A STUDY OF THE TOLERANCE OF VARIOUS CLADOCERA TO OXYGEN DEFICIENCY IN THE WATER.

by

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ABSTRACT

The importance of oxygen deficiency in the water as a factor limiting the distribution of Cladocera has been investigated.

A method has been devised by means of which Cladocera can be subjected to a constant degree of oxygen lack for any desired time.

A comparison was made between the ability of pond and lake dwelling Cladocera to survive under conditions of oxygen deficiency.

The results obtained indicate that Cladocera living in the open waters of large lakes die rapidly when placed in waters of low oxygen content, in which pond dwelling species can survive for many hours.

Animals living in small ponds experience a diurnal fluctation in the amount of dissolved oxygen in the water. In some ponds the amount of dissolved oxygen frequently falls to very low levels at which planktonic lake dwelling Cladocera are incapable of survival for 4 hrs. This may account for the absence of planktonic species from small ponds.

INTRODUCTION

I. The importance of various environmental factors in determining the distribution of the Cladocera.

Of the many factors involved in the distribution of the Cladocera, some of the most apparent are, pH, temperature, food and oxygen concentration in the waters where the animals are found.

pH has been studied by a number of workers, including Poulsen (1928), Hubault (1933) and Pacaud (1939). It has been shown to be of limited importance, but there is much disagreement among the various authors.

Temperature has been investigated in relation to longevity of Daphnia magna Straus, by MacArthur & Baillie (1929), Anderson & Jenkins (1942) and Pratt (1943), in relation to the production of males by Grosvenor & Smith (1913) and Banta (1939), also, more generally, in relation to the distribution of the Cladocera as a whole, by Brown (1929) and Johnson (unpublished work). Johnson showed that the Cladocera have, with very few exceptions, a wide degree of temperature tolerance; many can live and reproduce in ice covered waters, and are also capable of survival at temperatures of 30 - 35°C. Thus it seems unlikely that temperature can exert an appreciable influence on their distribution. Among the few exceptions to this generalisation is Moina macrocopa Straus, a warm water species found only in summer in England.

Food supply was found, by Johnson, to be an important factor in limiting cladoceran distribution.

The concentration of oxygen in the water, in relation to the species of Cladocera present, has been studied in the field, and also, in a preliminary way in the laboratory, by Wagler (1923) and Pacaud (1939). This work will be discussed later. The genus Daphnia has also been intensively studied by Fox (1948), Fox, Hardcastle & Dresel (1949) and Fox, Gilchrist & Phear (1951), in connection with its ability to synthesize haemoglobin when the oxygen content of the water is low.

II. Oxygen as an environmental factor - its effect on animal behaviour and metabolism.

A great deal of work has been done on the importance of oxygen concentrations both in air and water, as a factor limiting the distribution of animals. (This has recently been reviewed by Prosser, Brown, Bishop, Jahn & Wulff in their Textbook of Comparative Animal Physiology, 1950). It was discovered by Fox & Simmonds (1933) that the metabolic rates (as measured by oxygen consumption) of animals living in well aerated waters such as swiftly flowing streams, are higher than those of animals living in lentic habitats, lakes and ponds where the oxygen supply is limited, and at times very low. That is to say, those animals living in well aerated habitats do in fact need more oxygen than those which live in poorly aerated places. Fox & Simmonds (1933) measured the metabolic rates of various closely related arthropods from different habitats, and found that the oxygen consumption of those from running waters was always greater than that of their pond dwelling, or marine, relatives. In 1935 Fox, Simmonds & Washbourn published work on ephemerid nymphs from swiftly flowing and from still waters, which showed that not only is the demand of the swift water forms for oxygen far in excess of that of the still water genera, but in addition, the animals from swiftly moving waters die at oxygen concentrations at which those from still waters remain alive. Washbourn (1936) showed that trout fry reared in swiftly and slowly running waters had different metabolic rates, and Walshe (1948) showed that the oxygen consumption of stream living chironomid larvae is higher than that of nearly related pond dwelling larvae. She also showed that chironomid larvae from ponds are capable of survival under conditions of almost total oxygen lack, for a much longer time than stream living larvae. This, and other work[x] has shown that animals of many different phyla are physiologically adapted to meet the needs of their particular environments, and that their oxygen requirements are an important factor in limiting their distribution.

Most animals live in places where their particular oxygen requirements are easily met. Some, such as endoparasitic worms in the gut of vertebrates inhabit places where they are almost completely devoid of oxygen, but since they respire anaerobically they do not need

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[x] FOOTNOTE E.g. that of Krogh & Leitch (1919) on the oxygen affinities of the haemoglobins of carp and trout, Van Dam (1938) on the utilisation of oxygen and the regulation of breathing in aquatic animals, and Hall (1929) on the influence of varying oxygen tensions upon the rate of oxygen consumption in marine fishes.

to obtain oxygen from their environment. There are however all stages of gradation between the environment and oxygen requirements of the endoparasitic worm and the brook trout. The fauna of small ponds experiences a diurnal shortage of oxygen for a period of a few hours before dawn; this is caused by the failure of aquatic plants to produce oxygen during the hours of darkness when photosynthesis is at a halt, and the consequent exhaustion of oxygen from the water by the respiratory processes of both plants and animals - when dawn breaks, and photosynthesis commences again the oxygen content of the water rises rapidly. Bottom dwellers in large lakes experience a seasonal oxygen lack when a thermocline is established, cutting off their oxygen supply from the surface waters, during the period of summer stagnation. Yet other animals, living in swamps, experience conditions of permanent low oxygen because of the large numbers of bacteria found in such waters which not only lower the oxygen content by their own respiration, but also by saturating the water with the gaseous products of their metabolism. Animals colonising such habitats are found to have various mechanisms with which to combat these periods of oxygen lack. Such mechanisms may be either structural, as the lung of Protopterus, or behavioural as the swarming of Vorticella which according to Moldawskaja (1937) takes place under conditions of oxygen lack, and enables these normally sessile animals to move into regions where the amount of dissolved oxygen in the water is greater. There are also physiological modifications including the possession of a respiratory pigment, the ability to accumulate an oxygen debt, and the differences in metabolic rate mentioned above.

III. The adaptations of Cladocera to the amount of oxygen available in their environments.

Cladocera are found in all types of fresh water habitat. There are also three marine genera. Those which are planktonic in lakes do not experience an oxygen lack, neither in all probability do the marine ones; but those living on lake bottoms, and in small ponds, must be able to withstand varying degrees of oxygen deficiency. How they do this is not fully understood, except in the case of *Daphnia*, which is known to synthesize haemoglobin, and also to aggregate at the surface of the water when the oxygen content is low (Fox 1948, Fox, Gilchrist & Phear 1951). Red *Daphnia* can survive longer under conditions of oxygen lack than pale ones (Fox, Gilchrist & Phear 1951). This provides an interesting parallel with man, for when a man experiences oxygen lack at high altitudes, he is able to remain more active if the amount of haemoglobin in his body is increased by blood transfusion (Hall 1949). If he remains several days at a high altitude he synthesizes more haemoglobin himself, as do pale *Daphnia* in waters containing little oxygen. The species *Ilyocryptus sordidus* (Lièven), a mud burrowing cladoceran, is always red when taken from the field, owing to the haemoglobin in its blood; but young animals reared in the laboratory in well aerated water without mud, grow into pallid adults. If they are given mud they immediately burrow into it and within a week become bright red (Fox *et al.* 1951). The observations of Fox and his co-workers indicate that it is highly probable that all Cladocera, with the possible exception of the fresh water planktonic and marine genera, are capable of haemoglobin synthesis when the amount of dissolved oxygen in the water is low. Even Daphnia hyalina Leydig, a planktonic species found only in lakes, has been observed, in Pallanza, N.Italy, to have small amounts of haemoglobin in its blood. This occurred when D. hyalina was kept in cement tanks with abundant micro-organisms which at times lowered the oxygen content of the water to below 50% air saturation (Fox, Hardcastle & Dresel 1949).

Little is known about the metabolic rates of Cladocera from different habitats. The work which has been done on cladoceran metabolic rate has been chiefly concerned with variations during the life cycle, or between the sexes of various genera (MacArthur & Baillie 1929, Obreshkove 1930, Terao 1931 (computed from the data of Obreshkove)). Obreshkove & Banta (1930) studied the rate of oxygen consumption of different clones of Simocephalus derived from a single mother, to show the occurrence of physiological mutations within the Cladocera, and O'Connor (1948) devised a technique for measuring the oxygen consumption of small aquatic animals with a high degree of accuracy, using Daphnia as his experimental material. No attempts have yet been made to correlate metabolic rate with habitat as has been done with fish, mayflies and chironomids (see pp. 2 & 3).

Apart from the preliminary studies of Wagler (1923), Pacaud (1939) and various observations by Birge & Juday (1911, 1912), Woltereck (1932) and Aurich (1933) working on the fauna of lakes in N.America, nothing is known about the comparative ability of the planktonic and pond species of Cladocera to survive under conditions of severe oxygen lack.

The purpose of my work has been to investigate the importance of the amount of dissolved oxygen in the water as a factor limiting the distribution of Cladocera. The problem has been approached entirely as a laboratory investigation. Experiments were designed in which individuals of various species, in particular species of the genus *Daphnia*, were kept for a standard time in water of known, constant, low oxygen content; numbers of survivors were counted at the end of that time. The precise methods and techniques employed, and the results obtained are described below.

METHODS.

All analyses of dissolved oxygen content of the water throughout this work were made by the method of Fox & Wingfield (1938). This is a modification of the Winkler method whereby the sample of water needed for analysis is very small, 1 - 2 ml. Apart from the high degree of accuracy which it affords, the advantages of this method are obvious where the amount of water available for analysis is limited, as is the case in most laboratory experiments.

While being kept in the laboratory, animals were fed on the unicellular green alga *Chlorella vulgaris* Beij. *Chlorella* was grown on agar slopes which were illuminated by a mercury strip lamp for ten days after innoculation; they were then transferred to a window facing North, to avoid direct sunlight, where they remained suitable for use over a period of several weeks. The nutrient medium in the agar was that of Pearsall and Loose (1936). *Chlorella* was added to the cultures of Cladocera in suspension in water. *Chlorella* suspensions were produced by the addition of a little water - from the cladoceran culture to be fed - to the test tube containing the *Chlorella* covered slope; the tube was then shaken vigorously and emptied into the culture. No attempt was made to maintain a constant amount of available food in the stock cultures as this was thought to be unnecessary.

