

Phototactic and thermotactic responses facilitating survival of *Cloeon dipterum* (Ephemeroptera) larvae under winter anoxia

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About 10 d after the freezing up of a pond, and coincident with the total water-body becoming anoxic, larvae of *Cloeon dipterum* L. were observed to suddenly migrate to the underside of the ice. Larvae remained there for the winter. Laboratory experiments were performed to examine the factors responsible for causing this migration. In aerated water larvae, acclimated to 2.5°C and 120 lux, were negatively phototactic in a light gradient (25 to 1 lux), but positively thermotactic in a temperature gradient (4.1 to 1.9°C). Both responses were reversed under anoxia. Light was more directing than temperature under the prevailing conditions.

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Примерно через 10 дней после замерзания пруда и одновременно с аноксидацией всего слоя воды, у личинок *Cloeon dipterum* L. наблюдалась внезапная миграция к нижней стороне ледового покрова. Личинки оставались там на всю зиму. Проведены лабораторные эксперименты для исследования факторов, вызывающих эту миграцию. В аэрированной среде личинки, акклиматизированные к температуре 2,5°C и освещенности 120 люксов, проявляли отрицательный фототаксис на градиенте освещенности 25-1 люкс. У них обнаружен положительный термотаксис в температурном градиенте 4,1–1,9°C. Оба таксиса имели обратные проявления при аноксидации. В существующих условиях свет оказался более сильным фактором, чем температура.

Introduction

Small ponds in Sweden are usually completely anoxic for several months in winter when they are ice-covered (Nagell and Brittain unpubl.). Shortly after the pond freezes up, and coincident with the total water-body of the pond becoming anoxic, many *Cloeon dipterum* L. larvae migrate to the underside of the ice and remain there for the winter. In spring, with oxygenation of the surface water, larvae leave the ice even though most of the ice is still present. Nagell (1977) has shown that the larvae survive better on the underside of the ice than they do at greater depths. In a pond there is a light gradient with increasing intensity towards the surface. There is also a temperature gradient decreasing towards the surface in autumn and winter.

The present paper examines the factors responsible for inducing and directing this migration of larvae. Experiments were designed to determine whether light or temperature was the more directing within the actual limits of an ice-covered pond.

Material and methods

Larvae were collected in September and October from a small pond near Uppsala in central Sweden and acclimated to 2.5°C for at least 4 wk with a 12 h light-period at about 120 lux (fluorescent tube, Osram Daylight).

Experiments were performed in a covered polyethylene tray (125 × 20 cm and 7 cm depth) (Fig. 1). A light gradient was established by placing a 6 W bulb at one end of the tray. Elsewhere the tray was light proof and also insulated to prevent heat loss. The temperature gradient was established with a thermostat-controlled electric heater and a cold finger of a refrigerator. Five equal-size zones were marked out on the bottom of the tray. Light intensity and mean temperature in the middle of zone one was 25 lux (4.1°C), in the middle of zone

three 4 lux (2.5°C) and in the middle of zone five <1 lux (1.9°C). Controls showed that day to day variation was only 0.1–0.2°C.

In the experiments with aerated water larvae were introduced into the tray from a small chamber in the middle of zone three. Larvae were allowed to adapt for 1.5 h in the chamber before release.

In the anoxia experiments larvae were introduced into a 100 × 5 cm perspex tube sealed with two rubber stoppers (Fig. 1). The tube was placed in the tray so that experiments with aerated or anoxic water could be carried out simultaneously. One of the stoppers was pierced by a hypodermic needle through which nitrogen could be bubbled into the tube. In the middle of the tube wall there was a hole connecting with a small chamber open at its base. Oxygen was removed from the water by bubbling nitrogen through the vertically-held tube for 10 min. Fifty mg glucose was also added to stimulate respiration of bacteria in the water. After two days at room temperature when the bacteria completely had eliminated remaining oxygen the tube was placed in the tray. Following temperature equilibration, larvae were pipetted into the chamber in a small volume of nitrogen-bubbled water. The very negligible amount of oxygen introduced into the tube by this procedure was ignored. After 1.5 h the larvae were released into the tube by raising the small chamber. During the experiment both the needle and the hole in the tube were sealed. All experiments were performed in a constant temperature room at 3°C.

Migration of larvae outdoors was observed in a small pond with vertical concrete walls. Oxygen concentration of the surface water beneath the ice was determined frequently by drawing up water samples through a narrow electrically-heated steel tube (Nagell 1977), thereby avoiding undue disturbance to the pond. Larvae were observed and counted as they migrated from one of the walls of the pond onto the underside of an adjacent 100 × 50 cm area of the ice.

