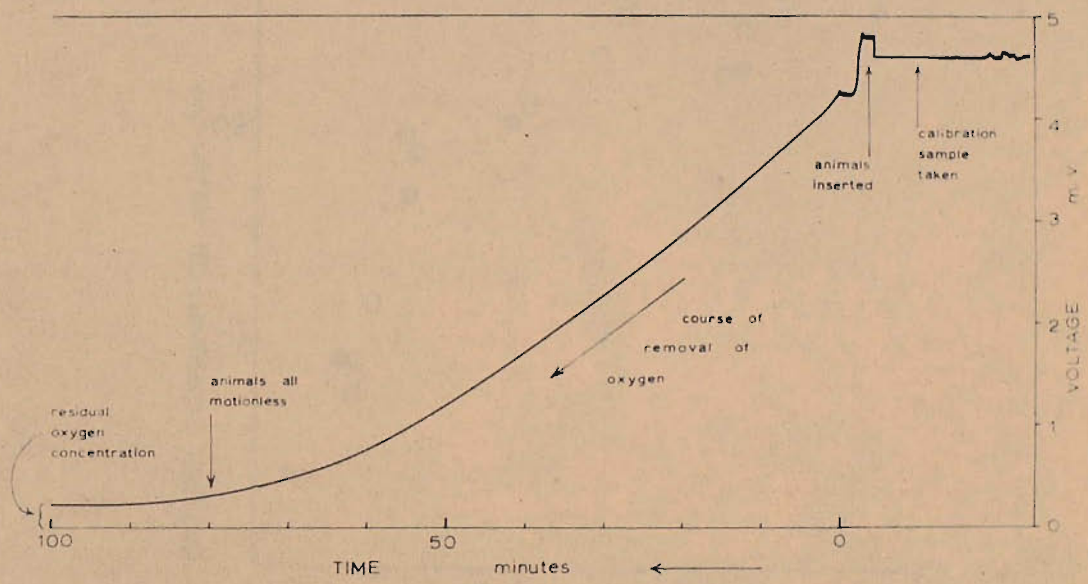
During a sudden change in oxygen concentration in the continuous flow apparatus, the values obtained from each electrode were plotted against time. A blank run, without animals, changing the oxygen concentration in the same way, was then traced. The electrode readings taken during the blank run were subtracted from those obtained in the experiment with animals. In this way curves tracing the oxygen consumption of C. diaphanus during a sudden change in the oxygen concentration of the surrounding water were obtained, and two are plotted in figure 13 .

The results showed that C. diaphanus requires little or no time to adapt to a sudden change in environmental oxygen concentration. The only response recorded was an immediate and almost direct rise or fall in its oxygen consumption to a new steady level, reached only a few minutes after the change.

The similarity of the curves in figure 12 also implies that the possible accumulation of toxic metabolites in the closed chamber was having no detectable effect on the measured rate of oxygen consumption in C. diaphanus. This may be due to the short length of the experiments, as a relatively small p.H. change from 7·9 to 7·2 was recorded during an exceptionally long experiment lasting 6 hours.

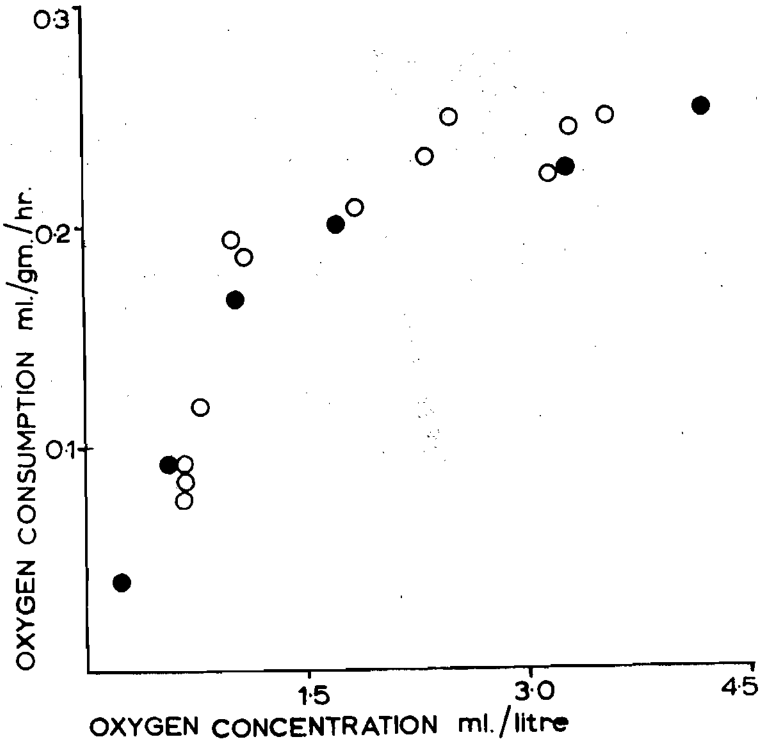
These results are, therefore, of technical importance as they demonstrate the validity of results obtained in a closed system. Accordingly use of the closed chamber respirometer was prefered for much of the study, because of its relative convenience in allowing for rapid measurement of oxygen consumption over the whole range of oxygen concentrations. Also it allows the effects of two environmental variables to be assessed simultaneously. This advantage was made use of in later work, when the effects of decreasing oxygen concentration were measured at a range of environmental temperatures.

The oxygen consumption of the aquatic larvae of Chironamus riparius was also measured in both respirometers, and the results are compared in figure 14. Once again the two curves are very similar in shape, indicating a situation comparable to that just described for C. diaphanus, though this was not investigated further. On the basis of these results it was considered valid to make further measurements of the respiration of C. riparius larvae in the closed chamber apparatus.



figure, 11. Diagram of a potentiometer trace obtained during an experiment in the closed chamber respirometer.

When the oxygen electrode gave a steady reading for air saturation it was calibrated and animals were inserted in the respiration chamber. Their oxygen consumption was followed as a drop in the current from the electrode, measured as a voltage drop across a resistance.

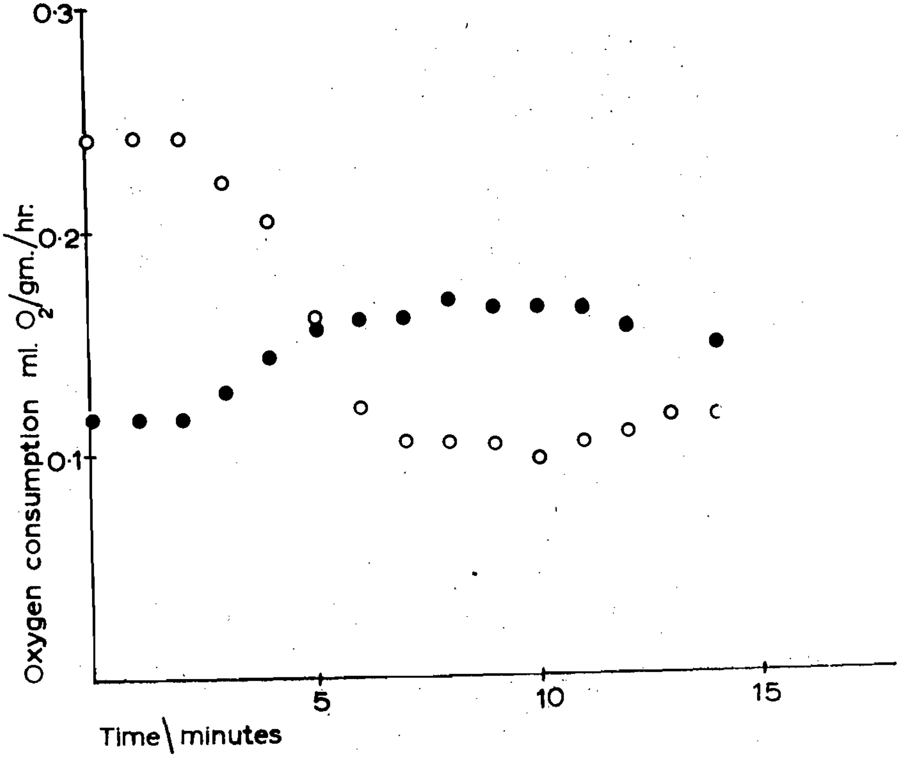


figure, 12. The oxygen consumption of C. diaphanus with decreasing oxygen concentration, at $18^{\circ}C$. Comparison of experiments in the continuous flow o and closed chamber ● respirometers.

The measured rate of oxygen consumption and Pc. were similar in both types of respirometer. The Pc. was at approximately 1·6 ml. O_2/litre.

Total of 4 experiments in the continuous flow respirometer,each involving the exposure of 3 animals to 4 different O_2 concentrations.

Single experiment in the closed chamber respirometer chosen from 4 experiments at the same temperature(@ I8 C).



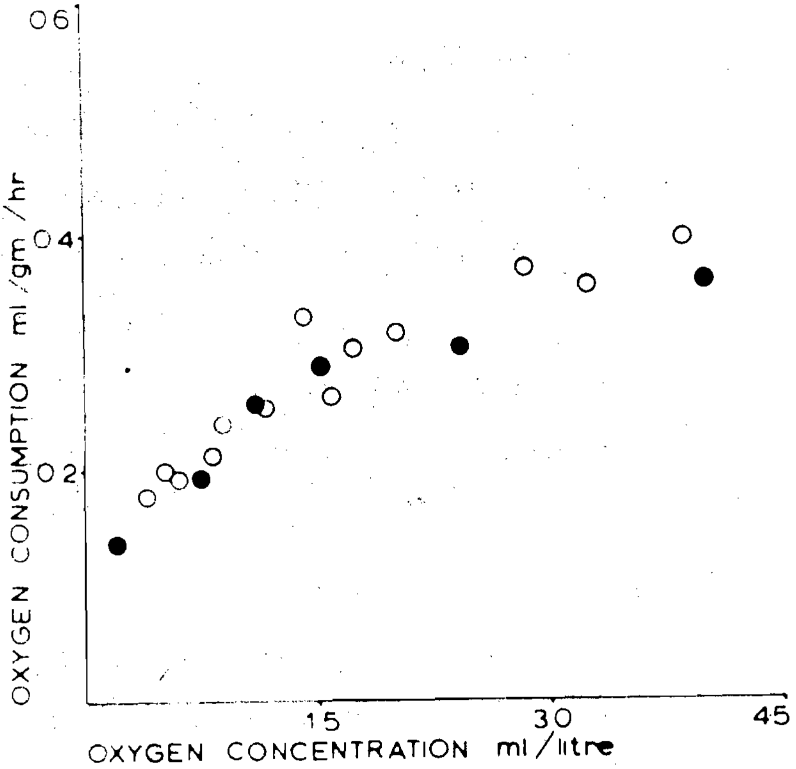
figure, 13. The oxygen consumption of <u>C. diaphanus</u> after a sudden change in environmental oxygen concentration.

○ change from 5 ml. O_2/litre to 1·65 ml./litre.

● " " 1·65 ml./litre " 4·2 ml./litre.

There was no marked overshoot or undershoot in its rate of oxygen consumption after the sudden change.

Plot of I experiment chosen from a total of 4.



figure, 14. The oxygen consumption of C. riparius larvae with decreasing oxygen concentration at 18°C. Comparison of experiments in the continuous flow o and closed chamber ● respirometers.

The measured rate of oxygen consumption and Pc. were similar in both types of respirometer. The Pc. was at approximately 1·5 ml. O_2/litre.

Total of 4 experiments in the continuous flow respirometer, each involving the exposure of 20 larvae to 4 different O_2 concentrations.
Single experiment in the closed chamber respirometer chosen from 4 experiments at the same temperature.

Respiratory dependence.

The curves shown in figures 12 and 14 trace the rate of oxygen consumption of the two species during a decrease in the oxygen content of the surrounding water. The shapes of these curves have both an ecological and physiological significance.

Many workers in the past observed that the rate of uptake of oxygen by organisms respiring in a closed vessel decreased as the oxygen content of the water fell. So widespread was this phenomenon that it received the name 'respiratory dependence', though the exact meaning of the term was not at first clear. In his review of the subject von Ledebur, 1939 remarked that the observed restriction in oxygen consumption could not be interpreted as a restriction of vital processes. Following this observation Fry, 1957 stated that respiratory dependence should be investigated in relation to active metabolism. He pointed out that if organisms are to remain in oxygen balance, then the restriction imposed on their respiratory rate by a decrease in the ambient oxygen level means a restriction of the activity of the organisms and not of their maintenance or resting metabolism.

The ability of an animal to be active is important in its relationships with its environment, and the oxygen concentration at which an animal first shows respiratory dependence will be an ecologically limiting factor.

The oxygen concentrations at which the two species first showed respiratory dependence in figures 12 and 14 may correspond to their "incipient limiting levels", defined by Fry, 1952 as "that level of oxygen tension below which the rate of oxygen uptake, at the maximum steady state of activity, begins to be reduced." The experimental conditions previously described were such as to stimulate the animals to high levels of activity, though it was not certain that they were reaching a maximum steady state. That their activity was approaching maximum may be suggested by the stability of results from duplicated experiments. Their activity during experiments appeared to be significantly higher than the average level of activity observed in animals living undisturbed in the stock tanks; defined as their random activity. Later results indicated that during experiments in the closed respirometer the activity of the chironomid larvae, which normally inhabit mud tubes, was approximately double their random level.

Fry stressed the importance of measurements on maximally active animals and stated, "much of the measure of success an animal may enjoy is due to its ability to be active". All measurements made in this study were taken from active animals. The terminology employed by Fry is too specific to be used in the present study, so for convenience the level at which the animal's oxygen consumption became dependent on the ambient oxygen concentration was designated their critical oxygen concentration, abbreviated as Pc. For the purpose of this study the Pc. was defined as:the environmental oxygen concentration at which the rate of metabolism of an active animal was first limited by oxygen depletion. This was observable as the level below which any further decrease in oxygen concentration resulted in a proportionate decrease in the animal's rate of oxygen consumption.

Though a line drawn through the points in figures I2 and I4 would probably trace a hyperbola, the pattern of oxygen consumption with decreasing oxygen concentration may be divided into two phases. As the oxygen concentration decreased the animals consumed oxygen at only a slightly lower rate until at low concentrations their consumption began to fall more rapidly,i.e.they became oxygen dependent. The point at which this change takes place,observable as the point of maximum change of direction on the curves,is their Pc. which can be seen to lie at approximately I.5 ml O_2/litre in figures I2 and I4. Neither animal will,therefore,be seriously limited by the low and variable oxygen concentrations recorded in temporary ponds.

This independence of environmental oxygen indicates that the respiratory physiology of the two species is specifically adapted for life in low and variable oxygen concentrations. A part of the present study comprised an attempt to isolate and analyse the relative importance of the various factors contributing to the specialisation of their respiratory systems.

The factors determining the respiratory dependence of C. diaphanus and the larvae of C. riparius .

I Tissue respiration.

In an investigation of the separate physiological factors involved in determining the respiratory dependence of the two species it seemed logical to consider first the internal concentration limiting the utilisation of oxygen at their tissues. Winzler, 1941 observed that oxidations catalysed by the cytochrome system are usually found to be independent of oxygen concentration down to extremely low levels. Prosser and Brown, 1961, did, however, point out that although the oxygen pressure needed for the saturation of enzymes is probably not often limiting, it cannot be ruled out. It was thought advisable, therefore, to check the respiratory dependence of a tissue preparation.

A saline suitable for use with C. diaphanus tissue was prepared on the advice of Dr. A. P. M. Lockwood, using the values for blood composition given by Croghan, 1958. The composition of this saline was:

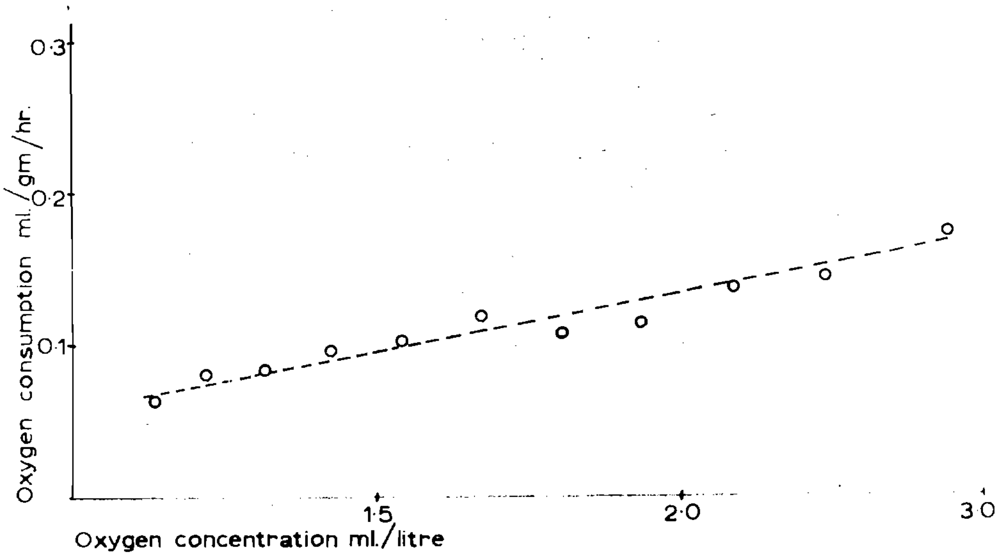
sodium chloride	0·362 gm./litre.
potassium chloride	0·037 gm./litre.
calcium chloride	0·142 gm./litre.
glucose	0·500 gm./litre.

A number of C. diaphanus were washed in the saline then macerated between glass surfaces. The oxygen consumption of this mixed tissue preparation was then measured in the closed chamber respirometer, full of the saline, at the temperature to which the whole animals were previously acclimated, which was 20°C. A result is given in figure 15 .

The absolute rate of oxygen consumption was unimportant and was expressed per gram wet weight of the whole animals. The important feature of this result was the shape of the respiratory dependence curve. The oxygen consumption of the tissues decreased quite rapidly with the oxygen concentration, but there was no definite Pc. like that detected in the respiration of whole animals. This linear decrease was probably due to a progressive decay in the condition of the preparation or to the accumulation of toxic metabolites, during the 4 hour experiment in unbuffered saline.

This result indicates that the oxygen consumption of a preparation of tissues from C. diaphanus is unaffected by decreased oxygen concentrations down to levels well below the measured Pc. of the whole animal. Oxygen utilisation at the tissues cannot, therefore, be involved in determining the measured respiratory dependence of C. diaphanus.

If their tissue respiration operates efficiently down to very low oxygen concentrations then the respiratory dependence of the two species must depend on the properties of their systems for the presentation, uptake and transport of oxygen. These processes are divisible into oxygen uptake from the environment, which depends on the nature of the respiratory surfaces and their ventilation, and oxygen transport within the animal's body, which depends upon the efficiency of its circulation and the possible possession of a respiratory pigment.



figure, 15. The oxygen consumption of a mixed tissue preparation of C. diaphanus with decreasing oxygen concentration.

The rate of consumption of the tissues decreased throughout the experiment, but did not show the definite Pc., characteristic of whole animals.

Selected from a total of 3 experiments.

II. The possible role of respiratory ventilation and the effect of size on the respiratory dependence of *C. diaphanus* and the larvae of *C. riparius*.

The possible limitations imposed on an animal's oxygen consumption by the efficiency with which it ventilates its respiratory surfaces may be important to species living in stationary water masses such as small ponds. *C. diaphanus* ventilates its body surface by the action of its eleven pairs of thoracic appendages. The movements of these appendages also serve~~s~~ to move the animal through the water and cause the currents from which it filters its food. The possible limitations imposed by this triple function of the appendages were first mentioned by Fox and Johnson, 1934. This problem together with the author's work on the frequency of ventilation movements and changes in locomotory ability with varying oxygen concentration, is discussed in greater detail in a later section.

Chironomid larvae ventilate their respiratory surfaces by undulating their bodies. This activity is designed to irrigate their mud tubes. The sensitivity of the animals to low oxygen concentrations, as shown by the onset and duration of this activity, has been studied by Walshe, 1950.

She found that in well aerated water the larvae spend about 50% of their time in respiratory behaviour, consisting of intermittent irrigation of their tubes, 35% of their time in filter-feeding, and the remainder in periods of rest. As the oxygen concentration in the water dropped, they spent a progressively larger proportion of their time in respiratory irrigation. Below 10% air saturation they no longer fed, and in completely anaerobic conditions they soon became immobile. This respiratory behaviour appeared to be a response to internal p.H. changes, and enabled the larvae to maintain an oxygen supply in their mud tubes adequate for their haemoglobin to function in oxygen transport to the tissues.

Shepard, 1955 working on Salvelinus fontinalis, found that the major factor producing respiratory dependence in this species is the maximum amount of water which can be presented to its gills by its ventilatory apparatus. Their small size, relatively large respiratory surfaces and observed ventilatory behaviour indicate that ventilation may be less important than other factors in determining the respiratory dependence of C. diaphanus and the larvae of C. riparius . The efficient ventilation of their respiratory surfaces may, therefore, be one factor enabling the two species to be independent of environmental oxygen concentration to low levels.

Individual C. diaphanus of different sizes were found to have different rates of oxygen consumption, though there was no comparable change in the position of their Pc.'s. This observation agrees with that made by Job, 1955 on Salvelinus fontinalis. The variation in the size of the C. diaphanus used in the present experiments was not very large; there was, therefore, little variation in the surface area they had available for respiratory exchange. This may explain the absence of a detectable effect of size upon the position of their Pc.

In the later larval instars of C. riparius their rate of oxygen consumption per gramme of tissue was found to decrease as their size increased. They also became more independent of oxygen, i.e. their Pc. decreased in their last larval instar. This is unlikely to be directly linked to their increase in size, which would be expected to have the opposite effect. Their decreased Pc. may follow the lower metabolic rate of the larger larvae or be due to an increase in the efficiency of their circulatory system, as their internal organisation changes in successive instars, whereas it stays virtually the same during the post-adult moults in C. diaphanus. The chironomid larvae may, therefore, have a more efficient oxygen transport system in the later instars, to compensate for their increased size.

III. The role of haemoglobin in determining the measured respiratory dependence of C. diaphanus and the larvae of C. riparius

Both species have body fluids circulating in an open haemocoel and because of their small size the relative efficiency of this system of distribution is unlikely to be directly involved in determining the position of their Pc.'s. The presence of a respiratory pigment in the circulating fluid may, however, have more influence on their respiratory dependence.

A respiratory pigment is a substance which forms a compound with oxygen by means of a reversible reaction, which enables it to take up oxygen at regions of high partial pressure of the gas and release it where the partial pressure is low. Ideally the loading and unloading tensions for a pigment correspond to the oxygen conditions at the animal's respiratory surface and its tissues, so that the maximum amount of oxygen is taken up at the former and delivered to the latter. In this way the possession of a respiratory pigment results in more oxygen being supplied to an animal's tissues than can be carried in simple solution in its blood. Prosser et al, 1952 stated that where there is a significant amount of pigment, as in Arenicola marina, the oxygen capacity of the blood is roughly ten times as much as it would be without a pigment. It is clear, therefore, that the possession of a respiratory pigment is likely to decrease the limits imposed by oxygen transport on an animals Pc.

C. diaphanus, when kept in well oxygenated conditions in the laboratory, at temperatures below 20°C, is predominantly greenish-blue in colour. When the oxygen concentration remains low for a prolonged period it turns red. This change is due to the synthesis of haemoglobin. The first reported identification of this pigment in the Crustacea was by Lankester in 1869, working on C. diaphanus. The ability to synthesise haemoglobin is shared by other phyllopod Crustacea, noteably Daphnia on which much work has been done by Munro Fox and his collegues (for a review see Fox, Gilchrist and Phear, 1951) and the Anostracan Artemia salina studied by Gilchrist, 1954, who has also done some recent work on the pigments in C. diaphanus. Gilchrist, 1962, stated that C. diaphanus, like other Anostraca, always has traces of haemoglobin in solution in the blood, but synthesises much more of the pigment when there is little dissolved oxygen in the surrounding water.

Experiments were designed to assess the importance of this apparent physiological adaptation to low oxygen concentrations, in determining the respiratory dependence of C. diaphanus. Initially its oxygen consumption was measured before and after prolonged exposure to low oxygen concentrations.

A population of adult C. diaphanus was transferred to a closed water circulation in which the oxygen content was kept below 2 ml./litre, by bubbling nitrogen and air through the head-tank. The animals were fed regularly on dried brewer's yeast, and the oxygen content of the water was checked daily. After approximately fourteen days their colour had changed from greeny-blue to a redder shade, indicating the presence of an increased concentration of haemoglobin in their blood. The same effect was achieved by culturing C. diaphanus in unaerated aquarium tanks at room temperature. The oxygen content of the water fell to well below 2 ml./litre, often down to 0·9 ml./litre. This oxygen depletion resulted from the activity of bacteria, which often formed a scum on the water surface, and the dense populations of Daphnia spp. and ostracods, which always accompanied C. diaphanus hatched from mud containing their eggs. The complete absence of photosynthesising algae in these yeast fed cultures prevented the oxygen replacement normally occurring in the environment, from counteracting this depletion. C. diaphanus survived quite well under these conditions, and assumed, together with the Daphnia population, a dark reddish colour, once again indicative of an increased haemoglobin concentration in their blood.

Members of both these C. diaphanus populations from conditions of low oxygen, and animals from well aerated conditions were exposed inside the closed chamber respirometer, to a decreasing oxygen concentration. Some results are shown in figure 16 .

The males and females from well aerated conditions have an identical pattern of oxygen consumption with decreasing oxygen concentration. This experiment was carried out at 21°C , and at this temperature the animals achieved only a partial independence of oxygen even at the higher concentrations, and had an indistinct Pc. above 2 ml./litre.

There is a distinct difference in response to decreasing oxygen in the two sexes from the populations kept at low oxygen concentrations. The females have a higher oxygen consumption per gramme of body weight than the males at all levels of oxygen concentration. Also the shapes of their respiratory curves are fundamentally different. These differences were illustrated by subtracting the levels of oxygen consumption of the animals kept in aerated water, from the levels of the males and females kept in low oxygen conditions. The resultant curves are plotted at the base of figure 16 .

These curves demonstrate the changes in the respiratory dependence of C. diaphanus after prolonged exposure to depleted oxygen concentrations. The females are completely independent of environmental oxygen down to a Pc. at approximately 1·5 ml./litre. In consequence their rate of oxygen consumption is higher at the lower oxygen concentrations than the measured rate of animals from aerated water. The difference between the two rates reaches a maximum at an oxygen concentration of 1·25 ml./litre. The males consume oxygen at much the same rate as the animals from aerated water over the whole range of oxygen concentrations. The plotted difference between the two rates is, therefore, relatively small but does rise to an indistinct peak again at an approximate oxygen concentration of 1·25 ml./litre.

The respiratory dependence of C. diaphanus appears, therefore, to change after prolonged exposure to low environmental oxygen concentrations. This change is, however, only really marked in the females. This difference in the extent to which the oxygen consumption of the two sexes is changed compares with an observation made by Gilchrist, 1962; she found that female

C. diaphanus always have a higher total haem content than males under the same experimental conditions, (c.f. plate 4). Gilchrist, 1954, also found that female Artemia salina synthesise more haemoglobin than males, after a given period in water with a low dissolved oxygen content. The functional significance of these different haem concentrations was, however, doubtful in this species, as she observed no difference in survival time between males and females in water deficient of oxygen.

The possible link between the reported sexual difference in haem synthesis in the Anostraca, and the observed effects of low oxygen on the respiratory dependence of male and female C. diaphanus, was investigated by poisoning the animals with carbon monoxide. This gas acts as a respiratory poison by combining with reduced haemoglobin to the exclusion of oxygen. In contrast to the production of oxyhaemoglobin, the reaction producing carboxyhaemoglobin is relatively irreversible. The haemoglobin is, therefore, rendered functionless in oxygen transport by carbon monoxide. Unfortunately carbon monoxide also poisons the cytochrome system, when present in high concentrations, (Wolsky, 1938) ~~relative affinity of Chironomus haemoglobin and cytochrome oxidase for carbon monoxide, relative to that for oxygen, was given as 400 and 0·1 respectively, by~~

~~Wyman, 1948~~. It is, therefore necessary to use a very dilute solution of carbon monoxide to avoid depressing tissue respiration. The dose levels recommended by Ewer, 1942 were found to markedly depress the oxygen consumption of *C. diaphanus* at air saturation levels of oxygen. This indicated that the cytochrome system in *C. diaphanus* is more sensitive to poisoning with carbon monoxide than was reported in *Chironomus plumosus* by Ewer. More dilute solutions, of approximately 2% saturation, were, therefore, used throughout the experiments, and even these concentrations had a measurable effect on the cytochrome system. The effect of carbon monoxide poisoning on the oxygen consumption of female *C. diaphanus* taken from water with a low oxygen concentration, is shown in figure 17 .

The rate of oxygen consumption of the poisoned animals was lower at all oxygen concentrations, possibly because the carbon monoxide was effecting their cytochrome systems. The important change was in the shape of the curves. The oxygen consumption of untreated females is almost completely independent of environmental oxygen down to a concentration of 1·7 ml./litre. After treatment with carbon monoxide their consumption is only partially independent down to an indistinct Pc. above 2·0 ml./litre. The line at the base of figure 17 traces the difference between the rates of oxygen consumption of untreated and poisoned animals; its maximum is at 1·5 ml./litre.

The shapes of the curves shown in figure 17 and the differences between them resemble the results obtained from normal female C. diaphanus taken from well aerated and stagnant water (see figure 16) . In both sets of experiments the oxygen consumption of animals with and without a functional blood pigment was compared. The reported synthesis of high concentrations of haemoglobin by female C. diaphanus when exposed to low oxygen concentrations (Gilchrist, 1962) is, therefore, responsible for the measured changes in their oxygen consumption. These results establish the functional significance of the occurrence of haemoglobin in C. diaphanus and, because of the measured changes in the position of their Pc.'s , demonstrate its importance in determining the respiratory dependence of this species.

Poisoning male C.diaphanus with CO(2expts.)resulted in an overall decrease in their respiratory rate without evidence of any change in their Pc. This further demonstrated the absence of functional haemoglobin in the males.

Using a hand spectroscope the author was able to confirm the presence of haemoglobin in female C.diaphanus acclimated to low O_2 and noted that it was deoxygenated in animals which had exhausted the available O_2 in the closed chamber respirometer.

The larvae of Chironomus riparius in common with many other chironomids but in contrast to C. diaphanus, always possess appreciable quantities of haemoglobin in their blood, regardless of environmental oxygen conditions. Several workers have investigated the functional significance of the blood pigment in chironomid larvae. Ewer 1942, working on larvae of the Chironomus plumosus group, found by carbon monoxide poisoning, that the haemoglobin was functionless as a transporter of oxygen in air-saturated water, but enabled them to stay relatively more independent of oxygen at concentrations below 3 cc. O_2/litre. Thus, by lowering the concentration at which the animals respiration became oxygen dependent, the haemoglobin was to a large extent responsible for their independent type of respiration curve. Ewer found, from her observations of the habitat of the larvae, that they were able to live in situations where an oxygen concentration of less than 2 cc. O_2/litre persisted for periods of at least 16 consecutive hours.

Harnisch, 1936, using carbon monoxide in very high concentrations, which were probably effecting the cytochrome system, claimed that the haemoglobin in Chironomus thummi functioned right up to air saturation, and was of particular importance in allowing for a rapid repayment of an oxygen debt; after prolonged immersion in deoxygenated water. Although his work is rightly criticized by Ewer and Walshe, because of his high concentrations of carbon monoxide and inadequate temperature control during experiments, his findings agree to some extent with those of Walshe 1948 (a) working on *C. plumosus*. She stated "in both Harnisch's larvae and mine the possession of haemoglobin enabled the larvae to show an increased degree of activity aerobically".