Several preliminary experiments were made with *Daphnia magna* Straus and *Daphnia obtusa* Kurz, during which a small number of animals (10 *D. magna* and 20 *D. obtusa*) was placed in a stoppered 3 litre glass bottle completely full of water with a known low oxygen content. The experiments were terminated when half the animals were dead, at which time a second oxygen determination was made. This method was found to have several disadvantages; they are listed below:-

1. The animals used up oxygen during the experiment. It was therefore not possible to determine with any accuracy what oxygen pressures were lethal and what were not. An initially sufficient percentage air saturation (e.g. 10% for D. magna) was in the course of 24 hrs. reduced to a lethal level (5 - 6%). There was no means of telling whether D. magna would survive indefinitely at, say 7 - 8%, or if this saturation pressure, maintained for a sufficient time, would have killed them.

2. The small number of animals used at a time necessitated too great a number of experiments in order to obtain results of any significance.

3. The numbers of animals dead and alive were very difficult to observe with accuracy. Many animals lying motionless at the bottom of the bottles were not dead, and revived when placed in aerated water.

In view of the above disadvantages, it was necessary to devise a method whereby the percentage saturation of air in the experimental water would remain constant throughout the experiment, many animals thus being able to be used at a time. It was also decided, for convenience, to fix a standard time for the duration of all experiments, at the end of which the animals would be removed from the bottles and survivors counted.

The maintenance of a constant percentage air saturation in the experimental water necessitated the passage of a continual current of gas, containing the required amount of oxygen, through the water during experiments. A simple apparatus (see fig. 1) was set up.

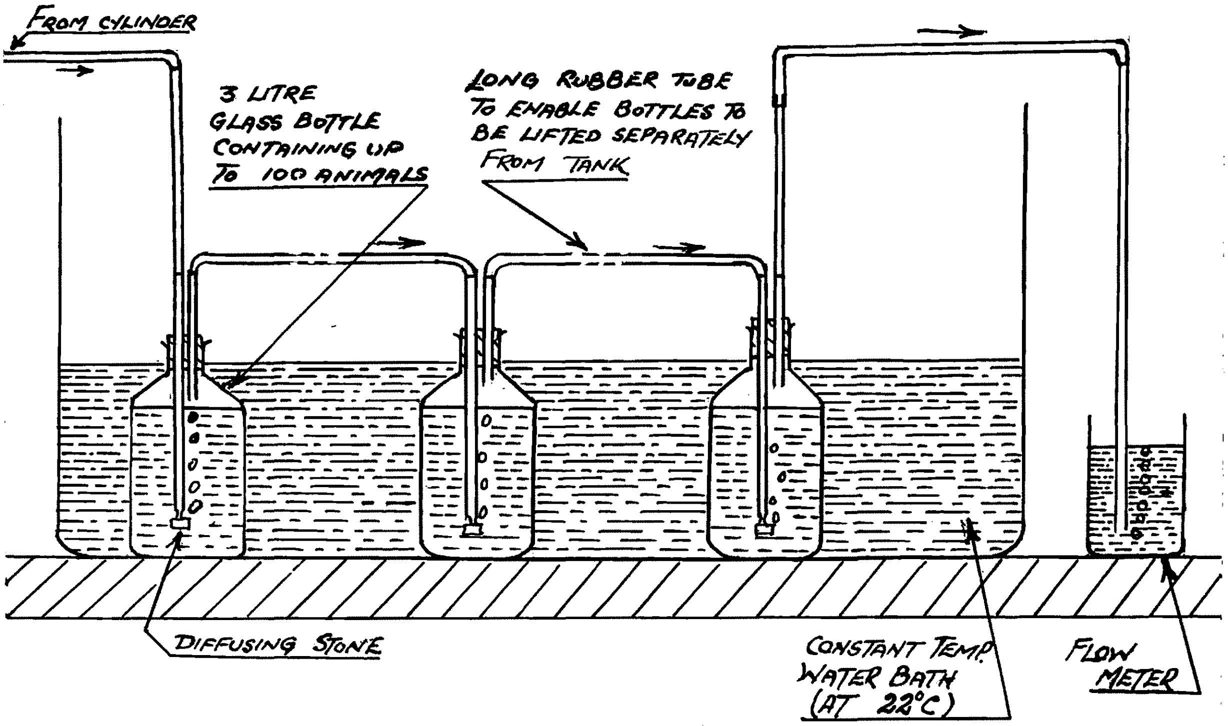

FIG I

The British Oxygen Company supply cylinders containing oxygen and nitrogen mixed in any specified proportions. A slow steady stream of gas from one of these cylinders flowing at sufficient speed to maintain an equilibrium between itself and the water, in spite of the metabolism of as many as 50 water fleas in a 3 litre bottle, did not seem to have any adverse mechanical effects upon the animals. They swam freely in and out of the stream of bubbles appearing quite unaffected by it. It soon became clear however, that in order to gain sufficient knowledge of the tolerance of various Cladocera to oxygen lack, many such cylinders, each containing different proportions of oxygen and nitrogen, would be needed. This would have been a costly and cumbersome procedure. I therefore decided to attempt to make my own gas mixtures as required for experiments. The method finally adopted, known as the aspirator method, is described below.

Description of aspirator method (see fig. 2).

Water in graduated aspirator (B), is displaced upwards into aspirator (A), by gas from cylinders entering (B) through 3 way tap (C) as shown on the diagram. (A) is placed as near to the ceiling as possible, and (B) on the floor, in order to provide the maximum head of water. The two aspirators are connected by rubber tubing on which is a screw clip (D). By turning the 3 way tap (C) it is possible to connect the aspirator (B) with the experimental bottles (E) in the constant temperature water bath (F). When (D) is released, the water from (A) flowing into (B) will force gas from (B) through the water in the experimental bottles. The rate at which the gas flows may be adjusted by turning (D).

When making a gas mixture, aspirator (B) which is graduated at intervals of 500 ml., is first filled with water. Appropriate volumes of nitrogen and oxygen are run in (with (D) open) displacing water upwards into (A). (C) and (D) are then closed, and the mixture is ready for use when required.

Practical details.

1. A convenient capacity for (B) is 10 litres. (A) must be larger than (B).

2. The surface of the water in (B) must not be allowed to come

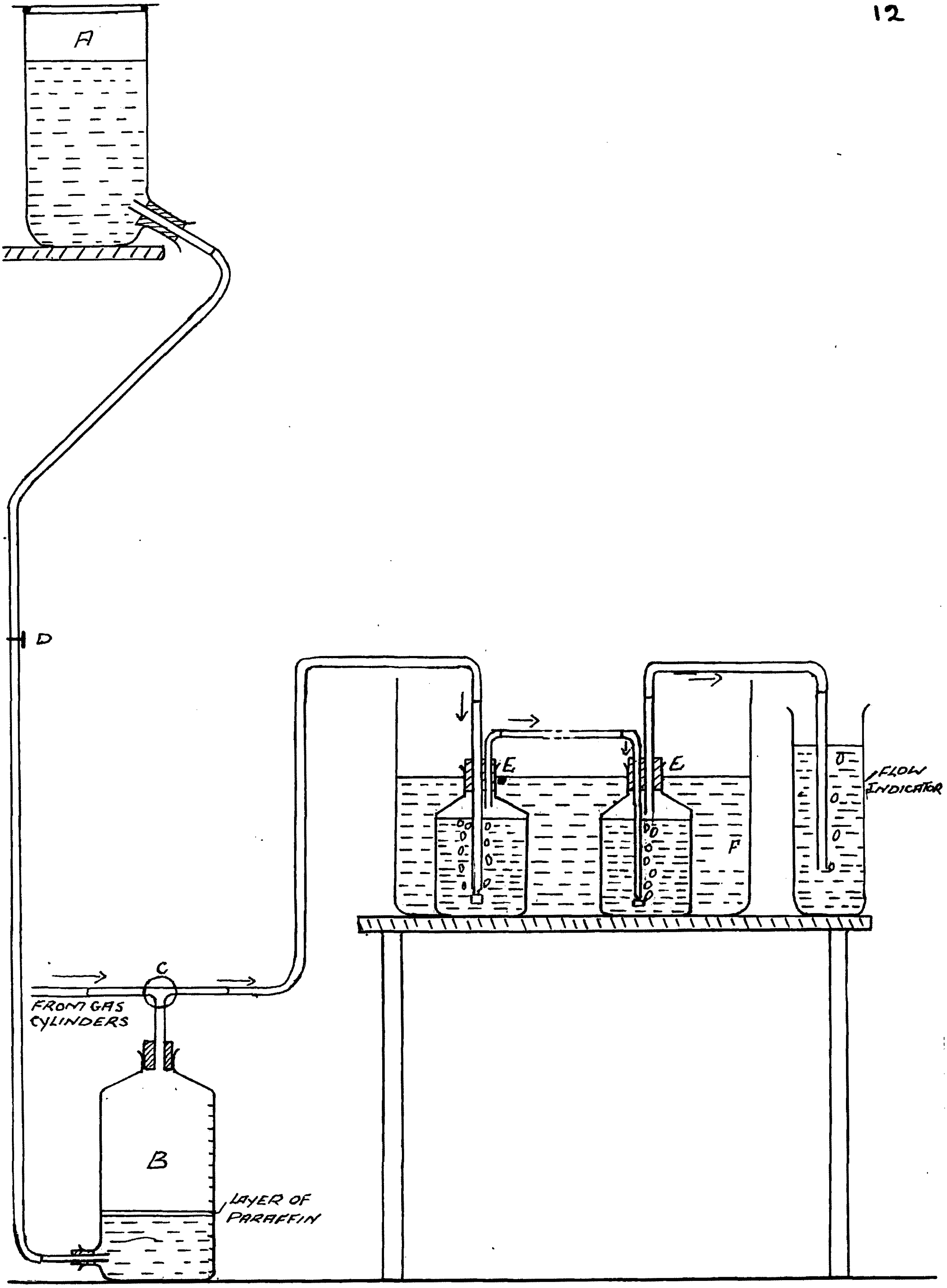
A
D
FROM GAS CYLINDERS
C
B
LAYER OF PARAFFIN
E1
E
F
FLOW INDICATOR

FIG. 2

into contact with the gas mixture, as diffusion across it would alter the mixture. This is prevented by a layer of liquid paraffin (odourless type) on the surface of the water in (B).

3. The greater the head of water, the more experimental bottles can be used in series.

4. It proves necessary that the rubber bung in the top of (B) containing the stem of the 3 way tap, be wired in. A certain amount of paraffin is bound to come in contact with it during the making of mixtures, and this seeps round it causing it to become loose, and rise out when under pressure if not wired.

