ASPECTS OF ECOLOGY AND DEVELOPMENT OF CHIROCEPHALUS DIAPHANUS PRÉVOST (CRUSTACEA: ANOSTRACA) IN THE NEW FOREST

by

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TO THE MEMORY OF MY

BELOVED MOTHER

A C K N O W L E D G M E N T S

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF PLATES

LIST OF TABLES

ABSTRACT

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ASPECTS OF ECOLOGY AND DEVELOPMENT OF CHIROCEPHALUS DIAPHANUS PRÉVOST (CRUSTACEA : ANOSTRACA) IN THE NEW FOREST

by Azwar Noaman Khalaf

The natural occurrence of the fairy shrimp Chirocephalus diaphanus Prévost in two temporary freshwater ponds was investigated. There was only one generation in each wet period. There are four factors which may determine the life span. These are (a) predation, (b) low oxygen concentration, (c) temperature and (d) desiccation. The growth curve had three distinct phases. The rate of growth was rapid in both the first and the third phases. In the second phase the rate was very slow and may be associated with the maturing of the gonads. The maximum body length was 36 mm. The maximum life span in a predator free pond was nearly eight months. Records of the limnology of the two ponds for the period of the study are given.

Under natural conditions the eggs sink to the bottom of the pond after laying, where low oxygen concentrations exist. Eggs either develop a little or no development takes place under these conditions. Then the eggs are exposed to moist soil when the pond dries up. This provides aerobic moist conditions which are the most favourable conditions for the embryonic development. Then hatching occurs after they are inundated with rainwater. The complete escape of the nauplius from its three membranes takes place in two stages. The breaking stage, which is

strictly an osmotic phenomenon, and the hatching process, which is caused by mechanical means.

The dried eggs were found to float, due to air spaces formed between the tertiary shell/chitinous membrane complex and the embryonic mass. The eggs can withstand a drying period of at least four months; however this depends on the relative humidity. The total water loss was less at 0% relative humidity than that at higher humidities up to 83% (inclusive), although the initial loss was more rapid. No embryonic development was found to have taken place at sub-freezing temperatures. The higher the temperature the shorter the time required for the egg to complete its development up to a limit. Above this limit, the higher the temperature of exposure, the shorter is the time required to harm and kill the egg.

The embryonic development was inhibited at very low oxygen concentrations. The eggs would hatch more readily at higher concentrations of oxygen.

Pond water having concentrations as high as ten times that of the natural habitat were found to have had no adverse effect on the embryonic development and hatching. Development and hatching were normal at osmotic pressures lower than 3 and 1·5 atmosphere respectively.

Depth of water had no influence on embryonic development and hatching. Pressures due to depth of water had no significant effect on these two processes. Low oxygen concentrations which exist at the mud-water interface and within the upper layers of the mud were responsible for retarding embryonic development and for inhibiting hatching.

SECTION ONE

GENERAL INTRODUCTION

SECTION 1

General introduction

The fairy shrimp Chirocephalus diaphanus Prévost is a member of the order Anostraca which belongs to the sub-class Branchiopoda, class Crustacea. The majority of the anostracan species live in temporary freshwater habitats; however, some species dwell in saline or brackish waters. Baird (1849) was the first to report that C.diaphanus lives in small freshwater pools. The water conductivity of temporary freshwater bodies varies with time. It increases by the evaporation and decreases through the addition of rainwater.

Hartland-Rowe (1968) reported a conductivity for the habitat of the freshwater fairy shrimp Artemiopsis stefanssoni Johanson of 159 micromhos/cm. Low conductivities for freshwater habitats were also reported by Beeton (1965); Carter (1972); Hutchinson (1957); Iverson (1971); and Ryder (1964). Other members of the order Anostraca such as Branchinecta salina Dad, Branchinecta spinosa and Branchinectilla salina Dad have been found in saline or brackish water. White (1967) reported that in western Canada the salt concentration of the habitat of Artemia salina L. ranges between 100,000 and 300,000 ppm. The fairy shrimp Branchinecta mackini Dexter, and Branchinecta gigas Lynch were found in brackish water having lower salt concentrations.

In general, all members of the order Anostraca are known to live only in temporary habitats; however, Relyea (1937) reported that Artemia was found in permanent habitats. Longhurst (1955) also reported that the fairy shrimp Branchipus vernalis occurs in permanent pools in North America. Those species which are confined to temporary habitats survive periods of desiccation or total freezing through their drought-resistant eggs.

Most of the published works concerning the anostracan species are restricted to general field observations and to the occurrence of the different species, Broch (1965); Coopey (1950); Dexter (1962); Dexter and Ferguson (1943); Dexter and Kuehnle (1948); Ferguson (1939); Hall (1961); Mathias (1937); Moore (1955); Nourisson (1964); and Prophet (1963).

Work on the rate of the embryonic development under natural conditions is scarce. No regular and detailed observations on development in the field have been made; however, Hall (1961) suggested that the embryonic development takes place some time before the filling of the pond, but whether the embryonic development takes place immediately after the eggs have been laid and before the habitat becomes dry, or during the dry period of the pond, is not known. This matter was investigated in the present study.

Rate of growth of anostracan branchiopods, as determined from field samples, has been reported for some other species by several workers, e.g. Dexter and Ferguson (1943); Moore (1963); and by Nourisson and Aguesse (1961). Lake (1967) studied the rate of growth of C.diaphanus in a laboratory culture. No reference was found in the literature to the rate of growth of this species under natural conditions.

The egg of C.diaphanus was first described by Baird (1849). It was re-described by Hall (1953), and by Mawson and Yonge (1938). Hall described briefly the complete hatching process and reported that this process takes place through two stages, the breaking stage and the hatching stage, but what are the causes for the hatching are not known. No previous work on the hatching mechanism in the anostracan branchiopods has been carried out. A part of the present study was directed to elucidating this mechanism.

Controversy exists concerning the development of the anostracan branchiopods in relation to the environmental factors. Nourisson (1964) concluded from his few field observations on C.diaphanus in France that the temperature of the habitat is the most important ecological factor controlling the development. Some workers also drew a similar conclusion on other anostracan species, e.g. Dexter and Ferguson (1943); and Moore (1959a). But Hall (1959c) concluded that hatching of the eggs may be related to the depth of water and that a depth of 20 cm or greater would clearly retard the embryonic development and hatching. Also the implication was clear in some other works that depth may have a retarding effect on the development and hatching, e.g. Avery (1939); and Castle (1938). On the other hand, Bernice (1972b), Broch (1965) and Moore (1967) reported that depth had no influence on embryonic development and hatching.

Little work has been done concerning the relationship between the oxygen concentration and the development of the egg. Moore (1967) suggested that low oxygen concentrations inhibited hatching of Streptocephalus seali Ryder. On the other hand Nourisson (1964) reported that he could not consider the oxygen as a limiting factor for the development of the egg. Part of this thesis concerns the influence of oxygen concentrations on embryonic development and hatching.

Laboratory studies on the role of osmotic pressure in controlling or affecting embryonic development and hatching in freshwater anostraca are scarce. It seems that the brine shrimp A.salina has received more attention than the freshwater species, e.g. Clegg (1962)(1964); Grainger (1956); and Croghan (1958). Hall (1953) suggested the possible existence of osmotic relationships between the egg contents and the surrounding medium. Whether osmotic pressure plays a part in breaking of the egg shell or in true hatching is not known. The present study also dealt with

this point.

The eggs of the anostracan branchiopods were reported to survive desiccation for different periods, e.g. Broch (1965); Hall (1953); and Mattox and Velardo (1950). Moore (1967) found that the desiccation influences both rate of hatching and total percentage hatch of S.seali. Hall (1953) found that short periods of desiccation would retard the embryonic development of C.diaphanus. He also suggested that some development had taken place while the eggs were dry, but that investigation on a large scale was necessary to substantiate this point.

It was clear that there was need for a programme of laboratory and field investigation designed to relate the knowledge of occurrence and distribution of C.diaphanus to observed fluctuations and variations in environmental factors. The present study is divided into two parts; the first part was conducted in the field and the second part consists of investigations into the effect of variations of environmental factors on the processes of development and hatching.

SECTION TWO

BIOLOGY AND HABITAT OF C.DIAPHANUS IN THE NEW FOREST TEMPORARY PONDS

Plate [1]. Adult male of Chirocephalus diaphanus Prévost.

Plate [2]. Adult female of Chirocephalus diaphanus Prévost.

Plate [1]

Plate [2]

SECTION 2

Biology and Habitat of C.diaphanus in the New Forest temporary ponds

2.1 Introduction

The fairy shrimp C.diaphanus occurs sporadically in some temporary freshwater ponds in the New Forest (Hampshire). Hall (1953) (1961) observed the appearance of C.diaphanus in three ponds in the New Forest. This species no longer exists in these three ponds.

The occurrence of newly hatched nauplii of anostracan branchiopods was observed soon after the filling of the pond with rainwater. The early hatching of some anostracan species was also reported by Coopey (1950); Ferguson (1935)(1939); and by Hall (1961). Other workers have reported that populations of anostracan branchiopods commence when the eggs hatch following the melting of ice in the winter or spring, e.g. Dexter and Ferguson (1943); Johansen (1921).

During the period of the present study, it was found that winter populations of C.diaphanus survived the periods during which the pond was covered with a layer of ice. Hall (1961) and Weaver (1943) reported the presence of some anostracan species while there was still ice covering the pond. The record of the occurrence in two temporary freshwater ponds in the New Forest for a period of nearly two and a half years is presented in this section, together with the rate of growth, sex ratio and the life span.

2.2 Culture and food

During the early stages of the present study two methods for the preparation of culture media for C.diaphanus were used. The two methods were described by Lake (1967) and by Moore (1957). In summary the first method involved the preparation of cultures of algae in a sterile medium containing the following constituents: 0·3 gm KNO_3, 0·05 gm $MgSO_4$, 0·1 ml micronutrient solution, 0·1 ml Citrate solution and 1 litre of distilled water.

Conical flasks containing 1 or 2 litres of this medium were inoculated with algae and exposed to daylight. When satisfactory algal growth was seen, portions of the culture were centrifuged. The algae were transferred and placed in aquaria containing aged tapwater. Hatched nauplii were placed in the aquaria. The depth in the aquaria at this time was 2-3 cm. This was increased as the animals grew. The second method involved the use of soil extract instead of the aged tapwater. The soil extract was prepared by autoclaving approximately one kilogram of soil (taken from Godshill pond) in one litre of distilled water. The liquid extract was decanted and filtered into bottles stoppered with cottonwool plugs. These bottles were kept in a cold room until use. The quantity of soil extract used was between 10-15 ml per litre of distilled water. The hatched nauplii of C.diaphanus were placed in aquaria containing this culture medium and were fed on algae and yeast suspension. Neither method was found to be very satisfactory, since the cultures showed high mortalities. However, the second method seemed to be better than the first. When Godshill and Eyeworth ponds were full, their water was used as a culture medium for C.diaphanus.

Plate [37]. Asbestos bowl used as artificial pond for culturing Chirocephalus diaphanus Prévost.

Plate [3]

2.2.1 A new culture technique for rearing C.diaphanus

Later and with the advancement of the study, a shortage in the numbers of the animals for the experimental purposes was felt, and a new method for culturing C.diaphanus was needed. Asbestos bowls having a diameter of approximately one metre and a depth of 25 cm. were placed outdoors and were filled with soil from the natural habitat of C.diaphanus to a depth of 5 cm. - Plate [3]. These bowls were then filled with either aged tapwater or with distilled water. The type of water used was not critical. The newly hatched nauplii of C.diaphanus were transferred from the hatching petridishes into these bowls. Sufficient food seemed to be provided by the natural growth of algae. This method was found to be highly satisfactory and C.diaphanus could live for as long as eight months under these conditions. Air and water temperatures, conductivity, depth, volume and surface area for these bowls were measured every two days for a period of nearly two months. The volume was calculated using the following equation:

$$V = \frac{1}{3} \pi d^2 (3a - d)$$

and the surface area was calculated using the following equation:

$$S.A. = a^2 \pi$$

where V = volume; d = depth; π = constant = 3·1416; S.A. = surface area and a = radius.

The fluctuations of the above factors during the two month period are summarized in Figs. [1], [2], [3], [4], for three of these bowls. Bowl (A) was shaded during the mornings, bowl (B) was unshaded all the day, and bowl (C) was shaded during the afternoons.

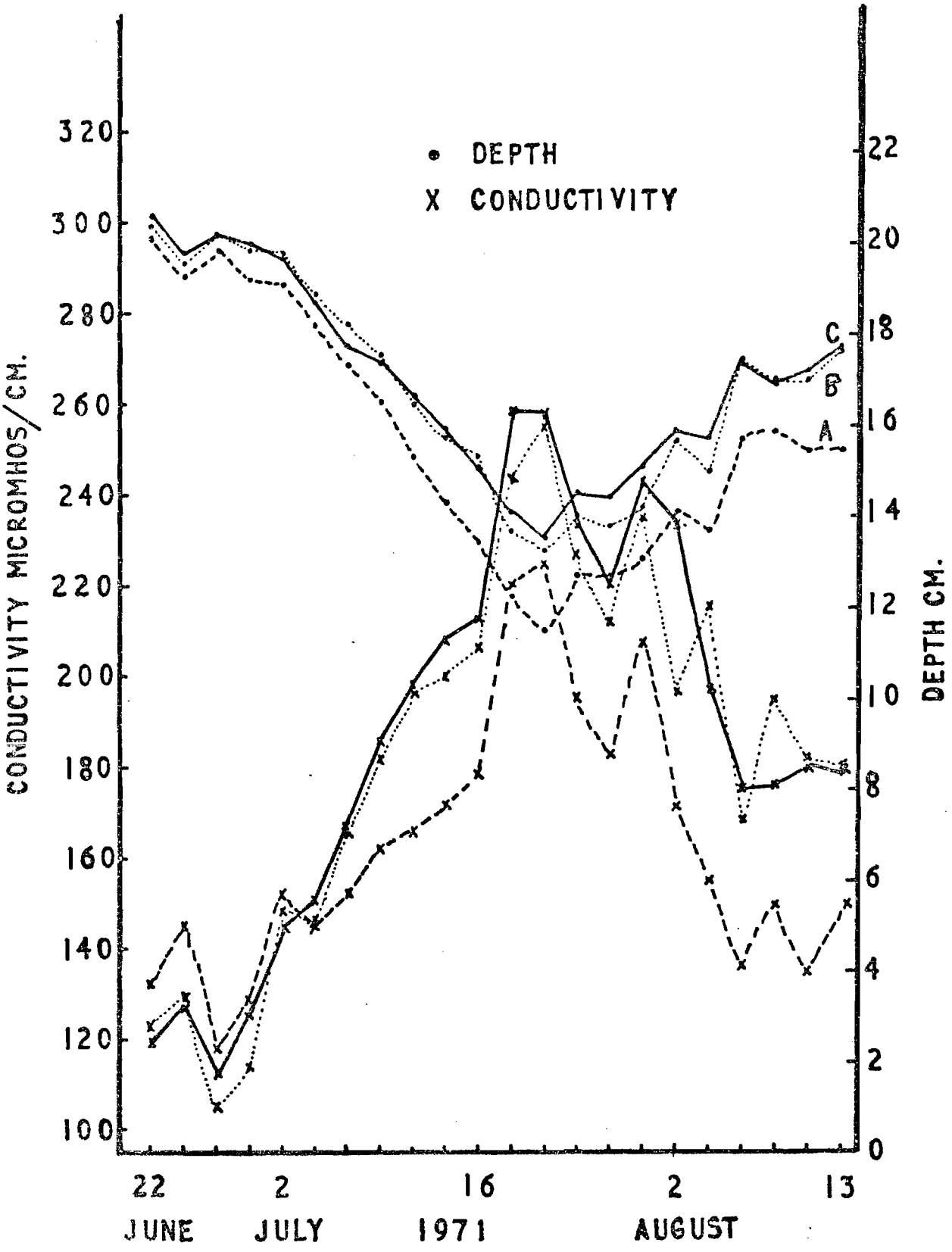


FIG.1: CONDUCTIVITY AND DEPTH OF THE ARTIFICIAL PONDS.

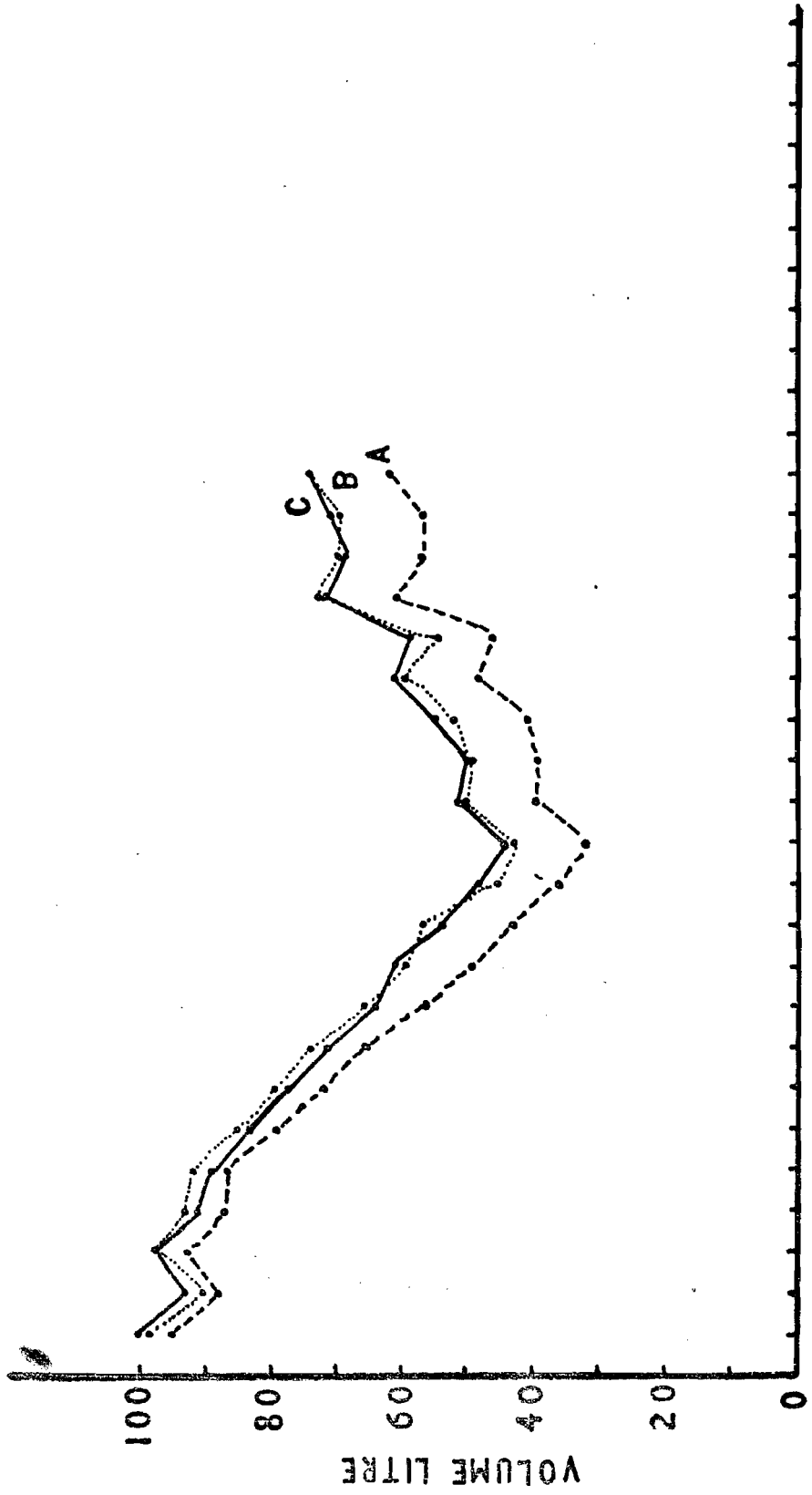


FIG.2 : VOLUME OF THE ARTIFICIAL PONDS.

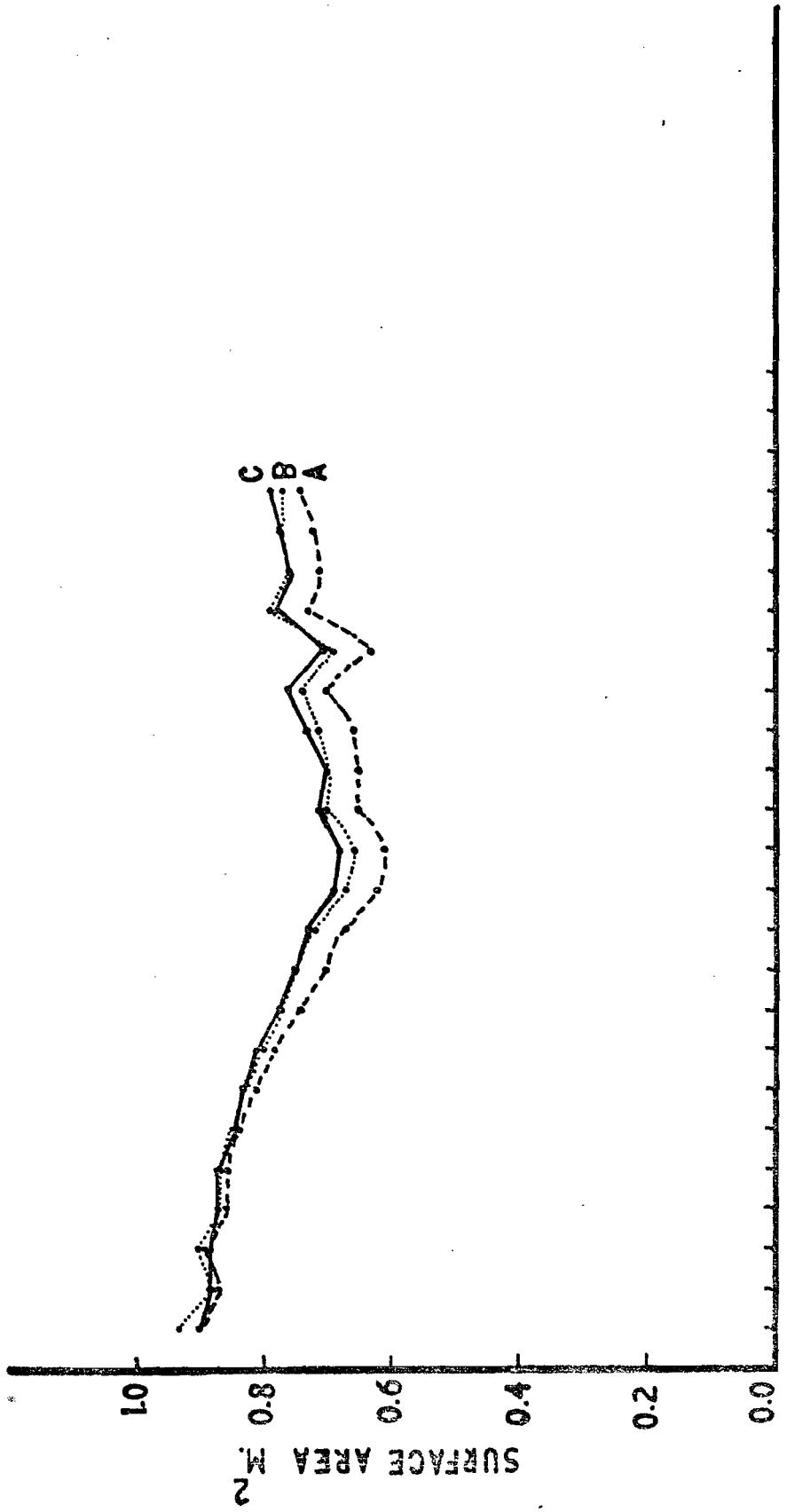
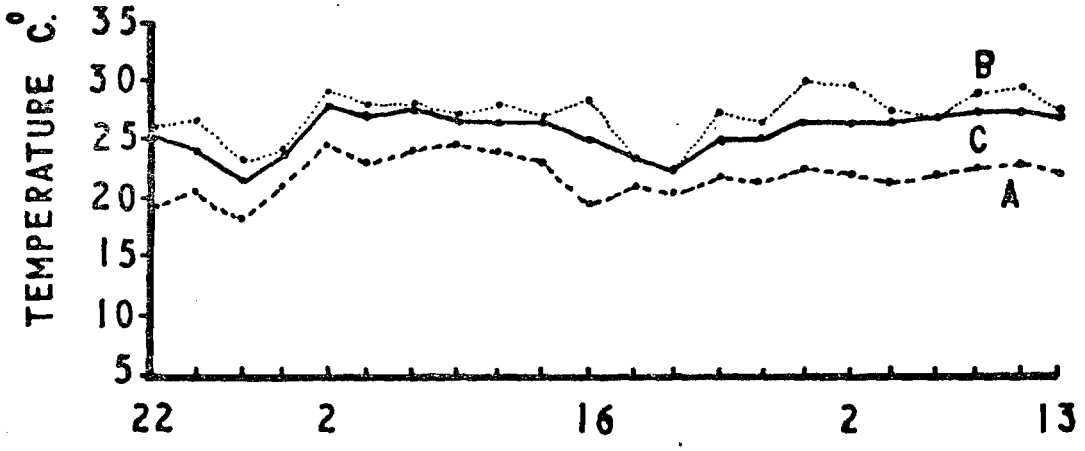
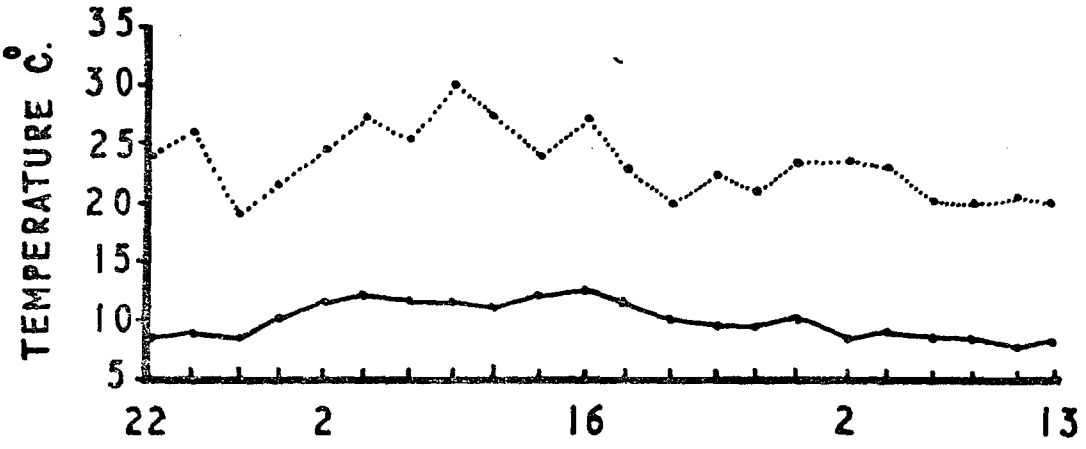


FIG.3 : SURFACE AREA OF THE ARTIFICIAL PONDS.

FIG. 4



WATER TEMPERATURE OF THE ARTIFICIAL PONDS.



MAXIMUM AND MINIMUM AIR TEMPERATURES.

2.3 Life cycle, seasonal occurrence and the existence of one generation

One advantage of regular and frequent visits to the field in the study of temporary habitats is that it is thus possible to ascertain the exact time of hatching and also to determine the cause of the elimination of the population. Both ponds studied were visited weekly for nearly two and a half years to give a clear picture of the life history of C.diaphanus. Hall (1953)(1961) carried out some work on the natural occurrence of C.diaphanus in Burley, Brockenhurst and Lee ponds in the New Forest. This species no longer exists in the ponds mentioned by Hall. The reason for the disappearance of C.diaphanus is not certain, but a possible explanation is to be given and discussed later.

2.3.1 Godshill pond

The first visit to this pond was made on 14th October 1970, when the pond was found to be dry. Samples from the soil were brought to the laboratory and covered with distilled water. Large numbers of C.diaphanus nauplii hatched within two days. During October 1970 there was insufficient rainfall to cause an accumulation of water in the pond, and thus no hatching could have occurred. The pond was visited again on 24th November 1970, and adult specimens of C.diaphanus were collected having a length of 14-16 mm. Presumably hatching took place in early November when heavy rainfall was recorded. The total rainfall in the first week of November was 57·7 mm. On subsequent visits C.diaphanus was found to be present in high densities until 15th February 1971, when a clear decline was observed. A month later only eight aged specimens of C.diaphanus were collected after a careful search. At the same time large numbers of predators were collected. The pond contained water until the end of April 1971, but no C.diaphanus were subsequently

found. The pond was found to be dry on 5th May 1971.

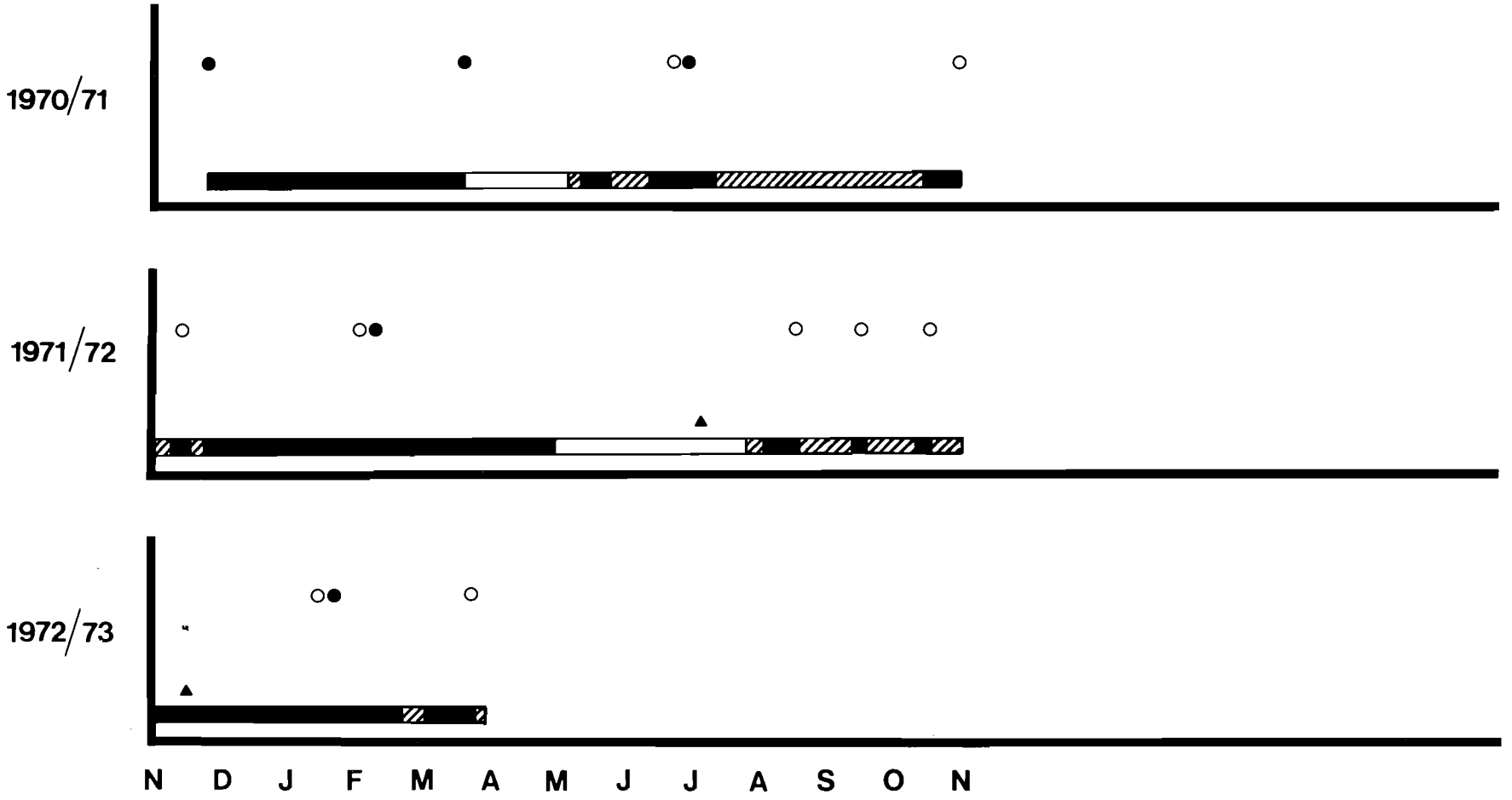
During the summer of 1971, two successive populations of C.diaphanus were eliminated by drying up of the pond before the animals could reach sexual maturity and so produce eggs.

The natural occurrence of C.diaphanus in Godshill pond during the period of the present study is shown in Fig. [5]. The exact hatching time and the time of the termination of the population of C.diaphanus are given elsewhere in this thesis.

The pond remained dry until 22nd November 1971, when a large number of newly hatched nauplii of C.diaphanus was collected following rainfall of 15·2 mm. However, a few specimens of C.diaphanus having a body length of 3-4 mm. were also caught in the net, together with the newly hatched nauplii. These bigger C.diaphanus must have been from the previous hatching which took place on 9th November 1971. These animals must have either survived the short dry period within the highly moistened soil until the next filling of the pond, or a few small puddles formed in the hoofmarks were missed during the previous visit. However, the second possibility is the more likely one. The possibility of the survival of C.diaphanus in water-saturated soil was examined by placing 40 animals,having an average length of 4-6 mm., in a plastic tray containing moistened soil. It was found that 12 hours later only 12 of these C.diaphanus had survived and these recovered when transferred to water again.

The pond was observed to be covered with a layer of ice on most of the visits which were made during January and February 1972. However, the pond was found to have a population of C.diaphanus until 2nd May 1972 when no individuals could be seen, although the pond contained plenty of water. On 12th July 1972 a few C.diaphanus, having an average length

FIG. 5 : SEASONAL OCCURRENCE OF C. DIAPHANUS IN GODSHILL POND



of 2 mm. were found swimming in small puddles formed in hoofmarks at the margin of the pond following rainfall of 12·8 mm., which occurred on 7th and 8th July 1972. This hatching suggests that the second generations sometimes found with the old generations are due to some hatching taking place in the marginal areas of the pond when the level of the pond is increased by rainfall. Hall (1961) reported that on all the occasions on which he has recorded the occurrence of C.diaphanus in Burley and in Lee ponds, there had been only two cases in which there was any evidence of the occurrence of a second generation in the same wet period. The author agrees with Hall's suggestion that it is possible that eggs laid near the margin would be subjected to drying or near drying, and that with refilling of the pond these would hatch and so give a second generation. The author does not consider the second hatching as a true second generation since (a) the occurrence of this hatching is not frequent and depends on the increment of the pond level and is not derived from the population living in the same wet period, and (b) the numbers of the hatched nauplii in the second hatching were very small. Dexter and Kuehnle (1951) reported that when the pond fills up rapidly early in the season there is only one generation of E.vernalis, but with gradual filling several generations were found. In his work with S.seali, Moore (1951)(1963) reported that there was no evidence of any new hatch. Moreover, Prophet (1959) found that the population of S.seali apparently represented a single brood. It seems that there is only one generation in each filling of the pond, although there are a few nauplii which hatch with the gradual filling of the pond. However, Dexter (1946) reported that he found specimens of E.vernalis of all sizes and in all stages of development in deep pools. He suggested that more than one generation might be present. The eggs

laid near the margin might have been one source of the new hatchings mentioned by Dexter.

During the summer of 1972, three successive populations of C.diaphanus were eliminated by drying up of the pond. The individuals of these populations did not reach sexual maturity and so did not produce eggs. It is of interest to note that the numbers of the liberated nauplii in the second and the third populations were less than those found in the first population killed by drying up of the pond. The reason for this may be that the summer populations, due to their premature elimination did not contribute to the egg store of the pond. Thus each successive summer population would have reduced the total numbers of eggs in the pond and so each successive population would consist of a smaller hatch.

On 31st October 1972 newly hatched nauplii of C.diaphanus, having an average length of 0·57 mm. were collected. The individuals of this population were found to have reached sexual maturity approximately 42 days after the hatching occurred. The animals remained available in good numbers until a decline was observed on 27th February 1973, when large numbers of predators were collected. On 20th March 1973 only one specimen of C.diaphanus could be collected after a careful search, although the depth of the water at that time was 8·0 cm. This again confirms the disappearance of the C.diaphanus populations with the appearance of predators.

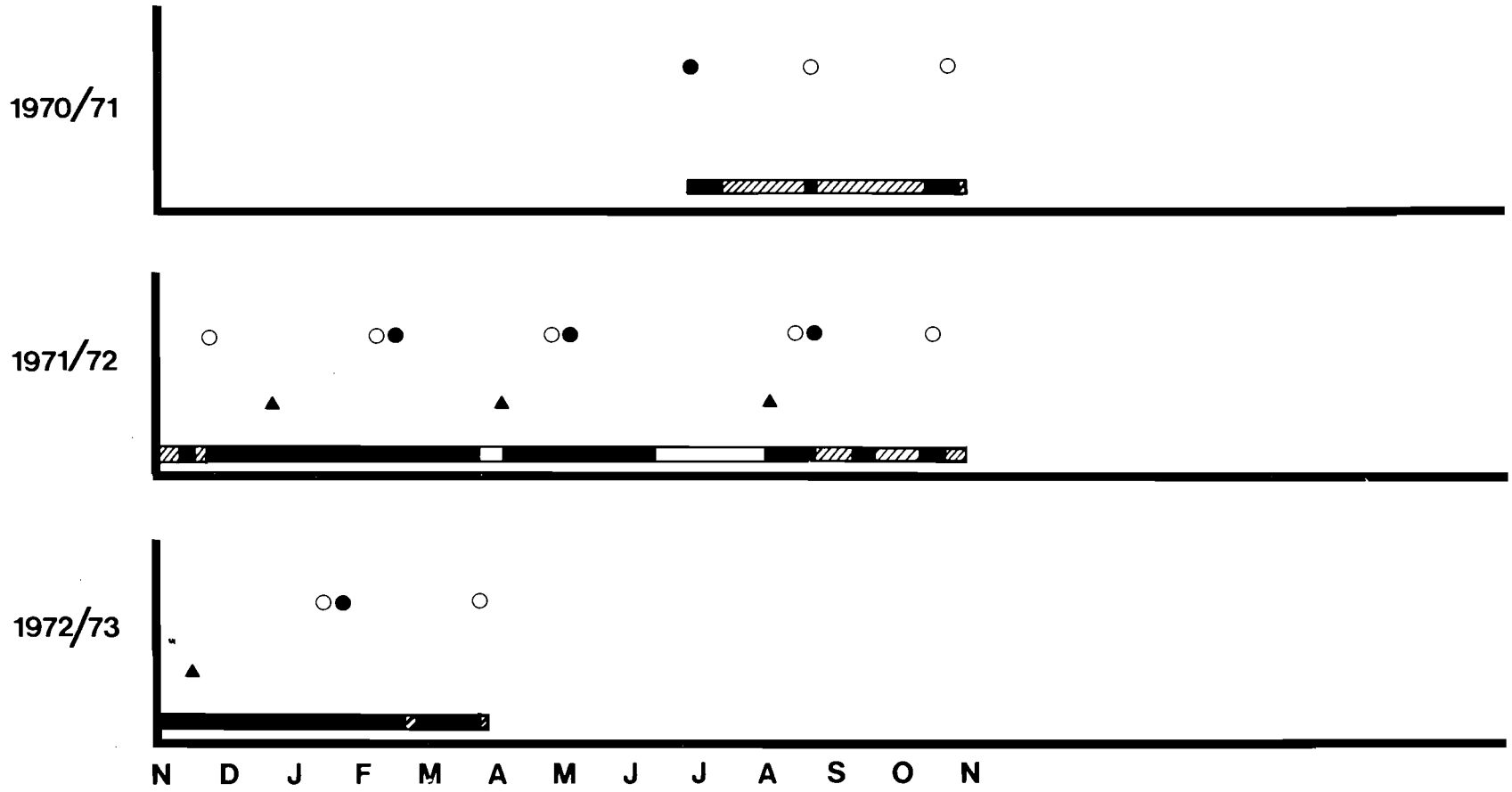
2.3.2 Eyeworth pond

The situation in Eyeworth pond was slightly different from that found in Godshill pond, since the former pond is shallower and smaller than the latter. Because of the shallowness of Eyeworth pond it was found that more hatchings occurred per year than in the Godshill

pond.

The pond was first visited on 29th June 1971, when it contained muddy turbid water to a depth of 10 cm. and supported a good population of C.diaphanus. The turbidity of the pond water was due to (a) the bottom of the pond being almost devoid of vegetation, and (b) the pond being frequently trampled by cattle. On a subsequent visit, a week later, the pond was found to be very shallow, but still contained a few C.diaphanus in obvious distress. The oxygen concentration was 1·5 mg/l. On 10th July the pond was found to be completely dry. The natural occurrence of C.diaphanus in this pond during the period of the present study is shown in Fig. [6] and the time of hatching and of the termination of the population are given elsewhere. During the summer of 1971 three successive populations of C.diaphanus were eliminated by the drying out of the pond before the animals could reach sexual maturity. Another population hatched on 9th November 1971. Newly hatched nauplii of C.diaphanus were collected from beneath a layer of ice. The pond was found to be completely frozen on 19th December 1971, and the entire population of C.diaphanus was killed. Newly hatched nauplii, having an average length of 0·45 mm. were collected two days later, although they were not as numerous as in the previous population. The small number of hatched nauplii in this population might be due to the fact that four previous populations had been killed before the animals could reach maturity and so produce eggs. It is of interest to mention that the pond was found to contain, on 8th February 1972, individuals of C.diaphanus of three different sizes. The first group of specimens had an average length of 9 mm., the second of 4 mm. and the third group had an average length of 1 mm. This variation might be due to the gradual filling of the pond, so that the eggs laid near the margin of the pond hatched when they were

FIG.6 : SEASONAL OCCURRENCE OF C. DIAPHANUS IN EYEWORTH POND



Indicated Chirocephalus present.
Water but no Chirocephalus.
pond dry
Immature Chirocephalus
Mature Chirocephalus
Hatching of a new generation

inundated with water. This is supported by the facts that a total rainfall of 44·9 mm. occurred during the period between 7th and 13th January 1972, and a total rainfall of 17·3 mm. occurred during the period between 23rd and 29th January 1972. The population of C.diaphanus disappeared completely from the pond from 27th March 1972. The water conductivity at this time was 112 micromhos/cm, and the water temperature was 7·5 C^{o}. The oxygen concentration was very low, having a value of 1·9 mg/l. The elimination of the population of C.diaphanus might be due in this case to the reduction in the oxygen concentration, especially since this pond is almost devoid of vegetation, and one would expect the oxygen concentration to fall to a very low value during the night.

From the field observations which have been carried out during the present study, it seems that no true second generation exists with an old generation. The new hatchings which add new specimens of C.diaphanus to the population take place from the eggs which were laid near the margin of the pond. The prevention of the hatching of a second generation is of considerable value to the survival of this species, since it ensures the hatching of a new generation only when favourable conditions are available. The prevention of hatching of a second generation was noted in aquaria containing adult C.diaphanus. Also it was observed in the artificial ponds.

During the summer of 1972, two successive populations of C.diaphanus were eliminated by drying up of the pond before the animals reached sexual maturity. The pond remained dry until 31st October 1972, when newly hatched nauplii were collected following 16·3 mm. of rainfall. Another hatching took place on 12th November 1972, following 16·9 mm. of rainfall on 12th November. A third hatching took place on 21st November after rainfall of 6·2 mm. on 19th November 1972. The hatched nauplii

in these new hatchings were few in numbers, since only those eggs laid near the margin hatched with the gradual filling of the pond. No further hatching took place, since the level of the pond was always high and the margin of the pond was continuously covered. The pond was found to be covered with a layer of ice during most of January and February 1973. Oxygen concentrations as low as 3 mg/l. were recorded below the ice. The animals reached sexual maturity approximately two months after hatching. The pond contained C.diaphanus in high densities until a slight decline was observed on 6th March 1973. At the same time a few dytiscid larvae and trichopteran larvae were collected. Only one adult C.diaphanus, in obvious distress, was collected on 20th March 1973. At this time the pond consisted of a collection of small puddles formed in hoofmarks. The winter population of C.diaphanus of 1972/1973 in Eyeworth pond reached sexual maturity two months after hatching, whereas that in Godshill pond took approximately 45 days to reach maturity. This is because of the shallowness of Eyeworth pond, which causes it to be covered with a layer of ice for a longer period than Godshill pond. However, the turbidity of the water might have affected the feeding of C.diaphanus.

These regular field observations on the natural occurrence of C.diaphanus in the New Forest indicate that there is a cycle of events in the temporary ponds studied which determines the appearance and the survival of this species. The eggs after being laid by the females sink to the bottom of the pond and stay there. No hatching of a second generation takes place until the filling of the pond following a dry period. However, a few nauplii may hatch when the marginal areas of the pond are covered by rainwater.

2.4 The causes of the termination of C.diaphanus populations

Analysis of the field observations showed that the reduction in the numbers of C.diaphanus in a certain population may be caused by one or more factors. These factors vary in their times of effect. The factors which may cause a reduction in the population density or the complete elimination of the population are predators, temperature, low oxygen concentration and finally desiccation. However, senescence may occasionally be of importance. The most important factor probably is predation. Predators were clearly of importance in reducing or eliminating the population of C.diaphanus in Godshill pond; more so than in Eyeworth pond. Being larger and deeper than the Eyeworth pond, the Godshill pond supports a larger population of predators. The disappearance of C.diaphanus with the occurrence of large numbers of predators may be more than a coincidence. On all occasions in which large numbers of predators occurred there was a parallel decline in the population of C.diaphanus. On 15th February 1971, a high population of C.diaphanus was present; at this time no predators were observed to be there. A month later on a subsequent visit only eight aged shrimps were collected after a careful search, but a few dytiscid larvae, trichopteran larvae, notonectids, dragonfly nymphs and a few tadpoles were collected. By the end of April, no C.diaphanus specimens could be seen in the pond when it was visited, although it contained water. Very large numbers of predators were collected at that time. On 8th February 1972, the number of C.diaphanus was quite high, but on a subsequent visit three weeks later fewer were caught. At the time remarkable numbers of predators were collected per sample. The relationship between the appearance of predators and the elimination of C.diaphanus population was also demonstrated by the winter population of 1973. The population of C.diaphanus was observed to be high until 27 February 1973

when a considerable number of predators was collected. At the same time a clear decline was seen in the number of C.diaphanus. On 20th March only one specimen of C.diaphanus was caught after a careful search, although the pond was still full to a depth of 8·0 cm. Fig. [7] shows the population density curves for the winter populations during 1972/1973 of C.diaphanus in both Godshill and Eyeworth ponds.

From the population density curves shown above, it can be seen that a marked reduction in population density of the nauplii occurs within the first week following the attainment of the maximum hatch. The causes of this decline are not known for certain, but may, in part, be due to the physiological limitations of the animals. The terminal decline of the population density of C.diaphanus is probably due to the effects of predation as has been discussed above. However, senescence must not be excluded in this case, but it is of interest to add that C.diaphanus was able to live for eight months in artificial ponds free of predators. The question arises as to why C.diaphanus did not survive for longer than five months in the field, whereas survived longer for up to eight months in artificial ponds having similar characteristics to the natural ponds. The answer to this is probably predation. The reason for the reduction in the numbers of C.diaphanus must be the presence of predators in large numbers during the late stages of the pond. On all occasions in which the winter populations of C.diaphanus disappeared, the oxygen concentration was very high and the temperature was within the acceptable range. The effect of the ionic concentration of the water can also be excluded, since low values for the water conductivity were recorded at these times. The effect of predation on the anostracan species was mentioned by Ardö (1947); Dexter (1967); Dexter and Ferguson (1943) and by Moore (1951). One of the reasons for the early hatching of C.diaphanus

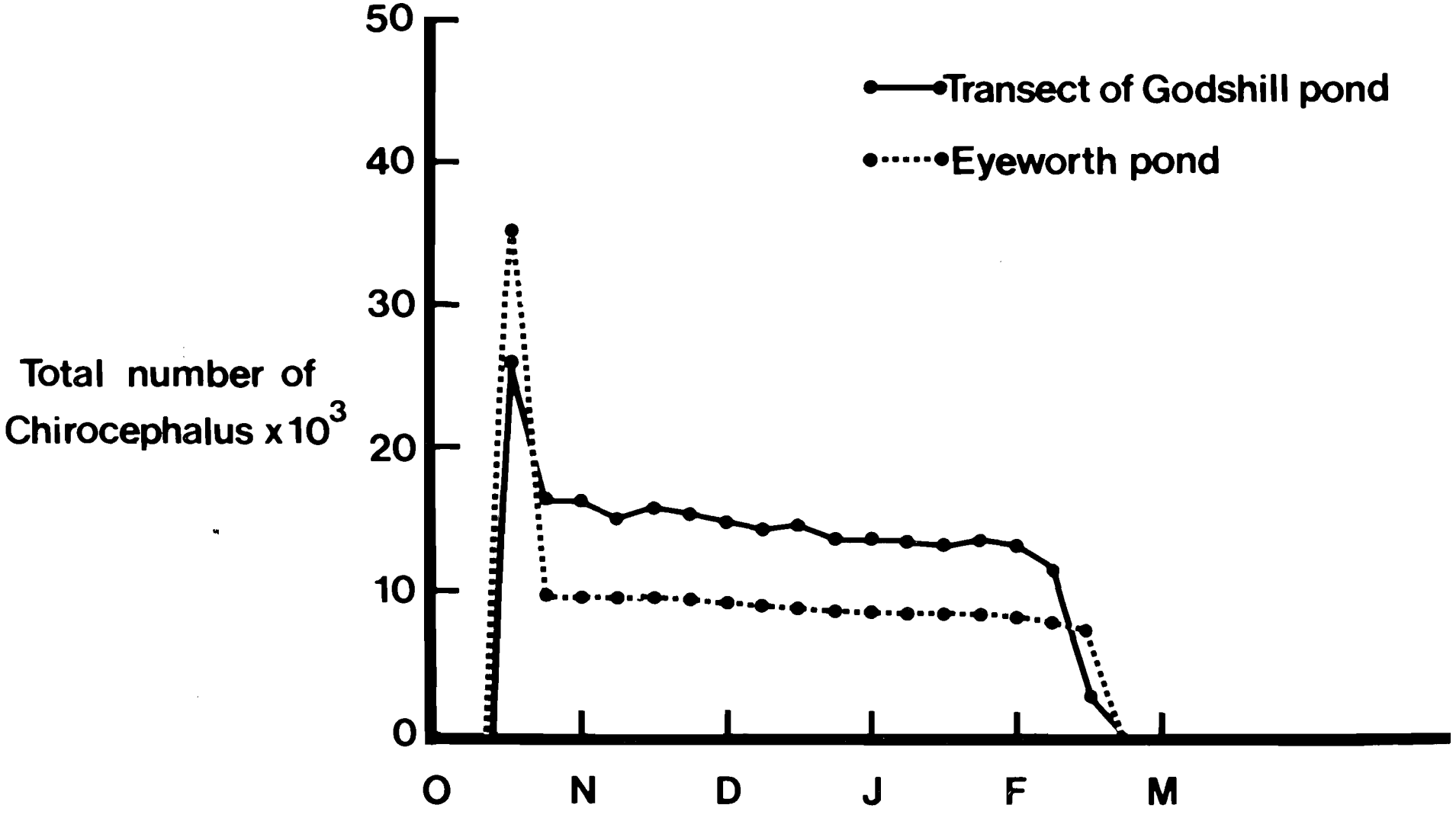


FIG. 7 : TOTAL NUMBER OF CHIRACEPHALUS DIAPHANUS IN THE WINTER OF 1972/1973

may be to allow them to reach sexual maturity and lay eggs before the appearance of predators, which increase in number with the increase in age of the pond.

Termination of the populations of C.diaphanus may also be caused by a great reduction in oxygen level. For example, on 5th July 1971 the oxygen concentration in Eyeworth pond at 10.30 a.m. was 1·5 mg/l. After a careful search, only a few specimens of C.diaphanus were collected. The animals were in obvious distress. At this time the water level was low and the water was muddy. Two days later, no C.diaphanus could be seen, although the pond still contained water. The pond was found to be dry on 10th July 1971. A decline in the numbers of C.diaphanus was noticed in Eyeworth pond on other occasions, when the oxygen level was very low. During the day a value of oxygen concentration as low as 1·8 mg/l. was also recorded in this pond; one may expect much lower values during the night. The specimens of C.diaphanus caught at those times were also in obvious distress.

A third factor which may reduce or eliminate the populations of C.diaphanus is temperature. From an analysis of the field observations it seems likely that this factor was instrumental in the elimination of C.diaphanus population on at least one occasion in Eyeworth pond. Thus, on 19th December 1971 one pond was found to be completely frozen and the entire population was killed. Low temperature may also have an indirect effect in that if temperatures are sufficiently low to cause the formation of an ice layer over the pond, oxygen concentration below the ice may fall to a low value. The upper extremes of temperature had no significant effect on the reduction or the elimination of the population of C.diaphanus, since exposure of the population to such temperatures was never prolonged. Experimentally the author has found that C.diaphanus can live as long as

one week at 25°C, three days at 30°C and more than one day at 33°C. A water temperature as high as 30°C was recorded in the artificial ponds, but this temperature was only maintained for a few hours, so it could have had no harmful effect on the population.

The final possible factor in the termination of C.diaphanus population is the drying out of the pond. The drying out of the pond eliminates the entire population, since neither the nauplii nor the adult C.diaphanus can survive desiccation. One adaptation of C.diaphanus and other anostracan species to the temporary habitat is the production of a drought-resistant egg by which the species can survive the dry periods of its habitat. In Eyeworth pond, desiccation killed four successive populations of C.diaphanus before they could reach sexual maturity during the summer season of 1972. The same phenomenon has been observed in Godshill pond. The ability to produce large numbers of eggs and the prevention of hatching at low oxygen concentrations may well be of considerable importance to the survival of the species. The production of a large number of eggs is of survival value to the species, since it enhances the ability of the species to survive in situations where hatching is rapidly followed by a drying out of the pond. If the females could produce only small numbers of eggs, successive repetitions of these situations would rapidly reduce the number of eggs in the pond to zero. The suppression of hatching at low oxygen concentrations is of survival value to the species, since if hatching occurred under these conditions the liberated nauplii would not survive due to the low oxygen concentrations and there would thus be considerable egg wastage. Dexter (1967) reported that termination of the populations of E.vernalis was caused by temperature, predation, or by drying out of the habitat. In his work with some anostracan species Moore (1963) mentioned that the chief

factors contributing to the decline and eventual elimination of S.seali and E.holmani from the pools appeared to be predation by invertebrates, senility and unfavourable high temperature.

In summary, there are four factors which may determine the life span of a population of C.diaphanus in a temporary pond. These factors are (a) predation, (b) low oxygen content of the pond, (c) temperature and (d) desiccation. However, senescence must not be excluded.

Hall (1953) reported the occurrence of C.diaphanus in two localities in the New Forest. These are Burley and Brockenhurst ponds. This species no longer exists in these two ponds. The reason for the complete disappearance of C.diaphanus is not certain, but it could be possible that two factors are responsible. The first reason is the heavy predation, which may have an obvious role in big ponds like these two, since these two ponds, in late stages, support high populations of predators. The second reason is that, because of the depth of these two ponds the laid eggs remain for long time within the mud where very low oxygen concentrations exist. These two ponds were observed to have contained water for as long as eight months. It was found experimentally, as will be discussed later, that low oxygen concentrations retard or inhibit embryonic development of C.diaphanus. The complete elimination of C.diaphanus from Burley and from Brockenhurst ponds might have been due to the action of these two factors mentioned above on many successive generations.

2.5 Rate of growth

Little work has been published concerning the rate of growth in the field of freshwater anostracans. Lake (1967) studied the rate of growth of a laboratory culture of C.diaphanus. He found in summary that the most favourable temperature range for both growth and reproduction in the laboratory was 10-20°C, and the most unfavourable temperatures were 25°C and 5°C, although C.diaphanus reached maturity at 25°C. rapidly, and at 5°C it was very slow. Nothing has been recorded about the rate of growth of C.diaphanus in the field.

In the present field investigations it was found that in winter populations, both females and males reached maturity approximately 45 days after hatching. Sexual maturity was determined by the appearance of white developing eggs in both oviducts and lateral pouches, and brown fully mature developed eggs in the ovisacs. In summer populations, sexually mature females were noted eighteen days after hatching. Unfortunately most summer populations were eliminated by desiccation before a complete study could be made on them. Hall (1953) reported that C.diaphanus reached sexual maturity in three weeks; also later he (1961) found that in summer it was possible to reach maturity in a period of 14 to 16 days. Moore (1957) reported that the rate of growth of S.seali was most rapid at 25°C and slowest at 18°C. This is in agreement with the field observations of the present study, in that the summer growth was more rapid than winter growth. Although sufficient data of the summer growth was not available, one might expect that the life span of C.diaphanus in summer would be shorter than that found in a winter population, since it was found that summer populations reach maturity before the winter populations. Winter populations in the present study lasted for more than five months.

The greatest body length of C.diaphanus found in the field collected

samples was 34 mm. On 5th March 1971 seven adult C.diaphanus were collected from Godshill pond by Professor H. Hewer, and these were given to the author. These specimens were five females and two males. The average length of these specimens was 33·5 mm. The length of the C.diaphanus reared in the artificial ponds, and which lived for nearly eight months, ranged between 34 and 36 mm. Hall (1959b) reported that he collected specimens of C.diaphanus having an average length of 40 mm. Lake (1967) reported that he collected individuals of C.diaphanus of 35-42 mm. long from a pond near Fordingbridge in the New Forest. The average length of the laboratory cultured C.diaphanus was less than that found in the field. The impairment of growth with laboratory reared individuals could be due to either (a) the animals were confined to a relatively small volume, and (b) the restriction of the diet to some algal species.

The growth curves of C.diaphanus obtained in the present study, as presented in Fig. [8] and Fig. [9], can be divided into three phases. The first phase was the period confined between hatching and sexual maturity. In this phase the rate of growth was rapid. The rates were 0·31 and 0·66 mm per day in the winter and summer populations respectively. The second phase was the period at which C.diaphanus attained sexual maturity. In this phase the rates were very slow. They were 0·20 and 0·43mm per day in the winter and summer populations respectively. The animals started to grow rapidly again after they reached maturity. The rates were 0·26 and 0·51 per day in the winter and summer populations respectively. Coopey (1950) found that E.oregonus has a rate of growth of 0.19 mm per day up to the time of egg production, 0.07 mm per day during egg production, and finally another rapid rate of growth of 0·18 mm per day after that. In his work with S.seali Moore (1955) reported that the rate of growth was 0·5 mm per day in the winter population in the first three weeks until

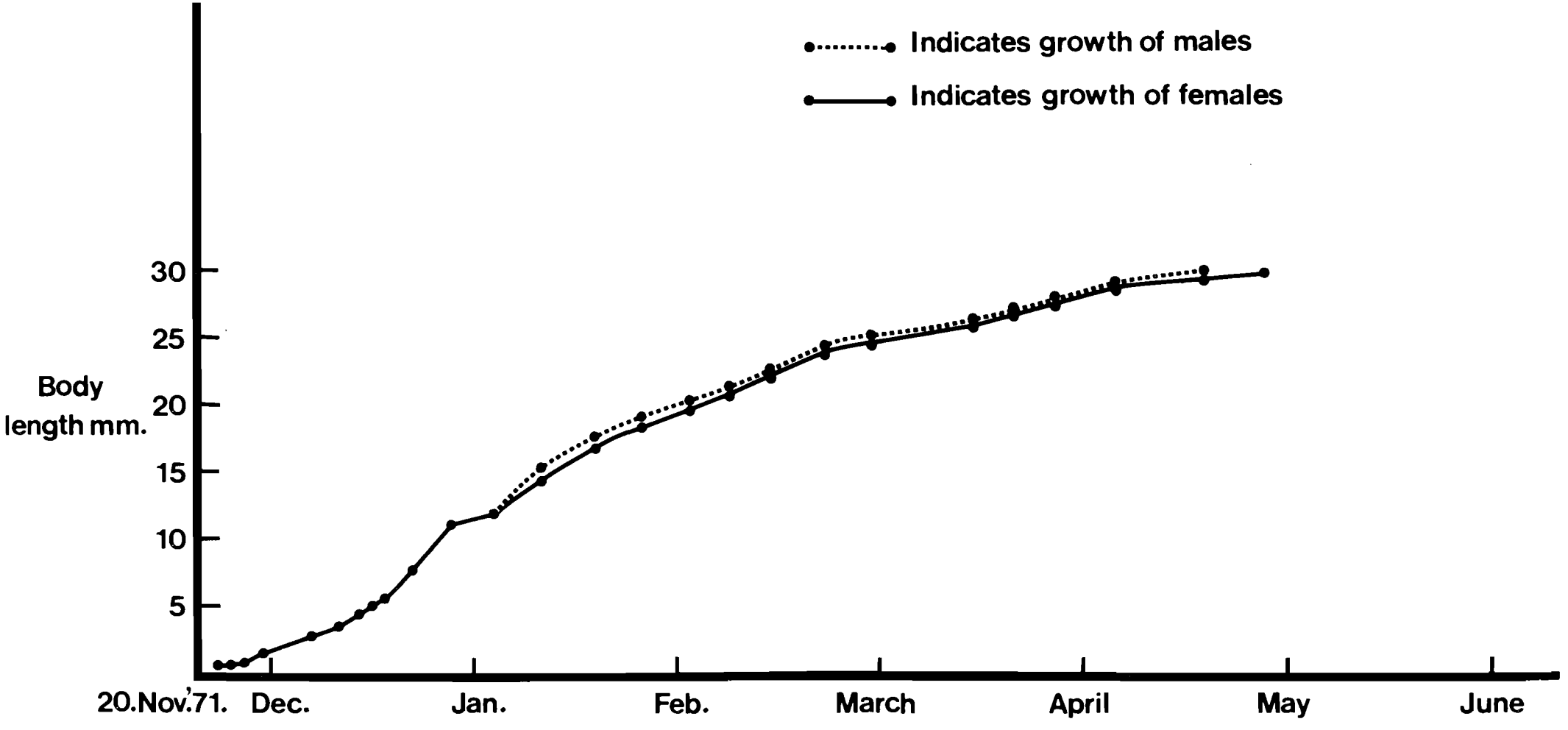
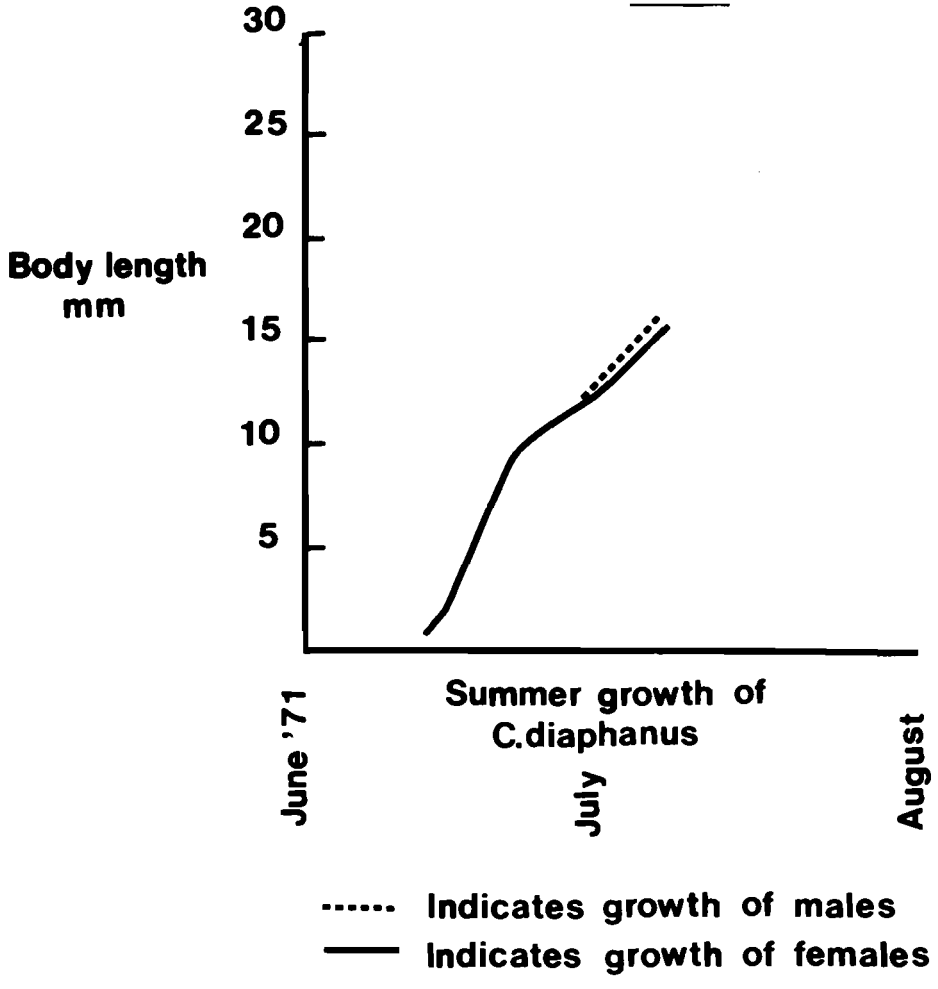


FIG. 8 : WINTER GROWTH OF CHIRACEPHALUS DIAPHANUS

FIG. 9


30
25
20
15
10
5
Body length
mm
June '71
July
August
Summer growth of
C.diaphanus
----- Indicates growth of males
—— Indicates growth of females

they started reaching maturity. This was followed by a slower rate of 0·1 mm per day, and finally in the third period the rate of growth was 0·4 - 0·5 mm per day. Moore (1955), White (1967) and all other workers mentioned above suggest that the slowing of the rate of growth of other anostracan species is near the attainment of sexual maturity of the gonads. It is probable that this is also the case in C.diaphanus. It was also noticed that the rate of growth of males is slightly more rapid than that found for the females.