Walshe, 1949, studied the respiration of several chironomids from still and moving waters. The larvae from stagnant waters became oxygen dependent in water between 15 - 44% (approx. I.5 - 4.5 ml./l.) saturated with air, while the species from streams were dependent at all oxygen concentrations up to air saturation. She pointed out that such different respiration curves from closely similar animals must have some special ecological or physiological importance. Her explanation of the physiological processes involved in the maintenance of these different degrees of oxygen dependence ruled out differences in the efficiency of oxygen transport to the tissues, since, "in the chironomid larvae, ... , the size and speed of blood circulation are the same in both members of the contrasted pairs". In an earlier paper Walshe, 1948 (b) working on *Tanytarsus brunnipes*, collected from a stream, questioned the functional significance of the haemoglobin in this species. After carbon monoxide treatment there was no change in their oxygen consumption until concentrations below 25% air saturation, at 17°C. *Tanytarsus* species are markedly sensitive to low oxygen concentrations and only occur in well-aerated waters (Theinemann, 1923) . It seemed unlikely, therefore, that the haemoglobin in these larvae was ever used in the environment. Moreover, Walshe pointed out that the tissues of the larvae of *Tanytarsus* had such a high oxygen demand that they became dependent on oxygen at blood oxygen tensions far higher than would cause dissociation of their oxyhaemoglobin.

Though there is no obvious function for the blood-pigment in animals from well oxygenated conditions, the different authors agree that the haemoglobin in chironomid larvae found in stagnant water is functional. It allows the larvae to be independent of oxygen down to low concentrations; so enabling them to live in depleted oxygen conditions. The extent to which the haemoglobin in the larvae of *Chironomus riparius* is responsible for their measured oxygen independence was, therefore, investigated.

A comparison was made between the respiratory curves of normal larvae and larvae poisoned with carbon monoxide, using the dose levels recommended by Ewer, 1942. A result is shown in figure 18 .

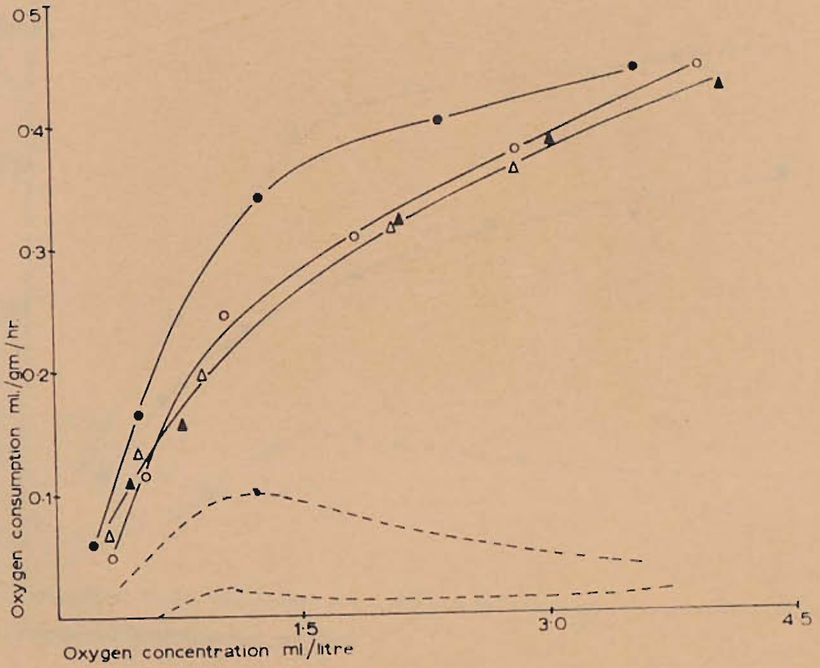
The oxygen consumption of the poisoned larvae remained the same as that of the normal ones, at the higher oxygen concentrations, indicating that the concentrations of carbon monoxide used were not effecting the cytochrome system, in this species. The untreated larvae were completely independent of oxygen down to a Pc. at 1·45 ml. O_2/litre, at 21°C. After treatment with carbon monoxide they were still completely independent of oxygen down to approximately 2·0 ml. O_2/litre. Their haemoglobin is, therefore, not functioning in oxygen transport at concentrations above 2·0 ml. O_2/litre, when there must be enough oxygen carried in simple solution in the blood to adequately supply the animal's tissues. Below 2·0 ml. O_2/litre, however, the haemoglobin plays an increasingly important part in maintaining a normal respiration rate down to very low levels of oxygen. This increased ability to respire at low oxygen concentrations is traced as the difference in oxygen consumption of poisoned and untreated larvae at the base of figure 18 . The increased oxygen uptake made possible by the presence of a functional blood pigment, increases as the oxygen content of the water decreases, down to very low levels, with a peak at approximately 0·5 ml. O_2/litre.

The presence of haemoglobin in the larvae of INSERT C. riparius arius is, therefore, of great importance in the determination of their relative respiratory dependence, and is probably the most important factor contributing to their marked resistance to low environmental oxygen concentrations. The poisoned larvae, were however, still independent of oxygen concentration down to approximately 2·0 ml. O_2/litre. Assuming that the whole of their blood-pigment was rendered functionless in this experiment, this indicates that other factors are involved in controlling respiratory dependence in this species. The transport of oxygen from their respiratory surfaces to their tissues seems to be relatively efficient even without haemoglobin. This compares with the measured oxygen independence of the larvae of Anatopynia varia, (Walshe, 1949). This species has a Pc. at approximately 3·0 ml. O_2/litre, and is able to live in stagnant ditch water, though its blood contains very little haemoglobin.

A factor which may possibly contribute to the measured oxygen independence of the chironomid larvae living in stagnant water is their relatively low level of oxygen consumption at high oxygen concentrations. Walshe 1949, comparing the respiration curves of larvae from still and moving waters, found that the totally dependent respiration of the species from well aerated conditions was linked to a correspondingly high level of oxygen consumption at the higher oxygen concentrations. Their basal metabolism appeared higher and she postulated a difference in "cellular respiratory systems" in the species from the two habitats. It is indeed possible that part of the process of physiological adaptation required for an animal to live in conditions of deficient oxygen, is a gearing of its enzyme systems to a new, lower basal metabolic rate. Prosser et. al. , 1957, found that goldfish acclimated for several days to low oxygen had a lower Pc. and standard metabolic rate than before the acclimation.

Plate, 4. Male and female C. diaphanus kept in low oxygen concentrations.

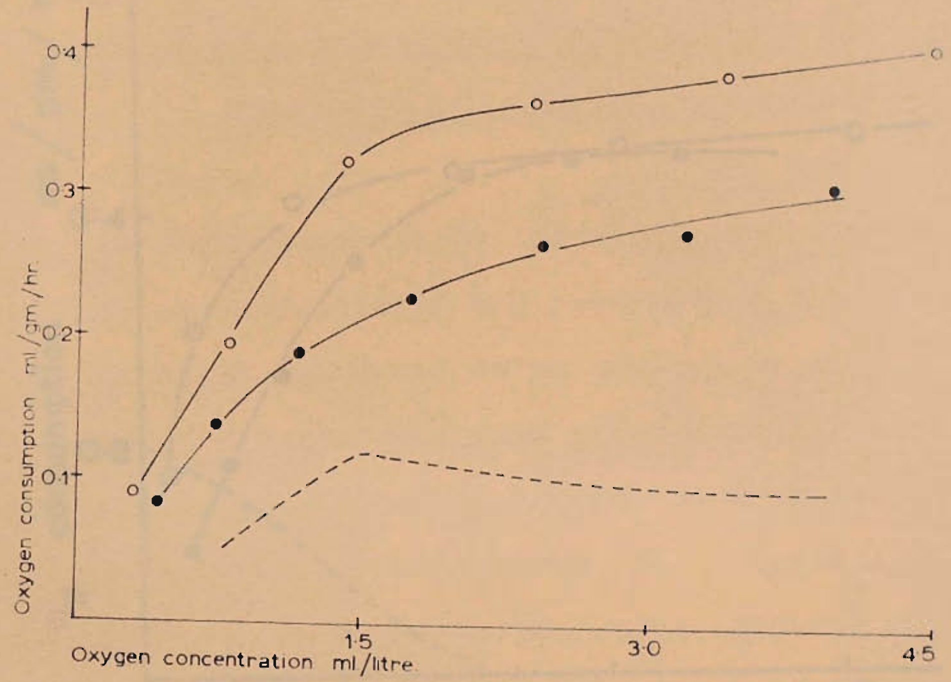
The female is more red in colour than the male, due partially to the higher concentration of haemoglobin in her body fluids.



figure, 16. The oxygen consumption of <u>C. diaphanus</u> in decreasing oxygen concentration, before and after acclimation to low oxygen concentrations.

▲ , female; Δ , male, from air saturated water.
● , female; o , male, from low oxygen concentrations.

The females acclimated to low oxygen concentrations had a lower Pc. and much higher rate of oxygen consumption than the animals from air saturated water. The difference between the rates is demonstrated by the upper divided line at the base of the figure. The males consumption did not change as markedly, the difference being traced by the lower divided line. 4 results for each curve(i.e.I6 expts.).

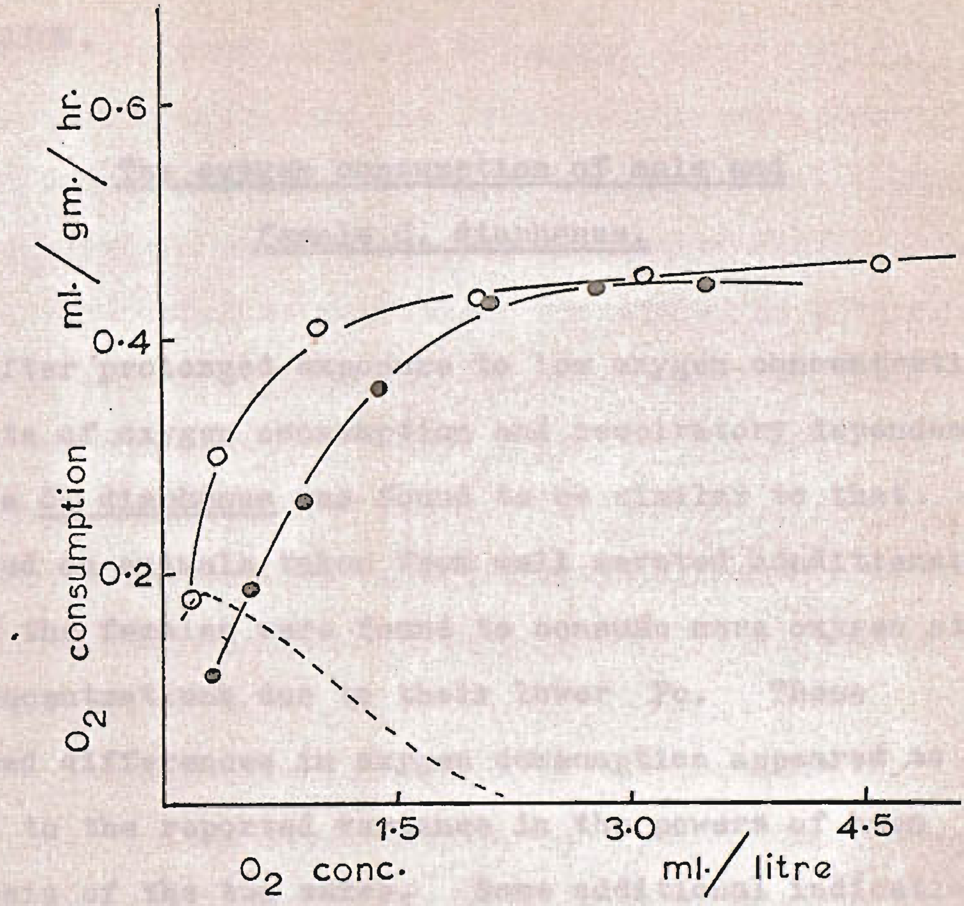


figure, 17. The effect of a 2% carbon monoxide solution on the oxygen consumption of female C. diaphanus acclimated to low oxygen concentrations.

o , normal females ● , females treated with CO.

The increase in its Pc. after poisoning indicated that the position of its normal Pc. depended partly upon the presence of functional haemoglobin in its body fluids. The divided line traces the difference between the two respiration rates and shows that the higher uptake made possible by a functional blood pigment was greatest at an oxygen concentration of approximately 1·5 ml./litre.

From 3 complete experiments tracing the consumption of different batches of females before and after CO treatment.



figure, 18. The effect of a 5% solution of carbon monoxide on the oxygen consumption of <u>C. riparius</u> larvae.

Their rate of oxygen consumption at high concentrations was unaffected. Their Pc. increased after poisoning demonstrating the functional significance of their haemoglobin in allowing a high rate of oxygen consumption at low oxygen concentrations. The divided line traces the low functional range of the blood pigment, which is most effective at approximately 0.5 ml. O$_2$/litre.

From 4 complete experiments.

DISCUSSION.

The oxygen consumption of male and female C. diaphanus.

After prolonged exposure to low oxygen concentrations the rate of oxygen consumption and respiratory dependence of male C. diaphanus was found to be similar to that measured on animals taken from well aerated conditions; whilst the females were found to consume more oxygen at low concentrations due to their lower Pc. These measured differences in oxygen consumption appeared to be linked to the reported variance in the powers of haem synthesis of the two sexes. Some additional indications of the different activity of the two sexes and its possible ecological significance were observed.

An experiment was conducted in which two males and two females were allowed to exhaust all the available oxygen in the same closed vessel. The males showed the first signs of distress, seen as a flexure of the body and a breakdown in the metachronal rhythm of their appendage movements, at 1·78 ml. O_2/litre. They were motionless at oxygen concentrations below 1·00 ml. O_2/litre, and did not survive the experiment. Meanwhile, the females showed no visible signs of distress right down to their residual level of oxygen concentration, below 0.5 ml. O_2/litre, and survived the experiment without apparent harm. In experiments on the locomotion of C. diaphanus, to be described in a later section, the females were found to move their appendages at a higher frequency and to swim faster than the males, at low oxygen concentrations. Female C. diaphanus, when adapted to life in conditions of depleted oxygen, are, therefore, more active and survive longer than males, when exposed to low oxygen concentrations; despite their extra burden of shelled eggs.

The ecological significance of these sexual differences in the resistance to low oxygen can only be a matter for conjecture. Oxygen concentrations low enough to limit the respiration of unadapted animals were recorded in the ponds studied, and are described in an earlier section. This adaptation is, therefore, potentially useful in the environment. One male may fertilize many females, so that it is of a greater survival value for the females to be resistant to these low oxygen concentrations, than the males, because of their greater individual importance in the maintenance of the population. This does not, however, constitute a reason for the males being largely incapable of adaptation. Also the females normally carry a supply of fertilised, and shelled eggs in the egg-pouch, and their possibly prolonged survival in bad conditions may serve to concentrate the distribution of the eggs in the deeper parts of the pond, before it dries up completely. When the pond is refilled the bulk of the eggs will then hatch immediately, without the necessity of the pond being completely full.

The ability of the males to survive for long periods in an oxygen concentration below 2·0 ml. O_2/litre, despite their apparent lack of adequate physiological adaptation, may be partially due to their not having to carry a large quantity of eggs. They do, however, show a behavioural response. Whenever the oxygen content of the water in the stock tanks became exceptionally low the males tended to congregate close to the surface. On occasions they were observed to swim right on the air/water interface, disturbing the surface with their appendages. This behaviour was not common in the females which swam in all parts of the tanks even when the oxygen concentration was very low. Fox, 1949 reported a similar response to low oxygen in Triops cancriformis (Breb) . Swimming very close to the surface probably serves to bring oxygenated water to the respiratory surfaces.

The functional significance of haemoglobin in C. diaphanus and the larvae of C. riparius.

Both C. diaphanus and the larvae of C. riparius have haemoglobin which functions at low oxygen concentrations and contributes to their marked independence of environmental oxygen concentration. There is, however, a difference in the functional range of the blood pigments in the two species. In female C. diaphanus oxygen consumption with functional haemoglobin is higher than without over a wide range of oxygen concentrations, with the greatest difference at approximately 1·5 ml. O_2/litre. The respiration of the chironomid larvae does not change after treatment with carbon monoxide, until approximately 2·0 ml. O_2/litre and their oxygen consumption with functional haemoglobin is progressively higher than without up to a peak at an external oxygen concentration of approximately 0·5 ml. O_2/litre.

The oxygen concentration at which maximum advantage is being gained from the possession of haemoglobin is, therefore, much lower in the chironomids than in C. diaphanus. This difference cannot yet be explained physiologically for these two species, though it is likely to be due to their possession of haemoglobins with different unloading tensions. Similar differences in the functional range of haemoglobin have been described in fish by Harnisch, 1951. He noted that the haemoglobin in *Cyprinus carpio* is still 95% oxidised at an oxygen pressure of 100 mm. Hg , whereas that of *Salmo trutta* is 50% dissociated at that pressure. These large differences in the oxygen tension at which the haemoglobins of these two fish are dissociated he believed would contribute to their very different respiratory dependence curves, and fit them for life in their characteristic habitats of still and moving water.

In the same way it is possible to speculate on the ecological significance of the differences between the respiratory dependence curves of *C. diaphanus* and the larvae of *C. riparius*, with and without functional haemoglobin. The larvae of *C. riparius* live in tubes in the mud at the bottom of the pond. Measurements made in small ponds by various workers, including the author, and described in an earlier section, showed that the oxygen concentration of the water often drops to very low levels. Local effects at the mud surface will, however, result in a drop in oxygen concentration to even lower levels, (Brundin, 1951) and the effect is especially marked in clumps of rooted vegetation (Buscemi, 1958) which are common in the ponds from which the larvae were collected. The larvae have, therefore, to live in extremely low oxygen concentrations and their markedly independent respiratory dependence curve shows that their metabolism has become adapted for this purpose. The synthesis of haemoglobin which functions at extremely low oxygen concentrations represents one of the ways in which they are adapted to life in these conditions.

C. diaphanus is a free-swimming filter-feeder usually found near the surface of the pond, or in the spaces between patches of aquatic vegetation. During the day it swims in water containing fairly high concentrations of oxygen. At night, however, the oxygen content of the whole pond may fall, as described in an earlier section. The physiology of C. diaphanus is, therefore, adapted to function in conditions of fluctuating, often quite low, oxygen concentrations. Exposure to low oxygen concentrations for prolonged periods, in the laboratory, led to a decreased respiratory dependence in female C. diaphanus (see figure 16). One of the major factors contributing to their lower Pc. was found to be the presence of an increased amount of haemoglobin in their blood. C. diaphanus has the facultative ability to synthesise haemoglobin in conditions of oxygen depletion, and high concentrations were observed in the blood of the natural populations during the periods of Summer stagnation. The haemoglobin in this species was found to be most effective at oxygen concentrations around 1·5 ml. O_2/ litre. This represents an adaptation to the low oxygen content occurring regularly in the water of the small ponds, but not to the very low concentrations found at the mud surface.

C. diaphanus swims freely around in the water mass and can easily avoid very low oxygen concentrations by moving into parts of the pond where it is higher. This ability is of especial importance to the males, because of their apparently less effective physiological adaptation to low oxygen concentrations. The chironomid larvae are, however, by no means trapped in the mud. They were observed to extend their tubes up from the mud surface when the water was particularly stagnant, and sometimes left their tubes and swam for short periods at the water surface. The activity required was, however, probably too high to give this behaviour the significance attributed to it by Pause, 1919, who believed that the larvae replenished an oxygen store in this way which would last them for several hours.

The different resistances of the two species to periods of anoxia.

The different physiological adaptations of C. riparius larvae and C. diaphanus, to their separate ecological niches within the temporary ponds is reflected in their reactions to periods of anoxia. These differences were first noted when, at the end of an experiment in the closed respirometer, the animals were left at their residual oxygen level for some time. Both sexes of C. diaphanus did not survive a period even as short as 30 minutes. Once their movements were arrested by oxygen starvation they seldom recovered; in the males this took place just before, in the females just after they reached their residual level. The larvae of C. riparius were, however, unharmed by long periods at their residual oxygen concentration, and survived, completely motionless, when kept overnight in deoxygenated water. When replaced in air saturated water they went through a period of intense irrigation activity, as described by Walshe, 1950.

An investigation was made of the effects on the two animals, of exposure to oxygen concentrations below their Pc.'s.

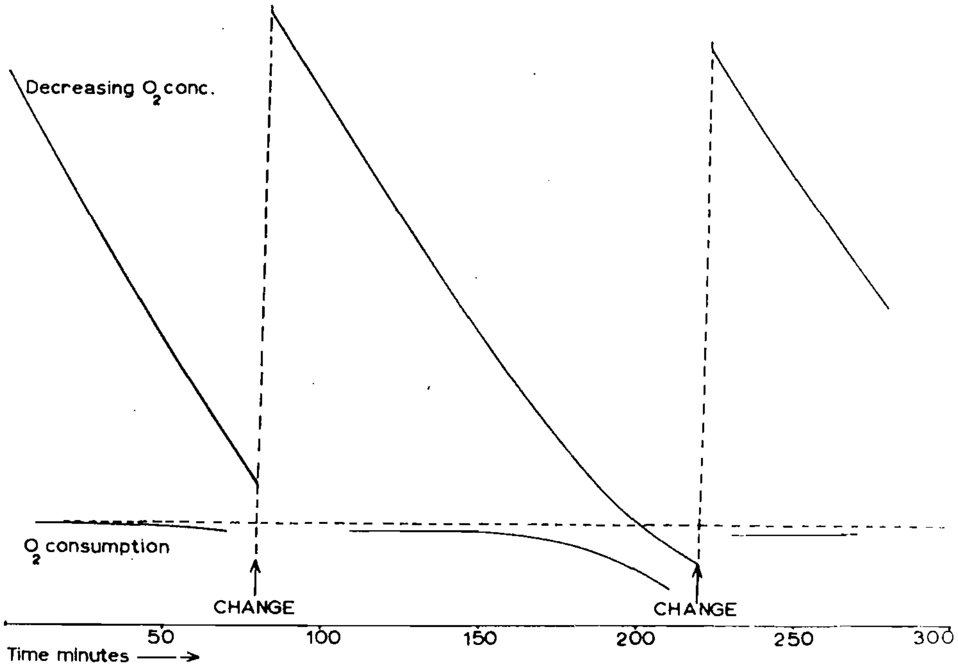
The animals were allowed to exhaust the oxygen in the closed respiration chamber, then oxygenated water was injected from a hypodermic syringe to increase the oxygen concentration to above their Pc.'s.

The oxygen consumption of C. diaphanus gradually decreased at each run, see figure 19 , indicating a deterioration in their condition, and there was no sign of an oxygen debt repayment. The increase in oxygen concentration had to be made before their residual level or the animals failed to survive the experiment.

When C. riparius larvae were exposed to a decreasing oxygen concentration, then to a sudden increase (see figure 20) their oxygen consumption increased markedly at first, indicating the repayment of an oxygen debt and increased activity similar to that described by Walshe, 1948(a) .

There is an obvious ecological significance in the ability of C. riparius larvae to survive prolonged anoxia, and to repay some part of an oxygen debt. They live in mud tubes where the oxygen concentration often falls to extremely low levels, and by ceasing activity, as described by Walshe 1950, and accumulating an oxygen debt, they can survive the periods when oxygen is particularly low. This ability enables chironomid larvae to be one of the few animals able to live in the deeper waters of eutrophic lakes, where they are often, in the absence of competition, very abundant (Harnisch, 1951) .

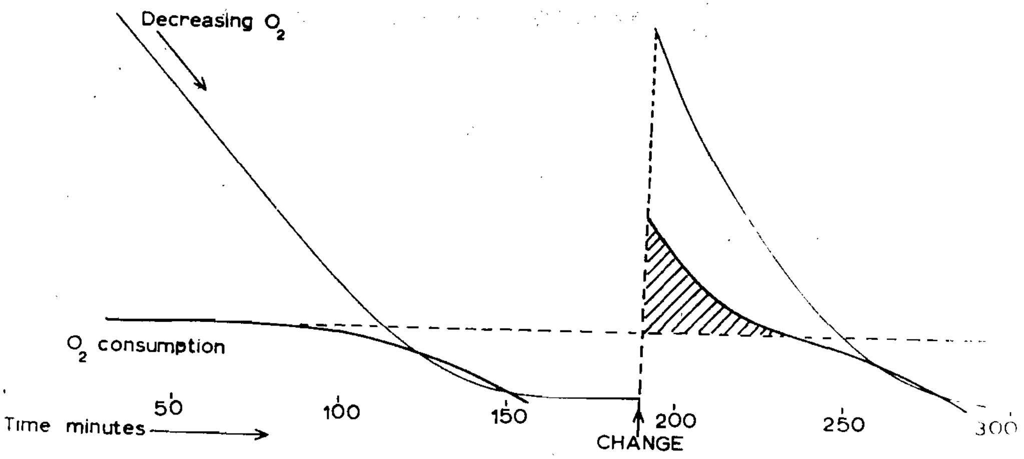
The low resistance of C. diaphanus to anoxia is possibly due to their continuous, high activity. The levels of activity produced in these experiments did not appear to be much above those normally found in animals swimming freely in the environment. The chironomid larvae respired during the experiments at approximately the same active rate per gram of tissue, as C. diaphanus, at room temperatures. When, however, they were protected from the effects of water movement by a layer of fine gauze above the stirring bar, and experiments were done in the dark, they had a much lower and irregular respiration rate which was independent of oxygen to a lower concentration. This lower level of oxygen consumption could be simulated by drugging the larvae with M.S.222 , and probably approximates to the random activity of the larvae when inside their tubes. Some experiments on randomly active larvae are described in a later section.



figure, 19. The oxygen consumption of <u>C. diaphanus</u> after exposure to very low oxygen concentrations.

The animals were allowed to exhaust the oxygen in the closed respirometer then returned to air saturated water. Their oxygen consumption decreased after each change indicating that they were unable to repay an oxygen debt and that exposure to very low oxygen concentration decreased their activity.

From 3 complete experiments.



figure, 20. The oxygen consumption of *C. riparius* larvae after exposure to very low oxygen concentrations.

The larvae survived prolonged exposure to their residual oxygen concentration and when returned to air saturated water showed an increased consumption (hatched area), indicating possible repayment of an oxygen debt and reflecting an increase in activity intended to ventilate their mud tubes.

From 5 complete experiments.

Oxygen consumption in the presence of high concentrations of carbon dioxide

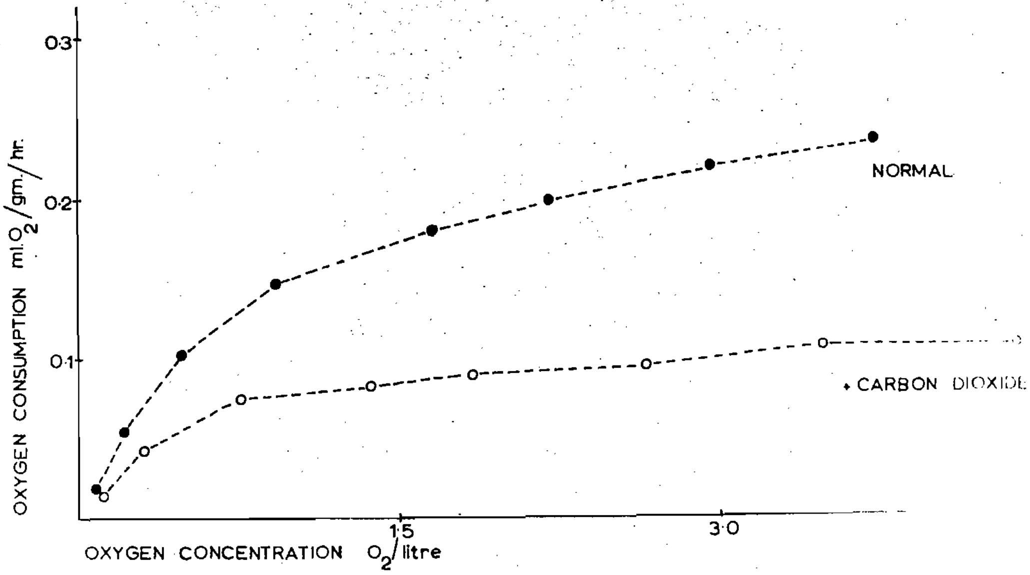
The recorded low p.H. and alkalinity titrations established that carbon dioxide concentrations of (total CO_2 + HCO_3 etc.) approximately 50 mg./litre were reached in temporary ponds during the summer. The oxygen consumption of many aquatic animals is reduced by high CO_2 concentrations (Prosser and Brown, 1961). One of the most important biological effects of high CO_2 in animals is the Bohr effect on the dissociation of blood pigments. Aquatic animals vary in their response to high concentrations of CO_2 and this variation seems to be correlated with their levels of activity and their characteristic environment. Scheer, 1963 reported that highly active fish such as mackerel and the salmonids, which live in well aerated conditions, show a large Bohr effect; whilst the effect is small in sluggish, still-water species such as the carp and catfish.

The two species from the temporary pond habitat both live in still-water which often contains low oxygen and high CO_2 concentrations. They differ, however, in their levels of random activity. C. diaphanus swims continuously whilst C. riparius larvae, when inside their tubes, undergo alternating periods of activity and rest (Walshe, 1950), resulting in a relatively low rate of oxygen consumption (see figure 34).

Immersion in a 10% solution of CO_2 in water with an oxygen concentration near air saturation level at room temperature, soon halted the swimming of C. diaphanus but had no visible effect on the C. riparius larvae. The effects of high CO_2 on the oxygen consumption of the two species are shown in figures 21 and 22.

When placed in a 5% solution of CO_2 (approximately 90 mg./litre) C. diaphanus consumed oxygen at approximately half the rate measured at air saturation. Later experiments detected a similar effect of high CO_2 on its swimming speed. The oxygen consumption of C. riparius larvae was relatively slightly decreased in a 10% solution of CO_2. In neither species was there any marked change in their Pc.'s at high CO_2 concentrations.

C. diaphanus with its relatively high level of activity is therefore, markedly sensitive whilst the less active C. riparius larvae are extremely resistant to high CO_2 concentrations. The unusually high resistance of the chironomid larvae to low oxygen and high CO_2 allows them to live in the richly organic detritus. C. diaphanus is able to escape the higher CO_2 concentrations near the substratum by swimming close to the water surface.

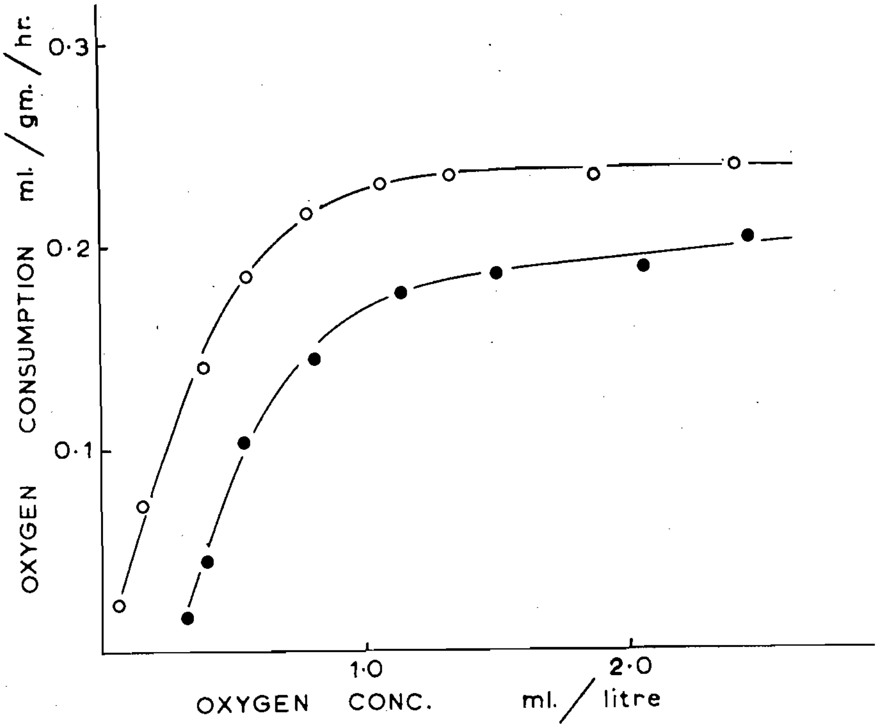


figure, 21. The oxygen consumption of <u>C. diaphanus</u> in high carbon dioxide concentrations.