5. As the volume of oxygen required is so much less than that of nitrogen, it is convenient to use cylinders of the British Oxygen Company's prepared oxygen-nitrogen mixtures, containing little oxygen. To these, nitrogen from another cylinder may be added (in aspirator (B)) to obtain the desired experimental mixture. Thus the volumes of gases run into the aspirator from the two cylinders are similar, therefore accurate mixtures are more easily made.

> For example, if a mixture which is 3% air saturated is required it is better to use a 6% B.O.C. mixture and pure N_2 in equal volumes, than to attempt to measure accurately 97 parts Nitrogen to 3 parts air or 99.4 parts N_2 to 0.6 parts O_2 which would give the desired mixture.

Experimental procedure.

1. The 3 litre experimental bottles were filled with tap water. Nitrogen was then bubbled through the water for about $\frac{3}{4}$ hr., to reduce its oxygen content almost to zero. The prepared mixture was next

bubbled through for 15 - 20 mins., sufficient time to allow the gases dissolved in the water to reach an equilibrium with those in the mixture.

2. The Cladocera were counted into groups of 50, and placed in small glass dishes.

3. The contents of the dishes were poured through a very small, fine mesh net, about 1" diameter.

4. The Cladocera retained in the net were transferred to the experimental bottles by dipping the net into them. This procedure ensured the transference of a minimum amount of water with the animals, thus disturbing the oxygen concentration of the water in the bottles as little as possible. It also caused the animals very little injury.

5. The bungs were inserted in the bottles containing the animals, after the withdrawal of a Winkler sample from each bottle. The flow of gas through the bottles was adjusted to a suitable slow steady rate (by turning screwclip (D)) and the experiment proceeded for 4 hours.

6. At the end of that time, a second Winkler sample was taken, and the contents of the bottles tipped into large pie dishes to facilitate the counting of survivors.

Duration of Experiments.

The choice of 4 hrs. as the length of experiments was influenced by the fact that it was necessary to work at percentage air saturations below that at which the haemoglobin of the animals can be used as an oxygen carrier, i.e. 15 - 20% air saturation. The haemoglobin of *Daphnia magna* becomes deoxygenated at 17% air saturation at 17°C (Fox 1945 b). It was found that with 4 hr. experiments the 50% death points of all species containing haemoglobin occurred at % air saturations well below 15%.

All experiments were carried out at 22°C, the lowest temperature easily maintained in the laboratory at all times of the year.

Culture of animals before experiments.

All animals used in my experiments (except those made at Pallanza) had been kept in the laboratory for a period of at least 14 days before their use. This was done for the following reasons:-

1. For convenience. When the source of animals was far from the laboratory, it could not be visited each day to obtain fresh ones.

2. As an attempt to standardise previous conditions: to avoid big differences in nutrition and fat stores of the animals which might have affected their ability to carry out anaerobiosis, and thus in turn have affected the experimental results.

3. To avoid the use of animals injured during capture. Injured animals either die or recover completely within two to three days after the injury. They might however be included in experiments made using animals immediately after capture. This might lead to an unnaturally high death rate after exposure to oxygen lack. Death owing to injury was particularly noticeable in the case of Daphnia hyalina after capture. This species was caught in a large plankton net drawn behind a boat. It is not unreasonable to suppose that capture in this manner would be more likely to injure the animals than capture in a small hand net from the edge of a pond. When Daphnia hyalina was brought into the laboratory and placed in aquaria to be kept for 14 days before use, there was always a high death rate during the first two days. Many of the dead animals had missing limbs. After first two days in the laboratory far fewer deaths occurred.

4. To avoid sudden great changes in temperature, which in themselves may prove fatal to Cladocera (Lefèvre 1942), occurring at the same time as the sudden exposure to very low oxygen concentrations. _Daphnia obtusa_ taken from the field in winter, when the temperature of the pond water was 4°C, and used immediately for experiments at 22°C, showed a greater mortality, at any given low oxygen concentration, on the day of capture than on subsequent days. This phenomenon did not occur in summer when the pond water was warmer. See table I.

Table I.

Susceptibility of _Daphnia obtusa_ to oxygen lack under various conditions.

Date	Previous temperature of the water.	Experimental temperature.	% air saturation at 22°C.	No. of survivors out of 50.
June 1st 1951	approx. 19°C	22°C	4	43
Nov. 6th "	4°C	22°C	8	9
Nov.16th "	17°C	22°C	4	45
Nov.16th "	17°C	22°C	5	50

The animals used on June 1st and November 6th were taken straight from the field. Those used on November 16th had been in culture for 10 days. All had similar egg numbers.

5. Some species thrive when cultured in tap water. _Daphnia hyalina_ does not. It therefore proved necessary to culture _D. hyalina_ in water from Regent's Park Lake.[x] Filtered water from this lake was

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FOOTNOTE [x] The reason why _D. hyalina_ thrives in water from Regent's Park Lake, but not in London tap water, remains obscure. Analyses were made of the iron content of both waters, also of their alkalinity and pH. Very little difference was detectable between them.

If a culture of water fleas flourished in tap water for 14 days before use, it was legitimate to neglect any possibility of tap water used during the experiments having an adverse effect on the animals. It would not be justifiable to disregard such a possibility if animals were used for experiment immediately after capture from the field.

RESULTS.

The following species were used in experiments:-

Daphnia obtusa Kurz.
Daphnia magna Straus.
Daphnia thomsoni G.O.Sars.
Daphnia pulex (De Geer).
Daphnia hyalina Leydig.
Simocephalus vetulus (O.F.Müller).
Simocephalus exspinosus (Koch).
Leptodora kindti (Focke).
Bythotrephes longimanus Leydig.

All data concerning the various species will be found in an appendix. Results are expressed collectively in Table II and Fig. 3.

The experiments with *Leptodora* and *Bythotrephes* and a few experiments with *Daphnia hyalina*, were conducted at the Istituto Italiano di Idrobiologia at Pallanza in N.Italy. The results of these are of a preliminary nature, since owing to technical difficulties it was

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also used in the experimental bottles when working with *D. hyalina*.

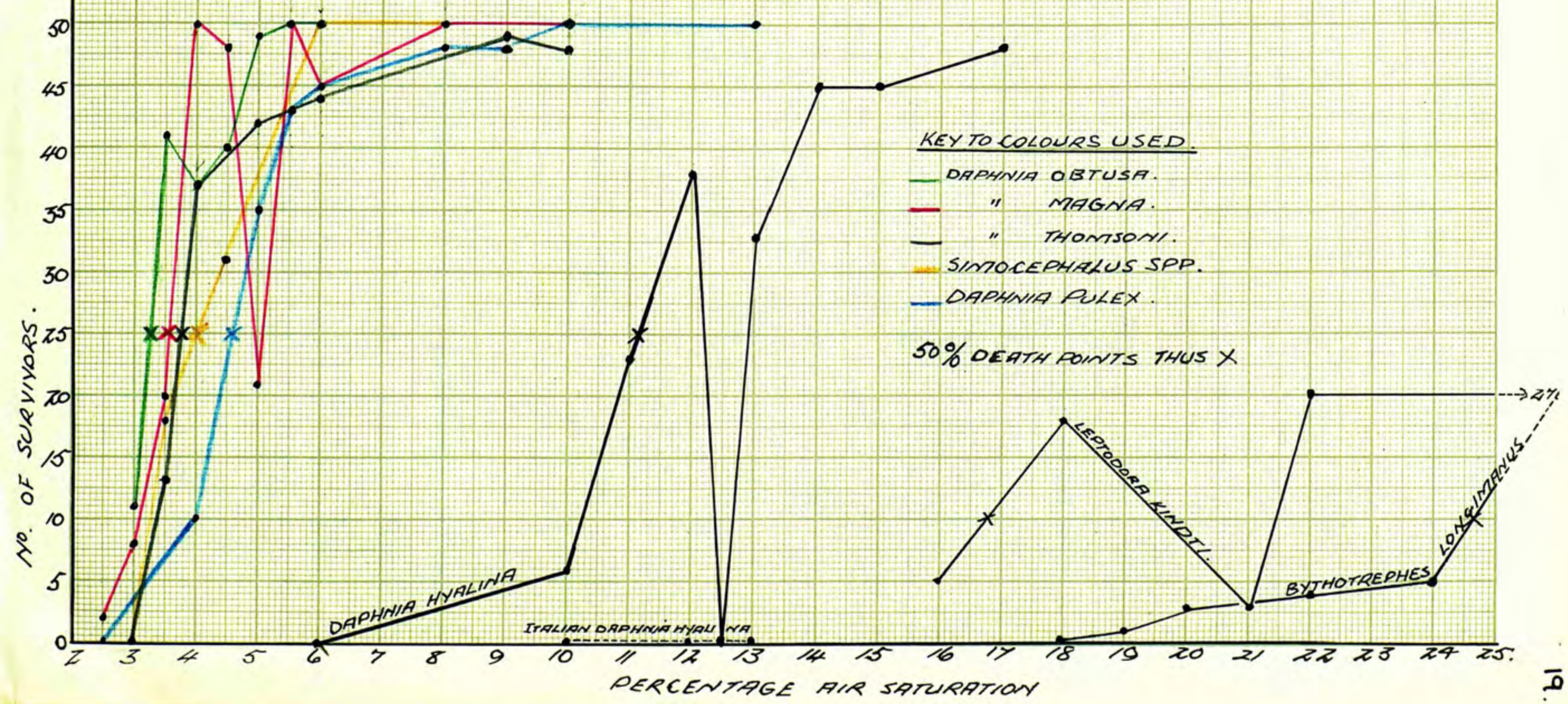
Table II.

Species	Source of animals	% air saturation at which 50% of the animals were dead in 4 hrs.	
Daphnia obtusa	Bedford College Botany Garden.	$3\frac{1}{4}$	at 22°C
Daphnia magna	King George VI reservoir, Staines, Middx.[x]	$3\frac{1}{2}$	
Daphnia thomsoni	Dried mud from New Zealand. Hatched in laboratory.	$3\frac{3}{4}$	
Simocephalus spp.	Viaduct pond, Hampstead Heath.	approx. 4	
Daphnia pulex	Viaduct pond, Hampstead Heath.	$4\frac{1}{2}$	
Daphnia hyalina	Staines South reservoir[x] Middx.	11	
Daphnia hyalina	Lago Maggiore	over 13	at 18°C
Leptodora kindti	Lago Maggiore	17	
Bythotrephes longimanus	Lago Maggiore	24	

[x] These animals were obtained by courtesy of the Biological Division of the Metropolitan Water Board. I am very grateful for the readily given help of all the members of staff in this Division during the collection of animals.