2.6 Egg production in the field

Nothing has been recorded about the production of the eggs of C.diaphanus in the field. However, Lake (1967) studied the egg production of a laboratory reared C.diaphanus. He found that the numbers of eggs per clutch varied between 5 and 178 eggs depending on the temperature. From the field observations it was found that the females of C.diaphanus can lay as many as 308 eggs per clutch, and this was recorded when the females were approximately three months in age. The females in the field started to produce eggs first as little as 8 eggs per clutch. Then the number of eggs started to increase. In the later stages of the life span the numbers of eggs per clutch started to decline again. One week before the disappearance of the population it was noted that the ovisacs of the females were devoid of eggs. This was presumably due to the absence of the males from the pond at that time. Such a phenomenon was also observed in the laboratory cultures of C.diaphanus. The reason for that is not known, but it is possible that the presence of the males acts as a stimulus for the production of the eggs.

2.7 Life span

The present study on the life span of C.diaphanus has shown that the females of this species live longer than the males in the natural habitat. This phenomenon was also noticed in the laboratory cultures. The regular field observations which have been carried out on two ponds suggest that C.diaphanus can live for up to five months under natural conditions: they then disappeared completely. Males were observed to disappear before females. This might be due to the fact that males of C.diaphanus are less robust than the females, Taylor (1965). The first appearance of the nauplii of C.diaphanus during the winter of 1971/1972 in Godshill pond was observed on 22nd November 1971. The average length of the nauplii at that time was 0·55 mm. Then on 27th April 1972, only adult females having an average length of 32 mm were collected. On a subsequent visit, a week later, a careful search was made but no C.diaphanus were found, although the pond contained water to a depth of 24·1 cm. Thus the life span for that population was five months. However, on 27th June 1972 one aged female was caught having a body length of 34 mm. If this female was from the previous population and escaped the sampling and predation, this means that it had lived for as long as seven months. Generally, it was found that females live longer than the males. The disappearance of the males with the occurrence of the predators before the females may suggest that the females can escape predators more easily than the males. In Eyeworth pond the winter population for the same year lasted for four months. Bernice (1972a) reported that the maximum life span for S.dichotomus was eighteen weeks during the winter, and no animals were present beyond that period, even though there was water. Moore (1959a) reported a life span of fourteen weeks for the fairy shrimp E.holmani. He (1951) observed that S.seali lived for 22 weeks, but Prophet (1959)

reported a life span of 23 weeks for this species.

In the winter population of 1972/1973, nauplii were first observed in Godshill pond on 31st October 1972. Only one specimen could be seen when the pond was visited on 20th March 1973. The pond was found to be dry on 27th March 1973. This population in Eyeworth pond lived for exactly the same period found in Godshill pond.

The summer populations in both Godshill and Eyeworth ponds were prematurely eliminated by drying up of the pond. Some of the populations were eliminated before the animals could reach sexual maturity and so produce eggs. Hall (1953) reported the disappearance of C.diaphanus from the ponds, although the ponds still contained water. On more than one occasion the disappearance of C.diaphanus was correlated with the appearance of predators in quite large numbers. This is discussed elsewhere in this thesis. Hall (1961) reported that C.diaphanus was present continuously for over four and a half months in Burley pond in the New Forest during the spring and early summer of 1954. Laboratory cultured specimens of C.diaphanus in the aquaria lived for as long as three months. But the author was able to keep C.diaphanus in the artificial ponds for a period of nearly eight months. The specimens were 34-36 mm long. It was observed that the females have greater longevity than the males when they were observed in the artificial pond. In a laboratory culture S.seali lived for up to five months, as reported by Moore (1955). Moreover Ardo (1947) reported that Tanymastix stagnalis lived for a period of one year.

2.8 Sex ratio

During the preliminary field observations on the late stages of the existence of a population of C.diaphanus, the author noticed that on 30th April 1971 no males could be observed in Godshill pond, whereas a number of aged females (32 mm) were collected. It was thought at first that this might be due to a sampling error. The pond was carefully searched but no males were found. Lake (1967) noticed that the males of C.diaphanus in laboratory cultures disappeared before the females. An interpretation of this observation may be that the males of C.diaphanus are less robust than the females, since Taylor (1965) found that the males were less tolerant of adverse oxygen conditions than the females. The premature disappearance of males of other anostracan species was also noticed by Coopey (1950); Creaser (1931); Dexter and Ferguson (1943). Weaver (1943). reported that there is a decrease in the number of the males of E.vernalis late in the season. On the other hand, Bernice (1972a) found that in the fairy shrimp S.dichotomus no such differences were seen, as both males and females occurred almost all through the season up to the end of the population in all the ponds in consistent proportions.

The present study has shown that populations of C.diaphanus start with a sex ratio of approximately 1:1 in both winter and summer populations. Subsequently the proportion of the males decreases. During the later stages of the population's life span the proportion of males was considerably reduced, and they finally disappeared before the females. This appears to be the usual phenomenon in C.diaphanus population. Four populations were carefully observed in the field from the hatching of the nauplii until the complete disappearance of the adults from the ponds. On 14th March 1972 the sex ratio in Godshill pond was 1·16:1·00 (Females:Males); later, on 27th March 1972, the sex ratio was 2·64:1·00. A sharp decline in the

proportion of males was then noticed on 18th April 1972 when the sex ratio was 8·8:1·0. Ten days later, on 27th April 1972, no males were seen to be in the pond, even after a very careful search. No C.diaphanus of either sex could be seen in the pond from 4th May 1972 onwards. Similar phenomena were observed for the other three populations studied in Godshill and Eyeworth ponds. It was observed that most of the ovisacs and lateral pouches of the females were empty when the males were no longer present in a large number. This phenomenon was also observed in the laboratory cultures. The presence of the males may stimulate the process of egg production by the females, so when no more males are around the females stop the production of eggs.

Weaver (1931) reported a 5:1 preponderance of females in E.vernalis early in the season, a subsequent increase in the proportion of males and finally a decrease in the relative numbers of males late in the season. The increase in the number of males in the middle stage of the population in Weaver's case might have been due to a sampling error. Dexter and Kuehnle (1948) found that the sex ratio of E.vernalis differs in different localities. In his study with S.seali, Moore (1955) found that the ratio of males to females in natural populations was approximately 1:1, and shows no change during the life cycle. The author noticed in his laboratory cultures that the numbers of males of C.diaphanus were always less than those of the females, especially in the late stage of the life span. The ability of the females to outlast the males is of considerable value to the species in ensuring maximal egg production, since the eggs are the means by which this species survives severe conditions. Moreover, Hall (1953) reported that he collected 110 specimens of C.diaphanus from Burley pond in the New Forest, eighty-six were females and twenty-four were males. The sex ratio in Hall's sample then is 3·58:1 (females:males). Table [17]

TABLE [1] Shows the sex ratio of C.diaphanus in Godshill and Eyeworth ponds

Winter populations			Summer populations		
Date	Number animals sexed	females males ratio	Date	Number animals sexed	females males ratio
Godshill p.			Godshill p.		
3.1.1972	63	1·25:1	28.6.1971	208	1·30:1
10.1.1972	250	1:1	5.7.1971	94	2·35:1
2.2.1972	88	1·40:1			
8.2.1972	170	1·25:1			
14.2.1972	53	1·33:1			
29.2.1972	133	1·40:1			
			Eyeworth p.		
9.3.1972	434	1·33:1	2.5.1972	28	1:1
14.3.1972	115	1·16:1	9.5.1972	21	1·33:1
20.3.1972	65	1·40:1	12.5.1972	20	1:1
27.3.1972	51	2·64:1	21.5.1972	26	0.86:1
5.4.1972	71	2·55:1	30.5.1972	17	2·40:1
11.4.1972	69	3·60:1	6.6.1972	7	2·50:1
18.4.1972	49	8·80:1			
27.4.1972	10	1:0			
Eyeworth p.					
29.2.1972	10	1:1			
9.3.1972	11	1·20:1			
14.3.1972	8	1·67:1			
20.3.1972	3	2:1			

shows the sex ratio of C.diaphanus in Godshill and Eyeworth ponds for four populations. The figures tabulated show the decrease in the numbers of males and then their complete disappearance before the females.

SECTION THREE

ECOLOGY OF TEMPORARY PONDS IN THE NEW FOREST

SECTION 3

Ecology of temporary ponds in the New Forest

3.1 Locality and description of the study area

The temporary freshwater ponds which have been selected for this study were both kindly recommended by Mr. R.E. Hall, to whom I am grateful. The fairy shrimp C.diaphanus was known to inhabit the two ponds. The two ponds studied were Godshill pond which is approximately two miles east of Fordingbridge, plate [4] and plate [5], and Eyeworth pond which is near Fritham, plate [6] and plate [7]; both are in the New Forest (Hampshire).

The Godshill pond is a grassy depression at the side of the road 25 x 20 metre in dimension. This pond is unshaded and cattle occasionally wander through the pond and use its water for drinking. The source of the water comes from the rains. The water is slightly acidic in reaction and pale greenish in colour. The maximum depth of the water in the winter, when the pond is full, is 60 cm. The conductivity varies between 21 and 332 micromhos/cm. The percentage moisture content of the soil when the pond is dry varies between 18 and 65 per cent of wet weight. Eyeworth pond is a muddy depression 10·3 x 6·0 metre in dimension and partly shaded with trees and shrubs. The bottom of this pond is almost devoid of vegetation, although its margins are covered with grass. The maximum depth when it is full is 18 cm. There are no records of C.diaphanus in this pond. The conductivity varies between 21 and 176 micromhos/cm. The water is slightly acidic in reaction. Cattle were noticed to wander through it frequently, and twice a pair of ducks was noticed to feed at the pond. The percentage moisture content of the soil varies between 23 and 52 per cent of wet weight. Both ponds were subjected to the same rainfall, since they are approximately five miles away from each other. In the late stages

Plate [4]. Godshill pond when dry.

Plate [5]. Godshill pond when full.

Plate [4]

Plate [5]

Plate [6]. Eyeworth pond when dry.

Plate [7]. Eyeworth pond when full.

Plate [6]

Plate [7]

before the ponds dry, there was a collection of small puddles formed in the hoofmarks of the trampling cattle.

Hall (1953) described two temporary freshwater ponds in the New Forest, from which he collected C.diaphanus. Both were described by him as roadside ponds, one was near Brockenhurst and was approximately 15 x 20 yards with a maximum depth of about 15 inches. The water was very muddy and it was used by ducks from a nearby cottage. The PH varied between 6·2 and 6·7. The bottom of the pond was almost devoid of vegetation. No C.diaphanus could be seen there. The second pond was near Burley. It is a grassy depression approximately circular in outline with a diameter of about 20 yards. The maximum depth when the pond was full was between 1·5 and 2 feet. These two ponds were visited several times by the author. It was found that the water in Burley pond was clear, pale greenish in colour, the pH varied between 6·4 and 8·3. It was noticed that it contained water continuously for a period of more than eight months. It was observed to be dry completely on 23rd August 1971. Predators were observed to exist in it, but no C.diaphanus could be detected. Dytiscid larvae and notonectids were abundant in large numbers. A few newts were also caught. Hall (personal communication) noticed the disappearance of C.diaphanus from these ponds a few years before the beginning of this study.

3.2 The associated fauna

The associated fauna occurring in the habitat of C.diaphanus was observed and samples from Godshill and Eyeworth ponds were collected for identification. The animals were classified using the keys given by Mellanby (1951), Ward and Whipple (1959), and the keys published by the Freshwater Biological Association. It seems that both ponds studied support very large populations of cladocerans, copepods and ostracods. It was observed that these animals occur within the first two days of the refilling of the pond. Like C.diaphanus these animals survive the dry periods of the pond by means of drought-resistant eggs. The notostracan tadpole shrimp Triops cancriformis Bosc. was also seen in Godshill pond on 28th June 1971. It remained until the pond dried out after 5th July 1971. Since that time no more Triops were seen in Godshill pond. The cladocerans were Daphnia pulex, Chydorus sp.; the copepods were Cyclops viridis, Canthocamptus sp., Diaptomus castor; and the ostracods were Cypridae spp. . After the establishment of the pond another group of animals occurred. These were Corixidae (Corixa sp.), Notonectidae (Notonecta sp.), Gerridae (Gerris sp.), Dytiscidae (Dytiscus sp.) larvae, Gyrinidae (Gyrinus sp.) tadpoles, trichopteran larvae, dragonfly nymphs, water mites, Gastropoda (Limnaea sp.), adult frogs, Analida (Tubifex) and Platyhelminthes (Tricladida). Chironomid larvae were observed in high populations in Eyeworth pond. It was noticed that whenever the numbers of the predators increased, the population of C.diaphanus started to decrease. Then a few days later the entire population disappeared. As has been mentioned before, the termination of the population of C.diaphanus before the drying up of the pond might be due to predation, temperature, low oxygen concentrations. However, senescence must not be excluded. Taylor (1965) divided the appearance of the animals in the Ringwood temporary

pond into three waves: (a) the first animals to appear hatched soon after the refilling of the pond by rainwater, that is to say at the same time as the hatching of C.diaphanus; (b) three weeks after the establishment of the pond, another wave of animals comes; these animals, such as the Gastropod Limnaea truncatula, are mainly from outside. By this time the C.diaphanus have reached sexual maturity and are able to breed and produce eggs; and (c) the third wave includes the predatory forms which consist of the large predators, both arthropod and vertebrate. The results of the field observations which were carried out during the present study showed the same general pattern of the occurrence of the animals in both Godshill and Eyeworth ponds. In his study, Moore (1951) reported that the associated fauna with the fairy shrimp S.seali were crawfish, midge and mosquito larvae, a few aquatic beetles and their larvae, and frog tadpoles, cladoceran and copepods. This generally seems to be similar to the fauna which was observed in both ponds studied. Prophet (1959) also reported a similar fauna in the habitat of S.seali.

3.3 The limnology of the temporary ponds studied

3.3.1 Methods

Samples of water from both Godshill and Eyeworth ponds were taken at weekly intervals during the morning of each sampling day for nearly two years. The samples were taken using a plankton net having a mesh size of 76 micron which caught even the nauplii if they were present. The water samples were brought to the laboratory and the numbers of animals caught were counted, and specimens were measured under the microscope. The temperatures of the air, water and the mud were measured using six probes of a "Grant thermistor thermometer [Grant Instruments]". The average of the readings was then calculated. Air temperature was measured at 25 cm above the ground. Water temperature was measured at approximately 5 cm below the surface of the water, and mud temperature was measured at a depth of 3-5 cm below the mud surface. The dissolved oxygen was measured by the "Dissolved oxygen meter model 15A [Electronic Instruments Ltd.]", and oxygen concentrations are given in mg/l. Oxygen values in the artificial ponds for the mud water interface, and a few cm below the surface of the mud, were measured using a "Laboratory oxygen analyzer model 777 [Beckman Instruments, Inc.]". Readings are given in mg/l. The depth of the water was measured with a metre stick with a flat base of wood. The pH of the water was measured using a direct pH meter model 23A [Electronic Instruments, Ltd.]. The conductivity of the water was measured by the "Conductivity bridge type E7566/3 [Mullard Equipment]". The conductivity of the water is given in micromhos/cm. at 25°C. Percentage moisture content of the soil during the dry phase of the ponds was determined by drying the soil samples overnight at 105°C in an oven, and is given as per cent wet weight.

3.3.2 Temperature

Because of the shallowness of both ponds studied, their water temperatures follow more or less those of the air. The fluctuations of the water temperature were less than that of the air, because of the specific heat of the water. No true thermal stratification was observed, although a difference of 1·0°C was observed between the surface temperature and the temperature near the bottom of Godshill pond sometimes, especially when the pond was visited late in the afternoon. However, the temperature was the same during most of the time. Yaron (1964) observed a difference of 2·0 - 2·7°C at sunset in a shallow pool between the temperatures at a depth of 20 cm and at a depth of 2 cm. The highest air temperature recorded was 26·5°C on 5th July 1971. Taylor (1965) reported a value of 28°C for the air temperature in the New Forest. Full records of daily minimum and maximum air temperatures based on monthly means are shown in Fig. [10]. Water temperature fluctuated markedly during the period of the study. The minimum water temperature in both Godshill and Eyeworth ponds was 0·5°C. when they were covered with a layer of ice. At that time, and during the subsequent visits, C. diaphanus were collected from beneath a layer of ice. Moore (1955) reported that the air temperature was -3°C when he collected S.seali from beneath a layer of ice. Hall (1961) observed that a population of C.diaphanus was present when a layer of ice was covering the pond. As is to be noted later, hatching of nauplii was observed to take place at very low temperatures. Taylor (1965) reported that a water temperature of 0·5°C was measured on 19 February 1963 in the Ringwood pond. Low values for the water temperature in the habitats of the anostracan branchiopods were also reported by Broch (1965), Coopey (1950) and by Prophet (1959). These low values of the temperature show that these animals can survive the changes in temperature throughout the

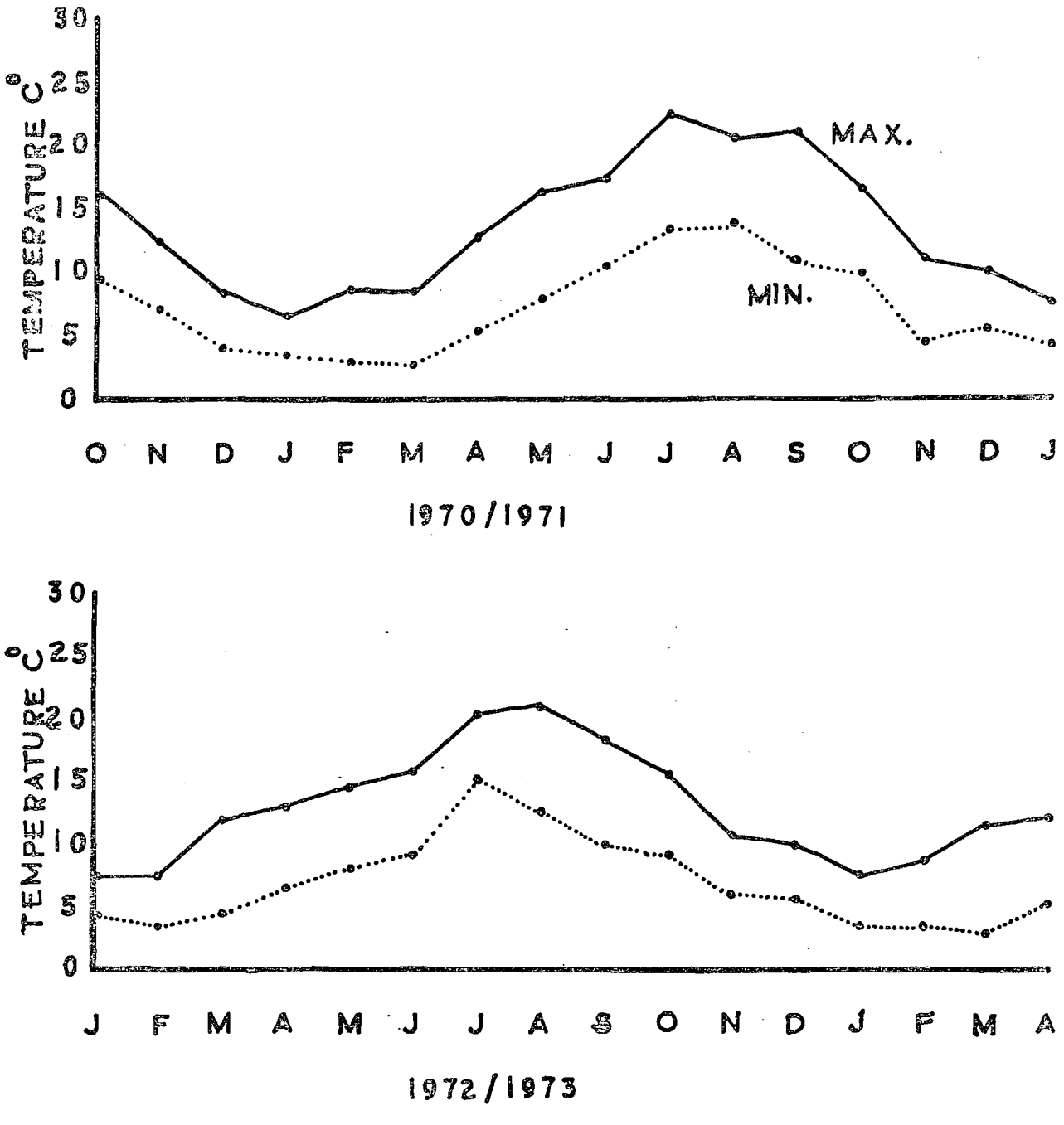


FIG. 10. DAILY MAXIMUM AND MINIMUM AIR TEMPERATURE BASED ON MONTHLY MEANS.

year.

The highest water temperature recorded during the present study was 28°C. At that time a good population of C.diaphanus was seen in the pond. This temperature seemed to have no harmful effect on C.diaphanus if maintained for a few hours, since it was possible to determine the lethal temperature for this species. It was around 40°C. Moreover, specimens of C.diaphanus were found to live for three days at 30°C and more than one day at 33°C. In the artificial ponds which were set outdoors, the highest water temperature recorded was 33°C. Taylor (1965) reported a water temperature of 30°C in the habitat of C.diaphanus in the New Forest near Ringwood. The oxygen concentration at that time was 1·8 ml/l. Moore (1955) collected S.seali from ditches when the water temperature was 35°C. He added that on no less than eleven separate occasions during the midsummer the maximum temperature recorded was 42°C. Extreme variability was found in the habitat of C.diaphanus through the year. The water temperature was found to vary between 0·5°C and 28°C.

The mud temperature was also measured in both ponds studied. The lowest mud temperature recorded was 0·5°C when the pond was covered with a layer of ice. The highest mud temperature was 26°C. Fig. [11] and Fig. [12] show the annual cycles of the air, water and mud temperatures throughout the study period in both ponds.

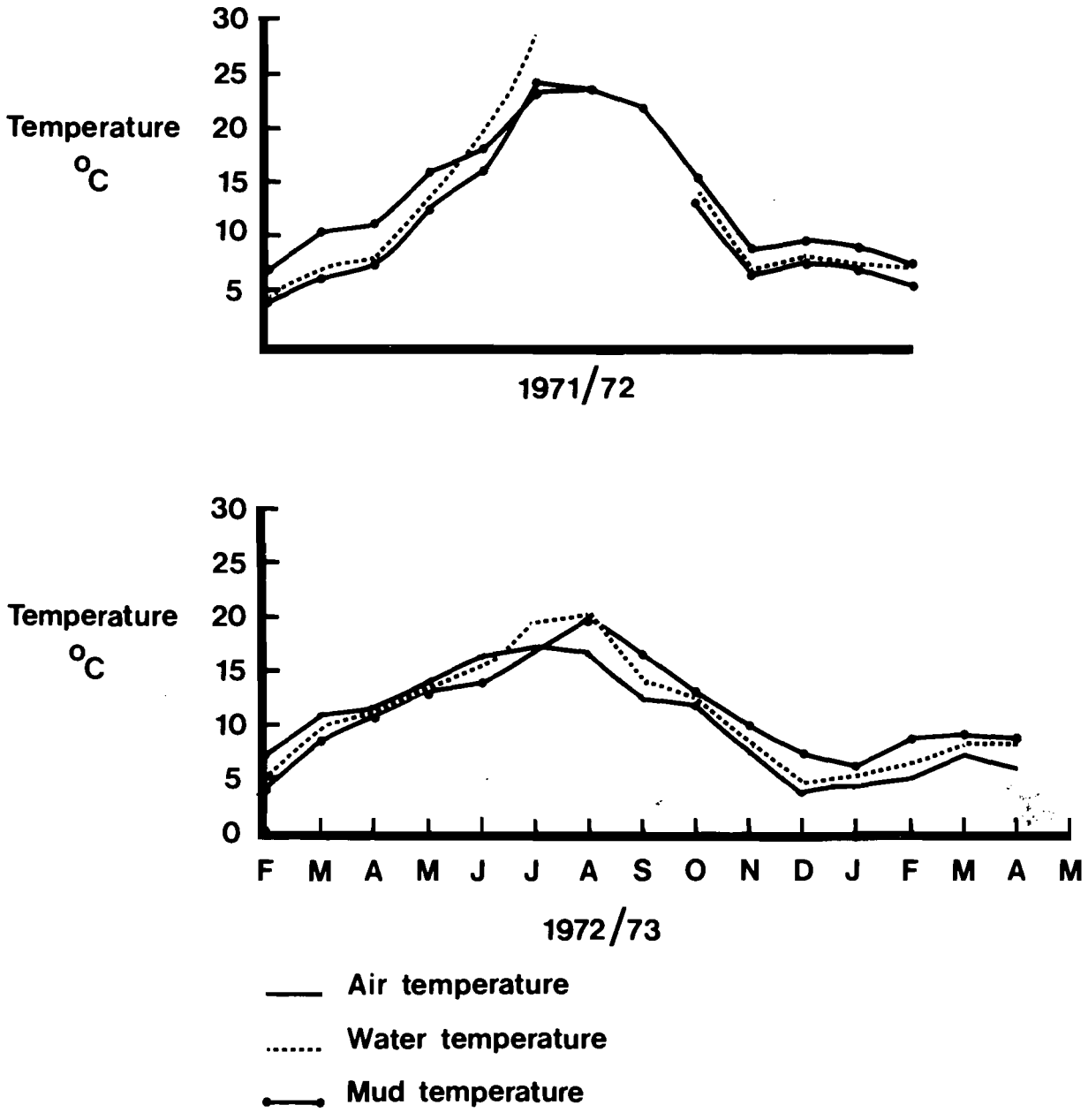


FIG.11 : ANNUAL CYCLE OF TEMPERATURE IN GODSHILL POND BASED ON MONTHLY MEANS

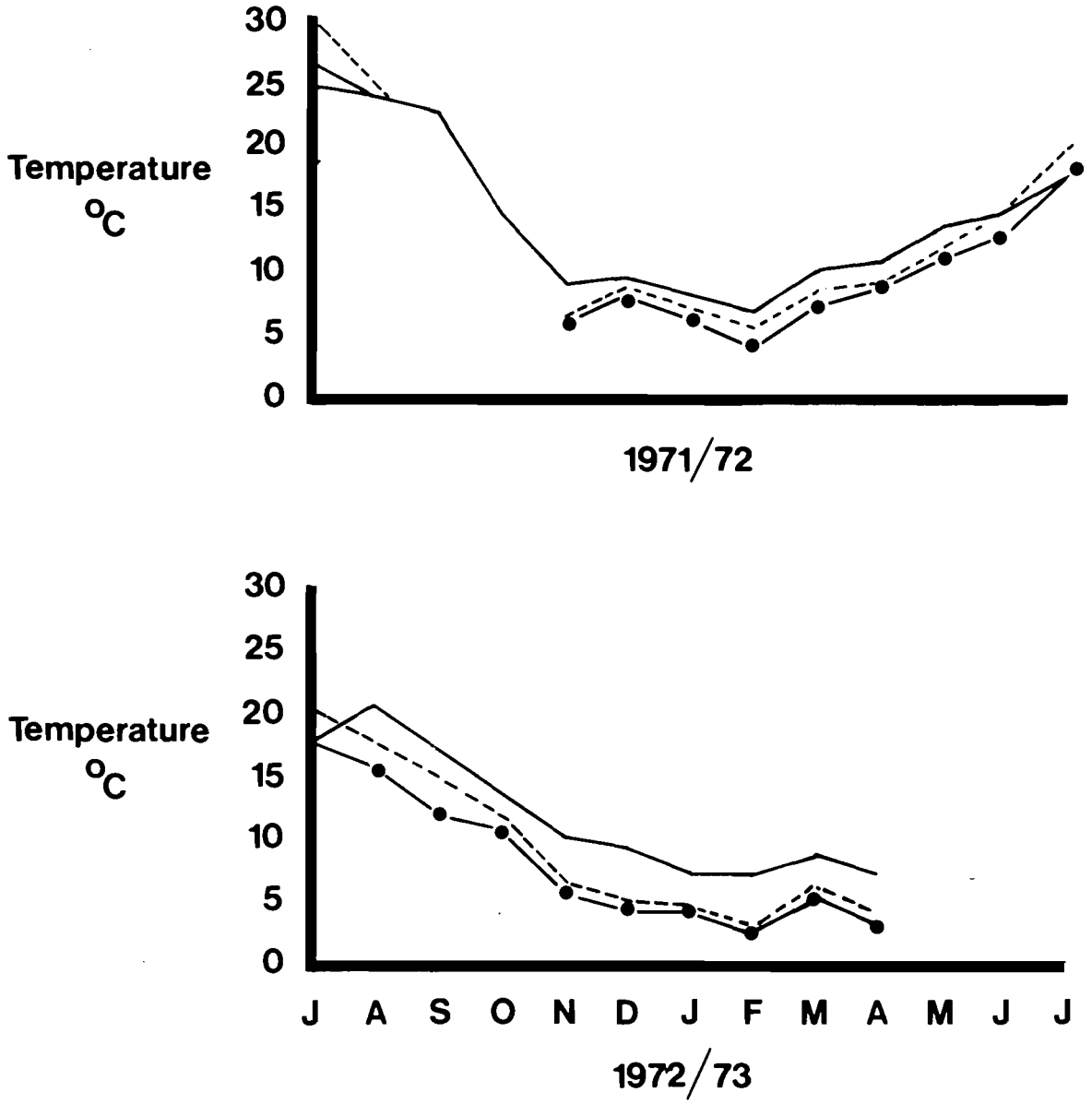


FIG.12 : ANNUAL CYCLE OF TEMPERATURE IN EYEWORTH POND BASED ON MONTHLY MEANS

3.3.3 Oxygen

Marked fluctuations in the dissolved oxygen concentration have been observed throughout the period of the present study in both ponds. The oxygen concentration ranged between 1·5 and 16·4 mg/l and between 1·5 and 13·1 mg/l in Godshill and Eyeworth ponds respectively during the period of the present study. Generally, the oxygen concentration in Godshill pond was found to be higher than that in Eyeworth pond. Eyeworth pond is almost devoid of vegetation. The water was noticed to be muddy and turbid most of the time, whereas that in Godshill pond was greenish in colour due to the high algal growth. It was noticed that the bottom of Eyeworth pond contained a very high population of chironomid larvae. Also Eyeworth pond is partly shaded with trees and shrubs which protect it from the direct action of the wind. In contrast, Godshill pond was exposed in all directions to the action of the wind. In both ponds it was found that towards the end of the life of the pond in the summer season, the oxygen concentration decreased to low values (1·5 mg/l). Yaron (1964) noticed that oxygen concentration fell to very low values (0·5 - 1·8 mg/l) in a temporary pool in Israel. These low values were recorded along the incipient drying of the pool. On 5th July 1971 the oxygen concentration in Godshill pond was 2·59 mg/l. At that time the water temperature was 26·5°C. The pond on that date was still supporting a good population of both C.diaphanus and T.cancriformis. At this time in Eyeworth pond the oxygen concentration was 1·5 mg/l. Only a few specimens of C.diaphanus, in obvious distress, were collected. The low values of oxygen concentration in summer are not surprising, especially when the ponds were very shallow and dense populations of animals were found. The greatest oxygen depletion was localized at the mud water

interface, presumably due to the direct respiratory action of the mud dwellers. Jones (1955) recorded values of 1·48 and 0·76 mg/l in Arenicola burrows, directly after they were exposed by the tide. Working in freshwater habitats, Cole (1932) found high oxygen contents in the surface layers of the water, whereas the oxygen concentration was 2·36 ml/l just above the mud surface, and between 0·13 and 0·6 ml/l four inches below the mud surface.

No regular field measurements have previously been made in the habitat of C.diaphanus in the New Forest. However, Taylor (1965) recorded on a few irregular visits the oxygen concentration for a temporary pond near Ringwood. He found a range of 1·5 - 6·5 mg/l. The oxygen concentration was also noticed to fall considerably during the period when the pond was covered with ice. Oxygen concentrations as low as 2·15 mg/l and 1·56 mg/l were measured at Godshill and Eyeworth ponds respectively when a layer of ice was covering both ponds. Fig [13] and Fig [14] show the annual cycles of the oxygen concentration in both ponds studied.

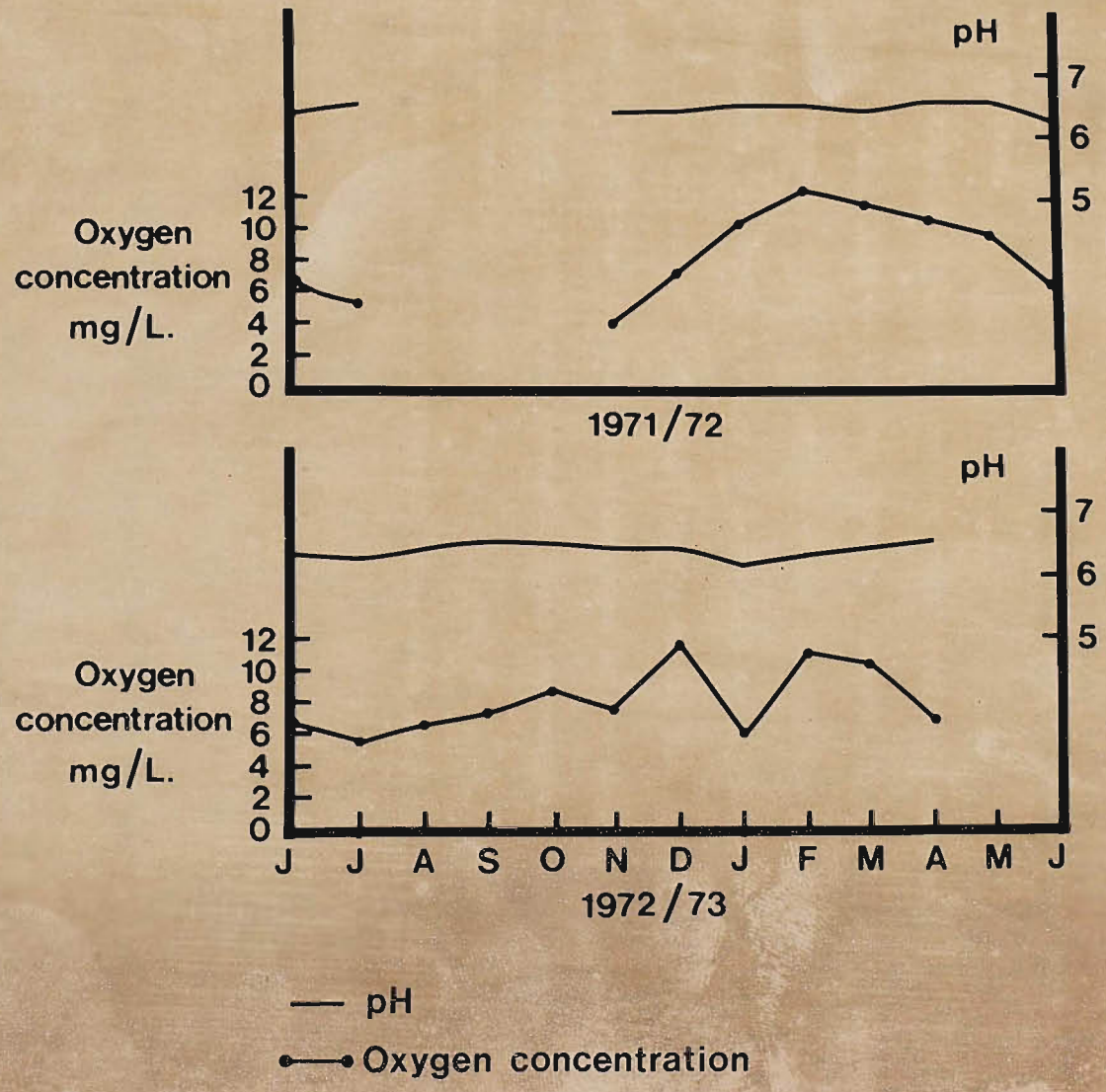


FIG.13 : ANNUAL CYCLES OF OXYGEN CONCENTRATION AND PH IN GODSHILL POND BASED ON MONTHLY MEANS

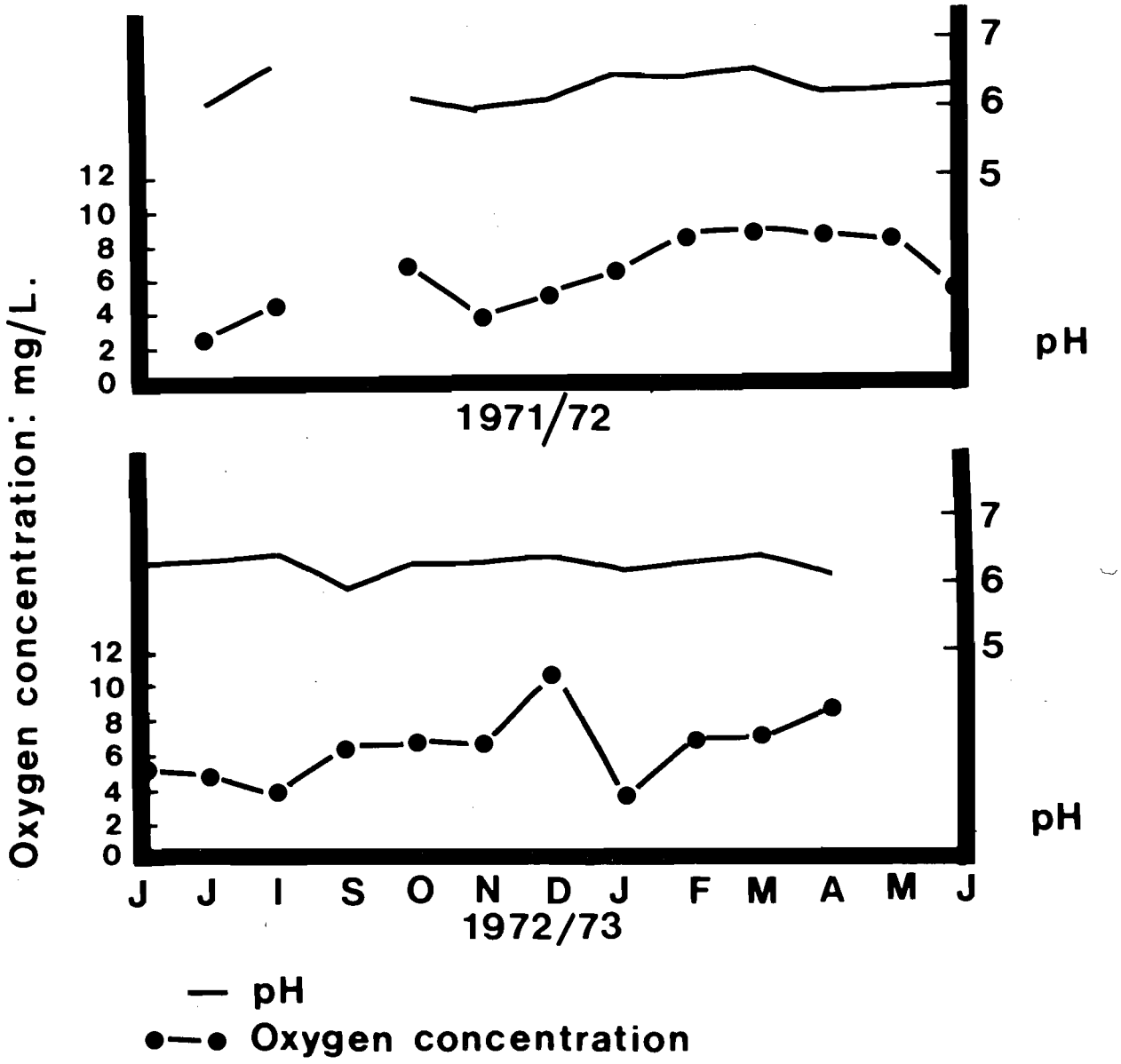


FIG.14 : ANNUAL CYCLES OF OXYGEN CONCENTRATION AND PH IN EYEWORTH POND BASED ON MONTHLY MEANS

3.3.4 Rainfall and Conductivity

As might be expected, conductivity was correlated ~~to~~ with the rainfall, as can be seen from Fig. [15] and Fig. [16] for both ponds studied. Data of the daily rainfall were obtained from the Southampton Weather Centre. Monthly means were then calculated and are given in mm per day. During the past two years the conductivity of the pond water was measured. The conductivity has long been considered as an indication of the total dissolved solids. This method is, of course, much easier to use than the gravimetric determination of the concentration of total dissolved solids. The conductivity varies with time in a temporary pond. It increases by the evaporation and decreases through the addition of rainwater. On a few occasions during this study, the conductivity of the rainwater was measured. It was found to be between 8 and 16 micromhos/cm. The minimum reading for the pond water conductivity in both Godshill and Eyeworth ponds was 21 micromhos/cm. Since the total concentration in ppm is approximately 56% of the conductivity at 25°C, the total concentrations of the above water can be estimated (Horne, 1967). Thus the minimal value of the pond concentration is approximately 11 ppm. Horne (1967) reported that some alpine phyllopods tolerate osmotic concentrations that are similar to that of distilled water, whereas other species are inhabitants of periodic prairie and desert ponds where evaporation rates are high and rapid fluctuations in salinity are frequent. He added that he never collected freshwater species from ponds with concentrations greater than 24 ppm. The highest measurement for the conductivity in Godshill pond was 332 micromhos/cm., whereas in Eyeworth pond it was 176 micromhos/cm. These values seem to fall within the range found for most freshwater habitats. Iversen (1971), working on the ecology of a mosquito population in a temporary freshwater pool, reported a range of conductivity between 119 and 279 micromhos/cm. Working with a related species Artemiopsis

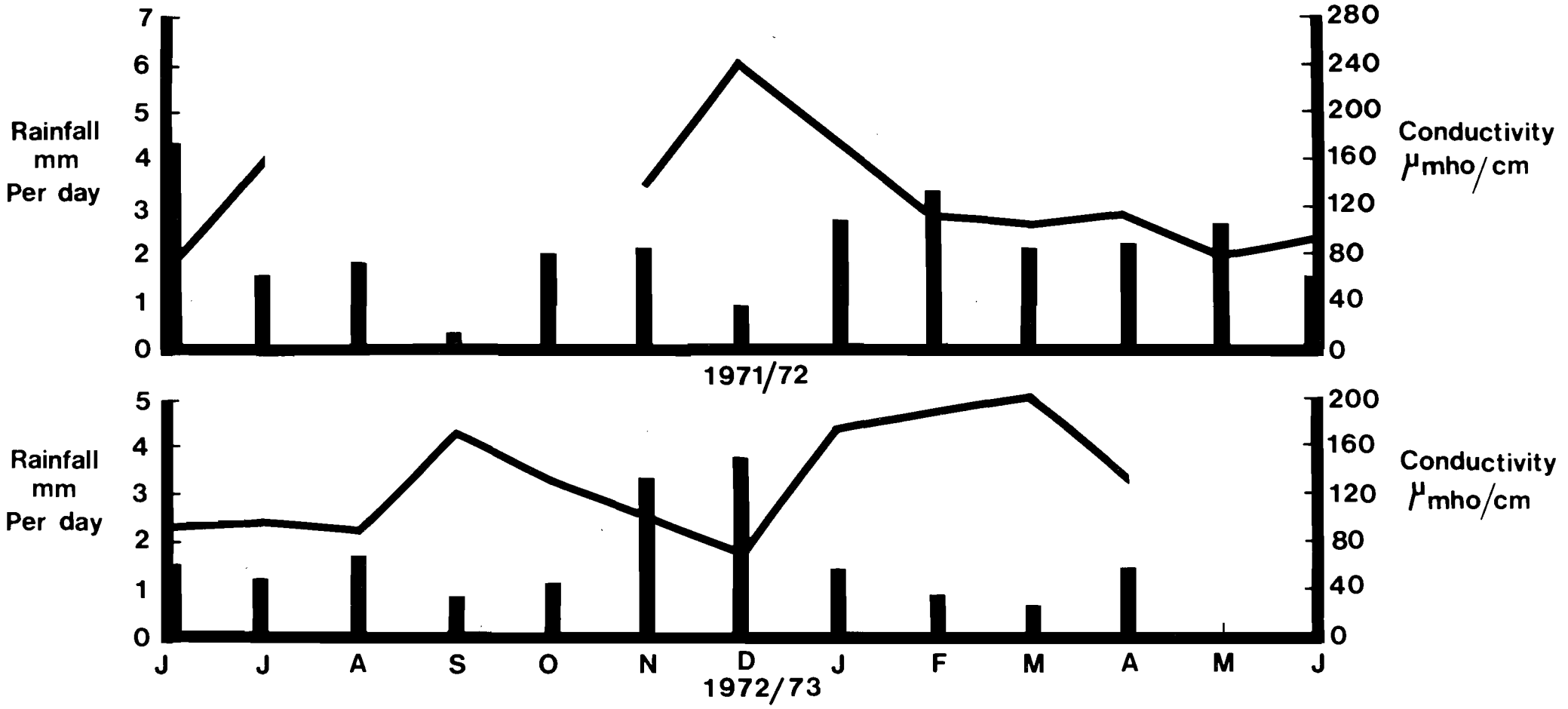


FIG. 15 : ANNUAL CYCLES OF CONDUCTIVITY AND RAINFALL IN GODSHILL POND BASED ON MONTHLY MEANS

Graphs show conductivity
Histograms show rainfall

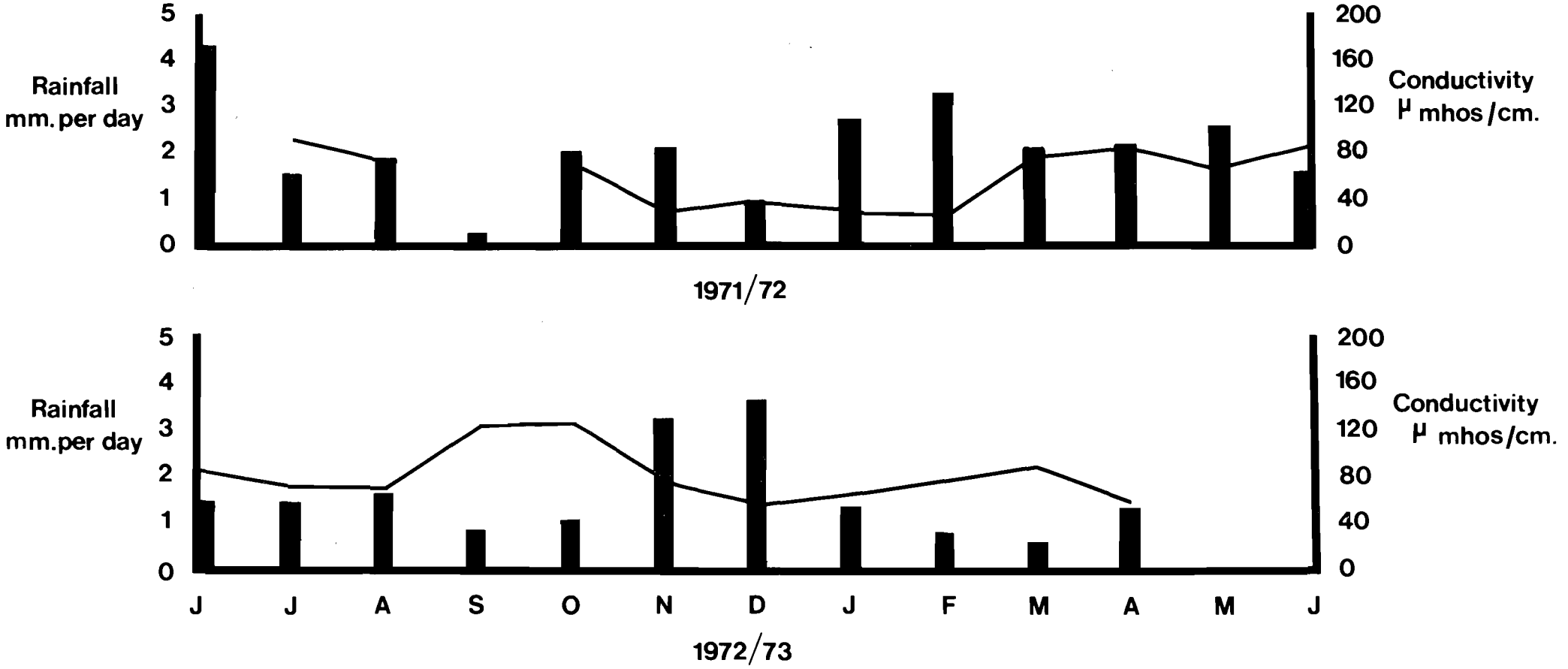


FIG. 16 : ANNUAL CYCLES OF CONDUCTIVITY AND RAINFALL IN EYEWORTH POND BASED ON MONTHLY MEANS

stefanssoni, Johansen, Hartland-Rowe (1968), reported a conductivity of 159 micromhos/cm. In his study on the distribution and abundance of 27 planktonic crustaceans, Carter (1972) found a range of conductivity between 64 and 194 micromhos /cm. He found that the small ponds showed the lowest values, usually less than 30 micromhos/cm. Ponds of greater area or depth have intermediate values, usually 30 - 60 micromhos/cm, the remainder had values of up to 178 micromhos/cm. The same relationship was found in Godshill pond and Eyeworth pond, i.e. Eyeworth pond being smaller and shallower had a lower range of conductivity than that of Godshill pond. Beeton (1965) and Ryder (1964) also reported low conductivities for the freshwater habitats. Finally one must add a few words about the water concentration of the habitats of the brackish water and saline water forms in order to give an idea about the difference between their habitats. White (1967) reported that in Western Canada A.salina lives in waters having salt concentrations of 100,000 to 300,000 ppm. He also added that Branchinecta mackini Dexter was found in ponds of salinities ranging from 85 to 1700 ppm, whereas Branchinecta gigas Lynch, from 1700 to 5000 ppm.

The author also measured occasionally the conductivity of the water in Burley and in Brockenhurst ponds in which C.diaphanus no longer exists. The range of the conductivity in Burley pond was 33 - 362 micromhos/cm and that of Brockenhurst pond was 28 - 278 micromhos/cm.

3.3.5 Depth and Volume

Both depth and volume were measured for the past two years for Godshill and Eyeworth ponds. Godshill pond was deeper than Eyeworth pond, and its dimensions were larger. So one would expect that the volume of Godshill pond will be larger than that of Eyeworth pond. The volume of the ponds was estimated using a method designed by MacDonald (personal communication). The volume in this method can be calculated from the measurement of the deepest point in the pond. The volume of Eyeworth pond was calculated weekly. Unfortunately only the volume of a transect in Godshill pond was calculated because of technical difficulties in surveying all the pond. The transect surveyed was 25 x 2 metre. The maximum depth in Godshill pond recorded during the winter, when the pond was full, was 60 cm., whereas the maximum depth for Eyeworth pond was 18 cm. The volume of the transect in Godshill pond at that time was 18,000 litre. The whole pond was approximately ten times the transect, so the total volume of Godshill pond when full is 180,000 litre. The total volume of Eyeworth pond when full was 6,880 litre. Fig. [17] and Fig. [18] show the annual fluctuations of depth and volume in both ponds studied throughout the period of the present study. Hall (1953) reported a maximum depth for Burley and Brockenhurst ponds of 60 and 38 cm. respectively.

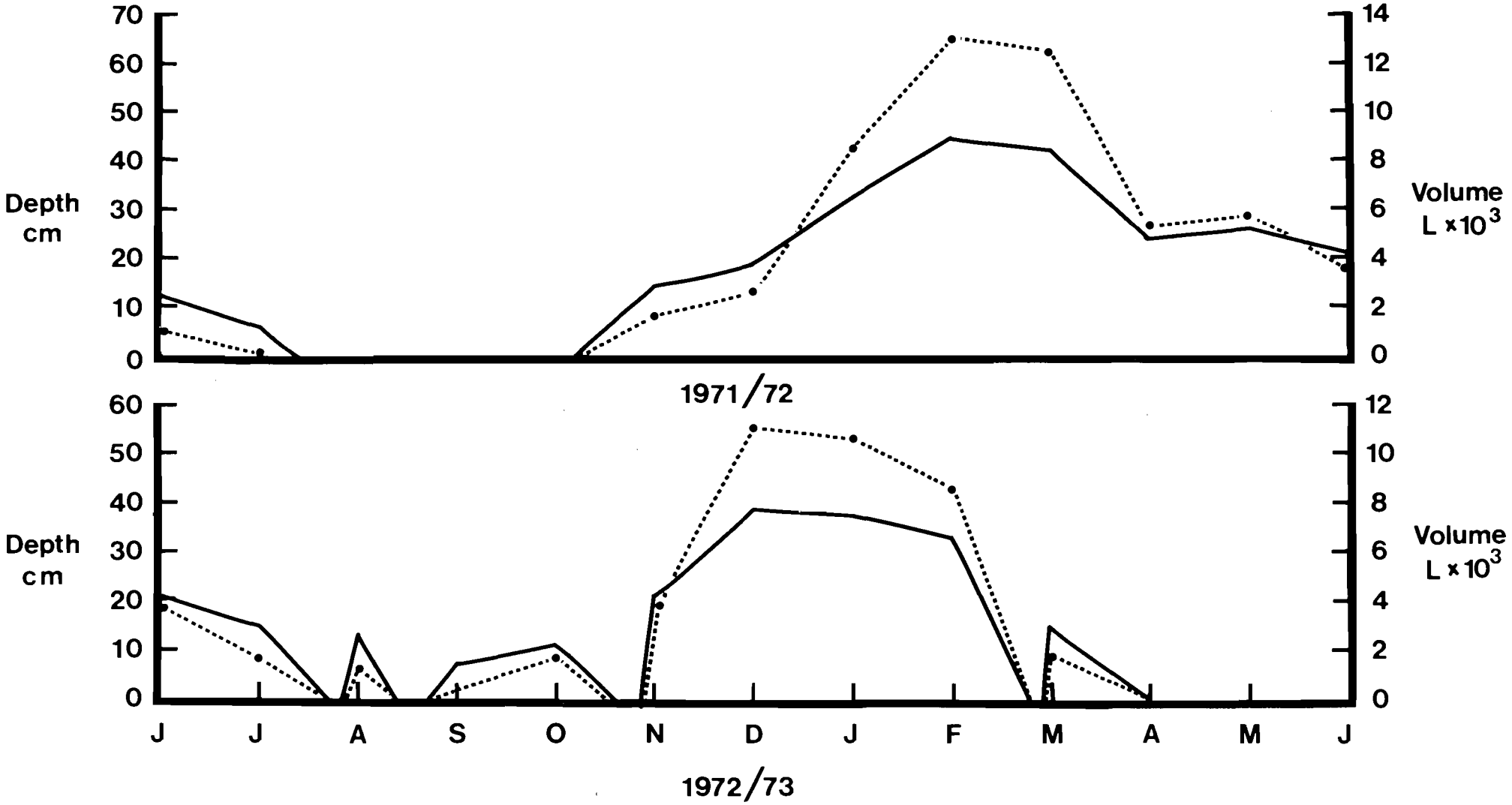


FIG.17 : ANNUAL CYCLES OF THE TRANSECT VOLUME AND DEPTH OF GODSHILL POND BASED ON MONTHLY MEANS

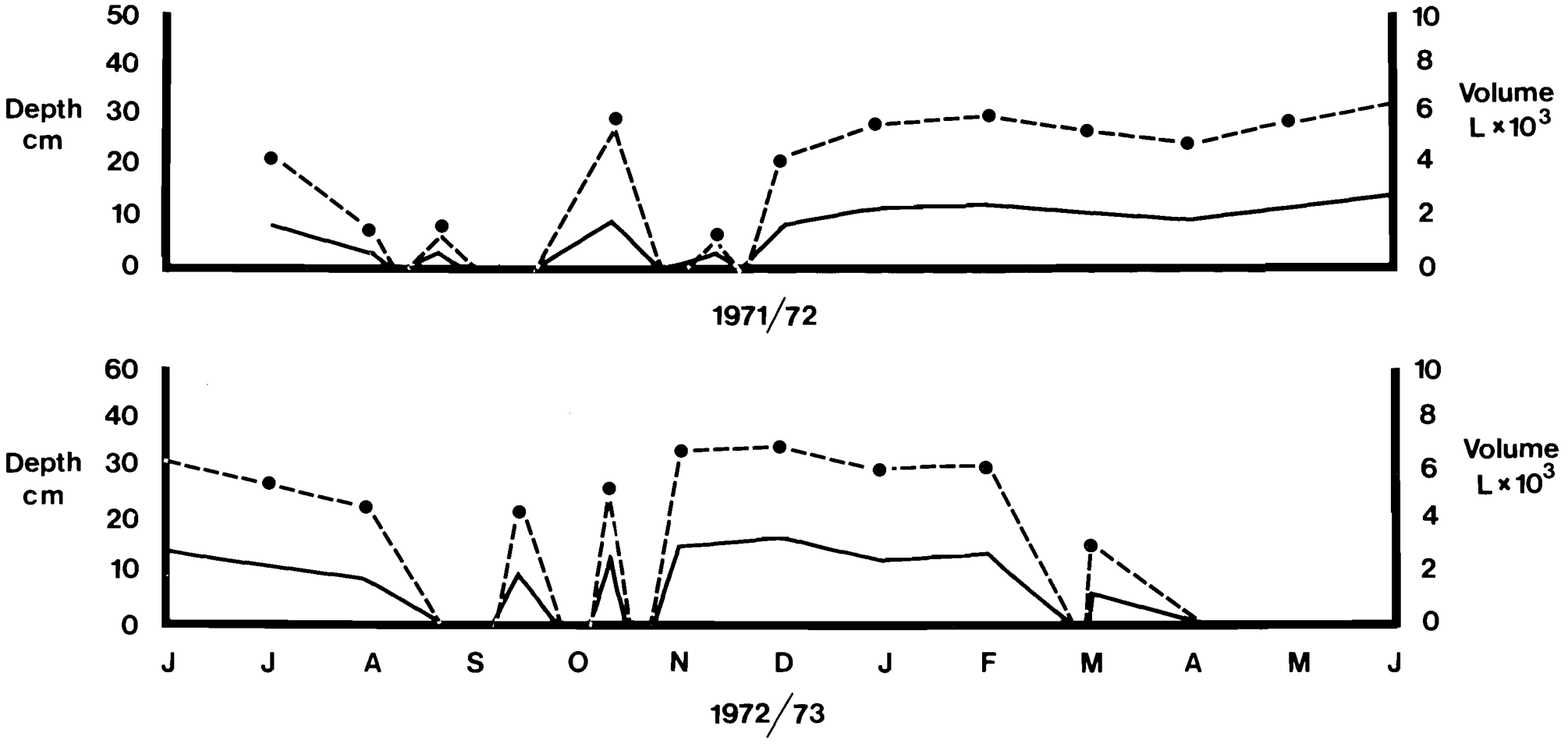


FIG.18 : ANNUAL CYCLES OF VOLUME AND DEPTH OF EYEWORTH POND BASED ON MONTHLY MEANS

•---• Volume

—— Depth

3.3.6 The Hydrogen ion concentration

The water in both Godshill and Eyeworth ponds was slightly acidic throughout the period of the study, although the pH values were variable on each visit. The pH was found to change following the change in the oxygen concentration. This is to be expected, since high oxygen concentrations are mainly the result of photosynthetic activity which will lead to a decrease in the carbon dioxide concentrations. The high concentrations of the carbon dioxide are responsible for the low values of pH. Hutchinson (1957) reported that the highest pH values were certainly due to the photosynthetic removal of carbon dioxide. Also it was noticed that immediately after rainfall there was a decrease in the pH values in both ponds studied. This is also to be expected since rainwater has quite low pH values. The range of the pH values found for Godshill pond was 5·9 - 6·8, whereas the range in Eyeworth pond was 5·7 - 6·7, Fig. [13] and Fig. [14]. These ranges are very close to each other. From the occasional observations which were carried out on both Burley and Brockenhurst ponds, which were known in the past to support populations of C.diaphanus, the water in the Brockenhurst pond was found to be slightly acidic in nature with a range of pH of 6·2 - 6·7. In Burley pond the pH was found to be between 6·4 and 8·3. Taylor (1965) reported pH values for a temporary pond inhabited by C.diaphanus near Ringwood in the New Forest of 6·6 and 6·7. Anostracan branchiopods are known to inhabit acidic waters as well as alkaline ones. Moore (1955) reported that the lowest pH recorded in the habitat of S.seali was 4·9, whereas the highest value was 6·2. In his work with E.vernalis, Dexter (1946) reported that this species was found in a pH range from 5·3 to 7·6, with instances for almost every graduation between these limits. Mathias (1937) reported

that phyllopods prefer alkaline water and do not withstand acid water. This seems untrue in the case of C.diaphanus and the species mentioned above, since their habitats were found to have acidic water rather than alkaline water. The writer was able to culture C.diaphanus in water having a pH of up to 8•5 without affecting their activities. So it seems that C.diaphanus and other related species are able to tolerate a wide range of hydrogen ion concentration. White (1967) reported that the habitat of B.mackini and B.gigas had a pH range of 8•5 - 9•2.