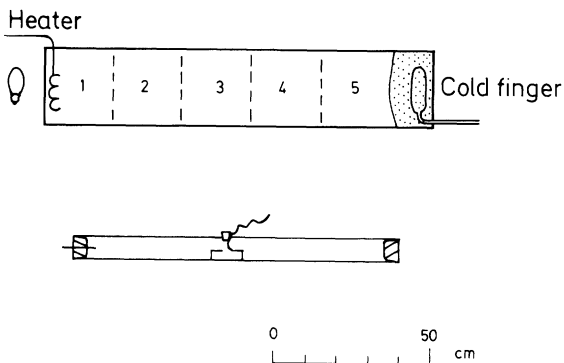


Fig. 1. Experimental tray with its five zones showing the thermo-regulated electric heater, the 6 W bulb and the cold finger surrounded by ice (stippled area). Below the tray is the perspex tube used for the anoxia experiments. This is a side view of the tube showing the small chamber into which the larvae were first introduced.

Results

Results from the control experiments with no light or temperature gradients are given in Fig. 2. The five curves, each representing the five zones of the tray, show the number of larvae in each zone as a percentage of the total. There was no tendency for directed movement.

Larvae in a light gradient with aerated water moved towards the dark part (Fig. 3A, B), although slight irritation greatly accentuated movement (Fig. 3A). But under anoxia the response was reversed and larvae moved towards the light (Fig. 3C).

Larvae in a temperature gradient with aerated water moved towards the warm part (Fig. 4A), but towards the cold part under anoxia (Fig. 4B).

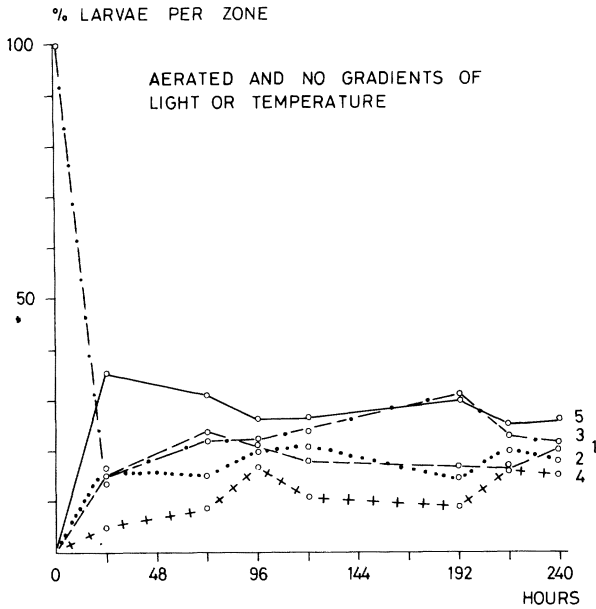
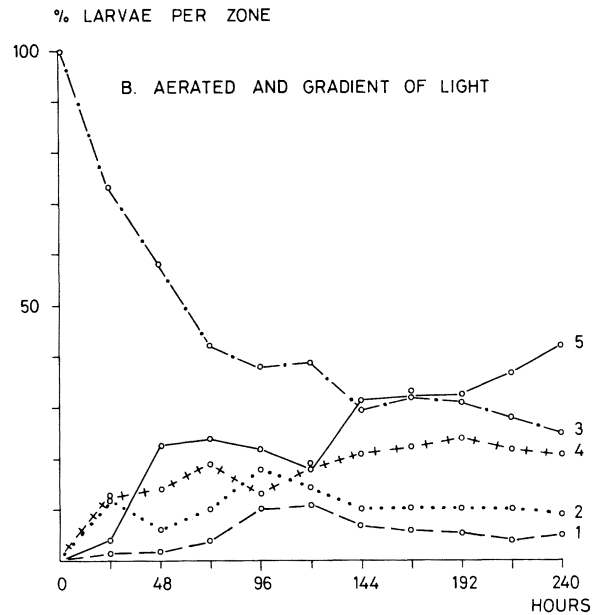