FIG. 3

A COMPARISON OF THE SURVIVAL OF DIFFERENT CLADOCERA AFTER EXPOSURE FOR 4 HOURS TO VARIOUS DEGREES OF OXYGEN LACK.



not possible to use the aspirator method at Pallanza. Experiments were made using stoppered bottles, completely full of water, without a continuous flow of oxygen-nitrogen mixture through the water. 20 animals per 3 litre bottle were used, instead of 50 as in other experiments, in order to lessen the risk of the accumulation of toxic waste products of the animals' metabolism having a harmful effect upon them. The temperature was 18°C instead of 22°C, for some Leptodora in control vessels at high air saturation pressures died at 22°C, but not at 18°C. The results do however clearly indicate the difference in ability to survive at low oxygen concentrations, between lake and pond dwelling Cladocera; for this reason they have been included in Fig. 3.

The apparently greater suceptibility to oxygen lack of the Italian Daphnia hyalina than the English ones is worthy of note. If more data were available for the Italian animals one might postulate the existence of different physiological races within the species, with varying abilities to withstand lack of oxygen; but since the number of experiments made with D. hyalina in Italy is so small, and the method not the same as that used in England, it appears wiser merely to record the difference between the results, without further speculation regarding their significance.

It was noticed that when Daphnia was first introduced into the experimental bottles, the animals swam near to the top, but after 5 - 10 minutes they were evenly distributed throughout the water. They remained thus until they became enfeebled by lack of oxygen, when they swam weakly near the bottom.

Some difficulty was experienced in deciding, at the end of an experiment, which animals were dead and which alive. All animals not swimming normally were examined under a microscope. Those which exhibited no movement at all were counted as dead. Those which exhibited any movement, of heart and/or limbs, however irregular, were kept in aerated water and examined again the next morning (i.e. after about 18 hrs.). Those which by then had fully recovered, were added to the number of survivors. The ones which were still in an enfeebled condition were counted as dead, for experience has shown that in all cases they would subsequently have died. Similar problems regarding the death of Cladocera were encountered by Pacaud (1939), Prosser (1942), Brown (1927a) and Johnson (unpublished work), the latter authors working on the temperature tolerance of the order: Prosser on the mechanisms controlling heart beat in arthropods.

It has been shown (by Ingle, Wood & Banta 1937, Banta 1939, Fox 1948 and Fox *et al.* 1949) that the number of eggs formed by *Daphnia* is proportional to the amount of food eaten, that is to say, the more eggs which are present in the brood pouches of adult females, the better fed is the population. It might be supposed that *Daphnia* with many eggs, being better nourished, would be more able to withstand oxygen lack than *Daphnia* with few eggs. A small number of experiments using *Daphnia magna*, comparing animals with many eggs and animals with few eggs, indicated that the effect of nutrition on ability to survive oxygen lack, if any, is negligible. (See tables III & IV). Ephippial and parthenogenetic females were also compared. No difference between their reactions to oxygen lack could be detected. I have not compared males with females, or tested the influence of age on survival under conditions

Table III.

Comparison of susceptibility to oxygen lack of Daphnia magna with many eggs, few eggs and ephippia.

Condition of animals	% air saturation	No. of survivors out of 50.
many eggs	6	50
ephippiate	6	45
few eggs	7	42
few eggs	7	43
ephippiate	7	42

Table IV.

Condition of animals	% air saturation	Time for 50% death
no eggs	5	3 hrs.
many eggs	4	3 hrs.
no eggs	7	23 hrs.
many eggs	5	23 hrs.
no eggs	4	6½ hrs.

The results in table IV were obtained with Daphnia magna from Bedford College Botany garden, enclosed in stoppered bottles with no air surface. The experiments were made before the development of the aspirator method. The results show the great variability of this species - the number of eggs appears to have little significance.

of oxygen deficiency. Such investigations may yield very interesting results, since male and young female Daphnia are known to have a higher metabolic rate than adult females (MacArthur & Baillie 1929).

In experiments with Daphnia magna I observed that some animals, when they had been in water of low oxygen content for 1 - 1½ hrs. began to swim erratically and in a few minutes sank to the bottom of the bottles where they lay motionless. Subsequently, after a further ½ - 1 hr., they revived and were seen to be swimming normally again. A similar phenomenon has been observed by Pacaud (1949) in the course of studies on the Ephemeroptera.

If the data given in the appendix, for the pond dwelling species of Daphnia are examined, it will be seen that D. magna gave very variable results.[x] The greater number of deaths at 5% air saturation than at 4½% is a mystery. Possibly if more experiments had been carried out with D. magna at 4½ and 5% air saturation, the average number of deaths would have been greater at 4½%. Possibly also the use of animals of one clone, bred in the laboratory under standard conditions would have yielded more uniform results. It was however considered of greater

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FOOTNOTE [x] In previous work (Fox, Gilchrist & Phear 1951) D. magna has yielded widely varying results when used to compare the survival of red and pale Daphnia in water deficient in oxygen. The results of these workers, expressed in their Table I, p.518, indicate that under similar conditions, the time taken for 50% of the pale Daphnia to die varied from 26 to 93 hours.

importance in the present work to establish a difference between pond and lake dwelling Cladocera regarding their tolerance of oxygen lack, than to investigate in detail the tolerance of any one pond dwelling species such as *Daphnia magna*.

DISCUSSION.

The results shown in Fig. 3 and table II indicate that those species of Cladocera normally found in small ponds are capable of living under conditions of greater oxygen deficiency than those inhabiting the open waters of large lakes. The genus *Daphnia* contains species which are found only in lakes, e.g. *D. hyalina*, and species which are normally confined to small ponds, e.g. *D. magna* and *D. thomsoni*, an inhabitant of temporary pools in New Zealand and S.Africa. It is of interest to record that many of my experiments with *D. magna* were made with animals collected from an exceptional habitat, namely the surface waters of King George VI reservoir at Staines, Middx. This reservoir is a large open body of water with a surface area of 350 acres, and a storage capacity of 4,450 million gallons. It has a flat bottom which is 52 feet below the surface when the reservoir is full; *Daphnia magna* and *Daphnia hyalina* were found together in the plankton. The 50% death point of *D. magna* from this reservoir did not differ from that of *D. magna* collected from small ponds such as those in Bedford College Botany Garden. This appears to indicate a genetical control of the tolerance of oxygen lack in the various species, and demonstrates conclusively that an acclimatisation does not take place when *D. magna* finds itself in an unusual habitat.

The difference between the 50% death points of *Leptodora* and

Bythotrephes from the Lago Maggiore is of interest. It has been observed by Professor V.Tonolli (personal communication) that *Leptodora* swims in deeper water in the Lago Maggiore, than *Bythotrephes*. At a depth of 30 metres in this lake, where *Leptodora* is most abundant, the temperature of the water is 10 - 15°C and it is not usually fully saturated with air. *Bythotrephes* on the other hand swims in the warmer surface water (up to 25°C) in which the amount of dissolved oxygen is greater. Thus the death of *Leptodora* but not of *Bythotrephes*, in air-saturated water at 22°C, and the death of *Bythotrephes* but not of *Leptodora* at percentage air saturations between 24 and 17, is clearly correlated with their distribution in the field.

It would be very interesting to know whether there is any difference in metabolic rate between the lake and pond species of *Daphnia*. The results of such an investigation would be comparable with those mentioned in the introduction to this thesis, regarding the metabolic rates of various arthropods and fishes from different habitats.

It would also be interesting to determine the percentage air saturation at which the marine Cladocera have their 50% death points, and the metabolic rates of these genera. Marine arthropods generally have a lower oxygen consumption than their relatives from well aerated fresh waters (Fox & Simmonds 1933). The reasons for this are not fully understood.

DISCUSSION OF PREVIOUS WORK.

The first laboratory experimental work known to me on the tolerance of Cladocera to oxygen deficiency in the water was that of Wagler (1923). He confined *Daphnia cucullata* Sars. in stoppered bottles with no air surface. He placed some in the light and others in the dark. When the animals began to die he removed the stoppers, and made analyses of the dissolved oxygen in the water. He states that animals began to die when the oxygen content of the water fell to 0.69 - 0.20 cc. O_2/litre. He also states that those in the light lived longer than those in the dark; (presumably because of the photosynthetic activity of algae in the water). The disadvantages of this experimental technique are many, and are pointed out by Pacaud (1939, p.210).

The method employed by Pacaud for the study of the same problem is a great improvement on that of Wagler. Pacaud devised an apparatus containing 250 ccs. water through which he maintained a constant circulation of nitrogen. The nitrogen removed all gaseous products of the metabolism of experimental animals. At the same time it steadily lowered the amount of dissolved oxygen in the water. Like Wagler, Pacaud terminated his experiments when the animals began to die. He then analysed the water to determine the amount of dissolved oxygen present. He used several different species at the same time, placing 10 individuals of each species in his apparatus together. Thus he was able to observe which species died first. This procedure however made his results rather difficult to interpret, since he had several variable factors in every experiment, viz.:-

1. The number of different species in the apparatus.
2. The total number of animals in the apparatus.
3. The rate at which the dissolved oxygen concentration in the water diminished.
4. The final dissolved oxygen concentration.

5. The number of dead animals.

6. The length of time for which the experiment ran.

In the description of his experimental technique (p.210) Pacaud states that he measured the total hardness of his experimental water. He does not however refer to this again in his tables of results. He also states that the pH of the water was adjusted to 7, and the nitrogen purified before coming into contact with the water. He exercised great care over his oxygen analyses (p.220). Possibly he would have obtained results of greater value had he concentrated on eliminating some of the variable factors listed above, and performed less complicated experiments in water of unknown total hardness.

CONCLUSIONS.

The investigation has shown that 4 pond species of *Daphnia*, *D. obtusa*, *D. magna*, *D. thomsoni* and *D. pulex* have a similar capacity for survival in oxygen deficient waters.

A small amount of data for *Simocephalus vetulus* and *Simocephalus exspinosus* indicates that the capacity of these species for survival in oxygen deficient waters is similar to that of the pond dwelling species of *Daphnia*.

Daphnia hyalina, a species found in the plankton of lakes, is much less resistant to lack of oxygen than the pond species. This may account for its absence from small bodies of water in which it would experience periods of severe oxygen lack.