3.3.7 Soil moisture and temperature

Naturally one would expect that the percentage moisture content of the soil varies with the rainfall. This was found to be the case in both ponds studied. The percentage moisture content of the soil in Godshill pond was found to vary between 18 and 65 per cent of wet weight, whereas that in Eyeworth pond was between 23 and 52 per cent. This slight difference is presumably due to the presence of gravel in the soil of Eyeworth pond, since it is at the side of a gravel road. Both air and soil temperatures were measured at each visit. All data obtained is presented in Table [2] and Table [3]. In a country like Britain which has a temperate climate, it would be expected that the soil would be moist most of the time during the year; however, moisture content may fall for a very short period to a low value. This is very important to the survival of the eggs within the moist soil. Unfortunately no direct relationship between the percentage moisture content of the soil and relative humidity is known to the author. It is not therefore possible to relate directly these percentages with the experimental findings found of the embryonic development in relation to relative humidity. However, the general pattern of the relationship is obvious. It was found on several occasions that the percentage moisture content of the upper layer of the soil was less than that found beneath the soil surface. For example in Godshill pond it was found that the percentage moisture content at the upper layer was as follows:

At soil surface	34	32	51	42	37	55	50
At few cm. below the surface	45	42	57	51	46	56	51

Since the eggs when laid by the females fall to the bottom of the pond and are eventually covered by a layer of mud, the upper layers of the

TABLE [2] Shows the percentage moisture content of the soil in Godshill pond during dry periods.

Date of soil sample collection	% moisture content	Air temp. C°	Soil temp. C°
1.6.1971	65.63	24.5	24.0
7.6.	50.30	24.0	23.0
12.7.	58.78	21.5	26.0
19.7.	46.32	23.5	25.5
26.7.	35.22	23.3	23.3
3.8.	30.45	23.8	24.0
12.8.	32.88	24.8	24.3
23.8.	33.24	23.1	25.3
31.8.	28.91	19.0	20.0
6.9.	18.78	26.8	28.8
13.9.	26.93	23.0	24.0
20.9.	20.80	24.4	22.2
27.9.	29.59	15.8	17.4
4.10.	31.97	16.0	16.0
11.10.	30.24	17.9	16.4
1.11.	54.16	12.1	12.3
17.11.	67.21	6.5	5.5
25.7.1972	53.74	16.6	18.0
22.8.	52.06	22.5	21.6
29.8.	40.02	24.5	24.1
5.9.	37.14	17.0	17.1
19.9.	54.52	15.7	17.5
26.9.	46.84	17.5	15.3
3.10.	41.65	15.8	16.2
17.10.	56.01	12.0	11.0
24.10.	50.18	12.0	10.0

TABLE [3] Shows the percentage moisture content of the soil in Eyeworth pond during dry periods

Date of soil sample collection	% moisture content	Air temp. C°	Soil temp. C°
12.7.1971	43·39	23·7	26·6
19.7.	31·28	26·5	27·5
26.7.	28·49	23·7	24·6
3.8.	30·57	23·9	24·5
12.8.	31·42	25·1	26·9
23.8.	52·10	25·0	24·5
31.8.	38·94	18·2	19·3
6.9.	30.82	27.9	28.9
13.9.	28.37	23·0	23·0
20.9.	23·90	24·0	23·0
27.9.	28·76	15·4	15·4
4.10.	37·18	15·3	14·4
11.10.	35·57	16·5	14·3
1.11.	40·98	13·8	10·8
17.11.	41·82	7·5	6·0
29.8.1972	40·16	24·4	25·7
5.9.	32·39	17·5	16·2
26.9.	40·08	21·0	20·6
3.10.	39·00	15·9	11·7
24.10.	45·74	12·0	8·5

soil after the drying out of the pond will protect the eggs from complete desiccation for some time. However, it was found experimentally that the eggs of C.diaphanus can survive a few months of severe desiccation. In a country like Britain such conditions may occur only at infrequent intervals. No previous work has been carried out concerning the moisture content of the microhabitat surrounding the eggs of the anostracan species, apart from that mentioned by Moore (1967). He reported that the soil moisture content of the habitat of S.seali varied between 9·5 and 60 per cent of dry weight of the soil.

Soil temperature rose to 28·8 and 28·9°C at Godshill and Eyeworth ponds respectively, on 6th September 1971, whereas the minimum soil temperatures recorded were 5·5 and 6·0°C on 17th November 1971.

3.4 Diurnal fluctuations

The fluctuations of the air, water and mud temperature, as well as the oxygen concentration and the pH were measured in 24 hour cycles on both a rainy, windy day during the winter and on a calm sunny day during the summer. This was carried out in order to show to what extent these environmental factors can vary within the habitat of C.diaphanus. Fig. [19], [20], [21] and Fig. [22] show the diurnal changes in the temperature, oxygen and pH in Godshill pond during 6th/7th March 1972 and during 11th/12th July 1972. On the winter day, the maximum air temperature recorded was 11·5°C, whereas the minimum temperature was 3°C. The temperature of the water rose from 3·5°C to 9·0°C, and that for the mud from 3·5°C to 7·5°C.

The oxygen concentration was always high, ranging between 9.8 and 14 mg/l. These high values for the oxygen might be due to the constant stirring action of the wind and also be due to the addition of the rain-water which is well aerated. It was noticed that even during the night the oxygen did not fall to very low values. Changes in the pH were irregular at that time, and one cannot draw any conclusions from them. The pH varied between 5·9 and 6·5.

During the summer (on 11th/12th July 1972), the changes in the environmental factors were clearer. The maximum air temperature was 23°C recorded at 2 p.m. and the minimum was 9°C recorded at 12 midnight. Water temperature rose from 10°C to 27°C, whereas mud temperature rose from 12°C to 22°C. The oxygen concentration generally was high during the day and low during the night. The range of the oxygen concentration was between 3·1 and 16·2 mg/l. The minimal value was recorded at 2 a.m. and the maximal value was recorded at 2 p.m. This is to be expected, since during the night the oxygen is depleted by both animals and plants

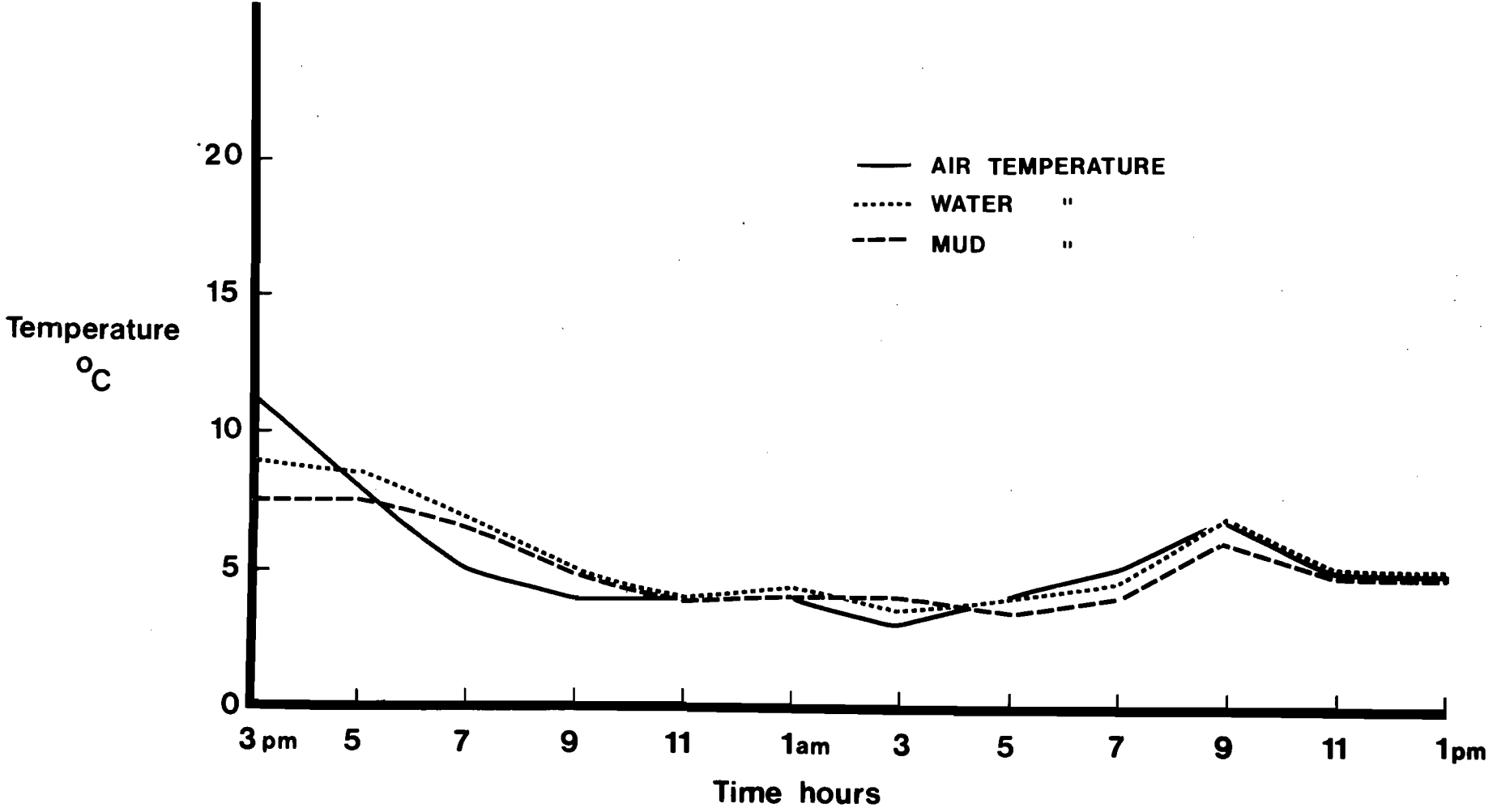


FIG.19 : DIURNAL FLUCTUATIONS OF AIR, WATER AND MUD TEMPERATURE IN GODSHILL POND 6/7 MARCH, 1972 (RAINY AND WINDY DAY)

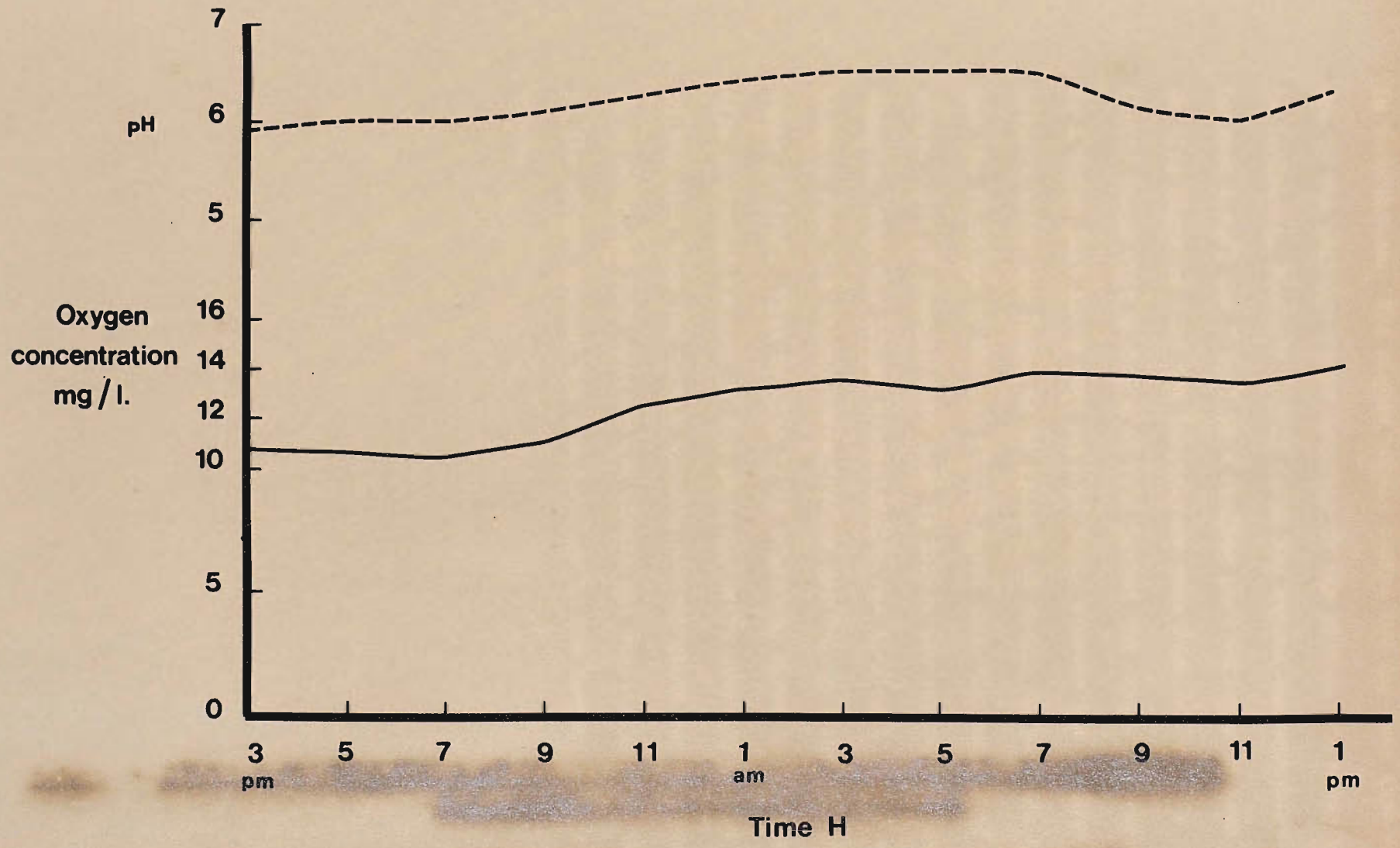


FIG.20 : DIURNAL FLUCTUATIONS OF OXYGEN AND PH IN GODSHILL POND 6/7 MARCH, 1972 (RAINY AND WINDY DAY)

in respiration. The higher values during the day were also expected due to the photosynthetic activity of the plants. On a day in May, in Louisiana, Bamforth (1962) found the oxygen concentration to vary between 1 mg/l and 9 mg/l in a shallow pond during a 24 hour period. He also reported that the temperature rose from 24 to 30°C. Barclay (1966) found that during a 24 hour period the oxygen concentration fell to a value just above zero mg/l at dawn, whereas the maximum oxygen concentration was found to be present at dusk. In general, these findings and the findings of Whitney (1942) agree with the results of the present study. The pH changes were seen to follow the oxygen changes. For example, when the oxygen concentration was 16·2 mg/l the pH was 6·4, whereas when the oxygen concentration was 3·1 mg/l the pH fell to a value of 6·05. This is also to be expected, since the oxygen concentration increased because of the photosynthetic activity of the plants; at the same time the carbon dioxide concentration was decreasing. Respiration of both animals and plants was responsible for the decreasing of the oxygen content and the increase in the carbon dioxide concentrations.

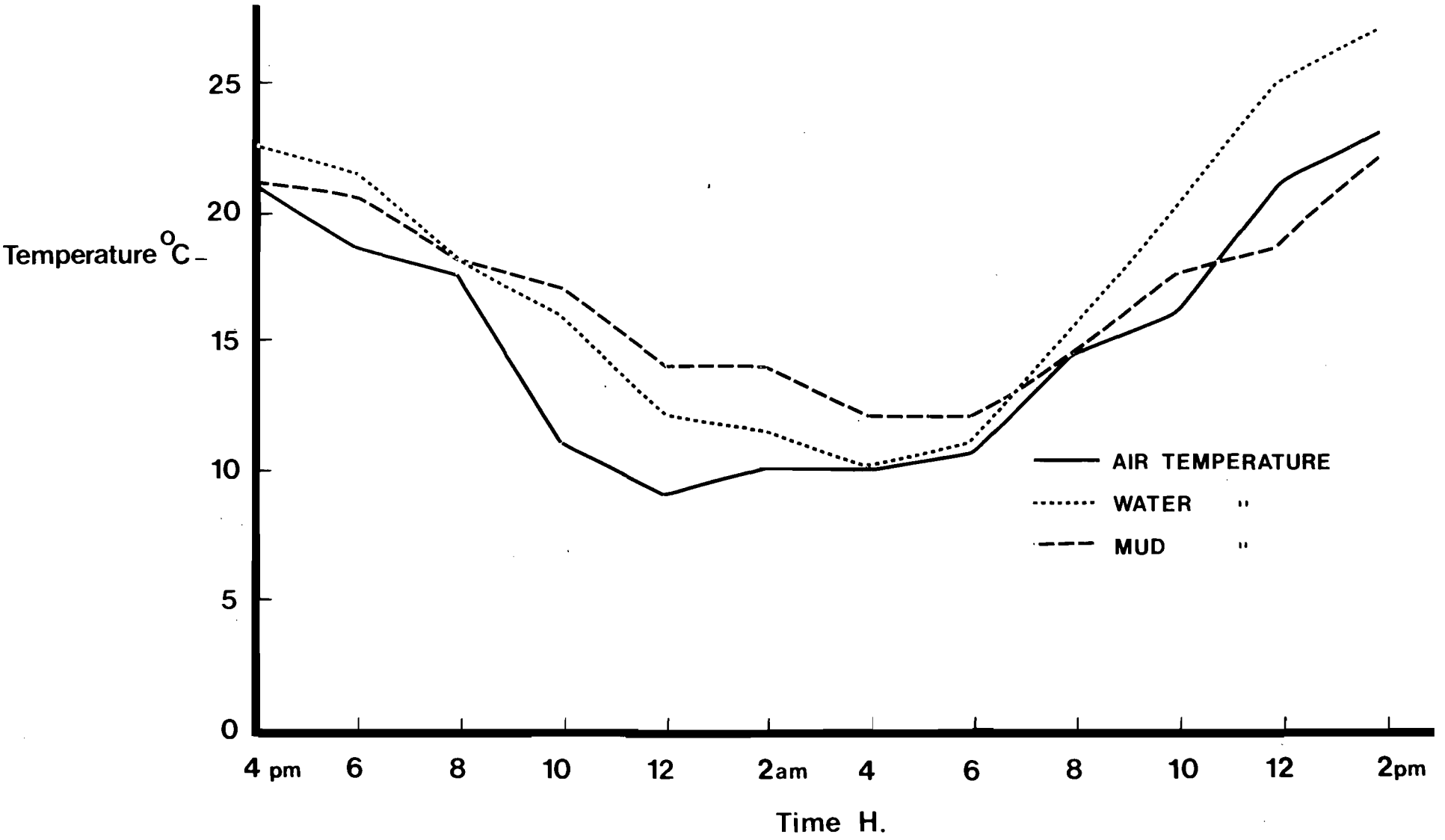


FIG.21 : DIURNAL FLUCTUATIONS OF AIR, WATER AND MUD TEMPERATURE IN GODSHILL POND 11/12 JULY, 1972 (CALM AND SUNNY DAY)

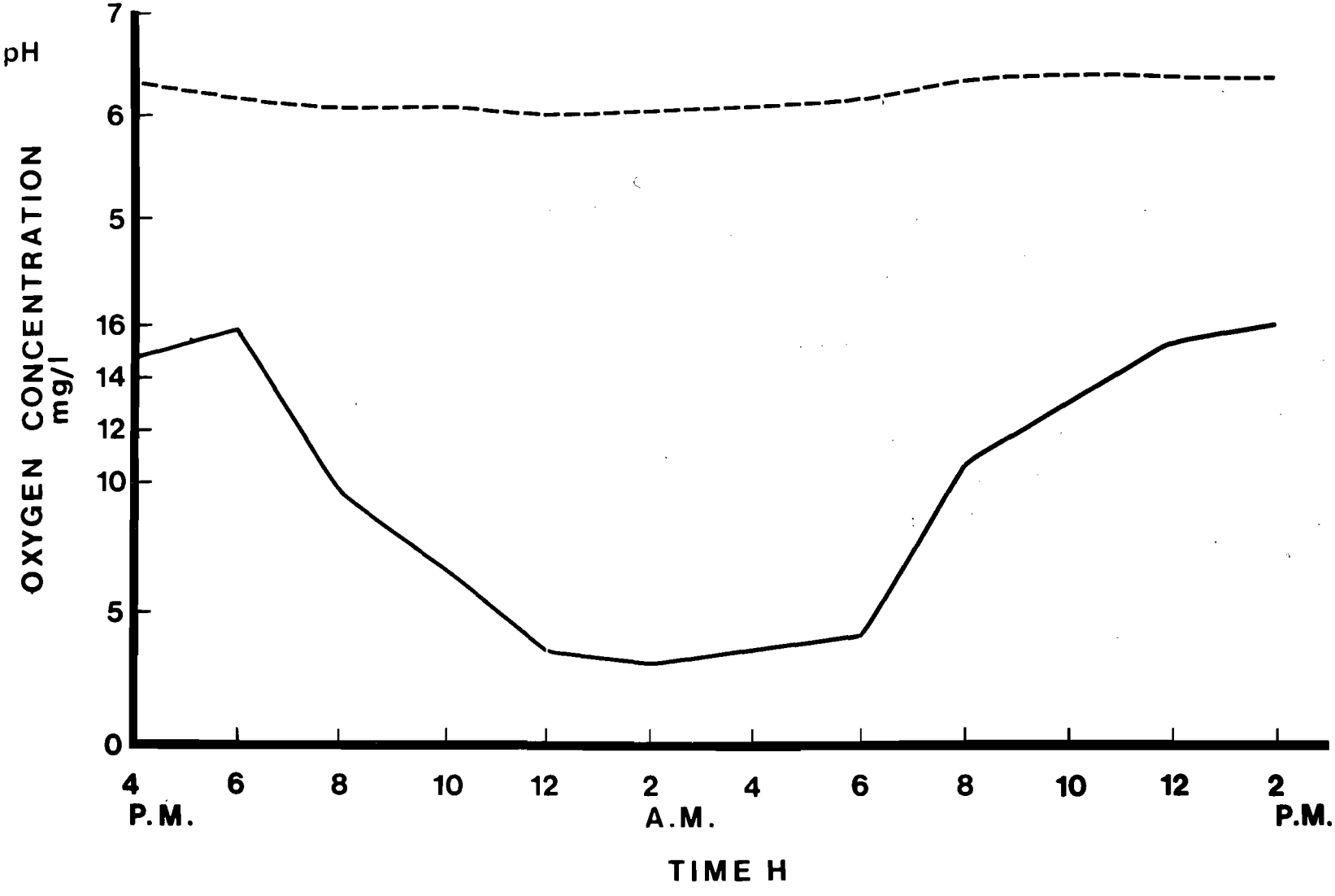


FIG. 22: DIURNAL FLUCTUATIONS OF OXYGEN AND PH IN GODSHILL POND 11/12 JULY, 1972 (CALM AND SUNNY DAY)

SECTION FOUR

EMBRYONIC DEVELOPMENT AND HATCHING OF THE EGG IN NATURE

SECTION 4

Embryonic Development and Hatching of the Egg in Nature

4.1 Introduction

Nauplii of *C. diaphanus* usually occur in nature soon after the refilling of the pond by rainwater. Regular field observations on the hatching time for many populations were made during the last two and a half years. These observations suggest that not all the eggs which have been laid are ready to hatch at the time of the next filling of the pond, since it was found that (a) further nauplii appear even though the previous population was eliminated by desiccation before reaching maturity and (b) it was found that some of the eggs which had been separated from the soil samples took 8-10 days to reach breaking stage. This means that for some reason no embryonic development seemed to have taken place in the eggs. One possible reason is the low oxygen content of the mud and the distribution of the eggs within the soil. However, quite large numbers of the eggs were in an advanced stage of development when they were separated from the soil, since they hatched within two days of their transfer to water. Thus this short time taken by the eggs to hatch and the early occurrence of the nauplii in the field suggest that embryonic development had taken place under natural conditions some time before the refilling of the pond. But whether the embryonic development takes place immediately after the eggs have been laid and before the habitat becomes dry, or during the dry period of the pond, was not known. Hall (1961) discussed this possibility briefly in the light of the absence of a frequent second generation, which suggested that some factor or factors may inhibit or retard the embryonic development. At the same time he added that in the two cases in which a

second generation developed without the complete drying of the pond, there was a period in which the water level fell considerably, so it is possible that eggs laid near the margin would be subjected to drying or near drying, and that with refilling of the pond these would hatch, and so give a second generation. Due to the distribution of the eggs within the soil, some of the eggs would not be able to hatch after the first coverage with rainwater, but then after another refilling they may have the opportunity to hatch. Dexter and Kuehnle (1951) reported that with gradual filling of the pond several generations of E.vernalis were found at the same time. In previous papers, Hall (1959b) (1959c) suggested that the delayed development is correlated with the depth of water overlying the eggs. No previous detailed study has been made on the embryonic development of anostracan branchiopods under natural conditions. So to determine whether embryonic development in nature occurs immediately after the eggs have been laid or later during the dry period, this study was carried out. The investigation was based on regular mud sampling and field observations.

4.2 Collecting eggs from adult females

Male and female adult shrimps either from laboratory cultures or field collected samples were separated into groups. Each group consists of 5-10 pairs. These groups were then each transferred into a finger bowl containing habitat water. Then next day the finger bowls were searched and the laid eggs were collected by means of a small pipette under the microscope at 35X magnification. To make egg collection more easy, the water was stirred clockwise or anticlockwise using a glass rod in order to drive the eggs towards the centre of the finger bowl. The collected eggs were transferred to a clean petridish containing water. The petridish was then placed under the microscope and the eggs were pipetted and transferred to another clean petridish containing water. This latter procedure was used in order to get rid of all unwanted material which had accidentally been transferred in the first step of this method. The eggs were then washed several times with distilled water to be ready for use.

4.3 Separating eggs from the soil

Three sieves with different mesh sizes were used in this technique. The sieves were placed one on top of each other so they would be arranged in an order of increasing mesh size. The mesh sizes of these sieves were 106, 300 and 850 micron. The sieves used were made by Endecotts (Test Sieves) Ltd. The lowermost sieve was that having the finest mesh, that is to say 106 micron. The uppermost sieve was that having a mesh size of 850 micron. The third sieve, which had 300 micron mesh size,was placed in between the two mentioned above. Since the egg of the fairy shrimp C.diaphanus has a diameter of less than 300 micron on average, the lowermost sieve will hold the eggs but at the same time will permit finer material to pass through. The upper two sieves, having mesh sizes of 300 and 850 micron, will hold all larger particles but at the same time will allow the eggs to pass through. The soil sample was placed in the upper sieve and a stream of tapwater was directed on it from above using a water hose. The material retained in the lowermost sieve,including the eggs, was washed out into a round glass dish. Then the water was stirred clockwise or anticlockwise using a glass rod in order to drive the eggs and other heavy particles to the centre of the dish. However, sometimes this latter step was not used. A small quantity of the material in the round glass dish was removed and placed in a petridish containing water. The contents of the petridish were carefully examined under the microscope at 35X magnification. The contents of the petridish were examined until no more eggs could be seen. The eggs were removed by means of a small pipette and placed in watch-glasses for the subsequent study.

4.4 The distribution of eggs in nature and its value for the survival of the species

The field observations indicate that the adults tend to accumulate mainly in the central parts of the pond, but the reason for this behaviour is not known. According to this it was thought that oviposition may take place mainly in the middle part of the pond. This view was supported by the field investigation on the distribution of the eggs in the soil. In this investigation, soil samples at weekly intervals were brought from the Godshill pond while it was dry. Surveys for eggs of C.diaphanus were conducted in the field at two areas within the natural bottom of the Godshill pond, namely the marginal area and the central area. The marginal area usually covered with water when there is plenty of rain, whereas the central area covered with water continuously as long as the pond holds water. So the marginal area is subjected to drying before the central area. One sample was taken from the marginal region of the pond and the other sample was taken from the central part of the pond. Then both samples were washed separately using the sieving technique described before. Then the material retained in the lower sieve was carefully examined for a fixed time of three hours in each sample. The number of eggs obtained at the end of three hours for both samples are shown in Table [4].

Table [4]. Number of eggs obtained from soil samples at the end of three hours

Number of eggs in each sample	
Central area	Marginal area
150	50
78	20
100	24
80	38
71	21
114	45
98	33

From the figures shown in the above table, it is obvious that the eggs were found in large numbers in the central area of the pond compared to the numbers found in the marginal area. This pattern of egg distribution for a species inhabiting temporary ponds may have an ecological significance, since the eggs require water or moist soil to complete their embryonic development, and nauplii require water for their hatching. Moisture will be provided in the central part of the pond longer than at the marginal part, since the latter dries earlier.

4.5 Investigation of the embryonic development and hatching under natural conditions

To determine the stage of embryonic development of the eggs separated from the soil samples, the following procedure was carried out. Eggs, after being separated and washed several times with water, were transferred to watch-glasses filled with distilled water. These watch-glasses were kept at laboratory temperature, which had a mean of 22°C at the time of the investigation. Daily observations were made on the eggs for the appearance of the break in the tertiary shell. The time required to show peak break was taken as a measure for the stage of the development.

To study the embryonic development of this species under natural conditions it was decided to divide the work into two parts. The first part was conducted in the natural habitat of this species, and the second was conducted in an artificial pond, which may be considered as typical of the habitat of this species. The artificial pond was established using a 110 x 25 cm asbestos bowl, filled with habitat water and having all the characteristic fauna and flora found in the natural pond.

4.5.1 General plan for the study

4.5.1.1 Godshill pond

Godshill pond was taken as a field site for this study. Soil samples were removed from two locations within the pond, each of which had been marked in advance. The first location was 3 x 1 metre at the margin of the pond (Station A). The second location was 2 x 2 metre at the central part of the pond (Station B). Soil samples were taken at ten day intervals. The samples were washed and eggs were separated using the method described before. The eggs were then transferred to watchglasses containing distilled water. The watchglasses were kept at laboratory temperature, to determine the time required by the eggs to reach the

breaking stage.

The study was started on 20th March 1972 and was stopped on 9 August 1972. The starting time was so arranged that most of the shrimps were observed to have reached maturity, and females were able to lay eggs. Station (A) was covered with water until 11th April 1972, then after this period onwards only station (B) was covered with water. On 25th July it was observed that the pond was completely dry. Weekly measurements of the physical and chemical factors were made throughout the time of the investigation. All measurements are given with the results of the second chapter, Fig. [11], [13], [15] and [17].

4.5.1.2 Artificial pond

The embryonic development was also studied using an artificial pond, which represented the natural one in its characteristics. Batches of 100 freshly laid eggs were separated and placed each in a small glass tube 1 x 2 cm., open at both ends. Then both ends were sealed with a nylon cloth having a mesh size of 80 micron. These tubes were placed at the bottom of the artificial pond, so that they were covered with a thin layer of mud. The tubes were removed at intervals of one week after placing them at the bottom of the artificial pond. The eggs were separated, washed and then placed in watchglasses containing distilled water to determine the time required to reach the breaking stage. Temperatures of the air, water and substratum were measured twice a month. Oxygen concentrations of the water, the mud-water interface and 2-3 cm. below the surface of the mud were measured. Conductivity and depth of the water were also measured. All the above measurements are given in Table [5]. On 10th September 1972 the remaining water was removed in order to subject the bottom of the artificial pond to a dry period. The

Table [5] Shows the temperature, oxygen concentration, depth and conductivity in the artificial pond.

Date	Air temperature C°		Water temperature C°		Oxygen concentration mg/l.			Conductivity micromhos/cm.	Depth cm.
	minimum	maximum	minimum	maximum	water	mud-water interface	mud		
5.7.1972	9·0	18·5	12·5	26·5	4·41	1·52	0·49	118	9·2
15.7.1972	9·5	22·5	11·5	29·5	4·16	1·10	0·55	180	8·8
28.7.1972	13·0	25·0	15·0	31·5	4·38	1·26	0·53	255	7·9
12.8.1972	9·5	21·5	12·5	29·0	4·58	1·71	0·53	262	7·6
29.8.1972	10·0	24·0	11·5	31·5	5·20	1·30	0·59	265	4·3
10.9.1972	9·5	22·5	10·5	28·0	5·10	1·54	0·48	265	3·5
20.9.1972	9·0	22·0	-	-	-	-	1·62	-	Pond dry
30.9.1972	8·5	17·5	-	-	-	-	2·40	-	Pond dry

glass tubes were also removed at weekly intervals and were subjected to the usual procedure in order to determine the time taken by the eggs to reach breaking stage, giving a measurement of the amount of development which had taken place up to this point.

4.5.2 Results of the investigation

Table [6] shows the peaks of the eggs showing the break in the tertiary shell each day at laboratory temperature. Dates of collection of the eggs from the field are given in the table. Eggs separated from station (A) samples are represented by (o) and the eggs which had been separated from station (B) samples are represented by (+). Generally, it can be seen from this table that the rate of embryonic development has greatly retarded throughout the period in which water was covering the bottom of the pond. At station (A) it can be seen that no embryonic development seemed to have taken place during the period between 22nd March 1972 and 11th April 1972. After this period had elapsed, the water disappeared from station (A), and a slow embryonic development seemed to have taken place, since it was found that eggs separated from this station took less time in laboratory conditions to reach the breaking stage. The embryonic development was slow at that time, presumably due to the low temperature found there.

At station (B) little embryonic development, or none, seemed to have taken place when the water was still covering the area. This is obvious during the period between 22nd March 1972 and 18th April 1972. Then, after this period had elapsed, the embryonic development was more rapid, since no water was then covering the area. It can be seen that embryonic development at station (B), after drying out of the pond, was more rapid than that observed at station (A); this is presumably because the temperature was higher at that time.

The findings of the investigations which have been carried out on the rate and on the pattern of the embryonic development in the artificial pond agree entirely with the results of the field investigations. From Table [7] it could be seen that the rate of the embryonic development was

Table [6]. Rate of embryonic development under natural conditions (Godshill pond)

Station (A) o
Station (B) +

Dates of collection from the field

Days at Lab.temp.	20.3.72	5.4.72	11.4.72	18.4.72	28.4.72	9.5.72	16.5.72	21.5.72	30.5.72	6.6.72	13.6.72	21.6.72	27.6.72	4.7.72	12.7.72	18.7.72	25.7.72	31.7.72	3.8.72	9.8.72
	No water at marginal area																	pond dry		
1													o							+
2											o	o		o	o			+	+	+
3									o	o			o	o	o			+	+	
4									o	o		o	o		o			+		
5							o				o								+	
6						o	o	o	o											
7							o	o	o											
8		o		o	o	o											+			
9		+	o	o	o	o					+	+	+	+	+	+	+			
10	o +	o		+	+	+		+		+	+	+	+	+	+	+				
11	o +		o +	o +	+	+	+	+	+	+	+									
12	+		o +				+		+											
13			o +																	
14																				
15																				
16																				

Table [7]. Rate of embryonic development in an artificial pond approximating to natural conditions.

Peak break o

Dates at which egg batches were removed from the artificial pond and placed in watchglasses

Days at Lab.temp.	1.7.72	7.7.72	15.7.72	21.7.72	29.7.72 Pond wet	5.8.72	12.8.72	25.8.72	8.9.72	20.9.72	27.9.72 Pond dry	10.10.72	17.10.72
1												o	o
2											o	o	
3											o		
4										o	o		
5										o			
6													
7													
8						o		o	o				
9			o	o	o		o	o	o				
10	o	o	o	o	o	o			o				
11	o	o		o									
12													
13													
14													
15													

nearly negligible during all the period between 1st July 1972 and 8th September 1972, since throughout that period the pond contained water. But after that period had elapsed, the pond was dry. The embryonic development, as can be seen from Table [7] was more rapid, since the eggs took only a few days to show the break in the tertiary shell when transferred to watchglasses containing water.

Table [5] shows the oxygen concentrations measured in water, mud-water interface and 2-3 cm. below the mud surface. From these measurements one can see how low was the oxygen concentration surrounding the eggs. The retardation of the embryonic development under such conditions is not surprising, since it was found experimentally that low oxygen concentrations would retard and inhibit the embryonic development of this species. On the other hand, there was an increase in the amount of oxygen surrounding the eggs when the pond dried out. Oxygen concentration as high as 2·4 mg/l was found in the soil when the pond was dry. At the time when there is an increase in oxygen level, the embryonic development was noticed to be rapid.

4.6 Breaking and hatching in nature

As has been shown, the eggs after being laid by the females sink to the bottom of the pond and stay there, and either develop a little or no development takes place at all. Then, during the drying period of the pond, eggs seem to develop rapidly up to the breaking stage. However, some of the eggs go further and show the break in the tertiary shell, especially if the soil has a high moisture content. Presumably small numbers of the broken eggs could survive for a longer time if there were no standing water. No hatching takes place while there is no free standing water in the pond. Hatching occurred as soon as the rain-water inundates those eggs which are ready to hatch. During the regular field observations for the last two years and a half it was noticed that in all cases nauplii of C.diaphanus were seen within two days after the refilling of the pond, or even less. The occurrence of C.diaphanus nauplii in the field was seen several times during the time of the present study. The hatching of C.diaphanus took place in Godshill pond on 12th July 1971, 14th October 1971, 12th November 1971, 22nd November 1971, 4th July 1972, 2nd August 1972, 12th September 1972, 10th October 1972, 31st October 1972, 14th November 1972 and 3rd April 1973.

In Eyeworth pond the hatching took place on 19th August 1971, 14th October 1971, 12th November 1971, 22nd November 1971, 21st December 1971, 5th April 1972, 11th April 1972, 2nd August 1972, 12th September 1972, 10th October 1972, 31st October 1972, 14th October 1972, 21st November 1972 and 3rd April 1973. However, the natural occurrence of this species in the two mentioned ponds has already been discussed before. On all occasions on which hatching was observed in the field, there have been three cases in Godshill pond and two cases in Eyeworth pond in which hatching took place while the pond still contained water.

4.7 Discussion

Under natural conditions, the eggs of the fairy shrimp C.diaphanus and other species of anostracan branchiopods, after laying, sink to the bottom of the pond, where low oxygen concentrations may exist. Then the eggs are exposed to moist soil when the pond dries up. This provides aerobic moist conditions, which may be the most favourable ones for the embryonic development of this species. The hatching occurs soon after they are inundated by rainwater which is well aerated. The early occurrence of the nauplii of C.diaphanus in the field would suggest that the embryonic development has taken place up to the breaking stage before the refilling of the pond, since nauplii occurred within two days after the refilling of the pond. But whether the embryonic development took place immediately after the eggs had been laid and before the habitat became dry, or during the subsequent drying period of the pond, was not known. In his work with C.diaphanus, Hall (1961) discussed this matter briefly in the light of the absence of a frequent second generation, which suggests that some factors may inhibit or retard the embryonic development. On all occasions on which hatching was observed in the field, there have been three cases in Godshill pond and two cases in Eyeworth pond in which hatching took place while the pond was still containing water. As has been suggested by Hall (1961), it is possible that eggs laid near the margin would hatch and give a second generation with the refilling of the pond. It was observed that the numbers of nauplii hatched as second generations after the gradual filling of the pond were very small. From the results of this study it seems that no embryonic development (or very little) takes place immediately after laying, since it was found that as long as the eggs were covered by water, they took the normal time or slightly longer to reach the breaking stage. Thus most of the embryonic development seems to have taken place during the dry

period of the pond. The eggs, as has been mentioned elsewhere, could withstand long periods of drought. So it seems that, during this period, the eggs are subjected to aerobic moist conditions. Under these conditions the eggs continue their embryonic development up to a point just before the occurrence of the break in the tertiary shell. At this stage of development the eggs are ready to hatch as soon as the pond is filled again with rainwater. Such a mechanism would contribute to the survival of the species, as it would ensure the hatching of the eggs in favourable environmental conditions.

SECTION FIVE

A STUDY ON THE EMBRYONIC DEVELOPMENT AND HATCHING MECHANISM IN THE EGG OF C.DIAPHANUS

SECTION 5

A study on the embryonic development and hatching mechanism in the egg of C.diaphanus

5.1 Introduction

The hatching process of anostracan branchiopod eggs has been of interest to some biologists for many years; however, most of the work which has been done was on the factors influencing this process. Detailed work on the hatching mechanism in this group is scarce. Complete escape of the nauplius from the egg membranes is a two stage process. The two stages - first described by Baird (1849) and then re-described in more detail by Hall (1953) - are namely the breaking stage and the hatching stage. The breaking stage is the stage at which a split occurs in the tertiary shell and the embryo contained in the hatching membrane protrudes through the split. The hatching process is the stage at which the hatching membrane is ruptured and the nauplius is set free. During the period between the breaking stage and the hatching process, the embryo seems to revolve about its longitudinal axis. The embryo also shows irregular movements of the appendages. The significance of these movements in the hatching process is not known. In his work with the fairy shrimp Chirocephalopsis bundyi Forbes, Broch (1965) reported that it is possible that the rupture of the hatching membrane may be due to a mechanical abrasion.

The breaking stage seemed to be strictly an osmotic phenomenon, since the embryo was not able to move at the time of the occurrence of the break in the tertiary shell. Clegg (1962)(1964), working with the brine shrimp Artemia salina L., stated that it seemed possible that glycerol might be involved in the shell-rupture because of its presence in high concentrations. Moreover, Hall and MacDonald (personal communication)

have demonstrated glycerol accumulation in the breaking process of C.diaphanus. This suggests that the breaking stage may be strictly an osmotic phenomenon.

Davis (1963) stated that the hatching process in some aquatic invertebrates may be by mechanical means or through the osmotic uptake of water. Yonge (1937) and Marshall and Orr (1954) also suggested an osmotic hatching in some marine forms of Crustacea. Thus from the above statements it is possible that the complete hatching process may be due to the embryonic movements and to osmotic action; however, the presence of a hatching enzyme must not be excluded.

5.2 Preparation of the embryos for the study

Batches of freshly laid eggs were washed several times with distilled water and each placed in watchglasses filled with distilled water. These watchglasses were kept at laboratory temperature. The mean laboratory temperature at the time of the investigations was 22°C. These batches were removed at intervals of twenty-four hours during the first five days of immersion, and at intervals of six hours during the subsequent time. The batches of eggs were then transferred to watch-glasses containing 5% Sodium hypochlorite solution in order to remove the egg shell and hence observe the morphological changes in the embryonic mass. Then after approximately five minutes, the eggs were removed, washed several times with distilled water and then examined under the microscope at 140X magnification. After the occurrence of the break in the tertiary shell, there was no need to use the sodium hypochlorite solution to remove the egg shell, since it was removed by means of a fine needle.

5.3 Embryonic development and occurrence of break

As has been shown elsewhere in this thesis, there is no visible increase in the diameter of the developing egg throughout the course of the embryonic development up to a point just before the appearance of the break. The author is interested mainly in the period between the occurrence of the break and the hatching process. The first external morphological change observed was the appearance of the blastopore two-three days after laying. However, plate [8] shows that within the first two days of laying, the embryo is a smooth yellowish white mass. No observable surface structures could be noticed. In the present investigations it was observed that, following this, little external morphological differentiation seemed to occur until the sixth day of incubation at laboratory temperature, that is to say approximately twenty-four hours before the appearance of the break in the tertiary shell, when the appearance of a transverse constriction (the constriction between the thoracic region and the abdominal region) was observed, plate [9]. Then after a further six hours more morphological differentiation could be seen, especially in the thoracic region where the rudiments of antennal appendages are apparent, although not fully developed, plate [10]. However, in this photomicrograph the abdominal thoracic constriction is not clear because of the orientation of the embryo and the lighting used at the time of the photography. Plate [9] shows the transverse constriction more clearly. Plate [11] shows the embryo twelve hours after the first six days of immersion. Rudiments of three pairs of appendages could be distinguished at this stage. In plate [12] the three pairs of appendages, namely, the first antennae, the second antennae and the mandibles are distinct. No compound or larval eyes could be distinguished before the breaking had occurred. A few hours before the occurrence of the break, the three pairs

Plate [8]. Embryo within the first two days of laying, after the removal of the egg shell. The embryo is a smooth yellowish white mass. No observable surface structures could be noticed.

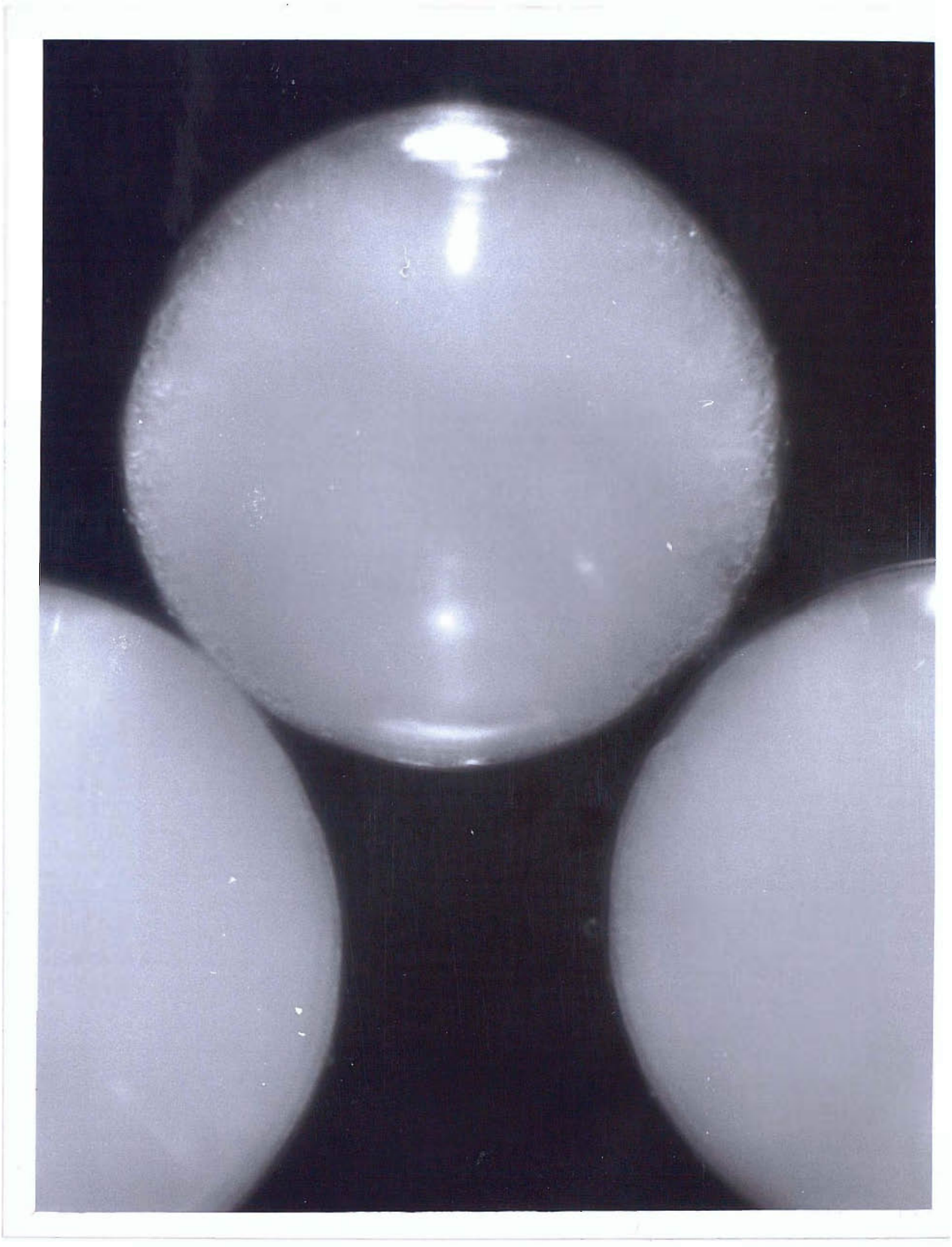


Plate [8]

Plate [9]. Embryo approximately twenty-four hours before the occurrence of the break in the tertiary shell (showing the constriction between the thoracic region and the abdominal region), after the removal of the egg shell.

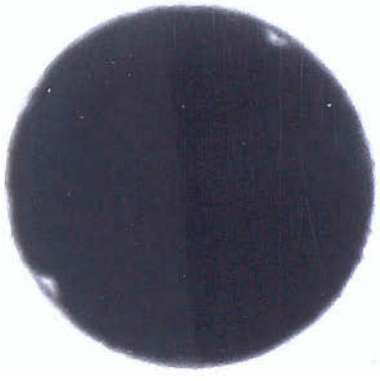


Plate [9]

Plate [10]. Embryo six hours after the first six days of immersion (showing the rudiments of the antennal appendages), after the removal of the egg shell.

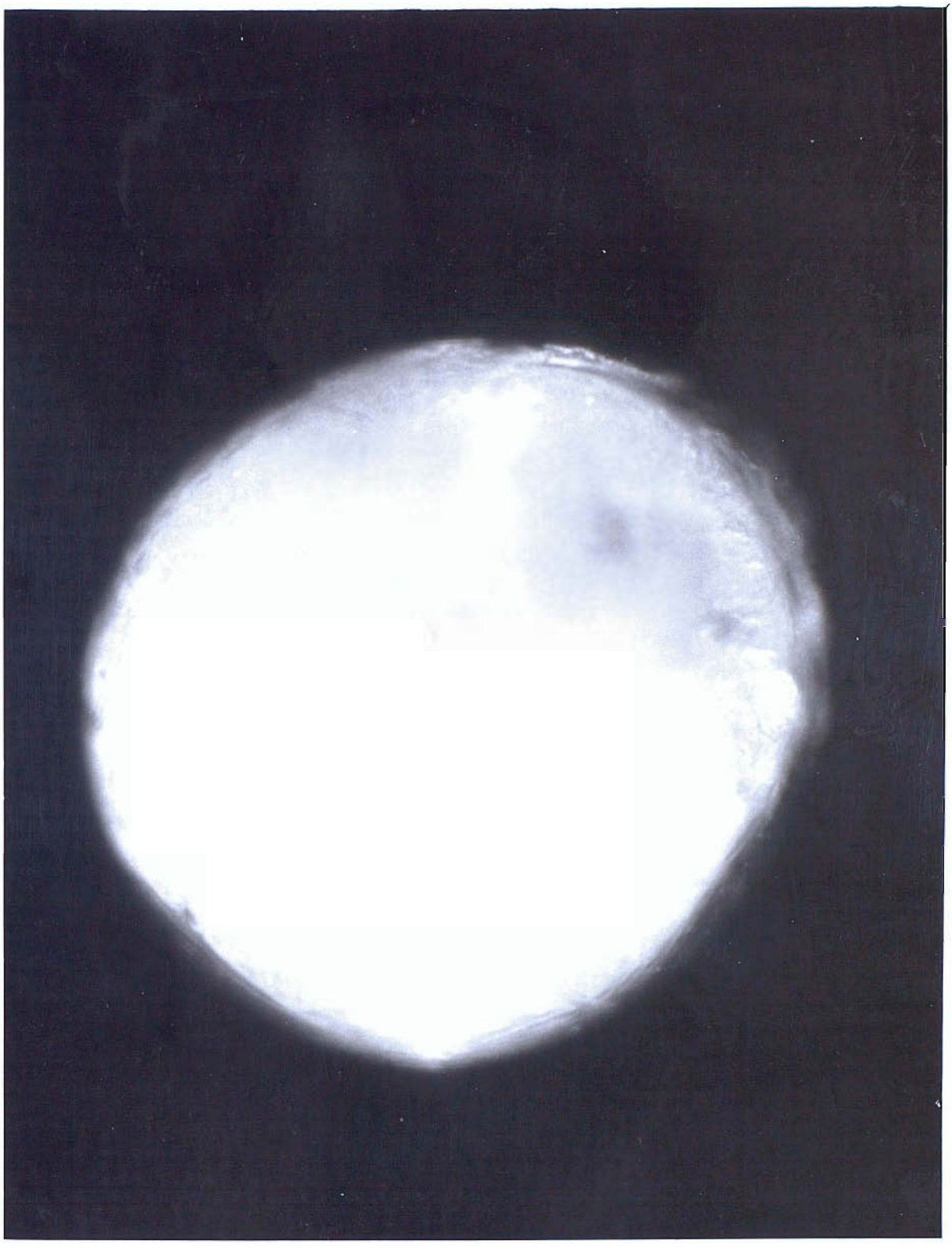


Plate [10]

Plate [11]. Embryo twelve hours after the first six days of immersion (showing the rudiments of three pairs of appendages more clearly), after the removal of the egg shell.

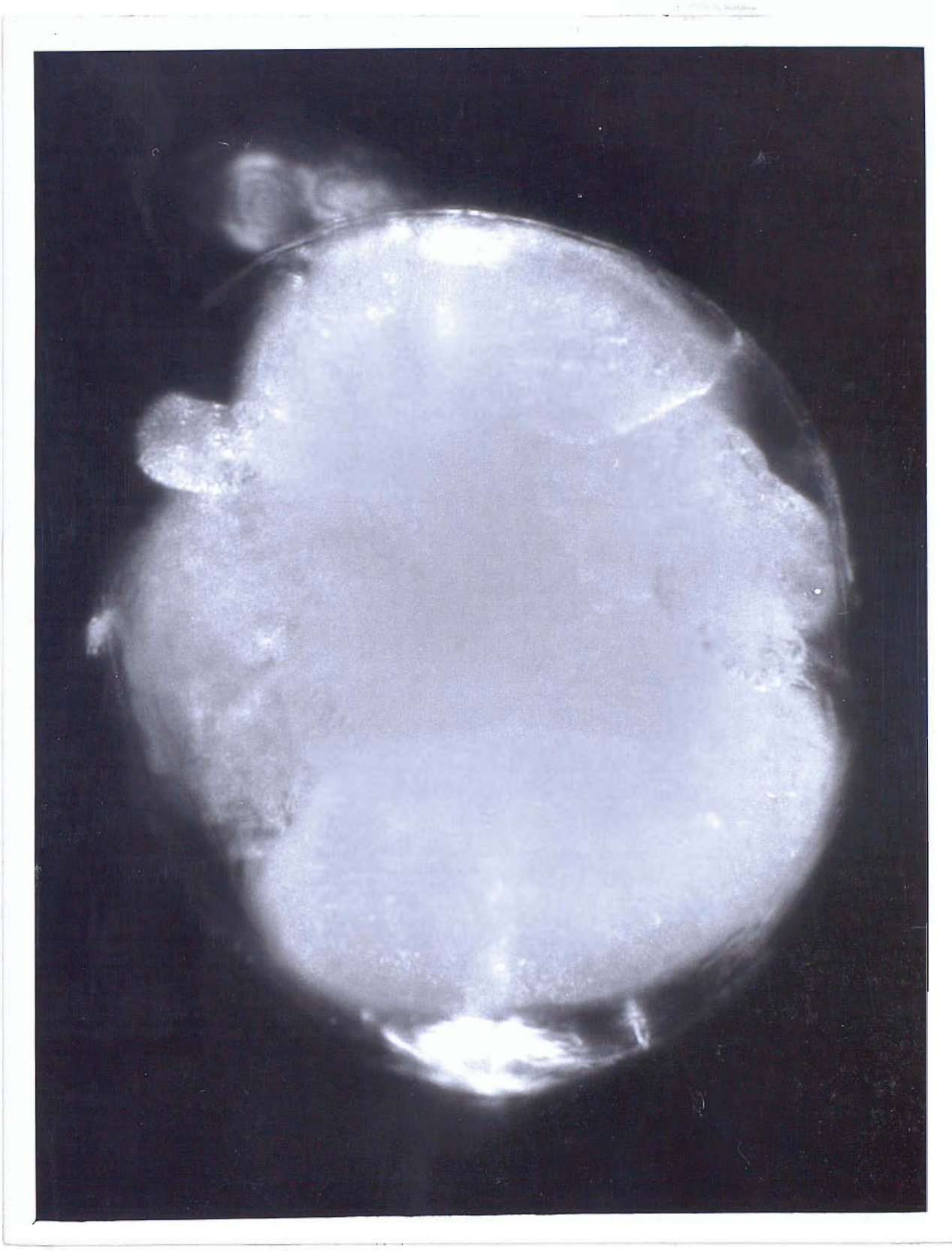


Plate [11]

Plate [12]. Embryo a few hours before the occurrence of the break showing the first antennae, the second antennae and the mandibles, after the removal of the egg shell.

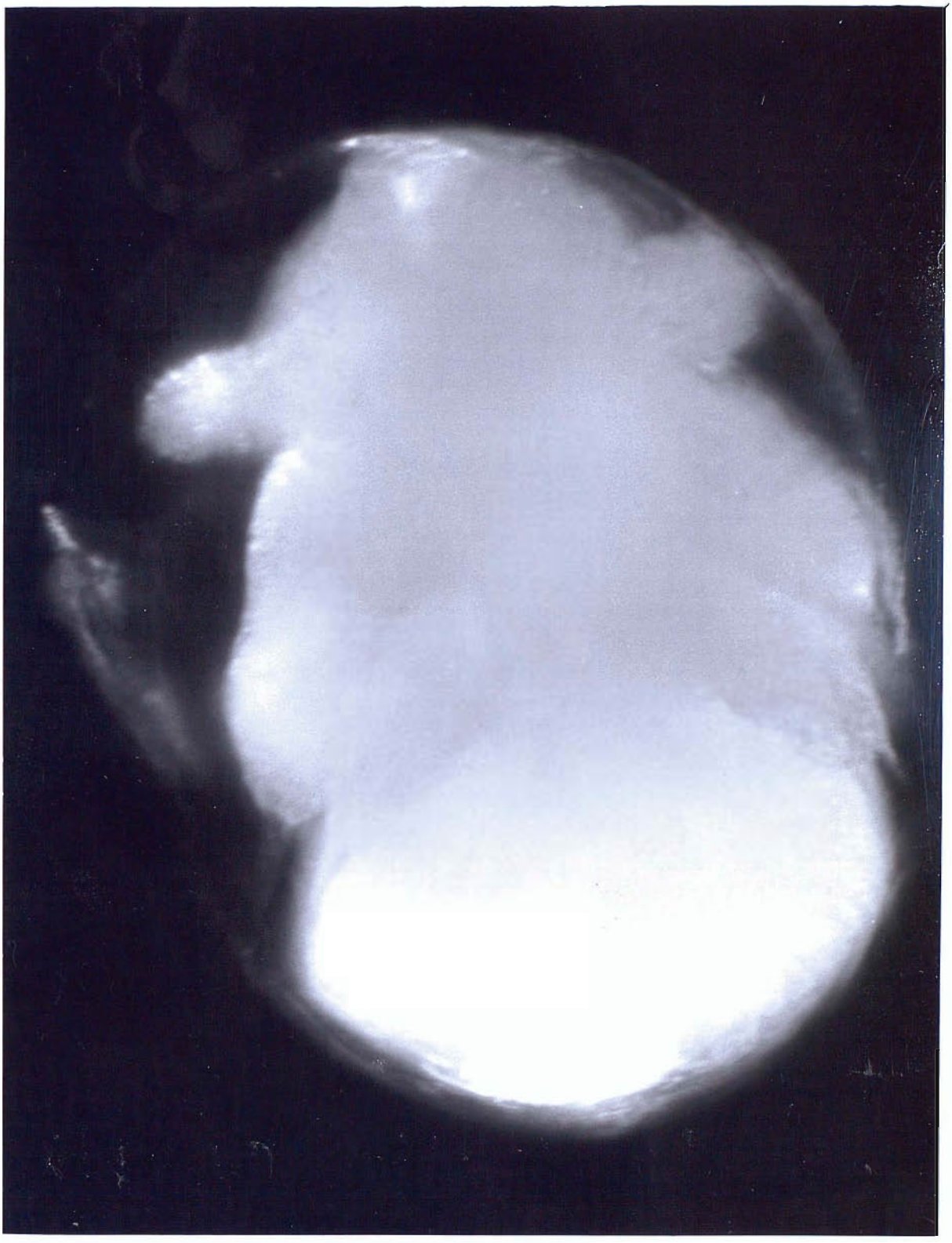


Plate [12]

of appendages are more distinct. At this stage the embryo does not show any movement.

Baird (1849) was the first to describe the egg-covering of this species. He referred to an inner, transparent envelope and a thicker, external opaque coat over it. In their work with C.diaphanus, Mawson and Yonge (1938) mentioned a thin inner membrane which is surrounded by a thicker, rugose outer membrane. Hall (1953) was able to distinguish a third membrane occurring between the two mentioned by Baird and by Mawson and Yonge. The re-description by Hall is the one which is more readily acceptable, since this third membrane was very easy to distinguish under the microscope. For convenience, the three membranes described by Hall (1953) will be referred to as tertiary shell, chitinous membrane and the hatching membrane. Plate [13] shows the egg of C.diaphanus before the occurrence of the break. The spiny appearance is due to the raising of the surface of the tertiary shell into a series of irregular shaped polygonal cells. The thick shell covering the egg of the anostracan egg is presumably to protect the embryo from (a) mechanical injury, (b) complete dehydration, and (c) high temperature, since it was found that undried C.diaphanus eggs could survive more than nine days at 35°C without any harmful influence.