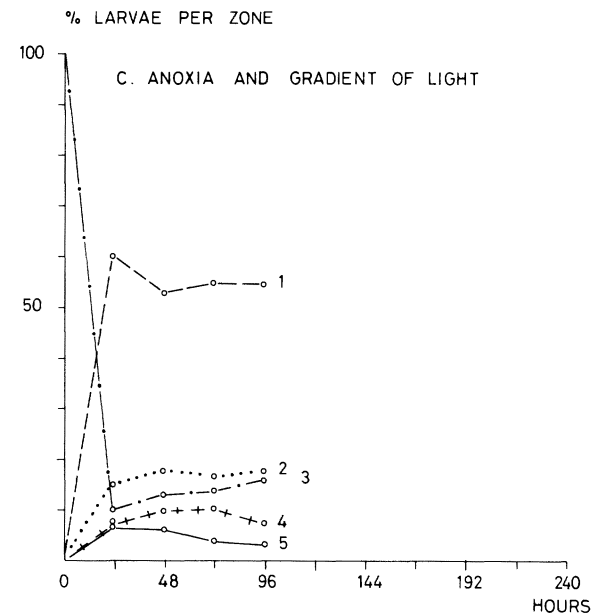
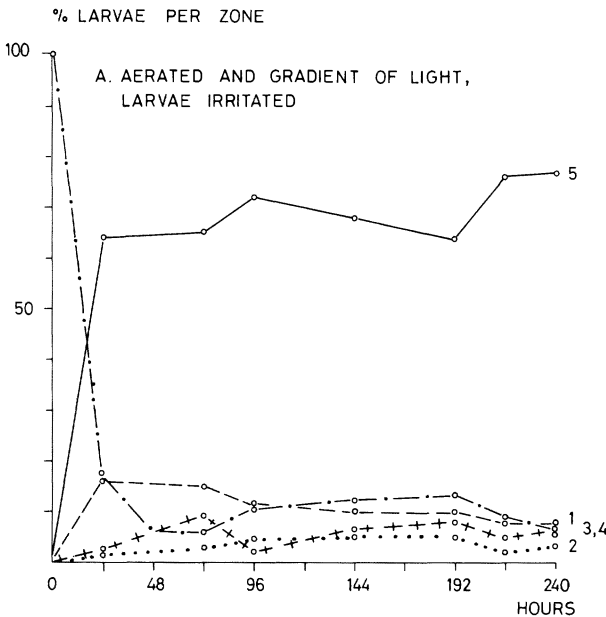
Fig. 2. Movement in aerated water without temperature or light gradients. Mean of two experiments with a total of 200 larvae. Experiments were performed in darkness at 3°C. The establishment of a temperature gradient was avoided by slowly circulating the water in the tray. The five curves represent the number of larvae in each zone of the tray (zones 1-5) as a percentage of the total. Zone one is the brightest and warmest.