Its rate of oxygen consumption was approximately halved by exposure to a 5% solution of CO_2.

(in Southampton tap-water.)

From 2 complete experiments.



figure, 22. The oxygen consumption of C. riparius larvae in high carbon dioxide concentrations.

Exposure to a 10% solution of CO_2 resulted in only a 20% reduction in their rate of oxygen consumption.

From 3 complete experiments.

THE EFFECTS OF TEMPERATURE ON THE OXYGEN CONSUMPTION OF C. DIAPHANUS AND THE LARVAE OF C. RIPARIUS.

INTRODUCTION.

In small bodies of freshwater, such as the temporary ponds inhabited by Chirocephalus diaphanus Prevost, environmental conditions are very variable. The wide range of temperature variation in these ponds is described in an earlier section. As the animals studied are relatively small invertebrates their body temperatures will fluctuate with the environment, particularly as they respire over external gill-like respiratory surfaces. Brett, 1956 stressed that among aquatic gill-respiring animals there is no escape from environmental temperature, as the tissue temperature and environmental temperature are constantly equilibrated at the gill surfaces. The internal temperature of the animals in the temporary pond habitat will, therefore, fluctuate widely, and their metabolism must compensate for, or be resistant to, these fluctuations for the animals to survive.

"Since temperature is a measure of molecular agitation, it limits the rate of chemical reactions, and is one factor in the control of growth and metabolism. Temperature limits the distribution of animals and at the same time determines their rate of activity." Prosser and Brown, 1961.

A large volume of literature exists on the temperature relations of animals. Much of it is concerned, however, with tolerance limits, rather than the effects of temperature on the rate functions of animals, and is not, therefore, directly applicable to the present study. Useful reviews of the work on the effects of temperature, on the distribution, survival and metabolism of animals are those of Brett, 1956 and Fry, 1957 referring only to the work on fish, and the more general reviews of Bullock, 1955 and Belehradek , 1957 .

The present study is concerned with the effects of temperature upon the respiration of C. diaphanus and the larvae of C. riparius . One intention was to investigate the effects of the measured environmental temperature range and fluctuations on the metabolism of the two species. This is of particular interest in this comparative study, as although the animals live in the same environment, because of their separate ecological microhabitats ~~niches~~, they experience very different temperature ranges. For instance in the measurements described earlier a diurnal fluctuation of 12°C in the water mass, during a June heat-wave, resulted in a measured fluctuation of only 5°C in the surface layers of the mud. The chironomid larvae may, therefore, be buffered against extreme temperature fluctuation by the high specific heat and low thermal conductivity of the surrounding mud and water.

Bullock, 1955 reported physiological differences in heart rate and gonad maturation in limpets and Mytilus from intertidal stations less than 1·5 metres apart. The significant perameter of the intertidal difference appeared to be temperature; the higher tide specimens acting as though adapted to higher average temperatures. Similar "microgeographic physiological differences" may be expected in the two species from the temporary ponds.

Macan, 1961 stressed the value of working at a series of temperatures when studying the oxygen requirements of animals. As much of this study is concerned with the respiratory dependence of the two species, the effects of temperature on their oxygen consumption is important. Temperature represents one of the most important external factors limiting the respiratory dependence of animals. Fry and Hart, 1948 in their work on the goldfish, found that the critical oxygen concentration and minimum tolerable oxygen concentration, are higher as the temperature rises. No study of the physiological adaptations responsible for an animal's independence of environmental oxygen is, therefore, complete without an investigation of the effects of temperature as it is the environmental factor most directly responsible for activity changes and possible physiological stress.

There were, therefore, two aims in this investigation; one ecological the other physiological. They were:

(a) to study the effects of measured environmental temperature conditions on the activity of the animals.

(b) to investigate the limiting effects of temperature upon the rates of oxygen consumption and the respiratory dependence of the two species.

METHODS.

All individuals of both species were similar in origin and condition to those used in the earlier work on oxygen consumption. C. diaphanus was maintained in well aerated tanks at all temperatures, to prevent the different physiological adaptations of the two sexes to low oxygen levels, from affecting the results. Previous to any experiment the animals were fully acclimated to the initial experimental temperature. This was achieved by maintaining them at this temperature until they reached a stable physiological state, as indicated by the stability of their rate of oxygen consumption. The time allowed was never less than fourteen days, though a much shorter time was found to be sufficient at the higher temperatures.

The effects of size variation on the oxygen consumption of both species was compensated for as described earlier. Their respiration was measured polarographically in either the open or closed systems, as indicated in each section.

RESULTS.

I. The relation of the oxygen consumption of active animals to environmental temperature and oxygen concentration.

The oxygen consumption of animals acclimated to a series of temperatures between 5° and 30°C was measured over a range of oxygen concentrations in the closed respiratory chamber. The usefulness of results from this type of experiment was discussed and vindicated in an earlier section. Both species were maintained at high levels of activity throughout the experiments, using the same conditions as were described in the section on respiratory dependence.

The rates of oxygen consumption of active animals in relation to oxygen concentration, found at the different temperatures investigated, are shown in figures 23 & 24 , where the rate of oxygen uptake is plotted against oxygen concentration. The most readily observable effect on both species is to alter their rates of oxygen consumption, which increase with temperature at all oxygen concentrations. In order to examine this effect of temperature on oxygen uptake, independent of the effects of oxygen concentration, it was measured at a level above the animal's critical oxygen concentration at all temperatures. The effect of temperature upon the rates of oxygen consumption at an oxygen concentration of 3·0 ml/litre, is shown in figures 25 and 26.

Figure 25 shows the oxygen consumption of C. diaphanus between 5° and 25°C. The scattered distribution of the points is due to the complication of the effect of size on respiration rates, discussed in an earlier section. The points through which the line is drawn represent the oxygen consumption of hypothetical animals with an individual weight of 0·05 gm. These values were obtained by inspection of the results for the effect of size on respiration shown earlier (see figure 9), and only represent an approximation of the size compensated rate of consumption at each temperature. This does, however, give a truer indication of the pattern of respiration with temperature than would be obtained from a mean line through all the points, though the size variation was not very large and the points are grouped around the line.

The effect of temperature on the rate of oxygen consumption of the larvae of C. riparius is shown in figure 26. There is less scatter of the points, as the size factor was easily eliminated by working on the final instar only, all the individuals being of very similar size.

In both species, the rate of oxygen consumption of active animals acclimated to the experimental temperature, increases with rising temperature. The rate of increase is, however, different in the two species. This is seen to be due to the relatively higher rate of oxygen consumption of *C. diaphanus*, at the low temperatures. This difference is emphasized in figure 27 where the two curves are plotted as the log velocity of the respiration rate, against temperature. The $Q_{10's}$ taken from these curves are 1·68 for *C. diaphanus*, and 2·99 for *C. riparius*. .

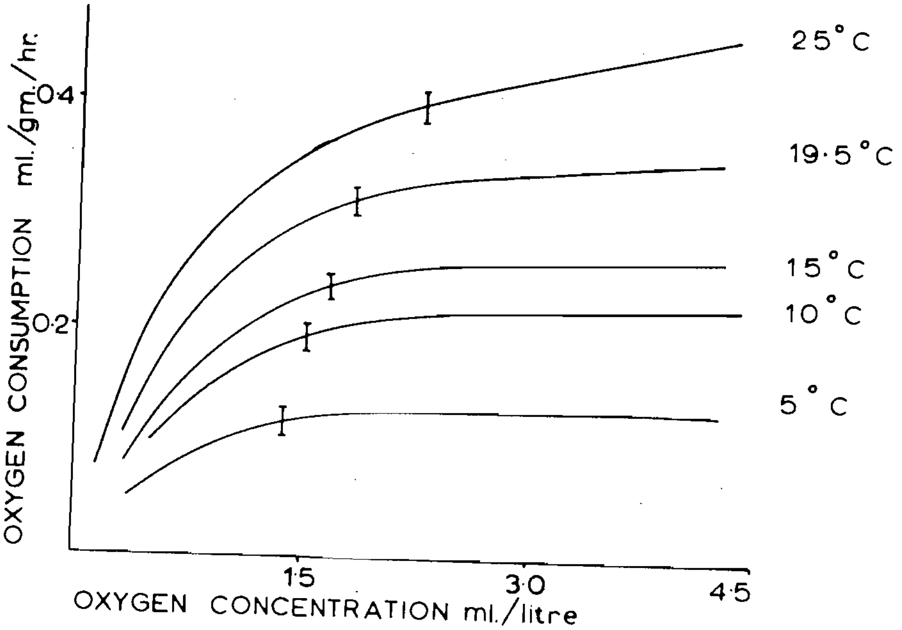
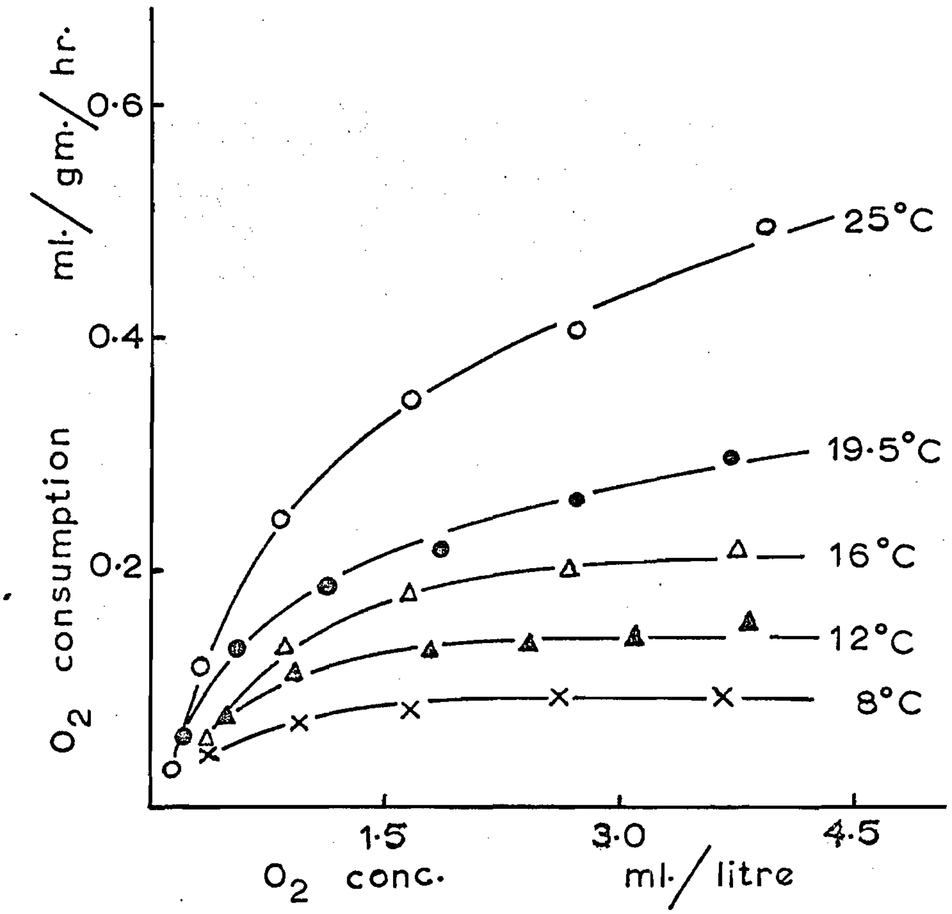


figure 23. The respiratory dependence curves of <u>C. diaphanus</u> at a range of temperatures between 5° and 25°C.

Its rate of oxygen consumption increased with temperature at all oxygen concentrations. It also became progressively more dependent on environmental oxygen concentration as the temperature increased, as is indicated by the approximate position of the Pc. at each temperature, shown as I.

The lines trace the range of oxygen consumption and Pc. predicted for animals of 0.05 gm. wet weight, after examination of 24 experiments in the closed chamber respirometer.



figure, 24. The oxygen consumption of C. riparius larvae at temperatures between 5° and 25°C.

Both their rates of oxygen consumption and Pc.'s increased with temperature.

5 curves illustrating the changes in oxygen consumption and Pc. with temperature, selected from 20 experiments in the closed chamber respirometer.

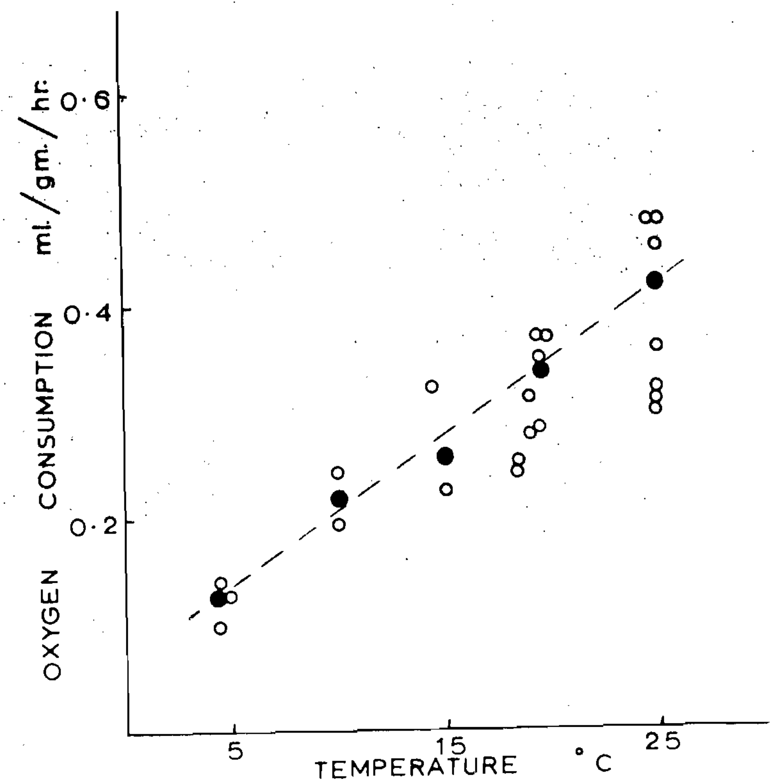


figure 25. The effect of temperature on the rate of oxygen consumption of C. diaphanus at an oxygen concentration of 3·0 ml./litre.

Its rate of oxygen consumption, estimated for animals of 0·05 gm. body weight, increased almost linearly with temperature. The Q_{10} was approximately 1·68.

Points taken from 22 experiments in the closed chamber respirometer

Mean at	25° C	.387 ± .082	ml/gm/hr.
	I9.5°C	.308 ± .050	ml/gm/hr.
	5°C	.I25 ± .046	ml/gm/hr.

The wide dispersion of probability about the mean is due to size variation. The line traces size compensated values(see pg. 9I).

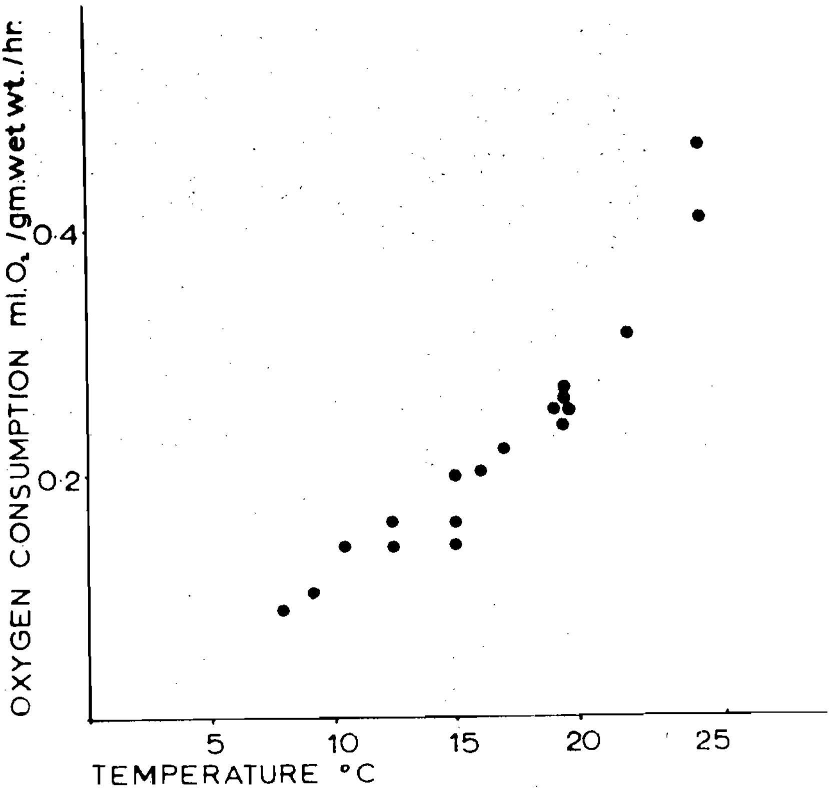


figure 26. The effect of temperature on the rate of oxygen consumption of the 4th. larval instar of C. riparius, at an oxygen concentration of 3.0 ml./litre.

Its rate of oxygen consumption rose sharply with temperature, from a low level at 8^{o}C, with an approximate Q_{10} of 2·99.

Points taken from I8 experiments in the closed chamber respirometer.

Mean at I9.5 C .2536 ± .0035 ml/gm/hr.

The narrow dispersion of probability obtained from 5 experiments at I9.5 C demonstrates the potential accuracy of this method for measurements on a large number of individuals of a similar size.

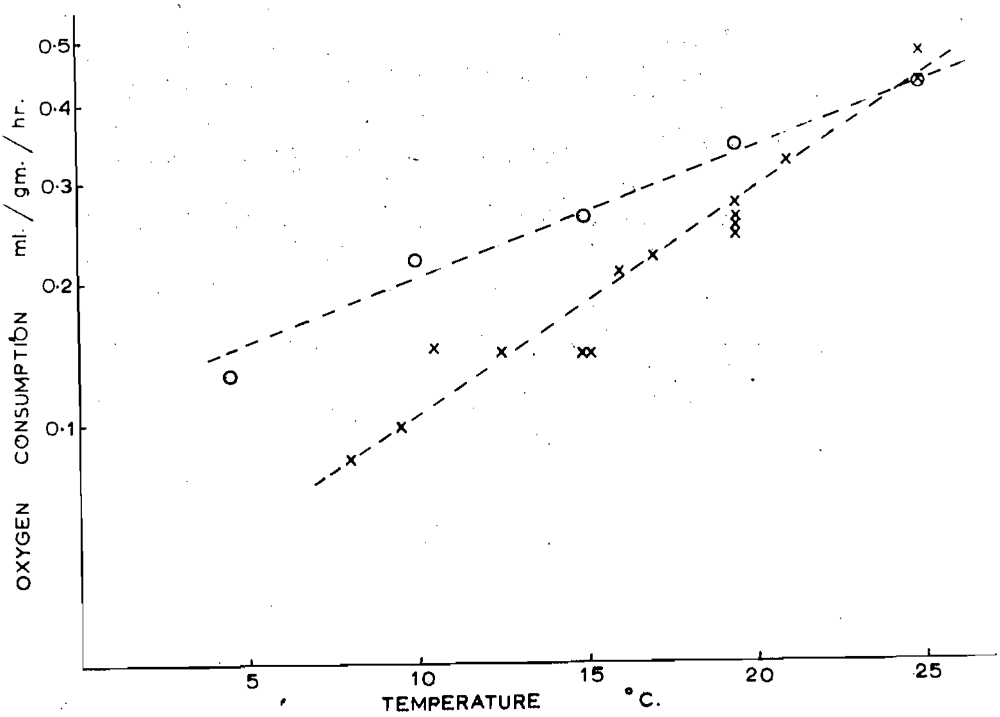


figure 27. The effect of temperature upon the rate of oxygen consumption of C. diaphanus and larvae of C. riparius, at an oxygen concentration of 3·0 ml./litre.

C. diaphanus, O has a lower Q_{10} than the C. riparius larvae, X due to its higher rate of oxygen consumption at the lower temperatures.

DISCUSSION.

This well defined difference may be important in attempting to clarify the physiological factors involved in oxygen utilisation in each species. "In general the physical properties of solutions are less sensitive to temperature change and have a Q_{10} between 1 and 2 ; chemical reaction rates are usually more than doubled per 10°C rise, hence the Q_{10} is between 2 and 3" (Prosser and Brown 1961) .

The high Q_{10} value obtained from the temperature acclimated chironomid larvae indicates that temperature is effecting their metabolism right at the enzymic level. This would account for the Q_{10} being characteristic of a chemical reaction. This implies that the supply of oxygen to the tissues is not a limiting factor in the respiration of this species, possibly due to the abundance of haemoglobin in their haemolymph. It also implies, however, that the larvae possess no mechanisms for temperature compensation at the cellular level, of the type described by Precht, 1951 . The steep drop in oxygen consumption at the lower temperatures is reflected in observations on their activity. C . riparius larvae collected from ponds at $0^{\circ} - 5^{\circ}C$ were motionless, but showed a high level of ventilation activity when their temperature was increased in the laboratory.

Conversely, *C. diaphanus* was observed to swim fairly actively at low temperatures. Measurements to be described in a later section, demonstrated that the swimming speed and appendage movements parallel the curve for oxygen consumption, in their response to temperature. Thus *C. diaphanus* is able to remain relatively active when acclimated to low temperatures. Evidence of a similar temperature compensation was described by Mayer, 1914 for *Aurel~~l~~ia aurita*. He found the pulsation rate of the jellyfish to be about the same in animals acclimated to 29°C and 14°C .

Bullock, 1955 reviewed the evidence for multiple levels of adaptive temperature regulation at biochemical, cellular, organ and behavioural levels in poikilotherms, any of which may partially account for this measured maintenance of high oxygen consumption and activity in *C. diaphanus*. This problem is discussed in greater detail in the later section on temperature acclimation.

This high rate of activity at low temperatures may be of ecological importance, as stocks of *C. diaphanus* survived particularly well in the laboratory at low temperatures, and appeared less prone to disease than those in warmer water. The high level of activity may be necessary in order to maintain a certain minimum speed of appendage movement to produce swimming and feeding currents of sufficient strength. Also the animals may require to ventilate their respiratory surfaces continuously as they seem incapable of building up an oxygen debt in poor conditions, in the way that the chironomid larvae do (see figures 19 & 20). The rapid ventilation movements of the larvae when first removed from cold conditions indicates that they were living in a state of internal oxygen depletion.

The low Q_{10} of the respiratory rate in C. diaphanus is not, however, due only to its high activity at low temperatures. There must be some reason why the animal does not show a greater increase in metabolism at the higher temperatures and so feed, reproduce and dominate its' habitat faster. This ability would be a particular advantage to an animal living in temporary habitats where the time available for growth and reproduction is often very short.

Bullock, 1955 discussed this question. He thought the simplest answer to this problem was one of balance between the separate regulatory processes; "something is not proceeding as fast as oxygen consumption, so that the whole machine is unable to keep up the high rate." In the case of C. diaphanus the low Q_{10} , below 2 , points to some physical process limiting the rate of metabolism at higher temperatures. As the specimens used in these experiments did not have high concentrations of haemoglobin in their blood, the limiting factor may be part of the process of oxygen uptake and distribution, which will be dependent on diffusion and simple solution in the body fluids. The role of the blood pigment in regulating oxygen uptake is discussed at greater length in the earlier section on the control of respiratory dependence.

RESULTS (continued) .

Inspection of the results shown in figures 23 and 24 reveals another factor in the animals' respiration which is affected by temperature. They show an increased respiratory dependence at the higher temperatures, observable as an increase in the level of their critical oxygen concentrations (Pc) . Fry and Hart, 1948 , described a similar effect of temperature on the maximum steady rate of oxygen uptake in relation to oxygen tension, in the goldfish.

The changes in Pc. with temperature are summarised for both C. diaphanus and the larvae of C. riparius in figure 28 . The values were obtained from a series of curves similar to those shown in figures 23 & 24. The point at which the respiration rate first deviated from a straight line drawn through the oxygen independent part of each curve, marked the point at which respiration first became oxygen dependent, and was taken as the Pc.

Figure 28 shows that the Pc. rises with increasing temperature. It is also evident that the rate of increase parallels the effect of temperature on the rate of oxygen consumption, in each species (see figures 25 & 26) . The implication is that the Pc's. are, in both species, directly related to the animals activity, as reflected in their rates of oxygen consumption.

This relationship between the rate of oxygen consumption and the Pc. at the different temperatures, is shown, for both species, in figure 29 . The values for the Pc's. obtained by inspection as described above, are not accurate enough for any quantitative relationship to be measured. The grouping of the points into a linear distribution does, however, signify that there is a non-random relationship between the rate of oxygen consumption, which is a measure of activity, and the Pc. in both species.

Having discussed respiratory dependence in the two species at some length it is important to reaffirm their relative independence of environmental oxygen. Reference to figure 28 shows that in neither animal will the rate of oxygen consumption be limited by the higher oxygen concentrations, until temperatures above 25°C . This implies that oxygen supply to the tissues is relatively efficient in both animals; the processes involved were investigated in the earlier section on respiratory dependence.

A point of incidental interest is that a line joining the values for the Pc's. of C. diaphanus in figure 28 would cross the measured air saturation concentration, which falls off with increasing temperature, at approximately 40°C. In an early exploratory experiment on the effects of temperature on C. diaphanus, the animals upper lethal temperature was measured in the continuous flow respirometer. Animals acclimated to 20°C were introduced into the respiratory chamber and the temperature was gradually increased. After each increase of about 3°C the water was equilibrated with air and the electrodes recalibrated. Several readings of the animals oxygen consumption were then taken. At 38°C their oxygen uptake was extremely high, then at 40°C they stopped respiring and did not survive the experiment. Temperatures which are lethal in a short time are more extreme than those in which death is delayed, and animals can build up a resistance to high temperatures if allowed some time to acclimate during the increase. As the animals in this experiment were only exposed to each temperature for approximately 30 minutes the value for upper lethal temperature is not an accurate one. It is, however, the same value as previously quoted from figure 28 . It is possible, therefore, that heat death in C. diaphanus is due to asphyxiation.

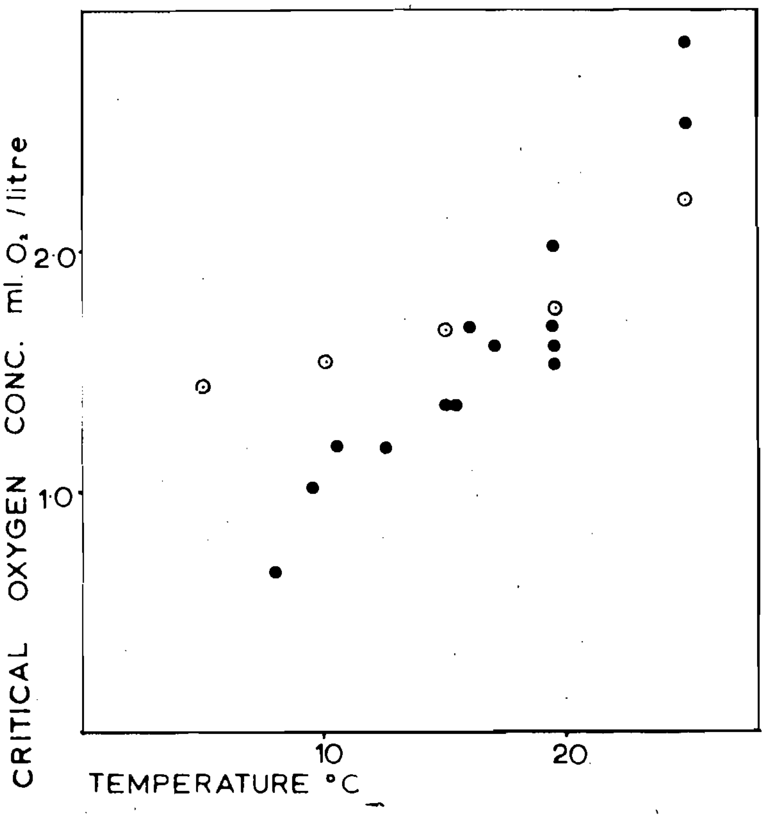
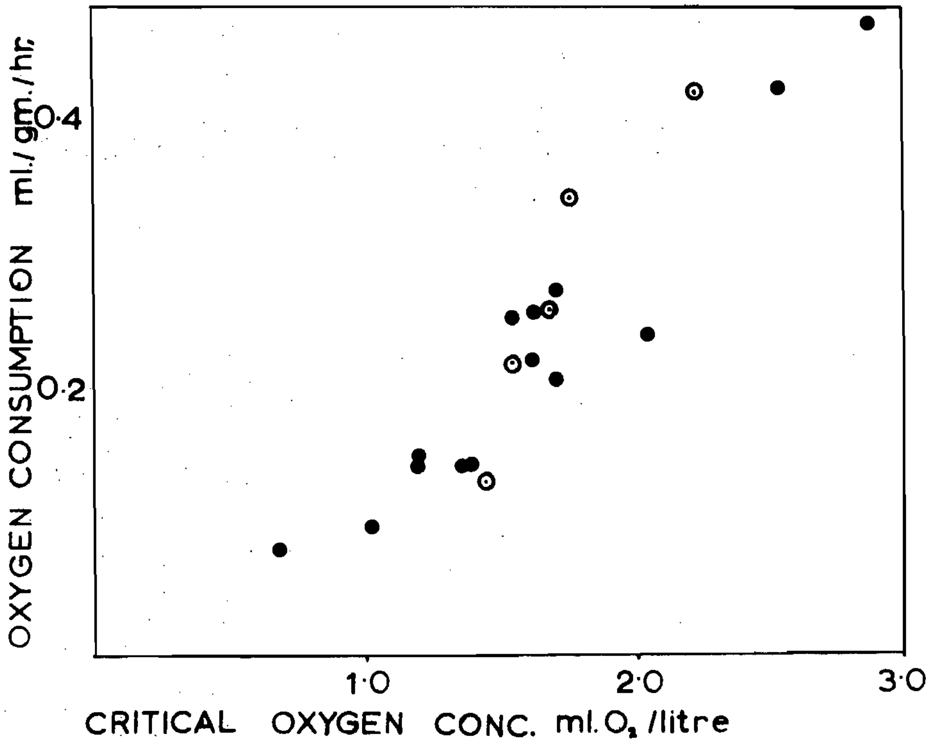


figure 28. The critical oxygen concentrations of *C. diaphanus* and larvae of *C. riparius* at temperatures between 5° and 25°C.

The Pc.'s of both species increase with temperature. The increase is greater for *C. riparius* ●, than *C. diaphanus*, 0 resembling the different responses of their rates of oxygen consumption.

Plot of Pc.'s from I4 experiments on *C.riparius* and the mean position of the Pc. at 5 temperatures, estimated from I8 experiments on *C.diaphanus*.



figure, 29. The relationship between rate of oxygen consumption and Pc. in C. diaphanus and the larvae of C. riparius.

o , C. diaphanus; ● , C. riparius larvae.

The approximately linear arrangement of the points for both species indicates that a direct relationship exists between their rates of oxygen consumption and Pc.'s, at different temperatures.

Replot of results shown in figures 25,26 and 28.

RESULTS.

II. Changes in the rate of oxygen consumption of the two species following a sudden increase in environmental temperature .

Poikilotherms show alterations in various functions, such as lethal temperatures, behaviour and biochemical activity, in accordance with their temperature of acclimation. When the temperature is raised or lowered abruptly all these functions undergo an immediate change, and eventually restabilize at a rate characteristic for the new temperature.

The measured diurnal temperature fluctuations in the temporary pond habitat, described in an earlier section, indicate the importance, to the members of the fauna, of their physiological process of temperature acclimation.

