

Adaptations and resistance to anoxia in *Cloeon dipterum* (Ephemeroptera) and *Nemoura cinerea* (Plecoptera)

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The survival of larvae of *Cloeon dipterum* L. (Ephemeroptera) and *Nemoura cinerea* (Retzius) (Plecoptera) was studied after exposure to anoxia for 40 h at temperatures between 0°C and 11.5°C. If the larvae were acclimated to 2°C for 6 wk *C. dipterum* survived temperatures close to 0°C better than *N. cinerea*. If they were acclimated to 10°C, most *C. dipterum* died at all experimental temperatures. *N. cinerea* died to 60% at 5°C; at higher or lower temperatures the mortality increased. A 6-d stay in 2°C of *C. dipterum* previously acclimated to 10°C was not long enough to induce resistance to anoxia. When exposed to anoxia *N. cinerea* became unconscious within 10 min, whereas larvae of *C. dipterum* did so first within 60 min.

It is concluded that a long term acclimation to low temperatures induces in *C. dipterum* but not in *N. cinerea* a high degree of resistance to anoxia at temperatures close to 0°C. This resistance is of great importance for *C. dipterum* since in cold temperated regions this species usually overwinters in anoxic conditions. The different rate at which the species lose consciousness in anoxia may be important for their survival in natural habitats.

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Исследовали выживание личинок *Cloeon dipterum* L. (Ephemeroptera) и *Nemoura cinerea* (Retzius) (Plecoptera) после выдерживания их в течение 40 ч в бескислородной среде при температуре между 0°C и 11,5°C. Если личинки акклиматизированы к 2°C в течение 6 недель, *C. dipterum* переносит температуры, близкие к 0°C лучше, чем *N. cinerea*. Если они акклиматизированы к 10°C, большая часть *C. dipterum* погибает при всех экспериментальных температурах. 60 % *N. cinerea* погибает при 5°C. При более высокой или низкой температуре смертность возрастает. Для *C. dipterum*, если они предварительно акклиматизированы к 10°C, 6-суточное пребывание при 2°C недостаточно для выработки их устойчивости к бескислородной среде. В бескислородной среде *N. cinerea* становится нечувствительным через 10 мин, а личинки *C. dipterum* – 60 мин. Сделано заключение, что долгосрочная акклиматизация к низким температурам вызывает у *C. dipterum* (но не у *N. cinerea*) высокую устойчивость к бескислородным условиям при температурах, близких к 0°C. Эта устойчивость имеет большое значение для *C. dipterum*, поскольку в районах холодного климата этот вид обычно зимует в бескислородных условиях. Различия в длительности периода, когда личинки нечувствительны к бескислородным условиям, могут иметь значение для их выживаемости в естественных местообитаниях.

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1. Introduction

Small, shallow ponds in central or north Scandinavia commonly are anoxic for several months every winter (Nagell and Brittain 1977). In autumn such ponds are often inhabited by larvae of *Cloeon dipterum* L. and sometimes also by larvae of *Nemoura cinerea* (Retzius). We observed in a pond containing both species in autumn that, immediately after the break-up of the ice in spring, *N. cinerea* was absent. This species seems thus unable to survive a long period of anoxia during winter conditions.

These observations seem to contradict experience from laboratory experiments (Nagell 1973) in which *N. cinerea* seemed to survive exposure to low oxygen concentrations better than *C. dipterum*. Those larvae were collected during late spring, i.e. from water with a temperature around 10°C.

The hypothesis was formed that *N. cinerea* is the more resistant of the two species to anoxia when acclimated to late spring temperatures, whereas *C. dipterum* is the more resistant when acclimated to winter temperatures. The purpose of this study is to test this hypothesis experimentally and to relate the results to the temperature and oxygen conditions in the habitats of the two species.