As has briefly been mentioned before, the complete hatching takes place through two distinct stages, namely the breaking stage (occurrence of break in the tertiary shell) and the hatching process (the hatching membrane being ruptured and nauplius set free). The breaking stage seems to be a strictly osmotic process, since the split in the tertiary shell occurs at the time when the embryo is not able to show any movement whatsoever. The unhatched embryo starts to move its appendages about 6-8 hours after the break has occurred. Moreover, the embryo begins to

Plate [13]. An egg of Chirocephalus diaphanus Prévost before the occurrence of the break. The spiny appearance is due to the raising of the surface of the tertiary shell into a series of irregular shaped polygonal cells.

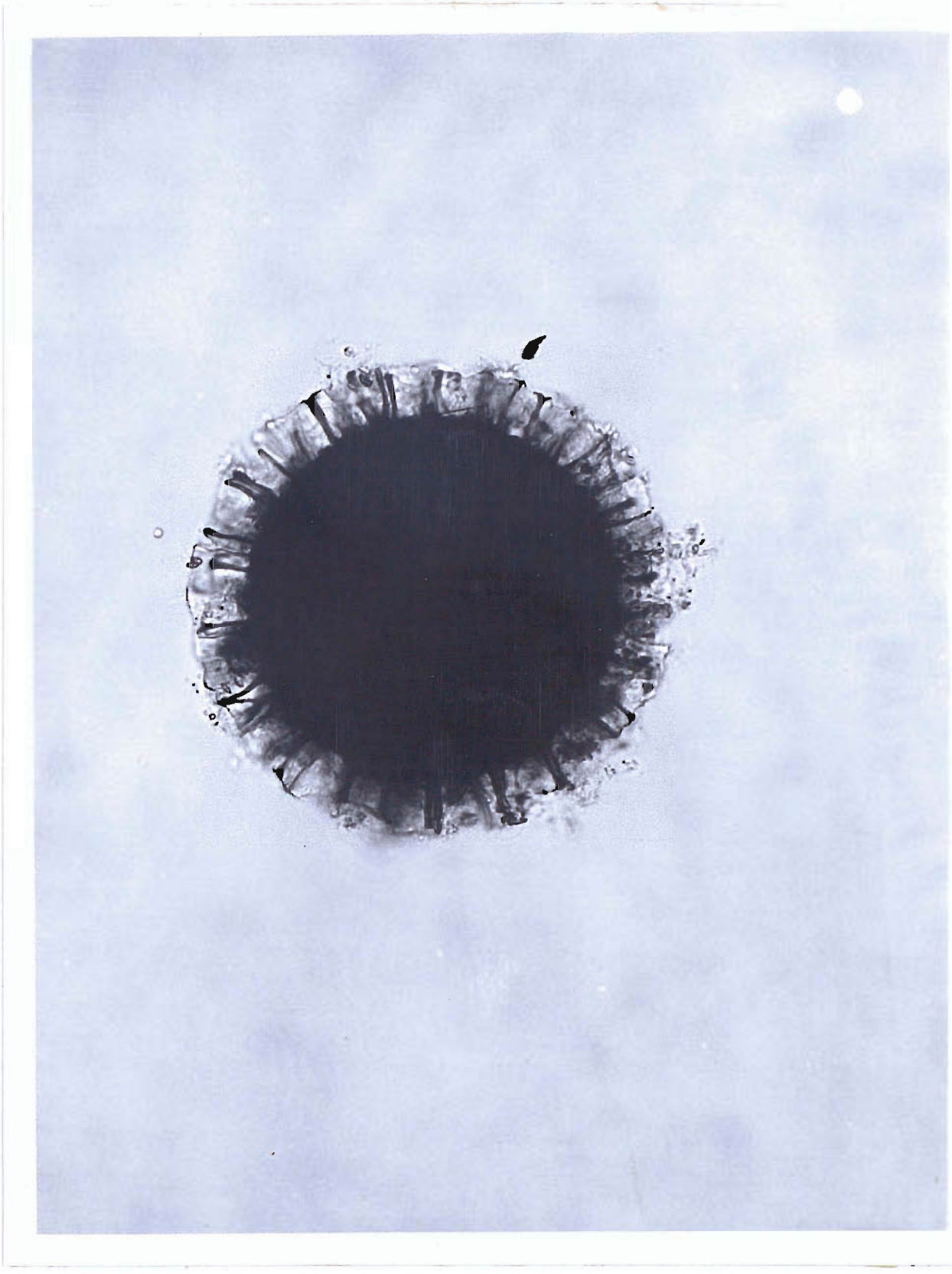


Plate [13]

Plate [14]. An egg showing the occurrence of the break in the tertiary shell.

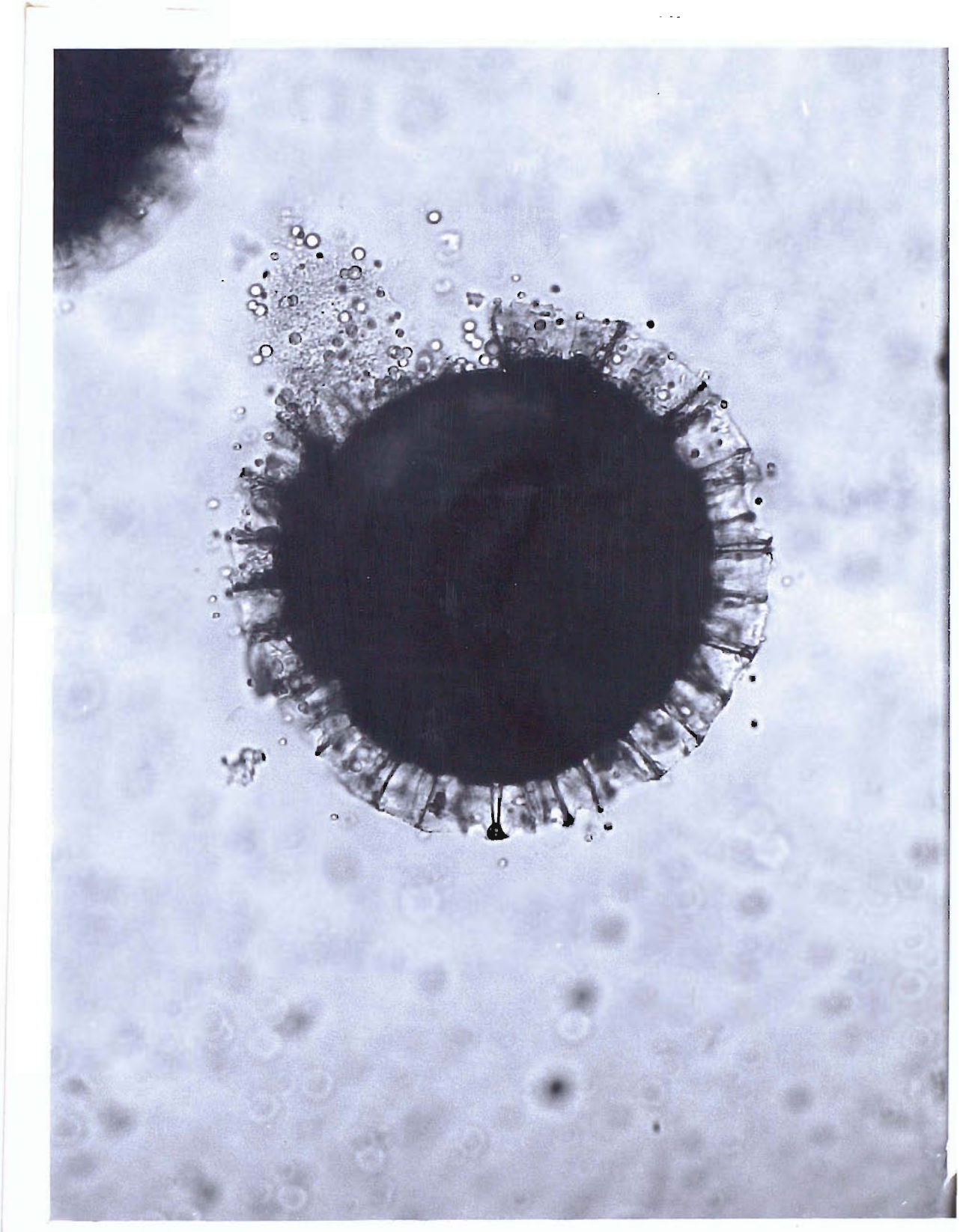


Plate [14]

Plate [15]. Unhatched embryo 6 - 8 hours after the break has occurred.
The unhatched embryo at this stage starts to stretch out its antennal appendages.

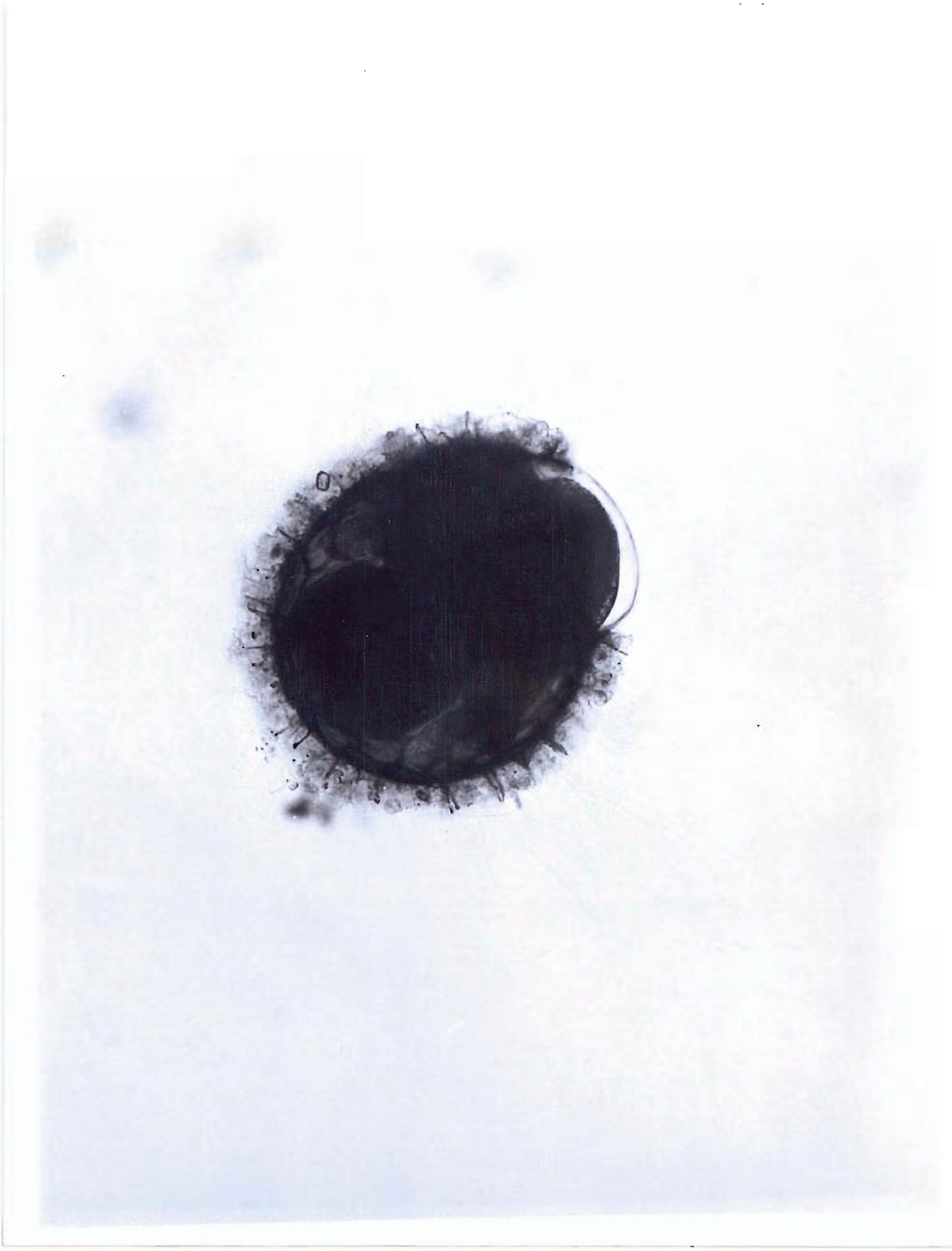


Plate [15]

Plate [16]. Unhatched embryo 8 - 10 hours after the break has occurred.
The embryo gradually elongates and becomes more active by moving its appendages and hence pushing the hatching membrane.

Plate [16]

move about its longitudinal axis (rotatory movement) about 8-10 hours after the break has occurred. Before the occurrence of the split in the tertiary shell, it seems possible that, due to the metabolism of the embryo, there is an accumulation of metabolic products which increase the osmotic pressure inside the tertiary shell. Then, at this stage, osmotic inflow of water starts to increase rapidly. As a result of the rapid inflow of water there is an increase in the pressure inside the egg shell. This increase in pressure inside stretches the tertiary shell and the chitinous membrane and leads to the appearance of the split, plate [14]. The accumulation of glycerol has been found to occur before the emergence of the embryo of A.salina from the shell (Clegg, 1962, 1964), and in the eggs of C.diaphanus, Hall and MacDonald (personal communication). The split in the outer covering of the egg starts to become bigger and bigger due to the osmotic inflow of water, which leads to an increase in the embryonic contents within the hatching membrane. The unhatched embryo starts to stretch out its antennal appendages 6-8 hours after the break has appeared, plate [15]. Then a few hours after that the embryo gradually elongates, plate [16], and becomes more active by moving its appendages and hence pushing the hatching membrane. At this stage the pigmentation of the larval eye is distinct, but there is still no evidence for the appearance of the compound eyes. Later the unhatched embryo begins to move around its longitudinal axis (rotatory movement). At this stage the segmentation of the abdominal region is very clear and the larval eye is apparent; however, no pigmentation of the compound eyes could be seen at this stage, plate [17] and plate [18]. Plate [19] shows the nauplius immediately after the hatching process.

Plate [17]. Unhatched embryo 16 - 18 hours after the break has occurred (larval eye is distinct but no compound eyes are detectable). The embryo at this stage begins to move around its longitudinal axis (rotatory movement).

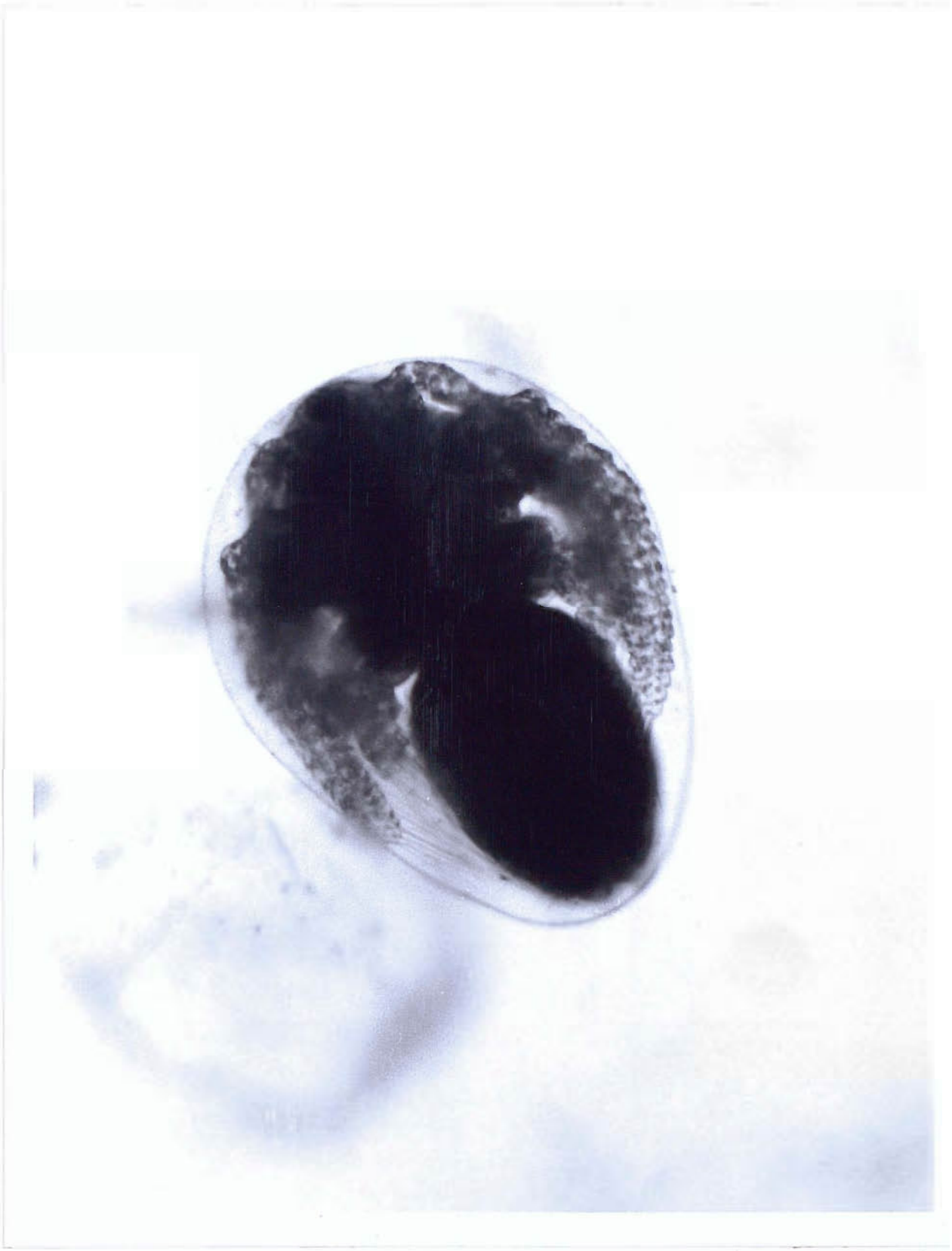


Plate [17]

Plate [18] Unhatched embryo approximately 24 hours after the break has occurred. Embryo is in its full size and moving actively, pushing and abrading the hatching membrane.

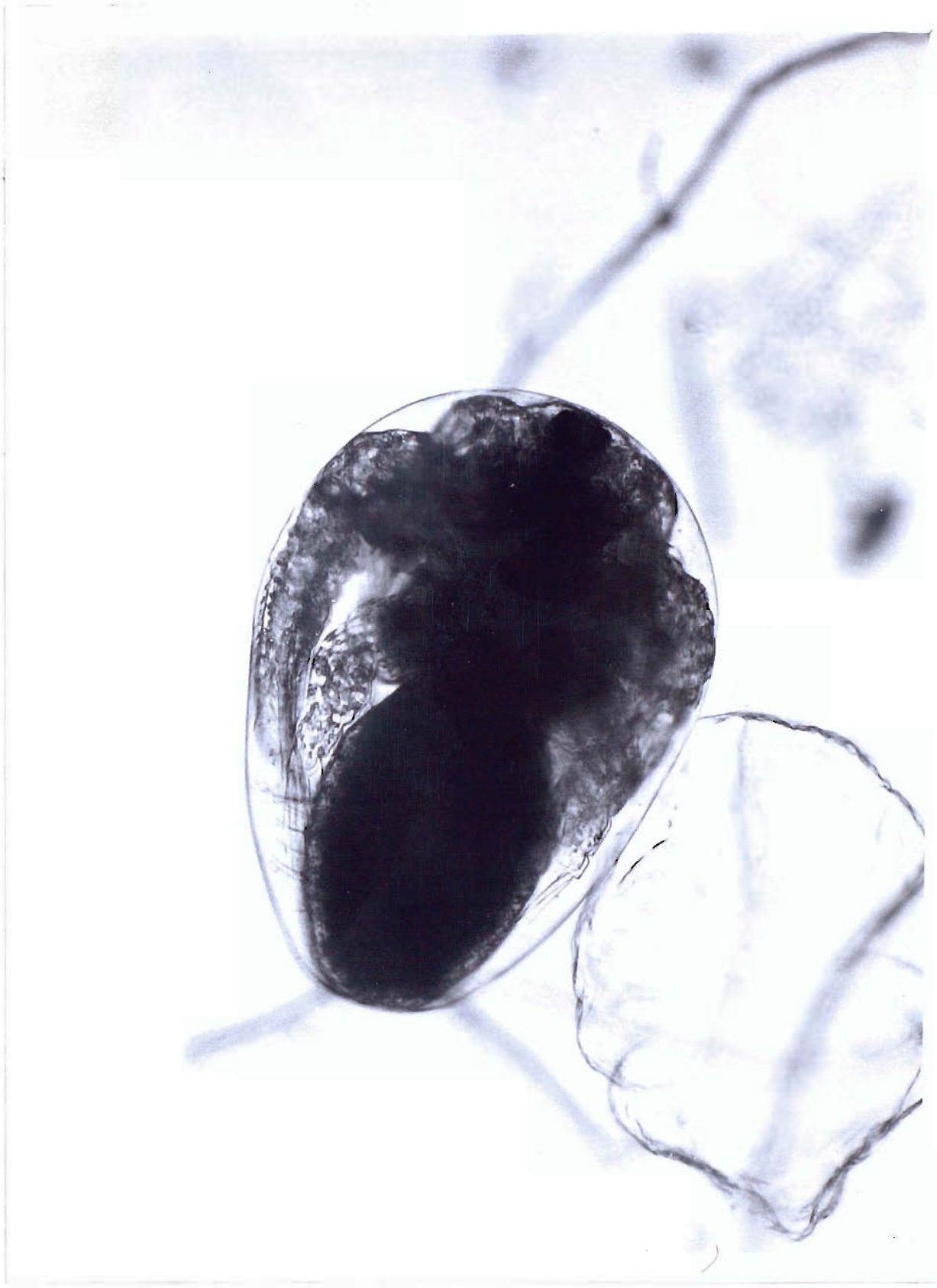


Plate [18]

Plate [19]. A nauplius immediately after hatching.

Plate [19]

5.4 The types of embryonic movements and possible existence of a hatching movement

From the long microscopical observations which have been carried out throughout the course of the present study, it was noticed that hatching occurred always when the unhatched embryo was highly active. The unhatched embryo was moving its appendages, actively pushing and abrading the hatching membrane. Very long and careful microscopical observations were carried out on a large number of embryos in order to examine the hatching process. These observations showed that in all the hatching cases which had been observed there was a sudden bursting of the hatching membrane, which always happened when the embryo was moving its appendages actively. Furthermore, the microscopical observations also have shown that there are spiny projections in the joints of the protopodites of the second antennae, Fig. [23] and plate [20]. These projections face the hatching membrane. The microscopical observations suggest that the antennal movement may have a major part in rupturing the hatching membrane through the abrading action of these spiny projections. Moreover, this would not be surprising because it is known that the second antennae are the chief organs of locomotion in the larval stages; furthermore, they are relatively powerful structures. Also it was noticed that in all cases of hatching the head region, and especially the second antennae, were the first parts to extrude. The sudden bursting of the hatching membrane always occurs at the time when the rate of the movements is maximal. The unhatched embryo, as has been said, executes another movement along its longitudinal axis (rotatory movement). The latter movement, however, is not as frequent as the antennal movements. The significance of the rotatory movement may be that it enables the embryo to locate the weak areas in the hatching membrane by changing its position through this movement. Plate [21] shows this rotatory movement.

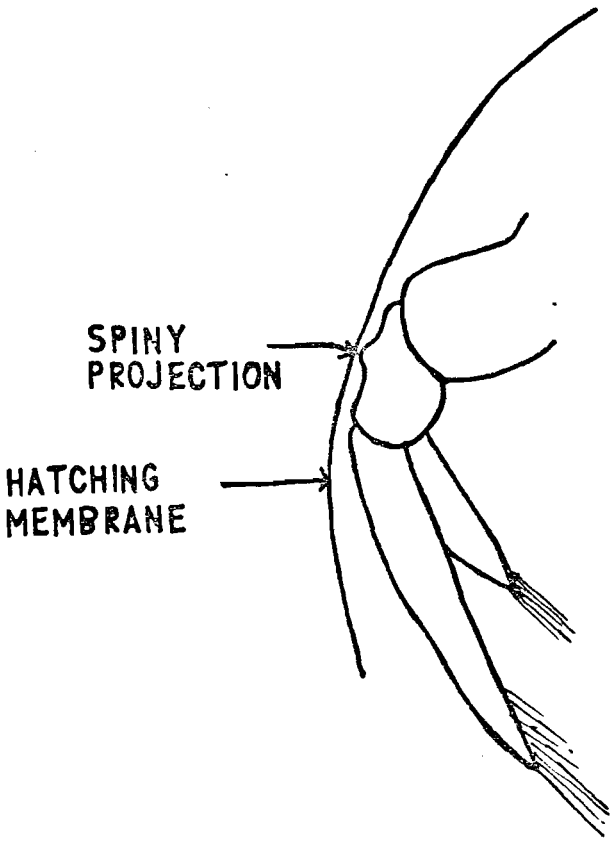


FIG. 23 : SECOND ANTENNA OF NAUPLIUS SHOWS THE SPINY PROJECTION.

Plate [20]. Hatched nauplius showing the spiny projections in the joints of the protopodites of the second antennae.

Plate [20]

Plate [21]. Shows the rotatory movement of the unhatched embryo along its longitudinal axis.

Plate [21]

Both the antennal and the rotatory movements were studied carefully from the time they first started to appear. The unhatched embryo starts to move its appendages 6-8 hours after the break has occurred, whereas it starts to move along its longitudinal axis 8-10 hours after the occurrence of the break. The rate of the embryonic movements at the beginning were 23 and 10 movement / 10 minutes for the antennal and the rotatory movement respectively. Then the rate of the movements was gradually increased with the time. The maximum number of the antennal movements was 120 movement / 10 minutes, whereas that for the rotatory movements was 40 movement / 10 minutes. Both maxima were recorded about 30 hours after the break has occurred. Fig. [24] and Fig. [25] show the increase in the rate of both embryonic movements from the beginning until 30 hours after the break has occurred, when the maxima were observed. Finally, it is possible to consider the antennal movement as a hatching movement aiding in the rupturing of the hatching membrane. However, one must not exclude the possible action of the osmotic pressure. Hall and MacDonald (personal communication) failed to detect glycerol in the post-breaking stage. The presence of an enzyme aiding in the hatching process is also a possibility. Broch (1965) stated that in all the hatches he had observed in C.bundyi, the embryo was extremely active.

It is of interest to add that the embryonic movements were also observed in those embryos which failed to hatch. The embryos started to be less active and the rate of their movements started to decline 4-5 days after the break had occurred. Ten days after the appearance of the break, the rate of the movements were 20 and 6 movement / 10 minutes for the antennal and the rotatory movements respectively. However, at that time the unhatched embryos within the hatching membrane continued their

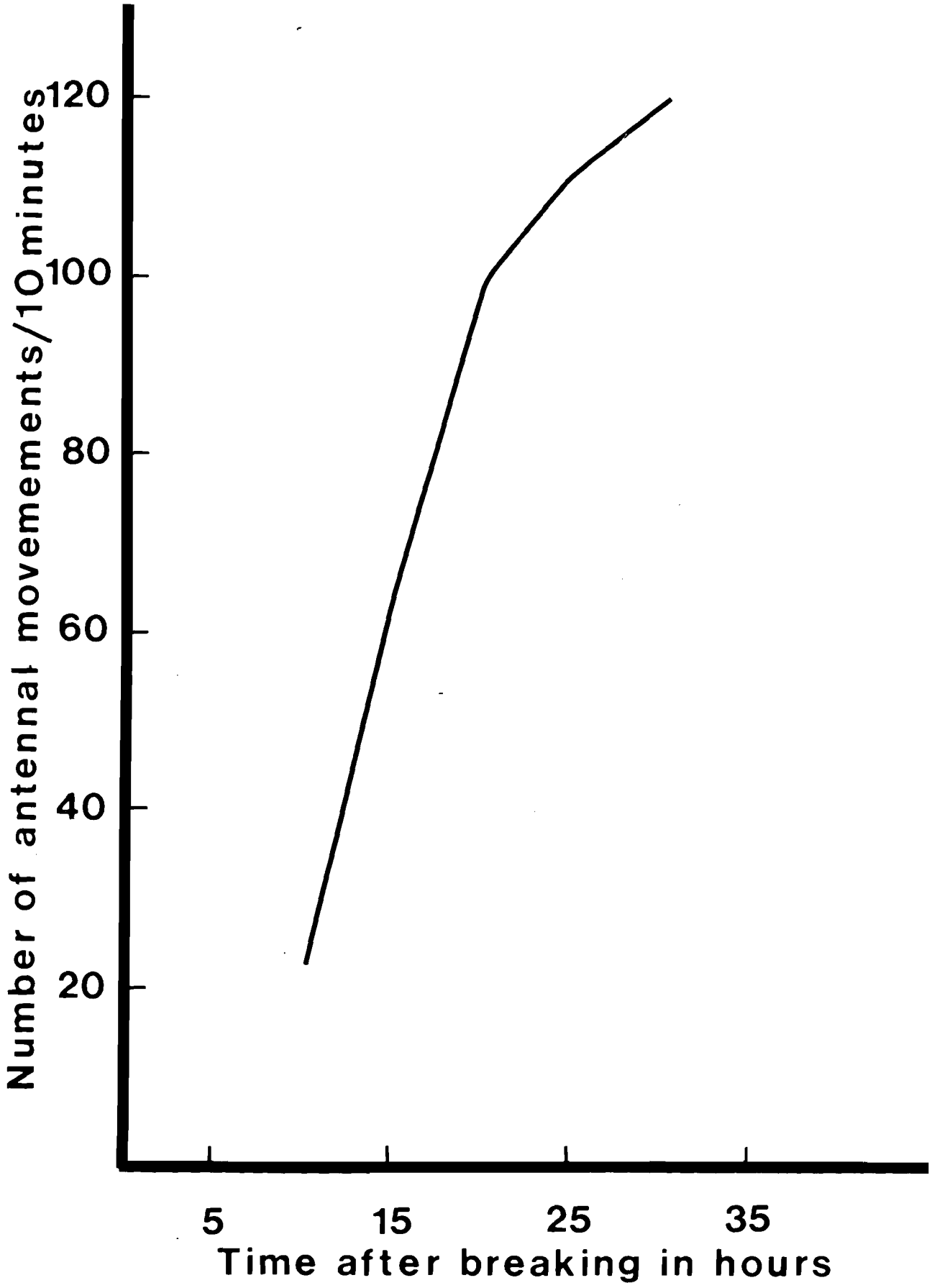


FIG. 24 : INCREASE OF ANTENNAL MOVEMENT WITH TIME AFTER BREAKING

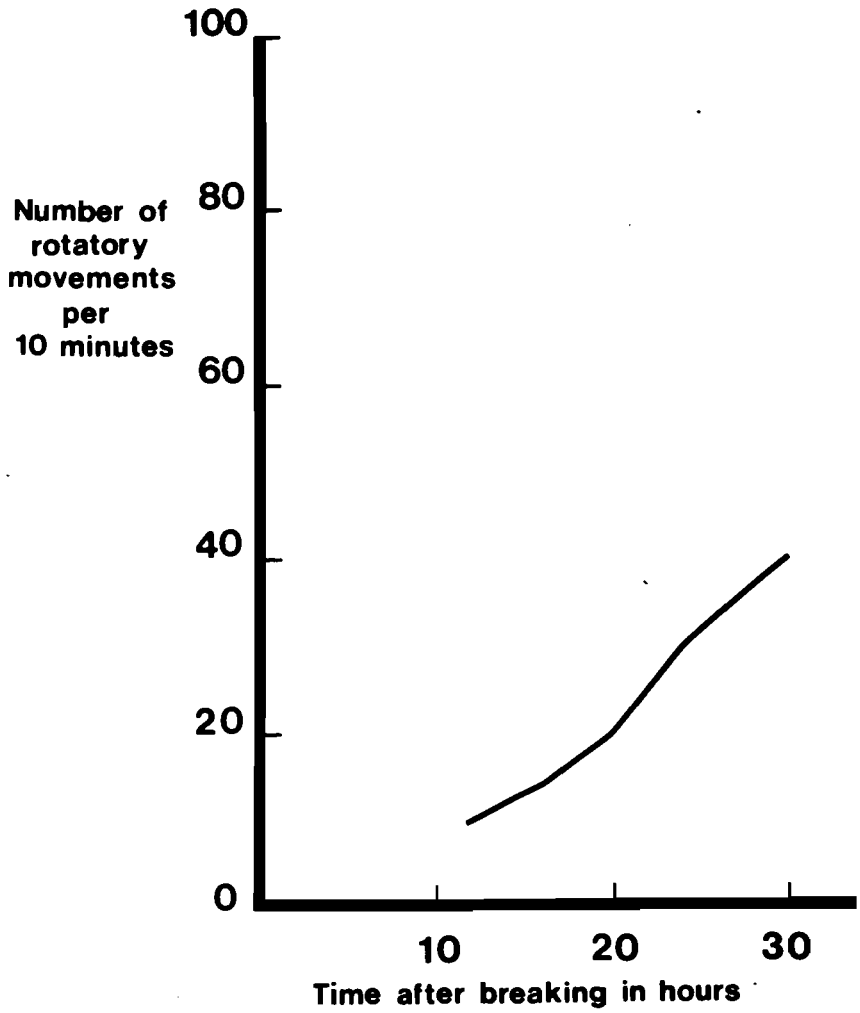


FIG. 25

INCREASE OF ROTATORY MOVEMENT WITH TIME AFTER BREAKING

development and developed the compound eyes. Moreover, the segmentation of the abdominal region was very distinct. Thus those embryos which have failed to hatch can be distinguished as second instar larvae, plate [22].

Plate [22]. Shows unhatched embryo which failed to hatch within its hatching membrane (compound eyes are very clear, together with the abdominal segmentations).

Plate [22]

5.5 Size increase of the unhatched embryo after breaking and its relation to the hatching process

There was no increase in the diameter of the developing egg throughout the course of the embryonic development up to a point just before the occurrence of the break in the tertiary shell. Immediately before the occurrence of the split, the egg content starts to swell, due to the osmotic inflow of water from the surrounding medium. The size of the embryo at the time of the occurrence of the break in the tertiary shell was 303 micron, whereas the size increased to 407 micron, twenty hours after the break has occurred. The latter was the maximum size reached by the unhatched embryo in distilled water. The egg diameter before reaching breaking stage varies between 250 and 300 micron. Fig. [26] shows the rate of size increase of the unhatched embryo from the first moment of the appearance of the break in the tertiary shell. Moreover, the photomicrographs shown in the previous section also show clearly the gradual size increase of the unhatched embryo. It is of interest to mention that in all hatches observed under the microscope, it was noticed that the rupture of the hatching membrane occurred when the unhatched embryo reached its maximum size. Moreover, by that time the embryo was highly active.

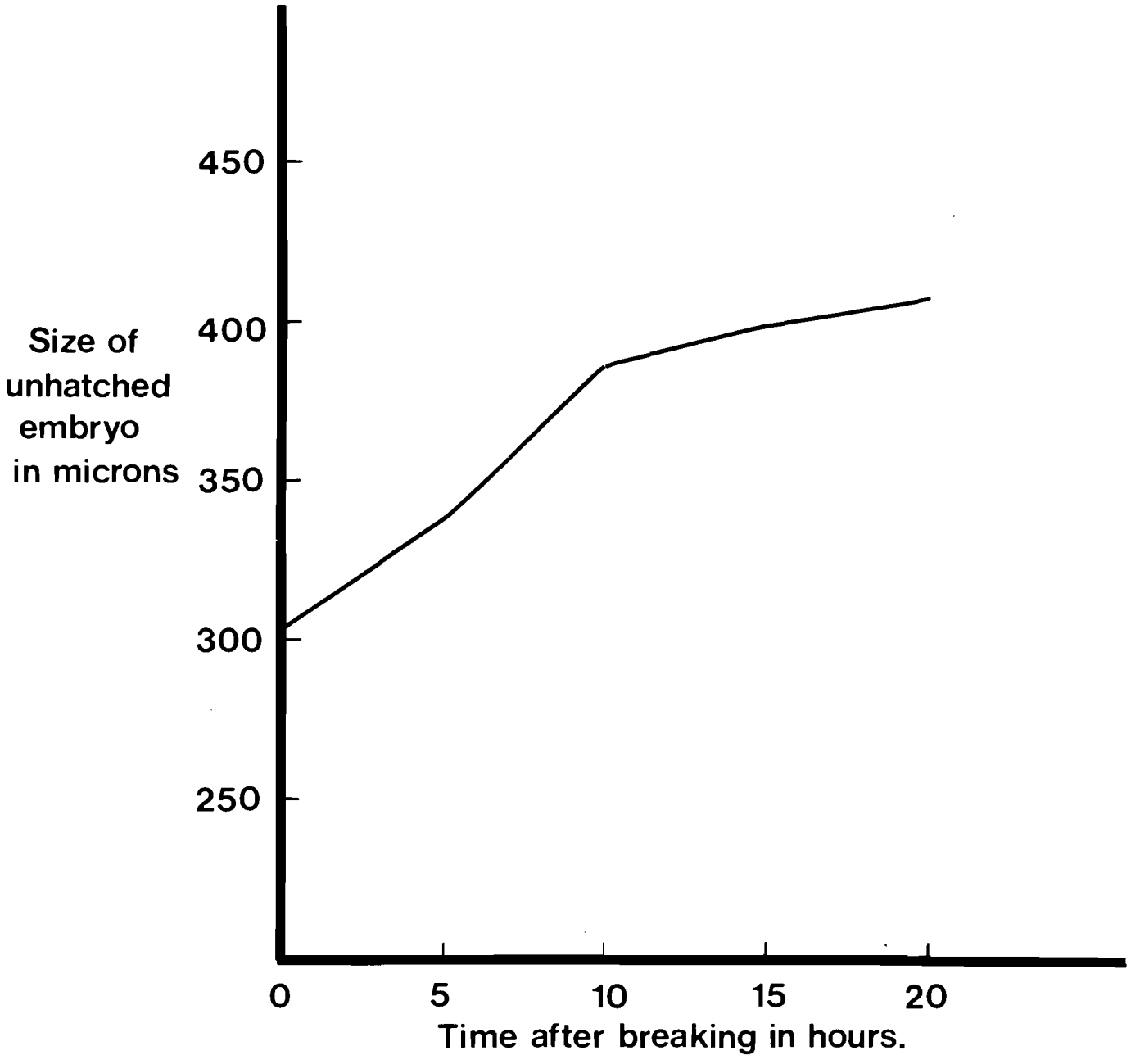


FIG. 26

INCREASE IN SIZE OF UNHATCHED EMBRYOS IN DISTILLED WATER

5.6 Discussion

In his work with C.diaphanus, Baird (1849) used the word rupture referring to the occurrence of the split in the tertiary shell. Then he referred to the actual escape of the nauplius from its hatching membrane as hatching. Hall (1953) re-described these two phenomena as breaking and hatching respectively. Meiklejohn (1929) used the word splitting to refer to the breaking, when he stated "after 15 days the coat of one of them (eggs) was discovered splitting". In his work with Chirocephalus nankinensis (shen), Hsu (1933) described the occurrence of the break in the tertiary shell as a hatching. No fixed terms were used to refer to these two processes; moreover, nothing has been said about the cause of these two processes in the anostracan species. There are two types of considerations which indicate that the occurrence of the split in the tertiary shell of the C.diaphanus eggs is caused by an osmotic action. The first evidence is the presence of an accumulation of metabolic substances such as glycerol in the breaking stage, which leads to an increase in the inner osmotic pressure and then aids in the process of the inflow of water. The second evidence is that the unhatched embryo is incapable of any movement whatsoever when the break occurs in the tertiary shell, since the embryo starts to move its appendages 6-8 hours after the occurrence of the break.

The true hatching is more likely to be caused by the movement of the embryo itself, although one must not exclude the possibilities of an osmotic action or the presence of a hatching enzyme. No embryo hatched before its rate of movement had reached a peak. This also confirms the important role played by the embryonic movements in rupturing the hatching membrane. The presence of spiny projections in the joints of the protopodites of the second antennae is further evidence to strengthen this

point of view. Moreover, it was demonstrated by Hall and MacDonald (personal communication) that there was no glycerol accumulation in the post-breaking stage. Although the hatching process in some crustacean species may be caused by the mechanical aid of the mother (Davis, 1964), this possibility is excluded in C.diaphanus, since (a) the eggs after being laid sink to the bottom of the pond and develop there, and (b) the eggs hatch in the absence of the adults. Thus in summary the complete escape of the nauplius from its membranes involves two stages, (a) the breaking stage which is strictly an osmotic phenomenon, and (b) the hatching stage, which is caused by the action of the embryonic movements.

SECTION SIX

A STUDY OF THE INFLUENCE OF DRYING ON THE EMBRYONIC DEVELOPMENT AND HATCHING

SECTION 6

A study of the influence of drying on the embryonic development and hatching

6.1 Introduction

The fairy shrimp C.diaphanus, as a species fitted for life in temporary freshwater ponds,must have some special means by which it can overcome drying out of the habitat. Drying out of temporary ponds is irregular depending on many factors. Because of this irregularity many successive generations may be eliminated from the pond by desiccation before the animals can reach sexual maturity. It is obvious in this species that nauplii, metanauplii and adults cannot survive drying. The egg then is the stage by which this species can survive the drying period. Mattox and Velardo (1950) noted that eggs of branchiopods could survive desiccation. They reported that dried eggs of the conchostracan branchiopod, Caenestheriella gynecia, would develop and hatch. The capability of the eggs to withstand a dry period is therefore seen to be the way by which such species are able to survive drying and repeat their generations. Hall (1953) found that short periods of desiccation would retard the embryonic development of C.diaphanus. He also suggested that some development had taken place while the eggs were dry, but that investigation on a large scale was necessary to substantiate this point. The necessity for a drying period for the development and hatching has been reported by several other investigators for many species of branchiopods. Hay and Hay (1889) and Weaver (1943) had failed to hatch eggs of the fairy shrimp without drying. Longhurst (1955) reported that anostracan species have an adaptation to the temporary pond through their drought-resistant egg, in which hatching occurs only after desiccation. On the other hand, Avery (1939) in his work on E.vernalis found that eggs of this species had hatched without drying. The same was observed for the same species by Castle (1938). Other

investigators who reported hatching without drying for anostracan branchiopods were Baird (1849), Broch (1965), Hall (1953), Mathias (1926), Moore (1951), Nourisson (1964) and Prophet (1963). Bishop (1968), working with Limnadia stanleyana King, reported that there were no significant differences in the proportion that hatched without drying and the proportion that hatched after drying, but no indication was found for the degree of desiccation other than complete dehydration on silica gel. Most of the work which has been done on the effect of drying on the embryonic development and hatching of the fairy shrimp dealt with the question of whether drying is necessary for development and hatching or not. Nothing has been said about the influence of different levels of desiccation. Only a few workers have taken the effect of different humidities into consideration, although eggs of the fairy shrimp are not subjected to a fixed condition of desiccation under natural conditions. The need for a comprehensive study was indeed suggested by Hall (1953). The question is whether the egg can develop and hatch if it is previously subjected to desiccation or not. If it does, what degree of desiccation can eggs withstand? For how long can eggs survive such conditions? And by what means can they retain their water? All these questions need answers in order to complete our understanding of this matter. Broch (1965) has briefly studied the effect of different relative humidities on the survival of the unhatched (prehatched) embryos of the fairy shrimp C.bundyi. He found that prehatched embryos which have been kept at relative humidities below 100% collapsed after two days. They did not recover again when transferred to water. However, he did not mention any study on the embryo before or at breaking stage. In his work on S.seali, Moore (1967) stored eggs for 40-60 days at different humidities. He found that the desiccation influences both rate of hatching and total percentage hatch. From this brief introduction it

seems that no comprehensive study has been carried out in this field. What happens to the egg after a prolonged period of desiccation is not known. Is there any influence of drying on the structure and size of the egg? Does the egg develop in the absence of free water? If it does, how far can it go? By what means does the egg survive severe desiccation? Does it lose some of its water? This present study was carried out to throw some light on this problem.

6.2 Methods and material

Eggs used throughout the experiments described in this section were collected from adult females brought from the natural habitat or from females from the laboratory culture. Techniques used in collecting and separating the eggs were described elsewhere in this thesis. Eggs were first washed with either distilled water or tap water to remove detritus and other suspended materials. The age of any egg that had been used was thus less than twenty-four hours after laying.

Various solutions of sodium hydroxide and sulphuric acid were used to provide different relative humidities, as described by Solomon (1945, 1951). Solutions providing relative humidities of 25, 50, 75, 83 and 96% were prepared. A humidity of 100% was provided using a beaker containing distilled water placed in the humidity chamber (desiccator). Complete desiccation of 0% relative humidity was established using dry silica gel as a desiccant. Estimation of humidities was made using cobalt thiocyanate papers (Solomon, 1957). For comparison with standards, a "Lovibond solids comparator" was used. The required solutions were placed in small beakers. These beakers were then transferred into different humidity chambers (desiccators). Batches of eggs were counted, washed and then placed in small watchglasses 2 cm. in diameter. Free standing water was pipetted off. Furthermore strands of filter paper were used to remove as much of the free water left as possible. This latter method has been carried out under the microscope to avoid any reduction in the number of eggs which might attach to the strands. The eggs were then allowed to dry at laboratory temperature for half an hour. These watchglasses were transferred into the humidity chambers. Desiccators were stored at different temperatures of 22, 15, 10, 5, 0 and $-5^{\circ}C$ ($\pm 2^{\circ}C$). Batches of eggs were removed at

fortnightly intervals from the humidity chambers to laboratory conditions, and were covered with water. They were kept under daily observation for any sign of breaking or hatching. To incubate eggs at 100% relative humidity they were placed in small watchglasses and these, in turn, were kept in humidity chambers containing beakers with distilled water. Some eggs, however, were placed on damp filter papers and these were kept in the humidity chambers. This method provides the egg with 100% relative humidity, but still some free water could be available. This is more likely the condition in nature where the eggs are surrounded by moist soil. Damp filter papers were kept wet throughout the course of the experiment.

To see if desiccation has any effect on the structure and size of the eggs, batches of eggs were placed in watchglasses. Water was pipetted off and all free water was withdrawn using filter paper strands. Then the eggs were left for half and hour in laboratory conditions to dry. Watch-glasses were kept at different humidities as described before. Again desiccators (humidity chambers) were kept at laboratory temperature of $22^{\circ}C$ ($\pm$ $2^{\circ}C$). Weekly observations were carried out. Diameters of the eggs were measured under the microscope using an ocular micrometer at 140X magnification. The egg shell was removed by soaking the egg in 5% sodium hypochlorite solution. These steps were carried out to see the change in the structure of the egg and to locate the air space formed after desiccation. In the sodium hypochlorite technique eggs were placed in solid watchglasses containing 5% solution of this substance for 5-15 minutes. This time depends on the degree of humidity in which the eggs were previously kept. After the time required to dissolve the tertiary shell had elapsed, eggs were removed, washed several times with water and then placed in other solid watchglasses. Another technique was also used to remove the egg shell, using concentrated solution of sodium hydroxide. In this latter method, the action of sodium hydroxide was much slower than sodium hypochlorite.

It took 3-5 days to remove the egg shell completely. However, the action of sodium hydroxide may be hastened if a boiling water bath was used.

To study the effect of different relative humidities on the unhatched embryo, and to determine its survival under these conditions, the following was carried out. To prepare unhatched embryos, freshly laid eggs were kept in distilled water for seven - eight days at laboratory temperature of 22°C ($\pm$ 2°C) until they showed a break in their tertiary shell. Then they were divided into groups. These groups were placed in small watchglasses of 2 cm. in diameter. All free water was pipetted off. Then the watchglasses were transferred and placed in humidity chambers having various relative humidities ranging from 0 to 100%.

To determine whether eggs are resistant to prolonged periods of desiccation, groups of a very large number of eggs were placed on filter papers. Then they were left to dry for half an hour at laboratory temperature. The filter papers containing the eggs were placed in plastic petridishes and stored for different periods of time under laboratory conditions.

Batches of eggs were also prepared to study the rate of water loss at different relative humidities. This was done by washing the eggs several times with distilled water, then dividing them into groups of 1000 eggs each. These groups were placed in small watchglasses which had previously been weighed empty. Free water was pipetted off and strands of filter paper were used to withdraw the remaining water. Watchglasses containing the eggs were then weighed immediately after being seen to have no more free water. Then they were kept at different humidities. The watchglasses with the eggs were removed and weighed at intervals of one hour after placing them in the humidity chambers. Unimatic balance, Stanton Instruments Ltd., Model C.L.1 was used.

6.3 Results

In all the experiments which were carried out in this series of studies, there was no evidence to suggest that drying is essential for development and hatching at least in this species, since in all investigations undertaken in this study eggs were collected from beakers containing adults, washed and placed in hatching chambers and kept for subsequent studies. Moreover, the routine hatching procedure used in this laboratory was to use eggs without drying. However, the small proportions of eggs which had failed to develop and hatch were dried and re-wetted again. None of them was noticed to develop or hatch. This method was to investigate the possibility of whether this small proportion needed a drying period to complete their development or not. It seemed to be that this was not the case.

6.3.1 Effect of desiccation on the structure, size and viability of the eggs

The egg of C.diaphanus was first described by Baird (1849), who referred to an external thick coat and inner transparent envelope. Hall (1953) re-described the egg of this species in more detail. He distinguished a third membrane occurring between the two referred to by Baird (1849) and by Mawson and Yonge (1938). The description of Hall (1953) is the more likely one to accept, since microscopical observations have revealed this third membrane. The size of the dried eggs has been seen not to differ significantly from those which had not been dried. However, it was noticed that dried eggs had slightly shrunk. Dissection of dried eggs showed that the embryonic mass inside the shell had collapsed. To locate the air spaces formed after desiccation two methods were used; firstly the dissection of the eggs, and secondly the removal of the egg shell using sodium hypochlorite solution. Both methods revealed that air spaces are

formed between the tertiary shell/chitinous membrane complex and the embryonic mass. After removing the egg shell the embryonic mass was found to be deeply indented on one side. The degree of indentation seemed to depend on the relative humidity at which eggs had previously been kept. Those eggs which had been kept at 100% relative humidity showed no indentation. All other eggs kept at relative humidities below 100% had shown such indentation. Plate [23] shows the indentation in a dried egg in comparison with an undried one. On the other hand the removal of the egg shell of the undried egg showed no indentation. It revealed a spherical embryonic mass, plate [23]. All eggs which have been kept at different relative humidities were found to float when placed again in water. Flotation time seemed to depend on the relative humidity at which eggs were previously kept. It has been found that eggs kept at 100% relative humidity did not float when returned again to water. For the eggs which have been kept at 83% relative humidity, flotation time was 12 hours approximately. For those kept at 25, 50 and 75% relative humidity it was 18-24 hours approximately. At the lowest relative humidity, that is to say 0%, the time was approximately 24-36 hours. However flotation time varies depending on the duration of desiccation. It may be longer than that mentioned before if eggs were kept dry for longer periods of time.

Plate [23]. Shows the indentation in the dried egg in comparison to undried egg after the removal of the tertiary shell (no indentation occurred in the wet egg).

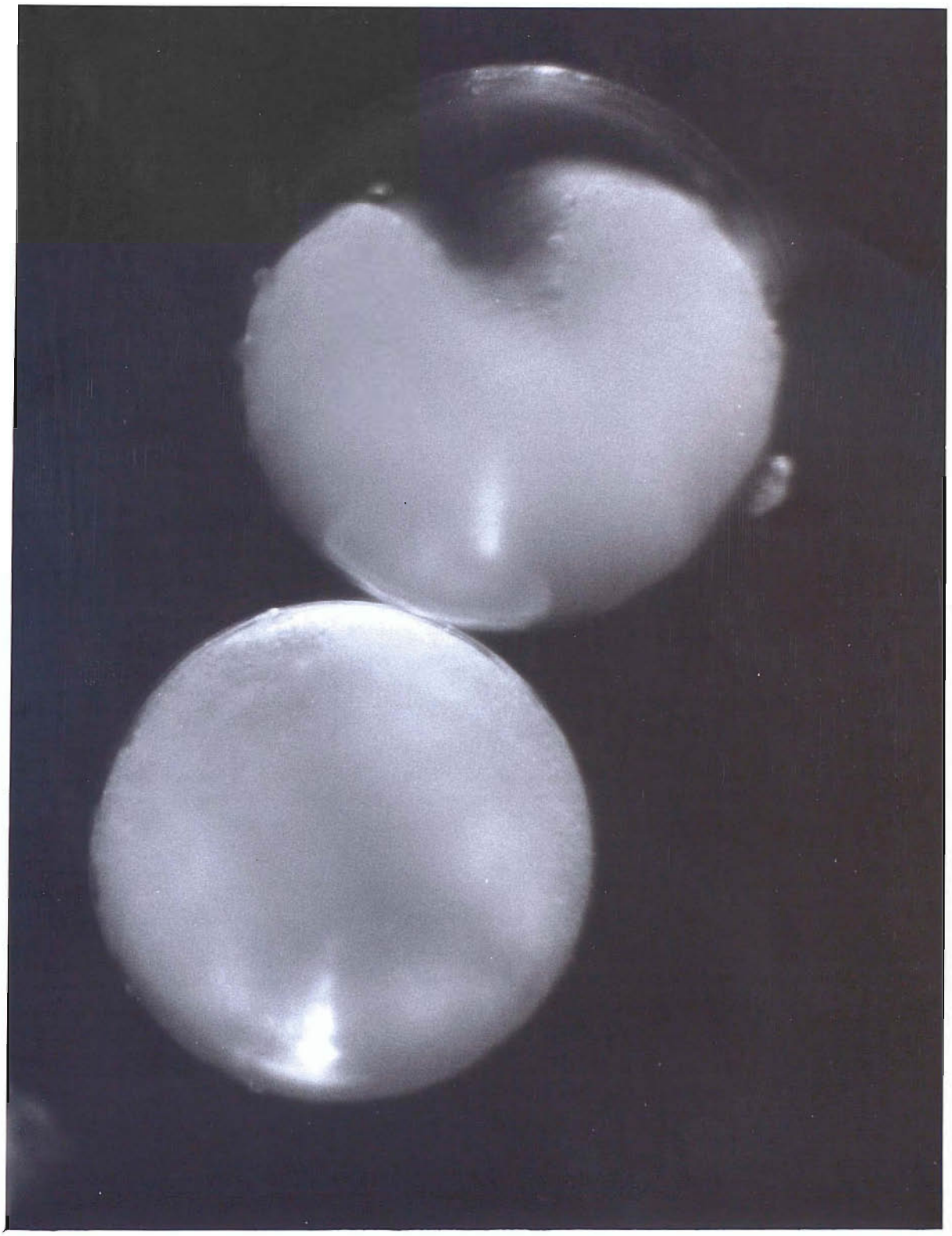


Plate [23]

6.3.2 Survival of unhatched embryos at different relative humidities

A very slight change was observed in the size of the unhatched embryos which had been kept at 100% relative humidity. It seemed that they did not lose a significant amount of water. They were seen alive for the next few days of observation. It was also noticed that their activities were less than those shown by embryos kept always in water. Those embryos which had been kept on damp filter paper also had changed in size slightly. However, their movements were observed to be less. No embryo had hatched in either case while they were under these conditions. Unhatched embryos which had been kept at less relative humidities were more affected and could not survive and stay alive for a long period of time. At relative humidities below 100%, that is to say at 83 and 96%, the hatching membrane had collapsed and the embryo had shrunk. However, they stayed alive for nearly half an hour. After this time had elapsed, it was noticed that they had died. No embryo had recovered after this treatment when transferred to water again. But when the unhatched embryos were kept for ten minutes only they recovered when returned to water again. Furthermore, some of them even had hatched after this treatment. Unhatched embryos kept at 25, 50 and 75% relative humidity had died 15-20 minutes after placing them in the humidity chambers, whereas at 0% relative humidity they did not survive a period of ten minutes.

6.3.3 The ability of the eggs to develop in the absence of free water

Eggs which had been kept on damp filter papers developed normally and reached breaking stage. These eggs started to show the break in the tertiary shell on the eleventh day after placing them on the damp filter paper at laboratory temperature. However, the peak break occurred on the twelfth day when 65 per cent of the eggs had shown the break in the tertiary shell. No hatching was observed in these eggs while they were still on the damp filter papers. Yet some had hatched when they were transferred and placed in water.

At lower temperature, that is to say 15°C, eggs which had been kept on damp filter paper took 20 days to show the break. The peak break in this case occurred on the twenty-second day after placing them on the damp filter paper. Slower embryonic development seemed to have taken place at a temperature of 10°C. At this temperature the eggs required approximately five weeks to reach breaking stage, whereas at a lower temperature of 5°C, eggs required nine weeks to show the break. Much slower embryonic development seemed to have taken place at the lower temperature of 0°C., since some of the eggs reached breaking stage 18 weeks after placing them on the damp filter papers. It was found that no embryonic development took place in those eggs which had been kept on damp filter paper at -5°C. It seemed that all eggs kept at -5°C were dead. This was tested when they were transferred into water and kept at laboratory conditions. None of them had reached breaking stage even after an immersion of one month.

The course of the occurrence of breaking was observed to be irregular in eggs kept on damp filter papers, especially at lower temperatures. It was also noticed that slower embryonic development seemed to have taken place at 100% relative humidity (without free water) than

that found in eggs kept on damp filter papers. This was seen when some of those eggs which had been kept at 100% relative humidity reached breaking stage within two days of their transfer to water. Moreover, some of them had reached breaking stage while they were still in the humidity chamber; they were however few in number. At the corresponding temperature, it was found that embryonic development which had taken place was slower than that found in those eggs kept on damp filter papers. It is of interest to mention that eggs which had been kept at 100% relative humidity completed their embryonic development although no free water was available. It is also interesting to note that most of the eggs which had been kept in humidity chambers having 100% relative humidity (without free water) at a temperature of $-5^{\circ}C$ stayed viable, whereas those eggs which had been kept on damp filter paper at the same temperature were found to be dead. In the latter case eggs could not survive even for twenty-four hours. Some fungal growth has been seen in many cases, but it seemed that this did not affect the viability of the eggs significantly if the latter were kept for short periods of time.

6.3.4 Embryonic development and hatching at different relative humidities

As has been mentioned earlier, two major stages of embryonic development were studied, namely breaking stage and hatching, in this series of experiments. No embryonic development seemed to have taken place under conditions of severe desiccation, since it was found that eggs which had previously been kept in desiccators having 0% relative humidity required the normal time to reach breaking stage (seven-eight days) or more when transferred to water. In those eggs which had previously been kept at 0% relative humidity for 2-5 weeks the time required for the break to appear in the tertiary shell was one-two days longer than in the case of eggs allowed to develop without drying. It was noticed that as the drying period increased, the delay in the appearance of the break became longer. For example, in those eggs which had previously been kept at 0% relative humidity for 7-19 weeks, the delay in the appearance of the break was 2-3 days. Not only this but the course of the occurrence of the break in the tertiary shell was irregular. This irregularity tended to be clearer as the desiccation period had increased. Furthermore, a clear reduction in the percentage break was noticed. For example, percentage of breaking as low as 40, 59, 34 and 36 were found for those eggs which had previously been kept for 10, 12, 15 and 19 weeks at 0% relative humidity respectively. However, the eggs which showed breaking are scattered over a period of 14-16 days. The latter pattern shows the irregularity in the appearance of the break after long periods of desiccation.

At higher relative humidities, that is to say 25, 50, 75 and 83%, a delay in the occurrence of the breaking stage was also observed. The time required for the break in the tertiary shell to occur was 1-2 days

longer than in the case of eggs allowed to develop without desiccation, so no development seemed to have taken place while the eggs were in the humidity chambers. Irregularity in the appearance of the break was also observed at relative humidities of 25, 50, 75 and 83%. This irregularity became clearer as the period of desiccation increased, and some of the eggs had reached breaking stage even after a period of two months. Irrespective of the desiccation level, a drying period as long as 42 days seemed to have no significant effect on the embryonic development, since percentage of breaking was always high. For example, 42 days after placing the eggs at 0, 25, 50, 75 and 83% relative humidity, the percentage of breaking was 90, 86, 89, 97 and 90 respectively. At higher humidities, that is to say 96% and 100% (without free water) high percentage of breaking was found even after a period of desiccation as long as 63 days. The latter period seemed to have no significant influence on the embryonic development if eggs were kept at higher humidities. Some embryonic development seemed to have taken place while eggs were in the humidity chamber having 96% relative humidity, since eggs reached breaking stage within a few days of their transfer to water. At relative humidity of 100% (without free water) the percentage of breaking was always high. Not only this, but even some of those eggs kept at such high humidity had reached breaking stage while they were in the humidity chambers. Periods of desiccation longer than 63 days seemed to affect the embryonic development at all levels of desiccation, since a decline in the percentage break was observed after this period. For example, 84 days after placing the eggs at 0, 25, 50, 75, 83 and 96% relative humidity the percentage of breaking was 59, 47, 22, 26, 37, and 56 respectively. Smaller numbers of eggs were seen to have reached breaking stage as the desiccation period had increased. After a desiccation period of 105 days at 0, 25, 50, 75,

83 and 96% relative humidity the percentage break was 34, 31, 21, 23, 28 and 44 respectively. No egg reached breaking stage at 50 and 75% relative humidity after a desiccation period of 133 days, whereas at 0, 25, 83 and 96% relative humidity the percentage of breaking was 36, 16, 4 and 31 respectively. One would expect that the percentage break in those eggs which had previously been kept at 0% relative humidity would be less than those kept at 50 and 75% relative humidity, but this was not the case. This matter will be dealt with and explained later on in this chapter, and a possible explanation will be given. Table [8] shows the percentage of breaking and percentage hatch at various relative humidities after different periods of desiccation.

The influence of varying the period of drying on the advanced stage egg seemed to follow generally the same pattern as those eggs which had been dried immediately after laying. It was found that advanced stage eggs seemed to withstand a desiccation period longer than those which had been dried after laying, since advanced stage eggs remained viable for a longer time. It was observed that the percentage break of advanced stage eggs started to decline 105 days after placing them in the humidity chamber, whereas the percentage of breaking of those eggs which had previously been dried immediately after laying started to decline 56 days after placing them in the humidity chambers. However, a great decline in the percentage break of those eggs which had previously been dried immediately after laying was observed 84 days after placing them in the humidity chambers. Eighty-four days after placing the advanced stage eggs at different relative humidities the following percentage of breaking was found 74, 66 and 80 at 0, 25 and 83% relative humidity respectively, whereas after a period of 105 days the percentage break was 66, 47 and 67 respectively. Longer period of incubation seemed to affect the viability of the eggs clearly,

TABLE [8]. Percentage break and percentage hatch of early stage eggs at various combinations of relative humidity and incubation time at 22°C.