Experiments were conducted to study the reactions of C. diaphanus and the larvae of C. riparius to sudden temperature change, as revealed by their rates of oxygen consumption. A large batch of animals of each species were removed from the stock tanks, where they were acclimated to a mean temperature of 15°C , and placed in water of a higher temperature. Their rates of oxygen consumption were then followed for a period of seven days after the change.

The immediate changes in oxygen consumption of <u>C. diaphanus</u> during a sudden increase in temperature from 15° up to 29°C , were recorded in the continuous flow apparatus, (see figure 30) . Its rate of oxygen consumption markedly increased immediately after the change, then dropped with minor oscillations, to a new level above that recorded at 15°C . This initial response lasted for approximately 1½ hours. The slow drop in rate over the next six hours indicates that this new level of oxygen consumption is not the fully acclimated one, characteristic for the new temperature.

The slower process of complete acclimation was studied by a series of experiments in the closed respiration chamber. The response of both species to an increase from 15° to 25°C was traced as a series of curves plotting oxygen consumption against oxygen concentration (see figures 31 and 32) . There are two observable effects upon the oxygen consumption of both species. Immediately after the increase in temperature

(a) consumption is high over the whole range of oxygen concentrations, and

(b) respiration is almost totally dependent upon oxygen concentration, right up to air saturation levels.

As the processes of compensation progress the recorded rates of oxygen consumption and levels of critical oxygen concentration decrease, until they reach the values characteristic for each species at the higher temperature (c.f. figures23 & 24).

The changes in oxygen consumption, with time, at a single oxygen concentration of 3•0 ml/litre. , are plotted in figure 33 .

The initial 'overshoot' reaction is larger in C. diaphanus. This short term effect of a sudden temperature change has been studied by Grainger, 1956 , 1958 . He believed the response to be partially behavioural, involving changes in the activity of the animals after stimulation of receptors. As he detected an overshoot in the oxygen consumption of Artemia immobilised with ether, there are other factors involved in this response.

Grainger, 1956 believed the widespread occurrence of the temperature overshoot reaction suggested that the effect may be inherent in the organisation of some organisms or tissues. The possibility of a direct effect of temperature on activity at the cellular level was later borne out by Grainger's experiments on the eggs of Astacus pallipes, 1958 and bakers yeast, protozoa and strains of bacteria, 1960, (b) . He later demonstrated by his work on the homogenised brain and muscle tissue of *Rana temporaria* (Grainger, 1960 (a)), and a simple model of an enzyme system: (Grainger, 1960 (b)) that a temperature overshoot was shown by the biochemical reactions in cells. Grainger, 1958 discussed the mechanism of this transitional effect and concluded that the cell was reacting as a steady state system, since such overshoot effects may be found in theory during the transition periods between two steady states.

Both *C. diaphanus* and the larvae of *C. riparius* respond to sudden temperature change by an overshoot in their rates of oxygen consumption. Although its overshoot is larger the time taken to reach a fully acclimated state after a sudden temperature change is less in *C. diaphanus* than the larvae of *C. riparius*. In both species the oxygen consumption falls close to the new acclimated level in the first twelve hours. Twenty hours after a 10°C increase in temperature, *C. diaphanus* reaches a fully acclimated state, (see figure 33), whilst the slope of the divided line traces the slower acclimation of the *C. riparius* larvae over the next five days. It is important to note that although the temperature change was the same for both species the relative effects upon their rates of oxygen consumption may differ because of their different Q_{10}'s .

The slower change in oxygen consumption, after the initial overshoot reaction, represents the process of physiological temperature compensation, reviewed by Bullock, 1955 . There is some evidence to suggest that this process of thermal acclimation at the cellular level may result from a type of enzyme induction. Bullock quotes the work of Precht who found that brei or homogenates of isolated tissues from many acclimated animals show, besides an altered oxygen uptake, changes in the activity of several enzymes, including dehydrogenases, alkaline glycerophosphatase and peroxidase.

This establishment of a new level of oxygen consumption, after a change in temperature which resulted initially in a period of increased consumption (see figure 33) is an example of the type of acclimation designated as pattern III by Precht, 1958 . In this pattern the acclimated rate lies between the first stabilised value, reached a short period after the change, and complete acclimation, i.e. there is a partial compensation, leading to a new level of oxygen consumption at the increased temperature.

In addition to the decrease in the rate of oxygen consumption after the initial temperature overshoot there is a gradual change in the respiratory dependence of both species. This is observable in figures 31 and 32 as a progressive recovery from complete conformity of their oxygen consumption to the environmental concentration, recorded at the peak of the initial overshoot response, to the pattern of dependence characteristic of animals acclimated to the new temperature (c.f. figures 23 and 24) . This progressively increased independence of environmental oxygen concentration, after a sudden temperature increase, probably follows indirectly from the decreasing rate of oxygen consumption shown in figure 33 . A similar relationship between respiratory dependence and rate of oxygen consumption was described in the earlier section on the acclimated rates of oxygen consumption at a range of temperatures (see figure 29) .

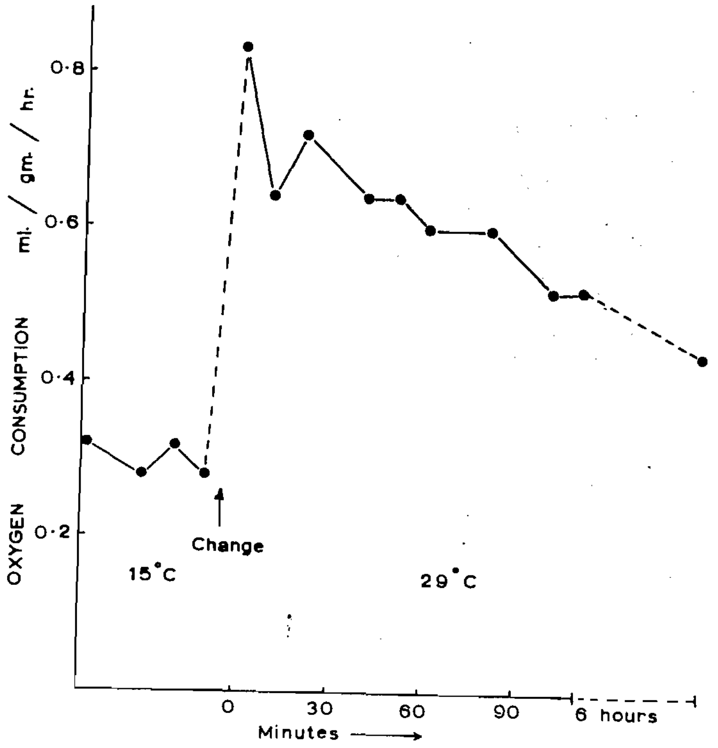


figure 30. Effect of a sudden increase in temperature from 15°C to 29°C on the oxygen consumption of <u>C. diaphanus</u>.

Experiment in the continuous flow respirometer tracing the overshoot response of <u>C. diaphanus</u> to a sudden temperature change. The initial overshoot lasted only 1½ hours; the new respiration rate was not the fully acclimated one, as can be seen from the change in rate over the next 6 hours.

One experiment on 3 female <u>C.diaphanus</u>. Total of 3 experiments.

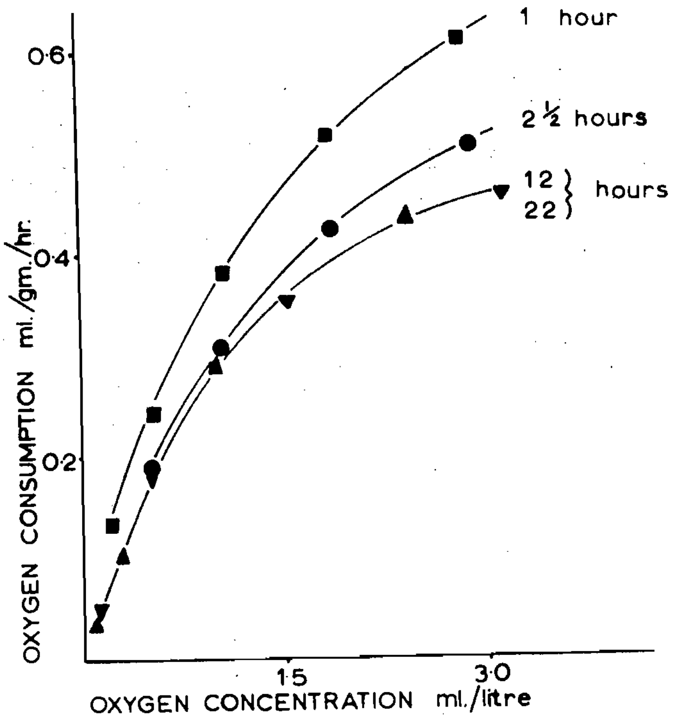


figure 31. The respiratory dependence curves of *C. diaphanus* at various times after a sudden increase in temperature from 15° to 25°C.

Both its rate of oxygen consumption and Pc. fell with time until after 12 hours the acclimated state for the new temperature was reached.

Four respiratory dependence curves selected from a total of 8 experiments.

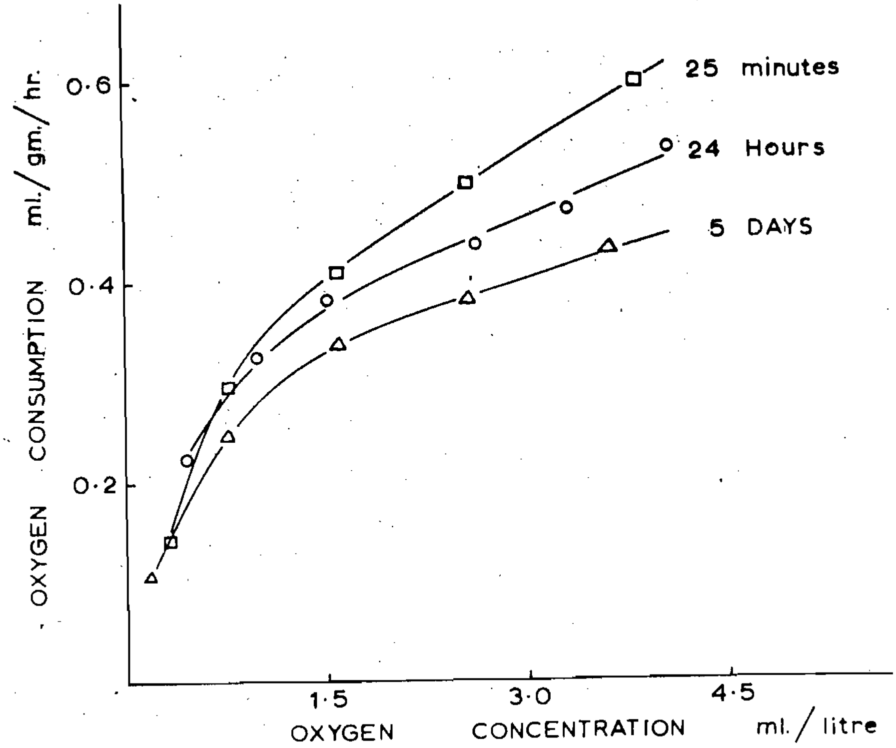


figure 32. The respiratory dependence curves of C. riparius larvae at various times after a sudden increase in temperature from 15° to 25°C.

Both its rate of oxygen consumption and Pc. fell slowly with time until it reached the acclimated state for the new temperature after approximately 3 days.

Selected from a total of 6 experiments.

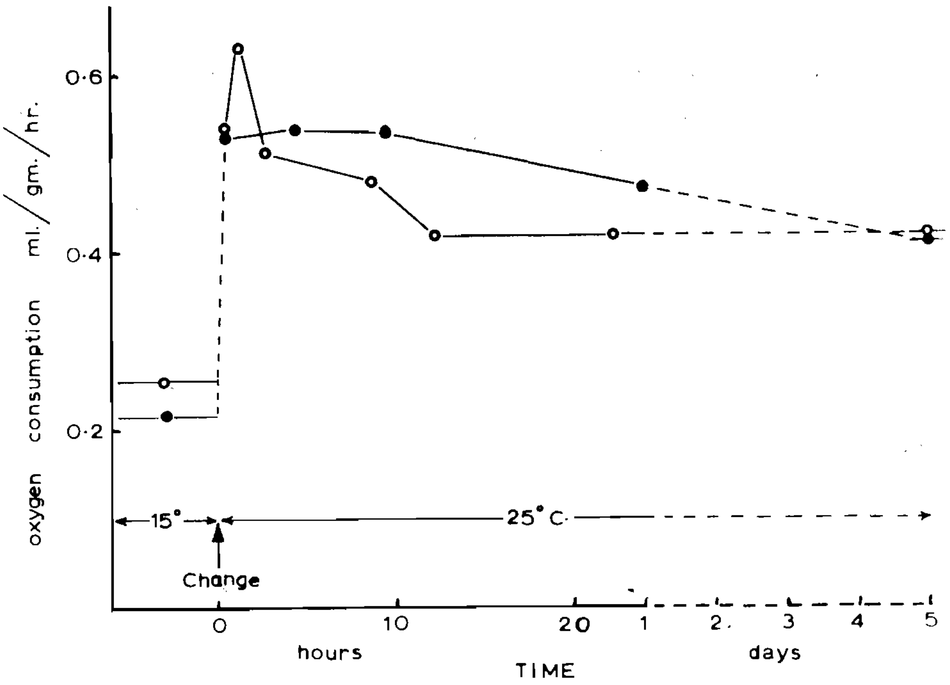


figure 33. The oxygen consumption of *C. diaphanus* and larvae of *C. riparius* at an oxygen concentration of 3.0 ml. O_2/litre, after an increase in temperature from 15°C to 25°C.

C. diaphanus O reached its acclimated rate of oxygen consumption at the higher temperature after 12 hours. *C. riparius* larvae●, although they showed a less extreme initial overshoot response, took over 3 days to reach their new acclimated rate.

Points taken from curves similar to those shown in figs.3I & 32.

DISCUSSION.

The most remarkable feature arising from this investigation into the temperature relations of the two species, is the apparent correlation between their rates of oxygen consumption and critical oxygen concentrations. This link between their levels of activity and respiratory dependence may throw some further light on the nature of the phenomenon of respiratory dependence, and its relationships to the animal's environment.

The direct link established between the two perameters of respiration may be via the processes of oxygen uptake and transport. The increased activity of poikilotherms at higher temperatures, or after a sudden increase in temperature, resulting from direct effects on the reaction coefficients of their metabolic reactions, may result in rapid depletion of the oxygen supply to their tissues. A faster uptake of oxygen from the environment will be required to maintain the increased level of activity. This will in turn be dependent upon the oxygen concentration in the surrounding water. As the temperature increases the affinity of blood pigments for oxygen is reduced: Black and Irving, 1937 found that in three species of trout the tension for 50% saturation of the blood pigment rises about 1 mm. Hg.

of oxygen partial pressure with each degree centigrade. Also the solubility of oxygen in the body fluids decreases at higher temperatures.

At higher temperatures, specially after a sudden increase in temperature, maintenance of an adequate supply of oxygen to the tissues will, therefore, be limited by a relatively small decrease in environmental oxygen concentration. Any decrease which results in oxygen lack at the tissues will prevent them from maintaining their maximum level of activity. In this way first oxygen supply, then tissue activity will be restricted, and the animal will become oxygen dependent.

Conversely, at low temperatures, or after temperature compensation, the oxygen requirements of the less active tissues are smaller. Respiratory uptake and transport will provide adequate oxygen for maximum levels of tissue activity, down to very low external oxygen concentrations. The animals will be, therefore, independent of environmental oxygen concentration down to these low levels.

This hypothetical link between the animals' rates of oxygen consumption and their levels of oxygen dependence, provides a possible explanation of the relationships observed in the above experiments. It is supported by the measurements, described in the previous section, of the limitations imposed on the animals' relative oxygen dependence by their processes of oxygen uptake and transport.

Further consideration of these possible links between the rates of oxygen consumption and relative respiratory dependence of the two species invites some discussion of the adaptive responses enabling them to remain relatively independent of variations in environmental oxygen concentration. The processes of temperature compensation may represent one of these responses. The decrease in oxygen consumption, after the initial overshoot reaction to a sudden increase in temperature, described above, is paralleled by the animals' critical oxygen concentration, to render them more independent of environmental oxygen. If the animals were not able to compensate for the effects of increased temperature then their critical oxygen concentration would increase, and they would have a respiratory curve similar to that obtained from animals at the peak of their overshoot response (see figures 31 & 32). C. diaphanus in particular, if its activity increased from the high levels recorded at low temperatures, at the same rate as the oxygen consumption of C. riparius larvae, with a Q_{10} of 2·99 , would become totally oxygen dependent at high temperatures and its activity would fluctuate widely with environmental oxygen. The function of temperature compensation in maintaining the respiratory independence of C. diaphanus at higher temperatures, may, therefore, account for its recorded Q_{10} of 1·68 (see figure 27).

Both species are perhaps able to remain fairly independent of oxygen concentration, even at high temperatures, by virtue of their relatively low acclimated oxygen consumption at these temperatures. Animals from habitats in which the environmental oxygen concentration is often low, characteristically have an oxygen independent type of respiration curve with the oxygen consumption reaching the maximum independent rate well below air saturation levels, (Berg, Jonasson and Ockelmann, 1962) . This is true of both C. diaphanus and the larvae of C. riparius (see figures 12 and 14) . One example of the differences in respiratory rates between animals from well aerated and poorly aerated habitats was provided by Walshe, 1949 working on chironomid larvae. At 17°C and 6 ml./litre oxygen concentration, Tanytarsus brunnipes, from a stream, respired at approximately 450 cu.mm.O_2/gm./hr. and was an oxygen conformer, while Chironomus longistylus, from a ditch, respired at approximately 315 cu.mm.O_2/gm./hr. and had a critical oxygen concentration at approximately 2·0 ml O_2/litre.

This interspecific example again provides evidence for a link between rates of oxygen consumption and relative respiratory dependence.

When discussing this point earlier, in the section on respiratory dependence, it was postulated that these differences may be due to different rates of activity at the tissue level in the two groups of animals. An important constituent of temperature compensation in an animal is, however, a modification of its behaviour, as was shown by overshoot experiments on Artemia (Grainger, 1958). It is, therefore, important to measure the level of activity which animals maintain in their natural surroundings to see if they can reduce their oxygen requirements so as to remain independent of environmental oxygen down to low concentrations.

ADDENDUM.

As stated earlier, all measurements were taken from active animals as this provided a conveniently constant and definable physiological and behavioural state, and gave repeatable results. As this study is partially an ecological one, however, I thought it advisable to take some measurements from animals in conditions which simulated their natural surroundings. This is particularly important with the chironomid larvae which normally live in mud tubes and show much lower rates of activity than the levels maintained during experiments. Consequently experiments were carried out on the larvae of *C. riparius* under conditions in which they exhibited a pattern of movement analagous to their random activity.

The experiments were carried out in the closed chamber with a layer of folded nylon net protecting the larvae from the action of the magnetic stirring bar, and only very subdued illumination. Under these conditions the larvae wriggled into folds in the netting and underwent individual cycles of activity similar to those described by Walshe, 1950 . Experiments were conducted under these conditions to measure their rates of oxygen consumption, respiratory dependence and compensation for a sudden increase in temperature.

Figure 34 shows a respiration curve from randomly moving larvae at 22°C, compared to one taken from active larvae. Their rate of oxygen consumption at 3·0 ml. O_2/litre is approximately 50% of the active level, and shows minor fluctuations, due probably to the periods of inactivity and intermittent ventilation behaviour observed in the randomly active larvae. Also their critical oxygen concentration was at 0·8 ml./litre as compared to 1·6 ml./litre for active animals. Thus once again there is a direct relationship between the levels of oxygen consumption and relative respiratory dependence.

The temperature overshoot and compensation after a change from 15° to 25°C, shown in figure 35, follows much the same pattern as for active animals except that the rate of oxygen consumption, especially immediately after the temperature change, is much lower.

It appears that in the environment the larvae will be much less dependent on ambient temperature and oxygen concentration than was indicated by the results from active animals, simply because they are less active. This observation agrees with that made by Hyman, 1932 on <u>Nereis virens</u>. She found that the worm respired at a lower rate and was less dependent upon environmental oxygen concentration when enclosed in a tube.

Walshe, 1950 described how the larvae of <u>Chironomus plumosus</u> became completely motionless when exposed to extremely low oxygen concentrations. Presumably this decreases their oxygen requirements and, enables them to survive periods of anoxia during which they may, when necessary, accumulate an oxygen debt.

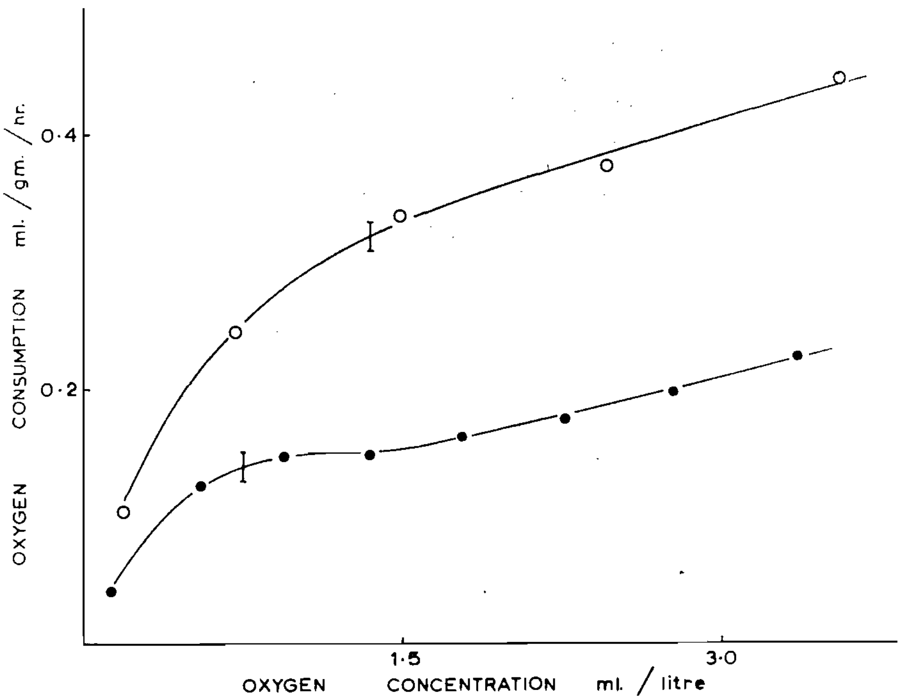


figure 34. Comparison of the respiratory dependence curves from active and randomly moving larvae of <u>C. riparius</u> at 22°C.

○ active Larvae
● randomly moving Larvae

The randomly moving larvae have a lower rate of oxygen consumption and a lower Pc. than the active larvae.

Results obtained from the same larvae under different experimental conditions. Total of 4 experiments.

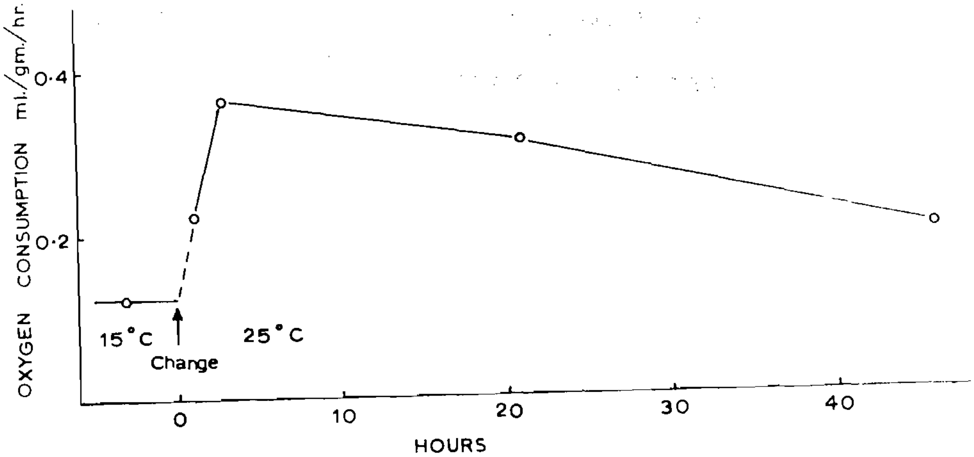


figure 35. The effect of a sudden increase in temperature from 15° to 25°C on the oxygen consumption of randomly moving C. riparius larvae.

There was very little initial overshoot response, but the recovery to a new acclimated rate was very slow. The rates of oxygen consumption at 15°C and 25°C were very low compared to those recorded from active larvae.

Points taken from 5 experiments, on separate batches of larvae, in the closed chamber respirometer.

DISCUSSION (continued) .

Having investigated some of the factors responsible for the animals' respiratory dependence and resistance to temperature variation it is worth while considering how these separate factors may effect their relations with the environment.

Both C. diaphanus and the larvae of C. riparius must be eurythermal in order to live in temporary ponds, with the measured fluctuations reported in an earlier section. The physiological adaptations and resistances developed by the two species to combat extreme temperature effects are, however, different. An animal's ability to withstand large changes in temperature, in small bodies of freshwater, is related to its resistance to fluctuations in environmental oxygen concentration, which always accompany these changes. As mentioned earlier, heat death in C. diaphanus appears to be due to asphyxiation rather than a direct result of the effects of temperature.

The factors contributing to the resistance of C. diaphanus to temperature variation are:

(a) physiological compensation for low temperatures, enabling the animal to maintain a high rate of activity and so move, feed and ventilate its respiratory surfaces efficiently at these low temperatures (see figures 25, 38 & 39).

(b) a fast recovery from the effects of a sudden temperature change, with an overshoot response lasting approximately 1½ hours, and complete temperature compensation in less than 20 hours. (see figures 30 & 33).

(c) the ability to synthesise haemoglobin and so become more independent of the low oxygen concentrations often encountered at the higher temperatures.

(d) possibly its relatively low oxygen consumption at the higher temperatures, though this is likely to result from a limitation of the animal's physiology rather than an adaptation.

The larvae of Chironomus riparius appear at first sight to be poorly adapted for life in fluctuating temperatures. Their activity drops right off at low temperatures, (see figure 26) , until they become completely motionless just above 0°C . Also their processes of compensation to a sudden change in temperature are very slow, (see figures 32 & 33) though they do not show an extreme overshoot reaction, due perhaps to the absence of a pronounced behavioural response.

The larvae may be able to live in the temporary ponds because they are partially insulated from extreme temperature variation when living in their mud tubes at the bottom of the pond. They are not able, however, to completely avoid unfavourable conditions in this way, as the temperature at the mud surface may become very high in bright sunlight, and the oxygen concentrations are often very low, as discussed in the earlier section on the environment.

When exposed to extreme conditions or a sudden change of temperature their ability to survive may be due to:

(a) their very low rates of activity and consequent oxygen requirements when inside their tubes (see figures 34 and 35) .

(b) their ability to remain completely motionless at low temperatures and oxygen concentrations, respiring anaerobically, when necessary, for several hours and accumulating an oxygen debt (see figure 20) .

(c) their possession of haemoglobin which is functional at very low levels of environmental oxygen concentration, even at high temperatures, (see figure 18).

The larvae are not, of course, imprisoned in their tubes, and were observed to swim near the water surface for short periods when the oxygen concentration was particularly low.

C. diaphanus is, therefore, eurythermal and resistant to low oxygen by virtue of its ability to compensate for and adapt to changes in the environment, while the larva of Chironomus riparius is able to survive because of its extremely high resistance to unfavourable conditions.

The marked effect of sudden temperature change upon the position of the critical oxygen concentration in both species is of importance in a consideration of their relationships with their environment. The results shown in figures 23 & 24 indicated that in neither species was oxygen consumption at all limited by the higher oxygen concentrations until temperatures above 25°C . This is, however, only true of animals completely acclimated to the environmental temperature. If the temperature is suddenly increased the Pc. increases in both species. As the temperature in the temporary ponds was shown to fluctuate widely, often over very short periods, this must represent a limitation on the activity of the animals at higher temperatures, which is not indicated by the respiratory dependence curves from temperature acclimated animals.

The relatively fast acclimation time of C. diaphanus (shown in figures 30 & 33), may enable it to live in the surface layers of the pond where temperature fluctuation will be maximal, while the chironomid larvae with their slower acclimation are partially insulated from fast temperature changes by their mud tubes.

An interesting piece of speculation arises from the observed relationship between temperature and oxygen concentration within the ponds. The oxygen concentration was found to fall to a minimum diurnal level in the early hours of each morning, after prolonged darkness had cancelled out oxygen replacement by photosynthesis (see figure 4). At this time the temperature of the water is falling, often quite rapidly (see figure 2). The animals may, therefore, be experiencing an undershoot in their oxygen consumption (Grainger, 1956) , and be more resistant to the decreasing oxygen concentration. Conversely, in strong sunlight the animals oxygen requirements will increase, due to the rise in environmental temperature, at the same time, however, photosynthesis will be at its height and the water may even be supersaturated with oxygen (see figure 4). This superimposition of ecological factors is a characteristic of these small aquatic habitats which may enable the animals to survive in conditions where if the highest temperature coincided with the lowest oxygen concentration their activity would be severely limited.

THE FUNCTIONING OF THE APPENDAGES OF C. DIAPHANUS IN SWIMMING AND VENTILATION.

INTRODUCTION.

In the earlier section on the oxygen consumption of C. diaphanus the possible role of the efficiency with which it ventilates its respiratory surfaces in determining its Pc. was mentioned, but not considered further. The inclement conditions often recorded in the stationary water mass of temporary ponds may accentuate the animal's need for an efficient system of ventilation of its respiratory surfaces. In investigating the importance of this factor in determining the relative respiratory dependence of C. diaphanus, the whole swimming behaviour of the animal has been studied, and the results compared with its known respiratory pattern.

Much of the observed behaviour and results for respiration and activity are discussed with reference to the phylogenetic position of C. diaphanus. The results obtained may provide evidence on the possible evolution of respiratory systems in the Crustacea, and the concept that phylogenetic 'primitiveness' is at all correlated with low powers of physiological adaptation.

The phylogeny and functioning of the anostracan appendage.

After much discussion in the nineteenth century, summarised briefly by Lankester, 1909, the Branchiopoda are now recognised as being primitive members of the Class ~~sub-phylum~~ Crustacea. In his paper (Lankester, 1881) on the appendages and nervous system of Apus cancriformis (sic.), he noted that its central nervous system was preserved in a "primaeval or archaic condition", as found in planarians and some annelids. On appendage structure he described the preoral appendages of Apus as "homogenous" with the appendages of the Chaetopoda (Annelida) , and noted similarities in the structure of its postoral appendages with those of Limulus, "the most archiac representative of the Arachnida." The characteristics Lankester described as primitive in Apus are all possessed by C. diaphanus as are other morphological characters, such as an elongate heart, which are also regarded as the primitive crustacean condition.

Cannon 1928, in his work on C. diaphanus briefly summarised the previous work, and reinvestigated the mechanism responsible for sweeping food particles towards the mouth, in the Anostraca. He considered his study to be important in relation to the origin of other Crustacean feeding methods, and hence the information it may yield as to the phylogeny of the group. He suggested "that the Crustacea inherited from their annelidan ancestors a metachron~~i~~al type of limb movement such as occurs in *C. diaphanus* today." The primitive limb structure, he thought, may have been a simple biramous paddle, easily derived from the annelid parapodium, of the type found in the posterior thoracic appendages of the fossil form *Lepidocaris*. He suggested that as the appendages nearest the mouth had to deal with most food a strong maxillary pump was developed. This emancipated the more posterior limbs from food gathering and led to their specialisation for locomotion in the Malacostraca. In the Anostraca, however, the food-gathering of the trunk limbs was at first subsidiary to that of the maxillae, but with increasing efficiency of the trunk limb system the maxillary apparatus became superfluous and the maxillae gradually disappeared.