Leptodora and *Bythotrephes*, other planktonic Cladocera, are less tolerant of oxygen deficiency in the water than any species of the genus

Daphnia. *Leptodora* from the Lago Maggiore can tolerate a lower percentage air saturation than *Bythotrephes* from the same lake. This is correlated with the vertical distribution of the two genera in the lake plankton. Like *Daphnia hyalina*, *Leptodora* and *Bythotrephes* may be confined to large bodies of water by their inability to survive under conditions of severe oxygen deficiency.

SUMMARY.

1. The factors involved in the distribution of Cladocera are discussed. Previous work is briefly summarized.

2. The importance of oxygen as a factor regulating the distribution of animals is described. Methods adopted by animals to combat periods of oxygen deficiency are reviewed briefly, with special reference to the Cladocera.

3. The aims of this work are stated.

4. A description of methods employed during the investigation is given.

5. Results obtained are tabulated and phenomena of interest occurring during the experiments are described.

6. The significance of the results of this investigation, and the methods of previous workers are discussed.

7. Conclusions drawn from the results are stated.

8. Full data are given in the Appendix.

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List of References

Anderson, B.G. & Jenkins, J.C. 1942. A time study of events in the life span of Daphnia magna. Biol.Bull. Wood's Hole, 83, 260.

Aurich, H. 1933. Zooplankton einiger Seen der Chiemgaus. Intern. Rev.Bd., 29, 295.

Banta, A.M. 1939. Studies on the physiology, genetics and evolution of some Cladocera. Publ.Carnegie Instn., 513.

Birge, E.A. & Juday, C. 1911. The inland lakes of Wisconsin. The dissolved gases of the water and their biological significance. Bull.Wisconsin Geol.Nat.Hist. Survey series 7, 12.

Brown, L.A. 1929 (a). The natural history of Cladocerans in relation to temperature I. Amer.Nat., 63, 248.

Fox, H.M. 1945 (b). The oxygen affinities of certain invertebrate haemoglobins. J.exp.Biol., 21, 161.

Fox, H.M. 1948. The haemoglobin of Daphnia. Proc.Roy.Soc.B, 135, 195.

Fox, H.M., Gilchrist, B.M. & Phear, E.A. 1951. Functions of haemoglobin in Daphnia. Proc.Roy.Soc.B, 138, 514.

Fox, H.M., Hardcastle, S.M. & Dresel, E.I.B. 1949. Fluctuations in the haemoglobin content of Daphnia. Proc.Roy.Soc.B, 136, 388.

Fox, H.M. & Simmonds, B.G. 1933. Metabolic rates of aquatic arthropods from different habitats. J.exp.Biol., 10, 67.

Fox, H.M., Simmonds, B.G. & Washbourn, R. 1935. Metabolic rates of ephemerid nymphs from swiftly flowing and from still waters. J.exp.Biol., 12, 179.

Fox, H.M. & Wingfield, C.A. 1938 A portable apparatus for the determination of oxygen dissolved in a small volume of water. J. Exp. Biol. 15, 437.

Grosvenor, G.H. & Smith, C. 1913 The life cycle of Moina rectirostris. Quart. J. micr. Sci. 58, 511.

Hall, F.G. 1929 The influence of varying oxygen tensions upon the rate of oxygen consumption in marine fishes. Am. Journ. Physiol. 88, 212.

Hall, F.G. 1949 The interval of useful consciousness at various altitudes. Journ. App. Physiol. 1.

Hubault, E. 1933 Le pH dans les lacs des Vosges. C.R. Ass. Franç. Av. Sci. 485.

Johnson, D.S. A study of the physiology and ecology of certain Cladocera. Thesis for Ph.D. degree, 1951. Unpublished.

Krogh, A. & Leitch, J. 1919 J. Physiol. 52, 228.

Lefèvre, M. 1942 L'utilisation des algues d'eau douce par les Cladocères. Bull. biol. Fr. Belg. 76, 250.

MacArthur, J.W. & Baillie, W.H.T. 1929 Metabolic activity and the duration of life I: Influence of temperature on longevity in Daphnia magna. J. exp. Zool. 53, 221.

Moldawskaja, E.A. 1937 Der Einfluss des O_2 Hungers auf die Entwicklung des unteren Wimperkranzes bei Vorticella nebulifera. Arch. f. Protist., 88, 353-365.

Obreshkove, V. 1930 Oxygen consumption in the developmental stages of a cladoceran. Physiol. Zool. 3, 217.

Obreshkove, V. & Banta, A.M. A study of the rate of oxygen consumption of different Cladocera clones derived originally from a single mother. Physiol. Zool. 3, 1.

Obreshkove, V. & Banta, A.M. A study ~~of the rate of oxygen~~ consumption of ~~different Cladocera clones~~ derived originally ~~from a single mother~~. Physiol.Zool., 3, 1.

O'Connor, R.J. 1948. Measurement of the oxygen consumption of Daphnia by a modification of the cartesian diver technique. J.exp.Biol., 25, 313.

Pacaud, A. 1939. Contribution à l'écologie des Cladocères. Suppléments au Bull.Biol. de Fr. et Belg., 25.

Pacaud, A. 1949. Contribution à l'étude de l'adaptation de divers organismes aquatiques à l'appauvrissement périodique du milieu en oxygène. Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie, 10, 357.

Pearsall, W.H. & Loose, L. 1936. The growth of Chlorella vulgaris in pure culture. Proc.Roy.Soc.B, 121, 451.

Poulsen, E.M. 1928. Faunistische und biologische Untersuchungen über die Cladocerenfauna von Danemark. Vidensk.Medd.naturh.Foren.Kbh., 86.

Pratt, D. 1943. Analysis of population development in Daphnia at different temperatures. Biol.Bull., 85, 116.

Prosser, C.Ladd. 1942. An analysis of the action of acetyl choline on hearts, particularly in arthropods. Biol.Bull., 83, 145.

Prosser, C.Ladd., Bishop, D.W., Brown, F.A.Jnr., Jahn, T.L. & Wulff, V.J. 1950. Comparative Animal Physiology. W.B.Saunders Co., Lond.

Terao, A. 1931. Change of vitality with age as based on the living unit of organisms. Proc.Imp.Acad.Tokyo, 7.

Van Dam. 1938. On the utilisation of oxygen and the regulation of breathing in some aquatic animals. Thesis, Groningen.

Walshe, B.M. 1948. The oxygen requirements and thermal resistance of chironomid larvae from flowing and from still waters. J.exp.Biol., 25, 35.

Washbourn, R. 1936. Metabolic rate of trout fry from swift and slow-running waters. J.exp.Biol., 13, 145.

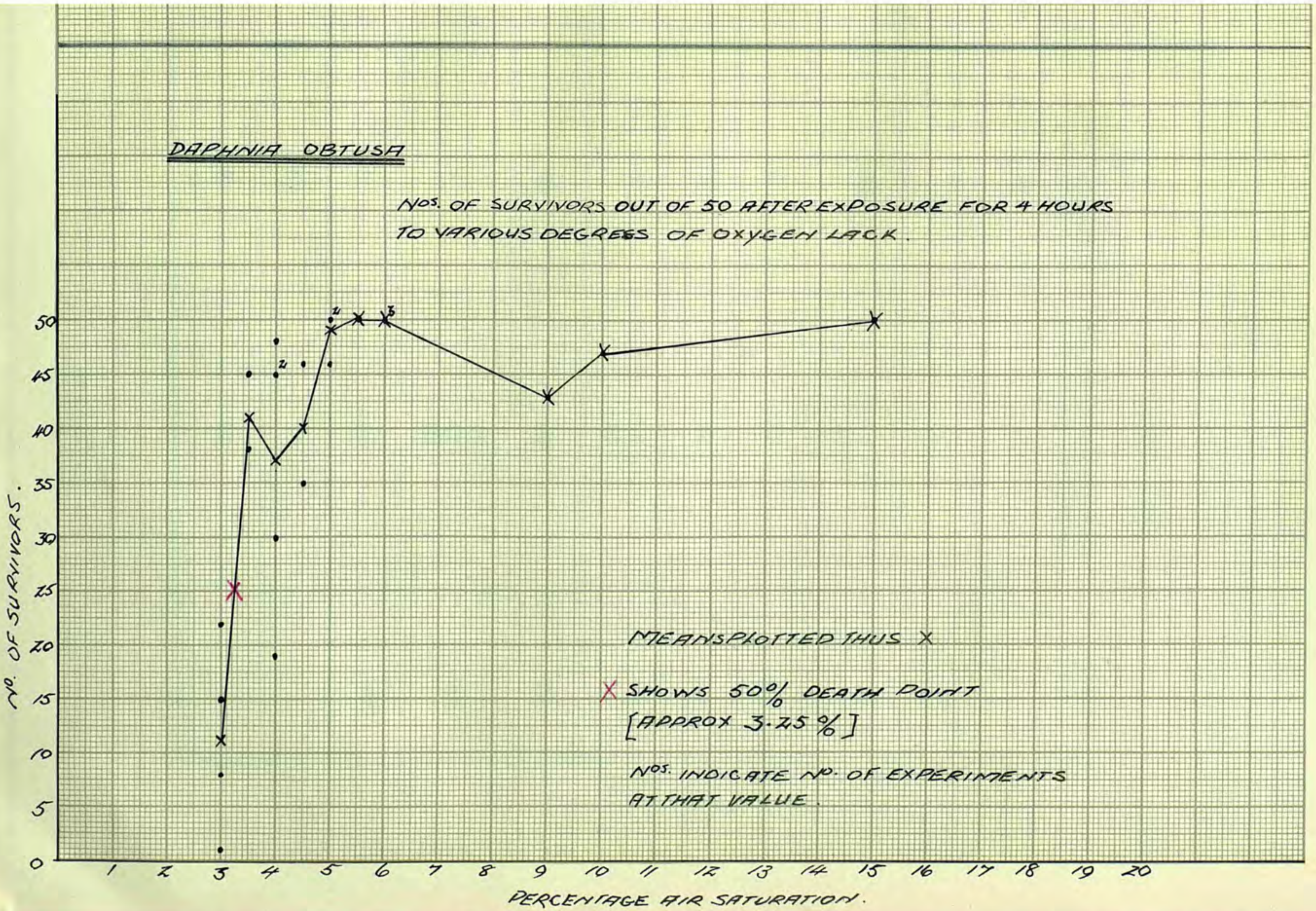
Woltereck, R. 1932. Races, associations and stratification of pelagic daphnids in some lakes of Wisconsin. Trans.Wis.Acad. Sci.Arts Lett., 27, 487.