Breaking B
Hatching H

Time weeks	Relative humidity 0%		25		50		75		83		96		100	
	B	H	B	H	B	H	B	H	B	H	B	H	B	H
1	94	77	85	66	95	58	98	59	90	63	85	68	96	81
2	88	69	95	66	95	52	94	51	92	73	98	71	99	77
3	98	68	95	42	95	45	87	31	89	65	95	75	89	68
4	98	70	80	64	93	75	98	68	88	71	96	76	93	70
5	96	60	78	41	90	58	98	62	80	57	98	70		
6	90	48	86	37	89	44	97	35	90	45	90	72		
7	80	57	72	24	66	23	86	25	83	33	78	49		
8	68	38	74	20	61	40	77	27	77	28	83	55		
9	68	32	54	20	55	21	57	27	66	23	87	50		
10	40	19	59	27	18	3	33	16	48	11	61	29		
12	59	17	47	11	22	5	26	12	37	4	56	21		
15	34	5	31	16	21	3	23	7	28	0	44	11		
19	36	2	16	0	0	0	0	0	4	0	31	15		

since 133 days after placing those eggs which had previously been dried immediately after laying, the percentage of breaking was 36, 16 and 4 at 0, 25 and 83% relative humidity respectively; whereas the percentage break for the advanced stage eggs after the same period of desiccation was 68, 30 and 46 respectively. It was also observed that the resulting unhatched embryos became weaker as the period of desiccation increased, since a reduction in their movements was noticed. The subsequent hatching of the embryos seemed to be more affected than the breaking by increasing the period of drying, since,in general, the percentage of hatching was much less than the percentage found for the breaking. For example, after a desiccation period of 42 days the percentage break was 90, 86, 89, 97, 90 and 90 at 0, 25, 50, 75, 83 and 96% relative humidity respectively, whereas the percentage of hatching was 48, 37, 44, 35, 45 and 72 respectively. Table [9] shows the percentage of breaking and hatching for the advanced stage eggs after different periods of desiccation at various

TABLE [9]. Percentage break and percentage hatch of advanced stage eggs at various combinations of relative humidity and incubation time at 22°C.

Breaking B
Hatching H

Time weeks	Relative humidity 0%		25%		83%		100%	
	B	H	B	H	B	H	B	H
2	87	76	92	66	96	76	96	84
4	86	66	84	72	84	72	96	60
6	88	56	76	42	82	46	92	70
8	88	54	72	10	78	26	90	82
10	94	36	84	32	79	14	96	66
12	74	22	66	25	80	10		
15	66	18	47	11	67	4		
19	68	10	30	8	46	0		

relative humidities. Irrespective of the level of desiccation, the highest percentage break recorded was 94, whereas that for hatching was 77 per cent. It is of interest to note that the relationship between peak break and peak hatch was observed to be the same as that found for the undried eggs, that is to say the peak hatch occurred two days after the appearance of the peak break. However, the percentage of hatching found for dried eggs was less than that found for undried ones. This was obvious when a very low percentage of hatching was found for those eggs which had previously been dried for 105 days. The percentage of hatching was 18, 11 and 4 at 0, 25, and 83% relative humidity respectively. A few eggs hatched when they were dried for a longer period. It was found that the percentage hatch was 10, 8 and 0 at 0, 25 and 83% relative humidity respectively. These figures cited above would indicate that eggs would stay viable at 0% relative humidity for a longer period than if they were kept at 25, 50, 75 and 83% relative humidity. This above finding will be explained later in this section. Very similar results were found at lower temperatures, although the rate of embryonic development observed at high relative humidities was slower. It was found that again no embryonic development seemed to have taken place at relative humidities below 83% (inclusive) while the eggs were still in the humidity chambers. The delay in the appearance of the break at lower temperatures of 15, 10,5 and $0^{o}C$ was found to be the same as that found at $22^{o}C$. At a temperature of $-5^{o}C$ it was found that most of the eggs which had been kept at 0% relative humidity were viable even after a period of 105 days. This is very interesting because no egg seemed to have survived at this temperature for such a long period of time when eggs were kept at 100% relative humidity. The survival of the dried eggs at such a low temperature might be due to the absence of a large amount of water, which may kill the egg through the formation of

ice. All results obtained at temperatures of 15, 10, 5, 0 and -5°C are shown in Tables [10], [11], [12], [13] and Table [14], or are given in histograms in the appendix.

TABLE [10]. Percentage break at various combinations of relative humidity and incubation time at 15°C.

Time weeks	Percentage break						
	0%	25%	50%	75%	83%	96%	100%
2	96	100	96	100	100	100	98
4	92	98	98	90	96	96	100
6	98	100	100	86	96	98	72 *
8	100	78	98	88	92	96	
10	86	64	68	22	34	82	
12	90	58	56	0	0	82 *	
15	62	40	6	0	0	42 *	

* Indicates fungal growth

TABLE [11]. Percentage break at various combinations of relative humidity and incubation time at 10°C.

Time weeks	Percentage break				
	0%	25%	50%	75%	100%
2	100	96	100	90	98
4	96	90	98	92	84
6	98	80	88	90	84
8	90	78	70	72	60 *
10	84	64	70	54	

* Indicates fungal growth

TABLE [12]. Percentage break at various combinations of relative humidity and incubation time at 5°C

Time weeks	Percentage break				
	0%	25%	50%	75%	100%
2	92	94	90	86	96
4	82	86	90	82	90
6	90	82	86	90	96
8	88	76	82	80	92
10	90	78	42	38	96

TABLE [13]. Percentage break at various combinations of relative humidity and incubation time at 0°C

Time weeks	Percentage break				
	0%	25%	50%	75%	100%
2	86	100	98	100	96
4	88	98	92	100	94
6	78	98	90	88	96
8	80	86	84	90	94
10	84	80	60	58	92

TABLE [14]. Percentage break at various combinations of relative humidity and incubation time at -5°C

Time weeks	Percentage break				
	0%	25%	50%	75%	100%
2	90	90	92	92	44
4	94	76	86	92	66
6	92	92	92	78	42
8	98	90	80	88	18
10	74	80	74	84	0

6.3.5 Effect of prolonged period of drying on the viability and development of the eggs

As has been seen from the results of the previous section, a great decline in the percentage break and percentage hatching was found as the drying period had increased. The decline was very clear, especially after a desiccation period of four months. The results obtained in this investigation are in complete agreement with those obtained in the previous experiments. All these findings give an indication that eggs of this species can withstand a drying period of at least four months. It was found that eggs which had been kept dry under laboratory conditions for nearly two years would not survive, since no embryonic development seemed to have taken place after their transfer to water. Removal of the shell of the dried eggs revealed that no morphological changes had taken place in the embryonic mass; it also showed that some of those eggs were empty. Of the 810 and 1128 eggs which had been kept dry for nearly two years, none was found to be still viable. Those eggs which had been kept dry since 26th January, 2nd February, 8th March and 4th May 1971 were returned to water again on 12th October 1972. None of them reached breaking stage or hatched. However, a few of those eggs which had been kept dry since 8th July 1971, 21st December 1971 and 18th April 1972 reached breaking stage when they were transferred to water again. Of the 1110, 1670 and 2156 eggs which had been dried since the dates mentioned above, only 22, 17 and 129 eggs reached breaking stage. However, in terms of percentage break, the above figures were 2, 1 and 6 per cent. Furthermore, none of the broken eggs had hatched. Table [15] shows the number of eggs which reached breaking stage after a prolonged period of drying.

Table [15]. Percentage break and percentage hatch following various periods of prolonged desiccation.

Date eggs were desiccated	No. of eggs used	No. of eggs broken	% Break	% Hatch
23.11.70	810	0	0	
21.12.70	1128	0	0	
26.1.71	3823	0	0	
2.2.71	2635	0	0	
8.3.71	671	0	0	
4.5.71	582	0	0	
8.7.71	1110	22	2%	0
21.12.71	1670	17	1%	0
18.4.72	2156	129	6%	0

On 12th October 1972, eggs were placed in distilled water and were kept at laboratory temperature 22°C.

6.3.6 Water loss at different relative humidities

All eggs which had been kept dry at various relative humidities lost water even at 100% relative humidity (without free water). But the amount lost in the latter was only 7·5% of wet weight. It was found that there was a close relationship between the relative humidity and the rate of water loss. For example, one hour after placing the freshly laid eggs in the humidity chambers, they lost 3·50, 31·00, 38·00, 43·25, 47·20, 49·30 and 52·80 per cent of their wet weight at 100, 96, 83 75, 50, 25 and 0% relative humidity. Fig [27] shows the curves of water loss for the freshly laid eggs kept at different humidities. It is very interesting to note that eggs which had been kept at 0% relative humidity lost less water than those eggs kept at 25, 50, 75 and 83% relative humidity, although the initial loss was greater. This may be due to the rapid hardening (tanning) of the tertiary shell at 0% relative humidity. The loss of water as percentage of original wet weight was 7·50, 47·69, 59·29, 70·39, 70·83, 68·31 and 54·92 at 100, 96, 83, 75, 50, 25 and 0% relative humidity respectively.

It was found that the pattern of water loss from the advanced stage eggs which had been kept at various relative humidities seemed to be similar to that found for those eggs dried immediately after laying, although the rate of water loss was slower. The rate of water loss was also closely correlated with the relative humidity. For example, one hour after placing the advanced stage eggs at different humidities, they lost 2·98, 27·37, 41·72 and 46·82 per cent of their original wet weight at 100, 83, 25 and 0% relative humidity. Again the total loss of water at 0% relative humidity was found to be less than that found at 25 and 83% relative humidity. The total loss of water, as a percentage of the original wet weight, was 6·38, 52·71, 65·11 and 49·00 at 100, 83, 25 and 0% relative humidity. Fig. [28] shows the representative curves of water loss for the advanced stage eggs kept at different relative

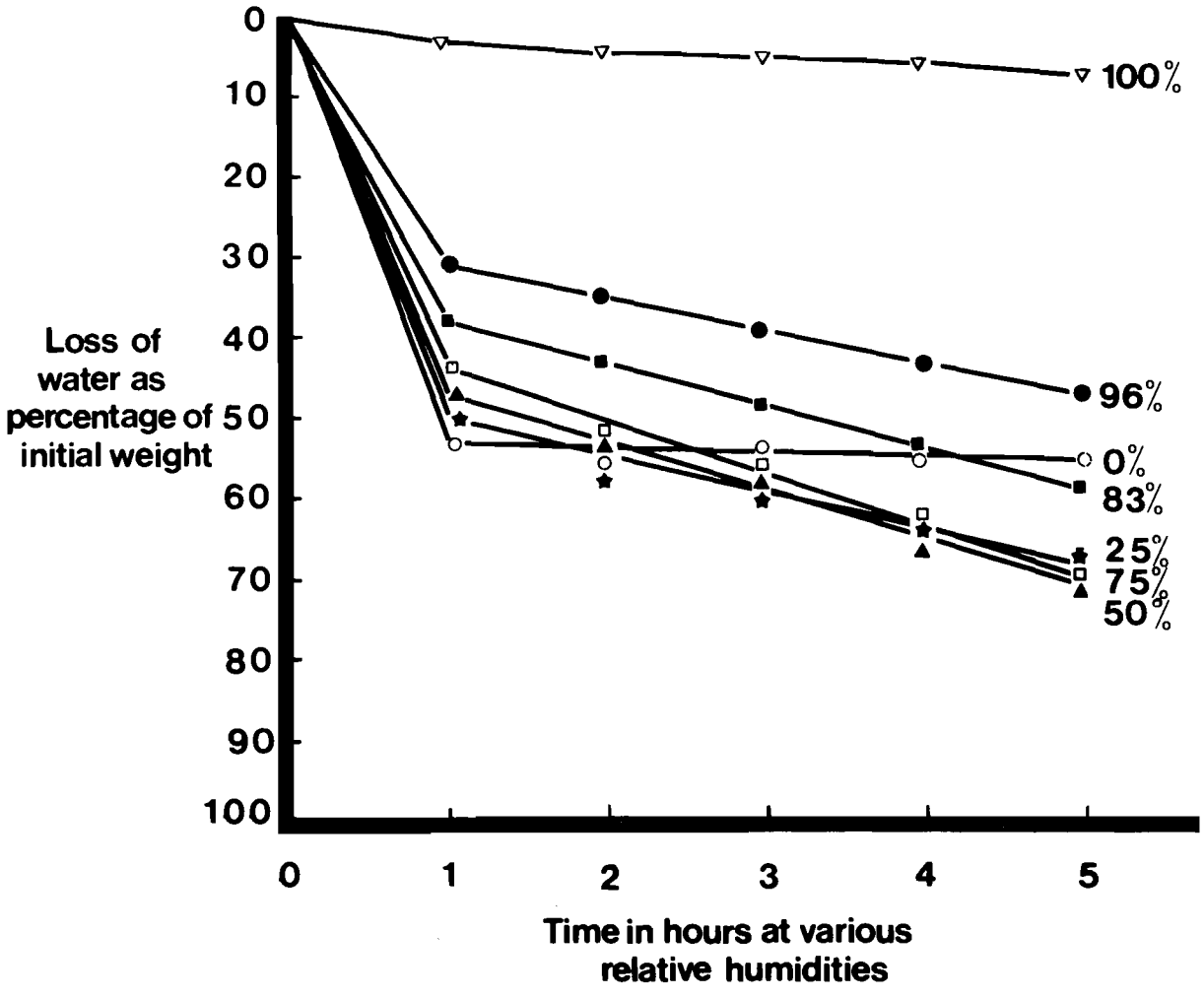


FIG. 27 : WATER LOSS OF EARLY STAGE EGGS AT VARIOUS RELATIVE HUMIDITIES

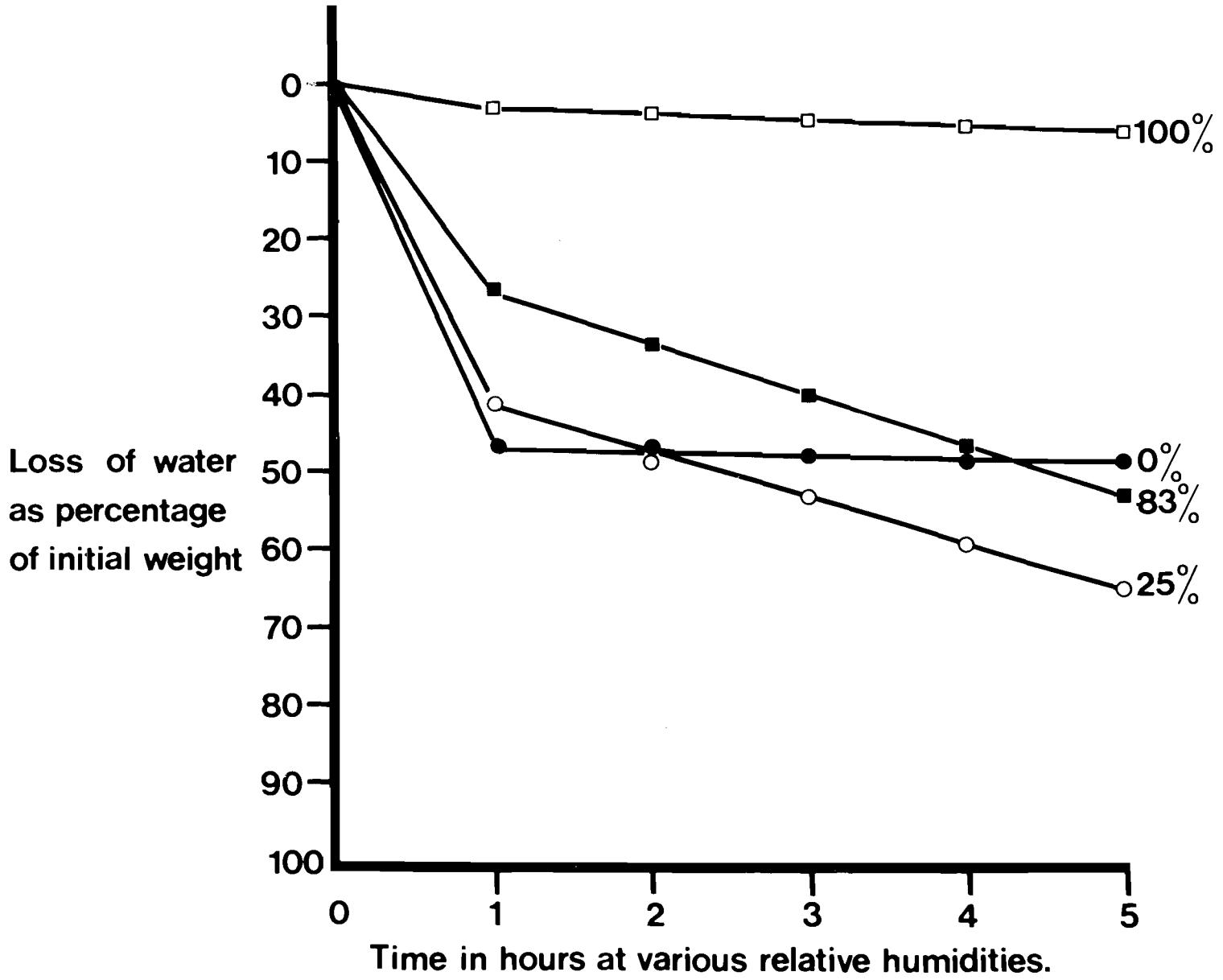


FIG. 28: WATER LOSS OF ADVANCED STAGE EGGS AT VARIOUS RELATIVE HUMIDITIES

humidities.

To see the amount of water left in the eggs after keeping them at various humidities, the eggs were dried in an oven at 105°C for twenty-four hours. Their water content is given as a percentage of their weight in Table [16]. It was found that water content of the eggs which had previously been kept at 0% relative humidity was more than that found in the eggs which had previously been kept at 50 and 75% relative humidity, but it was less than that found at 96% relative humidity. The water content of the eggs which had been dried at 105°C for twenty-four hours was 27·65, 21·74, 22·22 and 36·76 per cent of their weight at 0, 50, 75 and 96% relative humidity respectively.

TABLE [16]. Per cent water content of eggs at the end of the experiment shown in Fig. [27], as estimated by weight loss following heating for 24 hours at 105°C.

Relative humidity	wt. gm. 1000 eggs after 24h. at R.H.	wt. gm. 1000 eggs after 24h. at 105°C.	water content gm.	% water content
0	0·0047	0·0034	0·0013	27·65
50	0·0046	0·0036	0·0010	21·74
75	0·0045	0·0035	0·0010	22·22
96	0·0068	0·0043	0·0025	36·76

Eggs were dried at 105°C for 24 hours; their water content is given as a percentage of their weight.

6.3.7 Egg shell hardening and its ecological importance as a protective mechanism from complete desiccation

The time taken by the concentrated solution of sodium hydroxide to dissolve the egg shell of the egg was different depending on the level of desiccation in which eggs had previously been kept. At laboratory temperature, the time required to remove the egg shell of freshly laid eggs was one day, whereas the time required for those eggs which had been dried for one year was 4 days. Sodium hydroxide solution took 5 days to dissolve the egg shell of those eggs which had been kept dry at 0% relative humidity for 10 weeks, whereas for those eggs kept at 25, 50 and 75% relative humidity the time required to remove their egg shell was 4 days. Shorter time was found to be required to dissolve the egg shell of those eggs which had previously been kept at 83 and 96% relative humidity for 10 weeks. The time required in this case was 3 days. These results suggest that the hardening process (tanning) depends on the environmental humidity, since it was found that the lower the relative humidity, the longer the time required to dissolve the egg shell.

Applying sodium hypochlorite technique, nearly the same pattern of results was observed. For example, the time required to remove the egg shell of undried eggs was 5 minutes, whereas the time required for those eggs which had been dried at laboratory conditions for nearly one year was 8-9 minutes. Seven minutes were found to be required to dissolve the egg shell of those eggs which had previously been kept at 83 and 96% relative humidity, whereas 9 minutes were required for those eggs kept at 25, 50 and 75% relative humidity. Longer time (12 minutes) seemed to be required to dissolve the egg shell of those eggs which had previously been kept at 0% relative humidity. Table [17] shows the time required to dissolve the egg shell of dried and undried eggs. The present results and those obtained using sodium hydroxide solution suggest the

TABLE [17]. Shows the time required to dissolve the egg shell of both dried and wet eggs in sodium hydroxide and sodium hypochlorite solutions.

Drying conditions	Sodium hydroxide time days	Sodium hypochlorite time minutes
undried eggs	1	5
dry for one year at lab.temp.	4	8-9
83 - 96% R.H.	3	7
25 - 75% R.H.	4	9
0% R.H.	5	12

presence of some hardening process which is more likely to be a tanning process. As has been seen, the speed of this process seemed to be inversely proportional to the environmental humidity, so this relationship may give an interpretation for the longer survival of the eggs at 0% relative humidity than at higher humidities. It would also explain why eggs kept at 0% relative humidity lost less water than those kept at 25, 50, 75 and 83% relative humidity. It was noticed that some of the dried eggs had shown a crack in the tertiary shell when they were transferred again to water. This crack was completely different from the normal break which usually occurs in the tertiary shell. In this crack phenomenon the tertiary shell swelled when eggs were returned to water and then cracked. It was also observed that all cracked eggs were dead, since none of them developed. Plate [24] shows a photomicrograph of this abnormal crack. The number of cracked eggs was found to increase as the storage time at different relative humidities increased. Also the

Plate [24]. Shows the abnormal crack in the tertiary shell. This crack is completely different from the normal break which usually occurs in the tertiary shell. In this crack phenomenon the tertiary shell swelled when eggs were returned to water. All cracked eggs were dead, since none of them developed.

Plate [24]

number of cracked eggs seemed to be correlated with the environmental humidity. For example, eggs were removed 10 weeks after placing in the humidity chambers and were kept in water under daily observation. The percentage of cracked eggs for those eggs which had previously been kept at 83% relative humidity was 38 per cent, whereas percentage of cracked eggs for those kept at 75, 50, 25 and 0% relative humidity was 45, 34, 6 and 0 per cent respectively. Fig [29] shows the percentage of cracked eggs at various relative humidities 10 weeks after placing them in the humidity chambers. However, more will be said about the hardening process and its possible role in protecting the embryonic mass from complete dehydration.

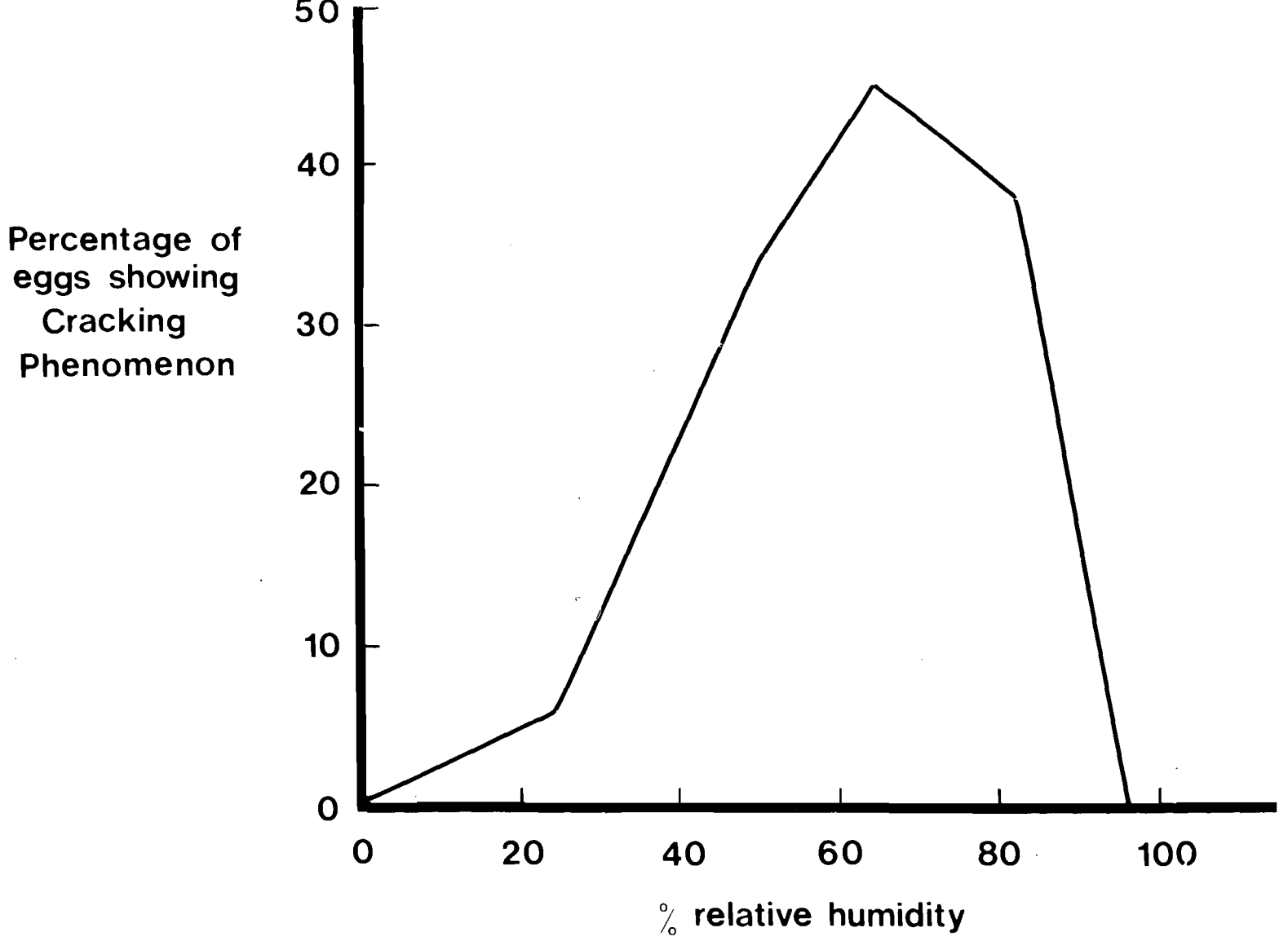


FIG. 29 : PERCENTAGE OF EGGS SHOWING ABNORMAL CRACKING IN THE TERTIARY SHELL AFTER A DESICCATION PERIOD OF 10 WEEKS AT VARIOUS RELATIVE HUMIDITIES

6.4 Discussion

It was commonly believed by some investigators that a period of drying is necessary for the egg of the anostracan fairy shrimp to be able to complete its embryonic development and then hatch. Indeed, one would think at the first time that this is the case, since most of the anostracan branchiopods species live in temporary habitats. Whether this is true or not is a debatable matter. Anostracan branchiopods inhabit ponds which are filled with water during some parts of the year but dry during others. Their eggs, therefore, are subjected to different periods of desiccation before they hatch, the period and conditions of desiccation being variable and irregular depending on many factors. The eggs seemed to hatch, and the fairy shrimps resume their activities after the establishment of the pond again due to rainfall. Some investigators have said that this period is necessary for the eggs to complete their development, others disagree. Among those who reported the essential requirement of a drying period were Hay and Hay (1889). They stated that eggs of B.vernalis must be dried before development and hatching will take place. In his work on some American phyllopods, Creaser (1931) has suggested that drying might be necessary for development and hatching. Weaver (1943) failed to hatch eggs of E.vernalis without drying. Furthermore Longhurst (1955) reported that anostracan species have an adaptation to the temporary pond through their drought-resistant egg, in which hatching occurs only after desiccation.

On the contrary, some other investigators have found that dried eggs have failed to hatch, but there is little detailed information on the drying period and degree of desiccation. Such information has, however, been considered only in a few works. Moist eggs of E.vernalis hatched, whereas dried eggs failed to do so (Avery, 1939). Dexter and Ferguson (1943) also failed to get dried eggs of E.vernalis to hatch, but they did not mention for how long eggs had been kept dry or in what degree of

desiccation they were kept. It seems that most of the workers have answered the question of whether drying is necessary for development and hatching or not, but only few mentioned the drying period which the eggs can withstand and stay viable. On the other hand, among the investigators who found a period of drying is not necessary for development and hatching were Baird (1849), Mathias (1926), Castle (1938), Avery (1939), Dexter and Ferguson (1943), Moore (1951), Hall (1953), Prophet (1963) and Broch (1965). Thus there are now two opinions for the necessity of drying or not. These are (a) drying is necessary, and (b) drying is not. The findings of the present study have shown that both dried and undried eggs can develop and hatch. But it seems that as the drying period increases, egg viability is affected more. A desiccation period of more than six months under laboratory conditions seemed to have affected the egg viability severely, since only a few eggs reached breaking stage. A percentage of breaking as low as 6% was found for those eggs which had previously been dried at laboratory conditions for six months. However, a longer period of desiccation would bring percentage break down. Furthermore, eggs of this species seemed not to be able to survive a desiccation period exceeding one year, whereas eggs of the brine shrimp A.salina have been found to resist longer than this period; they may be dried and kept for several years and then hatched when returned again to sea water (Green, 1967). Moreover, Bishop (1968), working with L.stanleyana, and Moore (1967), working with S.seali, reported that both dried and wet eggs had hatched. The latter also found that percentage of hatching would be reduced if eggs were kept dry for longer periods. He also found that complete mortality would occur if eggs were stored for nearly two months in a moisture free environment. This is in agreement with the results of the present study, yet eggs of C.diaphanus would survive longer periods of desiccation. The findings of Hall (1953), who observed delayed hatching for the dried eggs of this species, would also agree with the present results. He found that hatching was delayed, although the expected

relationship between the appearance of the break and hatching was noticed. Moreover, he suggested that some factor may be responsible for preventing the hatching of a considerable proportion of the eggs at the normal time. However, in this present study, a reduction in the activities and embryonic movements were observed, especially if eggs were dried for longer periods, so this reduction in the rate of movements may be responsible to some extent for the lack of hatching.

The dried eggs float when placed in water. It was found that eggs which had been subjected to desiccation lost some of their water which, in turn, caused the lowering of their density. This floating phenomenon may be due to the formation of air space between the tertiary shell / chitinous membrane complex and the embryonic mass. It was found that in all eggs which had been kept at all relative humidities below 100% the hatching membrane was found to be drawn in. The indentation seemed to be correlated with the amount of water lost. Furthermore, dissecting the eggs under the microscope revealed that the air space is formed between the tertiary shell / chitinous membrane complex and the embryonic mass. The removal of the egg shell using 5% sodium hypochlorite solution also confirmed the previous finding, since the embryonic mass was found deeply indented on one side. Plate [23] shows the collapsed embryonic mass and the air space formed after the egg was dried.

The situation seemed not to be the same for the unhatched embryos. These embryos were more sensitive to even a very short period of desiccation at various humidities, including that of 96% relative humidity. These findings suggest that survival of the unhatched embryos in nature depends on the presence of standing water or at least on very high environmental humidities. This is obvious because their hatching membrane had collapsed within less than an hour at all relative humidities below 100%, whereas at 100% relative humidity the unhatched embryos were found to live for many

days. However, their survival at 100% relative humidity (without free water) for such a long time may be due partly to some water condensation. The collapse of the hatching membrane and the contraction of the embryonic mass also demonstrate the permeability of this membrane to water, so one would imagine the great protective value of the egg shell from complete dehydration. The ecological significance of the survival of the unhatched embryos at high relative humidities is that some of the eggs may reach the breaking stage under natural conditions in the moist soil, even if there were no standing water. This happens especially when there are short periods of intermittent rain which moisten the soil but are not sufficient to fill the pond. Under such conditions, therefore, the broken eggs (unhatched embryos) could survive and stay alive until the filling of the pond. The ability of some eggs to reach the breaking stage at 100% relative humidity was, indeed, observed under laboratory conditions also. The findings of the present study are in harmony with the field observations. In nature it was found that eggs after being laid by the females sink to the bottom, then either develop a little and very slowly or not at all. The eggs are then exposed to moist soil after the pond dries up. This provides an aerobic moist condition which may be the most favourable condition for the embryonic development. The significance of this is not due to the drying up of the pond, but rather to the consequent increase in oxygen concentration, since it was found that drying is not essential for the embryonic development and hatching of this species. The ability of the eggs to proceed and complete their embryonic development at very high relative humidities, and in the absence of free water, is very important to the survival of this species, since if they proceed with their development up to the breaking stage in moist soil and before the refilling of the pond by rain water, they will be able to hatch immediately after the establishment of the pond. This adaptation will let the hatched nauplii

reach sexual maturity very soon and give an opportunity to the adults to breed and lay eggs before the elimination of the population from the pond, either by shortage in rainfall and drying up of the pond or by the appearance of the predators.

In the laboratory it was found that eggs were able to proceed and complete their embryonic development at higher humidities up to the breaking stage. However, some had shown the break in the tertiary shell under such conditions. Hall (1953) suggested that in the eggs of this species some embryonic development may have taken place during the desiccation period. At that time the storage place in Hall's experiments might have been of quite high humidity. In this case the findings of Hall agree with the present results, since some embryonic development was found to have taken place at 96% relative humidity.

At lower humidities it was found that no embryonic development seemed to have occurred. Although the eggs survived quite long periods of drought, nevertheless they required free water or at least very high humidity in order to complete their development. In his work on S.seali, Moore (1967) has also found that the degree of desiccation influences both rate of hatching and total percentage hatch. Moreover, he found that complete mortality of eggs occurs after 40 - 60 days' storage in a moisture-free environment. The results of the present study agree with his findings, but it seems that the eggs of C.diaphanus can survive at severe desiccation longer than S.seali.

Data from the habitat of C.diaphanus showed that soil moisture expressed as per cent wet weight may fall to a value as low as 18%, especially in the upper layer, although it was noticed to be high most of the time. Moreover, since it was found, as stated elsewhere, that the eggs are deposited a few centimetres below the surface, the upper layer of the soil protects the eggs from severe desiccation to some extent. Unfortunately, no direct relationship is known to the author between the

relative humidity and percentage moisture content of the soil, but one can see that a very long period of desiccation will lead to the reduction of the percentage of breaking, and in turn to the percentage hatch.

Eggs of this species then must be provided with enough water to complete their embryonic development and if there were standing water (or very high moisture) in the environment, eggs would show the break in the tertiary shell. Furthermore, availability of sufficient moisture in the soil will prevent the death of the eggs, which in turn survive and stay viable until the refilling of the pond. If more rainwater becomes available then hatching will take place.

Linder (1960) reported that the hardening and darkening of the egg shell of the fairy shrimp C.bundyi could be due to the formation of quinone-like substances. He postulated that no quinone could be detected in his study. This might be because he used the techniques which detect the presence of the free quinone and not the combined one, since it is known that the quinone tanning process is the combination of the protein (free amino groups) and the quinone. Then the detection of the presence of the quinone should be made using a technique which can reveal the combined rather than the free quinone. However, he gave some evidence, although not direct ones, for the presence of quinone tanning system; among these is the predominance of the aromatic cross linkages in the lipoprotein complex, and the solubility of aromatic tanned protein in sodium hypochlorite, which is known to dissolve such a substance. The results of the present study have shown that the egg shell dissolved within a few minutes in a solution of 5% sodium hypochlorite. Moreover, the time required to dissolve the egg shell in the sodium hypochlorite solution depends on the environmental humidity and duration of storage. It was observed that the time required to dissolve the egg shell of dried eggs was longer than that required for undried ones. Then one

could say that the lower the envionmental humidity the faster the hardening of the egg shell, and the longer the period of desiccation the harder the egg shell will be. Hardening process (tanning) seemed to be faster in the egg shell of dried eggs; this might be because the eggs were uncovered by water and exposed to more oxygen. Also it seemed to be faster at 0% relative humidity than that at higher relative humidities because one would expect a free moisture environment at 0% relative humidity. The necessity of the oxygen for the hardening process of the soft insects' cuticles has been shown by Pryor (1940a, 1940b), Dennell (1958) and has been confirmed by Linder (1960) in his work on the egg shell of C.bundyi. Pryor (1940a, 1940b) reported that J.Dewitz (1916) noted that the darkening of the puparia of Calliphora could be prevented by covering them with water.

As has been seen, there is a relationship between the rate of water loss and relative humidity. It was also found that eggs lost water in all humidities tested, including that of 100% relative humidity (without free water). The same correlation has been observed by Martin and Cooper (1972) in annuran eggs. Freshly laid eggs lost water more rapidly than advanced stage eggs, but still the rate of the water loss is correlated with the relative humidity. Birch and Andrewartha (1942) have found that the susceptibility of grasshopper eggs to desiccation, as measured by the rate of loss of water in atmospheres of different humidities, varies according to the stage of development of the embryo; being greater for newly laid eggs, and least for diapause eggs. A similar pattern of water loss was found in the eggs of Gryllulus commodus Walker by Browning (1953).

The total water loss for the freshly laid eggs was more than that found for the advanced stage eggs. There are two possible explanations for this: (a) eggs in their late stage of development may have an ability to hold their water more than the freshly laid ones, and (b) the condition of the tanned egg shell might have been different. It was also found that

advanced stage eggs were more resistent to desiccation than those eggs in their early stages of development. Hunter-Jones (1964), in his work on the desert locust Schistocerca gregaria Forsk, has found the same pattern of relationship. He reported that eggs that had been less than four days in moist sand were unable to withstand twenty-four hours in dry conditions, whereas eggs that had been four or more days in moist sand (advanced stage) were able to withstand up to 48 hours in dry sand and still remain viable and hatch when returned to moist sand. It was found that the total water loss was less at 0% relative humidity than that at higher humidities up to 83% (inclusive), although the initial loss was more rapid. This may be due to the hastening of the hardening (tanning) process at 0% relative humidity because of the complete absence of environmental moisture. In 1916, Dewitz (Pryor, 1940a, 1940b) noted that the darkening of the puparia of Calliphora could be prevented by covering them with water.

The hardening process (tanning) may have a very important ecological value as a protective mechanism to the eggs from complete dehydration if their habitat is exposed to a quite long period of drying, especially to those eggs which are deposited on the upper layer of the soil, since the eggs after being laid are deposited a few centimetres below the surface.

SECTION SEVEN

THE EFFECT OF EXTERNAL OSMOTIC PRESSURES ON THE EMBRYONIC DEVELOPMENT AND HATCHING

SECTION 7

The effect of external osmotic pressures on the embryonic development and hatching

7.1 Introduction

The ~~crustacean~~ anostracan branchiopods live generally in freshwater ponds which have a temporary nature. A few, however, have been found in saline water, e.g. A.salina and Cyzicus maxicanus Claus. The brine shrimp A.salina is even found in habitats of a high salt content (Cole et al., 1967). Branchinecta lindahli and Streptocephalus texanus have been found in ponds containing 405 mmoles/l. (21572 ppm) and 320 mmoles/l. (16455 ppm) respectively. These are quite high concentrations. Triops longicaudatus on the other hand is a species found in ponds having low concentrations. The fairy shrimp C.diaphanus is an inhabitant of certain temporary freshwater habitats. These habitats may be subjected to fluctuations in environmental factors. One of these factors is the action of evaporation, which leads to changes in the total concentration of the pond water. In a number of examples pond water diluted by rainwater reached a value of concentration of 9 ppm. This value is as low as distilled water. Pond water is also concentrated by evaporation and a value for total concentration of 143 ppm has been recorded. At the same time Horne (1967) reported that some freshwater species of phyllopods were never found in habitats exceeding a total concentration of 24 ppm.

Laboratory studies on the role of osmotic pressure in controlling or affecting embryonic development and hatching in anostracan branchiopods are scarce. However, some work has been done on A.salina (Clegg, 1964). Nothing has been reported about C.diaphanus, apart from that mentioned by Hall (1953), who mentioned the possible existence of osmotic relationship between the egg contents and the surrounding medium. But whether osmotic pressure plays a part in the breaking of the tertiary shell or a

true hatching mechanism was not known in this species. It has been shown that one of the most important factors leading to hatching of the eggs of many crustacean species is the dilution of the habitat water. Some metabolic substances, as has been found in some species, may take a part in the osmotic breaking of the tertiary shell. Substances such as glycerol have been found in considerable amounts in the dormant cysts of A.salina (Clegg, 1964) and in this species (Hall and MacDonald, personal communication). Accumulations of such substances have been noticed at the breaking stage. It has been thought that high concentrations of pond water may delay embryonic development or inhibit the hatching process, and so may prevent a second generation occurring in nature. Whether this is true or not is uncertain. Ramult (1925) found that the more the water evaporated, the more the eggs showed lack of development power. Furthermore, some have attributed the death of the population of some species in the summer to increasing ionic concentration. Horne (1967) found experimentally that B.lindahli could hatch in high salinity (66 mmoles/l. or 2996 ppm), whereas T.longicaudatus egg hatchability was affected by osmotic concentrations of more than 22 mmole/l. or 999 ppm. In his work on Daphnia Ramult (1925) reported that concentrations of 100 mmoles or above would produce a "closed development". Furthermore, Hall (1953) suggested that the explanation of delayed hatching may be found in the osmotic relationships of the egg and the surrounding fluid. Broch (1965) has also mentioned the possible influence of osmotic pressure in hatching of C.bundyi.

Because of the lack of detailed information and to contribute in filling the gap in our knowledge of this problem, a series of experiments and observations were made to see the effects of various osmotic pressures on embryonic development and the hatching process of the egg of this species.

7.2 Methods and Materials

Eggs of C.diaphanus were collected from adult females by the method already described. Therefore eggs used in this series of studies were not older than twenty-four hours. They were washed several times with distilled water to get rid of all suspended detritus and exuviae of the adults. Then eggs were rinsed thoroughly with the experimental solutions before starting the experiments. This latter is to eliminate possible dilution of the media. The external media were aerated every two days. The experimental solutions were renewed whenever fungal growth started to appear. Distilled water was added whenever necessary to keep the concentrations constant throughout the course of the experiment. Distilled water was used as a diluent in the preparation of media, rather than using tap water or habitat water as some workers have done. However, the concentration of pond water is negligible if compared to the solutions used.

Preparation of different concentrations of sodium chloride (M.W. = 58·44) was carried out using initial concentrated solution. This solution is 1 Molal (Molal : concentration of a solution formed by dissolving a molecular weight of a substance in one Kilogram of water). This molality was diluted with distilled water in order to produce the required concentrations. A wide range of sodium chloride solutions was prepared. This range was from 1 mM to 300 mM. The latter was the highest concentration used.

The osmotic pressures of NaCl solutions were estimated using the formula $\pi = M\nu RT$, where π is the osmotic pressure in atmospheres; M, the molality (which has been multiplied by 2 to obtain the effective concentration of ions); ν, the molal activity coefficient (values for ν were obtained from Robinson and Stokes, 1959); R, the gas constant

(0.0821 l.atm.mole^{-1} degree^{-1}); T, the absolute temperature. Distilled water, tap water and pond water were used as controls.

Sugar solutions of mannitol and sucrose of different osmotic pressures were also used in these studies, to see if the results obtained using sodium chloride are due to its chemical effect or due to the increase in osmotic pressure. A series of experiments was carried out like the one carried out with sodium chloride. Solutions of mannitol and sucrose of known osmotic pressures were prepared from data given by Garner (1928) and Clegg (1964).

Different concentrations of sodium chloride solutions in mmoles and their osmotic pressures in atmosphere are listed below:

Concentration in mM	Osmotic pressure (atmosphere)
10	0·376
20	0·754
30	1·131
40	1·508
50	1·884
60	2·261
70	2·672
80	3·015
90	3·435
100	3·769
110	3·916
120	4·272
130	4·628
140	4·984
150	5·340
160	5·696
170	6·052
180	6·408
190	6·765
200	7·121
250	8·901
300	10·681

The complete process of hatching falls into two stages, as has been shown elsewhere. For convenience, the first stage will be called breaking and the second is hatching. The effect of various solutions having different osmotic pressures on embryonic development and on both stages mentioned will be studied.

To study the relationship between rate of embryonic development and different osmotic pressures, batches of one hundred freshly laid eggs each were placed in petridishes 10cm. in diameter as hatching chambers containing the required solutions. Hatching chambers of this size were selected to ensure that no oxygen shortage occurred, and to facilitate the aeration of experimental solutions, since it has been found that oxygen is necessary for both embryonic development and hatching of this species.

To see the influence of different osmotic pressures on the breaking stage and to explain the possibility of the presence of osmotic emergence of the nauplii (breaking), eggs were kept first in distilled water or aged tap water up to the stage at which a split was about to occur in the tertiary shell. The time for this is usually six - seven days at a temperature of 22°C ($\pm$ 2°C). Then batches were separated and placed in the hatching chambers at various concentrations of sodium chloride.

Other lots of unhatched embryos were prepared for another series of experiments. This is to provide some knowledge of the role by which osmotic pressure may play its part in the hatching mechanism, and percentage hatch at various pressures. Unhatched embryos were prepared by keeping eggs in distilled water or aged tap water for six - eight days at laboratory temperature. This time is required for eggs to complete their development and show a break in the tertiary shell. Daily observations were carried out for the appearance of any sign of break or hatch. The

number of eggs showing the break and the number hatching were recorded each day.

Studies on possible size decrease or increase of the egg at different osmotic pressures were also carried out. This was done by keeping groups of eggs in different sodium chloride solutions. These eggs were then examined daily under the microscope and their diameters were measured with an ocular micrometer at 140X magnification.

It has been seen before that there is a relationship between embryonic movements and the hatching process. The rates of movements were noticed to be slower at higher concentrations. It was decided to investigate this matter and to try to relate it to the hatching mechanism and decrease in per cent hatch. Movements of the embryo were observed and their rates were calculated at time intervals.

Habitat water is subjected to dilution and increase in total concentration in nature, by both rainwater and evaporation - such increase in concentration may have an effect in delaying embryonic development or may inhibit hatching. To explain this problem and contribute in throwing some light on it, pond water having different concentrations was prepared. Preparation was carried out by placing habitat water in plastic trays 40 x 20 x 5 cm. and leaving them outside. Evaporation takes place, so increasing concentrations. The resulting media were aerated using an oxygen generator operated for a reasonable time. Habitat water with total concentrations as high as 1560 ppm was prepared. Batches of 100 eggs each were placed in watchglasses. They were washed several times with distilled water. Then they were rinsed with experimental concentrations. Watchglasses were kept at laboratory temperature of $22^{\circ}C$ ($\mp 2^{\circ}C$). Daily observations were carried out for the appearance of any sign of break or

hatch. The number of eggs showing the break and the number hatching were recorded every day.

Conductivity at 25°C was measured with "Conductivity bridge" type E7566/3 (Mullard Equipment). Total concentration in ppm is approximately 56% of the conductivity at 25°C (Horne, 1967), thus total concentrations of the solutions obtained can be estimated. Dissolved oxygen concentrations were measured using a "Laboratory Oxygen Analyzer" Model 777 (Beckman Instruments, Inc.); values are given as mg/l.

7.3 Results

Since these three types of water were used as controls, there have been no differences in the time required by the eggs to reach breaking stage in distilled water, tap water and pond water. Rates of development for eggs kept in them are all the same. It was 12·50 - 14.28 per cent development per day. Eggs passed through their development normally. The duration of a normal development at laboratory temperature 22°C (∓ 2°C) from laying until the occurrence of the break in the tertiary shell was seven - eight days. However, sometimes breaking starts to occur after five or six days of immersion. It is of interest to say that in some cases, whether experimental or controlled, it has been noticed that a small portion of eggs do not complete their development or hatch. It was found that they were dead. This phenomenon is sometimes to be noticed even in natural habitat water and under favourable conditions.

7.3.1 Effect of different osmotic pressures on embryonic development and hatching

The development of the embryo seems to be normal and requires the same time in distilled water as in sodium chloride solution having an osmotic pressure of 0·376 atmosphere or slightly more. Eggs started to show breaking in the sixth day of immersion in distilled water and in 0·376 atmosphere of sodium chloride. Peak break occurred on the eighth day. The mean per cent development per day was 12·50. At higher concentrations, that is to say 20, 30, 40, 50 and 60 mM NaCl (0·754, 1·131, 1·508, 1·884 and 2·261 atm.respectively), the peak of break occurred in the ninth day of immersion. At these concentrations the eggs seem to have a mean per cent development per day of 11·11. This is less than that found in the control. But still embryonic development was normal, and morphological changes were as usual and no differences were noticed.

Percentage break at the concentrations mentioned above were high and very close to that found in the control. In distilled water a figure of 98% has been recorded. At concentrations of 10, 20, 30, 40, 50 and 60 mM NaCl, the percentage break was 90, 92, 90, 88, 90 and 88 respectively. From these results it appears that osmotic pressures as high as 2·261 atmosphere have no effect on embryonic development or percentage break. Even at higher pressure, 2·672 atm. (70 mM NaCl) there was no significant effect, since 86 per cent break has been found. Number of eggs reaching breaking stage started to be less in more concentrated solutions. This was noticed at 80 mM (3·015 atm.), when only 78% reached breaking stage. Reduction was very obvious at a concentration of 110 mM (3·916 atm.). The obtained percentage was only 58. Delay in the occurrence of break was only one day at all concentrations up to 90 mM NaCl (3·435 atm.). The rate of development was 11·11 per cent development per day. At higher concentrations, that is to say 100, 110 and 120 mM NaCl (3·769, 3·916 and 4·272 atm. respectively), the delay was two days. So the rate of development was 10·00 per cent per day. The breaking stage seems to be clearly affected by osmotic pressures higher than 3·769 atm. (100 mM NaCl). Very low percentages of broken eggs have been found at osmotic pressures of 4·272, 4·628, 4·984 and 5·340 atm.(120, 130, 140 and 150 mM NaCl respectively). They were 30, 20,14 and 2% respectively. No egg had reached breaking stage at osmotic pressures higher than 5·340 atm. even after immersion of one month.

Delay in the occurrence of break was three and four days at osmotic pressures of 4·628 and 4·984 atm. (130 and 140 mM NaCl). Rates of development were 9.09 and 8·33 per cent per day respectively. Rates of development at various osmotic pressures are shown in Fig. [30]. Most of the eggs which failed to show break did so when transferred to distilled water.

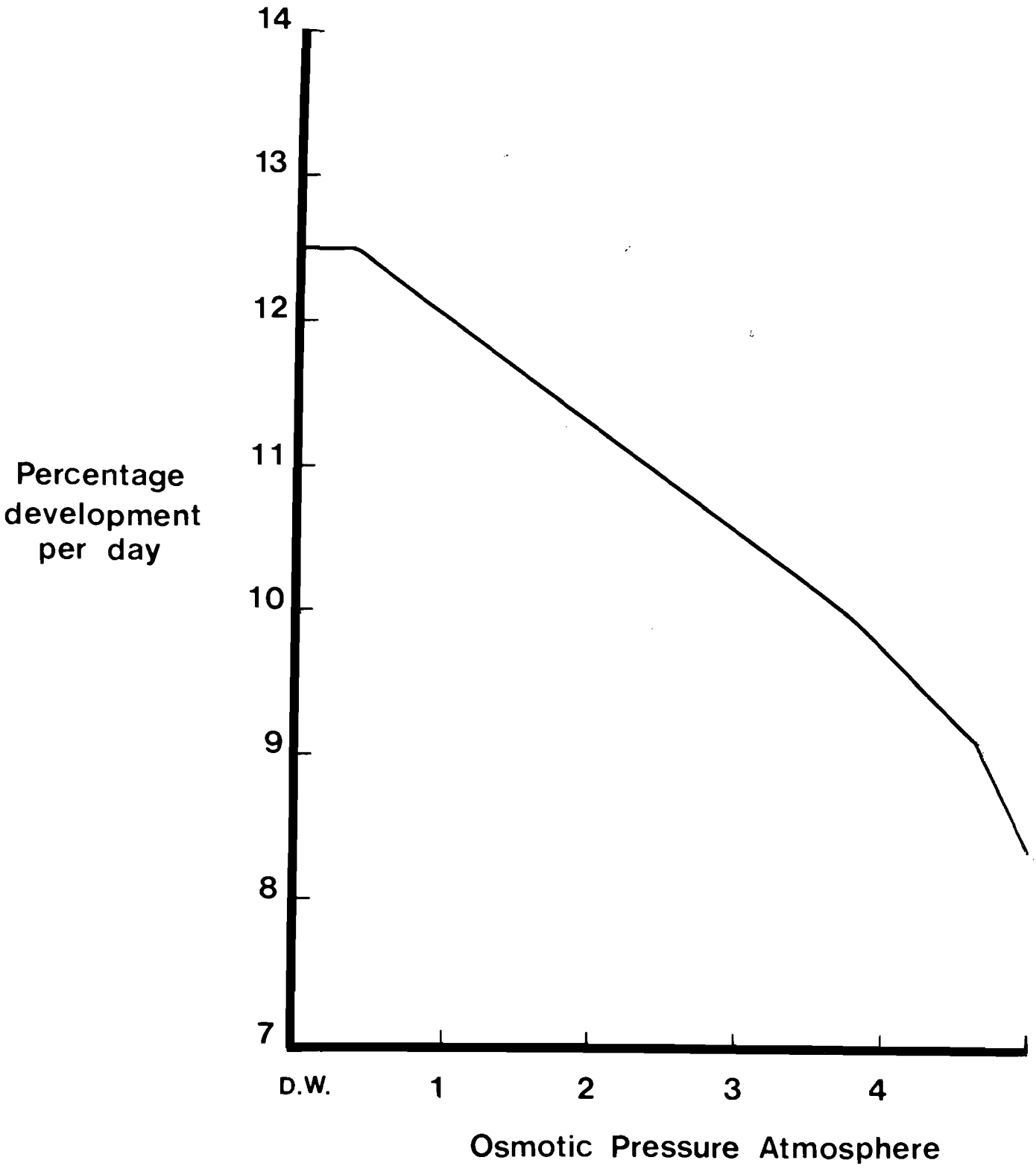


FIG. 30

RATE OF DEVELOPMENT AT VARIOUS OSMOTIC PRESSURES

(Sodium chloride solutions)

At no time in these experiments have any changes been seen in the diameter of the eggs at all concentrations used. But it was observed through close microscopical observations that the embryonic mass had shrunk inside the tertiary shell. This was because of the great differences in the osmotic pressures between the egg contents and the external media.

Morphological changes were examined under the microscope. This examination was carried out after the removal of the tertiary shell using 5% sodium hypochlorite, which is known to dissolve aromatic tanned proteins. Changes were normal at all concentrations up to 100 mM NaCl (3·769 atm.) or slightly more. The perivitelline fluid which exists between the unhatched embryo and its hatching membrane had decreased in volume. This volume decreases with the increase of the osmotic pressure. At the same time rates of embryonic movements were also affected by increasing the pressures. A remarkable reduction in rates of movement has been observed at concentrations higher than 70 mM (2.672 atm.). Although little influence has been seen on the embryonic development and breaking stage at concentrations below 100 mM (3·769 atm.), the hatching process seems to be affected by these concentrations. From the observations during this experiment it was seen that some of the hatched nauplii were very weak and morbid. This phenomenon becomes clear at higher osmotic pressures. Some of the embryos died a few hours after being hatched. Nauplii were slightly smaller than those hatched in distilled water. So at higher concentrations well-formed dwarf embryos were produced.

As concentration went up, that is to say higher than 110 mM or 120 mM (3·916 and 4.272 atm.), it was found that some of the nauplii were dead within their hatching membranes. In some cases exuviae of the

first instar larvae were visible inside the hatching membranes. This has been found at concentrations of 130 mM NaCl (4·628 atm.) upwards. Unhatched embryos which had failed to hatch at higher concentrations were seen to be in an advanced state of morphological differentiation and to have developed compound eyes while they were still within their hatching membranes.

In distilled water 88 per cent of the eggs had hatched. They lived normally when transferred to the culture media. A high percentage hatch was also found at concentrations of 10, 20, 30 and 40 mM NaCl (0·376, 0·754, 1·131 and 1·508 atm. respectively). They were 86, 86, 84 and 72 per cent respectively. Most of them lived normally when transferred to culture media or habitat water. But they could not survive for a long time if left in the experimental media. Percentage hatch was rather less at higher concentrations. Fifty-eight per cent only had hatched at 50 mM NaCl (1·884 atm.). At concentrations of 60, 70, 80, 90, 100 and 110 mM NaCl (2.261, 2.672, 3.015, 3.435, 3.769 and 3.916 atm.) per cent hatches were 28, 20, 18, 12, 8 and 4. No embryo had hatched at higher concentrations. All results are shown in Fig. [31] and Fig. [32].

Very similar results to those found with sodium chloride solutions were obtained for the sugar solutions (Mannitol and Sucrose). Sugar solutions of mannitol and sucrose, having osmotic pressures of 0.238 and 0.260 atm., had very little influence on either the embryonic development or the hatchability of the eggs. At these osmotic pressures 96 and 94 per cent break was found respectively. For hatching it was 90 and 88% respectively. Osmotic pressures higher than 3 atmospheres started to affect embryonic development and breaking stage clearly. At pressures of 3.818 and 3.915 atmosphere of mannitol and sucrose,

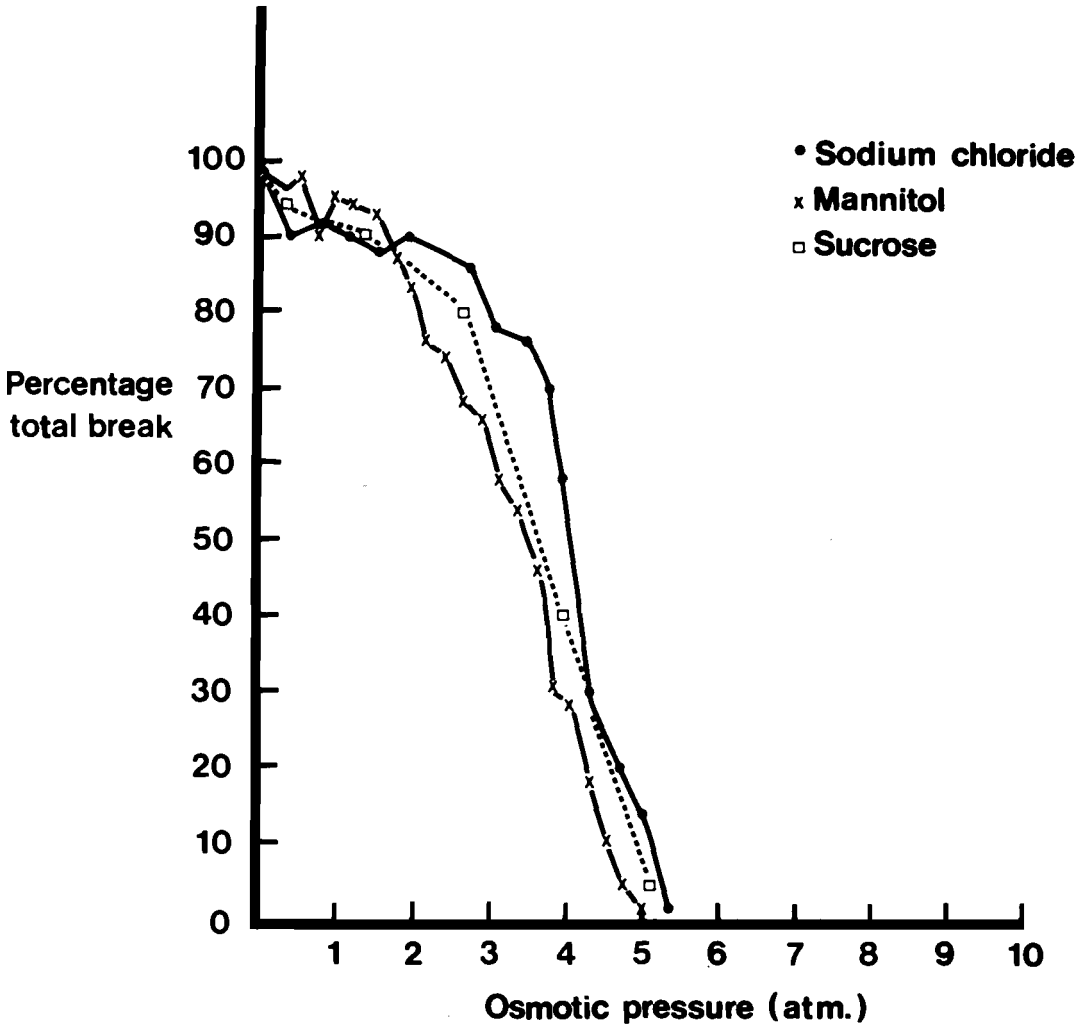


FIG.31 : EFFECT OF OSMOTIC PRESSURE ON BREAKING

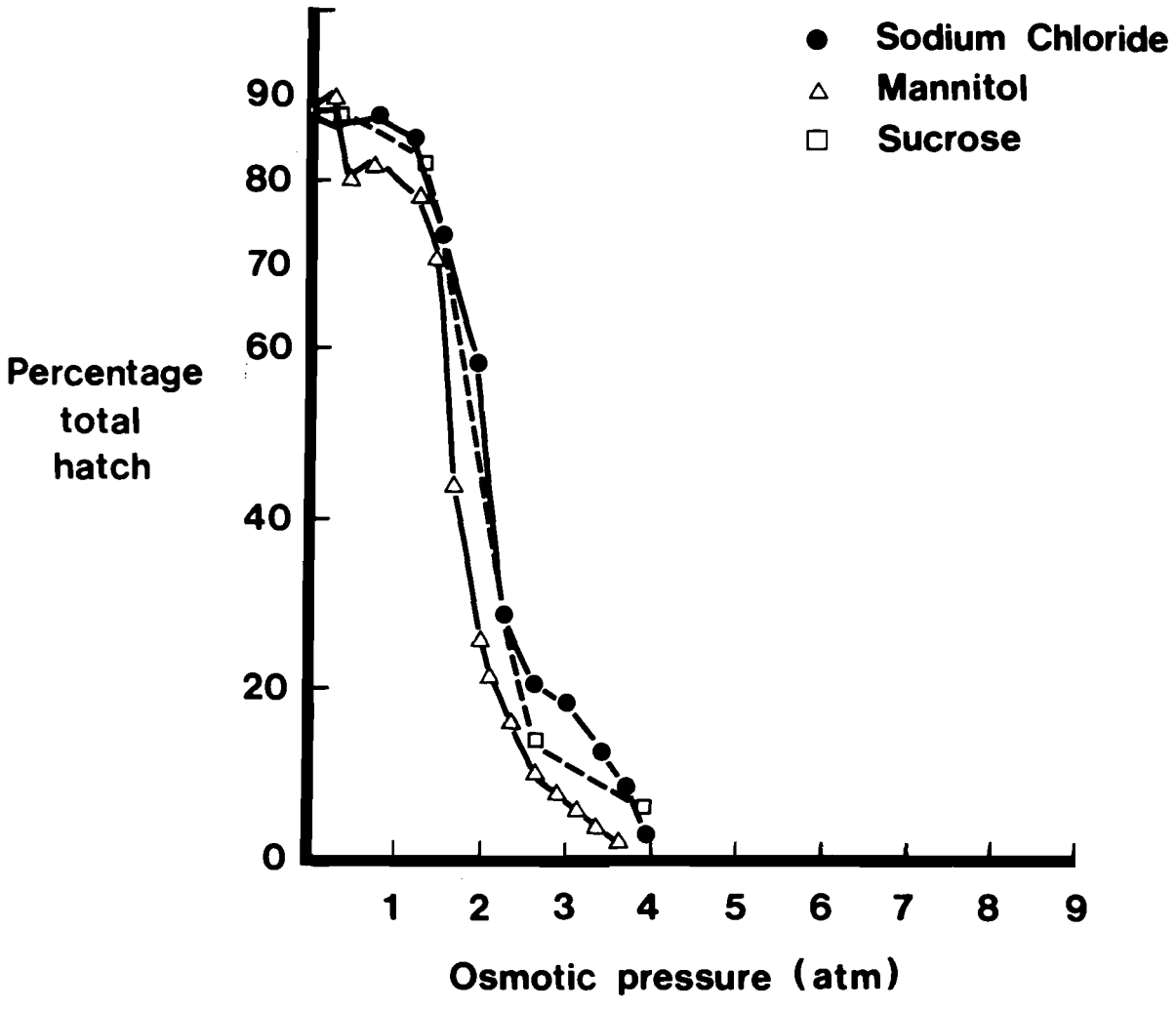


FIG.32 : EFFECT OF OSMOTIC PRESSURE ON HATCHING

the per cent break was 30 and 40 respectively. No egg had shown break in the tertiary shell in solutions having osmotic pressures higher than 5·966 atmosphere. This is true for all substances used. Hatchability of embryos seems to be more affected by increasing the osmotic pressure, solutions having values of osmotic pressure less than those which exert no influence on embryonic development seem to disturb the hatching process. Osmotic pressures below 1·432 atmosphere (inclusive) had high per cent hatch in comparison to that of the control. As osmotic pressure goes up, percentage hatch starts to decline more clearly. For example,in mannitol solutions having osmotic pressures of 3·103, 3·341 and 3·580 atmosphere, percentage hatch was 6, 4 and 2 only. No embryo hatched in solutions having osmotic pressure higher than 3·916 atmosphere. Fig.[31] and Fig. [32] show the detailed data obtained in this series of investigations.