In this way both the Branchiopoda and the Malacostraca may be derived from a hypothetical Proto-anostracan type, with simple paddle-like limbs, and the present day Anostraca may have undergone as much structural divergence, in their feeding mechanism from this common ancestor, as have the Malacostraca.

The immediate importance of Cannon's work to this present study is that he demonstrated the double role of the anostracan trunk appendages in locomotion and feeding, the two processes arising automatically from the metachron~~i~~al beat of the appendages. As the water current over the body is the animals source of oxygen, the movement of the appendages has a triple function as discussed by Johnson 1936.

In the present study on C. diaphanus it was this third function of the appendage movements in ventilating the respiratory surfaces, which was reinvestigated. The object was to guage the importance of the appendage activity in controlling or causing the previously measured respiratory pattern of C. diaphanus, in response to varying environmental conditions. Special consideration was given to the possible limitations imposed on the functioning of the appendages, by their triple role.

The relative specialisation of the appendages to their role in ventilation was first investigated by Fox and Johnson 1934. They were interested in the possible physiological and ecological reasons for the different sensitivity of animals to changes in oxygen and carbon dioxide concentrations. This they investigated by working on a selected range of Crustacea from various aquatic habitats. They measured the frequency of movement of the appendages producing the respiratory current by direct observation, and recorded them with a tapping key on a kymograph drum.

Their experiments on C. diaphanus showed that exposure to water with a low oxygen content of 2·2 cc./litre caused no change in 1½ hours, nor did water with an abnormally high oxygen content have any effect. Also the rhythm was not accelerated by gradually increasing the carbon dioxide content of the water to a concentration which began to slow the appendage movements.

These results are immediately open to criticism on the basis of the measured oxygen consumption of C. diaphanus, described in the earlier sections of this present work. Fox and Johnson do not describe the conditions from which their specimens of C. diaphanus were taken. This is important to their study, as if the animals were adapted to water with a low oxygen content the metabolism of the females at least, would not be effected by prolonged exposure to an oxygen concentration of 2·2 cc./litre, as, at room temperature, their Pc. is well below this level. The Pc. has, however, been shown to depend on temperature, and as Fox and Johnson did not give their experimental temperature, it is impossible to say whether or not they were working in a range of oxygen concentrations likely to have any effect upon their animal's oxygen dependence. I think it advisable to use oxygen concentrations known to effect the animals' metabolism, when attempting to detect any effect upon their processes of ventilation, and for this reason their work has been repeated.

In a later paper Johnson, 1936, extended the investigation, into the frequency of ventilation movements, to more species of Crustacea. In his discussion he cited the results from the earlier paper and stated that the insensitivity of C. diaphanus can probably be considered primitive as in this animal the limbs concerned in forming a respiratory current are not specialised for this purpose.

The concept that primitiveness should be necessarily correlated with insensitivity or inefficiency is of course erroneous, and Johnson was not implying that this is so. I believe, however, that even though the general anatomy of the group is accepted as representing the primitive crustacean pattern, the actual primitiveness of the structure and functioning of the anostracan appendage is questionable. Even when the phyllopodium is accepted as the primitive form of the crustacean appendage, the detailed arguments of Cannon, outlined above, indicate that the appendage in the Anostraca is a very specialised structure when compared to the probable ancest~~o~~ral form. If, however, Garstang's theory (Garstang and Gurney, 1938) on the relationships between the trilobite fossil *Protaspis* and the present day crustacean nauplius is accepted; the primitive structure of the crustacean appendage must then be considered to have been stenopodial rather than phyllopodial. Also the method of feeding in the primitive Crustacea, as indicated by the structure of the fossil trilobites and the habits of the present day genus *Limulus*, may have been grubbing around on the surface of the substratum. In this respect the feeding behaviour of *T. cancriformis*, of the related order Notostraca, as described by Fox, 1949, is closer to this primitive pattern than the anostracan method.

Whichever theory is accepted the structure, and presumably the functioning, of the anostracan appendage cannot be regarded as representing the truely primitive crustacean pattern. It is possible, therefore, that the appendages, having developed a specialised mode of functioning, have the ability to serve not only their feeding and locomotary functions, as described by Cannon, but also are able to ventilate their respiratory surfaces efficiently. To accomplish this they must, because of the conditions characteristic of their environment be resistant and possibly able to adapt to low oxygen and high carbon dioxide concentrations.

Another potential weakness in the work of Fox and Johnson was exposed by the recent work of Arudpragasam and Naylor, 1964 (b) on Carcinus maenas. Johnson measured the rate of scaphognathite beat in this species, and could detect no correlation between his measurements and the external oxygen and carbon dioxide concentrations. He concluded that the shore-crab was insensitive to the respiratory gases, and thought it possible that this was correlated with its' amphibious habits. Arudpragasam and Naylor measured the actual ventilation volume, that is the quantity of water passing through the gill chamber in a given time. They found that it increased when the crab was exposed to decreasing oxygen and temporary increases in carbon dioxide concentration. They considered that measurements of ventilation volume should give a more reliable indication of respiratory responses than counts of scaphognathite beat. The limitation on the usefulness of Fox and Johnson's results was that no measure was taken of amplitude changes in the movements of the ventilatory appendages; the method used to increase the ventilation volume of the branchial pump in many teleost fishes, van Dam, 1938.

Taking account of these several objections to Fox and Johnson's work, and the possible role of ventilation in controlling the respiratory dependence of *C. diaphanus*, their experiments on this species have been repeated and extended.

Retrospective literature research revealed the extremely important work of Lowndes, 1933 on the feeding mechanism of *C. diaphanus*. His excellent photography and critical analysis of his own and previous author's results contributed much to the consideration of the adaptability of the anostracan appendage.

Fortunately his conclusions agreed to a large extent with those introduced above. Thus he considered that much of the work outlined above represented an oversimplification of the problem. He presented convincing arguments indicating that the primary function of the foliaceous anostracan appendage was respiratory and pointed out that the variable metachronial beat of the row of slightly dissimilar appendages could not result in the constant pattern of feeding currents postulated by Cannon. 1928 . This laggardly discovery of Lowndes' work served, therefore, to reinforce the author's view that the appendage action in *C. diaphanus* is likely to be adapted to function efficiently in respiratory ventilation.

METHODS.

I The measurement of the frequency of appendage movements.

The work of Fox and Johnson on the frequency of the appendage movements in C. diaphanus was repeated. Measurements on animals swimming freely in large volumes of water were difficult to carry out and the results were rather variable. An alternative method had, therefore, to be found. It was noticed that whenever the water in the stock-tanks was disturbed C. diaphanus tended to swim actively against any current produced. Use was made of this observation in all measurements of its appendage movements.

The animals were introduced into a horizontal glass tube along which ran a slow stream of water. They headed into this current and its velocity was adjusted so that they were held stationary, relative to the sides of the tube, swimming against the water stream. Their movements were then observed through a magnifying lens and timed with a stop-watch. Under these conditions the animals swam with a constant, possibly maximum frequency of appendage movements (A.P.B.R.) , and this enabled repeatable results to be obtained.

This method proved satisfactory for measurements below room temperature; at and above 20°C , however, the counts became very inaccurate due to the difficulty of counting beats at the relatively high speeds of approximately 250 beats per minute. Accordingly stroboscopic illumination was used to slow the apparent beat rate, and enable it to be measured. An Evans Electroselenium Ltd. 'Stroboflash' unit, which gave a flashing light source, with a continuously variable speed, was used, and was calibrated against a metal reed vibrating at 50 cycles per second on the A.C. mains. The experiments were done in complete darkness except for the stroboflash light. The appendage movements were accurately measured by setting the flash at slightly below the synchronous speed and counting beats. which when added to the speed of the stroboflash gave the actual speed of appendage movement. This calculated speed was then checked by setting the stroboflash at the same value and observing the appendages to see if they appeared stationary. This process was repeated several times on each animal before a value for its appendage beat frequency was finally accepted.

It was found that the easiest appendages to observe were the last pair, and the movements of this 11th. pair of thoracic limbs were counted in both sexes. Complete beats, that is the number of movements to and from the rear-most position of the appendage sweep, were counted for a measured length of time.

C. diaphanus swims with its appendages uppermost in its natural surroundings, and exhibits a strong ventral light reaction (Seifert, 1932) . In these experiments the animals were illuminated from above, as if the light was shone from one side they tended to turn towards it. This made counting difficult, and it was thought that this change in orientation may effect the frequency of their appendage movements.

II The measurement of relative swimming speed.

Arudpragasam and Naylor, in their work on *Carcinus*, exposed the limited usefulness of frequency measurements in a study of changes in ventilatory activity. Their work on ventilation volumes cannot be repeated on *C. diaphanus*, which does not possess a carapace or discrete gill chamber, as it is impossible to measure directly the amount of water passed over the respiratory surfaces in a given time. Measurements were, therefore, made indirectly by measuring the animal's swimming speed against a stream of water. The results had a direct relationship to ventilation volume as they represented a measure of the volume of water passing over the animal's body in a given time.

The method used was that described by Ryland 1963, and first used by Bishai, 1960 in which the animals were made to swim against a water current of known velocity passing through a long glass tube. The apparatus (see figure 36), was essentially similar to that figured by Ryland, except that the experimental tube was enclosed with a water-jacket. The water in the header-tank was heated to the required temperature and its gas content adjusted by bubbling nitrogen, carbon dioxide and air in the required proportions. At the same time it was circulated round the water-jacket to maintain the desired conditions within the experimental tube.

The mode of operation was exactly as described by Ryland, and will only be briefly outlined here. Water from the header-tank entered the system via tap 1. and flowed through a T - piece to either end of the experimental tube, which was 25 cm. long and had an internal diameter of 1·0 cm. The direction of the water flow was controlled by appropriate use of clips 3 and 4 or 2 and 5 , and its velocity by the adjustment of 2 or 3 , and the height of the water head above the tube.

An animal was selected from one of the stock tanks, transferred with a wide-bore pipette to a funnel fitted near one end of the experimental tube, and tap 6. was opened. Screw clip 5 was then slowly released and the animal was carried in a stream of water down into the apparatus. A slow stream of water was then passed down the tube for a few minutes, while the animal settled down. The tube was mounted in front of a plain white background so that the animal received no visual stimulation when moving along the tube. The only response in these conditions was an orientation to the direction of water flow, followed by active swimming against it. This avoided the observed hyperactivity of an animal being washed backwards past a fixed object visible through the sides of the tube. This activity was far greater than the maximum recorded swimming speed in the absence of visual stimuli, and was too erratic to allow consistent measurement.

No difficulties such as were described by Ryland working on plaice larvae, were experienced in measuring the swimming speed of C. diaphanus. The animals were continuously active, and when faced with a water current in the apparatus swam vigorously against it, often for many minutes. Their behaviour was similar to that described in herring larvae by Bishai. They to inclined their bodies downwards at 45° to the water current, though they tended to swim in the top half of the tube, rather than along its axis. In consequence their heads were positioned in the fastest water flow, at the centre of the tube, and their abdomens in the region of minimum velocity, close to the side of the tube. (see figure 37) . For this reason it was assumed that their appendages were distributed evenly through the range of current velocities between the sides and middle of the tube, and the mean water flow was taken as their swimming speed. This value was measured by collecting the water in a graduated cylinder, for a period timed with a stop-watch. The mean flow was the volume per second divided by the cross-sectional area of the tube.

The careful measurements carried out by Ryland, who applied equations to his results allowing for the flow pattern inside the tube, was not thought necessary in this case as it was not essential to obtain the animal's exact swimming speed when comparing the effects of different environmental conditions. It was necessary, however, to check that the animals were swimming in the same region of the tube at each estimation. This was not at all difficult in practice, as when they were exerting their maximum effort, the animals always took up their characteristic position in the tube, shown in figure 37 .

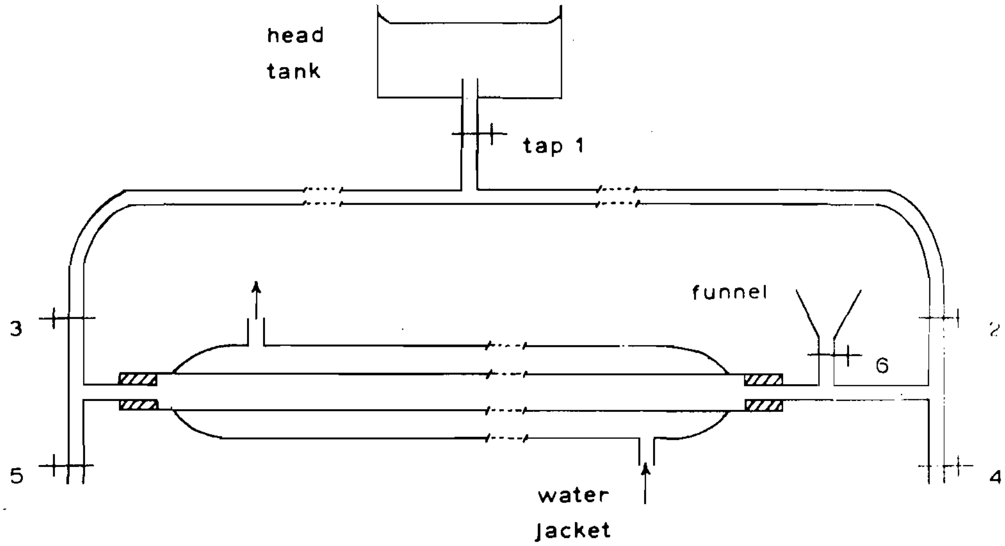


figure 36. Apparatus for measuring the swimming speed of <u>C. diaphanus</u> against a water current.

In the figure the horizontal tubes have all been shortened. The way in which the apparatus was used, including the functions of the numbered taps, is explained in the text.

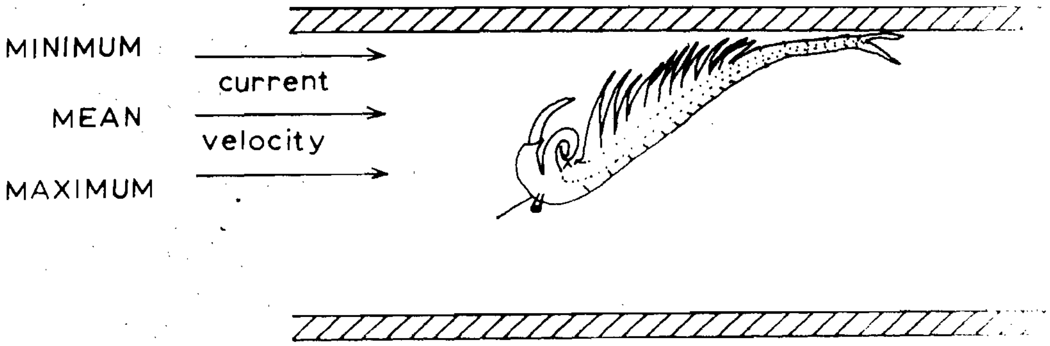


figure 37. The position adopted by C. diaphanus when swimming actively against a water current passing along a tube.

It swam in the top half of the tube with its body inclined at an angle to the water stream. Its 11 pairs of appendages were, therefore, distributed evenly through the current gradient across the tube.

RESULTS.

I *The effects of temperature variation.*

The measurements of environmental conditions, described in an earlier section, showed how important the temperature relationships of the animal's metabolism must be in their extremely variable habitat. Following the fairly detailed study of the temperature effect on the oxygen consumption of *C. diaphanus*, described earlier, the first set of experiments on limb functioning comprised an investigation into the effect of temperature on the frequency of appendage movement and swimming speed of *C. diaphanus*. These experiments also provided a convenient trial of the techniques involved, prior to the work on the effects of variations in the respiratory gases.

As when measuring oxygen consumption, the animals were first acclimated to each experimental temperature for at least fourteen days. Experiments were then carried out to measure their appendage beat frequency and swimming speed at five temperatures between 0^{o} and $30^{o}C$. The experiments below $15^{o}C$ were done in a cold room with an ambient temperature of $4^{o}C$, and the water in the head-tank and water jacket was accurately controlled at the experimental temperature. Each animal was weighed and measured after the experiment, and its sex was noted.

The results for appendage beat frequency (A.P.B.R) and relative swimming speed are plotted in figures 38 & 39. The large variations in the values obtained, especially at the higher temperatures, is partially due to the inevitable errors in observational measurements of animal behaviour, but also represents a size effect. The larger animals were observed to have a slower A.P.B.R., no doubt correlated with their lower metabolism, previously reflected in their oxygen consumption. They also had a slower swimming speed than the smaller individuals, though this difference could be correlated with their different resistances to and positions in the water flow, as well as their lower metabolic rate. In neither set of experiments were sufficient results obtained to enable the size effect to be analysed quantitatively. As, however, the animals did not differ greatly in size and accurate quantitative figures were not required, the results were considered satisfactory.

With increasing temperature both the frequency and effectiveness in swimming of the animal's appendage movements rises almost exponentially. There is a marked similarity in the effect of temperature increase on these two perameters of activity and the oxygen consumption of C. diaphanus.

This relationship indicates that, with changing temperature, the speed at which C. diaphanus swims is directly related to the rate at which its appendages beat, and that the resultant energy output is directly reflected in its oxygen consumption.

The similarity in shape of the three curves for the effect of temperature on the activity of C. diaphanus, indicates a close similarity in the Q_{10} value for each curve. The approximate $Q_{10's}$ between 10° - 20°C are respectively

for oxygen consumption: 1·65

for appendage beat rate: 1·67

for swimming speed: 1·69

All three values are below 2, indicating, as discussed earlier, that some physical process, such as oxygen transport, is limiting the activity of this species. (Bullock 1955) .

Exploratory experiments indicated that after a sudden increase in temperature the appendage movements of C. diaphanus experienced an overshoot reaction similar to that detected in its oxygen consumption. After a change from 10°C to 25°C the A.P.B.R. of animals swimming freely in a bowl showed a marked overshoot for 1½ hours. Immediately after a change from 10°C., when the acclimated swimming speed is approximately

16 cm. per minute, to 20°C., when the mean is approximately 28 cm. per minute, a batch of C. diaphanus were found to swim at approximately 40 cm. per minute. This is further evidence of a direct relationship between the changes in A.P.B.R., swimming speed and oxygen consumption, with temperature variation.

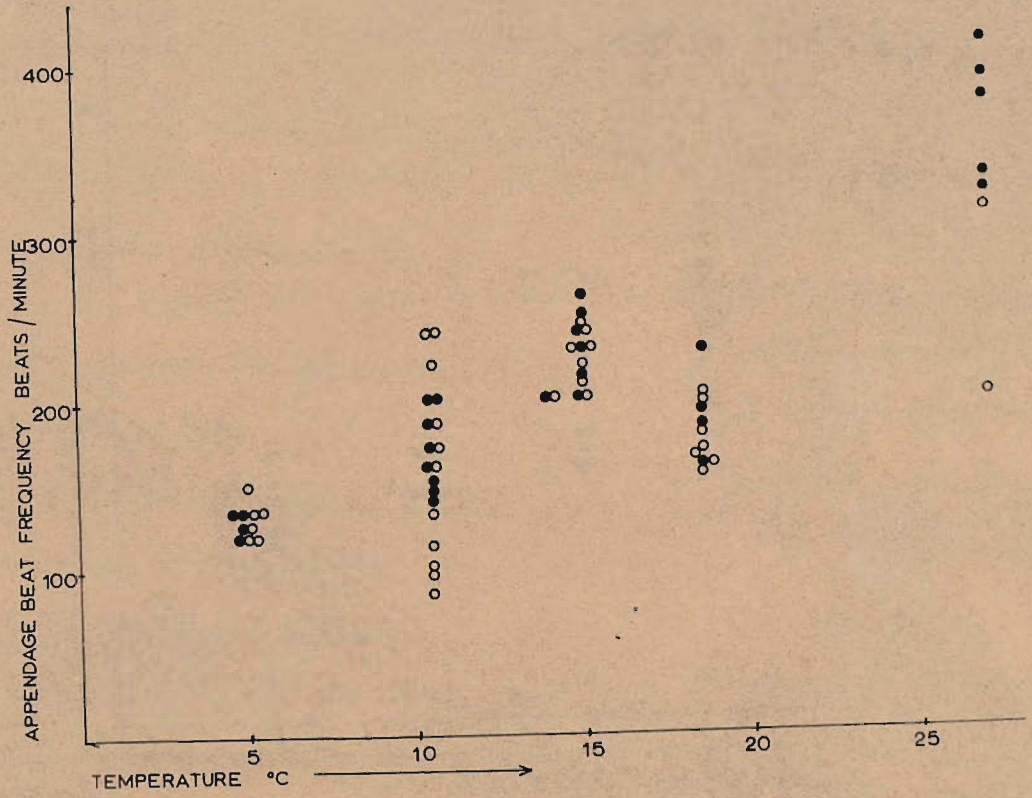


figure 38. The frequency of appendage movements in *C. diaphanus* at temperatures between 5° and 30°C.

· Its A.P.B.R. was relatively high at 5°C and increased with temperature having a Q_{10} of approximately 1·67.

Each point is a result for one animal and represents the mean of between 5 and 10 measurements of its A.P.B.R.

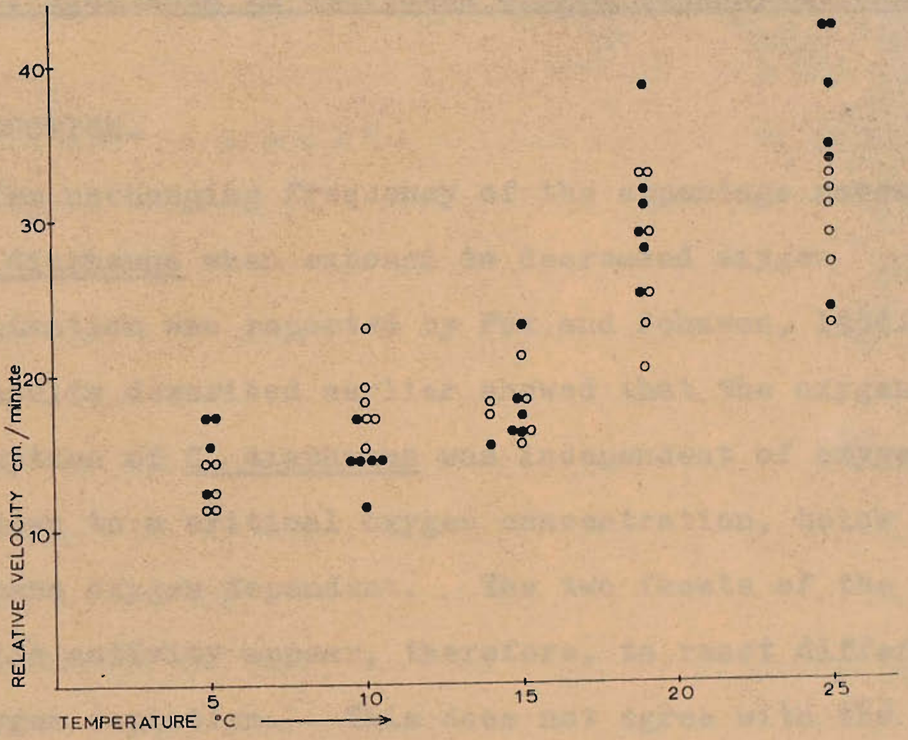


figure 39. The swimming speed of C. diaphanus at temperatures between 5° and 25°C.

The animals swimming speed was relatively rapid at 5°C and increased with temperature, having a Q_{10} of approximately 1·69.

Each point is the mean of between 5 and 10 estimations of swimming speed on a single animal.

II The frequency of appendage movements in C. diaphanus in decreased oxygen concentrations.

INTRODUCTION.

The unchanging frequency of the appendage movements in *C. diaphanus* when exposed to decreased oxygen concentration was reported by Fox and Johnson, 1934. The results described earlier showed that the oxygen consumption of *C. diaphanus* was independent of oxygen only down to a critical oxygen concentration, below which it became oxygen dependent. The two facets of the animal's activity appear, therefore, to react differently to oxygen depletion. This does not agree with the measured effects of temperature variation, which was found to change both the oxygen consumption and the frequency of appendage movements in very similar ways. As the work of Fox and Johnson was considered inconclusive, for the reasons outlined earlier, it was thought advisable to repeat their experiments before this duality in the effects of decreased oxygen concentration was discussed further.

METHOD.

The frequency of the appendage movements in C. diaphanus were measured by the method described earlier, and the animals were again acclimated to the experimental temperature for approximately fourteen days. The oxygen content of the water was adjusted by bubbling nitrogen and air through the header-tank at various rates, and was measured by taking regular water samples for analysis by the modified Winkler technique (Fox and Wingfield, 1950) . Each animal, before being inserted into the apparatus, was placed in the header-tank for approximately thirty minutes to allow it some time to acclimate to the experimental oxygen concentration. This short time was considered adequate for the oxygen content of the water to have full effect on the animals activity, on the basis of the respiration experiments described earlier, see figure 13 . Once in the tube the animals were allowed a few minutes to settle down, then the frequency of their appendage movements was measured. The whole experiment was carried out at 25°C. as more accurate results could be obtained using the stroboflash, at the relatively high A.P.B.R. reached at this temperature. Also the respiratory dependence of C. diaphanus was previously shown to be affected by temperature, and at 25°C the animals Pc. was at

2·25 ml./litre. Any disparity between the effects of oxygen on their respiration and the activity of their limbs is, therefore, best measured at 25°C because a relatively high ambient oxygen concentration is known to limit the animal's metabolism at this high temperature.

RESULT.

The results are plotted in figure 40 .

The measured frequencies markedly vary at all oxygen concentrations, due possibly to faults in the method, but chiefly because of individual variation in the animals used. One major source of variation, not mentioned by Fox and Johnson, is the different A.P.B.R.'s of the two sexes, particularly marked at the low oxygen concentrations. At all the oxygen concentrations where measurements were taken the appendages of the females had a significantly higher beat rate than the males. These different A.P.B.R.'s at low oxygen concentrations paralleled the higher oxygen consumption of females acclimated to low environmental oxygen concentrations (see figure 16) . This may indicate an important functional correlation between the two facets of activity as the animals used in the present study were maintained in unaerated aquarium tanks, and appeared to possess high blood concentrations of haemoglobin.

The results shown in figure 40 vindicate Fox and Johnson's reported observation. The frequency of appendage movement of C. diaphanus is relatively unaffected by a decrease in oxygen concentration in the surrounding water. There was actually a slight increase in frequency, most marked around 2·8 ml O_2/litre, when the oxygen concentration was only moderately decreased. This is not, however, likely to represent a major adaptation in the animal's ventilatory behaviour, though it does approximate to the position of their Pc. at 25°C.

These results do not, of course, allow for the possible effects of fatigue or the accumulation of an oxygen debt after prolonged exposure to low oxygen. C. diaphanus enclosed in a small volume of water and allowed to exhaust all the available oxygen stopped swimming near their residual oxygen concentration. This result does show, however, that, in the time and over the concentration range known to effect their rate of oxygen consumption, the animals do not adapt to lowered oxygen by an appreciable change in the frequency of their appendage movements.

Having shown the findings of Fox and Johnson to be substantially correct, the different effects of oxygen concentration on the oxygen consumption and frequency of appendage movement require further consideration. The fact that C. diaphanus maintains an unchanging A.P.B.R. whilst its oxygen consumption is decreasing, poses the problem of where they are getting the excess energy to maintain high activity while the oxygen supply to their tissues is falling. It is possible that they are accumulating an oxygen debt, though earlier experiments detected no repayment of such a debt (see figure 19). The answer may be connected with the limitation inherent in Fox and Johnson's work, i.e. perhaps the frequency of appendage movement is not a reliable measure of the activity or ventilation behaviour of C. diaphanus.

Accordingly, the study was extended by measuring the swimming speed of C. diaphanus with decreasing oxygen concentration. This not only gives an indirect measure of the animal's ventilation volume, as discussed earlier, it also is a measure of the work done by the animal on its surroundings, in moving the water past itself, and as such represents a true measure of activity.

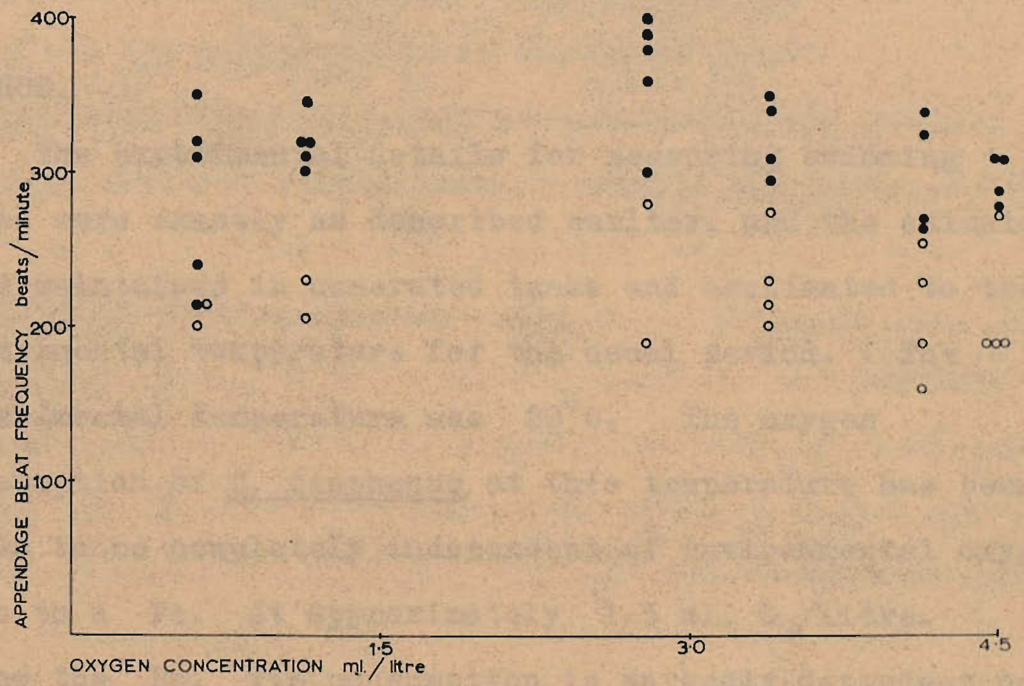


figure 40. The frequency of appendage movements in C. diaphanus at low oxygen concentrations.

●, females; o, males.

The females had a higher A.P.B.R. than the males at all oxygen concentrations. No significant change in A.P.B.R. with decreased oxygen concentration was detected during the experiments. A.P.B.R. is therefore, independent of oxygen down to very low concentrations for short periods.

Each point is the mean of between 5 and IO estimations of A.P.B.R. on a single animal.

III The swimming speed of C. diaphanus in decreased oxygen concentrations.

METHOD.

The experimental details for measuring swimming speed were exactly as described earlier, and the animals were maintained in unaerated tanks and acclimated to the experimental temperature for the usual period. The experimental temperature was 20°C. The oxygen consumption of C. diaphanus at this temperature has been shown to be completely independent of environmental oxygen down to a Pc. at approximately 1·5 ml. O_2/litre. Below the Pc. its consumption is markedly dependent on the environmental oxygen concentration (see figure 23). This sharp division of the animals metabolism into oxygen independent and dependent phases at a relatively high oxygen concentration, makes this temperature ideal for studying the effects of oxygen on the animal's swimming speed.

The measured respiratory pattern at 25°C. (see figure 23) , the temperature used in the measurement of the effects of oxygen on the frequency of appendage movements, was considered to render this temperature unsuitable for the present study, as there was not such a clear division into independent and dependent phases.

The one disadvantage of these different experimental temperatures is that direct comparison of the results from the two measurements of appendage action is impossible. The different temperatures will, however, tend to minimise rather than accentuate any differences between the two results. The high temperature used in the frequency measurements would have tended to lead to increased oxygen dependence, yet complete independence was detected. The lower temperature used in the present study will favour the swimming speed remaining relatively more independent of oxygen, any tendency to dependence will, therefore, be more significant. Indirect comparison of the two results is of course readily available via the measured pattern of respiratory dependence at both temperatures, shown in figure 23.