APPENDIX

TABLE 5

Daphnia obtusa

% air saturation at 22°C	No. of survivors out of 50 after 4 hrs.	Average
3	1 8 15 22	11
3½	38 45	41
4	19 30 45 45 48	37
4½	35 46	40
5	46 50 50	49
5½	50	50
6	50 50 50	50
9	43	43
10	47	47
15	50	50



DAPHNIA OBTUSA

Nos. OF SURVIVORS OUT OF 50 AFTER EXPOSURE FOR 4 HOURS TO VARIOUS DEGREES OF OXYGEN LACK.

MEANS PLOTTED THUS ×

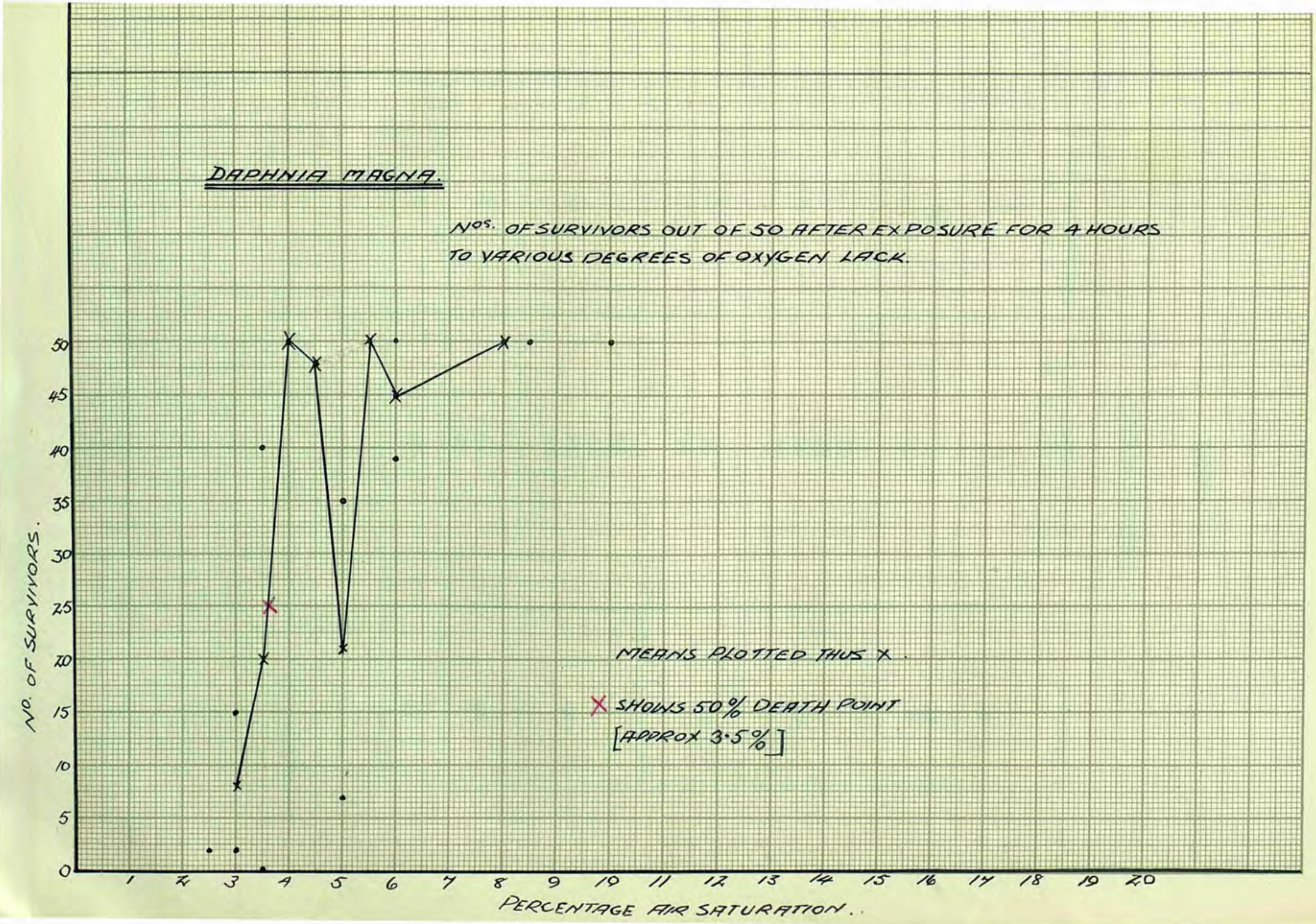
× SHOWS 50% DEATH POINT
[APPROX 3·25%]

Nos. INDICATE No. OF EXPERIMENTS AT THAT VALUE.

TABLE 6

Daphnia magna

% air saturation at 22°C	No. of survivors out of 50 after 4 hrs.	Average
2½	2	
3	2 15	8
3½	0 40	20
4	50	50
4½	48	48
5	7 35	21
5½	50	50
6	39 45 50	45
8	50	50
8½	50	
10	50	



DAPHNIA MAGNA.

Nos. OF SURVIVORS OUT OF 50 AFTER EXPOSURE FOR 4 HOURS TO VARIOUS DEGREES OF OXYGEN LACK.

MEANS PLOTTED THUS ×.

× SHOWS 50% DEATH POINT [APPROX 3·5%]

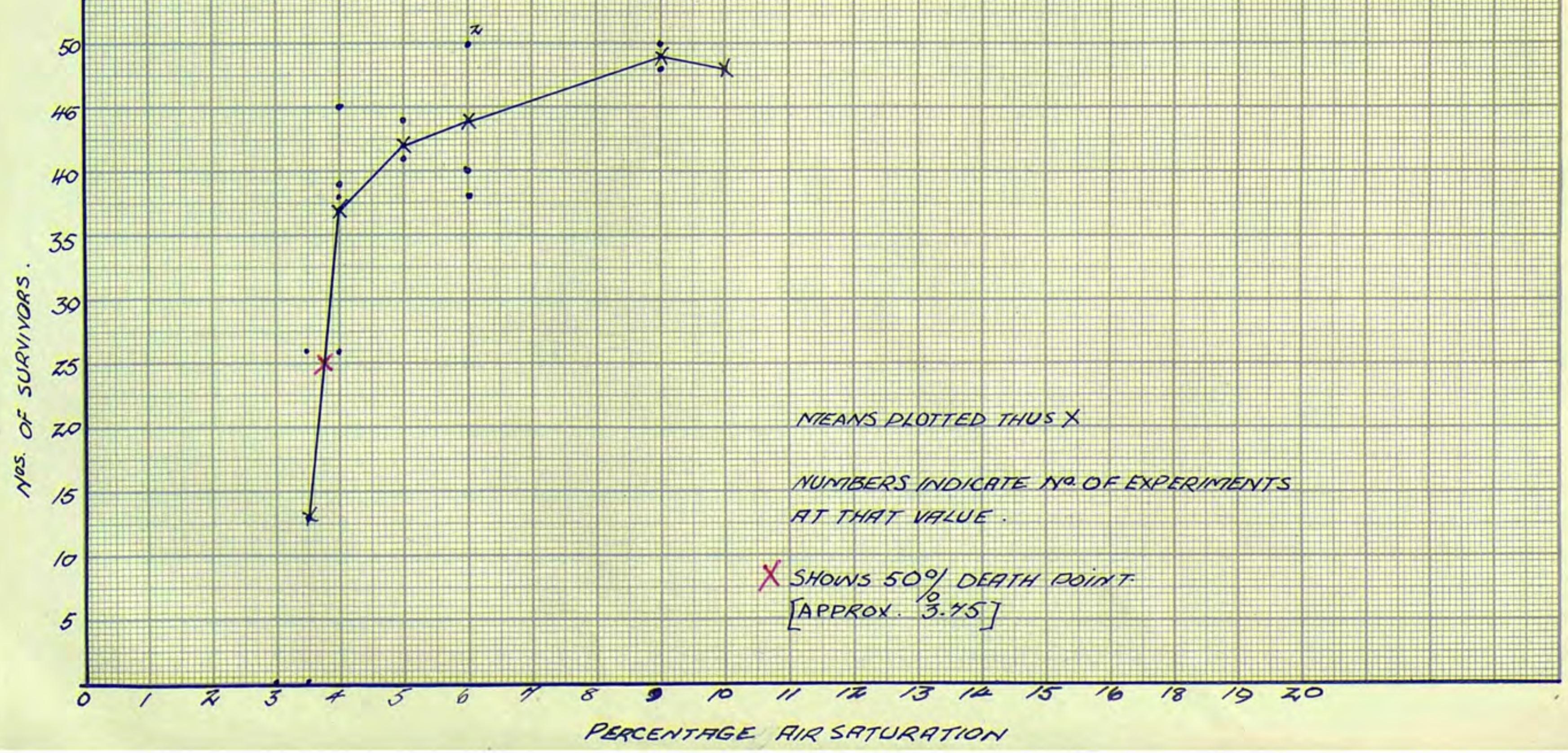
TABLE 7

Daphnia thomsoni

% air saturation at 22°C	No. of survivors out of 50 after 4 hrs.	Average
3	0	0
3½	0 26	13
4	26 38 39 45	37
5	41 44	42
6	38 40 50 50	44
9	48 50	49
10	48	48

DAPHNIA THOMSONI.

NOS. OF SURVIVORS OUT OF 50 AFTER EXPOSURE FOR 4 HOURS TO VARIOUS DEGREES OF OXYGEN LACK.