7.3.2 Delayed breaking and hatching at higher osmotic pressures and effect of addition of distilled water

Delay in the occurrence of break and hatching was very clear at higher concentrations (osmotic pressures). Delay of one day only has been seen in the occurrence of break at all concentrations below 60mM NaCl (2·261 atm.). In more concentrated solutions, that is to say 100, 110 and 120 mM NaCl (3·769, 3·916 and 4·272 atm.), the delay was two days. One would imagine that slower development had taken place. Three and four days of delay have been found for eggs kept at 130 mM NaCl (4·628 atm.) and 140 mM NaCl (4·984 atm.) respectively.

Transference of unbroken eggs and unhatched embryos into distilled water had a remarkable effect in letting the eggs proceed with their development and reach breaking stage. Eggs which had failed to reach breaking stage and had previously been kept at concentrations of 120 mM NaCl (4·272 atm.) up to 140 mM NaCl took two - three days to show a break in the tertiary shell. Those previously kept at all other concentrations below 200 mM NaCl (7·121 atm.) required four days to do so. This means that although eggs could not reach breaking stage at these higher concentrations, clearly some development had occurred. On the other hand, no development seems to have taken place in eggs previously kept at 250 and 300 mM NaCl (8·901 and 10·681 atm. respectively), since they took seven - eight days to reach breaking stage. Removal of the egg shell of eggs previously kept at 250 and 300 mM NaCl had revealed that no morphological changes had taken place in such highly concentrated solutions.

Addition of distilled water had less effect on hatchability. Twenty-thirty per cent of unhatched embryos, which failed to hatch at concentrations below 100 mM NaCl (3·769 atm.) did hatch after being transferred into distilled water, whereas only a few did hatch at higher

concentrations. Also it has been noticed that addition of distilled water caused the resumption of embryonic activities. The volume of the perivitelline fluid had increased as well.

7.3.3 Increase of the size of the embryo during the course of development at various osmotic pressures

As has been mentioned briefly before, there is no significant change in the diameter of the egg during the course of embryonic development. But immediately before the appearance of the break in the tertiary shell, the contents of the embryo started to swell gradually. It has been noticed that the rate of increase is different at different osmotic pressures. The higher the osmotic pressure of the surrounding medium, the lower is the rate of swelling. At the time of the appearance of the break, embryos had means of 303, 290, 285, 278, 268 and 268 microns in length, in distilled water, 10, 50, 100, 120 and 130 mM NaCl (0·00, 0·376, 1·884, 3·769, 4·272 and 4·628 atm.) respectively. Maximum lengths for the unhatched embryos have been recorded twenty hours after they had shown break. Maximum lengths were 407, 405, 381, 327, 326 and 324 microns in distilled water, 10, 50, 100, 120 and 130 mM NaCl respectively. However, Fig. [33] shows the rate of swelling of unhatched embryos at various osmotic pressures.

7.3.4 Relationships of embryonic movements with external osmotic pressures

Rates of embryonic movements: both the antennal (hatching movement) and rotatory movement (movement of embryo along its longitudinal axis), tended to decrease with the increase of external osmotic pressure. In distilled water the average rate of antennal movement was 120 mov./10 minutes both in the first and second days, in which the embryos were

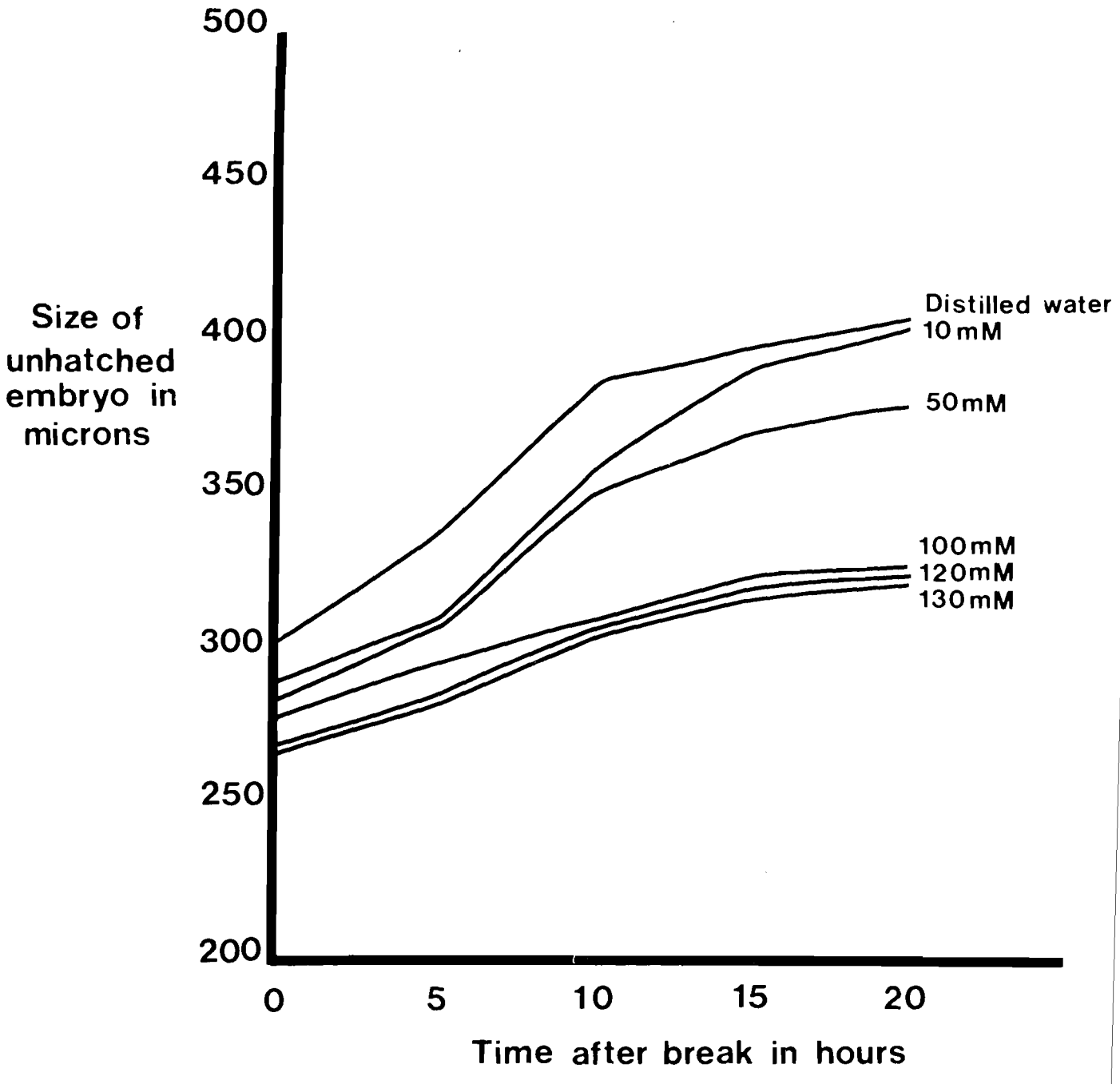


FIG. 33 : INCREASE IN SIZE OF UNHATCHED EMBRYOS AT VARIOUS CONCENTRATIONS OF SODIUM CHLORIDE

highly active. Some of the unhatched embryos in this experiment hatched before the study could be finished; so counts in the second day relied on the remaining embryos. At osmotic pressure of 1·131 atmosphere (30 mM NaCl) the rates of movement were less. They were 106 and 77 mov./10 minutes in the first and second days respectively. A clear reduction was found at osmotic pressures above 1·884 atmosphere (50 mM NaCl). Rates of 70 and 60 mov./10 minutes were found at 1·884 and 2·672 atmosphere (50 and 70 mM NaCl) in the first day. In the second day they were 60 and 43 mov./10 minutes respectively. A very low rate was seen at 4·272 atmosphere (120 mM NaCl). The rates were 8 and 6 mov./10 minutes in the first and second days respectively. Only 6 mov./10 minutes and 4 mov./10 minutes were recorded for embryos kept at 5·340 atmosphere (150 mM NaCl). Fig [34] shows the rate of antennal movement at various osmotic pressures.

In distilled water the rate of rotatory movement was 40 mov./10 minutes in both first and second days. Embryos kept at 1·131 atmosphere (30 mM NaCl) had rates of 30 and 20 mov./10 minutes in the first and second days respectively. Rates of movement in the first day were 23, 20, 10, 3, 3 and 2 mov./10 minutes at 1·884, 2·672, 3·015, 3·769, 4·272 and 5·340 atmosphere respectively (50, 70, 80, 100, 120 and 150 mM NaCl). Details of all results are given in Fig. [35].

7.3.5 The possibility that the oxygen is a limiting factor for development and hatching at higher osmotic concentrations

Although there were very slight differences, there was no significant reduction in oxygen concentration in any of the concentrations of the salt solutions tested. Measurements were taken, both at the beginning of the experiment and ten days later. However, a slight relationship was found between salt concentrations and dissolved oxygen. This may be due to the

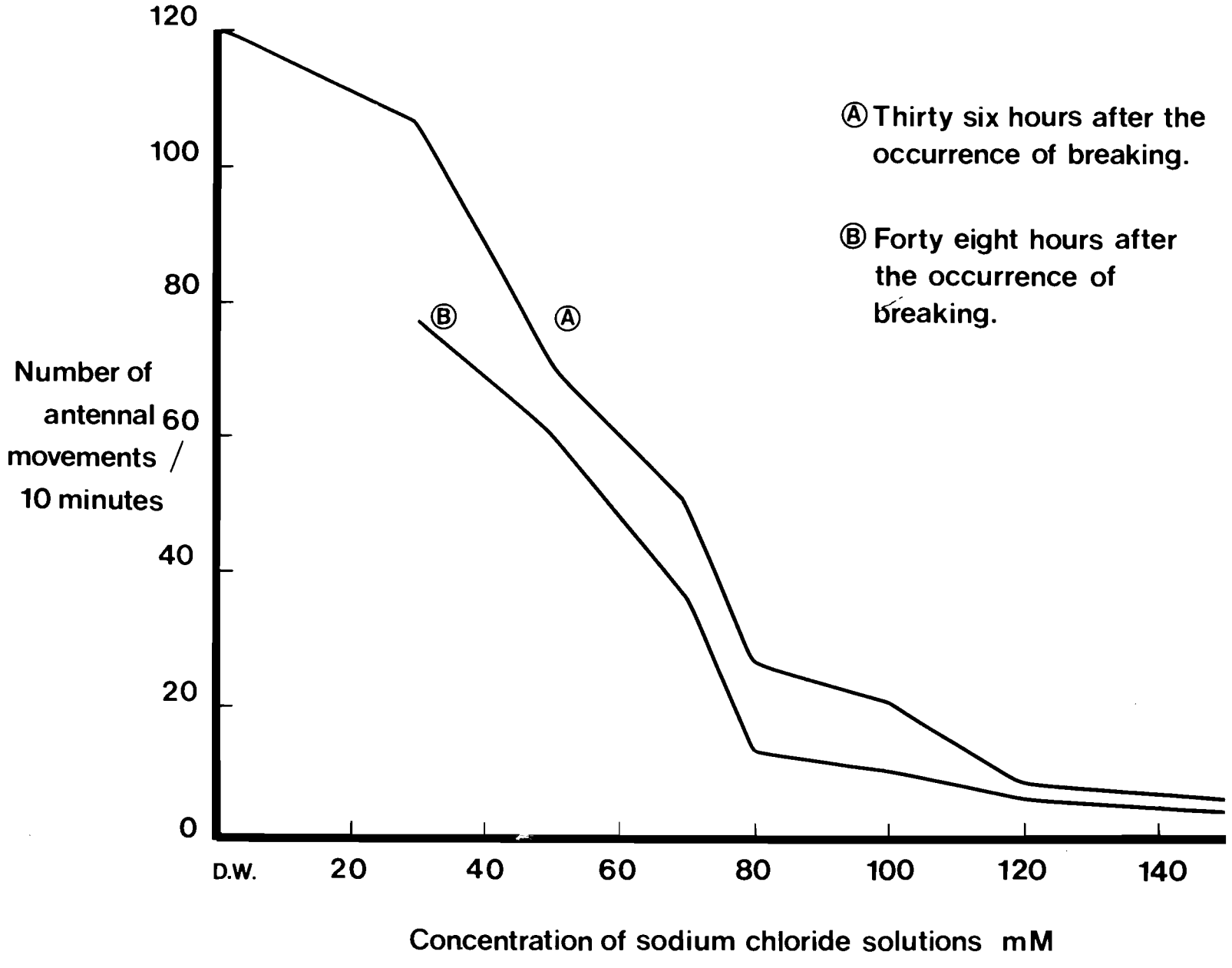


FIG.34 : NUMBER OF ANTENNAL MOVEMENTS IN RELATION TO VARIOUS CONCENTRATION OF SODIUM CHLORIDE SOLUTION

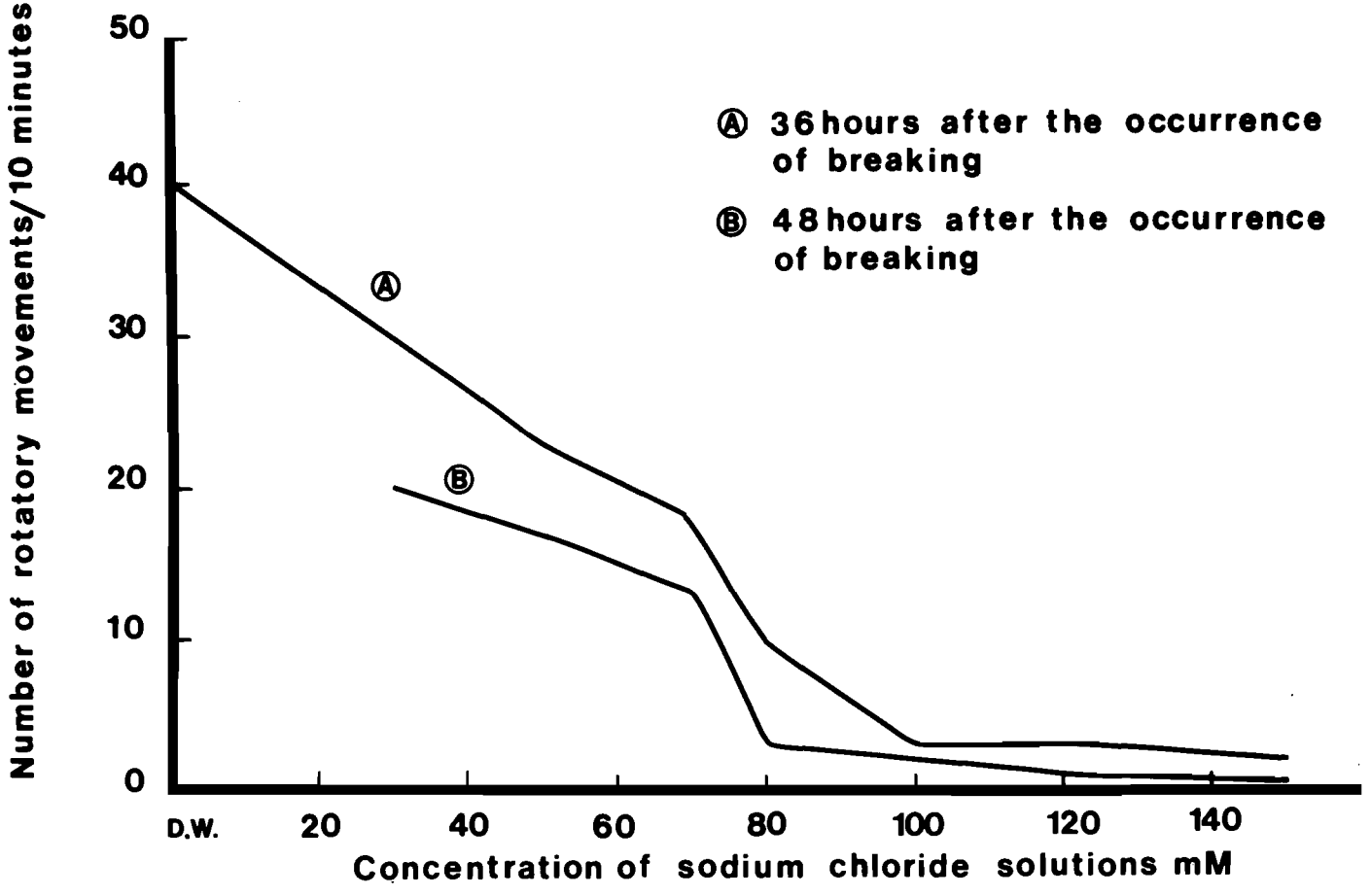


FIG.35: NUMBER OF ROTATORY MOVEMENTS IN RELATION TO VARIOUS CONCENTRATION OF SODIUM CHLORIDE SOLUTION

solubility of oxygen which tends to decrease with increasing osmotic concentration. The highest oxygen concentration which has been measured was 4·9 mg/l. This was found at both concentrations of 1 and 60 mM NaCl at the beginning of the experiment. Ten days later, the highest value for oxygen concentration was 4·5 mg/l. This was measured in both distilled water and 60 mM NaCl solution. The lowest values were 3·0 and 3·1 mg O_2/l. These occurred ten days later with sodium chloride of 180 and 200 mM. The obtained values for dissolved oxygen suggest that oxygen may play no part in retarding embryonic development or inhibiting hatching at higher osmotic concentrations. Table [18] shows dissolved oxygen concentrations at various sodium chloride solutions.

7.3.6 Some investigations on the effect of increasing total concentrations of habitat water on embryonic development and the hatching process

Eggs had developed normally in habitat water with total dissolved solids as high as 1560 ppm. The time required for the eggs to complete their development up to the breaking stage was seven - eight days. This is the same time as that required by eggs kept in distilled water or ordinary pond water. All concentrations of total dissolved solids between 112 ppm and 1560 ppm had no significant effect on either embryonic development or hatching. Percentage break at all concentrations tested was very high and close to that found in distilled water. One hundred per cent was found in distilled water and in a concentration of total dissolved solids of 134 ppm. Percentage breaks of 97 and 95 were recorded at concentrations of 624 and 1560 ppm respectively. The latter is the highest concentration tested.

The same is true for hatching. Percentage hatch was always high at all concentrations. Embryonic movements were normal and had high rates. Unhatched embryos were extremely active. No change has

TABLE [18]. Shows the oxygen concentrations at various sodium chloride solutions.

Sodium chloride concentration mM	At the beginning of the experiment	After ten days of immersion
Distilled water	4·8	4·5
1	4·9	4·1
10	4·7	4·2
20	4·8	4·0
30	4·4	3·9
40	4·7	4·1
50	4·7	4·2
60	4·9	4·5
70	4·5	3·9
80	4·7	4·2
90	4·5	3·9
100	4·3	3·7
110	4·4	3·8
120	3·9	3·7
130	4·0	3·6
140	3·9	3·7
150	3·7	3·5
160	3·8	3·3
170	3·5	3·2
180	3·5	3·0
190	3·1	3·1
200	3·2	3·1

been noticed in the volume of the perivitelline fluid surrounding the embryo at all concentrations. Table [19] shows the percentage break and percentage hatch at different concentrations of habitat water.

TABLE [19]. Effect of increasing concentrations of total dissolved solids of habitat water [Godshill pond] on development and hatching

Concentration of total dissolved solids in ppm	Percentage break	Percentage hatch
Distilled water	100	86
112	100	84
122	94	81
134	100	80
199	99	86
287	95	81
312	96	80
437	94	86
468	92	84
530	90	84
624	98	97
936	97	86
1560	98	95

7.4 Discussion

In his work, Hall (1953) has noticed a delay in the embryonic development and hatching of the fairy shrimp C.diaphanus. He mentioned that this may be found in the osmotic relationship of the egg contents and the surrounding fluid. Since that time nothing has been done concerning this matter. So it was felt that it was worthwhile to contribute to solving this matter, or try to throw some light on it.

As a dweller in temporary freshwater ponds, C.diaphanus must be subjected to various environmental changes throughout its life cycle. One of these changes is the action of natural evaporation on such a habitat. Because of this, temporary ponds may last for a week, a month or may-be a few months. In this case there must be an increase in the concentration of the pond water. This increment in concentration may have an effect on the embryonic development, or may delay or inhibit hatching. During the last two years it was necessary to measure the conductivity of the pond water, which is an indication of the concentration of the total dissolved solids, in order to see the fluctuations which happen under natural conditions. Also to record the highest concentration which may occur at such a habitat. It has been found that a value of 143 ppm was the highest concentration for the total dissolved solids at both ponds studied. However, Horne (1967) reported that some freshwater species of phyllopods were never collected from ponds with greater than 24 ppm. In this study it was observed that a wide range of concentrations would occur in the habitat of this species. This range is between 9 ppm and 143 ppm. A value of 9 ppm is very similar to that of distilled water. It is of interest to add that constituents of pond water, organic and inorganic substances, have some osmotic pressure, and then may affect embryonic development or hatching. However, their low concentrations in pond water

studied suggest that this effect is negligible. Habitat water with concentrations much higher than the one mentioned above were tested. It was seen that these concentrations, indeed, have no effect on embryonic development or hatchability of the eggs, since morphological changes would take place normally in pond water having a total concentration as high as 1560 ppm. This concentration is ten times that of pond water. Such a high value had never been observed or measured in the ponds studied, and it may never occur under natural conditions. Also it has been seen that eggs kept at all concentrations of pond water required the same time as that in distilled water. This again is an indication of the normal development which took place at these relatively high concentrations. It shows also that fluctuations of concentrations which happen under natural conditions have no influence in delaying embryonic development or inhibiting hatching process. This is obvious because it has been seen that percentage break and hatch as high as 98 and 95 were observed for these concentrations. The high percentage of hatching seen at these concentrations may suggest also that the hatching process is not strictly an osmotic phenomenon. Because, if it is so, one would expect that the highest percentage of hatch would occur always in distilled water. On the other hand, it should be said that osmotic action may contribute in hastening the hatching process, especially under natural conditions where there is continuous dilution of the habitat water through the addition of rain-water. Davis (1963) suggested the possible presence of an osmotic hatching mechanism in Palaemonetes vulgaris Say.

As has been mentioned elsewhere, complete hatching takes place through two distinctive stages, namely, the breaking stage (occurrence of break in the tertiary shell) and the hatching process (the hatching membrane

being ruptured and nauplius set free). The breaking stage seems to be a strictly osmotic phenomenon since the movements of the embryo, as already described, do not begin until about six - eight hours after the break has occurred. The unhatched embryo starts to stretch out its antennal appendages and becomes more active. Then the embryo gradually elongates. The rate of this enlargement depends on the external osmotic pressure. The greater the external osmotic pressure, the slower is the rate of this enlargement. Then the unhatched embryo starts to move its appendages pushing the hatching membrane. Later the unhatched embryo begins to move around its longitudinal axis (rotatory movement). Before the occurrence of the split in the tertiary shell, it seems possible that, due to the metabolism of the embryo, there is an accumulation of metabolic products which increase the osmotic pressure inside the tertiary shell. Then at this stage osmotic inflow of water starts to increase rapidly. As a result of the rapid inflow of water, there is an increase in the pressure inside the tertiary shell. This increase in the pressure inside stretches the tertiary shell and leads to the appearance of the split. This split becomes bigger and bigger due to the swelling of the embryo. The speed of water inflow depends also on the external osmotic pressure. It seems likely that osmotic inflow of water is of vital importance for the normal development of this species.

The presence of metabolic substances such as glycerol, in significant amounts, has been found before emergence of the embryo of A.salina from the shell (Clegg, 1962, 1964). It has also been found in the eggs of this species. An accumulation of glycerol was also found at the breaking stage of C.diaphanus. This glycerol may be the basis of the mechanism which enables the embryo to rupture the tertiary shell. Clegg (1964) also reported that the amount of glycerol in the cysts of A.salina

increases with the increase of the osmotic pressure of the surrounding medium. The ability to increase glycerol production (or other substances) with the increase of external osmotic pressure might be a very good method of overcoming the difference in osmotic pressures. The results of the present study suggest that the resistance of the embryo of C.diaphanus to quite high osmotic pressures is through this mechanism. The high percentage break obtained at high osmotic pressures may also be considered as evidence for this mechanism. So at higher osmotic pressure the embryo may become able to synthesize more metabolic substances which, in turn, increase internal osmotic pressure. However, further investigations are needed to fully explain such a mechanism. The author hopes to continue this work later on.

It has been seen that both embryonic development and hatching were normal at low osmotic pressures. But, as it increases, breaking would be affected and percentage break would be less. This is obvious, especially above osmotic pressures higher than three atmospheres. On the other hand percentage hatch starts to decline beyond an osmotic pressure of 1.5 atmosphere. This means that the hatching process is more sensitive to increase in external osmotic pressure than the breaking stage. This is possibly because of the absence of the ability to synthesize metabolic substances which may increase internal osmotic pressure at the hatching process. However, no significant amounts of such substances have been found before hatching. The tolerance of the embryo and the occurrence of breaking and hatching at these high osmotic pressures indicate that normal development and hatching take place within a wide physiological range.

The similarity of the results obtained with mannitol and sucrose to those obtained with sodium chloride solutions suggest that they were not due to the chemical effect of NaCl. It is rather due to the increase

in osmotic pressure. The gradual increase in the volume of the perivitelline fluid surrounding the unhatched embryo seems to depend on the external osmotic pressure. Not only this but the final size of the unhatched embryo depends on the osmotic pressure of the surrounding medium as well.

It has been seen that there is a decrease in the percentage break and hatch with the increase of the osmotic pressure. The latter also affected the activities and movements of the embryo very clearly. Inactivity of the embryo probably interferes with hatching, since it has already been shown elsewhere that the hatching process may be due to a combination of mechanical action of the embryonic movements and osmotic pressure. Furthermore, it has been found in all instances of hatching watched that the embryos were highly active, moving their appendages in an apparent attempt to push the hatching membrane. Thus osmotic pressure may play a role in affecting the hatching process through (a) its action in decreasing the volume of the perivitelline fluid, which in turn decreases the pressure inside the hatching membrane, leaving also a small space for the embryo; (b) its action in affecting the activities of the embryo which decrease the rates of the movements, thus decreasing the effect of the mechanical abrasion. However, Broch (1965) observed the relationship between the embryonic movement and hatching in C.bundyi. At higher osmotic concentrations the unhatched embryos are weakened, the movement slowed down and then after a few days it stopped, especially at very high osmotic pressures. Then the embryos die inside the hatching membrane. This can be prevented sometimes by decreasing external osmotic pressures. This was done by adding distilled water to the experimental solution. The transference of eggs and unhatched embryos, which had failed to show the break or hatch when previously kept at very high osmotic pressures, into distilled water was a stimulus for some of them to do so. This may be

because of decreasing the external osmotic pressure, which in turn increases the osmotic inflow of water. Because of this, embryonic contents swell, exerting pressure on the tertiary shell or hatching membrane. This osmotic swelling and increase in pressure through the inhibition of water, results in rupturing the tertiary shell and bursting the hatching membrane. When unhatched embryos, previously kept in pond-water or distilled water, have been transferred to concentrated salt solutions, they have shrunk. Shrinkage is due to the loss of water because of the difference in the osmotic pressure between embryonic contents and the surrounding medium. At higher concentrations it was observed that both egg and unhatched embryo would float first and then after a few minutes they became heavier and began to sink.

Oxygen concentrations measured both at the beginning and the end of the experiments seem not to be critical for the embryonic development or hatching, since much lower than the obtained values would delay or inhibit embryonic development or hatching. This excludes the possibility of the lack of oxygen and its influence at higher osmotic concentrations. However, experimental solutions were always in equilibrium with the atmosphere, and this provides a sufficient amount of oxygen for the development and hatching. Finally, one would conclude that even if there is an effect of the osmotic pressure beyond a certain limit on embryonic development or hatching, such value may never be found under natural conditions in the habitat of this species. So it is possible to say that under natural conditions osmotic pressure of pond water has no significant effect on development and hatching, and has no role in preventing the occurrence of a second generation.

SECTION EIGHT

TEMPERATURE EFFECT ON THE DEVELOPMENT AND HATCHING OF C.DIAPHANUS

SECTION 8

Temperature effect on the development and hatching of C. diaphanus

8.1 Introduction

Owing to the variability of air temperature and shallowness of the temporary ponds, the water temperature shows a great variation through the period of the pond's existence. The egg of C.diaphanus may be exposed in the field to varying temperatures throughout its development. The temperature may range from below zero in the winter to quite high levels during the summer. Temperatures taken at the anostracan branchiopods habitat may reach 42°C (Moore, 1955). He also said that water temperatures showed a very wide range. The minimum temperature was 8°C. At the other extreme, he collected S.seali from shallow ditches exposed to full sun with water temperatures of 35°C or higher on no less than eleven separate occasions. The maximum temperature recorded was 42°C. The freezing of the habitats of C.diaphanus was recorded by Hall (1961).

A controversy exists concerning the development of the anostracan branchiopods. Some workers have mentioned the necessity of freezing for the egg to develop; others say freezing is not necessary. Hay and Hay (1889) reported that both drying and freezing were necessary for development of B.vernalis. On the other hand, experimentally Weaver (1943) found that eggs of this species hatched both with and without freezing. Dexter (1946), working with the same species, could not find hatching after the eggs had been frozen. The requirement of freezing of the eggs of Branchipodopsis affinis Sars was reported by Bond (1934). He also said that some eggs of Artemia required freezing to hatch.

Mattox (1946) tested the influence of temperature on hatching of

the eggs of Cyzicus, and he showed that they develop between 10°C to 38°C. Also he said in his work with the same species in 1950 that hatching of the eggs would occur in nature and the laboratory only when the temperature had reached above 10°C. Chaigneau (1958) reported that eggs of Lepidorus apus L. did not hatch at temperatures below 6°C or above 20°C. Mathias (1934) reported that the dried eggs of A.salina could survive exposure up to 80°C for four days. He also reported that a small rise in temperature slightly reduced the time required for development. In his work with C.diaphanus, Hall (1959a) said that eggs kept at low temperature (2°C) developed slowly. Hinton (1954), working with A.salina, found that dried eggs heated at 103.5°C for 75 minutes gave a percentage hatch equal to 91% of the hatch of eggs kept at 25°C. For the dried eggs of Branchipus stagnalis L. and T.concriformis, Mathias (1929) found that the eggs remained viable when subjected to a temperature of 80°C, for 75 minutes, and no visible effect on subsequent development was seen. But when he heated the eggs in contact with water, the eggs withstood 42°C for two and a quarter hours.

Although enough quantitative data are not available, some investigators who have worked with related species suggest that warm temperatures are perhaps inhibitory to egg hatching. Thus, studying the effect of temperature on the eggs of a species dwelling in changeable temporary habitats is an important matter. In this study a further detailed investigation of the effect of keeping eggs in water at different temperatures may throw some light on this matter and contribute to the understanding of the relationship between temperature and rate of development. The influence of these temperatures was studied both on embryonic development and on hatching.

8.2 Embryonic development and its rate at different temperatures

Hall (1953, 1959a) and Mathias (1934) suggested that some slow development takes place at low temperatures. Hall found that the rate of development at 2°C appears to be about 1/7 (0.14) of the rate at 15°C. Effects of temperatures between -5°C to 20°C on the embryonic development were studied in the following series of experiments.

Hatching in some experiments was delayed, so that the appearance of the break is used for purposes of comparison.

8.2.1 Methods

Batches of freshly laid eggs, each was placed in a glass tube 2.5 cm in diameter containing aged tap water to a depth of approximately 1.5 cm. All tubes were kept in a cold room or a refrigerator. The experiments were carried out at temperatures of -5, 0, 5 and 10°C (± 1°C) The number of batches in each experiment were 6, 8, 6 and 3 respectively. One batch in each case was kept at laboratory temperature as a control. The mean laboratory temperature at that time was 20°C. Fortnightly one of the batches was removed and placed in a watchglass filled with aged tap water. The watchglasses were kept at laboratory temperature under daily observation for any sign of breaking in the outer covering of the egg or of hatching. Another four batches of freshly laid eggs were placed each in one of four glass tubes containing aged tap water to a depth of approximately 1.5 cm. Two of them were kept at 15 and 18°C (± 1°C). The other two tubes were kept at laboratory temperature as controls. The mean laboratory temperature during the time of the experiment was 20°C. All batches were examined daily for any breaking or hatching. All controls of the previous series were held at laboratory temperature.

8.2.2 Results

No embryonic development took place at -5°C. All eggs were found to be dead when they were examined one week later. Their death was demonstrated when they were transferred to watchglasses filled with water and kept at laboratory temperature. No development could be detected in this case. This sub-freezing temperature seems to be lethal to the eggs of C.diaphanus in wet conditions. The death of the eggs at such sub-freezing temperature could be due to the mechanical effect of the formation of ice upon the embryonic mass or to the complete arrest of the physiological processes, or to both effects.

At 0°C the batches were transferred to laboratory conditions at fortnightly intervals. The peak breaking occurred in the eighth, seventh, sixth, fourth and third days after 2, 4, 6, 8, 10 and 12 weeks respectively. One of the batches was left at 0°C. Eggs in this batch started breaking after about 16 weeks of immersion at this temperature. This indicated that the eggs required approximately 112 days to reach breaking stage at 0°C. This suggested that some slow development was taking place under these conditions. The mean percentage of development per day in relation to control was 0·062.

At higher temperature, that is to say 5°C, after 2 weeks, the eggs started breaking in the sixth day when they were transferred to laboratory conditions. The peak breaking occurred in the fourth and second days after 4 and 6 weeks of exposure at 5°C respectively. One more batch was left at this temperature in order to see the time required for the eggs to complete their development. The eggs in that batch started breaking after approximately 7 weeks of immersion at this temperature. Slow development seems to take place at this temperature, but it is clear that the rate of development was more than that at 0°C. The mean percentage of development per day at this temperature in relation to control was 0·142. The

eggs kept at 10°C for 2 weeks started breaking in the second day when they were transferred to laboratory conditions. However, peak breaking occurred in the third and fourth days. One more batch was left at 10°C. The eggs in that batch started to show breaking when 22 days had elapsed. But the peak of breaking occurred in the 24th day of immersion. The rate of development at this temperature was much faster than that at 5°C. The mean per cent development per day in relation to control was 0·291.

At 15°C the eggs required about 16 days to complete their development. Eggs kept at this temperature started breaking in the fourteenth day of immersion. Peak breaking occurred in the sixteenth day. At this temperature the mean percentage of development per day in relation to control was 0·437. The eggs kept at 18°C reached breaking earlier than those kept at lower temperatures. They started breaking in the tenth day of immersion, but the peak occurred in the thirteenth day. The mean per cent of development per day in relation to control was 0·538.

In the control which was held at mean laboratory temperature of 20°C, the eggs started breaking in the sixth day, the peak occurring in the seventh and eighth days of immersion. The mean per cent of development per day at this temperature was 14·28.

From these previous results it could be seen that if eggs were kept for longer periods at a low temperature, the time subsequently required for development in water at laboratory temperature was reduced. Again it was observed that as temperature increased, the time to complete development decreased. For this species of fairy shrimp, whose eggs were studied, no significant differences were observed between per cent hatch after incubation for different periods of time at 0, 5 and 10°C and those kept at 15, 18 and 20°C. Mean percentage of development per day at different temperatures for eggs of C.diaphanus in relation to control are given in

Table [20]. Table [21] shows mean days for C.diaphanus eggs to reach breaking after exposure to various low temperatures for different periods of time. Results are also given in the appendix and expressed as histograms.

TABLE [20]. Mean percentage development per day for egg of C.diaphanus at different temperatures

Temp. °C.	Time required for egg to complete develop. at diff. temp.	Mean develop. per day	Mean % develop-ment per day	Mean % develop-ment per day in relation to control
-5	-	0	0	0
0	112	0·0089	0·89	0·062
5	49	0·0204	2·04	0·142
10	24	0·0416	4·16	0·291
15	16	0·0625	6·25	0·437
18	13	0·0769	7.69	0·538
20	7	0·1428	14·28	-

TABLE [21]. Mean days required for egg of C.diaphanus to reach breaking after exposure to various low temperatures for different periods of time.

Mean days of breaking at control (20°C)	Exposure temp. °C	Exposure duration (days)	Mean days to break at lab.temp. after exposure period	Per cent break
8	0	14	8·0	99
		28	7.0	99
		42	6.5	98
		56	6.0	100
		70	4.0	99
		84	3.0	100
		112	started to show breaking	98
8	5	14	7.0	100
		28	4·0	97
		42	2·0	97
		49	started to show breaking	96
7	10	14	3	89
		21	started to show breaking	96

8.3 Survival and hatching of unhatched embryos at lower temperatures

The unhatched embryo is the embryo which protrudes through the split in the outer covering of the egg but is still contained in the inner, transparent third membrane. As mentioned before, the breaking in the outer covering of the egg could take place under natural conditions before the filling of the pond, especially when there is highly moistened soil surrounding the egg and favourable temperature exists. Moreover, it has been seen experimentally in a preliminary study that the egg could reach breaking stage after some time when placed in 100% relative humidity. The unhatched embryo may be exposed to lower temperatures in the field. For this reason it is relevant to study its survival and hatchability at lower temperatures.

8.3.1 Methods

Five hundred freshly laid eggs were placed in a petri dish filled with well aerated distilled water. The petri dish was kept at a mean laboratory temperature of 20°C. After seven days the eggs reached breaking stage. These unhatched embryos were divided into five batches of 100 eggs each. Four of them were kept at -5, 0, 5, and 10°C respectively. The fifth batch was kept at laboratory temperature as a control. The mean laboratory temperature at that time was 20°C. All batches were examined daily for any hatching.

8.3.2 Results

All the unhatched embryos kept at -5°C were found to be dead when examined the next day. They were removed and placed in laboratory conditions to examine the possibility of their recovering again; none of them recovered.

Embryos kept at 0, 5, 10°C and control (20°C) hatched within 2-3

days. At 0°C only 55% hatch was observed. Unhatched embryos hatched significantly less at 0°C in comparison with 5 and 10°C temperatures. However, no significant differences were observed between the per cent hatch at 5, 10 and 20°C.

The per cent hatch at different temperatures mentioned above are tabulated in Table [22]

TABLE [22]. Percentage hatching at different temperatures

Temperature °C.	Number embryos used	Per cent hatch
Control (20°C)	100	78
10	100	73
5	100	81
0	100	55
-5	100	0

8.4 Embryonic development at higher temperatures

Temperatures as high as 30°C or more may occur in the natural habitat of C.diaphanus in Britain. Such temperatures may be reached when the pond is full of water or subsequently when it dries up. These batches of eggs in this experiment were incubated at different but constant temperatures.

8.4.1 Methods

Five batches of freshly laid eggs were placed each in one of five glass tubes containing aged tap water to a depth of approximately 1·5 cm. Four of them were incubated in water baths at constant temperatures of 22, 23, 25 and 30°C for more than one month. Daily observation was carried out on these tubes. They were then transferred to watchglasses filled with well aerated aged tap water in order to see their viability and capability for further development. The fifth tube was kept at laboratory temperature as a control. The mean laboratory temperature at that time was 20°C.

8.4.2 Results

Twenty-one per cent of the eggs incubated at a constant temperature of 22°C reached breaking stage after 18 days of immersion. No more eggs showed breaking in the outer covering even after 34 days. After 34 days at temperatures of 23, 25 and 30°C, no egg reached breaking stage. The eggs were transferred to watchglasses filled with well aerated aged tap water. Peak breaking occurred in the 1st, 1st, 3rd and 4th days for those eggs which were previously incubated at 22, 23, 25 and 30°C respectively. When eggs were incubated at constant temperatures of 22 and 23°C a slight difference occurred in the rate of development in comparison with that of the control (20°C), although these temperatures prevented breaking

from taking place. The rate of development at 25 and 30°C was slower than that at temperatures of 22 and 23°C. The reason is, when the eggs were transferred to laboratory temperature, peak breaking occurred in the 3rd and 4th days at 25 and 30°C, while at 22°C and 23°C the peak occurred in the 1st day. At these treatments only about half of the embryos did hatch when they were transferred to laboratory conditions.

The prevention of the break in the outer covering was confirmed and demonstrated by further investigation. This prevention seems to have a significance important in the protection and survival of the embryos in nature, as will be discussed later on. No significant influence was found on the embryonic development or breaking of the eggs if incubated alternately at 20 and 22, at 20 and 23, at 20 and 25, and at 20 and 30°C. This matter will be discussed later.

8.5 Prevention of breaking at higher temperatures

To confirm the findings of the previous experiment, eggs in the late stage of development were incubated at constant temperatures of 23, 24, 30 and 35°C.

8.5.1 Methods

Five hundred freshly laid eggs were kept in a petri dish filled with aged tap water. These eggs were left for six days at laboratory temperature until they reached a point just before the breaking stage. They were then divided into five batches, 100 eggs each. Each batch was placed in a glass tube containing aged tap water to a depth of 1·5 cm. approximately. Four of them were incubated at constant temperatures of 23, 24, 30 and 35°C for about 8 days. The fifth batch was kept at laboratory temperature as a control. Daily observation was carried out and the mean laboratory temperature at that time was 20°C.

8.5.2 Results

No breaking in the outer covering of the egg was seen at any of the above temperatures, even after 8 days of incubation. But when the eggs were transferred to watchglasses filled with well aerated water and kept at laboratory conditions, peak breaking occurred in the 1st, 1st, 2nd and 4th days at temperatures of 23, 24, 30 and 35°C respectively, while in the control no delay in break was seen. The prevention of breaking at these temperatures could be caused by both the physical effect of these temperatures and by oxygen depletion. However, the second cause is more likely.

8.6 Effect of varying exposures to high temperatures on development and hatching

A long series of experiments were performed to investigate the influence of exposure to high temperatures for different periods of time as well as to ascertain the threshold temperature that allows development. Little investigation has been done concerning exposure of eggs of anostracan branchiopods to high temperatures and their subsequent effect on embryonic development and hatching.

8.6.1 Methods

Batches of freshly laid eggs were placed each in a glass tube containing distilled water to a depth of approximately 1·5 cm. These tubes were incubated at temperatures of 35, 40, 45, 50 and 55°C for different periods of time. Then they were removed and placed in watchglasses filled with well aerated distilled water. The watchglasses were kept at laboratory temperature. The mean laboratory temperature at that time was 20°C.

The exposure durations were varied from 30 minutes to 9 days, as shown in Table [23].

8.6.2 Results

Exposure to temperature of 35°C for a period of time as long as 9 days seems to have little effect on subsequent development of the eggs. The eggs exposed to this temperature showed a peak breaking in the 8th to 11th days after they were transferred to laboratory temperature. Delay in the occurrence of the peak breaking of 1-3 days could be seen. The percentage of breaking in each exposure with the duration to which the egg can withstand each temperature, together with the per cent hatch are given in Table [24]. The hatching process seems to be affected by the previous exposure of the eggs to high temperature, although little influence

TABLE [23]. Exposure durations to high temperatures

Number of batches	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Temp. °C	Exposure duration																
35	1h.	2	3	6	9	24	42	48	65	68	105	110h.	5 days	6	7	8	9
40	1h.	2	3	4	5	6	12.30	19	25.30	43	48	66	72	75	90	98	-
45	30 min.	45	60	90	2h.	2.30	3.30	4	18.30	24h.							
50	45 min.	60	75	90													
55	5 min.																

TABLE [24]. Time required for the eggs to reach breaking stage and percentage of broken eggs after they were transferred to laboratory temperature, together with the per cent hatch.

Exposure Temp. °C	Exposure duration	Days for peak breaking to occur	% Broken eggs	% Hatch
Control (20°C)		7, 8	94	80
35	1h.	10	92	54
	2	10	88	66
	3	9, 10	98	48
	6	10	96	82
	9	10	94	58
	24	10, 11	92	46
	42	8, 9	88	62
	48	7, 8	90	66
	65 68	7, 8	90	28
	105	8	70	56
	110 h.	8	46	44
	5 days	8	84	
		9, 10		32
	6	9, 10, 11	96	62
	7	9, 10	92	32
	8	9, 10, 11	92	36 8
	9	9	96	20
40	1h.	9, 10	100	68
	2	9, 10	92	70
	3	9, 10	94	24
	4	9, 10, 11	98	62
	5	9, 10, 11	94	50
	6	9, 10	94	66
	12.30	10	98	88
	19	8, 9	94	58
	25.30	8	96	44

TABLE [24] contd.

Exposure Temp. °C	Exposure duration	Days for peak breaking to occur	% Broken eggs	% Hatch
40	43	8	98	22
	48	8	88	22
	66	10	16	2
	72	-	20	2
	75	-	24	0
	90	-	8	0
	98	-	10	0
45	30 min.	7, 8	98	58
	45	7, 8	96	52
	60	7, 8	94	46
	75	7, 8	100	64
	90	8	96	38
	2 hours	8	94	74
	2.30	8	92	74
	3	8	94	64
	3.30	8, 9	88	44
	12.0	11	92	6
	18.30	12, 13	64	18
	24 hours	-	0	0
50	45 min.	8	72	24
	60	-	38	2
	75	-	8	0
	90	-	0	0
55	5 min.	-	0	0

on subsequent embryonic development has been seen. From the observations during this experiment it has been noticed that some of the hatched nauplii were very weak and morbid; some of them dying within a few hours after being hatched.

At a temperature of 40°C the eggs were exposed for periods of 1, 2, 3, 4, 5, 6, 12·30, 19, 25·30, 43 and 48 hours. This exposure did not significantly affect the subsequent development or percentage of broken eggs. Exposure for a longer time caused a sharp reduction in the percentage of broken eggs. This could be easily seen from the comparison of the previous results with the results obtained when the eggs were exposed for periods of 66, 72, 75, 90 and 98 hours. Only 16, 20, 24, 8 and 10 per cent respectively of the eggs reached breaking stage in the above experiment. Breaking in these exposures was also delayed. The reduced percentage hatch suggests that some inhibitory influence on the hatchability of the embryos results from these exposures.

The eggs exposed to 45°C for 30 minutes, 45, 60, 75, 90 min., 2 hours, 2·30, 3, 3·30 and 12 hours showed peak breaking in the 8th to 11th days. Again no significant differences between the percentage of broken eggs in comparison with that of the control were seen, but exposure for 18·30 hours resulted in only 64% breaking. None of the eggs had developed after exposure for 24 hours to 45°C. Some hatching also was inhibited owing to these exposures to this temperature.

The exposure of the eggs to 50°C for 45 min. seems to have little effect on subsequent development. After exposure for 60, 75 and 90 minutes, per cent break was 38, 8 and 0 respectively, but only a few had hatched. This is presumably due to the morbidity of the embryos. It has been generally observed that all embryos which could not hatch were very weak

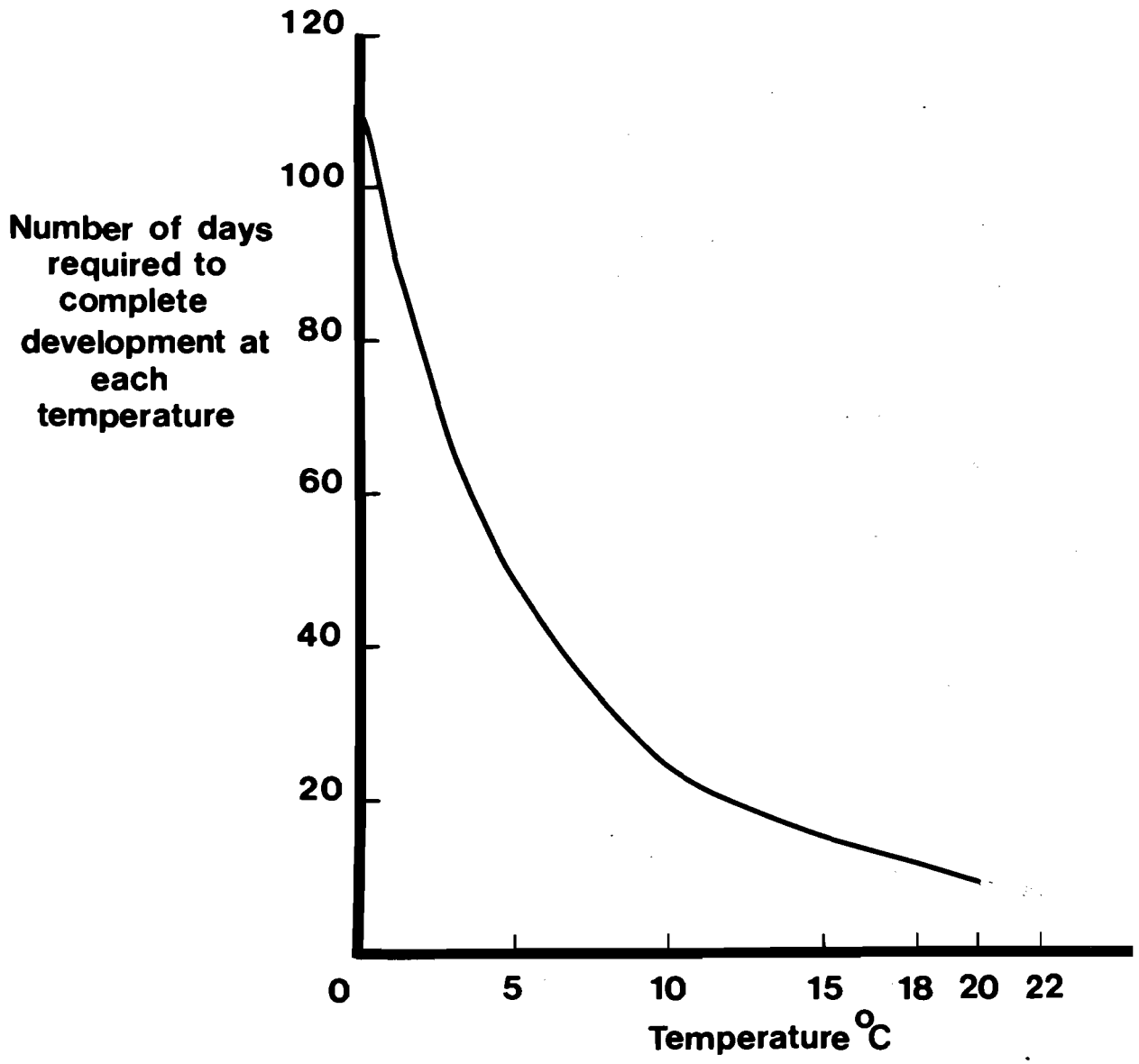


FIG.36

NUMBER OF DAYS REQUIRED FOR THE COMPLETION OF DEVELOPMENT OF C.diaphanus AT VARIOUS TEMPERATURES

and their characteristic movement around their longitudinal axis was very slow, and most of them were moving their appendages very feebly.

Higher temperature, that is to say 55°C seems to be fatal to the eggs even for a short time of exposure. No development could be noticed even after 5 minutes of exposure.

In general, short exposure to any temperature below 55°C did not significantly affect percentage break or hatching. Prolonged exposure resulted in a significant decrease in hatchability at all constant temperatures above 22°C, although per cent break at these treatments resulted in little significant differences in comparison with that of the control. Fig. [37] shows the time required to kill 50% of the eggs at various temperatures.

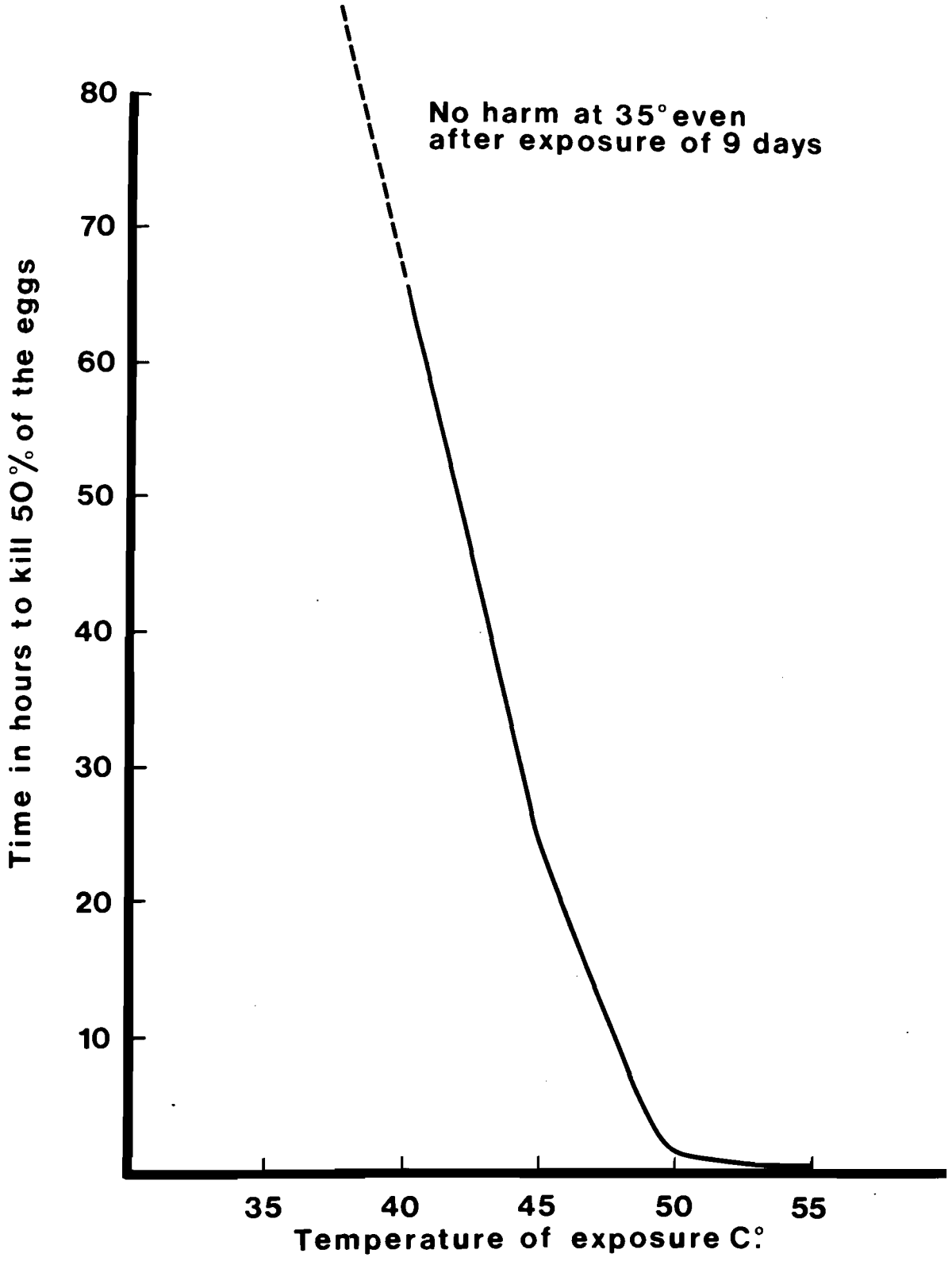


FIG.37 : TIME (HOURS) REQUIRED TO KILL 50% OF THE EGGS OF C.diaphanus AT VARIOUS TEMPERATURES

8.7 Alternating temperature

It was noticed from the previous section that the embryonic development was slow in the eggs incubated constantly at 22, 23, 25 and 30°C. Also it was observed that these temperatures prevented the occurrence of the break in the tertiary shell. However, when the eggs were transferred to laboratory temperature most of them reached breaking stage within one to four days of their transfer. This is of interest because it indicates that eggs could stay viable for some time if they were exposed to quite high temperatures in the field. It has already been reported that water temperature in the habitat of C.diaphanus may reach 30°C or higher during the summer period. It was decided to examine this point.

Batches of freshly laid eggs were incubated at temperatures alternating every twenty-four hours between laboratory temperature (20°C) and 22, 23, 25, 30 and 35°C respectively. The time required by the eggs incubated on alternate days at 20 and 22, and at 20 and 23°C was 7-8 days, which is the normal time usually taken by the eggs to show the break at laboratory temperature having a mean of 20°C. At alternating temperatures of 20 and 25°C the peak break occurred on the 9th day of incubation. This suggested that embryonic development took place at a slower rate; this was presumably due to the partial incubation at 25°C. At higher temperatures, that is to say at 30 and 35°C, the time required by the eggs to show the peak break was 10-11 days. These results are of interest because they suggest that, although eggs were prevented from reaching breaking stage at constant temperatures of 23, 25, 30 and 35°C, they did so when they were exposed alternately to these temperatures and 20°C. The eggs at these temperatures completed their embryonic development, although they did not show the break in the tertiary shell. This latter phenomenon may have considerable value for the survival of the embryos, since if they reached

the breaking stage and then hatched, the hatched nauplii might face unfavourable conditions during the summer when the pond exists only for short periods.

8.8 Discussion

No investigation of the quantitative relationship between temperature and the rate of development of C.diaphanus Prevost and other related species has been done in detail. However, Hall (1959a) and Mathias (1934) were able to observe this relationship. Both investigators have mentioned that there was a slow development at low temperature. In this study, a further detailed investigation was carried out in order to throw some light on, and elucidate, such an important matter. A long series of experiments were carried out in which different temperatures were studied. At very low temperature ($-5^{\circ}C$), no development was observed. It was noticed that all eggs exposed to such sub-freezing temperature were found to be dead when examined one day later. This temperature seems to be lethal for the eggs of this species in wet conditions. The death of the eggs could be, in this case, due to the mechanical effect of the formation of ice upon the embryonic mass or to the complete arrest of the physiological processes, or to both reasons. It seems likely that low temperature is likely to prove lethal only when all the water surrounding the eggs in the habitat freezes. When there is an adequate supply of water below the ice, there is no evidence that freezing of the surface will cause a complete freezing of the habitat. However, a preliminary study on freezing of the eggs in dry conditions suggested that the eggs could survive for a considerable time at a temperature of $-5^{\circ}C$. Dexter (1946), reported that the frozen eggs of E.vernalis would not develop or hatch. However, the requirements of freezing for the eggs of Branchipodopsis affinis, were reported by Bond (1934). He did not mention if the eggs were wet or dry. Exposure for 48 hours at $-5.5^{\circ}C$ kills all the eggs of Aedes aegypti and exposure at $0^{\circ}C$ kills some (Davis 1932).

At temperatures of 0, 5, 10, 15, 18 and $20^{\circ}C$ it has been observed that the rate of development increases with the increase of temperature

up to a limit. This limit seems to be at a constant temperature of 22°C. At this temperature 21% of the eggs reached breaking stage after eighteen days of immersion. However, most of the unbroken eggs did so when they were removed to laboratory temperature within two days.

Broch (1965) reported that early primary development of C.bundyi was retarded by low temperature (7°C). However, C.bundyi differs from C.diaphanus in that only early primary development is accelerated by temperature increase in C.bundyi, and further development occurs only under low temperature conditions. In the controls of this series of experiments, eggs reached breaking in the 7th or 8th day of immersion. The slight difference in the controls of all experiments may be due to either or both of two causes: (a) the eggs may have been different in their physiological processes, or (b) the environment in which they developed may have been different.

As has been seen from the results, the higher the temperature the shorter the time required for the egg to complete its development, Fig. [36]. The egg required 112, 49, 24, 16, 13 and 7 days to reach breaking stage at 0, 5, 10, 15, 18 and 20°C respectively. Table [21] shows the duration of the incubation period at different low temperatures and the subsequent time required for the eggs at laboratory temperature to reach breaking stage, as well as the percentage breaking in each case. Duration of different periods (at all temperatures above) did not significantly affect the hatchability of the eggs when they were transferred to laboratory temperature.

The development per day at each temperature was estimated by calculating the reciprocal of the duration period required for completion of development. This was multiplied by 100 in order to get the percentage of development per day. A simple formula could be used for this purpose.

This formula is 1/d x 100, where d is the time required for the egg to complete its development at each temperature. Table [20] shows the mean percentage of development per day at each temperature together with the time required for the egg to reach breaking stage. Rate of development, at a temperature of 2°C, of this species was obtained by Hall (1959a); in general, his results are in agreement with the results of this study.

