OVERWINTERING OF LARVAE OF *CLOEON DIPTERUM* (L.) IN AN ICE COVERED AND ANOXIC POND

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1. Introduction

Lakes and ponds in Sweden with the exception of those in the most southern and western parts are ice-covered during the winter for at least 3 to 4 months. For many small and shallow ponds this results in anoxic conditions and formation of hydrogen sulfide in the whole of the water body. Such conditions often prevail for several months and require that animals living in these biotopes must be particulary resistant to the effects of anoxia. Investigations of the winter conditions in ponds are relatively few. DINEEN (1953) observed anoxic conditions in a small Minnesota pond, SIKOROVA (1968) in a park pond, PENNAK (1968) in some alpine lakes with prolonged ice cover and ELGMORK (1959) in some ponds in southern Norway. DANKS (1971) has studied the chironomide fauna and the winter conditions of some ponds in northern temperate areas of Canada.

The larvae of *Cloeon dipterum* (L.) often live in great numbers in such ponds. During studies of oxygen consumption by larvae of *Cloeon dipterum* (NAGELL, 1973, 1974, 1975) it was observed that larvae collected during the winter were quite resistant to anoxic conditions at 0°C. They could, without adverse effects survive anoxic conditions for more than 6 weeks. During wintertime it was observed that the larvae were gathered on the underside of the ice. In order to survey more thoroughly the conditions prevailing during the winter, chemical analyses were performed during three winters in some ponds with rich abundance of *Cloeon dipterum*. Subsequently, survival experiments were performed in one of the ponds. The purpose was to find out whether the observed resistance to anoxia and the tendency to accumulate on the underside of the ice were adaptations to the special conditions in the ponds.

2. Material and Methods

2.1. Ponds investigated and analyses performed

Four ponds were investigated. Three of them are located near Uppsala, 70 km north of Stockholm, in a mixed forest dominated by conifers. They contained some vegetation but were rather low-productive with somewhat brownish water. The first pond, which is the most eutrophic contained the largest population of *Cloeon*, and has a volume of about 9 m^3 and a maximal depth of 1.5 m.

* The experimental part of the investigation was made at the Institute of Zoophysiology, University of Uppsala.

The second pond has a volume of about 6.5 m^3 and a maximal depth of 2.0 m. In the third pond the volume is about 800 m^3 and the maximal depth is 1.2 m. The fourth pond was made of concrete 2.4×1.6 m and 1.25 m deep with a volume of 4.8 m^3. The walls of this pond were overgrown with about 1 cm long algae and in the center of the pond there was a barrel with a water lily (*Nuphar*). In this pond regular water sampling and survival experiments were performed. Water samples were also taken from the other ponds which served essentially as material for controlling that the chemical conditions in the concrete pond were not widely divergent from those in the natural ponds.

Samples were taken regularly at intervals of about 10 days in the concrete pond during the winters of 1965/66, 1966/67 and 1968/69. In the other ponds samples were taken once a month. The samples were analyzed for oxygen concentration, conductivity, temperature, pH, oxidation-reduction potential and concentration of ferrous ions and hydrogen sulfide. They were taken through a 6 mm tube of stainless steel (Fig. 1). The tube was placed permanently in the pond. Before sampling it was heated electrically by an accumulator. It could then be turned around and the vertical position shifted. The shape of the tube permitted samples to be taken close to the underside of the ice. From an analytical point of view the arrangement was satisfactory, as it permitted sampling with only minute disturbances of the stratification in the pond. Moreover analyses vulnerable to oxygen could be performed without exposure to air.

In order to find out whether water from melting snow could percolate through the ice experiments were performed in which coloured water was supplied to the upper surface of the ice.

2.2. The experimental cages and their location

The survival experiments were performed in net cages made of 1×1 mm mesh stainless steel. Three different types of cages were used, all with a diameter of 15 cm. One type was open at the top and 45 cm high, one closed at both ends and 40 cm high and one was closed at both ends and 20 cm high. Experiments were performed during two winters.