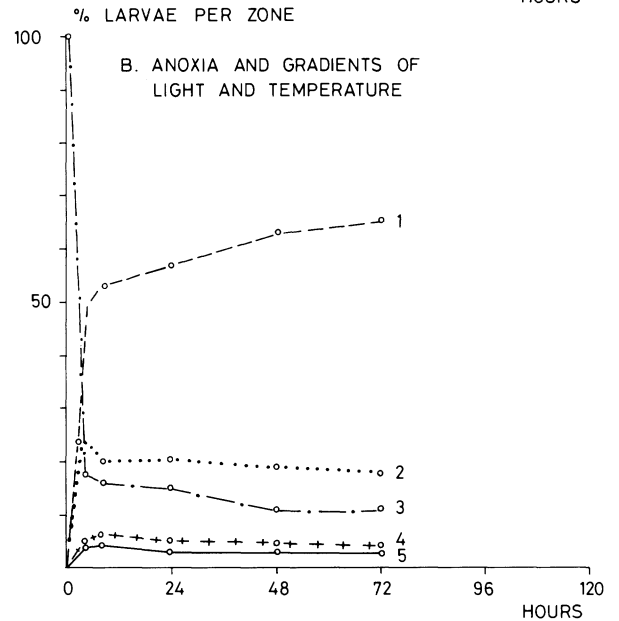
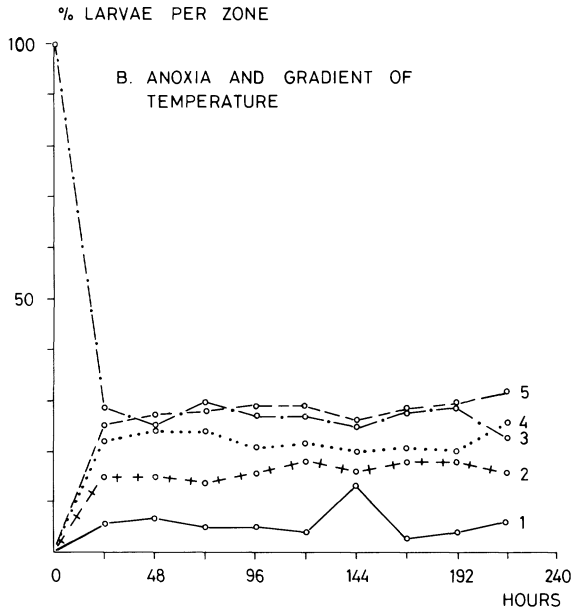
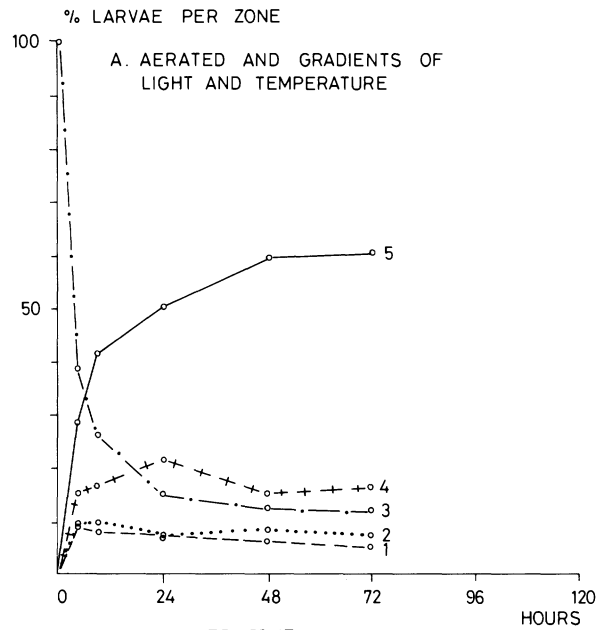
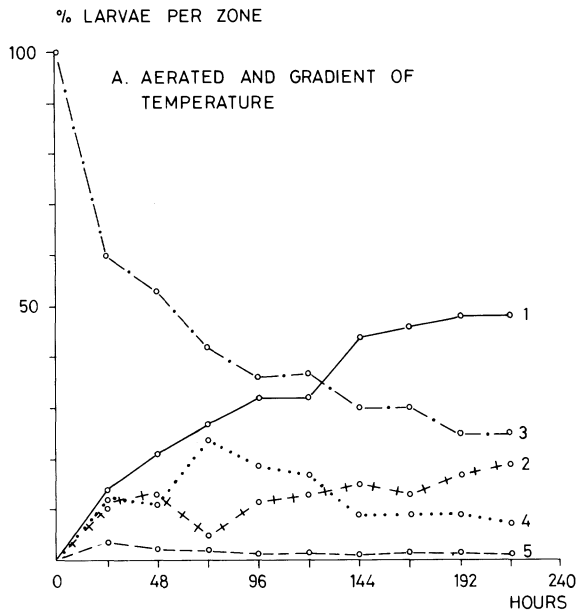
Larvae in combined light/temperature gradients in aerated water moved towards the dark, cold part (Fig. 5A), but under anoxia towards the light, warm part (Fig. 5B).

Distribution of larvae in the experiments after 72 h (Tab. 1) was compared with a theoretical distribution with evenly-spread larvae, using a χ^2 test in which the five zones of the gradient were treated as three (zones 1 + 2, 3 and 4 + 5). Experiment 2 was very close to the theoretical distribution and larvae showed no tendency for directed movement. In the other experiments the distribution differed from the theoretical one at the



Figs. 3A, B, C. Movement in a light gradient. Larvae in 3A were irritated on the first day by a few slight taps on the tray. Each figure is based on the mean of two experiments with a total of 200 larvae. The experimental temperature was 3°C.





Figs. 4A, B. Movement in a temperature gradient. Each figure is based on a mean of two experiments with a total of 300 larvae.

Figs. 5A, B. Movement in combined gradients of light and temperature. The brightest part of the gradient is also the warmest. Each figure is based on five experiments with a total of 500 larvae.

χ^2 values

		Experiments						
2	3A	3B	3C	4A	4B	5A	5B	
0.13	45.9	36.0	22.9	13.9	52.9	57.9	95.3	
0.43	64.6	54.0	76.3	49.9	14.1	60.3	30.7	
						56.1	48.2	
						31.6	50.2	
						81.7	22.2	

Tab. 1. Distribution of *Cloeon dipterum* larvae after 72 h from the start of the experiments, as tested by χ^2 test. Experiments are provided with the same designation as the figures in which they are shown. In experiment 2 the probability level is >95% and in the other experiments <0.1% (critical value 13.8).