It has been seen from the results that the temperature of -5°C was lethal to unhatched embryos. This may be due to the mechanical action of the formation of the ice upon the embryo. Unhatched embryos would survive and hatch at low temperature of 0°C. The per cent hatch at this temperature was 55%. However, unhatched embryos kept at 0°C hatched significantly less in comparison with 5, 10 and 20°C. The percentage hatched at 5, 10 and 20°C (control) were 81, 73 and 78% respectively. (Table [22]). Mattox (1950) reported that eggs of Cyzicus would occur in nature and laboratory only when the temperature had reached above 10°C. Eggs of Lepidurus apus (L.) did not hatch at temperatures below 6°C or above 20°C as reported by Chaigneau (1958). However, preliminary observation on the habitat of C.diaphanus suggested that hatching would occur earlier when the temperature was higher.

Embryonic development at constant temperatures higher than 20°C seems to be slower. No egg reached breaking stage at those temperatures even after 34 days of incubation. However, when the eggs were transferred to laboratory conditions peak breaking occurred in the 1st, 1st, 3rd and 4th days at 22, 23, 25 and 30°C respectively. This suggests that the embryonic development which has taken place at constant temperatures of 25 and 30°C was slower than that at 22 and 23°C. This could be associated with the amount of oxygen consumed and to the depletion of oxygen as well as to the physical effect of those temperatures. The above temperatures

seem not only to affect the early embryonic development but also to prevent the breaking in the outer covering of the egg. This has been shown from the results of the incubation of eggs at later stages at these temperatures. However, these eggs showed breaking when they were transferred to laboratory conditions. Although the incubation at these temperatures resulted in a significant decrease in hatchability, this indicates a good tolerance to high environmental temperatures which may occur in their habitat. The prevention of the break in the outer covering of the egg may have a significant importance in the field. High temperatures may occur in nature. If they do, and so allow breaking to take place, this will lead to the risk that broken eggs will face unfavourable conditions in the summer, especially when the eggs are still incubated in moistened soil after the pond dries up. This prevention may provide an opportunity to those eggs which have completed their development to live and survive until the occurrence of favourable conditions and the establishment of the pond. However, it has been observed from a preliminary investigation that eggs could reach breaking stage when incubated in 100% relative humidity. Water temperature in the habitats of the anostracan branchiopods may reach to a value as high as 35°C or higher (Moore 1955).

The exposure of the eggs to a constant temperature of 35°C for at least 9 days showed no significant effect on subsequent development. When the eggs were transferred to laboratory conditions, a high percentage of them reached breaking stage. But only some of the broken eggs had hatched.

At a temperature of 40°C eggs withstood exposure for 48 hours without noticeable influence on subsequent development. However, there was a delay of 1-3 days in the occurrence of the peak breaking. Exposure of eggs for more than this time would inhibit development clearly.

At higher temperatures, that is to say 45°C and 50°C, the time required to inhibit the subsequent development and kill most of the eggs was 24 hours and 1 hour respectively.

Complete inhibition of embryonic development was caused at a temperature of 55°C when eggs were exposed for 5 minutes only. This may be due to their death after this exposure. The cause of the death is more likely to be the physical effect of this temperature.

It can be concluded that short periods of exposure to high temperatures did not significantly affect subsequent development, although these exposures resulted in a decrease in per cent hatch. Increasing the exposure time, however, resulted in a sharp reduction in hatchability as well as percentage breaking at temperatures higher than 35°C.

The results of this experiment suggest that higher temperatures have a harmful effect on the embryonic mass and on development. The degree of this harmfulness seems to depend on both temperature and duration period of exposure. As has been seen from these results, the higher the temperature of exposure the shorter is the time required to harm and kill the egg (Fig. [37]). However, no disruptive effect on the egg-shell could be seen at these exposures.

SECTION NINE

OXYGEN AS A FACTOR AFFECTING EGG DEVELOPMENT AND HATCHING OF THE EMBRYO

SECTION 9

Oxygen as a factor affecting egg development and hatching of the embryo

9.1 Introduction

A little work has been done concerning the relationship between the oxygen concentration and the development of the egg. The eggs remain at the bottom of the pond after being laid by the females, and then in the moist soil when the pond dries up. Since this is the case, elucidation of such relationship is required. Field observations showed that the hatching of the nauplii of C.diaphanus occurs in the pond within 24-36 hours (or even less) after refilling by the well aerated rain-water. After the inundation and before the reduction of oxygen concentration in the mud - water interface (e.g. Brown and Carpelan, 1970, and Judson 1960), the rapidity of the hatching process may be due to the exploitation of the newly formed favourable conditions. Dutrieu (1960), working with A.salina reported that the dissolved oxygen is indispensable for the development and hatching of the eggs of this species. Moore (1963) suggested that low oxygen concentrations inhibited hatching of S.seali in the winter pools, but he did not mention its influence on the embryonic development. On the other hand, Broch (1965) found that the embryos of C.bundyi would hatch if placed in water of low oxygen concentration; at the same time he said that the early stages of development would be inhibited by such a low oxygen concentration.

The establishment and formation of low oxygen concentration in the aquarium probably prevents embryonic development. Hall (1959b) noticed such a phenomenon and he added that when he removed those eggs and placed them in shallow water they hatched. Hall interpreted this inhibition of the development as an influence of the depth of the water in the aquarium rather than the low oxygen concentration which may exist

there. Dexter (1946) also reported the inhibition of the egg development in the aquarium. In 1950 Mattox stored the eggs of Caenestheriella gynecia in water in a bottle at room temperature for about eight and a half years. This storage led to complete suspension of development and hatching. But when he transferred them to fresh tap-water they hatched after six days. Hatching of Limnadia stanleyana King was markedly repressed at lower oxygen concentrations, as stated by Bishop (1967). He also said that maximal hatching would occur at conditions of higher oxygen concentration.

9.2 Methods

A series of different oxygen concentrations was prepared in 10 litre reservoir bottles containing 8 litres of distilled water. The water was partially de-oxygenated by subjecting it to a vacuum produced by a powerful vacuum pump. De-oxygenation was hastened by heating the bottles in a warm-water bath while evacuation was carried out (Moore, personal communication). The de-oxygenated water was cooled to laboratory temperature before use. Another method was used to produce lower oxygen concentrations. In this method the reservoir bottle was connected to a nitrogen gas cylinder by a glass tube. At the time of introducing the nitrogen gas, the bottles were heated in a warm-water bath, to hasten the de-oxygenation process. After using this method for different periods, two layers , one of oil and one of liquid paraffin, were added to prevent any gaseous exchanges with atmospheric air. Water was then transferred to the hatching tubes and also to stoppered bottles for subsequent measurement of the dissolved oxygen by the Winkler method (Amer.Pub. Heal.Assoc., 1965), through a tube connected to the stopcock of the reservoir bottle which was introduced into the bottom of the bottles and allowed to overflow from the neck for some time, to avoid contaminating the water with air.

The influence of different oxygen concentrations on the early stages of embryonic development up to the breaking of the outer covering of the egg was studied. Similar observations were made on the breaking and hatching processes. (Breaking is the process in which the embryo protrudes through the split in the outer covering of the egg but is still contained in the inner, transparent third membrane. Hatching is the process by which the nauplius is set free by the rupturing of the inner transparent membrane.)

To examine the effect of low oxygen concentrations on the early stages of embryonic development, batches of freshly laid eggs were made up; each was pipetted into one of the test tubes below the oil and paraffin layers. The test tube was 2·5 cm. in diameter, containing distilled water to a depth of 1·5 cm (this is to eliminate the influence of the depth, if there is any). The concentrations of the oxygen in the tubes were 13·0, 9·8, 7·7, 6·8, 4·4, 3·8, 2·3, 2·0, 1·7, 0·9, 0·5 and 0·2 mg/l. Another batch of eggs was placed in a watchglass containing well aerated distilled water and kept as a control (open system). All the tubes were kept at 22°C (∓ 2°C) under daily observation for any sign of breaking or hatching of the nauplii. The eggs were left for seven days in these conditions, then they were removed and placed at standard aerobic conditions (open system). (Seven days is the time required for the egg to reach breaking stage at 22°C.)

To examine the influence of different oxygen concentrations on the breaking stage, different concentrations of oxygen were prepared by the same method as used before. A large number of freshly laid eggs was kept in a petridish filled with well aerated distilled water for six days at 22°C, until they reached a point just before breaking stage. The eggs were divided into ten groups. Nine of them were pipetted into nine test tubes, each containing different oxygen concentrations. These concentrations were 8·1, 5·8, 2·6, 1·9, 1·6, 1·4, 0·8, 0·4 and 0·3 mg/l. respectively. The tenth group was left in the petridish as a control (open system). All these groups were kept at 22°C under daily observation for any sign of breaking or hatching.

The effect of varying oxygen concentrations on the hatching process was studied. This was demonstrated through the incubation of a batch of freshly laid eggs in a petridish filled with well aerated distilled water

at 22°C for seven days until they showed breaking, although they were still contained in the third transparent inner membrane. These unhatched embryos were divided into eight groups; seven of them were pipetted into one of seven glass tubes containing 3.8, 2.2, 1.5, 0.8, 0.6, 0.4 and 0.3 mg/l. respectively. The eighth group was placed in a watchglass containing well aerated distilled water as a control. The mean laboratory temperature throughout this experiment was 22°C. Daily observation for the appearance of hatching of nauplii was carried out.

9.3 Results

In the first set of experiments, after the eggs have spent seven days in different oxygen concentrations at 22°C, all batches were removed and placed in watchglasses filled with well aerated distilled water (open system). A peak breaking occurred in the first day at concentrations of 13.0 - 6.8 mg/l. Some of the eggs were found to be already in the breaking stage, while the peak occurred in the third day at concentrations as low as 2.3 mg/l. No egg had reached the breaking stage in this case. In much lower oxygen concentrations (0.9, 0.5 and 0.2 mg/l.) the peak occurred in the fifth and sixth days. It seems that oxygen concentrations as low as 2.0 mg/l or even less did not inhibit the embryonic development completely; in fact it was very slow. But at lower concentrations (0.9 mg/l) and below the inhibition was obvious. After fourteen days had elapsed since the transference of the eggs to the watchglasses, the percentage of the eggs that showed breaking at different oxygen concentrations are given in Table [25].

In the second set of experiments, the eggs completed their development in aerobic conditions for six days at 22°C, until they reached a point just before the occurrence of the break in the outer covering of the egg. Then these eggs were transferred to different concentrations of oxygen. In the control (open system) and at high oxygen concentrations (8.1 and 5.8 mg/l.), a peak breaking occurred in the next two days after the transfer of the other batches to anaerobic conditions. At the oxygen concentration of 2.6 mg/l the eggs started to show breaking in the third day. In the fourth and fifth days other lots reached breaking. But at lower concentrations, that is to say 1.6, 1.4, 0.8, 0.4 and 0.3 mg/l., eggs did not start to show breaking until the seventh and eighth days of immersion. Breaking percentage at different

TABLE [25]. Shows the percentage of the eggs that showed breaking at different oxygen concentrations, together with the day of peak break.

Oxygen concentration mg/l.	Eggs broken		Day of peak break
	Number	Per cent	
Open system (control)	48	96	
13·0	49	98	1
9.8	44	88	1
7·7	49	98	1
6.8	49	98	1
4·4	46	92	1,2
3·8	41	82	2,3
2·3	42	84	3
2·0	32	64	4
1·7	29	58	4,5
0·9	30	60	5
0·5	33	66	5
0·2	28	56	6,7

oxygen concentrations fourteen days after immersion is shown in Table [26]. Most of the eggs which had failed to reach breaking stage in a low oxygen concentration did so later when they were transferred to aerobic conditions (open system), but still some did not. Unfortunately, due to some technical difficulties, the number of breaks occurring each day in this experiment could not be observed. It is noticeable that anaerobic conditions retard or prevent the development of the egg of this species.

In the control test tube of the third set of experiments, the unhatched embryos started to hatch in the next day. Seventy-eight per cent of them hatched within 24 - 48 hours. At low oxygen concentrations of 0·4

TABLE [26]. Breaking percentage at different oxygen concentrations after fourteen days of immersion.

Oxygen concentrations mg/l.	Eggs broken		Day of peak break
	Number	Per Cent	
open system (control)	42	84	
8·1	49	98	1,2
5·8	48	96	1,2
2·6	26	52	3,4,5
1·9	34	68	3,4,5
1·6	13	26	5,6
1·4	23	46	6,7,8
0·8	15	30	6,7,8
0·4	13	26	6,7,8
0·3	14	28	7,8

and 0·3 mg/l., only 10 and 4 per cent of them hatched. Percentage of hatched nauplii after one week is given in Table [27].

TABLE [27]. Percentage of hatched nauplii after one week at different oxygen concentrations.

Oxygen concentration mg/l.	Eggs broken	
	Number	Per cent
Open system (control)	39	78
3·8	30	60
2·2	26	52
1·5	26	52
0·8	13	26
0·6	9	18
0·4	5	10
0·3	2	4

In order to test the ability of the embryos to hatch, the unhatched embryos were transferred after seven days from the closed systems to watchglasses filled with well aerated distilled water. About 20-40 per cent of the embryos hatched.

9.4 Discussion

One of the most important factors which has a great influence on the life cycle and the biology of any species inhabiting a temporary freshwater habitat is the oxygen concentration. Such a microenvironmental factor plays its role together with the temperature on the stages through which this species passes to complete its life cycle, especially on the egg and its development. It is obvious that the eggs are usually exposed to different concentrations of oxygen in the natural conditions at different embryonic stages.

In the field three distinct phases of environmental factors which can influence the development of the egg could be differentiated. Firstly, after the laying of the eggs by the females, they sink to the bottom of the pond and stay there. At this time, the eggs pass through a condition of very low oxygen concentration. Webster (1962) and Brown and Carpelan (1970) reported very low oxygen concentrations at the bottom of the pond. Very little, if any, embryonic development seems to take place under these conditions. Two generations were never observed in the pond at the same time. Apparently this is due to the inhibition of the egg development at such low oxygen concentrations as existed in the mud-water interface. These concentrations may reach values as low as 0·22 mg/l. e.g. Webster (1962) and Cole (1932).

From the results of the first set of experiments it has been seen that the embryonic development was inhibited at very low oxygen concentration (below 0·9 mg/l.). At an oxygen concentration of 2·0 mg/l. embryonic development occurred, although very slowly. At higher concentrations the development was more rapid. This indicates the necessity of oxygen for the early stages of the development of C.diaphanus, at least in reasonable concentrations. Hall (1959b) attributed the inhibition of

embryonic development at the bottom of the aquarium to the depth of the water rather than to the low oxygen concentration which might exist there. The reduction in oxygen concentration at the bottom of the aquarium could be due to the accumulation of carbon dioxide and other poisonous gasses, resulting from the decomposition of the organic matter found there. The inhibition of the embryonic development in anaerobic conditions was shown experimentally by Broch (1965) in his work with C.bundyi. He found that the development was stopped by such conditions. Bishop (1967) observed the same effect on the development of the eggs of L.stanleyana, which inhabits temporary freshwater pools. The necessity of oxygen to the embryonic development of this species can be considered as a kind of adaptation which enables the species to survive and repeat itself in a changeable temporary habitat. Because, if any embryonic development takes place at the bottom of the pond, there will be hatching of nauplii which may face desiccation many successive times before they reach sexual maturity and be able to produce eggs. This will lead to the complete elimination of the species from its habitat. Thus, the hatching and the early stages of development seem to depend largely on the presence of a sufficient amount of oxygen in its environment.

The second phase of microenvironmental factors is the period when the pond dries up. This provides conditions which are more favourable for the embryonic development. But the significance of this phase is not due to the drying up of the pond but to the consequent increase in oxygen concentration. A moist soil surrounding the eggs, which can provide aerobic conditions after the pond dries up, enables the embryonic development to proceed and continue until the embryos reach a point just before the breaking of the outer covering of the egg. Some of the eggs may go even further in their development and show breaking if the moisture is quite high. In the second set of experiments, it was observed that

some of the eggs reached breaking stage after three - five days at a concentration of 2·6 mg/l., but they did not reach this stage at lower concentrations even after seven days. But when they were transferred to aerobic conditions, some showed breaking within 24 hours or more. Others failed to do so. The reason for the failure of some embryos to reach breaking after the transfer to aerobic conditions could be due to their death at such very low concentrations. When the oxygen concentration (as low as 2·8 mg/l.) is in equilibrium with atmospheric air, it may have no inhibitory effect on the embryonic development and hatching. Thus, the partial inhibition and delay of development in this case could be due to the reduction in the oxygen concentration. This reduction may be due to the prevention of any gaseous exchange with the atmospheric air. It can be concluded from these results that even the breaking of the outer covering depends on the presence of a sufficient amount of oxygen in the microenvironment of the egg. Broch (1965) has shown in his work with C.bundyi that pre-hatching (breaking and early stages of development) was inhibited at low oxygen concentrations, which is in agreement with the above results.

The third and final phase of ecological factors which influence the egg development seems to be the refilling of the pond by the well aerated rain-water. The embryos at this phase will reach the breaking stage or they may be at a point just before this stage, so they are ready to reach it and then hatch. The availability of favourable conditions for the hatching process, such as high oxygen concentration, presence of free standing water and probably low osmotic concentration are provided by the rain-water soon after the rainfall and before the rapid reduction in the oxygen concentration at the mud-water interface. Such reduction in oxygen level may take place a few hours, or maybe more,

after the establishment of the pond (Brown and Carpelan 1970, and Judson, 1960). It was observed that hatching occurred early in the field. This is in agreement with observations by Broch (1965) and Bishop (1967). This early hatching enables the embryos to exploit the temporary existence of the high oxygen concentration, but some hatching may occur during the process of reduction in oxygen level and before it reaches a very low concentration. The results of the third set of experiments indicate that the eggs of C.diaphanus would hatch more readily at higher concentrations of oxygen, and that at low oxygen concentrations it was clearly inhibited (10% hatched at 0·4 mg/l. and 4% hatched at 0·3 mg/l.), while 78% hatched in the open system (control). Bishop (1967) found that low oxygen concentration inhibited hatching of L.stanleyana. In their work on B.mackini, Brown and Carpelan (1970) reported that an oxygen concentration as low as 1 mg/l. would completely inhibit hatching. Moore (1963) suggested that eggs of S.seali hatch only in well aerated rainwater, soon after the refilling of the temporary pools. The necessity of oxygen for hatching was also reported by Dutrieu (1960) in his work on A.salina. On the other hand, Broch (1965) has shown that C.bundyi would occur more readily in water having a low oxygen concentration, which was in agreement with the findings of Borg and Horsfall (1953) with Aedes eggs, while Judson (1960) described an inverse relationship in Aedes aegypti L. and A.nigromaculis Ludlow. Brewer (1964), in his work with the copepod Diaptomus stagnalis Forbes, reported that the lowering of the oxygen concentration was the hatching stimulus. Finally, Mattox (1950) stored some eggs of C.gynecia with water in a bottle at room temperature for a period of nearly eight years. No development or hatching could be seen. After that they were removed to fresh tap-water. The eggs hatched after a period of six days. The suspension of the development and hatching for

such a long time could be due to the depletion of the oxygen concentration; so when he transferred the eggs to well aerated fresh tap-water they resumed their development and then hatched because of the sufficient amount of oxygen provided by the fresh tap-water.

SECTION TEN

THE INFLUENCE OF WATER DEPTH ON THE DEVELOPMENT AND HATCHING OF C.DIAPHANUS

SECTION 10

The influence of water depth on the development and hatching of C.diaphanus

10.1 Introduction

Little previous work has been carried out upon the relationship existing between the oxygen content and depth of water in the anostracan branchiopods' habitat. However, Hall (1959b, 1959c) mentioned the phenomenon of delayed development in the eggs of C.diaphanus and suggested that there was a correlation between this and the depth of water overlying the eggs. In his work with E.vernalis, Castle (1938) found that if the eggs of this species were kept in an aquarium, then after the water depth had been reduced the eggs hatched. Eggs of the same species were kept by Avery (1939) in finger bowls until they were needed. Weaver (1943), working with the same species, found that when the eggs had been transferred from aquaria to plain water they remained unhatched for as long as nine months. Moreover, Moore (1951), working with S.seali, reported that if eggs were immersed for several weeks in water they hatched after the volume of the water covering them had been reduced to half by evaporation. Other workers reported that there was no correlation between depth of water and embryonic development and hatching of anostracan branchiopods (Bernice, 1972b; Broch, 1965; and Moore, 1967).

Finally, it was noticed in a preliminary observation that a slow development seemed to have taken place while the eggs were at the bottom of aquaria containing pond water which had a depth of 20 cm. approximately. Oxygen concentrations of a value as low as 0·85 mg/l. were measured at the bottom of the aquaria mentioned above. The low oxygen values might have been the cause of the retardation in the embryonic development.

10.2 Methods and materials

Two methods were used in this series of experiments. The first method involved the use of glass tubes 33 x 3 cm. filled with water to different depths. In the second method, small glass cylinders 2 x 1 cm. containing eggs were suspended in a large tank of water at different depths.

Adult animals were placed as groups in 500 ml. beakers of habitat water. The beakers were inspected the next day and the deposited eggs collected. Then these eggs were washed several times with distilled water, counted and separated into batches of 50 or 100 eggs each. The water used as hatching medium was distilled water, aged tap water and pond water. Both distilled and pond water were used in this series of experiments in order to determine if there was any difference in the results between the two types of water, due to the possible influence of the decomposition of the organic matter present in the habitat water. In the first method, glass tubes were filled with distilled water to different depths ranging between 1 and 25 cm. Eggs, after being washed and counted, were pipetted into the bottom of the glass tubes containing distilled water. In the other replicates eggs were pipetted into the bottom of glass tubes containing aged tap water or pond water instead of distilled water. In the replicate in which pond water was used as a hatching medium, it was observed after a few days that the eggs were covered partially with algal and faecal material. Such a phenomenon was also noticed by Hall (1959c). The glass tubes were kept in the laboratory under daily observation, and the dates on which the break appeared in the tertiary shell of the eggs were noted. Hatched nauplii were removed carefully using a long capillary pipette without disturbing the water in the tubes. The mean temperature of the laboratory throughout the investigation was 22°C.

In the second method batches of eggs were placed in small glass cylinders 2 x 1 cm. These cylinders were then suspended by means of thin wires at the respective depths in a large tank of water. The water tank was kept in the laboratory under daily observation. The mean laboratory temperature at that time was 22°C.

In both methods used eggs were removed and placed in watchglasses containing water after most of them had reached breaking stage. On the tenth day of immersion oxygen concentrations were measured at different depths in both tanks filled with distilled water and pond water. The measurement of the oxygen concentration was made using the probe of the "Laboratory Oxygen Analyzer Model 777, Beckman Instruments Inc.". The measured values are shown in Table [31].

The low oxygen concentrations found at greater depths of habitat water, and which might be caused by the decomposition of the detritus present there, could be the reason for the retardation of the embryonic development observed by some investigators. It was decided therefore to investigate this factor. Two batches of 200 eggs each were pipetted into the bottom of two glass tubes filled with habitat water to depths of 15 and 25 cm. Some detritus and faecal materials were collected from the bottom of aquaria containing adult animals and were pipetted into the bottom of the experimental glass tubes covering the eggs which lay there. Another batch of eggs was placed in a watchglass containing filtered pond water as a control. The tubes and the watchglass were kept at laboratory conditions under daily observation. On the tenth day of immersion, oxygen concentrations near the bottom of the tubes were measured. Values of the oxygen concentrations are given in Table [31].

10.3 Results

In both replicates in which distilled water was used as a hatching medium high percentage of breaking was observed in comparison with that of the control. Even at the greatest depths appreciable percentage break was still found. For example, it was found that at depths of 15, 20 and 25 cm. water the mean percentage break was 93.5, 87.0 and 81.5 per cent respectively, whereas at depths of 1, 5 and 10 cm. water the percentage break was 93.5, 83.5 and 92.5 per cent respectively. From the above figures it seemed that depth of water had no significant effect on the embryonic development of this species. The percentage of hatching obtained for those eggs kept at the depths mentioned above also suggests that depth has no significant influence on this process; nevertheless percentage hatch was less than the percentage break. For example, the mean percentage of hatching at depths of 5, 10, 15, 20 and 25 cm. water was 69.0, 72.5, 70.0, 53.5 and 61.5 per cent respectively, whereas for a depth of 1 cm. water (control) the percentage hatch was 79 per cent. Table [28] shows the total percentage break and the total percentage hatch found in distilled water at different depths using both methods mentioned before. However, it was noticed that at greater depths, especially 15 cm. water upwards, the period required for the eggs to reach breaking stage was one - two days longer than that required in the control (shallow water).

In the second series of investigations, pond water was used as a hatching medium instead of distilled water. The results in this case were very interesting, since smaller numbers of eggs had reached breaking stage and hatched than was found in distilled water in the first series of experiments. For example, the percentage of breaking twelve days after immersion in water ranged from 68 - 32 per cent at depths ranging from 5 - 25 cm. of pond water, whereas the percentage hatch was 38 - 25 per cent.

TABLE [28]. Percentage break and percentage hatch twelve days after placing at various depths of distilled water.

Depth of water in cm.	Percentage break	Percentage hatch
1 cm. (control)	94 (93)*	78 (77)*
5	83 (84)*	72 (66)*
6	92	70
7	88	72
8	91	72
9	98	62
10	91 (94)*	68 (77)*
11	100	74
12	91	59
13	87	58
14	89	64
15	93 (94)*	72 (68)*
16	91	52
17	89	54
18	84	54
19	78	46
20	88 (86)*	44 (63)*
21	80	72
22	83	56
23	88	58
24	85	54
25	83 (80)*	56 (67)*

* Second trial

Both the percentage of breaking and hatching were calculated twelve days after immersion. Moreover, a delay of one - two days was noticed for those eggs which had been kept at depths of 5 and 10 cm. pond water, whereas a delay of two - three days was found in those eggs which had been kept at greater depths, that is to say 15, 20 and 25 cm. pond water. However, it was noticed that most of the remaining eggs reached breaking stage within two days of their transfer to the watchglass. All the results are presented in Table [29].

TABLE [29]. Percentage break and percentage hatch twelve days after placing at various depths of pond (habitat) water

Depth of water in cm.	Percentage break	Percentage hatch
1 cm. (control)	99	70
5	68	38
10	66	31
15	58	25
20	44	32
25	32	25

Table [30] shows the results obtained when aged tap water was used as a hatching medium. In general, one can see that these results are very similar to those obtained when distilled water was used as a hatching medium.

Those eggs which had been kept in habitat water at depths of 15 and 25 cm. and were covered with a layer of detritus did not show the break even after an immersion period of five weeks. However, when they

TABLE [30]. Percentage break and percentage hatch twelve days after placing at various depths of aged tap water

Depth of water in cm.	Percentage break	Percentage hatch
1 cm. (control)	97	73
5	83	72
10	92	68
15	87	62
20	88	53
25	63	44

were removed and placed in watchglasses filled with distilled water, most of them reached breaking stage within 4-6 days after their transfer from depths of 15 and 25 cm. water. This indicates that slow embryonic development seems to have taken place under those conditions. Moreover, the oxygen concentrations measured at the bottom of the tubes ten days after setting up the experiment were 1·1 and 0·9 mg/l. at 15 and 25 cm. water respectively. Higher oxygen concentrations were found at the bottom of those tanks which were filled with distilled and habitat water. The oxygen concentrations measured are given in Table [31].

TABLE [31]. Shows oxygen concentrations measured at various depths of distilled water, habitat water and habitat water with a layer of detritus at the bottom of the tube.

Depth cm.	Distilled water	Habitat water	Habitat water with a thin layer of detritus at the bottom
1	3·8	3·15	-
5	3·8	3·0	-
15	3·1	2·3	1·1
25	2·4	1·8	0·9

During the course of this study it was felt that it might be interesting to measure the oxygen level found at the bottom of the aquaria which contained adult fairy shrimps, in order to see the variation of the oxygen in the microhabitat of the eggs after being laid. It was found that the oxygen concentration varies, ranging from 2·5 mg/l. to a value as low as 0·85 mg/l.

10.4 Discussion

One of the environmental factors which was thought to have some influence on the embryonic development and hatching of the anostracan branchiopods is the depth of the water. Depth may exert its effect through three possible factors. These factors are (a) the pressure of the column of the water, (b) oxygen depletion and increase in poisonous substances, and (c) osmotic concentration.

Oxygen concentrations vary greatly depending on several factors, such as temperature, population densities, type and efficiency of the flora through their photosynthetic process, and the concentration also depends on the amount of organic matter present in the pond. It was previously found elsewhere that a very low oxygen concentration would inhibit the embryonic development and hatching of this species. The third possibility can be eliminated, since it was found elsewhere that the eggs of this species could complete their embryonic development and hatch at osmotic concentrations much higher than that of the experimental media.

The oxygen concentrations found at the mud-water interface of the pond were very low, as has been seen elsewhere. This low value and depletion of oxygen may be due to several factors, such as (1) decomposition of organic matter through the action of the micro-organisms, and (2) respiratory activities of the organisms inhabiting the mud or living close to the mud-water interface. The respiratory activities of the animals and plants in the pond should also be mentioned which contribute to the reduction of the oxygen in the pond water. This reduction, in turn, will affect the amount of oxygen found in the mud-water interface. It was noticed that oxygen concentration in the upper layer of the mud had fallen to a value as low as 0·5 mg/l. Moreover, an oxygen concentration of 0·85 mg/l. was found at the bottom of an aquarium having a depth of 20 cm.

Hall (1959b) suggested that there was a correlation between delayed development and depth of water overlying the eggs of C.diaphanus. Later (1959c) he concluded that the period required for development and hatching at greater depths is greater than that required in shallow water, and that the time required increases with the increase of depth. The results of the present study indicate that, using distilled water or aged tap water, the depth seemed to have no significant influence on the embryonic development and hatching of this species, at least up to a depth of 25 cm. water. Moreover, Moore (1967) found that a depth of water as high as 30 cm. seemed to have no significant influence on the hatching of the fairy shrimp S.seali. The delayed development shown in Hall's investigation might be due to the low oxygen concentration at the bottom of the glass tubes. The low value of the oxygen might have been due to the decomposition of the organic material and detritus accumulated there. Moreover, Hall (1959c) stated that he used water from the aquarium as a hatching medium, and also he noticed that the surface sculpturing of the eggs was somewhat obscured by faecal material and the eggs were "dirty". This supports the interpretation given to the delayed development observed in Hall's work. It has been seen that when habitat water was used as a hatching medium a delay of two - three days was found in the development of those eggs which had been kept at greater depths. Broch (1965) also found that depth had no significant effect on the development and hatching of C.bundyi. In her work with S.dichotomus, Bernice (1972b) reported that depth of water seemed to have no influence on the development and hatching of this species, since she found that a depth as great as 120 cm. did not affect the development and hatching.

On the other hand those eggs which had been kept at depths of 15 cm. and 25 cm. of habitat water, and were covered with a layer of

detritus, showed the delayed development mentioned by Hall (1959c), since a very slow embryonic development was found to have taken place under these conditions. This delayed development might be due to the low oxygen concentrations existing there and to the accumulation of poisonous gasses resulting from the decomposition of the organic material. Moreover, it has been seen that oxygen concentration as low as 0·9 mg/l. was found under these conditions.

In his work with E.vernalis, Castle (1938) found that eggs were kept in an aquarium for an unspecified time; then hatched after the water depth had been reduced. Eggs of the same species were kept in finger bowls until they were needed by Avery (1939). Furthermore, Moore (1951), working with S.seali, reported that eggs which had been immersed for several weeks in water hatched after the volume of the water covering them had been reduced to half by evaporation. It seems entirely possible that in all cases mentioned above the cause of the delayed development was the low oxygen concentrations resulting from the decomposition of the organic material and detritus accumulated at the bottom of the aquaria, especially as all the investigators mentioned above have reported that they used habitat water in their observations. Moreover, Moore (1963) stated that his routine method for hatching involves immersion of eggs under a depth of water equal to 7 cm.

From the discussion, it seems that depth of water has no significant effect on the development and hatching of the anostracan branchiopods, and if there were any delay, this might be due to the reduction of the oxygen level rather than due to the depth itself.

SECTION ELEVEN

THE DEVELOPMENT OF EGGS OF C.DIAPHANUS IN RELATION TO PRESSURE OF WATER

SECTION 11

The development of eggs of *C.diaphanus* in relation to pressure of water

11.1 Introduction

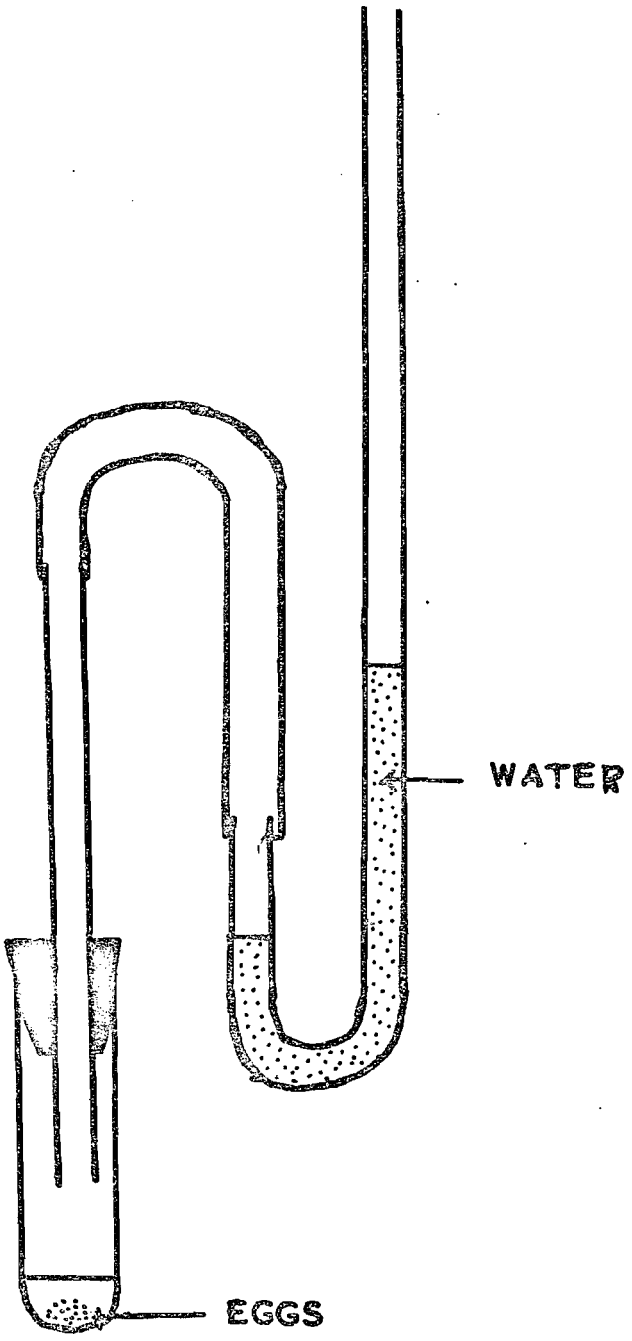
As has been mentioned in the previous section the depth of water may exert its influence through three possible factors. These factors are (a) oxygen variation, (b) osmotic concentration and (c) the pressure exerted by the column of the water. It was noticed from the results of the previous studies that oxygen concentration varies through the bottom of the natural habitat and also varies at the bottom of the experimental glass tubes with the depth of the water. Also it has been seen that eggs require sufficient amount of oxygen in their environment in order to be able to complete their embryonic development and hatch.

The second possibility can be eliminated, since embryonic development and hatching of this species were both found to be normal at osmotic concentrations much higher than that of the experimental medium. The third possibility is investigated in the present section.

No work has been done concerning this point in the anostracan branchiopods, although the pressure exerted by a column of water in a shallow freshwater pond may be considered as low enough to be of a little influence on the embryonic development. For example, a column of water 100 cm. in height has a pressure of 0·0967 atmosphere. Moreover, the highest value recorded for the depth of the freshwater ponds studied was 60 cm.

11.2 Methods and materials

Eggs were collected from the bottom of 500 ml beakers of pond water containing adult males and females. Eggs were then washed several times with distilled water in order to get rid of all suspended particles and faecal materials. Then the washed eggs were counted and separated into batches. Six glass tubes of 2·5 cm. in diameter were filled with well aerated distilled water to a depth of 2 cm. This depth of water was used so no oxygen deficiency would be expected. The batches of eggs were then pipetted into the bottom of each tube. Five of those batches were tightly connected to the apparatus used to prevent any air passage. The apparatus used in this experiment is illustrated in Fig. [38]. The pressures inside the apparatus were created by different columns of water. The systems were kept air-tight throughout the course of the experiments. The pressures applied were of 5,10, 15, 20 and 25 cm. water. The sixth glass tube was kept at laboratory conditions as a control. The mean laboratory temperature throughout the experiments was 22°C. The eggs were examined daily for any sign of break or hatching. The apparatus was disconnected and the pressures were released after seven or eight days, since after these periods more than 50 per cent of the eggs were found to have reached breaking stage.



FIG[38]. PRESSURE APPARATUS.

11.3 Results

It seems that the pressures exerted by columns of water as high as 25 cm. do not exercise any significant influence on the embryonic development and hatching of this species. It was found that at all pressures tested the percentage of breaking and hatching was high in comparison with that of the control. In the first trial, for example, at pressures exerted by columns of water of 5, 10, 15, 20 and 25 cm. the percentage break was 93·3, 93·3, 100, 96·6 and 93·3 respectively, whereas the percentage hatch was 70, 76·6, 83·3, 66·6 and 73·3 per cent respectively. The percentage of breaking and hatching of the control was 96·6 and 80 per cent respectively. From the above figures it seems clear that these pressures were too low to exercise any visible influence on the embryonic development or hatching. Moreover, in the second trial, batches of 100 eggs each were used. The results also were in full agreement with the results of the first trial. Furthermore, a pressure exerted by a column of water having a height of 35 cm. was also tested. The results of the latter treatment also indicate that pressure does not influence the embryonic development and hatching, since it was found that the percentage break and percentage hatch were 92 and 71 per cent respectively. All results are given in Table [32] and Table [33].

TABLE [32] Percentage of breaking and hatching under various pressures (Trial no.1)

Pressure as column of water	Number eggs used	% Break	% Hatch
1 cm. (control)	30	96·6	80
5	30	93·3	70
10	30	93·3	76·6
15	30	100	83·3
20	30	96·6	66·6
25	30	93·3	73·3

TABLE [33]. Percentage of breaking and hatching under various pressures (Trial no.2)

Pressure as column of water	Number eggs used	% Break	% Hatch
1 cm. (control)	100	92	77
5	100	91	81
10	100	86	69
15	100	99	79
20	100	89	67
25	100	96	81
35	100	92	71

No delay in the occurrence of the break could be noticed at any of the pressures tested, although the peak break in the first trial occurred in the seventh day of immersion, whereas in the second trial the peak break occurred in both the seventh and eighth days of immersion. This slight difference in the occurrence of the break might be due to the variation in the laboratory temperature during the period of the experiment rather than attributed to the influence of the pressure.

TABLE [34]. Shows the calculated pressures exerted by various depths of water

Depth of water cm.	Pressures exerted as atm.
1	0·00097
5	0·00484
10	0·00967
15	0·01451
20	0·01935
25	0·02419
35	0·03388
100	0·09675

11.4 Discussion

The delay in the occurrence of the break in the tertiary shell in those eggs which had been kept at the greater depths, that is to say 15, 20 and 25 cm. of pond water, is attributed to the low oxygen concentrations found there, as discussed in the previous section, rather than to the pressures of the columns of water or the variation in the osmotic concentrations. To confirm this view it was intended to apply pressures equal to the pressures exerted by the columns of water used in the previous section. In the apparatus used, no oxygen deficiency would be expected, so the possibility of the lack of oxygen was excluded in this case. The second possibility of the osmotic concentration was also excluded, since eggs of this species were already found to develop and hatch normally at osmotic concentrations much higher than that of the experimental media.

The third possibility left is the influence of the pressure exerted by the column of the water. The results of the present study have shown that at all pressures applied the embryonic development was normal and the break occurred within the expected time. Moreover, high percentages of breaking and hatching were found at all pressures examined. The normal relationship between the peak break and peak hatch was also observed in all cases. But it was noticed that in the second trial the peak break and the peak hatch had occurred in the eighth and tenth days of immersion respectively, whereas in the first trial they had occurred in the seventh and ninth days of immersion respectively. This means that there was a delay of one day in the appearance of the break and hatch in the eggs used in the second trial. This delay might be due to the variation in the laboratory temperature rather than to the influence of the pressure, since it was found that this delay had occurred in all treatments including that of the control. The results of the two trials, in general, confirmed the view of excluding the possible influence of the

pressure exerted by the column of the water when some retardation in the embryonic development was observed at greater depth of habitat water in the previous section. Such retardation was also observed by Hall (1959b, 1959c). However, the fact that the pressure experiments did not show any positive results is not surprising, since pressures as low as 0·0338 atmosphere may have no physical or mechanical influences on the embryonic mass or the egg membranes. So it could be entirely possible that the retardation of the embryonic development associated with the depth of habitat water might be due to the low oxygen concentrations existing there. This may also explain why Hall (1959b, 1959c) found that the period required for development and hatching increases with the increase of the depth of water. This could also give an explanation for the occurrence of the slight delay noticed in those eggs which had been kept at depths of 15, 20 and 25 cm. of habitat water in the previous section.

SECTION TWELVE

GENERAL DISCUSSION AND CONCLUSION

SECTION 12

General discussion and conclusion:

During the period of the present study it was found that there was no true second generation of C.diaphanus existing with an old generation. The new hatchings which add new specimens of C.diaphanus to the population take place from the eggs which were laid near the margin of the pond. The author does not consider the second hatching as a true second generation since (a) the occurrence of this hatching is not frequent and depends on the increment of the pond level and is not derived from the population living in the same wet period, and (b) the numbers of the hatched nauplii in the second hatching were very small. Hall (1961) reported the occurrence of C.diaphanus in Burley and in Lee ponds; there had been only two cases in which there was any evidence of the occurrence of a second generation in the same wet period. The prevention of the hatching of a second generation is of considerable value to the survival of this species, since it ensures the hatching of a new generation only when favourable conditions are available. The prevention of hatching of a second generation was noted in aquaria containing adult C.diaphanus. Also it was observed in the artificial ponds. From the analysis of the field observations it was found that there are four possible factors which may contribute in eliminating the populations of C.diaphanus from a temporary habitat. These are (a) predation, (b) low oxygen content of the pond, (c) temperature and (d) desiccation. However, senescence must not be excluded. The predation plays its part usually in the late stages of the life of the pond. By this time the animals have reached sexual maturity and are able to produce eggs.

The success and survival of C.diaphanus in its habitat depends mainly on the environmental conditions which permit (a) the population

to live and breed in a short period, (b) the embryonic development to be completed and (c) the ability of the egg to adapt to the changes of the environmental conditions. The field observations showed that this species can reach sexual maturity within approximately two weeks during the summer and six weeks during the winter. The short time by which the animals reach sexual maturity is of significance to the survival of this species, since the appearance of the predators will be a great danger to the population. The ability of the females to produce large numbers of eggs is also of considerable value to the survival of the species, since it enhances the ability of the species to survive in situations where hatching is rapidly followed by a drying out of the pond. If the females could produce only small numbers of eggs, successive repetitions of these situations would rapidly reduce the number of eggs in the pond to zero. The ability of C.diaphanus to live at low temperatures under a layer of ice and at temperatures as high as 30°C for a considerable period would also contribute to the success of this species in its habitat. One would expect such changes in the water temperature in temporary ponds in a country like Britain. As has been said, the upper extremes of the temperature had no significant effect on the reduction or the elimination of the population of C.diaphanus, since these high temperatures were only maintained for a few hours, so they could have had no harmful effect on the population. Another ability which has a survival value to this species is that they could withstand quite low oxygen concentrations. Taylor (1965) reported that the critical oxygen concentration for this species is 1.5 mg/l. However, during the present study it was found that this factor may play a part in eliminating the population if it falls to very low values.

The growth curves of C.diaphanus showed that there are three distinct phases. In the first phase, the rate of growth is very rapid.

Then there is a second phase in which the rate of growth is very slow. Cooper (1950) attributed the reduction in the rate of growth of E.oregonus in this phase to "some influence associated with the maturing of the sex products". Other workers also associated this reduction in growth to the maturing of the gonads. This might be also the case in C.diaphanus, since it occurs at the time in which both females and males attain sexual maturity. In the third phase the rate of growth is also rapid.

The anostracan branchiopods are known to inhabit temporary ponds which depend chiefly on the rainfall. Any shortage in the rainfall may result in drying up of their habitat. Because of this changeable habitat, and the severe conditions which they may endure, there must be a stage in the life cycle which is adapted to such unfavourable conditions. This species survive dry periods by means of drought-resistant eggs which hatch when they are inundated with rainwater. The nauplii of C.diaphanus were found to occur in the field within two days after the inundation of the pond with rainwater. This early hatching suggests that the embryonic development must have taken place some time before the refilling of the ponds. But whether the embryonic development takes place immediately after the eggs have been laid and before the habitat becomes dry, or during the dry period of the pond, was not known. The eggs of this species, after being laid by the females, sink to the bottom of the pond, where low oxygen concentrations may exist. No embryonic development (or very little) occurs at this time because of the oxygen concentration found there. This was supported experimentally when it was found that low oxygen concentrations would retard or inhibit the embryonic development and hatching of the eggs. Later the eggs are exposed to moist soil when the pond dried up. This provides aerobic moist conditions which are the most favourable ones for the embryonic development. It seems that most of the embryonic development takes

place during the dry period of the pond when plenty of oxygen is available. The eggs continue their development up to a point just before the occurrence of the break in the tertiary shell. At this stage of the embryonic development the eggs are ready to hatch as soon as the pond is filled again with rainwater. Such a mechanism would contribute to the survival of the species, as it would ensure the hatching of the eggs in favourable environmental conditions.

The complete escape of the nauplius from its three membranes takes place in two stages. The first stage is the breaking stage which seems to be strictly an osmotic phenomenon, since (a) there is an accumulation of metabolic substances such as glycerol in the breaking stage which leads to an increase in the inner osmotic pressure and then aids in the process of the inflow of water, and (b) the unhatched embryo is incapable of any movement whatsoever when the split appears in the tertiary shell. There is no increase in the diameter of the developing egg throughout the course of the embryonic development up to a point just before the occurrence of the break in the tertiary shell. Immediately after the appearance of the break, the embryonic contents start to swell gradually and the unhatched embryo starts to move later on.

The second stage is the hatching process, which is caused by the movements of the embryo itself, although one must not exclude the possibilities of an osmotic action and of the presence of a hatching enzyme. The hatching takes place always when the embryo is very active pushing and abrading the hatching membrane. The presence of spiny projections in the joints of the protopodites of the second antennae is further evidence to strengthen the mechanical hatching. The absence of an accumulation of glycerol in the post-breaking stage also supports this point.

It seems that after the completion of the embryonic development,

the eggs await an accumulation of rainwater to hatch, since the hatching process takes place only when there is standing water, whereas the breaking stage may take place even when there is no free water in the soil. The ability of the occurrence of the break in the outer covering of the egg in the absence of free water has been seen both in nature and in the laboratory. In nature, eggs at the breaking stage were isolated from the soil, especially when the soil was highly moist. Experimentally, some eggs kept at 100% relative humidity (without free water) reached breaking stage within two-three weeks at laboratory temperature. The ability of the eggs to complete their embryonic development at very high humidities is very important, since it enables them to reach a stage at which they are ready to hatch within a few days after being inundated with rainwater. The occurrence of hatching only in the presence of standing water is also very important to the survival of the species, since it would ensure the appearance of nauplii when free water is available. This is because both nauplii and adults cannot survive dry periods. This adaptation will let the hatched nauplii reach sexual maturity very soon and give an opportunity to the adults to breed and lay eggs before the elimination of the population from the pond, either by shortage in rainfall and drying up of the pond or by the appearance of the predators.

It seems that oxygen concentration is a very important factor in controlling the embryonic development and hatching of C.diaphanus. It was found that low oxygen concentrations cause retardation or inhibition of the development of the egg, and also hatching occurs only when a reasonable amount of oxygen is present. The necessity of oxygen to the embryonic development of this species can be considered as a sort of adaptation which enables the species to survive and repeat itself in

a changeable temporary habitat. Because, if any embryonic development takes place in the bottom of the pond, there will be hatching of nauplii which may face desiccation many successive times before they reach sexual maturity and so produce eggs. This will lead to the complete elimination of the species from its habitat. Thus, the embryonic development and hatching of C.diaphanus seems to depend largely on the presence of sufficient amount of oxygen in its micro-environment. The trigger of the hatching, as has been discussed, is thus the increment in the oxygen concentration immediately after the rainfall.

Prevention of a new hatching was also noticed in aquaria which contained adult C.diaphanus. This is presumably due to the low oxygen concentrations which exist there and not because the eggs have not yet been subjected to a dry period, since these eggs proceeded in their development and hatched normally when transferred to well aerated water. Thus the significance of the drying out of the pond is, in fact, due to the consequent increase in the oxygen concentration which enables the eggs to complete their embryonic development.

The dried eggs float when placed in water, since they have lost some of their water, which in turn causes the lowering of their density. This floating phenomenon is due to the formation of air space between the tertiary shell/chitinous membrane complex and the embryonic mass. The total water loss was less at 0% relative humidity than at higher humidities up to 83% (inclusive), although the initial loss was more rapid. This may be due to the hastening of the hardening (tanning) process at 0% relative humidity because of the complete absence of environmental moisture. The hardening process may have a very important ecological value as a protective mechanism to the eggs from complete dehydration of their habitat when it is exposed to a quite long period

of drying, especially to those eggs which are deposited on the upper layer of the soil. The field observations showed that the soil moisture fell to a value as low as 18 per cent of wet weight in the upper layer of the soil. Experimentally the eggs of C.diaphanus, in both the early and advanced stages of development, were found to survive a period of a few months in a moisture free environment.

Although some workers concluded from their work on other species of anostracan branchiopods that the temperature of the habitat is the most important ecological factor controlling the embryonic development and hatching, this seems not to be the case in C.diaphanus, since development and hatching would take place in a wide range of temperature. This indicates a good tolerance to low and high environmental temperatures which may occur in their habitat. However, higher temperatures were found to retard the embryonic development and prevent breaking and hatching, although these temperatures were not harmful to the adult C.diaphanus. The prevention of the occurrence of the break in the outer covering of the egg may have a significant importance in the field, since high temperatures may occur in nature. If they do, and so allow breaking to take place, this will lead to the risk that broken eggs will face unfavourable conditions in the summer, especially when the eggs are still incubated in moist soil after the pond dries up. This also gives an opportunity to those eggs which have completed their embryonic development to live and survive until the occurrence of favourable conditions and the establishment of the pond.

Hall (1953) noticed a delay in the embryonic development and hatching of C.diaphanus. He suggested that this may be found in the osmotic relationships of the egg contents and the surrounding fluid. Habitat water with concentrations much higher than that found in the

ponds studied had no effect on the embryonic development and hatching. The highest concentration tested was ten times that of the pond water. Such a high value had never been observed in both ponds studied. It was found that eggs kept at these concentrations of pond water required the same time as that in distilled water. This is an indication of the normal embryonic development which took place at these relatively high concentrations. The percentage break and percentage hatch were very high. It seems that the fluctuations of concentrations which happen under natural conditions have no part in delaying embryonic development or inhibiting the hatching process. Furthermore, this is also supported experimentally, when it was found that a normal embryonic development took place in solutions of quite high salt and sugar concentrations. It can be concluded that even if there is an effect of the osmotic concentration beyond a certain limit on embryonic development or hatching, such values may never be found under natural conditions in the habitat of C.diaphanus. The delayed embryonic development and the prevention of hatching of a second generation in nature must be due, then, to a factor or factors other than the increment in the osmotic concentration.

The depth of the water overlying the eggs was thought to retard the embryonic development and hatching of anostracan species. The retardation of the development of the eggs in aquaria was also attributed to the depth of the water. As has been seen, depth of water had no effect in retarding or inhibiting the embryonic development or hatching of this species. It seems entirely possible that the oxygen, which is usually found in very low concentrations at the bottom of the pond, was the factor responsible in retarding or inhibiting the embryonic development and hatching. Oxygen concentrations as low as 0·5 and 0·85 mg/l. were found in the upper layer of the mud and at the bottom of an

aquarium having a depth of 20 cm. water. As has been discussed before, depth may exert its effect through three possible factors. These factors are (a) the pressure of the column of the water, (b) oxygen depletion and increase in poisonous substances and (c) osmotic concentration variation. Both possibilities (a) and (c) can be excluded, as has been mentioned before, since they have no significant influence on development and hatching. Finally, it can be said that the oxygen is the most important factor controlling the embryonic development and in determining the hatching of nauplii in nature.

SECTION THIRTEEN

SUMMARY

SECTION 13

Summary:

(1) The natural occurrence of the fairy shrimp C.diaphanus in two temporary freshwater ponds in the New Forest was investigated for a period of two and a half years. During this period, it was found that there was only one generation in each wet period. No second generation seems to have occurred with the old generation. However, the few nauplii occurring sometimes are due to some hatchings taking place in the marginal areas of the pond when the level of the pond is increased by rainfall. Populations of C.diaphanus were found to survive under a layer of ice as well as at quite high temperatures. There are four factors which may determine the life span of a population in a temporary pond. These are (a) predation, (b) low oxygen concentrations of the pond, (c) temperature, and (d) desiccation. However, senescence must not be excluded.

(2) The curves of the growth of C.diaphanus can be divided into three phases. The first phase had a rapid rate, the second phase was of a slow rate and the third phase had a rapid rate. The growth in summer was more rapid than that in winter. Individuals reached sexual maturity in summer earlier than in winter. Females can lay as many as 308 eggs per clutch. In nature, this species was found to live for as long as five months. In artificial ponds, free of predators, C.diaphanus lived for approximately eight months. Populations start with a sex ratio of 1:1. Subsequently the proportion of the males decreases.

(3) Description of the study area was given together with the associated fauna and flora. Records of the limnology of the temporary ponds

(3) contd.

studied for a period of two years are also given. The highest temperatures recorded were 26·5, 28 and 26°C for the air, water and mud respectively. The lowest temperatures were 0, 0.5 and 0.5°C respectively. The oxygen concentration ranged between 1.5 and 16.4 mg/l. and between 1.5 and 13·1 mg/l. in Godshill and Eyeworth ponds respectively during the period of the present study. The minimum reading for the water conductivity in both Godshill and Eyeworth ponds was 21 micromhos/cm. The highest measurements for the conductivity in both Godshill and Eyeworth ponds were 332 and 176 micromhos/cm respectively. The maximum depth in Godshill pond recorded during the winter when the pond was full was 60 cm, whereas that for Eyeworth pond was 18 cm. The total volume of the former was 180,000 litre, whereas that for the latter was 6,880 litre. The range of the PH values found for Godshill pond was 5·9 - 6·8, whereas the range in Eyeworth pond was 5·7 - 6·7. It seems that C.diaphanus can live in acidic as well as alkaline waters. The percentage moisture content of the soil in Godshill pond when it was dry was found to vary between 18 and 65 per cent wet weight, whereas that in Eyeworth pond was between 23 and 52 per cent. The fluctuations of the air, water and mud temperatures, as well as the oxygen concentration and the PH were measured in 24 hour cycles on both a rainy windy day during the winter and on a calm sunny day during the summer.

(4) The embryonic development and hatching of the egg under natural conditions were studied. Under natural conditions the eggs sink to the bottom of the pond after laying, where low oxygen concentrations exist. Eggs either develop a little, or no development takes place under these conditions. Then the eggs are exposed to moist

(4) contd.

soil when the pond dries up. This provides aerobic moist conditions which may be the most favourable conditions for the embryonic development. Then hatching occurs after they are inundated with rainwater which is well aerated.

(5) A study on the embryonic development and hatching mechanism in the egg was carried out. The complete escape of the nauplius from its three membranes takes place through two distinct stages, namely the breaking stage and the hatching stage. The former seems to be strictly an osmotic phenomenon, and the latter is more likely to be caused by mechanical means. A detailed study on the embryonic movements was carried out. The possible presence of a hatching movement was discussed. The presence of spiny projections aiding in the hatching process was also discussed.

(6) A drying period is not essential for development and hatching of this species. The effect of desiccation on the structure, size and viability of the eggs was studied. The dried eggs were found to float due to air spaces formed between the tertiary shell/ chitinous membrane complex and the embryonic mass. The eggs were able to continue their development at higher humidities up to the breaking stage. However, some had shown the break in the tertiary shell under such conditions. No embryonic development seemed to have taken place at low humidities while the eggs were still in the humidity chambers. The eggs of this species can withstand a drying period of at least four months. A great decline in the percentage break and percentage of hatching was found as the drying period was increased. However, the survival time depends on the relative humidity of incubation. The eggs which had been kept at 0% relative humidity lost less water than those eggs kept at

(6) contd.

humidities lower than 83% (inclusive), although the initial loss was greater. The rate of water loss from the advanced stage eggs was slower than that found in those eggs dried immediately after laying. The rate of water loss was closely correlated with the relative humidity.

(7) No embryonic development was found to have taken place at sub-freezing temperatures. The eggs were killed at those temperatures in wet conditions, but survived in dry conditions. At higher temperatures, the rate of embryonic development increases with the increase of temperature up to a limit. The higher the temperature the shorter the time required for the egg to complete its development. Unhatched embryos would survive and hatch at temperatures of $0^{\circ}C$ and above. Eggs survived a period of two days at $40^{\circ}C$ without any harmful effects. The exposure of the eggs to a constant temperature of $35^{\circ}C$ for at least nine days showed no significant effect on subsequent development. As has been seen from the results, the higher the temperature of exposure the shorter is the time required to harm and kill the egg.

(8) The embryonic development was inhibited at very low oxygen concentrations. At higher concentrations the development was more rapid. Breaking of the outer covering of the eggs depends on the presence of a sufficient amount of oxygen in the micro-environment. The eggs would hatch more readily at higher concentrations of oxygen, and at low concentrations hatching was clearly inhibited.

(9) Pond water having total concentrations as high as ten times that of the natural habitat were found to have had no adverse effect on

(9) contd.

the embryonic development and hatching. Both embryonic development and hatching were normal at low osmotic pressures. But as it increases, breaking would be affected and percentage break would be less. This is clear at osmotic pressures higher than 3 atmosphere. Percentage hatch starts to decline beyond an osmotic pressure of 1·5 atmosphere. The hatching process is more sensitive to an increase in external osmotic pressure than the breaking stage. The possibility of the lack of oxygen and its influence on the development at higher osmotic concentrations was discussed and excluded.

(10) The influence of the depth of water on the embryonic development and hatching was examined. Depth had no influence on these two processes. The low oxygen concentrations which may exist at the bottom of the ponds and aquaria might be the factor which is responsible for retarding or inhibiting the development and hatching. Very low oxygen concentrations were found at the bottom of the aquaria at greater depths and at the bottom of the ponds.

(11) Pressures exerted by columns of water do not have any significant influence on the embryonic development and hatching.