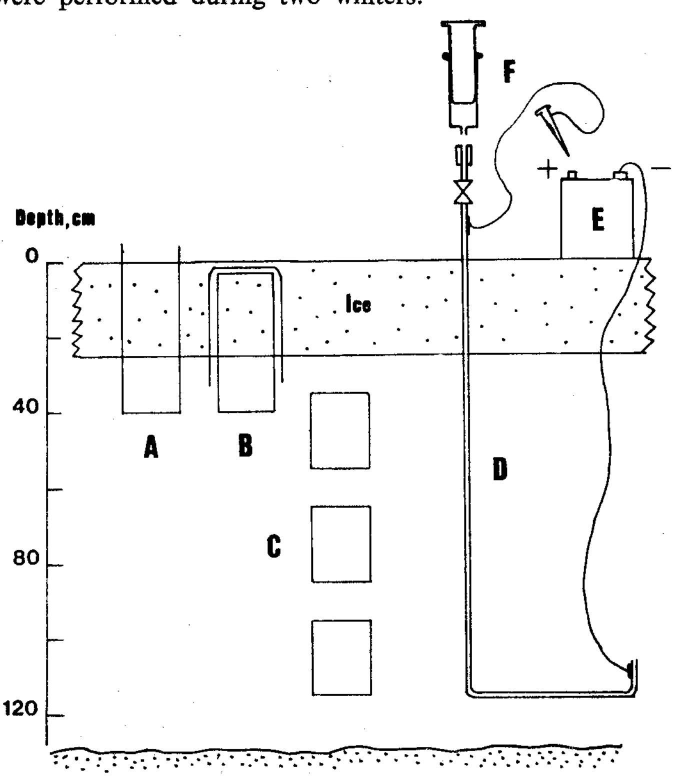

Fig. 1. The position of the cages and the water sampling equipment of the pond. A: open cage, B: closed cage covered with a polyethylene bag, C: cages for experiments below the surface, D: the sampling tube, E: accumulator, F: all glass syringe

During the first winter, 1972/1973 the influence on the survival of location at different depths of water was studied. Cages were placed at the following depths: on the surface, at depths of 35 cm, 65 cm and 105 cm (Fig. 1, A and C). At each depth three replicates containing 150 larvae were used. The cages at the surface were of the open type. They were placed so that the edges protruded 5 cm above the water. Thereby the larvae were prevented from leaving the cage, although they had an unbroken connection through the ice with its upper surface.

The following winter, experiments were designed to study to what extent an unbroken connection through the ice with the upper surface of the ice was of importance for survival. In this experiment, closed cages as well as open ones were used (type A and B in Fig. 1). The cages of type B were covered with a polyethylene bag extending 30 cm down the sides, which prevented percolating water from moving into the cage. Five replicates of each type of cage with 200 larvae in each were used. In order to minimize possible differences between the series, caused e. g. by substances dissolved from the polyethylene, a piece of polyethylene film with the same surface area as the bag was placed in each cage of the A type. Only a few water samples were taken during these winters in order to avoid disturbing the stratification in the pond. Samples were taken only to make sure that the conditions were comparable to those occuring during the preceeding winters.

3. Results

3.1. The water chemical conditions in the ponds

The water analysis showed that the conditions in the concrete pond were comparable to those in the other three ponds. The samples taken in the pond during the survival experiments showed that conditions during these two winters were the same as those prevailing during the preceeding winters.

The results obtained from the collective measurements gave the following picture of the conditions. The ponds were anoxic during 3–4 months, with reducing conditions. In the surface water the maximum concentration of hydrogen sulfide occurred in February or March. The concentration of undissociated hydrogen sulfide varied between 0.02 and 0.17 mg/l.

The experiments designed to study the percolation of water through the ice showed that percolation can take place, if the temperature of the surface of the ice is above the freezing-point. The percolation occurs through tiny irregular cracks in the ice. These cracks are found at intervals of a few centimeters in old ice. In one week old ice such cracks also occur though considerably less frequently. In the ice inside the open experimental cages such cracks could also be observed. It seems probable that cracks might appear along the vertical sides of the cages. No such cracks were however observed. Percolation took place rather quickly. Through 21 cm thick, 4 week old ice it took 15 to 20 minutes.