MEANS PLOTTED THUS X

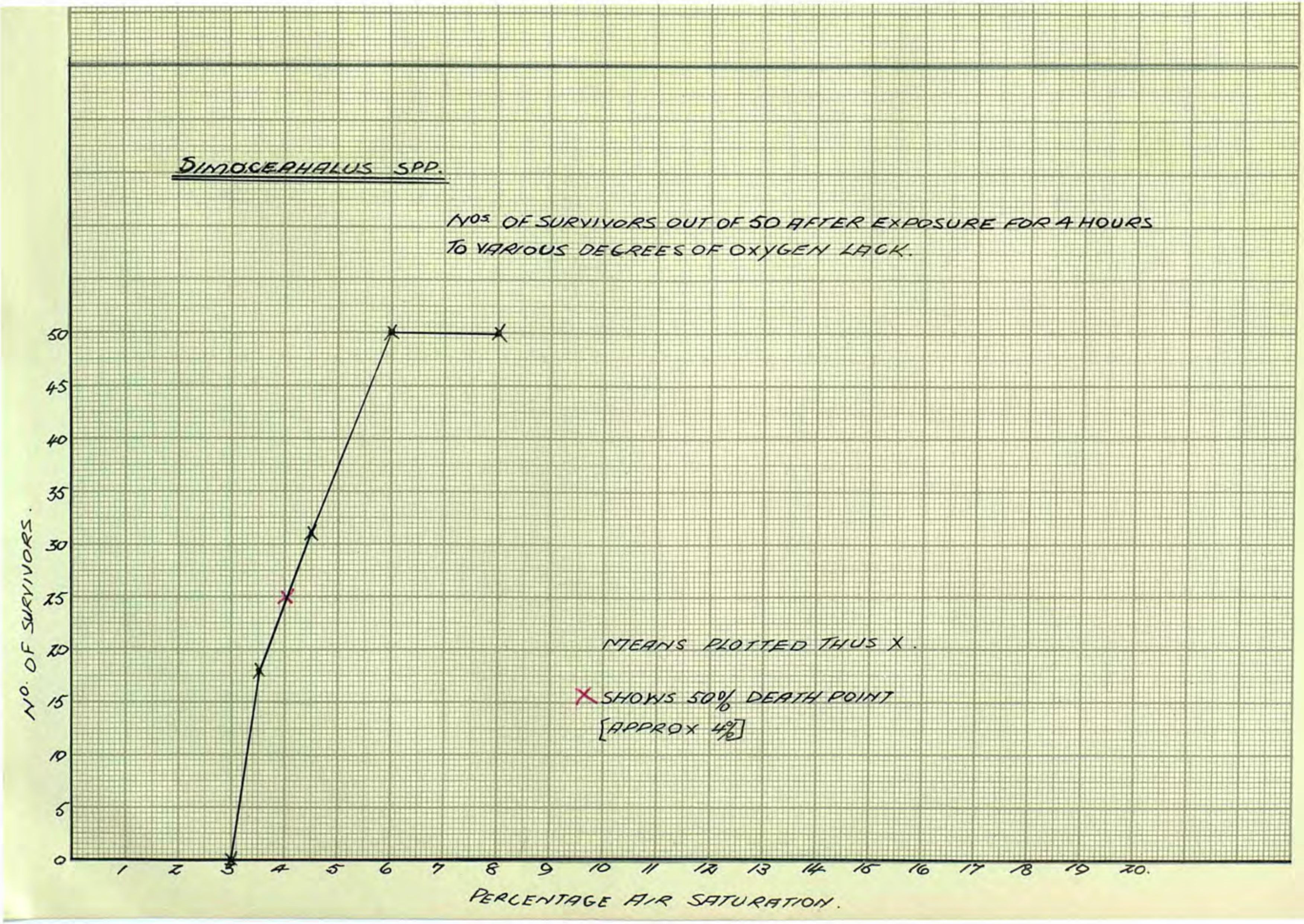
NUMBERS INDICATE No. OF EXPERIMENTS AT THAT VALUE.

X SHOWS 50% DEATH POINT [APPROX. 3.75]

TABLE 8

Simocephalus sp.

% air saturation at 22°C	No. of survivors out of 50 after 4 hrs.
3	0
3½	18
4½	31
6	50
8	50



SIMOCEPHALUS SPP.

Nos OF SURVIVORS OUT OF 50 AFTER EXPOSURE FOR 4 HOURS TO VARIOUS DEGREES OF OXYGEN LACK.

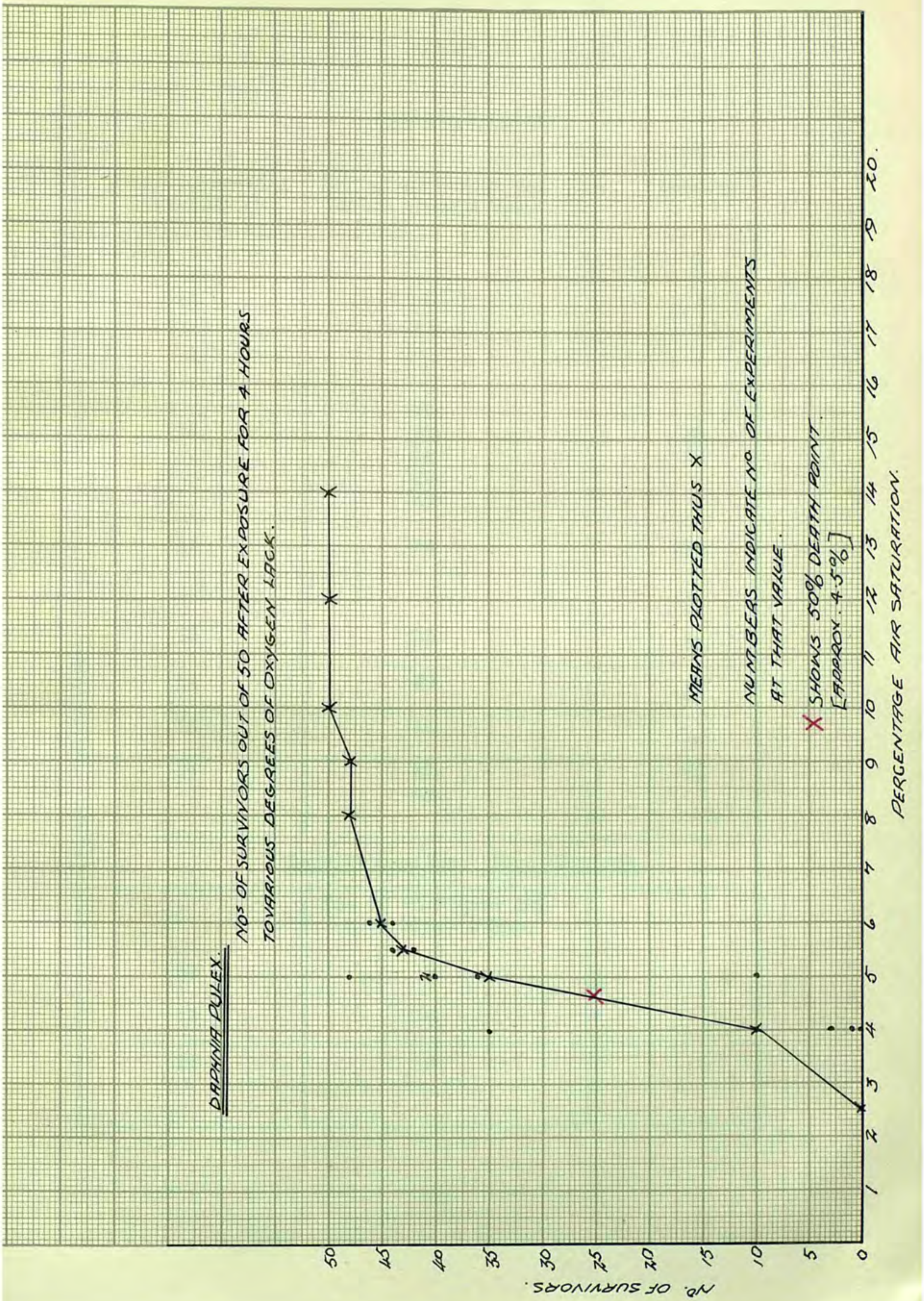
MEANS PLOTTED THUS X.

X SHOWS 50% DEATH POINT [APPROX 4%]

TABLE 9

Daphnia pulex

% air saturation at 22°C	No. of survivors out of 50 after 4 hrs.	Average
2½	0	0
4	0 1 3 35	10
5	10 36 40 40 48	35
5½	42 44	43
6	44 46	45
8	48	48
9	48	48
10	50	50
13	50	50
15	50	50



DAPHNIA PULEX

Nos OF SURVIVORS OUT OF 50 AFTER EXPOSURE FOR 4 HOURS TO VARIOUS DEGREES OF OXYGEN LACK.

TABLE 10

Daphnia hyalina

% air saturation at 22°C	No. of survivors out of 50 after 4 hrs.	Average
6	0	0
10	2 11	6
11	0 3 14 31 34 39 40	23
12	38	38
12½	0	0
13	33	33
14	45	45
15	45	45
17	47 49	48
	Daphnia hyalina. Lago Maggiore.	
	No. of survivors out of 20 after 4 hrs.	
10	0	
12	0	
13	0	

DAPHNIA HYALINA.

NOS OF SURVIVORS OUT OF 50 AFTER EXPOSURE FOR 4 HOURS TO VARIOUS DEGREES OF OXYGEN LACK.