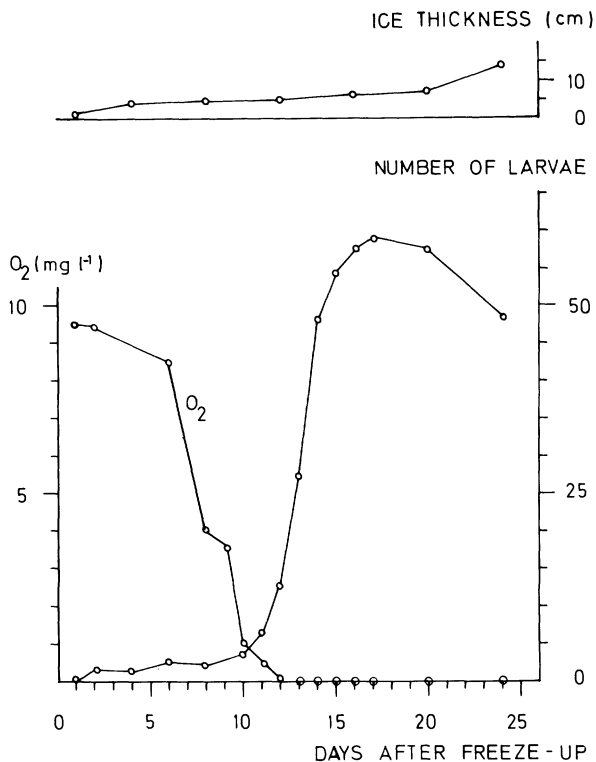


Fig. 6. Oxygen concentration and number of larvae on the ice against time. The left vertical axis shows the oxygen concentration of the water just beneath the ice. The right vertical axis shows the number of larvae migrating into a 0.5 m² area on the underside of the ice.

0.1% significance level indicating a great tendency for directed movement.

Ice thickness, oxygen concentration and number of larvae on the underside of the ice are shown in Fig. 6. There was a close correlation between the occurrence of larvae on the underside of the ice and anoxic water just beneath the ice. When the water became anoxic most larvae migrated to the ice. At freeze-up the pond was 4°C at 8.0 cm depth and 0°C just beneath the ice. Light intensity varied: in December with 4 cm ice covered by 1 cm snow, it was 80 lux at the underside of the ice during sunshine and 14 lux when overcast.

Discussion

Cloeon dipterum larvae in aerated water moved towards darkness or warmth, but under anoxia towards light or cold. Therefore anoxia may be regarded as the trigger causing larvae to reverse their response to light and temperature.

Experiments with combined gradients showed that light was more directing under the experimental conditions. Light often serves as stimulus directing to some-

thing else than light (i.e. a token stimulus) and is particularly effective because its source is usually well-defined as to direction. This does not usually apply to temperature (Fraenkel and Gunn 1961) because the thermal pattern around an animal is usually irregular and dependent on many variable factors.

In the experiments the gradients were horizontal, thereby excluding the directing effect, if any, of changing hydrostatic pressure with depth. Apart from the pressure gradient, the experimental light and temperature gradients were close to those in a natural pond. Probably larvae respond in the same way in the laboratory experiments as in nature and support for this is provided by the observations from the pond (Fig. 6). In nature light and temperature gradients both direct larvae towards the ice, so that the tendency for movement towards the ice is probably greater in nature than in the laboratory experiments.

The number of larvae in the observation area declined after 17 days (Fig. 6) and this was probably due to larvae leaving the walls and spreading themselves out along the ice.

The observed behaviour of larvae is most probably ecologically significant. In oxygenated water, without ice-cover, larvae move towards the dark vegetation where there is food and shelter; movement being accentuated when the larvae are disturbed. But under anoxia, movement to the ice greatly aids survival, especially during long winters. During thaw periods, oxygenated melt water can percolate through the ice and is probably used by the larvae (Nagell 1977).

In spring larvae left the ice even though most of the ice still remained. This may be due to the larvae, when sufficient aerated water is available for several days, reversing their response and moving towards dark and warmth and away from the ice. But this does not seem to occur when larvae come into contact with oxygen in percolating melt water during winter. However, this oxygenated melt water is only present as a thin layer and the oxygen is rapidly consumed by H₂S coming from beneath. Thus oxygen is probably available under these circumstances for only a relatively short period.

Similar movements towards the water surface at low oxygen concentrations occurs in other animals and this is often associated with a change from a negative to a positive phototactic response, although other directing stimuli, such as hydrostatic pressure, may be involved. Surfacing of fish in response to the light gradient may be triggered by the stress of low oxygen concentration (Fry 1971). Surfacing behaviour and penetration of the water surface also occurs in dragonfly larvae *Aeschna* sp. (Wallengren 1914) and for zygopteran larvae of *Calopteryx* species (Zahner 1959). It is also to be noted that some species of mayfly larvae from running water lose their negative phototactic and positive thigmotactic responses in still water (Elliot 1968); a response probably associated with reduced oxygen supply as the water becomes stagnant.

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