3.2. The survival of the larvae

As shown in Tab. I, the survival is greatest for larvae remaining on the underside of the ice. The frequency of survival diminishes greatly with increasing depth. Of the larvae that were found on the underside of the ice, 92.0$\pm$1.2% survived the 85 days of anoxia. At a depth of 35 cm, measured from the upper surface of the ice, 63.4$\pm$6.1% survived, and at a depth of 105 cm, only 22.7$\pm$10.2%. From the table it can be seen that the mean temperature also increases with depth. The mean temperature is used only as a provisional measure of the temperature conditions, because during the experimental period the temperature decrease was not linear.

The results from the experiment in which open and covered cages were compared are shown in Tab. II. Anoxic conditions lasted for 138 days. The survival in open cages was 59.5$\pm$4.8%, while in covered cages it was 18.1$\pm$2.5%.

Table I

Survival at different depths, 85 days of anoxia

Position of cage, depth, cm	Number of cages	Larvae per cage	Mean temp °C	% survival ± S. E.
Surface, open	3	150	0.0	92.0±1.2
35	3	150	0.6	63.4±6.1
65	3	150	1.4	32.7±9.1
105	3	150	2.7	22.7±10.2

3.3. Observations on migration of the larvae to the underside of the ice

At the beginning of freezing the ice is so transparent that *Cloeon* larvae attached to its underside may be counted. For some time after freezing of the pond, the oxygen concentration was recorded daily. At the same time the number of larvae migrating from one of the walls of the pond onto the underside of the ice was recorded. It then became apparent that the rate of migration increased considerably during the time immediately after the conditions in the pond became anoxic. After freeze up, during the 12 days which were required for conditions in the pond to become anoxic, about 20% of the larvae had migrated onto the underside of the ice. During the following 4 days an additional 75% migrated up to the underside of the ice.

Table II

Survival in open cages and cages covered with polyethylene, 138 days of anoxia

Position of cage	Number of cages	Larvae per cage	Mean temp °C	% survival ± S. E.
Surface, open	5	200	0.0	59.5±4.8
Surface, covered	5	200	0.0	18.1±2.5

During the overwintering period, the intestines of the larvae are empty, they do not eat and no growth takes place. This has also been observed by Bretschko (1965). However the larvae are in a potentially active state and are easily provoked into creeping or swimming.

4. Discussion

These results confirm that the anoxia is of very long duration and that reducing conditions prevail in the ponds investigated. During periods in which air temperatures are below the freezing point, such conditions prevail throughout the water body of the pond. At temperatures above the freezing point percolation of oxygen-rich melt water takes place. After percolation this water stratifies immediately below the lower surface of the ice. Because of the higher density of the pond water, water percolating through the ice probably never reaches deeper than a few centimeters beneath its lower surface.

There are strong indications that in most parts of Sweden such conditions occur commonly in ponds of the type investigated.

The results from the survival experiments of the first winter show that larvae attached to the underside of the ice survive to a greater extent than the others. From the results it can be concluded that larvae forced to remain at depths exceeding about 35 cm will not survive a 3 to 4 month long winter, i. e. the normal length of winter in central Sweden.

External factors that presumably have a great influence on survival are the temperature, the presence of undissociated hydrogen sulfide and the reduced conditions. Laboratory experiments indicate however, that the concentrations of undissociated hydrogen sulfide present in the pond do not adversely influence the survival (NAGELL, in prep.). This would mean that the diminishing survival with increasing depth in the pond is mainly an effect of the higher temperature and the more reduced conditions.

The experiments carried out during the second winter demonstrate clearly the strong positive influence of the percolating water on the survival of the larvae. Some of the larvae can survive without the percolating water. The mortality of the population as a whole will, however, be three times greater. Presumably the larvae survive by using an anaerobic metabolism; when oxygen is present they will change to an aerobic metabolism. Such mechanisms are known in different types of animals (BEADLE, 1961).