Each animal was, as in the earlier experiments, acclimated to the experimental oxygen concentration for thirty minutes in the header-tank, and allowed to settle down in the tube before any measurements were taken.

RESULTS.

The results are plotted in figure 41. Each point represents the mean of several measurements at the same oxygen concentration. The four sets of points, joined by divided lines for easier interpretation, represent results from males and females when the oxygen concentration was decreased, from air saturation, in four steps and increased, from a low concentration, in three steps. This precaution was taken to ensure that any apparent decrease in swimming speed at low oxygen concentrations was not due to fatigue or injury of the animals during the experiment.

In all four sets of results the swimming speed of the animals was shown to fall with decreasing oxygen concentration. The partial dependence on environmental oxygen resembles the pattern established for the oxygen consumption of C. diaphanus. The agreement is not, however, exact. When the concentration was reduced from air saturation the females were not completely independent of oxygen above 1·5 ml./litre, and the males appeared to be dependent over the whole range of concentrations. Starting at low concentrations, however, a distinct division into oxygen dependent then independent phases can be observed, particularly in the females. Combination of the two sets of results, which cancels out the possible effect of fatigue and injury, shows that certain definite similarities exist between the patterns of swimming speed and oxygen consumption of C. diaphanus from poorly aerated conditions. Comparing figure 41 with figure 16 it can be seen that:

(a) the females consume oxygen and swim at a higher rate than the males at all oxygen concentrations;

(b) the females are independent of oxygen concentration to a lower level, and show a more complete independence above their Pc.

(c) the approximate positions of the different Pc.'s of the two sexes are similar for their oxygen consumptions and swimming speeds.

This obvious relationship between the results for swimming speed and rate of oxygen consumption in C. diaphanus indicates that the former is a more meaningful measure of activity in this species than its A.P.B.R. A similar relationship was reported by Gibson and Fry, 1954, who found that the maximum cruising speed of the trout paralleled its oxygen consumption as a function of environmental oxygen.

These results also suggest that the ventilation volume in C. diaphanus may decrease with lowered oxygen concentration, assuming that it is directly dependent upon the animal's swimming speed.

The results for the frequency of appendage movement and swimming speed in C. diaphanus may now be compared. Both sets of experiments were on animals exposed to the experimental oxygen concentration for thirty minutes before each measurement. The results are, therefore, directly comparable, except for the different experimental temperatures which necessitate reference to their separate relationships with the measured pattern of respiratory dependence at each temperature.

All three effects of decreasing oxygen concentration are summarised and drastically simplified, for ease of comparison, in figure 42 . All sexual differences have been ignored in drawing the mean lines, and the curve for oxygen consumption is a hypothetical one simulating the respiratory dependence of the animals at a temperature midway between 20° and 25°C.

This figure emphasises the way in which the swimming speed of C. diaphanus parallels its oxygen consumption while the frequency of its appendage movements remains oxygen independent down to much lower oxygen concentrations.

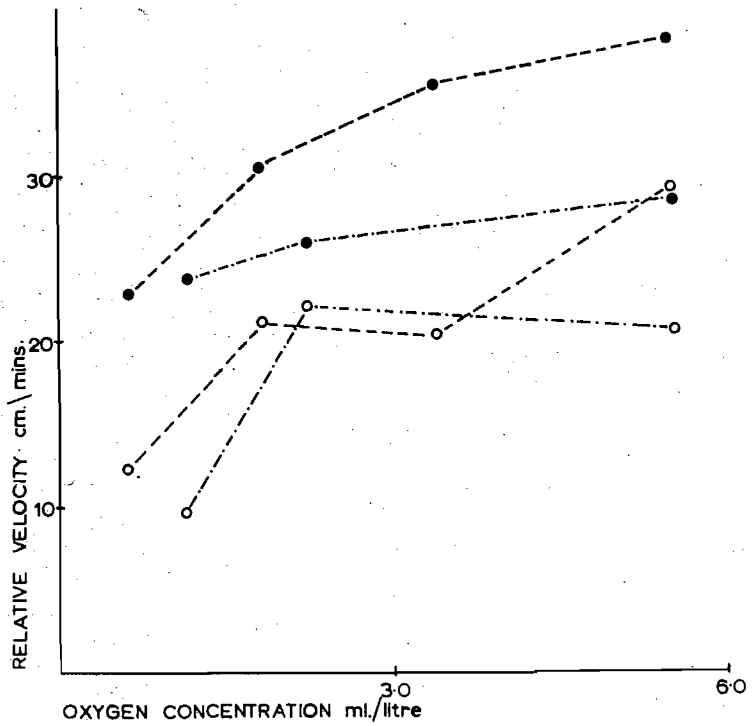


figure 41. The swimming speed of C. diaphanus at low oxygen concentrations.

---- experiments started at high concentrations.

-.-.-. " " " low "

●, females; o, males.

The females swam faster than the males at all oxygen concentrations. Swimming speed decreased with decreasing oxygen concentration, but was relatively independent at concentrations above 2.0 ml./litre.

Each point is the mean of 5 determinations of swimming speed at one oxygen concentration.

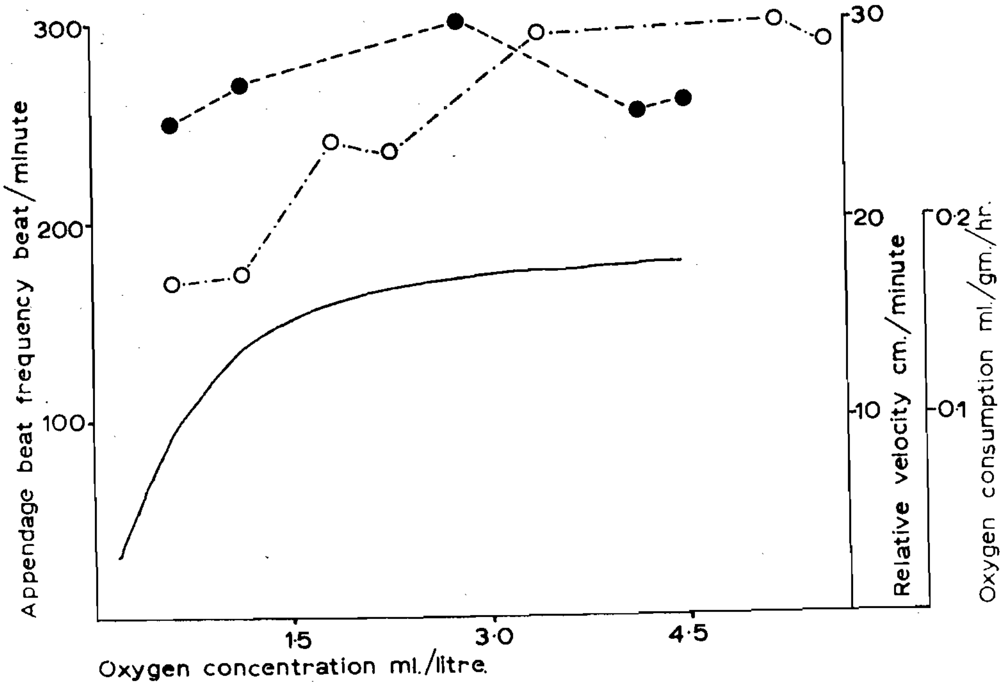


figure 42. The rate of oxygen consumption, swimming speed and frequency of appendage movements in C. diaphanus at low oxygen concentrations.

o, swimming speed; ●, A.P.B.R; –, oxygen consumption.

Its A.P.B.R. remained independent of oxygen down to very low concentrations. Under similar conditions its oxygen consumption and swimming speed were oxygen dependent at concentrations below 2·0 ml./litre.

IV The appendage action of C. diaphanus in increased carbon dioxide concentrations.

Fox and Johnson, 1934, also detected no change in the A.P.B.R. of C. diaphanus with increased carbon dioxide concentrations. Carbon dioxide was, however, shown earlier to have a marked effect on the animal's oxygen consumption, see figure 21 . Consequently the effect of high carbon dioxide concentrations on the A.P.B.R. and swimming speed of C. diaphanus was investigated.

The concentration of carbon dioxide in the water flow apparatus was increased by bubbling the gas through the water in the header-tank for short periods. The concentration obtained was measured, using the method described by Ruttner, 1953, with a p.H. meter and alkalinity titrations, and then checked at regular intervals with the p.H. meter, throughout the experiments. Both the frequency of appendage movements and swimming speeds were measured as before, and both results are summarised in figure 43 .

Each point is the mean of several measurements, and the four lines represent the two results for the separate sexes. Once again the two sexes differ in their A.P.B.R. and swimming speed, the females having the higher activity at all concentrations of carbon dioxide. This result shows that the animal's A.P.B.R. is less affected than their swimming speed, by increased carbon dioxide, though the levels used were rather high, and were enough to begin to slow the appendage movements. There is a distinct parallel between these results and the observations previously made on the effects of decreased oxygen concentration.

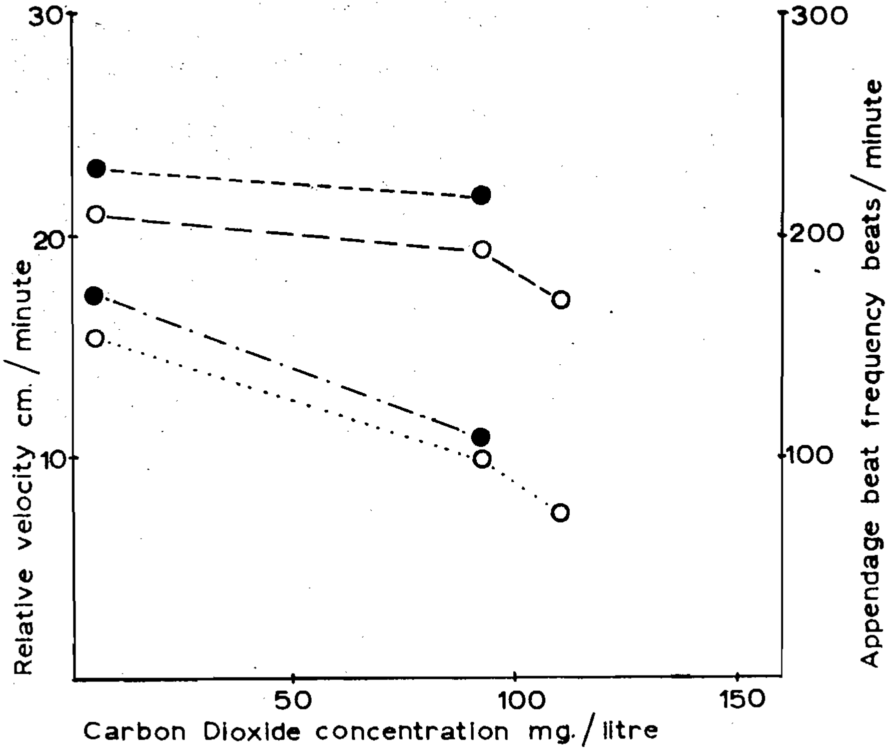


figure 43. The swimming speed and frequency of appendage movements in _C. diaphanus_ exposed to high concentrations of carbon dioxide.

●, females; o, males.

The females swam faster and had a higher A.P.B.R. than the males. The animals' swimming speed (lower two traces) was more markedly depressed by high CO_2 than their A.P.B.R.'s (upper traces).

Each point is the mean of 5 determinations of swimming speed or A.P.B.R. at each concentration of carbon dioxide.

DISCUSSION.

A brief synopsis of the results so far is that the swimming speed and oxygen consumption of *C. diaphanus* are depressed by a decrease in oxygen and an increase in carbon dioxide concentration in the surrounding water, whilst the frequency of their appendage movements is relatively unaffected.

The importance of these results to the present study is the bearing they may have on the activity and possibly the ventilation volume of *C. diaphanus*, as both factors will effect the animal's respiratory dependence. If *C. diaphanus* is incapable of adapting its appendage movements to changes in the concentrations of the respiratory gases, as is suggested by its unchanging A.P.B.R., this will limit its independence of environmental oxygen concentration.

Arudpragasam and Naylor, 1964, found that some other factor or factors than frequency were operative in determining the ventilation volume in *Carcinus*. Explanation of the recorded changes in swimming speed with unchanging A.P.B.R. necessitates the involvement of other factors in the locomotion, and consequently the ventilation volume, of *C. diaphanus*.

There are two possible ways in which these changes may come about:

(a) the amplitude of the appendage movements may change with the environmental oxygen and carbon dioxide concentrations. Bainbridge, 1958 detected in the goldfish a linear relationship between the amplitude of tail movement and the distance travelled.

(b) The appendages may be twisted or 'feathered' during their effective, backwardly directed stroke. This would decrease their angle of attack, resistance to passage through the water, and consequent effectiveness in producing forward movement.

Use of either or both methods would result in the observed drop in the swimming speed of C. diaphanus with changing oxygen and carbon dioxide concentrations, and would require less energy than normal movement, thus explaining the decreased oxygen consumption.

The relative roles of these two possible factors in determining the swimming speed of C. diaphanus were investigated by a close study of its appendage movements, using high speed cine-micrography.

A PHOTOGRAPHIC ANALYSIS OF THE APPENDAGE MOVEMENTS OF C. DIAPHANUS.

METHOD.

The animals were inserted into a tube along which water flowed from a header tank, as in the previous experiments. The tube was made of 'Perspex' and mounted in a small optically sided water bath. This avoided the scattering of light from the cylindrical sides of the tube. 'Perspex' was used because its refractive index is close to that of water, and this cuts down refraction which could distort the image. The water bath was mounted on the stage of a microscope which was swung over into the horizontal position, see figure 44 . The animals were filmed under low power, using a X 5 eyepiece and a X 3 objective, with a 'Vinten' 16 mm. high-speed camera, which filmed at 50 to 100 pictures per second. During experiments the animals were observed through a 'Wild' 'Watching-eyepiece' interposed between the microscope and the camera. This was a beam-splitting device allowing the microscope field to be observed while the camera was being operated. Using this device the animals could be observed swimming freely against the water current, and filmed whenever their appendages passed through the field covered by the camera. At the magnification used only a few appendages were visible at one time, and the eleventh or last pair were the ones studied because they were easier to pick out from their neighbours in the photographs, and also because they were used in the stroboscopic frequency measurements described earlier.

The animals were illuminated during experiments by a 250 watt lamp behind a diffusion screen of tracing paper, inserted between the lamp and the sub-stage condensor on the microscope. Careful focussing and adjustment of the condensor produced a bright, even illumination of the microscope field, ideal for filming at these high speeds. With this arrangement the animals were illuminated from the side, and because of their strong ventral light reaction they tended to twist towards that side whilst swimming. In this position the appendages on the side nearer the camera were brought into focus and photographed without any confusion of the picture by appendages from the other side. I found a clearer picture of single appendage movement was obtained from animals showing this orientation modified by the light source and considered that the advantage of the greater clarity in this position overode any possible effect on their movements.

One disadvantage of this form of illumination was that some of the strong background light was transmitted magnification used only a few appendages were visible at one time, and the eleventh or last pair were the ones studied because they were easier to pick out from their neighbours in the photographs, and also because they were used in the stroboscopic frequency measurements described earlier.

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One disadvantage of this form of illumination was that some of the strong background light was transmitted through the translucent appendages, particularly at their edges. In consequence their outlines were rather indistinct and the long fringe of bristles on the exopodite and endites of the appendages were not visible, on the photographs obtained. This is a severe limitation on the usefulness of the method and more work, possibly using dark background illumination, is required, before this study will be complete.

Despite these limitations a clear enough record of the appendage movements was obtained for some useful measurements to be made, see plate 5. These filmed records were analysed by feeding them through a photographic enlarger and drawing around the superimposed appendage positions. Each picture was lined up with the others by using the outline of the abdomen and the modified twelth pair of reproductive appendages as fixed points of reference. Alternate pictures from two consecutive movements were superimposed to avoid recording a single, possibly atypical movement.

There are two phases in the swimming movements of C. diaphanus. During the effective stroke the appendage is brought backwards, fully extended as a rigid plate and slightly concave in the direction of the stroke. Cannon, 1928, stated that this movement pushes water from the inter-limb spaces out postero-laterally, and produces the swimming stream. In the recovery stroke the basal part of the limb is rotated forwards by muscular action, dragging the distal and lateral lobes with it, folded back on the limb behind. In this way the limb offers the least possible resistance to the water flow in reaching its forward position, ready for the next swimming stroke. A fuller description, with diagrams, of these movements is given in the detailed analysis by Cannon, 1928.

The use of high-speed photography with transmitted light limited the present study to an analysis of the main components of these two phases of movement, as the smaller lobes of the appendage were not clearly resolved on the 16 mm. frames, under these conditions. On these films the exopodite or flabellum at the tip, and the backwardly projecting bract or branchia are more easily discernible than the rest of the appendage. I have, therefore, concentrated in this preliminary study on the movements of these two parts.

As discussed earlier, two main factors may be responsible for the recorded drop in the swimming speed of C. diaphanus with decreased oxygen and increased carbon dioxide concentrations. Both the amplitude and degree of feathering of the appendages during the effective stroke were investigated by filming the appendage movements in various concentrations of oxygen and carbon dioxide. As in the earlier activity experiments the animals were acclimated to the experimental temperature for several days, and to the experimental gas concentration for approximately 30 minutes before they were filmed.

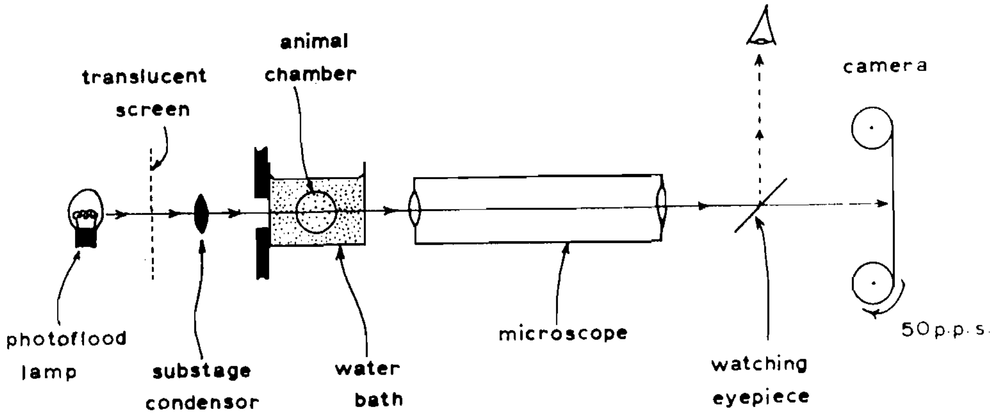


figure 44. Apparatus for the cine-micrography of the appendage movements in C. diaphanus.

The animal was held stationary, relative to the camera, swimming against a stream of water along a glass tube. The tube was enclosed in an optically-sided water bath mounted on the stage of a microscope tilted into the horizontal position. The tube and water bath are shown in transverse section on the figure.

Plate, 5. The movements of the 11th appendage of a male *C. diaphanus*; a) in water with a low oxygen content, and b) in water with a high carbon dioxide content.

1 part of an effective swimming stroke.
11 part of a recovery stroke.

RESULTS.

I Changes in amplitude of appendage movements in C. diaphanus related to variations in respiratory gas concentrations.

The amplitude of the appendage movements was recorded by projecting one frame of a filmed sequence onto a sheet of paper and drawing round the outline of the abdomen and reproductive appendages. This outline was then aligned with the projected image of alternate frames from two consecutive swimming movements, as described earlier. At each alignment a spot was drawn marking the position of the tip of the flabellum. In this way the path described by the tip of the appendage during its swimming movements was recorded.

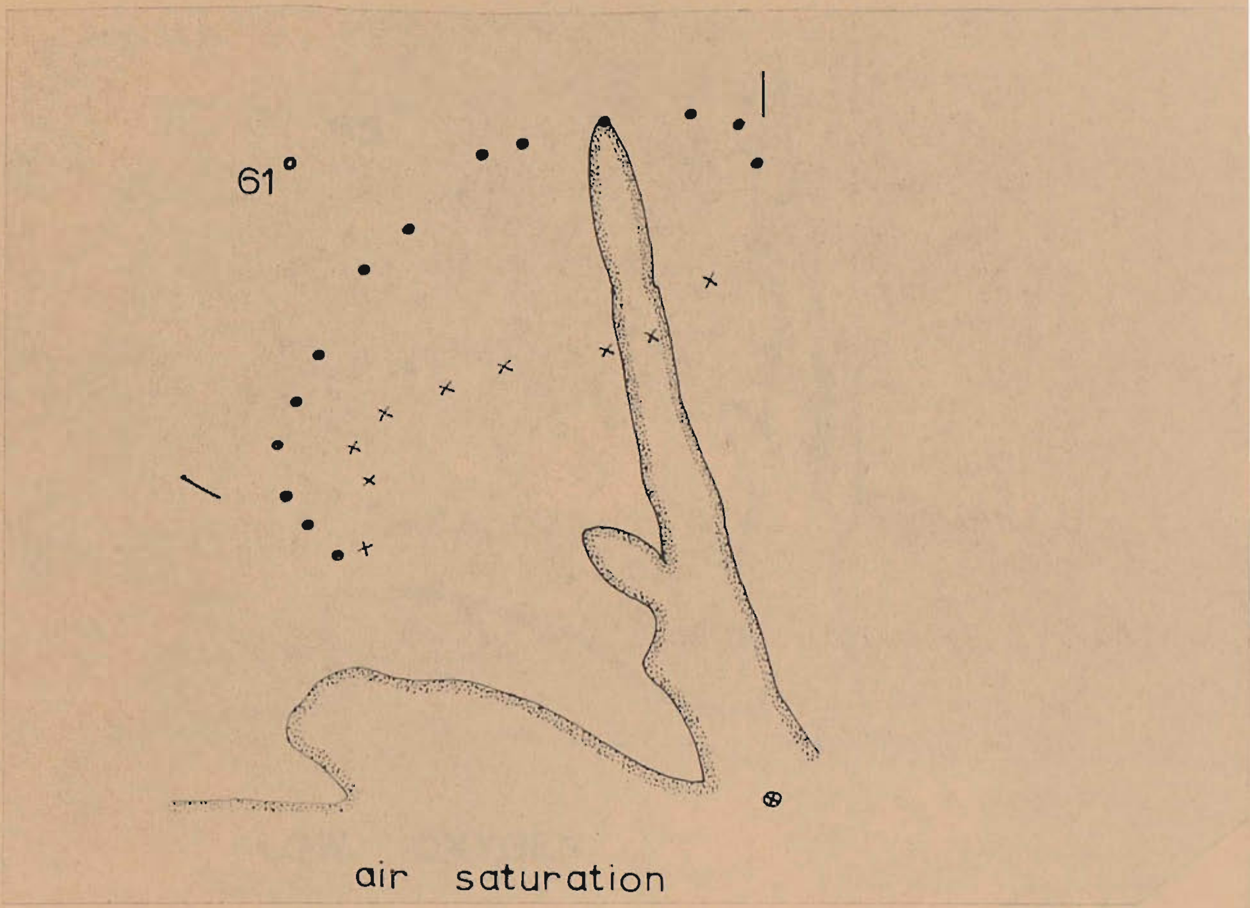
The pattern of movement in air saturated water is shown in figure 45 . The extension of the appendage during the effective stroke, and its folding during recovery are clearly followed. The amplitude of movement of the tip of the flabellum relative to the marked axis of the appendage is 61° . The important features to be noted about the movement is that the appendage does not go back close to the abdomen, and there is no pronounced lag at either end of the beat.

Figure 46 traces the movement of the tip of the appendage after about 40 minutes exposure to a low oxygen concentration of approximately 1·0 ml./litre. The amplitude has scarcely changed, there is only a slight increase to 65°. The position of the beat is, however, different. The effective stroke begins further back, relative to the limb axis and body, and ends closer to the abdomen with an obvious lag in this rear most position.

Figure 47 shows the effect of exposing the animals for about 30 minutes to a carbon dioxide concentration of approximately 75 mg./litre. At this concentration the frequency of their appendage movements was slightly decreased, but this provided the extreme effect required for this preliminary study. There is a pronounced increase in amplitude to 81°, with the appendage swinging right back to rest on the abdomen and lagging there for a relatively prolonged period.

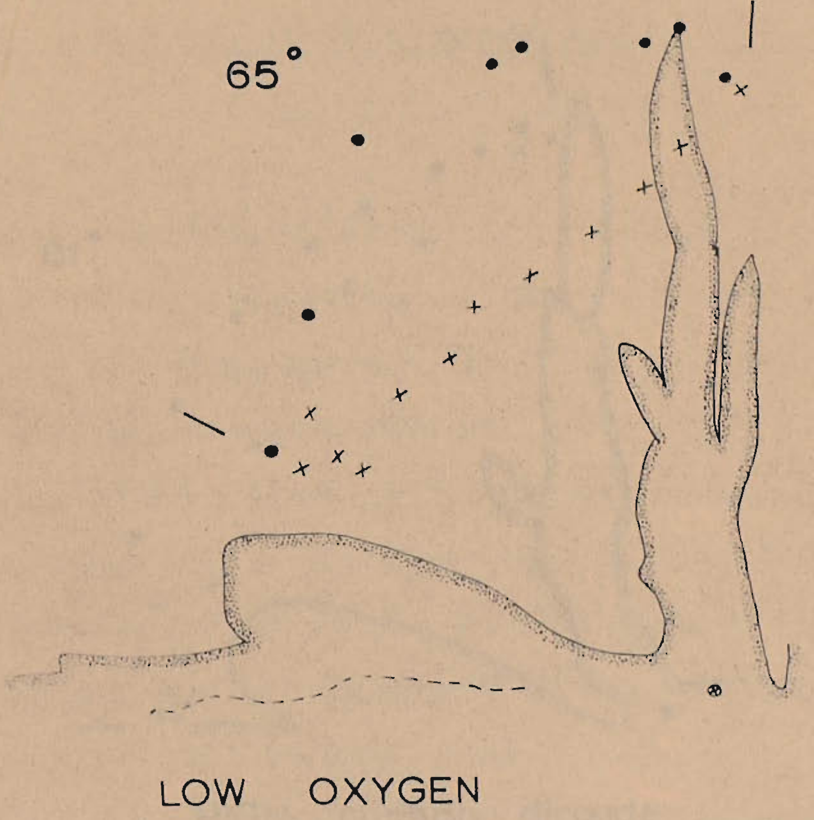
These results show that unfavourable environmental oxygen and carbon dioxide concentrations result in a maintained or increased amplitude of the appendage movements in C. diaphanus. The measured drop in its swimming speed with decreased oxygen and increased carbon dioxide concentrations cannot, therefore, be due to a decrease in the amplitude of its appendage movements. Some other factor, must, therefore, be involved in controlling the swimming speed of C. diaphanus.

The effectiveness of appendage movements in swimming depends on their frequency, amplitude and angle of attack. Changes in this third factor may explain the recorded drop in the swimming speed of C. diaphanus with changing respiratory gas concentrations.



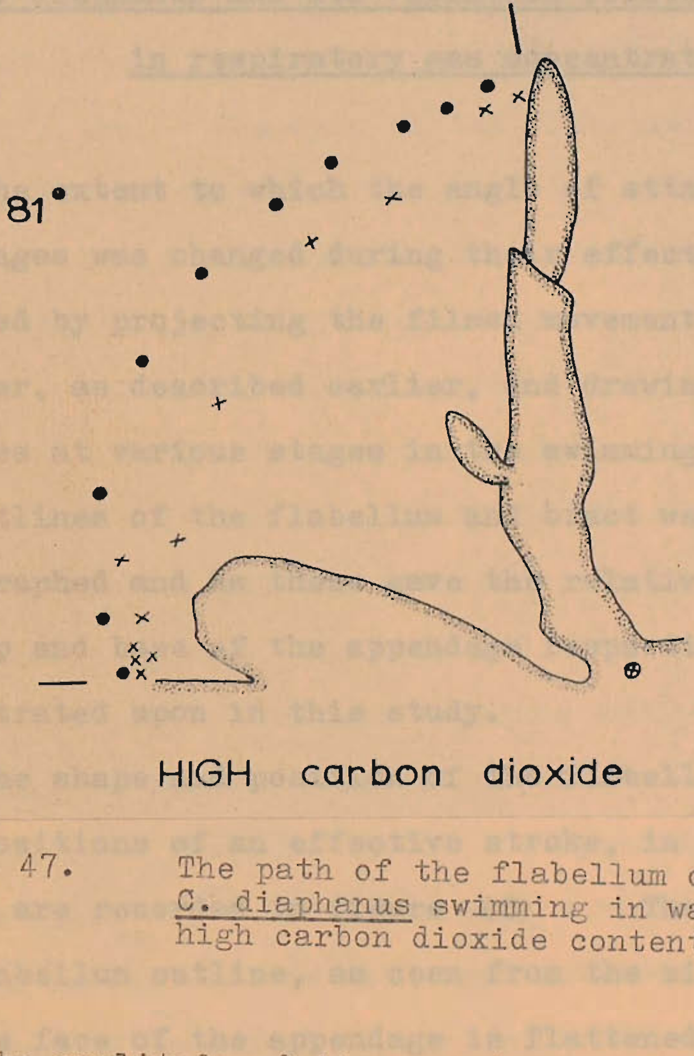
figure, 45. The path of the flabellum tip on the eleventh appendage of a male C. diaphanus swimming in air saturated water.

The effective swimming stroke ● and recovery stroke x were followed for two consecutive movements. The amplitude of the whole stroke was 61°.



figure, 46. The path of the flabellum of a male C. diaphanus swimming in water with a low oxygen content.

The amplitude of its appendage movements was 65°.



figure, 47. The path of the flabellum of a male *C. diaphanus* swimming in water with a high carbon dioxide content.

The amplitude of its appendage movements was 81°.

The angle of attack of the appendage movements in C. diaphanus and its variation related to changes in respiratory gas concentration.

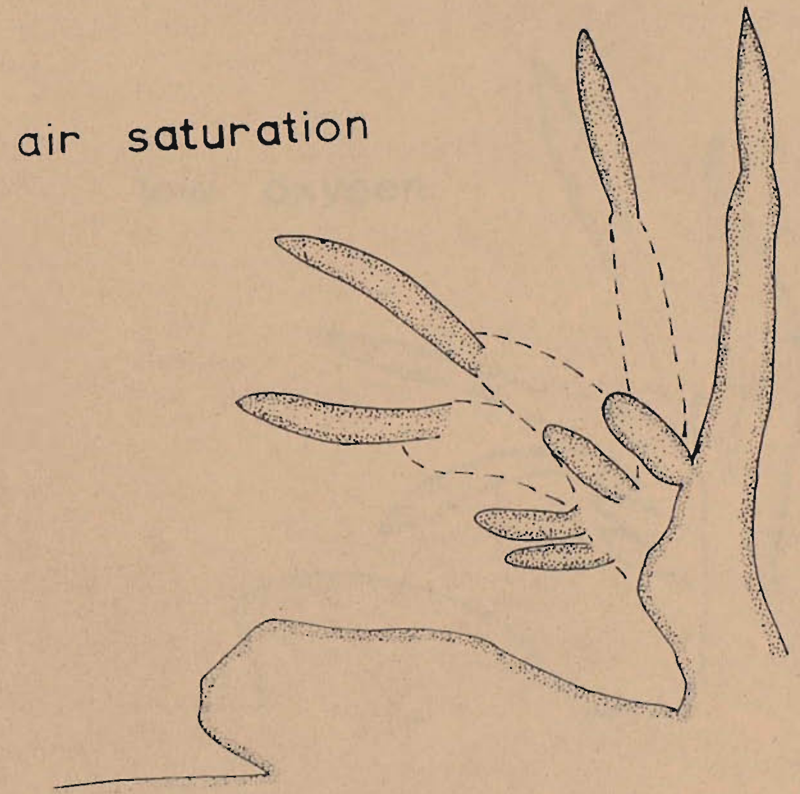
The extent to which the angle of attack of the appendages was changed during their effective stroke was measured by projecting the filmed movements onto a sheet of paper, as described earlier, and drawing superimposed outlines at various stages in the swimming movement. The outlines of the flabellum and bract were most clearly photographed and as these gave the relative positions of the tip and base of the appendage respectively, they were concentrated upon in this study.