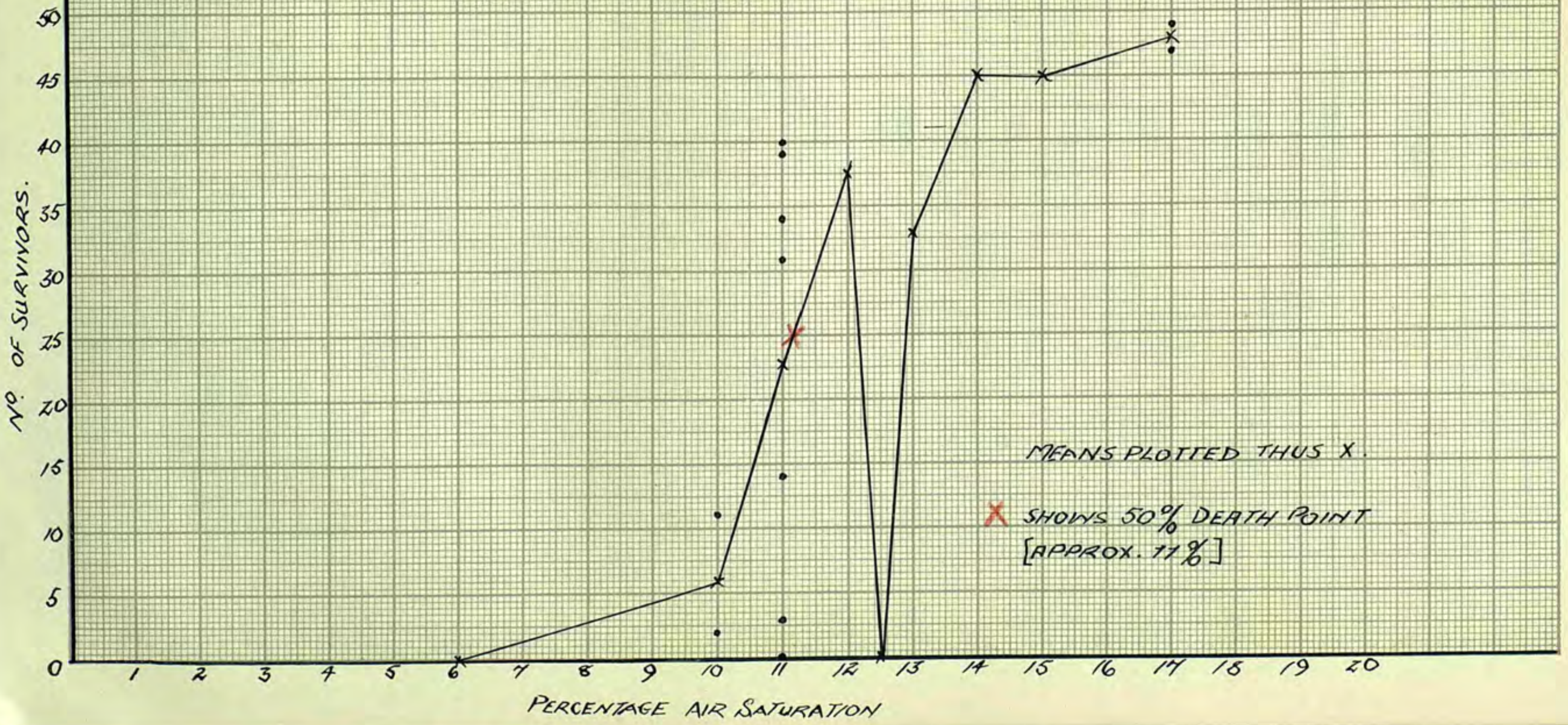
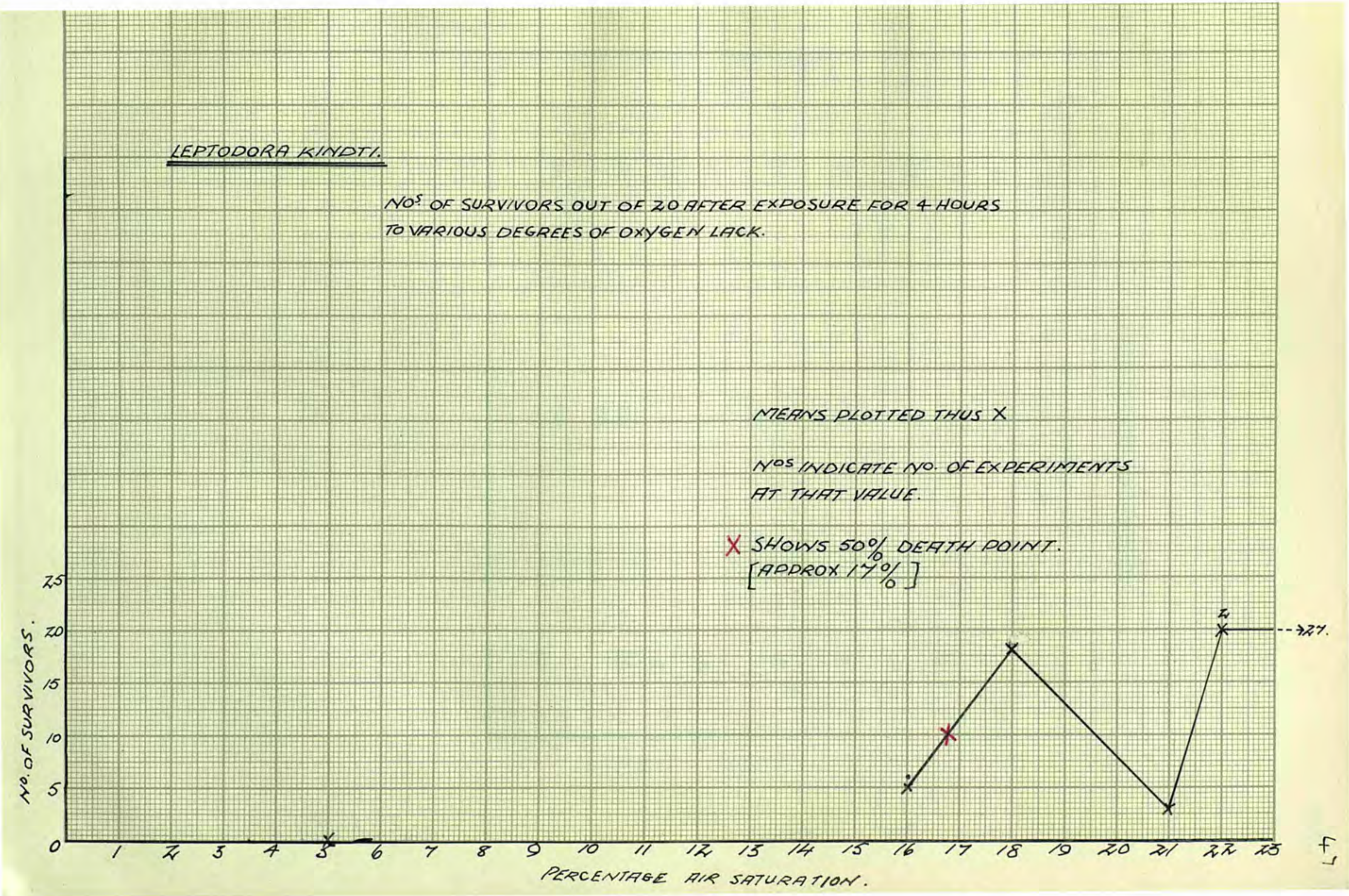


TABLE 11

Leptodora Kindti

% air saturation at 22°C	No. of survivors out of 20 after 4 hrs.	Average
5	0	0
16	5 6	5
18	18	18
21	3	3
22	20 20	20
27	20	20



LEPTODORA KINDTI.

NOS OF SURVIVORS OUT OF 20 AFTER EXPOSURE FOR 4 HOURS TO VARIOUS DEGREES OF OXYGEN LACK.

MEANS PLOTTED THUS X

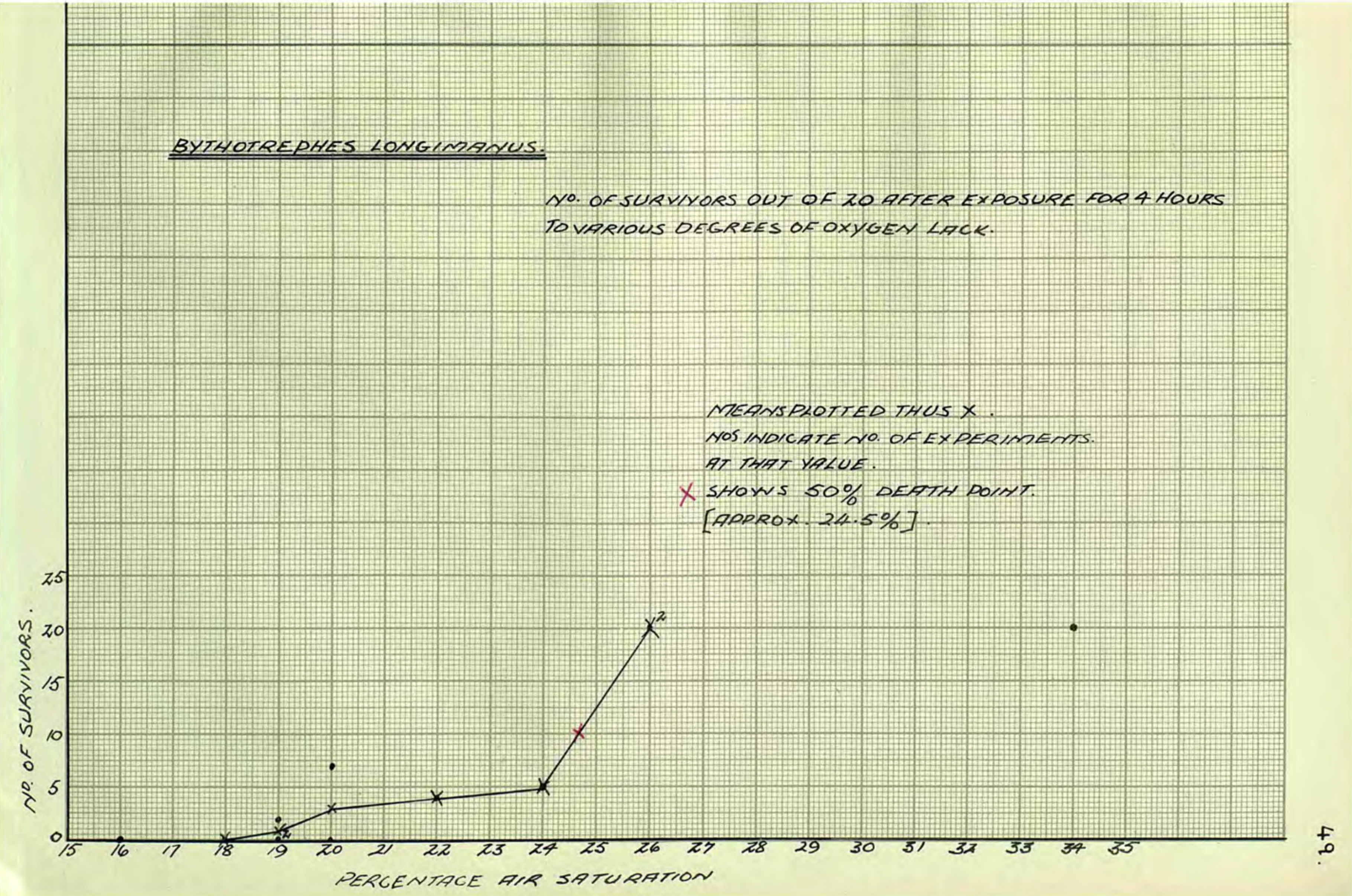
NOS INDICATE NO. OF EXPERIMENTS AT THAT VALUE.

X SHOWS 50% DEATH POINT. [APPROX 17%]

TABLE 12

Bythotrephes longimanus

% air saturation at 22°C	No. of survivors out of 20 after 4 hrs.	Average
8	0	0
16	0	0
18	0	0
19	0 0 2	1
20	0 7	3
22	4	4
24	5	5
26	20 20	20
34	20	20



BYTHOTREPHES LONGIMANUS.

No. OF SURVIVORS OUT OF 20 AFTER EXPOSURE FOR 4 HOURS TO VARIOUS DEGREES OF OXYGEN LACK.

MEANS PLOTTED THUS X.
NOS. INDICATE No. OF EXPERIMENTS. AT THAT VALUE.
X SHOWS 50% DEATH POINT.
[APPROX. 24.5%].