The observed migration of larvae to the underside of the ice is apparently an adaption which results in greatly increased survival of the larvae during overwintering.

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SUMMARY

Overwintering of larvae of Cloeon dipterum (L.) in an ice covered and anoxic pond

In some small ponds in central Sweden, observed over several years, anoxic conditions were observed to occur shortly after the ponds were covered by ice. In spite of these anoxic conditions great numbers of *Cloeon dipterum* larvae were observed to overwinter in the ponds. During the winter the larvae were attached to the underside of the ice. Experiments were designed to study the survival of the larvae during overwintering. During a period of 85 days the fraction surviving was 23% at a depth of 105 cm. During the same time period 63% survived at a depth of 35 cm. The largest number of survivors (92%) was observed among larvae on the underside of the ice. In another experiment, lasting for 138 days, the survival of two groups of larvae attached to the underside of the ice was compared. One of the groups was screened during the winter by a polyethylene film placed in the ice between its upper and lower surface. This arrangement impeded melt water to percolate through the ice at this spot. The other group was not screened. Of the first group 18% survived compared with 59% for the unscreened group. The results suggest that the position of the larvae on the underside of the ice increases their chance of survival. Two main reasons for this are put forward: the occurrence of a low temperature (close to 0°C) and accessibility to the larvae of small amounts of oxygen-rich water percolating through the ice during thaw periods.

DISCUSSION

U. HUMPESCH: Did you distinguish between the stages of the larvae in the experiments?

B. NAGELL: I tried to use the most common stage present in the pond at the start of the experiment in December. Larvae smaller or bigger than those were dismissed. Their size was ca. 5–8 mm. Which size by BRETSCHKO (1965) is called a half grown stage.

U. HUMPESCH: Did you feed the larvae in the cage?

B. NAGELL: No. The larvae do not feed during the winter in nature.

U. HUMPESCH: What plants and algae did you find in the water? Could there be an assimilation beneath the ice?

B. NAGELL: I have not studied that directly. The ice is opaque and covered with snow most of the winter. Thus there is little light beneath the ice. During one winter I studied water samples microscopically. During most of the winter there were no planctinic algae. About 2 weeks before the broke of the ice there were a few but as I believe not in sufficient numbers as to provide oxygen for the larvae.

U. HUMPESCH: BRETSCHKO (1965) pointed out that there is a stoppage in growth in his *Cloeon* population.

T. E. BRITTAIN: In Norway we have a similar situation in whereby nymphs of *Leptophlebia vespertina* survive in humic ponds devoid of oxygen during the period of winter ice cover.

J. P. NILSSEN: Did you find any evidence that the animals survived the winter conditions in any of the physiological states discussed by MANSINGH (Can. Ent. 103:983–1009, (1971)) e. g. quiescence, oligopause or diapause?

B. NAGELL: I think that the larvae are in a state of dormancy i. e. a physiological adaptation to overcome adverse conditions during the winter. The definition of diapause given by MANSINGH seems most suitable for describing the overwintering state of the larvae.

J. P. NILSSEN: Did you find any correlation between the depth of the snow cover or the type and thickness of the ice and the survival of animals immediately below the surface of the ice?

B. NAGELL: I have not specifically investigated this possibility though I did observe an inverse relationship between the depth of the snow cover and the thickness of the ice. For all types of ice which I have examined, there have been cracks through which melt water could percolate: the number of such cracks varies considerably for different types of ice. For successful survival of the larvae, the number of such cracks is less important than the accessibility of the larvae to oxygen-rich melt water. The degree of survival is thus dependent on the number of thawing periods during the winter. I believe therefore that the existence of cracks in the ice is the most important factor for survival.

J. P. NILSSEN: Have you measured the concentration of sulphate and heavy metals such as Zn, Pb and Hg immediately below the surface of the ice during periods of thawing?

B. NAGELL: No, I have not carried out such measurements.

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