The shape and position of the flabellum and bract at four positions of an effective stroke, in air saturated water, are recorded in figure 48 . The narrowness of the flabellum outline, as seen from the side, indicates how the face of the appendage is flattened and directed against the surrounding water during its swimming movement. Once again the position of the effective stroke should be noted; it starts at an angle of 90° to the body and travels down through 60° to an angle of approximately 30° to the abdomen. Also important is the fact that the appendage is rotating right from the base, as indicated by the movement of the bract.

Figure 49 shows the effective, swimming beat of the appendage in an animal exposed for approximately 40 minutes to an oxygen concentration below 1·0 ml./litre. The area of the flabellum is slightly wider, particularly at the top of the stroke, than that recorded in air saturated water. This may indicate a slight feathering of the appendage during the effective stroke. The major change is, however, in the position of the beat. In the first position outlined on figure 49 the appendage is fully 'feathered' as in a recovery stroke, and the effective stroke proper starts at an acute angle to the body and ends with its tip close to the abdomen. The main twisting action of the appendage is about a point on or above the position of the bract, so that the tip of the appendage bends over in an arc while the bract moves only a short distance. This is in contrast to the rigid movement of the appendage of an animal in air saturated water, which is rotated right from the base.

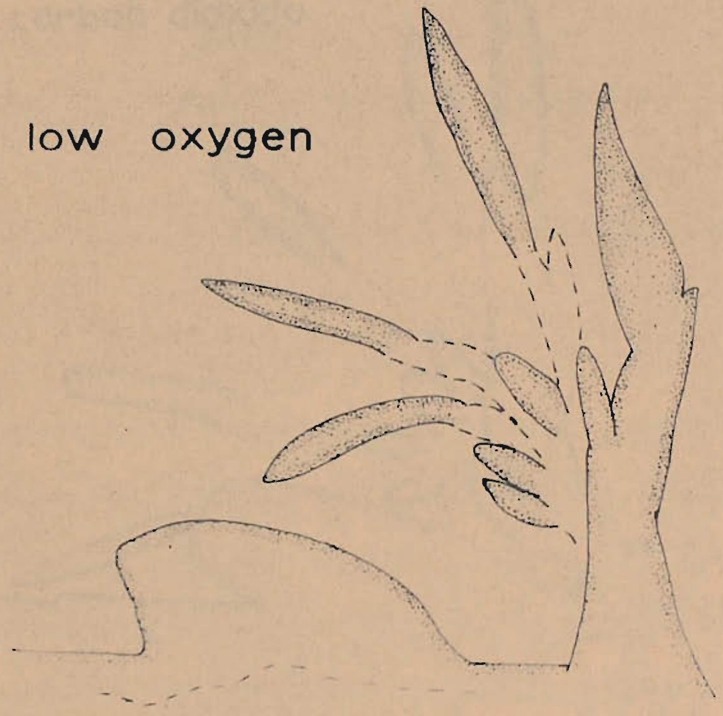
This modified beat with its apparently shorter true effective stroke and bending action may be expected to result in a drop in swimming speed. Also moving only the distal part of the appendage through what appears to be a less effective backward stroke may require less energy than the type of movement recorded in air saturated water.

Figure 50 is a record of the effective stroke in an animal exposed for approximately 30 minutes to a carbon dioxide concentration of 75 mg./litre. The area of the flabellum, as viewed from the side, is definitely greater than at air saturation, indicating an appreciable feathering of the appendage throughout the swimming movement. This would of course reduce its effectiveness in locomotion and also the work done in moving it through the water. The most obvious feature is once again the marked increase in the amplitude of the beat; the whole appendage is bending down to lie flat on the abdomen where it lags for an appreciable time before beginning the recovery stroke.



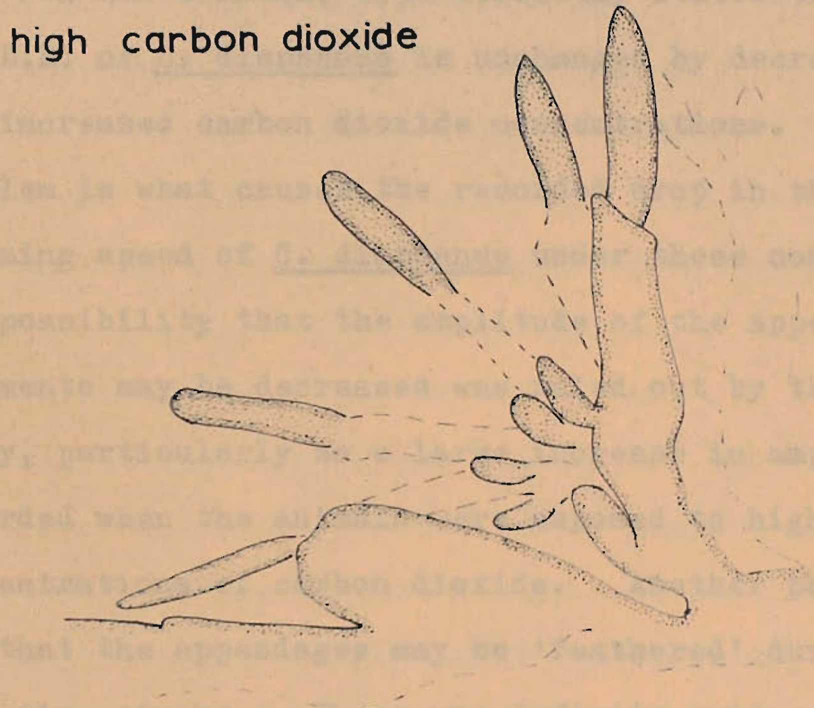
figure, 48. The outline of the flabellum and bract during an effective swimming stroke of the eleventh appendage of a male C. diaphanus in air saturated water.

The appendage moved from 90° back to an angle of approximately 30° to the body. The movement of the bract indicates that the appendage was held rigid during the swimming stroke. The narrowness of the flabellum, as seen from the side, indicates that its flattened surface was working directly against the surrounding water.



figure, 49. The outline of the flabellum and bract of an animal swimming in water with a low oxygen content.

The effective swimming stroke appeared to start at less than 90° to the body. The bract did not move through as wide an angle as the flabellum, and the appendage appeared to flex during the swimming stroke. The flabellum appeared to be twisted at the beginning of the stroke, giving it a wide outline.



figure, 50. The outline of the flabellum and bract of an animal swimming in water with a high carbon dioxide content.

The swimming stroke started at approximately 90° to the body and the movement of the bract indicates that the appendage was held rigid until it bent right down to meet the abdomen. The blunt, wide outline of the flabellum, as seen from the side, indicates that it was twisted or 'feathered' during the swimming stroke.

DISCUSSION.

Fox and Johnson, 1934 correctly stated that the A.P.B.R. of *C. diaphanus* is unchanged by decreased oxygen and increased carbon dioxide concentrations. The problem is what causes the recorded drop in the swimming speed of *C. diaphanus* under these conditions. The possibility that the amplitude of the appendage movements may be decreased was ruled out by the present study, particularly as a large increase in amplitude was recorded when the animals were exposed to high concentrations of carbon dioxide. Another possibility was that the appendages may be 'feathered' during their effective stroke. There was definite evidence in these results of some 'feathering' of the appendages, particularly under the stress of increased carbon dioxide concentrations.

There is an indication that the drop in swimming speed may result from different modifications of the appendage movements with changes in oxygen and carbon dioxide concentration. The effect of a decrease in oxygen was apparently to shorten the actual effective part of the stroke, though the entire movement was of much the same amplitude as at air saturation. Also the appendage was bent into an arc during its effective stroke and not held rigid as in the movements at air saturation.

With increased carbon dioxide the marked feathering of the appendages probably accounts for the loss of swimming speed, despite the increased amplitude of the effective stroke. The prolonged lag over the abdomen at the end of the effective stroke, may, however, play some part in this loss of effectiveness, and also may explain the slight drop in beat frequency measured at this concentration (see figure 43) .

The decrease in oxygen consumption recorded at the concentrations of oxygen and carbon dioxide used in these experiments, (see figures 16 and 21) probably results from a limitation of the animal's respiratory system. It is reflected in their activity as a decrease in the energy available for movement. This results in a less effective movement of the appendages against the resistance of the surrounding water, and so leads to a decrease in the animal's swimming speed. By changing the pattern of their beat, however, the animals are able to move their appendages at the same frequency and with a similar or greater amplitude than at air saturation, despite the decrease in available energy.

As stated earlier; for the ventilatory apparatus to function efficiently it must be resistant to, and possibly able to adapt to the low oxygen and high carbon dioxide concentrations, found in the environment. If the ventilation volume of *C. diaphanus* depended directly on the animal's swimming speed, then it would be progressively less efficient as the concentration of the respiratory gases became less favourable. This may, however, not be the case, and the present results indicate how the ventilation of the respiratory surfaces may be maintained in poor conditions.

The effectiveness of an appendage beat in moving an animal through water depends partially on its angle of attack. The actual volume of water passing over the appendage surface will, however, depend solely on the frequency and amplitude of its movements, which will be related by some sort of reciprocal function. As the exposure to changing concentrations of the respiratory gases has been shown to have little effect on the frequency of the appendage movements in *C. diaphanus*, then the volume of water sampled by its appendages will be directly proportional to the amplitude of their movements.

The observed effect of carbon dioxide on the appendage movements in *C. diaphanus* was to cause their angle of attack to change, so producing the measured decrease in swimming speed. The volume of water passing over the appendage surfaces was, however, increased by the greater amplitude of their movements. The feathering of the appendages under these conditions may, therefore, be part of a behavioural response necessary for efficient ventilation. The resultant reduction of drag will enable the amplitude of the appendage movements to be increased despite the decrease in available energy, indicated by the measured decrease in the animal's oxygen consumption. The water sampled by the respiratory surfaces will be fresh because the animal is moving through the water at only a slightly slower speed than at air saturation, (see figure 43). The ventilation volume will, therefore, be increased when the animal is exposed to high concentrations of carbon dioxide. This increased ventilation may be partially responsible for the efficient maintenance of metabolism shown in figure 21 , where the Pc. is 0·8 ml/litre, and may account for the known ability of C. diaphanus to survive in water containing relatively high concentrations of carbon dioxide.

With decreased oxygen concentration the ventilation volume remains potentially the same as at air saturation because the amplitude of the whole appendage movement does not change. The amplitude of the effective part of the swimming stroke appears, however, to decrease, and as this will result in less energy being required for movement, it may allow the animal to withdraw sufficient oxygen from the water to satisfy its metabolic requirements. This maintenance of ventilation volume by an unchanging frequency and amplitude of appendage movements may account for the apparent incompleteness of the oxygen independence of C. diaphanus above its Pc. (see figure ~~23~~ 2) . The modified action of the appendages at the lower oxygen concentrations requires less energy, as is indicated by the measured drop in swimming speed and this may result in a slight drop in the animal's oxygen requirements with decreased oxygen until at the Pc. its consumption decreases more rapidly. Some benefit may be derived from the changed appendage action, as the decreased activity and stable ventilation volume will together result in an increased oxygen independence, so that the Pc. falls at a lower level. Below this level some factor other than ventilation, probably in this case oxygen transport to the tissues, must limit the animal's oxygen consumption causing it to become oxygen dependent.

The effects of the two respiratory gases on the ventilatory behaviour of C. diaphanus would appear, therefore, to differ. High carbon dioxide concentrations produce an increase in the ventilation volume, while with decreased oxygen the animals merely maintain their ventilation volume at the same level, despite the drop in swimming speed.

CONCLUSION.

The ventilation of the respiratory surfaces in C. diaphanus depends on the action of its eleven pairs of thoracic appendages, which are also responsible for moving the animal through the water and providing currents from which food is filtered (Cannon, 1928). It is possible that this triple function will limit the adaptation of the appendage movements to any one of their roles. This limitation may be regarded as primitive (Johnson, 1936).

The results described above indicate that C. diaphanus is able to modify the action of its appendages in response to changes in the environmental concentrations of the respiratory gases. These modifications are likely to represent specific adaptations to changes in environmental conditions. Ventilation may, therefore, play an important part in the regulation of respiratory dependence in C. diaphanus. Certainly its place in the list of factors determining the Pc. in C. diaphanus (see pages 55 - 58) seems indisputable.

The similarity of the effects of oxygen lack and accumulation of carbon dioxide on the oxygen consumption and swimming speed of C. diaphanus indicates that the measured decrease in swimming speed results directly from a decrease in available energy. The recorded changes in appendage movements under unfavourable concentrations of the respiratory gases may, therefore, represent an adaptation allowing the animal to conserve energy by moving its appendages less forcefully against the surrounding water. By feathering its appendages C. diaphanus is able to reduce the work done moving them against the surrounding water whilst maintaining or increasing its ventilation volume. The reduced activity and increased ventilation will both make for greater independence of environmental oxygen and carbon dioxide concentrations.

It is equally possible that C. diaphanus is able to detect changes in environmental gas concentration, and the recorded changes in limb movements may be imposed by the central nervous system acting on information received from peripheral sense-organs. Whatever the mechanism is, it seems certain that no limitation is imposed on the ability of C. diaphanus to use its appendages to ventilate its respiratory surfaces efficiently, under changing environmental conditions.

Discussion of the possible limitations imposed on the action of the appendages in C. diaphanus by their triple function cannot be conclusive until some information on filter-feeding rates is available. The efficiency of filter-feeding in excess carbon dioxide or oxygen lack has not yet been measured. As C. diaphanus survived for long periods in the laboratory in tanks of oxygen depleted water where the carbon dioxide concentration was often quite high, and their guts were observed to be consistently full, it may be assumed that the feeding function of their appendages is not seriously impaired under these conditions. It is hoped that future experiments may include some work on filter-feeding rates, enabling the changes in all three appendage functions, under respiratory stress, to be examined. The present results indicate that the possible effects of their triple function may have been exaggerated in the past.

C. diaphanus is classified as a primitive crustacean on the basis of its anatomy; its physiology cannot, however, be regarded as primitive. It is a moderately successful species able to survive by virtue of specific adaptations to the conditions characteristic of its temporary pond habitat. This specificity of physiological adaptations to environmental conditions and not the animal's phylogenetic position is stressed by a consideration of the apparent pattern of ventilation in C. diaphanus compared to the known patterns in other animals.

The response to both oxygen lack and carbon dioxide excess by increasing the volume of water or air pumped over the respiratory surfaces has been reported in man (Haldane, 1922), and since in an extremely wide range of species. The way in which the ventilation volume is increased varies between species, but an increase in the amplitude of the ventilatory movements, as observed in C. diaphanus, is common, particularly among aquatic animals, (van Dam, 1938 ; Shelton, 1962) .

The presence of a continuous rhythmic movement of the ventilatory apparatus, controlled by centres in the central nervous sytem, is a widespread phenomenon, reported in mammals (Salmoiraghi and Delisle Burns, 1960) , fish (Shelton, 1959) insects (Miller, 1960 I ; Myers and Retzlaff, 1963) . It is probable that a similar autonomous rhythmicity, somewhere in the nerve cord of C. diaphanus, is responsible for the measured frequency of its appendage movements, causing it to remain unchanged despite extensive fluctuations in the concentrations of the respiratory gases.

Another physiological characteristic known to occur as an adaptation to environmental conditions is the possession of a blood pigment. The occurrence of haemoglobin in C. diaphanus, Arenicola marina, chironomid larvae, and vertebrates is explained by their high oxygen demand related to their oxygen supply, whether it be due to their high activity or a low environmental oxygen concentration, and has no bearing on their phylogeny.

Although the results obtained so far do not allow for a detailed analysis of respiratory control in *C. diaphanus*, the resemblences to the patterns established in other species present a basis for future work. This may take the form of an investigation of the origin of the constant frequency of appendage movements, in the central nervous system. Whatever future work might show, the present results are sufficient to demonstrate the relative efficiency of the respiratory system in *C. diaphanus*. The possible effects of the triple function of the appendages and its alleged "primitiveness" seem, therefore, to be unimportant.

Lowndes, 1933 noted that the swimming speed of *C. diaphanus* was variable, despite the constant frequency of its appendage movements, This he attributed to a variable twisting of the exopodite (flabellum), which he believed acted more like a propellor than a simple paddle. This observation is borne out by the author's measurements: the flabellum was observed to be ''feathered`` when the animal was swimming in water with a high carbon dioxide content and this resulted in the measured drop in its swimming speed.

SUMMARY OF RESULTS.

Measurements and observations taken from temporary ponds in the New Forest showed that their faunistic and physico-chemical properties varied widely, often over remarkably short periods of time. In common with other members of the restricted temporary pond fauna *Chirocephalus diaphanus* Prévost has drought resistant eggs. Its other physiological adaptations to life in this environment were investigated by measuring its oxygen consumption polarographically under a variety of conditions. Many of the experiments were repeated on the larvae of *Chironomus riparius* which were numerous in the New Forest ponds. Much of the study was, therefore, comparative.

The two species showed certain similarities in their patterns of oxygen consumption, indicative of similar physiological adaptations to their common habitat. For example their rates of oxygen consumption were independent of environmental oxygen concentration over a wide range i.e. they both had critical oxygen concentrations **of about** 1·5 ml. O_2/litre at 20°C .

They also possessed certain characteristics in common which could be responsible for this measured resistance to environmental fluctuations in oxygen concentration.

(a) They were both relatively small animals so that the efficiency of distribution of oxygen from their respiratory surfaces to their tissues may never limit their activity.

(b) They both had the respiratory pigment haemoglobin dissolved in their body fluids.

(c) There were indications that both species reacted to changes in the environmental concentration of the respiratory gases by a behavioural response affecting the water flow over their respiratory surfaces.

(d) They both were observed to be eurythermal though they were found to respond differently to temperature variation.

The two species invade the pond at different times and live in different parts of it, they may, therefore, be considered to live in separate ecological ~~niches.~~ microhabitats. The conditions in these ~~niches~~ microhabitats were found to differ. The mud in which the chironomid larvae build their tubes fluctuates less in temperature but is more prone to extreme oxygen depletion than the water mass in which C. diaphanus swims. These different environmental conditions were found to be reflected in the observed resistances and measured physiological adaptations of the two species.

The chironomid larvae were observed to survive prolonged oxygen depletion in the laboratory where the experimental animals were maintained in bowls of mud with oxygen concentrations often below 1·0 ml./litre in the covering water layer. In these conditions they extended the entrances of their tubes up from the mud surface and were able to survive. Under similar conditions of extreme oxygen depletion C. diaphanus died.

The observed physiological adaptations of the two species to low oxygen concentrations were found to correspond to their different resistances.

(a) When they were returned to air saturated water after a prolonged period of anoxia the rate of oxygen consumption of the chironomid larvae increased for a short time. This response resembled that demonstrated in the larvae of Chironomus plumosus by Walshe, 1948(a) Most of the increased oxygen consumption she attributed to a behavioural response resulting in increased activity, but considered that it may also represent the repayment of part of an oxygen debt. C. diaphanus showed no increase in oxygen consumption after exposure to low oxygen levels and did not survive even a short period at its residual oxygen concentration.

(b) The larvae of Chironomus riparius were observed to possess high concentrations of haemoglobin in their blood the whole time whereas C. diaphanus synthesises appreciable quantities of the blood pigment only after prolonged exposure to low oxygen concentrations (Gilchrist, 1962) .

(c) The haemoglobins were found to differ functionally in the two species. At 21°C the blood pigment in the chironomid larvae functioned in oxygen transport only at concentrations below 2·0 ml./litre and was most effective at approximately 0·5 ml./litre. At the same temperature the blood pigment in female <u>C. diaphanus</u> appeared functional up to 3·5 ml./litre and was most effective at approximately 1·4 ml./litre. These differences appear to fit the two animals for their respective habitats.

(d) The chironomid larvae were found to be very much more resistant to very high carbon dioxide concentrations, likely to be found only in the mud. A 10% solution of carbon dioxide resulted in a decrease of only 20% in their rate of oxygen consumption. A similar resistance to high concentrations of carbon dioxide was noted in stratiomyid larvae collected from dung heaps, by Miss Shiela Brydon (personal communication) . A 10% solution of carbon dioxide almost completely immobilised <u>C. diaphanus</u> and a 5% solution caused a 50% decrease in its rate of oxygen consumption.

The temperature in temporary ponds was observed to vary over a wide range; it was considered of interest, therefore, to measure the reactions of the two species to temperature variation. Though both species are eurythermal the chironomid larvae did not survive well in the laboratory at temperatures above 20°C. They may be shielded from excessive temperature variation by the water and mud, when in the mud. Since high environmental temperature was often found to coincide with low oxygen concentrations in the temporary ponds, the ability of the animals to survive variations in temperature is necessarily linked to their adaptations for withstanding varying oxygen concentrations.

Experiments indicated that C. diaphanus compensates metabolically for the effects of low temperature and so maintains a relatively high level of activity. This compensation may be necessary for efficient filter feeding at low temperatures and may partially explain its low Q_{10} of 1·68. Also the overshoot in the rate of oxygen consumption of C. diaphanus, produced by a sudden increase in temperature, is relatively short-lived. After an increase of 10°C the initial overshoot lasts only 1½ hours and it reaches its normal rate for the new temperature in approximately 12 hours.

In response to low oxygen concentrations C. diaphanus synthesises haemoglobin which makes it more resistant to the conditions likely to result during the hot summer months, and represents a long-term adaptation linked to the annual temperature variation.

C. diaphanus appears to adapt to varying temperatures; the larvae of *C. riparius* seem unable to adapt in the same ways. They show no ability to compensate for the effects of low temperatures: their rate of oxygen consumption is low and they become completely motionless below 5°C . This is illustrated by their high Q_{10} of 2·99 . Also they appear to recover very slowly after a sudden increase of 10°C in the environmental temperature; taking over two days to reach their acclimated rate of oxygen consumption at the new temperature.

Their ability to survive the large temperature fluctuations found even in the mud of the temporary ponds is probably dependent on:

(a) their low levels of random activity when in their tubes, which will decrease their oxygen requirements even at high temperatures;

(b) their ability to survive by accumulating an oxygen debt in the conditions of complete anoxia found in the mud at high temperatures and at low temperatures, below ice. This function was demonstrated by larvae when they were first warmed after collection from an ice covered pond. They were hyperactive for some time after the change in a very similar way to that previously observed in larvae recovering from prolonged anoxia.

(c) The low oxygen concentrations at which their haemoglobin is functional, even at high temperatures, may contribute to their survival in the summer months.

Whilst C. diaphanus is able to adapt to variations in temperature, the larvae of C. riparius appear to depend on their great tolerance of all environmental change, to survive temperature variation. Thus a sudden increase in ambient temperature is quickly compensated for by C. diaphanus but in the case of the chironomid larvae results in a prolonged increase in their rate of oxygen consumption. This causes them to deplete their supply of oxygen to a level at which they are only able to survive by virtue of their great tolerance of oxygen lack.

In addition to this comparative study, based on environmental measurements, an attempt was made to investigate the factors involved in controlling the respiratory dependence of the two species. To this end their critical oxygen concentrations (Pc.) were measured under a variety of environmental conditions and physiological states.

Measurements of the effects of temperature on the oxygen consumption of the two species established a link between their levels of activity and their Pc.'s. As the temperature rose and their oxygen consumption increased to provide for their rising level of activity, their Pc.'s increased proportionately. Further evidence of this link was provided by the effects of three other environmental variations.

(a) After a sudden increase in environmental temperature the animals experienced an overshoot in their rate of oxygen consumption. During the period of increased consumption they became oxygen dependent at higher concentrations than acclimated animals.

(b) When the water flow in the respiratory chamber was decreased the chironomid larvae became much less active. Consequently their oxygen consumption decreased and they became independent of environmental oxygen concentration to a lower level.

(c) Chironomid larvae subjected to water containing 2 parts per thousand of Sandoz M.S.222 anaesthetic respired at a lower rate than untreated larvae, and had a much lower Pc.

The characteristic Pc. of any species will depend on its physiology but will be affected by its behavioural response to environmental conditions. Environmental factors appear to effect the respiratory dependence of a species in two possible ways:

(a) by direct, short-term effects on the animals activity, e.g. the temperature overshoot;

(b) by long-term effects leading to physiological adaptation and the establishment of a greater independence from environmental oxygen concentration, e.g. the processes of temperature compensation and the effects of oxygen depletion on the synthesis of haemoglobin by C. diaphanus.

The physiological factors likely to be involved in determining an animals characteristic respiratory dependence curve were investigated in turn for the two species.

It was not considered likely that oxygen utilisation by the tissues would be involved in determining their Pc.'s. Winzler, 1941 stated that oxidations catalysed by the cytochrome system are usually found to be independent of oxygen concentration down to extremely low levels. Experiments on a preparation of C. diaphanus tissue indicated that this was in fact the case.

The limiting factors must, therefore, be some part or parts of the processes of oxygen uptake from the environment and transport around the body.

In the stationary water mass of temporary ponds, especially with the low oxygen concentrations often prevalent at high temperatures, it is necessary for the fauna to be able to actively ventilate their respiratory surfaces. The efficiency of this process may be a factor determining their Pc.'s. C. diaphanus ventilates its whole surface by the action of its eleven pairs of thoracic appendages, the same movements being used for swimming and feeding. As it apparently swims continuously it is always ventilating its respiratory surfaces. The work on the functioning of the appendages in C. diaphanus indicated that the amount of water passed over its respiratory surfaces increased in times of respiratory stress; particularly under the stimulus of an increased carbon dioxide concentration.

Walshe, 1950 showed that the larvae of Chironomus plumosus spent more of their time irrigating their mud tubes as the oxygen concentration decreased. Similar behaviour was observed in the larvae of C. riparius. When large numbers of larvae were rendered completely motionless by exposure to 20 parts per thousand of M.S.222 their oxygen consumption was markedly decreased but their Pc. remained high. As their heart rate appeared to be little affected by the drug it was assumed that in the absence of ventilation currents oxygen uptake was limited by the rate of diffusion of oxygen through the tightly bunched mass of larvae.

The control of their ventilation volumes, i.e. the supply of oxygenated water to their respiratory surfaces, seems, therefore, to be an important factor in the respiratory regulation of both species.

The diffusion of oxygen to the tissues of untreated individuals of both species is unlikely to be limiting as they are both relatively small animals with an open circulation bathing all their organs, so that the diffusion distances are likely to be short.

The possession of a blood-pigment seems a definite advantage in the temporary pond habitat, as is indicated by its widespread occurrence in members of the fauna. Chironomus riparius larvae always have haemoglobin dissolved in their blood. C. diaphanus synthesises more haemoglobin when kept in low oxygen concentrations (Gilchrist, 1962) ; this is an indication of its functional significance. The experimental removal of a functional blood pigment by poisoning with carbon monoxide, and experiments on C. diaphanus from well aerated water demonstrated the importance of haemoglobin in maintaining the low Pc.s of both species.

Without functional haemoglobin both species were still independent of environmental oxygen down to low concentrations. It would seem, therefore, that their whole processes of oxygen uptake and distribution are efficient and adapted to function at low oxygen concentrations. When their observed behavioural responses to oxygen depletion are considered (e.g. male C. diaphanus swim close to the water surface, C. riparius larvae build their tubes up from the mud and may leave them for short periods to swim near the surface) , both species would seem to be adequately adapted for life in the unfavourable conditions of their habitat.

Gilchrist, 1962 found that female C. diaphanus synthesised more haem than the males after prolonged exposure to the same low oxygen concentrations.

The experiments on the oxygen consumption and appendage action of C. diaphanus revealed other differences between the two sexes.

The present study revealed that both sexes had similar rates of oxygen consumption per gramme of tissue and Pc.'s when taken from well aerated water. After prolonged exposure to low oxygen concentrations, however, the females had a higher oxygen consumption at the lower concentrations and a lower Pc. than the males, and unadapted females.

When females kept in low oxygen concentrations were poisoned with carbon monoxide their respiratory dependence curves appeared to change back to the shape obtained from animals kept in well aerated water. The development of the different patterns of oxygen consumption at low oxygen concentrations may, therefore, be attributable to the different powers of haem synthesis in the two sexes.

The later work on appendage movements revealed that the females had a higher A.P.B.R. and faster swimming speed than the males, particularly at low oxygen concentrations. The different rates of oxygen consumption are, therefore, reflected in the motor activity of the animals.

The females were found to survive exposure for longer periods to lower oxygen concentrations than the males and were generally to be found last in field collections when the population was dying out. This may be due directly to their superior physiological resistance to low oxygen concentrations or their higher activity may allow them to avoid predators. In either case the measured superiority of the females would seem to have some ecological significance. The functional significance of their different resistances and survival times is a little difficult to determine. It may enable the females to carry their sac of fertilised, shelled eggs and to distribute them in the pond just before it finally dries up by which time the males may have succumbed to the poor conditions. The males did, however, seem able to survive low oxygen concentrations in open water, possibly due to their behavioural response of swimming close to the surface.

A fairly extensive study of the appendage action in C. diaphanus formed the final section of the present work. It was intended to investigate the role of ventilation in determining the oxygen dependence of this species. Special attention was given to the possible limitation imposed on the effectiveness of the appendages in any one role by their triple role in swimming, filter-feeding and ventilation.

Initially the frequency of the appendage movements (A.P.B.R.) and the animals swimming speed were measured. The effects of temperature on both these peremeters of movement resembled the results obtained for oxygen consumption, including the presence of an overshoot response to sudden temperature change. The effects of decreased oxygen and increased carbon dioxide concentrations on the A.P.B.R. agreed with the result given by Fox and Johnson, 1934 , i.e. the frequency was unaffected by changes in respiratory gas concentration. The swimming speed was found to be markedly affected, however, resembling the animal's oxygen consumption in its sensitivity to changes in oxygen and carbon dioxide concentration. Swimming speed appears to be a better measure of motor activity and effectiveness of the appendage movements than the A.P.B.R.

The absence of any direct correlation between A.P.B.R. and swimming speed, under conditions of respiratory stress, was found to be due to the appendages being 'feathered' during their effective stroke. Meanwhile the amplitude of the appendage movements tended to increase, particularly in the presence of high concentrations of carbon dioxide. In this way the appendage action in <u>C. diaphanus</u> may be specialised to produce an increased ventilation volume under unfavourable respiratory gas concentrations. It seems able to accomplish this despite its lower level of motor activity, which may in part result from an adaptation designed to conserve the available oxygen.

The action of the appendages in swimming and ventilation is summarised below.

1. SWIMMING SPEED = Volume of water moved past the body surface.

Volume of water moved past the body surface = MOTOR ACTIVITY.

MOTOR ACTIVITY = A.P.B.R. + AMPLITUDE + ANGLE OF ATTACK. of appendages.

2. VENTILATION VOLUME = Volume of water moved over the respiratory surfaces of the appendages.

Volume of water moved over the respiratory surfaces of the appendages. = A.P.B.R. + AMPLITUDE.

From this summary it can be seen that a change in the angle of attack of the appendages may reduce the animals swimming speed and motor activity, thus conserving its energy, whilst its ventilation volume may be increased by an amplitude change, its A.P.B.R. remaining constant throughout.

This possible pattern of adaptation of the appendage action in *C. diaphanus* to changes in respiratory gas concentration, indicates the importance of its ventilation volume in determining the respiratory dependence of this species. It also appears to contest the existence of the alleged limitation put upon the action of the appendages by their triple role.

The role of the appendages in filter-feeding has not been investigated in the present study and cannot be summarised in a simple form like the other two functions. The indication is, however, that *C. diaphanus* is able to feed satisfactorily in poor conditions so that the modified action of its appendages may have little effect upon its feeding currents.