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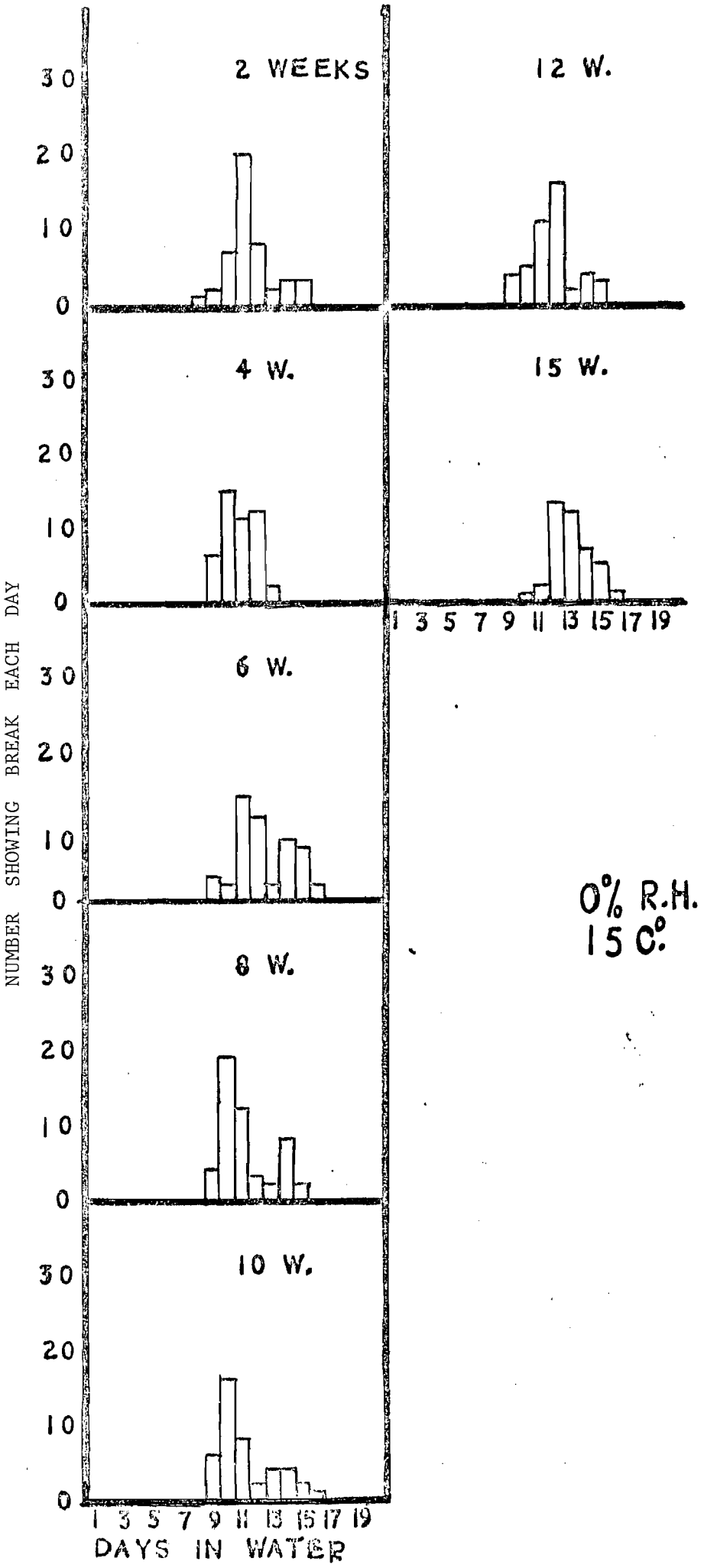
APPENDIX I

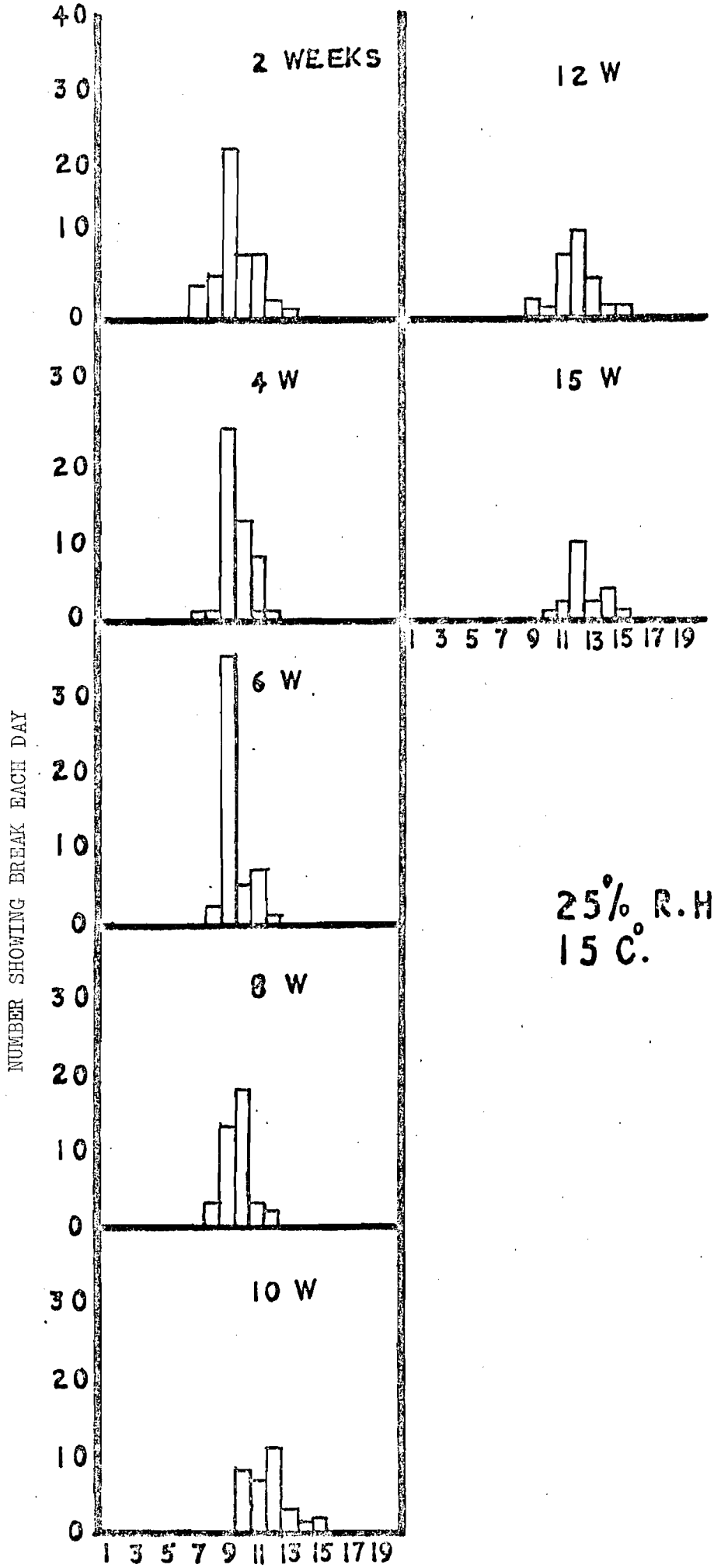
Shows the time required for the eggs of C. diaphanus to reach breaking after they have been subjected to various combinations of relative humidity and incubation time at different temperatures.

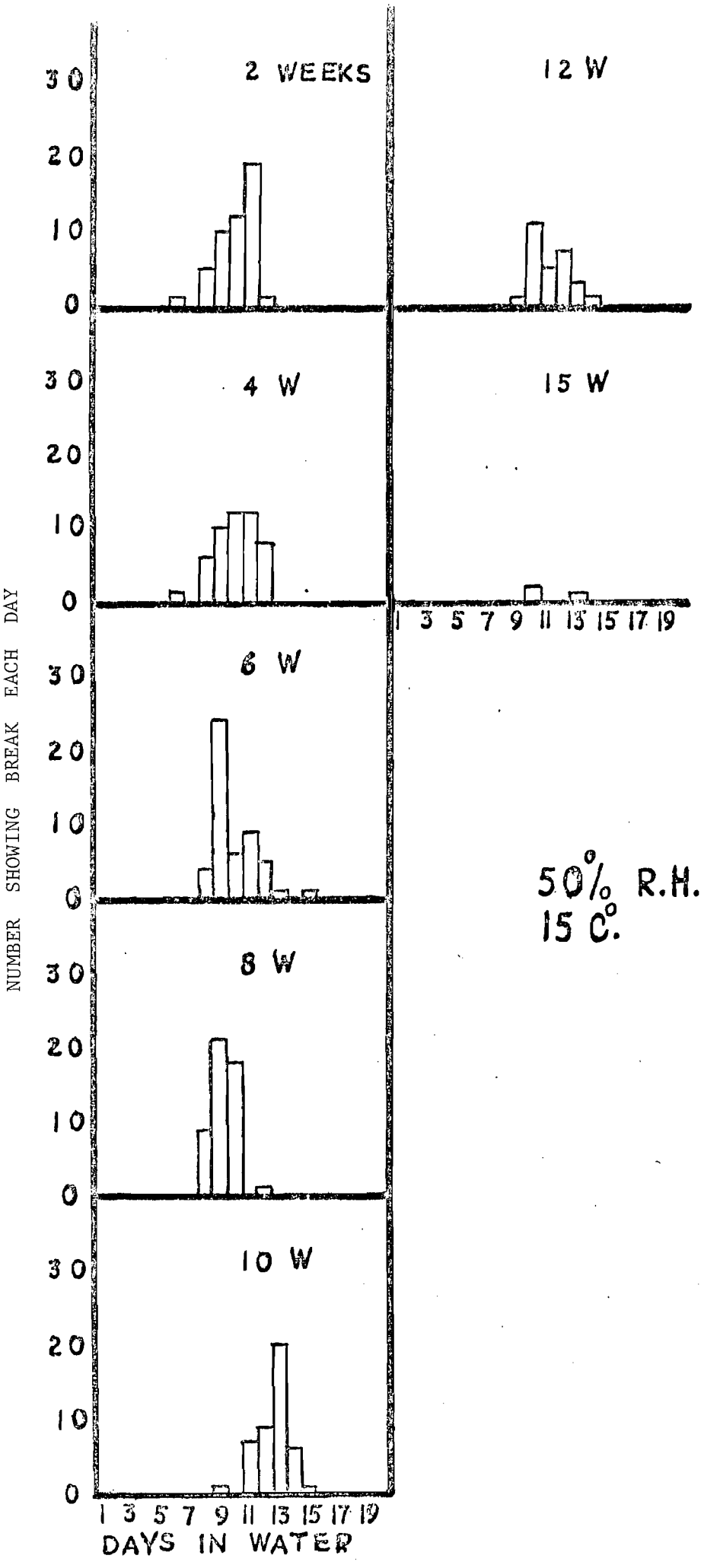
(a) As the drying period increased the delay in the appearance of the break became longer.

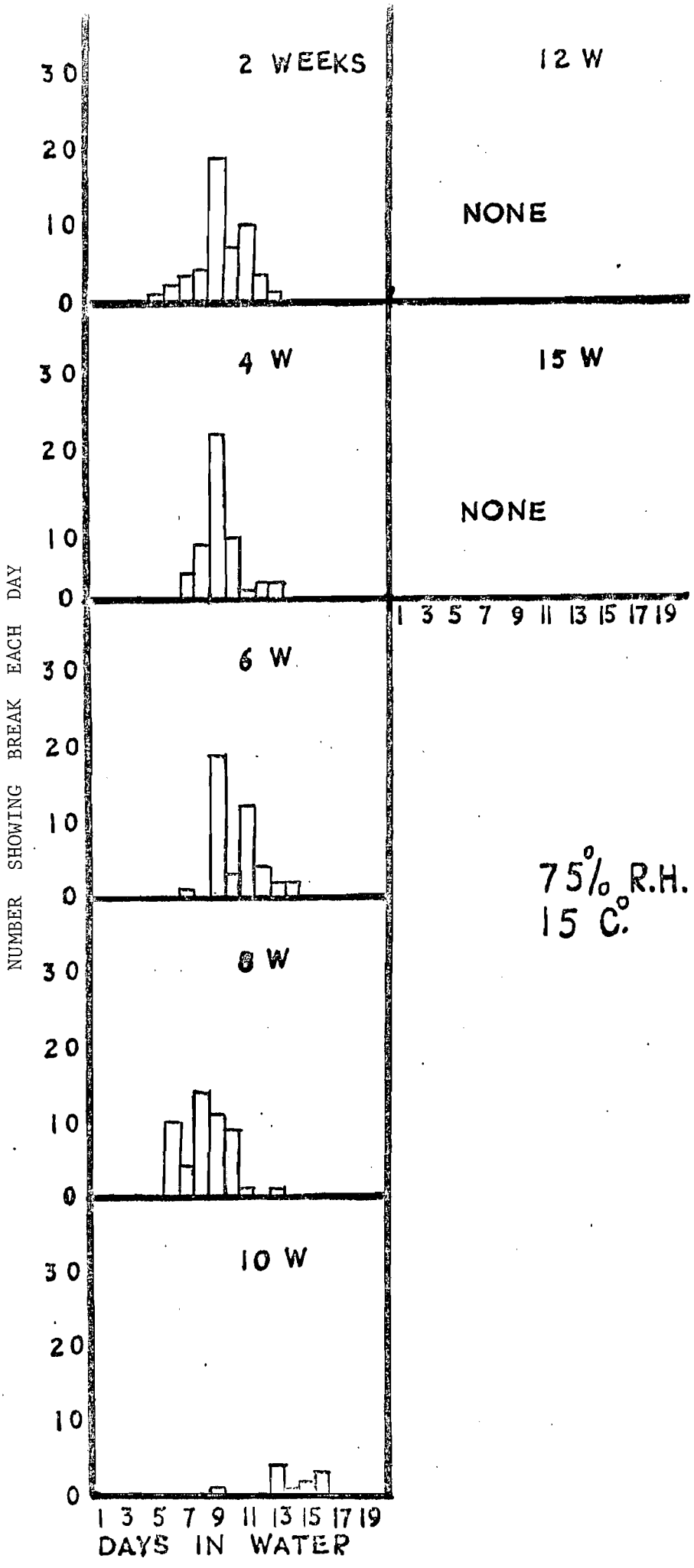
(b) Irrespective of the desiccation level, a drying period as long as 42 days seemed to have no significant effect on the embryonic development, since percentage of breaking was always high.

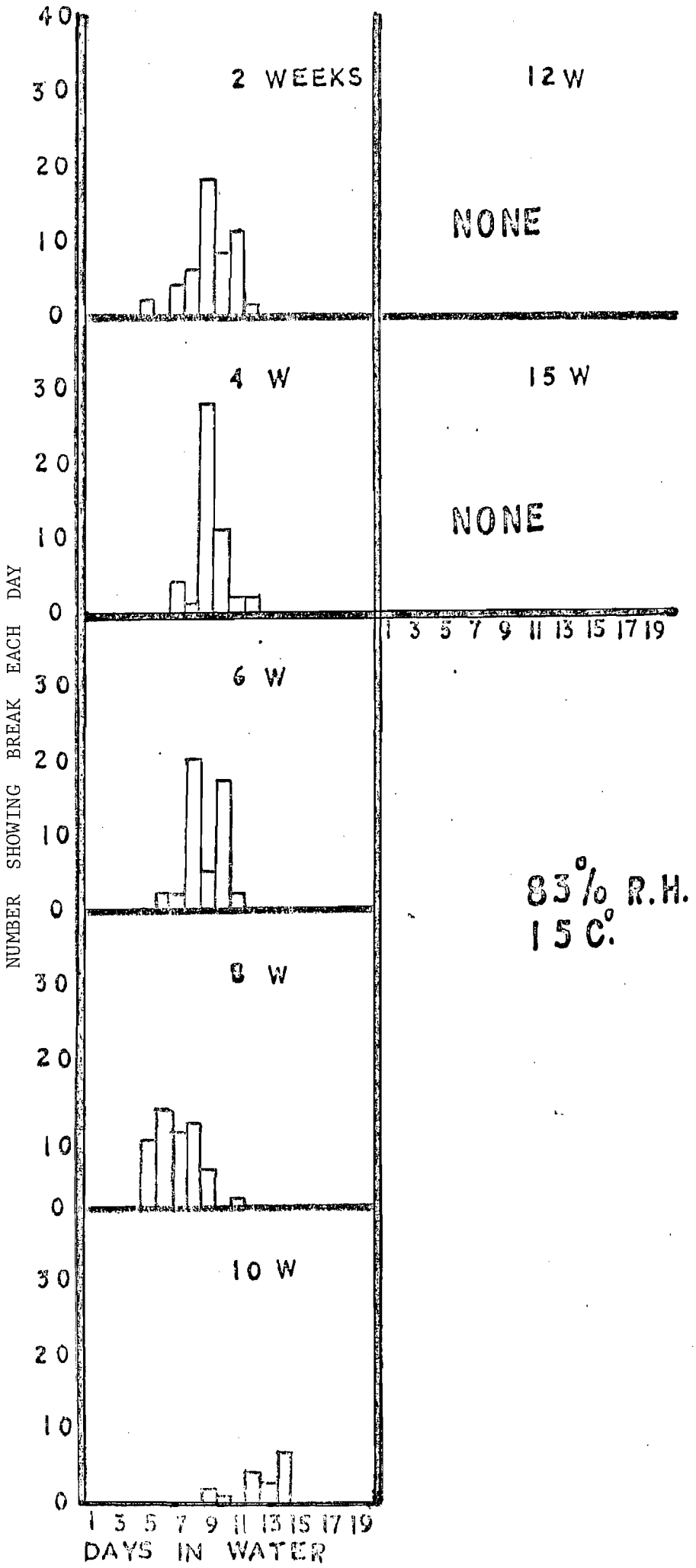
(c) At relative humidities below 83% (inclusive), no embryonic development seemed to have taken place while the eggs were in the humidity chambers. At higher humidities some development seemed to have taken place.

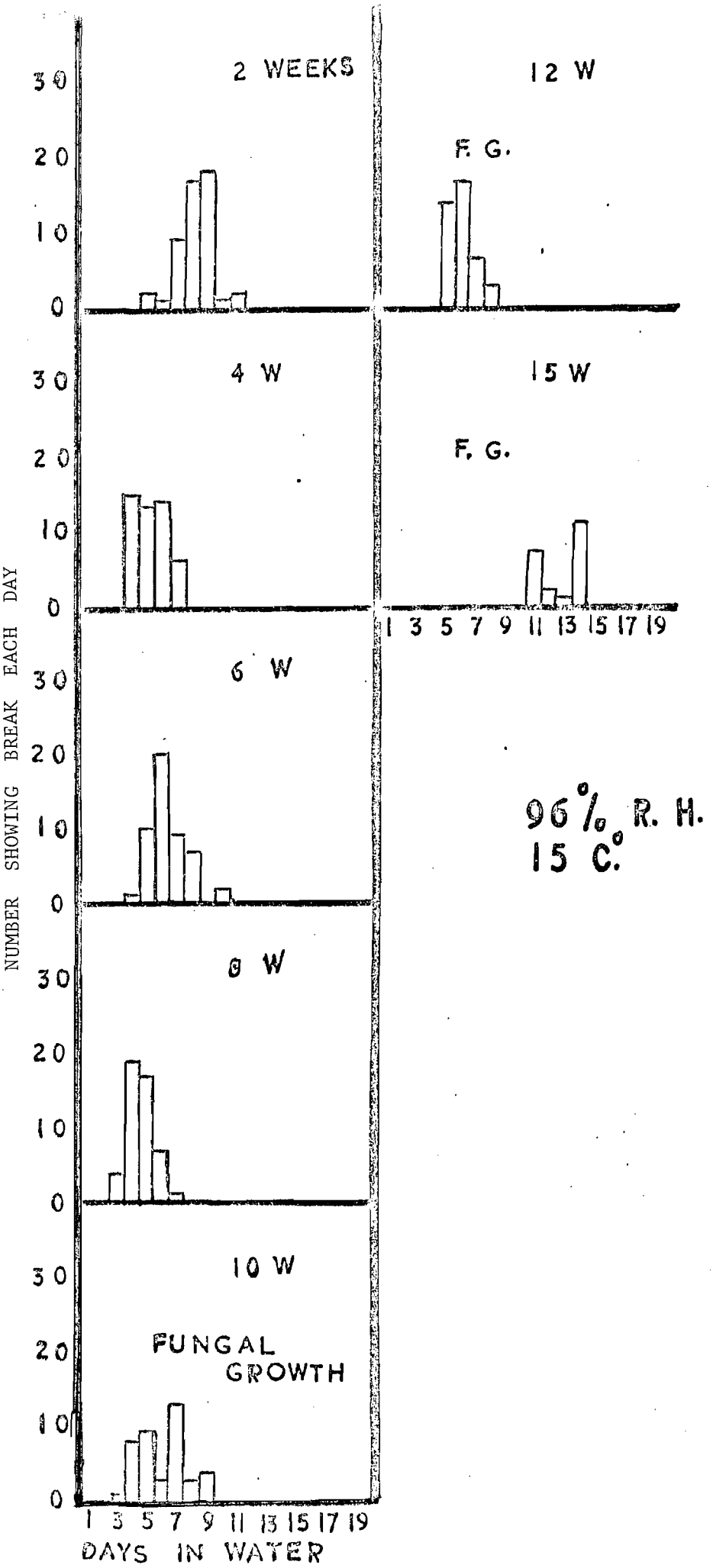

2 WEEKS
12 W.
4 W.
15 W.
6 W.
8 W.
10 W.
0% R.H.
15 C°
NUMBER SHOWING BREAK EACH DAY
0
10
20
30
1 3 5 7 9 11 13 15 17 19
DAYS IN WATER

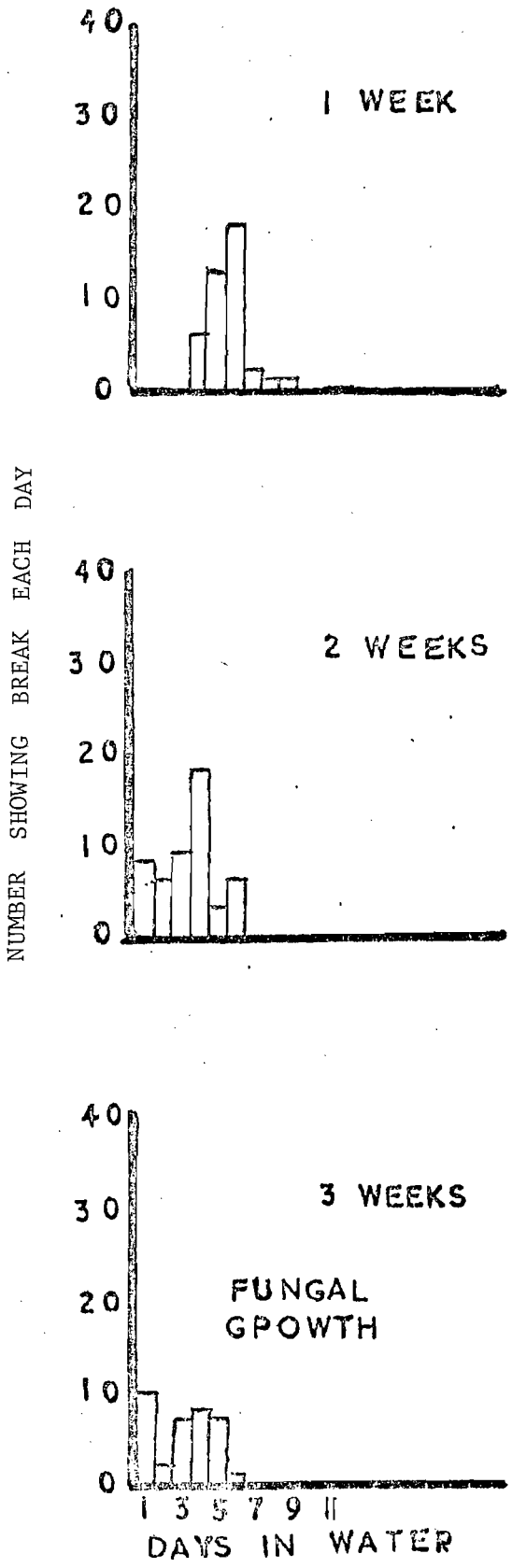

2 WEEKS
12 W
4 W
15 W
6 W
8 W
10 W
NUMBER SHOWING BREAK EACH DAY
0
10
20
30
40
1 3 5 7 9 11 13 15 17 19
25% R.H.
15 C°.


2 WEEKS
12 W
4 W
15 W
6 W
8 W
10 W
30
20
10
0
1 3 5 7 9 11 13 15 17 19
DAYS IN WATER
NUMBER SHOWING BREAK EACH DAY
50% R.H.
15 C°.

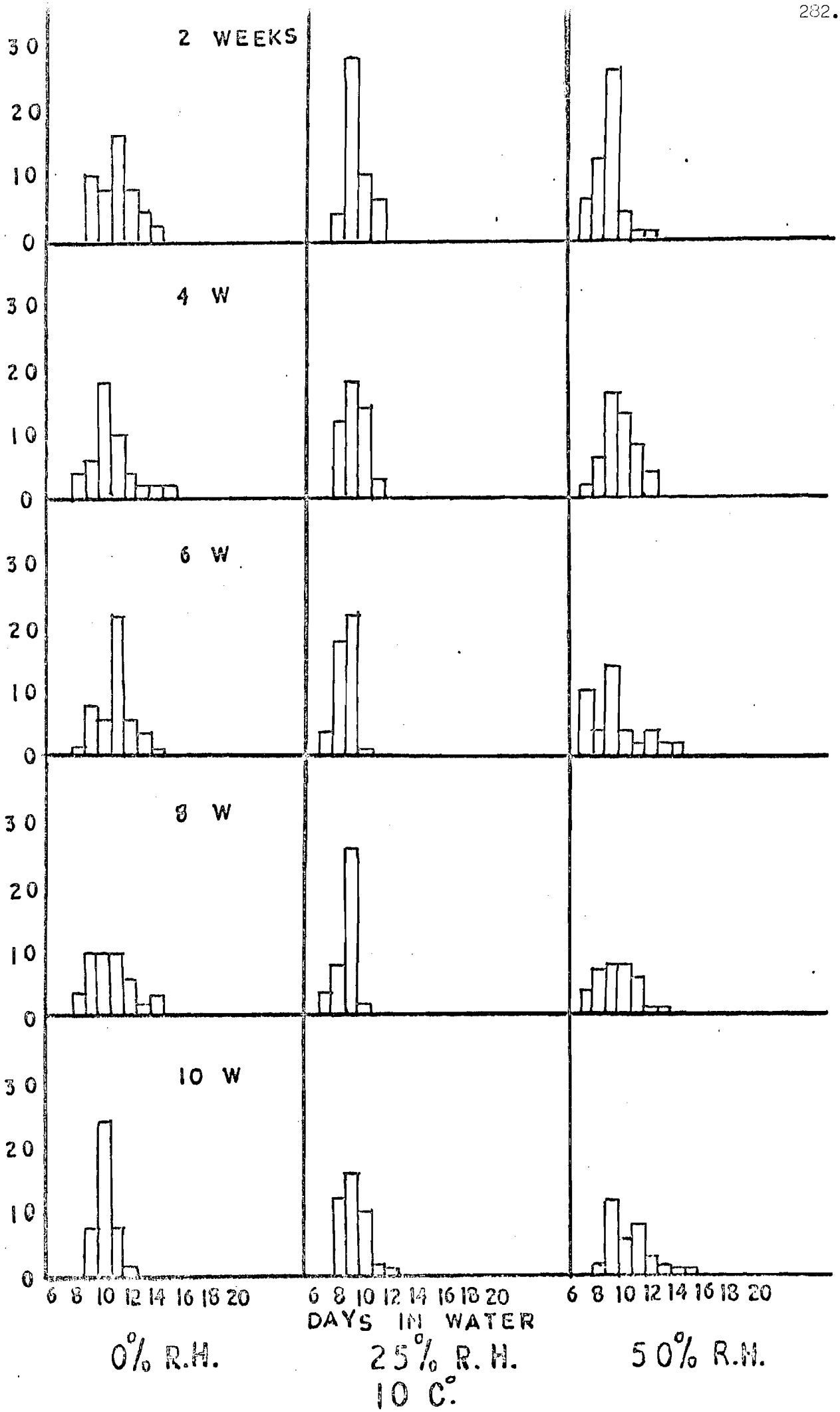

2 WEEKS
12 W
NONE
4 W
15 W
NONE
6 W
8 W
10 W
75% R.H.
15 C°
NUMBER SHOWING BREAK EACH DAY
0
10
20
30
1 3 5 7 9 11 13 15 17 19
DAYS IN WATER

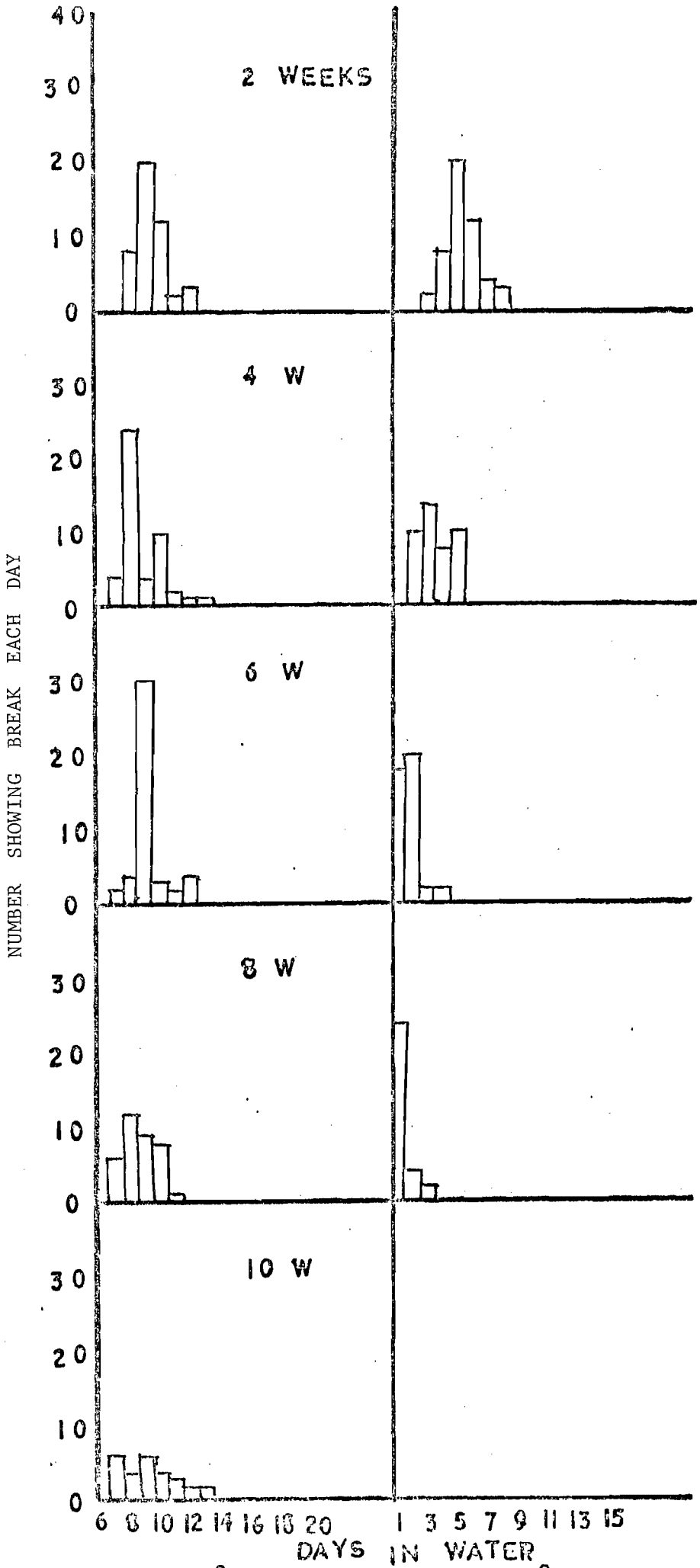

2 WEEKS
12 W
NONE
4 W
15 W
NONE
6 W
8 W
10 W
83% R.H.
15 C°.
NUMBER SHOWING BREAK EACH DAY
0
10
20
30
1 3 5 7 9 11 13 15 17 19
DAYS IN WATER

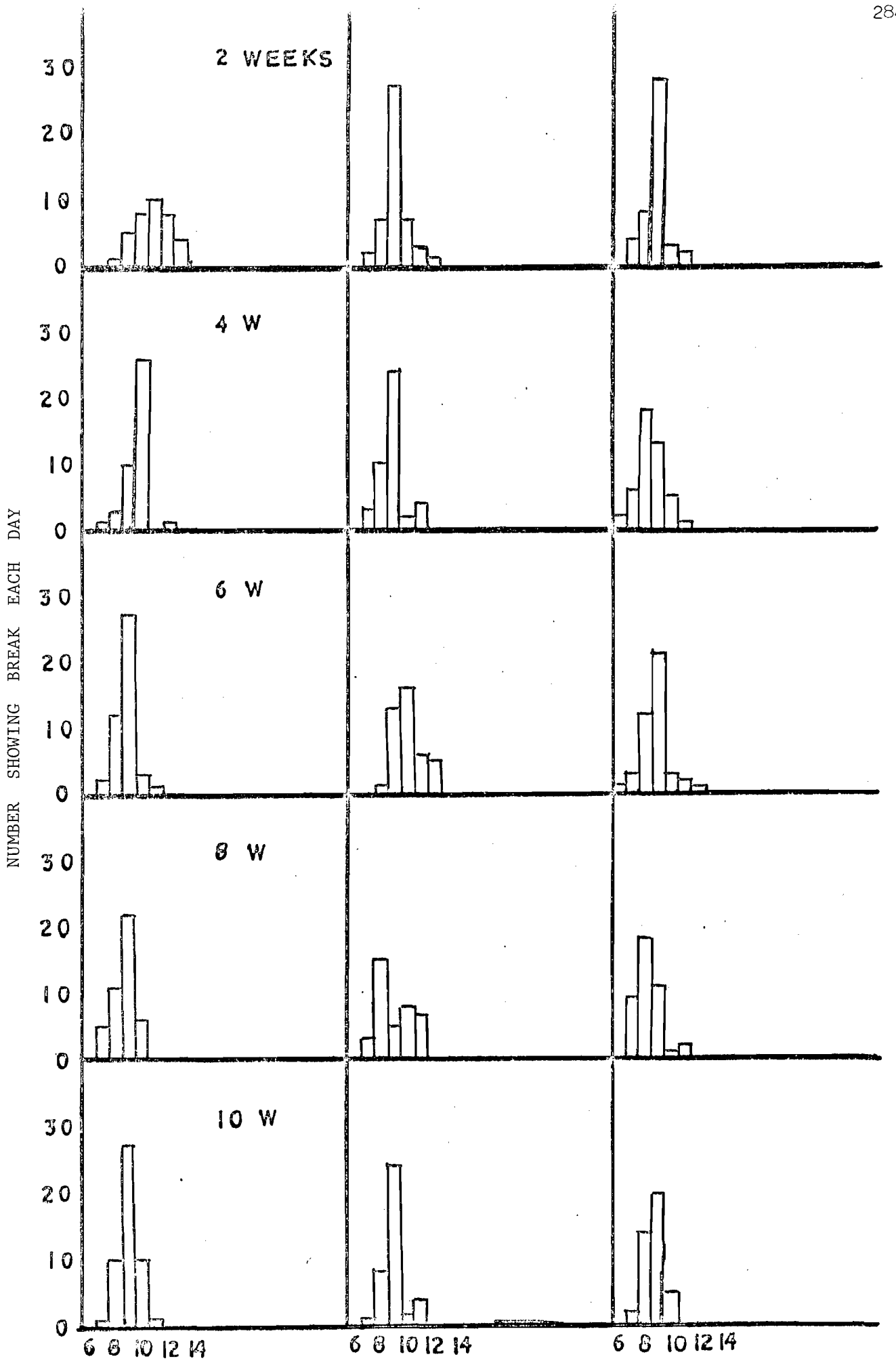

2 WEEKS
12 W
F. G.
4 W
15 W
F. G.
6 W
8 W
10 W
FUNGAL GROWTH
NUMBER SHOWING BREAK EACH DAY
0
10
20
30
1 3 5 7 9 11 13 15 17 19
DAYS IN WATER
96% R. H.
15 C°.

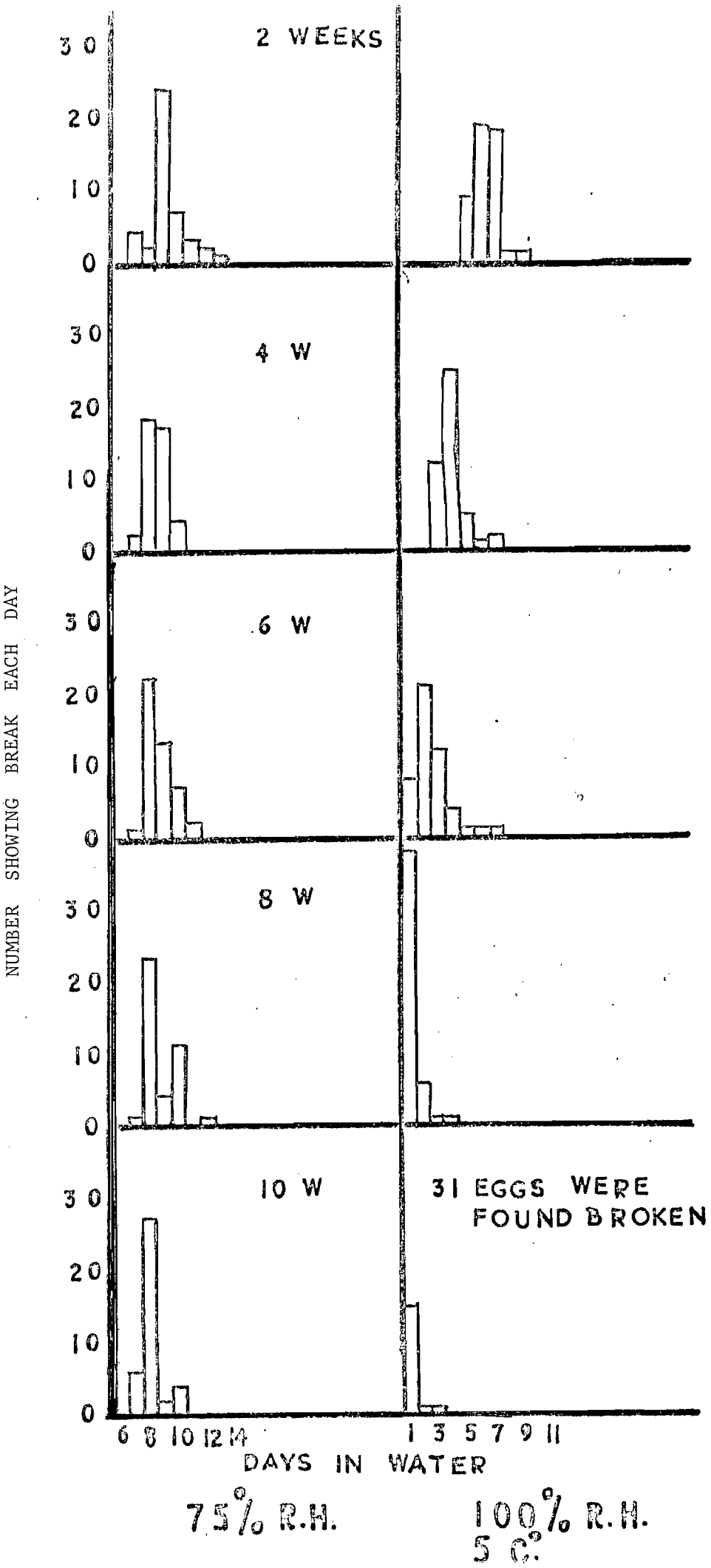

NUMBER SHOWING BREAK EACH DAY
40
30
20
10
0
1 WEEK
2 WEEKS
3 WEEKS
FUNGAL GPOWTH
1 3 5 7 9 11
DAYS IN WATER

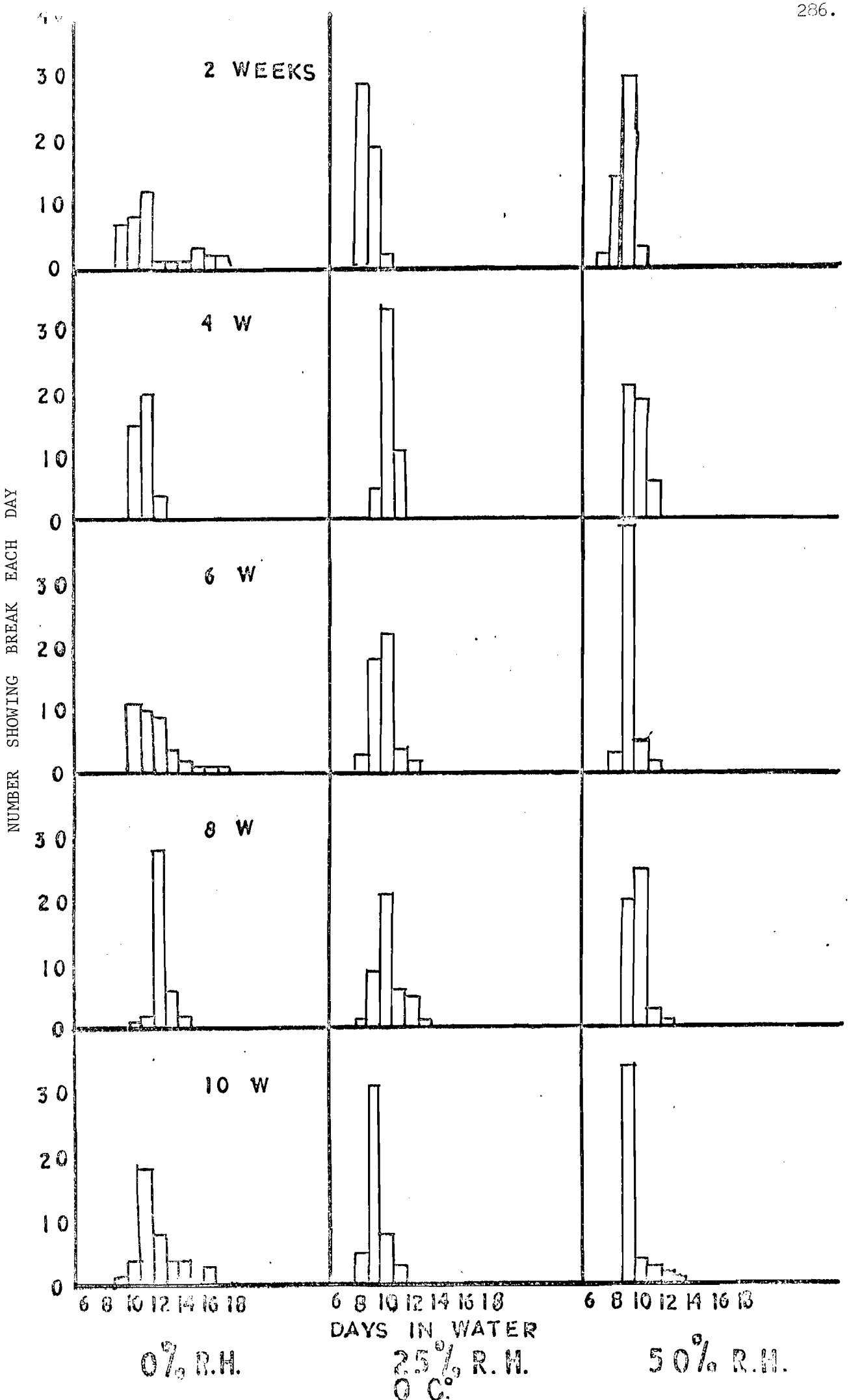
100% R.H.
15 C°.

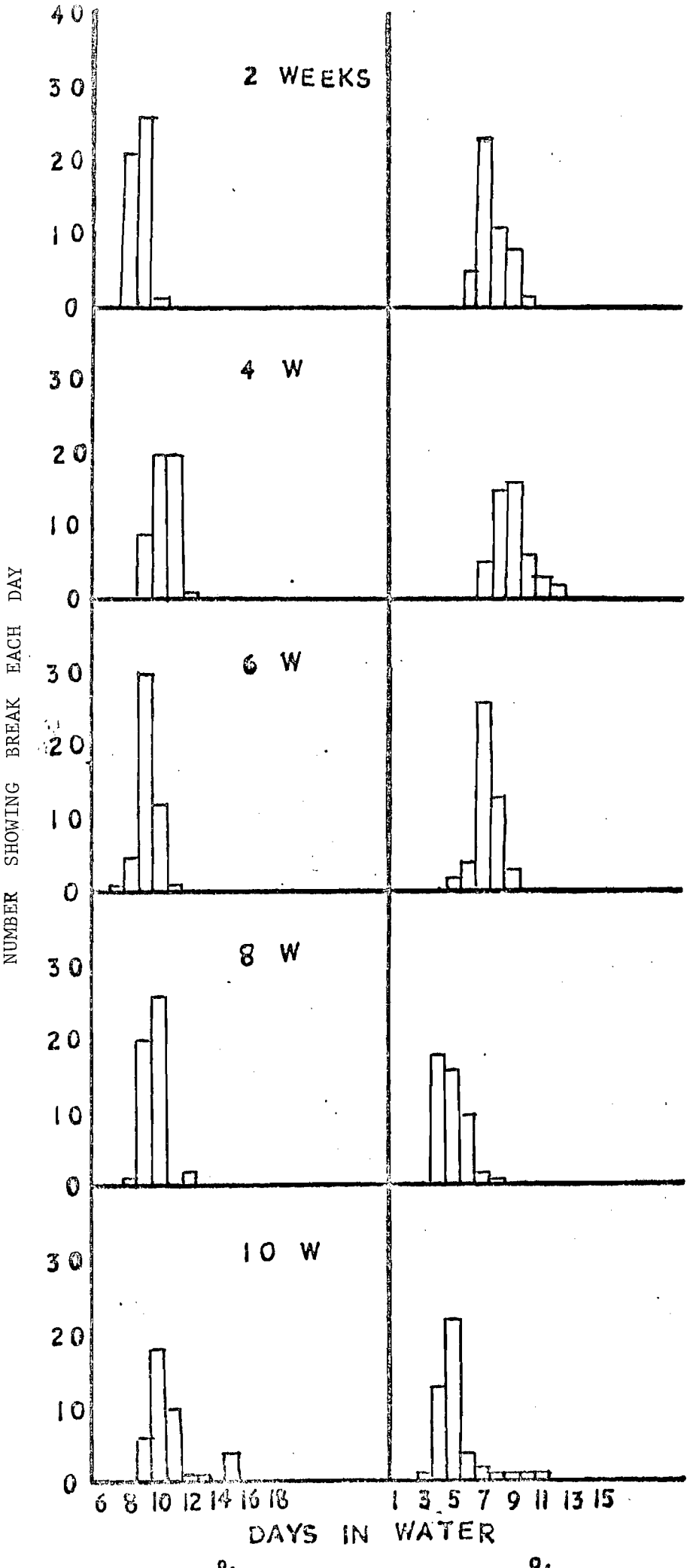

2 WEEKS
4 W
6 W
8 W
10 W
30
20
10
0
NUMBER SHOWING BREAK EACH DAY
6 8 10 12 14 16 18 20
DAYS IN WATER
0% R.H.
25% R.H.
50% R.H.
10 C°

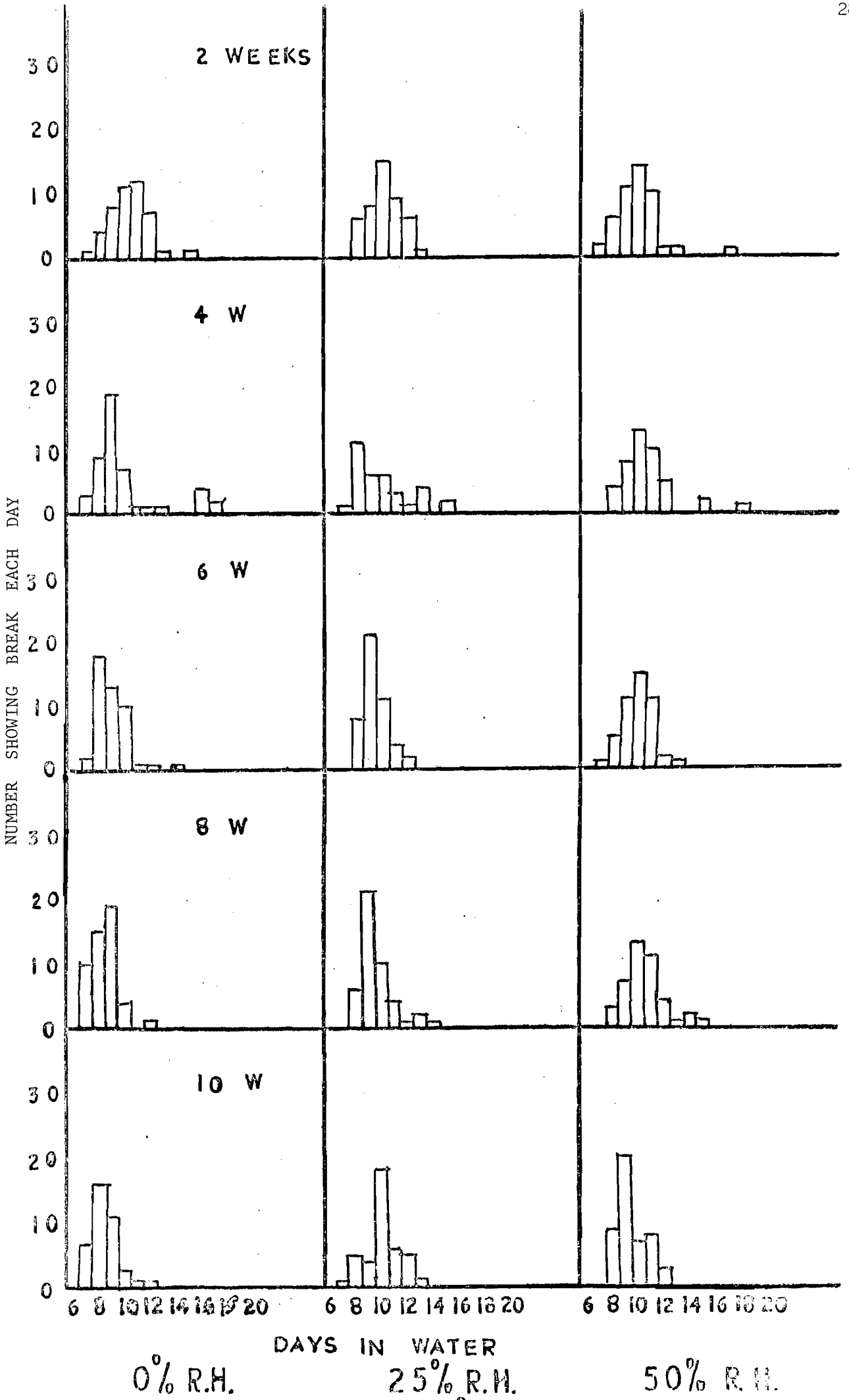

2 WEEKS
4 W
6 W
8 W
10 W
NUMBER SHOWING BREAK EACH DAY
0
10
20
30
40
6 8 10 12 14 16 18 20
1 3 5 7 9 11 13 15
DAYS IN WATER
75% R.H.
100% R.H.

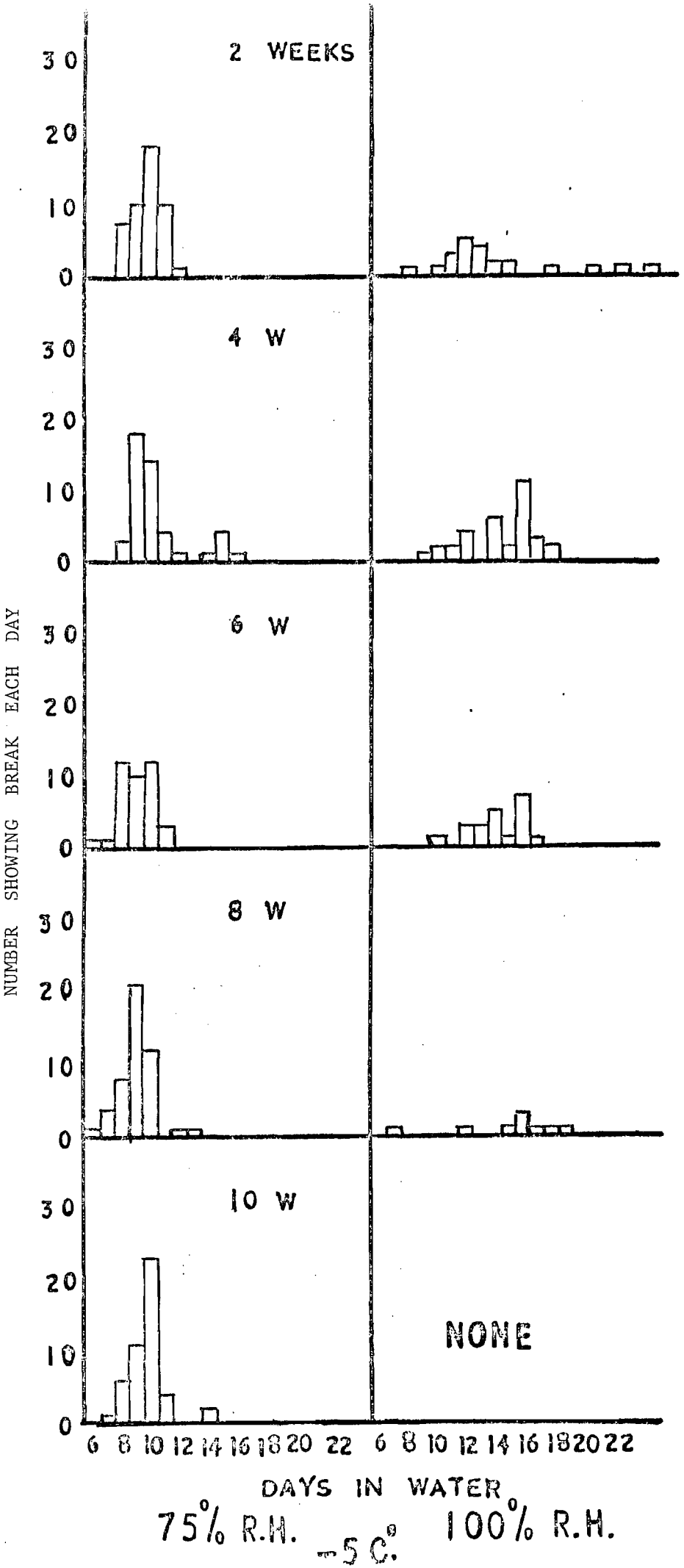

2 WEEKS
4 W
6 W
8 W
10 W
30
20
10
0
6 8 10 12 14
NUMBER SHOWING BREAK EACH DAY
0% R.H.
25% R.H.
5 C°.
50% R.H.


2 WEEKS
4 W
6 W
8 W
10 W
30
20
10
0
NUMBER SHOWING BREAK EACH DAY
31 EGGS WERE FOUND BROKEN
6 8 10 12 14
1 3 5 7 9 11
DAYS IN WATER
75% R.H.
100% R.H.
5 C°.


2 WEEKS
4 W
6 W
8 W
10 W
0
10
20
30
NUMBER SHOWING BREAK EACH DAY
6 8 10 12 14 16 18
DAYS IN WATER
0% R.H.
25% R.H.
0 C°
50% R.H.

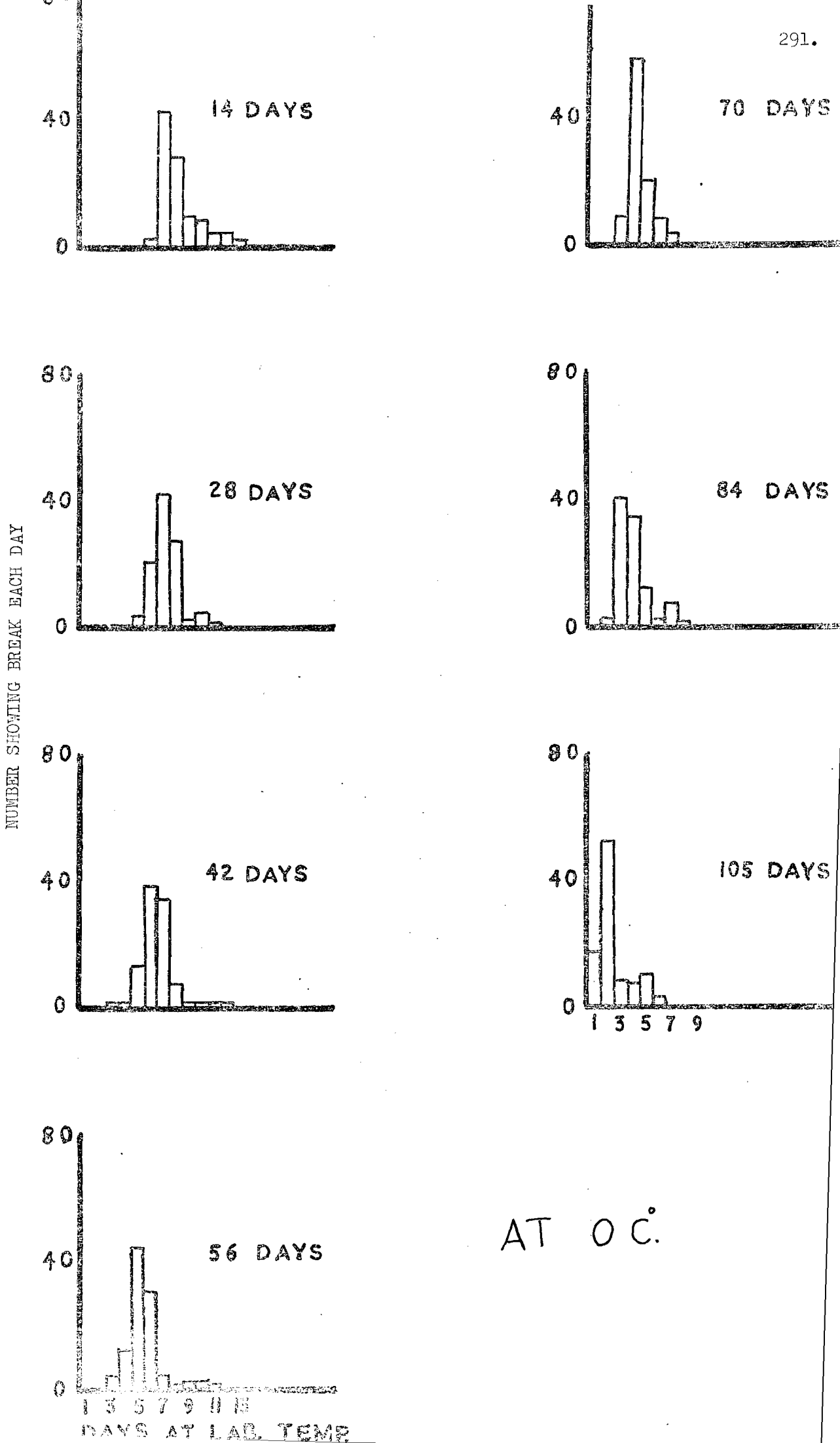

2 WEEKS
4 W
6 W
8 W
10 W
NUMBER SHOWING BREAK EACH DAY
0
10
20
30
6 8 10 12 14 16 18
1 3 5 7 9 11 13 15
DAYS IN WATER
75% R.H.
100% R.H.


2 WEEKS
4 W
6 W
8 W
10 W
NUMBER SHOWING BREAK EACH DAY
0
10
20
30
6 8 10 12 14 16 18 20
DAYS IN WATER
0% R.H.
25% R.H.
50% R.H.

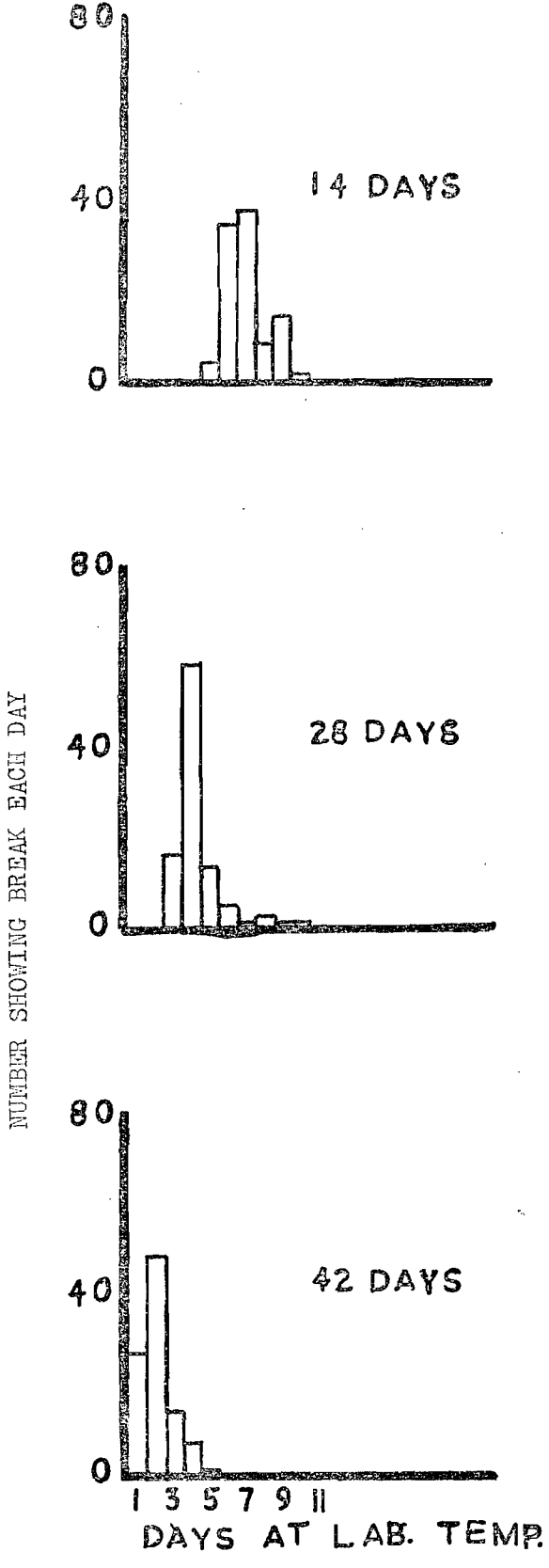

2 WEEKS
4 W
6 W
8 W
10 W
NONE
30
20
10
0
NUMBER SHOWING BREAK EACH DAY
6 8 10 12 14 16 18 20 22 6 8 10 12 14 16 18 20 22
DAYS IN WATER
75% R.H. -5 C° 100% R.H.

APPENDIX II

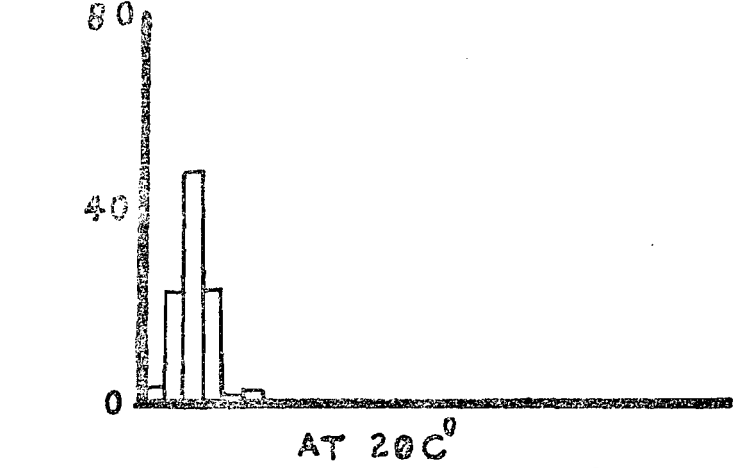
Shows the time required for eggs of C.diaphanus to reach breaking after exposure to various temperatures for different periods of time. No embryonic development took place at $-5^{\circ}C$. All eggs were found to be dead when they were examined one week later. At temperatures of 0, 5, 10, 15, 18 and $20^{\circ}C$, it has been observed that the rate of development increases with the increase of temperature.

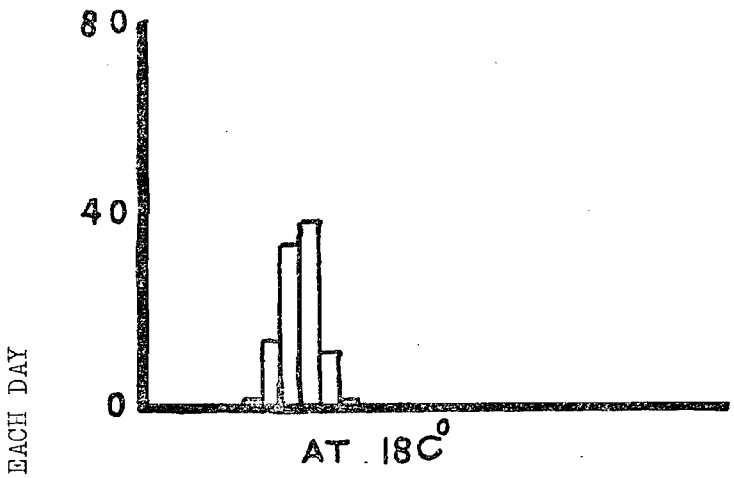

14 DAYS
70 DAYS
28 DAYS
84 DAYS
42 DAYS
105 DAYS
56 DAYS
80
40
0
1 3 5 7 9
NUMBER SHOWING BREAK EACH DAY
DAYS AT LAB. TEMP.

AT 0 C°.


80
40
0
14 DAYS
80
40
0
28 DAYS
80
40
0
42 DAYS
NUMBER SHOWING BREAK EACH DAY
1 3 5 7 9 11
DAYS AT LAB. TEMP.

AT 5°C.





NUMBER SHOWING BREAK EACH DAY