THE DISCOVERY OF TRIOPS CANCRIFORMIS (BOSC) IN THE NEW FOREST.

In June 1964 a single individual of the species Triops cancriformis (Bosc.) hatched from some mud collected from a pond near Fordingbridge in the New Forest, Hampshire. Regular observations were made on this pond through 1963-4 as it was a site for the collection of Chirocephalus diaphanus Prévost, which was the subject of a research project. No T. cancriformis were observed or collected in the pond during this period.

Fox, 1949 described the rediscovery of Apus (Triops cancriformis) in the New Forest, near Fordingbridge. He hatched a number of specimens out of dry mud collected in September 1947 from the site of a pool in which Triops had been previously recorded in 1934 (see Hobson and Omer-Cooper, 1935) . It is probable that the recent collection by the author was from the same pond as these earlier records.

In his paper Fox summarised the five previously published records of T. cancriformis in Great Britain, discussed its nomenclature and the reasons for its rarity in this country, and described its appearance, feeding habits, blood pigments, locomotion, and vision.

The great rarity of occurrence of this species in Britain was considered to make its rediscovery worthy of further comment, and a few additional observations on its habits were made.

T. cancriformis belongs to the order Notostraca, regarded as one of the most primitive crustacean groups (see Lankester, 1881) , closely related to the order Anostraca of which *Chirocephalus diaphanus* Prévost is a member. Both species are interesting because of their rarity and phylogenetic primitiveness, and also because of their occurrence together in temporary ponds, to which they are limited by predation. A study of the conditions in these ponds is included in an earlier section (pgs. 5 to 28) .

Having noted these close phylogenetic and ecological relationships, it was decided to repeat on *T. cancriformis* some of the work done by the author on *C. diaphanus*. The intention was to investigate any physiological similarities between the two species. Its respiratory dependence curve was determined polarographically using a method described earlier (see pgs. 39 to 41) . Briefly, the animal was exposed to a decreasing oxygen concentration inside a closed container, and its oxygen consumption measured over the whole range of oxygen concentrations below air saturation, at 20°C . A result is given in figure 51.

The oxygen consumption of *T. cancriformis* is independent of environmental oxygen concentration down to low levels (approximately 2·2 ml./litre at 21°C.) , after this concentration it becomes markedly dependent. This curve is very similar to that determined for *C. diaphanus* at the same temperature (see figure 12) , and indicates that the two species have a similar respiratory physiology. *T. cancriformis* consumes oxygen at a much faster rate per gramme of tissue, however, and its high activity is reflected in its observed rate of movement, with an appendage beat rate of approximately 250/minute at 20°C , considerably faster than that reported by Fox, 1949 and measured in *C. diaphanus* (200 /minute at 20°C) , and its very fast growth rate, to be described below.

Other physiological and behavioural similarities between these two species have been observed and reported. Fox, 1949 described the presence of haemoglobin in the blood of *T. cancriformis*, and stated that the quantity varied inversely with the dissolved oxygen content of the water. The presence of this pigment is responsible for the red colouration of the specimen in plate 7 . A similar occurrence of haemoglobin has been noted in *C. diaphanus* (see pgs. 60 - 64) , and reported by Lankester, 1870 and Gilchrist, 1962 .

T. cancriformis normally swims with its dorsal surface uppermost (see plate 6) . Fox, 1949 reported that when the oxygen content of the water became particularly low, it swam "slowly upside-down along the water surface" (see plate 7) . This modified behaviour was more prevalent in animals with little haemoglobin in their blood. Similar behaviour was noted in *C. diaphanus*, by the author, particularly in the males which are known to synthesise less haemoglobin than the females (Gilchrist, 1962) and are less resistant to extreme oxygen depletion.

In order to live in temporary ponds an animal must have a drought resistant stage and must mature rapidly. Both species have drought resistant eggs, and Fox, 1949 reported the fast growth rate of *T. cancriformis* in his laboratory. The specimen studied by the author lived for 23 days after it was first observed, and during this time grew from 0·86 cm. to 2·83 cm., measured from the front of the carapace to the base of the caudal furcae, at a mean water temperature of approximately 20°C . The exuviae were collected after each moult, and were measured, the measurement being made along the dorsal, sagittal line of the shield-shaped carapace, which is raised into a 'keel' (see plate 6). The increased length of the carapace at each moult, against time, is given in Figure 51 in which the figures are compared to some taken from a collection of Triops made in Africa and presented to the department by Mr. M. W. Paskin . I wish to acknowledge his thoroughness in noting the dates of his collections, taken from a single pool, and the air temperatures.

The increase in size over such short periods is exceptional in both cases and the growth rate seems to be temperature dependent. This fast growth rate is an important factor in the survival of these relatively large organisms as it allows them to reach maturity in ponds of a very temporary nature. This will be particularly advantageous in Africa where the ponds may only last for a few days.

The specimen taken from the New Forest finally moulted to a carapace length of 2·03 cm., and remained this size for 6 days. The carapace became thickly covered with matted detritus and an epizoic fauna including rotifers. At this time two egg sacs full of bright red, shelled eggs were seen on its ventral surface. Whilst the animal was being observed it caste one of these batches of eggs as a compact mass. Many eggs were later recovered from the detritus at the bottom of the bowl in which the animal had been kept. Soon after this observation T. cancriformis died without moulting again. Its final length, including the caudal furcae, was 6·26 cm.

T. cancriformis does differ from C. diaphanus in some important aspects of its physiology. Thus T. cancriformis feeds by stirring up the detritus with its strong anterior appendages and filtering off the rich organic suspension produced, whilst C. diaphanus filters off smaller particles, mainly algae, suspended in the water. There is little real difference between these two methods, however, as one could easily be derived from the other. The method used by T. cancriformis is similar to that thought to have been employed by the fossil trilobites and closely resembles the feeding behaviour of the present day genus Limulus. It may, therefore, represent the primitive arthropod feeding mechanism. Whenever overcrowding led to a depletion of suspended organic matter C. diaphanus was observed to feed on the detritus in a similar way to T. cancriformis, producing characteristic tracks across the substratum. This behaviour is variously developed in the different genera of the Anostraca, some feeding predominantly on the surface of the substratum (Gilchrist, personal communication.)

Fox, 1949 described T. cancriformis feeding avidly on other macro-invertebrates which it chewed up with the endites of the thoracic limbs. I observed it manipulating a string of chironomid eggs with its thoracic appendages and apparently devouring them. This habit will supplement its diet with high protein food and together with the large quantities of filtered detritus, will provide for its rapid growth rate.

When swimming free in the water T. cancriformis has a strong dorsal light reaction (Seifert, 1930) and C. diaphanus an equally strong ventral light reaction (Seifert, 1932). They are, however, both capable of reversing this orientation; T. cancriformis in response to oxygen deficiency (Fox, 1949 and see plate 7) and C. diaphanus to absence of suspended food particles (see above).

In Great Britain, T. cancriformis is always hermaphrodite and self-fertile (Longhurst, 1954). This could be an advantage in isolated temporary habitats as it allows a single surviving individual to re-establish a population. C. diaphanus is always bisexual, the sexes occurring in approximately equal numbers. The females always carry a store of fertilised eggs in the egg sac, however, and were observed to survive longer than the males in poor conditions, possibly due to their superior physiological adaptations.

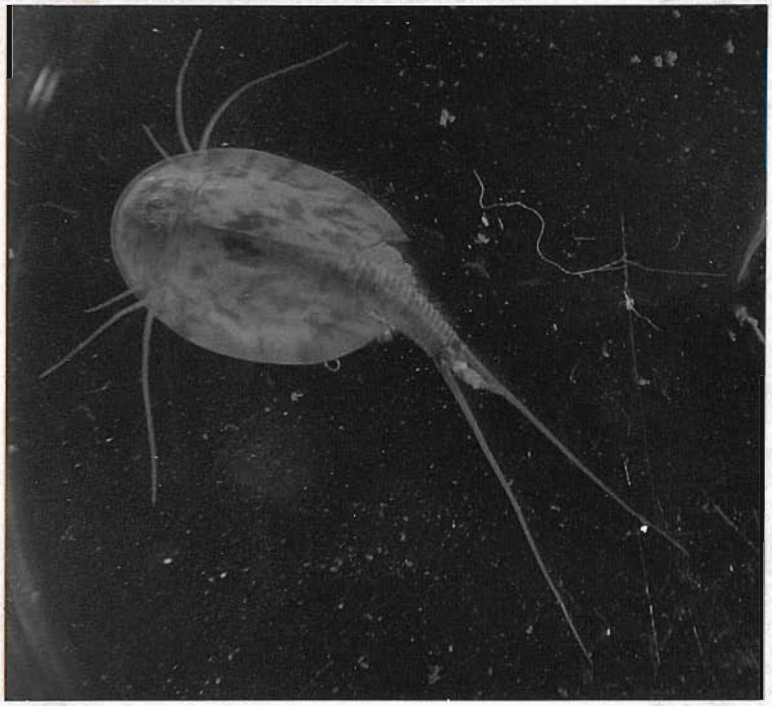
DISCUSSION.

Following upon the observations reported by Fox, 1949 in 1934, 1947, 1948 and 1949, the rediscovery of *Triops cancriformis* (Bosc) in this New Forest pond in 1964 would seem to establish the permanent, if somewhat sporadic, nature of the settlement of this species in this area. It seems probable that this prolonged maintenance of the population is due to eggs surviving in the mud at the site of the pond for long periods, rather than depending on repeated chance invasions from an outside source. Fox, 1949 observed that the hatching of *T. cancriformis* eggs is encouraged by a period of desiccation and that more may hatch from a second or third drying than from the first. This may explain the infrequent occurrence of *T. cancriformis* in the New Forest pond.

The discovery of *T. cancriformis* establishes a link between the locality studied by Fox and the one from which this recent discovery was made, and enables some earlier records of the occurrence of *Chirocephalus diaphanus* Prevost to be used. Hobson and Omer-Cooper, 1935 stated that *C. diaphanus* was known to occur in this locality in 1934. Fox, 1949 observed large numbers of

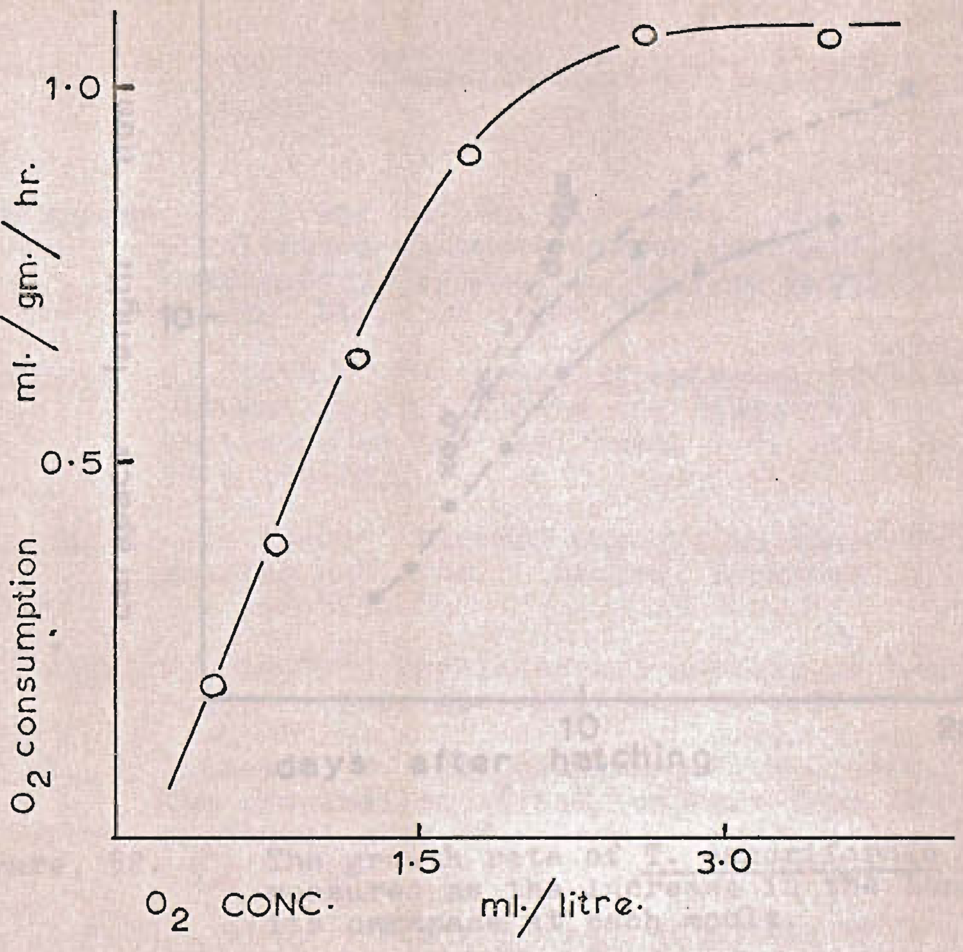
C. diaphanus in the same locality in February, 1948. Mr. R. E. Hall collected C. diaphanus from the pond in 1958 and my observations on the collection of this species from the same pond in 1963 and 1964 are given above (see pages 8 - 11). This species is very obviously endemic in this particular locality, though Hall, 1961 has reported the apparently discontinuous nature of the occurrence of C. diaphanus in other ponds in the New Forest. The continued existence of both the C. diaphanus and T. cancriformis populations in this single locality is of undoubted significance and may be explained by the more truly temporary nature of this pond, which was less deep and more often dry, than the other New Forest ponds observed by the author.

It was interesting to find two such closely related and uncommon crustacean species occurring in the same locality, and it is likely that they both owe their continued existence to their ability to live in this unusual and specialised habitat. Their physiological, morphological and behavioural adaptations to this habitat seem very similar. Thus, they both have drought resistant eggs and a relatively fast growth rate, leading to early maturation; their oxygen consumption is independent of environmental oxygen concentration over a wide range, they show the same behavioural response to short-term oxygen depletion, and they are both able to synthesise more haemoglobin when the oxygen concentration remains very low for prolonged periods. The great similarity between their physiological adaptations may be partially explained by the close phylogenetic ties between the two species which could govern the ways their organisation is able to adapt to environmental stress. It is, however, more likely to have evolved as a series of advantages enabling the animals to meet the demands of the extreme and unusual environmental conditions in temporary ponds.



Plate, 6. Triops cancriformis swimming in its normal position.

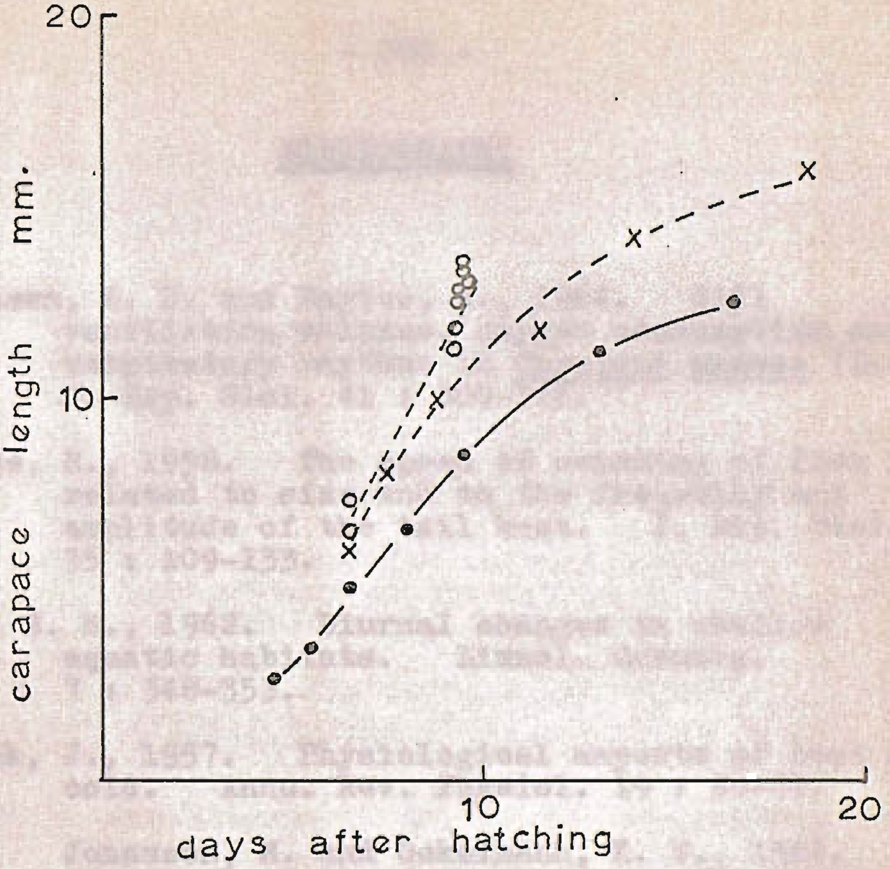
Plate, 7. T. cancriformis swimming ventral side uppermost. It is red in colour due to the presence of haemoglobin in its body fluids.



figure, 51. The oxygen consumption of *T. cancriformis* in decreasing oxygen concentrations at 21°C.

It has a much higher rate of oxygen consumption than *C. diaphanus* and a correspondingly high Pc. at approximately 2·2 ml./litre. It is however, considering its high level of activity, relatively independent of environmental oxygen concentration.

Only I experiment in the closed chamber respirometer.



figure, 52. The growth rate of T. cancriformis. measured as the increase in the length of its carapace at each moult.

x , mean water temperature 21°C (June, 1964).
● , " " " 19°C (January, 1965).
o , " " " 27°C (African species collected by M.W. Paskin, Nov. 1961).

The day of hatching could only be estimated for the African species.

Its initial rapid growth rate lasted for approximately two weeks, when it reached sexual maturity and grew less rapidly. Its rate of growth appears to depend on water temperature.

BIBLIOGRAPHY

Arudpragasam, K. D. and Naylor, E., 1964. Gill ventilation volumes, oxygen consumption and respiratory rhythms in Carcinus maenas (L.). J. Exp. Biol. 41 : 309-322.

Bainbridge, R., 1958. The speed of swimming of fish as related to size and to the frequency and amplitude of the tail beat. J. Exp. Biol. 35 : 109-133.

Bamforth, S. S., 1962. Diurnal changes in shallow aquatic habitats. Limnol. Oceanog. 7 : 348-353.

Belehradek, J., 1957. Physiological aspects of heat and cold. Annu. Rev. Physiol. 19 : 59-82.

Berg, K., Jonasson, M. and Ockelmann, K. W., 1962. The respiration of some animals from the profundal zone of a lake. Hydrobiologia 19 : 1-39.

Bishai, H. M., 1960. The effect of water currents on the survival and distribution of fish larvae. J. Cons. Int. Explor. Mer. 25 : 134-146.

Black, E.C., and Irving, L., 1937. The effect of carbon dioxide upon the oxygen capacity of the carp (Cyprinus carpio L.). Tr. Roy. Soc. Canada, Biol. Sci. 31 : 29-32.

Brafield, A.E., 1964. The oxygen content of interstitial water in sandy shores. J. Anim. Ecol. 33 : 97-116.

Brett, J. R., 1956. Some principles in the thermal requirements of fishes. Quart. Rev. Biol. 31 : 75-87.

Brundin, L., 1951. The relation of oxygen microstratification at the mud surface to the ecology of the profundal bottom fauna. Rep. Inst. Freshw. Res. Drottning. 32 : 33-42.

Bullock, T.H., 1955. Compensation for temperature in the metabolism and activity of poikilotherms. Biol. Rev. 30 : 311-342.

Buscemi, P. A., 1958. Littoral oxygen depletion produced by a cover of Elodea canadensis. Oikos 9 : 239-245.

Cannon, H. G., 1928. On the feeding mechanism of the fairy shrimp, Chirocephalus diaphanus Prevost. Trans. roy. Soc. Edinb. 55 : 807-822.

Cole, A. E., 1932. Method for determining the dissolved oxygen content of the mud at the bottom of a pond. Ecology, 13 : 51-53.

Croghan, P. C., 1958. The osmotic and ionic regulation of Artemia salina (L.). J. Exp. Biol. 35 : 219-233.

Dexter, R. W. and Sheary, L. E., 1943. Records of anostracan phyllopods in northeastern Ohio. Ohio J. Sci. 4 : 176-179.

Dexter, R. W., 1953. Studies on North American fairy shrimps with the description of two new species. Amer. Midl. Nat. 49 : 751-771.

Edwards, R. W., 1958. The effect of larvae of Chironomus riparius Meigen on the redox potentials of settled activated sludge. Ann. appl. Biol. 46 : 457-464.

Ewer, R. F., 1942. On the function of haemoglobin in Chironomus. J. Exp. Biol. 18 : 197-205.

Fox, H. Munro and Johnson, M. L., 1934. The control of respiratory movements in the Crustacea by oxygen and carbon dioxide. J. Exp. Biol. 11 : 1-10.

Fox, H. Munro, 1949. On Apus: its rediscovery in Britain, nomenclature and habits. Proc. zool. Soc. Lond. 119 : 693-702.

Fox, H. Munro and Wingfield, C.A., 1938. A portable apparatus for determination of oxygen dissolved in a small volume of water. J. Exp. Biol. 15 : 437-445.

Fox, H. Munro, Gilchrist, B. M. and Phear, E. A., 1951. Functions of haemoglobin in *Daphnia*. Proc. roy. Soc. B. 138 : 514-

Fry, F. E. J., 1947. Effects of the environment on animal activity. Univ. Toronto Stud. Biol. Series 55. Pub. Ont. Fish. Res. Lab., 68 : 62 pp.

Fry, F. E. J. and Hart, J. S., 1948(a). Cruising speed of goldfish in relation to water temperature. J. Fish. Res. Bd. Can. 7 : 169-175.

Fry, F. E. J. and Hart, J. S., 1948(b). The relation of temperature to oxygen consumption in the goldfish. Biol. Bull., Wood's Hole 94 : 66-77.

Fry, F. E. J., 1957 Chap. I in "The physiology of fishes.", edited by M. E. Brown. New York.

Garstang, W. and Gurney, D., 1938. The descent of Crustacea from trilobites, and their larval relations. In "Evolution" edited by G. R. de Beer. Oxford.

Gibson, E. S., and Fry, F. E. J., 1954. The performance of the lake trout, *Salvelinus namaycush*, at various levels of temperature and oxygen pressure. Can. J. Zool. 32 : 252-260.

Gilchrist, B. M., 1954. Haemoglobin in *Artemia*. Proc. roy. Soc. B. 143 : 136-146.

Gilchrist, B. M. and Green, J., 1962. Bile pigment in *Chirocephalus diaphanus* Prevost (Crustacea : Anostraca). Comp. Biochem. Physiol., 7 : 117-125.

Grainger, J. N. R., 1955. Ph.D. Thesis, London.

Grainger, J. N. R., 1956. Effects of changes of temperature on the respiration of certain Crustacea. Nature, Lond. 178 : 930-931.

Grainger, J. N. R., 1958. First stages in the adaptation of poikilotherms to temperature change. 'Physiological Adaptation' edits. C. L. Prosser. Amer. Physiol. Soc. Washington.

Grainger, J. N. R., 1960(a). The early stages in the adaptation of the frog Rana temporaria to higher temperatures. Verh. dtsch. zool. Ges. 1960 : 60-72.

Grainger, J. N. R., 1960(b). The early phases in the adjustment of respiratory rates of unicellular organisms to new temperatures and the simulation of these in a model. Z. wiss. Zool. 163 : 317-341.

Haldane, J. S., 1922. Respiration. New Haven, Yale University Press.

Hall, R. E., 1953. Observations on the hatching of eggs of Chirocephalus diaphanus Prevost. Proc. zool. Soc. Lond. 123 : 95-109.

Hall, R. E., 1959. The development of eggs of Chirocephalus diaphanus Prevost in relation to depth of water. Hydrobiologia 14 : 79-84.

Hall, R. E., 1961. On some aspects of the natural occurrence of Chirocephalus diaphanus Prevost. Hydrobiologia 17 : 205-217.

Harnisch, O., 1936. Primare und sekundare Oxybiose der Larve von Chironomus thummi. Z. vergl. Physiol. 23 : 391-419.

Harnisch, O., 1951. Hydrophysiologie der Tiere. Stuttgart.

Hobson, A. D. and Omer-Cooper, J. 1935. Apus cancriformis in Great Britain. Nature, Lond. 135 : 792.

Hughes, G. M. and Shelton, G., 1962. Respiratory mechanisms and their nervous control in fish. Advances in Comp. Physiol. Biochem. 1 : 275-364.

Hyman, L. H., 1932. Relation of oxygen tension to oxygen consumption in Neneis virens. J. exp. Zool. 61 : 209-221.

Job, S. V., 1955. The oxygen consumption of Salvelinus fontinalis. Univ. Toronto Studies Biol. Ser. no. 61. Publ. Ontario Fisheries Research Lab. 73 : 39 pp.

Johnson, M. L., 1936. The control of respiratory movements in Crustacea by oxygen and carbon dioxide. J. exp. Biol. 13 : 467-475.

Jones, J. D., 1955. Observations on the respiratory physiology and on the haemoglobin of the polychaete genus Nephthys, with special reference to N. hombergii (Aud. et. M. - Edw.) J. exp. Biol. 32 : 110-125.

Kanwisher, J., 1959. A polarographic oxygen electrode. Limnol. Oceanog. 4 : 210-217.

Lankester, E. Ray, 1869. Spectroscopic examination of certain Animal Substances. J. Anat. Lond. 4 : 119.

Lankester, E. Ray, 1881. Observations and reflections on the appendages and on the nervous system of Apus cancriformis. Quart. J. micro. Sci. 21 : 343-376.

Lankester, E. Ray, 1909. A treatise on zoology. Part VII. (W. T. Calman on Crustacea) London. Adam and Charles Black.

Lowndes, A. G., 1933. The feeding mechanism of *Chirocephalus diaphanus* Prevost, the fairy shrimp. Proc. zool. Soc. Lond. 1933 (ii) 1093-1118.

Macan, T. T., 1961. Factors that limit the range of freshwater animals. Biol. Rev. 36 : 151-198.

Mann, K. H., 1956. A study of the oxygen consumption of five species of leech. J. exp. Biol. 33 : 615-626.

Mathais, P., 1926. Sur la biologie d'un crustace phyllopode (Chirocephalus diaphanus Prevost) C. R. Soc. Biol., Paris 94 : 1193.

Mathias, P., 1937. Biologie des Crustaces Phyllopodes. Actualites sci. industr. 447 : 1-107.

Mayer, A. G., 1914. The effects of temperature upon tropical animals. Pap. Tortugos Lab. 6 : 3.

Mellanby, H., 1951. Animal life in fresh-water; a guide to British fresh-water invertebrates. (4th edition). London.

Miller, P. L., 1960. Respiration in the desert locust. I The control of ventilation. J. exp. Biol. 37 : 224-277.

Moore, W. G., 1951. Observations on the biology of *Streptocephalus seali*. Proc. La. Acad. Sci. 14 : 57-65.

Moore, W. G., 1955(a). The life history of the spiny-tailed fairy shrimp in Louisiana. Ecology 36 : 176-184.

Moore, W. G., 1955(b). Observations on the heat death of the fairy shrimp, *Streptocephalus seali*. Proc. La. Acad. Sci. 18 : 5-17.

Moore, W. G., 1957. Studies on the laboratory culture of Anostraca. Trans. Amer. micro. Soc. 66 : 159-173.

Moore, W. G., 1959. Observations on the biology of the fairy shrimp, Eubranchipus holmani. Ecology. 40 : 398-403.

Nourrison, M., 1960. Effet de l'assechement sur le developpement des oeufs de Chirocephalus stagnalis Shaw. C. R. Acad. Sci., 249 :

Pause, J., 1919. Beitrage zur Biologie und Physiologie der Larve von Chironomus gregarius. Zool. Jb. 36 : 339-

Pearce, A. S., 1913. Observations on the behaviour of Eubranchipus dadayi. Bull. Wisc. nat. Hist. Soc. 10 : 109-117.

Precht, H., Christopherson, J. and Hensel, H., 1955. Temperatur und Leben. Berlin, Springer-Verlag, 514 pp.

Precht, H., 1958. Concepts of the temperature adaptation of unchanging reaction systems of cold-blooded animals. Physiological Adaptation (edit. C. L. Prosser). Washington, American Physiological Society.

Prosser, C. L., Brown, F. A., Bishop, D. W., Jahn, T. L. and Wulff, V. J., 1950. Comparative Animal Physiology. Philadelphia, Saunders.

Prosser, C. L., 1958. The nature of physiological adaptation. Physiological Adaptation (edit. C. L. Prosser). Washington, American Physiological Society.

Prosser, C. L. and Brown, F. A., 1961. Comparative Animal Physiology. Philadelphia, Saunders.

Ruttner, F., 1953. Fundamentals of Limnology. Univ. Toronto Press.

Ryland, J. S., 1963. The swimming speeds of plaice larvae. J. exp. Biol. 40 : 285-300.

Salmoiraghi, E. C. and Delisle Burns, B., 1960. Localisation and patterns of discharge of respiratory neurones in the brain-stem of the cat. J. Neurophysiol. 23 : 2-13.

Scheer, B. T., 1963. Animal Physiology. Wiley, New York.

Seifert, R., 1930. Sinnesphysiologische untersuchungen am Kiemenfuss (Triops cancriformis Bosc.) Z. vergl. Physiol. 11 : 386-436.

Seifert, R., 1932. Raumorientierung und Phototaxis der Anostraken Euphyllopoden. Z. vergl. Physiol. 16 : 111-184.

Shelton, G., 1959. The respiratory centre in the tench (Tinca tinca L.). I. The effects of brain transections on respiration. J. exp. Biol. 36 : 191-202.

Shepard, M. P., 1955. Resistance and tolerance of young speckled trout (Salvelinus fontinalis) to oxygen lack, with special reference to low oxygen acclimation. J. Fish. Res. Board, Can. 12: 387-446.

Teal, and Halcrow, K., 1962. A technique for measurement of the respiration of single copepods at sea. J. Con. explor. mer. 27 : 125-128.

Theinemann, A., 1923. Die beiden Chironomusarten der Tiefenfauna der Norddeutschen Seen. Ver. int. Ver. Limnol. Kiel. Stuttgart, 1 : 108.

van Dam, L., 1938. On the utilization of oxygen and regulation of breathing in some aquatic animals. Dissertation, Groningen.

von Ledebur, J. F., 1939. Der Saurestoff als okologischer Faktor. Ergeb. Biol. 16 : 173-261.

Walshe, B. M., 1948(a). On the function of haemoglobin in Chironomus after oxygen lack. J. exp. Biol. 24 : 329-342.

Walshe, B. M., 1948(b). The function of haemoglobin in Tanytarsus. J. exp. Biol. 24 : 343-351.

Walshe, B. M., 1949. The oxygen requirements and thermal resistance of chironomid larvae from flowing and from still waters. J. exp. Biol. 25 : 35-44.

Walshe, B. M., 1950. The function of haemoglobin in Chironomus plumosus under natural conditions. J. exp. Biol. 27 : 73-95.

Ward, H. B. and Whipple, G. C. 1959. Fresh-water biology. (2nd. edition. edit. W. T. Edmondson). New York.

Wells, N. A., 1935. The influence of temperature upon the respiratory metabolism of the Pacific killifish, Fundulus parvipinnis. Physiol. Zool. 8 : 196-227.

Weymouth, F. W., Crisman, V. E., Hall, H., Belding, S. and Field, J., 1944. Total and tissue respiration in relation to body weight. A comparison of the kelp crab with other crustaceans and with mammals. Physiol. Zool. 17 : 50-70.

Winzler, R. J., 1941. The respiration of bakers' yeast at low oxygen tension. J. Cell. Comp. Physiol. 17 : 263-276.

Wolsky, I938. The effect of carbon monoxide on the oxygen consumption of Drosophila melanogaster pupae. J.exp.Biol. I5: 225-