2. Material and methods

2.1. Review of experiments performed

Larvae of both species were acclimated for 6 wk to either 2°C or 10°C, corresponding to late autumn and late spring temperatures, respectively. Thus, four groups of experimental larvae were obtained. The following experiments were performed:

- (1) All groups were exposed to anoxia during 40 h at temperatures ranging from 0°C to 11.5°C. The aim was to investigate the survival of the two species as a function of experimental temperature and to see whether this function was altered by the acclimation temperature.
- (2) Larvae of *C. dipterum*, acclimated to 10°C, were transferred to 2°C six days before being exposed to anoxia. The aim was to see if this relatively short acclimation to late autumn temperatures would change their resistance to anoxia.
- (3) Larvae of both species, acclimated to 10°C, were exposed to anoxia at 10°C for 7 h. Thereafter, they were allowed to recover in aerated water. The aim was to compare the time-course according to which they became unconscious as well as that of their subsequent recovery.

2.2. Details of experimental procedure

The larvae were collected in September in a brook (*N. cinerea*) and a pond (*C. dipterum*) just outside Uppsala in central Sweden. They were acclimated for at least 6 wk in tap water (composition see Höglund 1961), given a light period of 12 h a day (Osram, Daylight fluorescent tube) and having free access to food (dead leaves of maple, *Acer platanoides* L.). At the end of the acclimation period the majority of *C. dipterum* was in stage L V (2°C) or L VI (10°C), as defined by Cinciara (1978). *N. cinerea* after acclimation may be characterized as 1/2-grown (2°C) or 3/4-grown (10°C).

The experiments were performed in an apparatus (Fig. 1), where the larvae were exposed to a constant flux of anoxic water. The reservoir was filled with filtered experimental water (prepared from distilled water and chemicals of p.a. quality, cf. Tab. 1) to which 10 mg glucose l⁻¹ was added. Nitrogen gas was bubbled slowly through the water to remove most of the oxygen. The pH was re-adjusted by bubbling carbon dioxide through for one or two minutes. The water was left overnight to permit bacterial respiration to consume remaining traces of oxygen. Before the start of an experiment water samples were taken and Winkler reagents added; if no blue colour could be observed the water was considered anoxic (estimated detection limit, 0.015 mg O₂ l⁻¹).

Each test chamber was supplied with 20 larvae of each species, the animals being allowed to cling to a piece of cotton netting. After 40 h of exposure the larvae were allowed to recover for 96 h in aerated tap water at their acclimation temperature. This length of recovery was found necessary to permit a precise de-

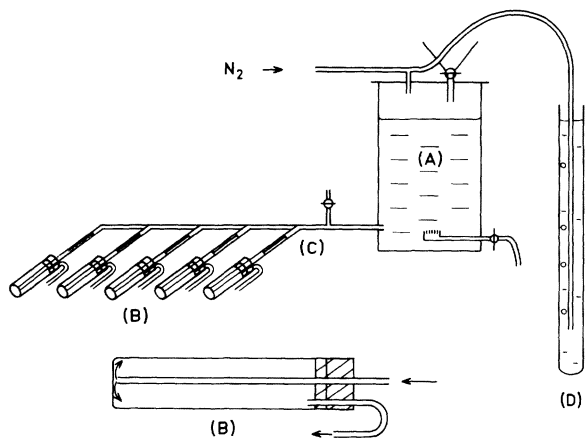


Fig. 1. The experimental apparatus. A glass container (A) (volume, 22 l) serves as the reservoir for the experimental water. This is forced by pressure into the test chambers (B) (volume, 10 ml) through five capillaries (C) giving equal fluxes (100 ml h⁻¹, passing the larvae with a speed of 0.1 mm s⁻¹). The pressure over the capillaries is held constant through compensating the decline in hydrostatic pressure in the glass container (A) by adjusting the position of the orifice of the tube in the container D.

Tab. 1. Composition of experimental water.

Ion	Concentration (mg l ⁻¹)
Ca ²⁺	7.3
Mg ²⁺	10.6
Na ⁺	18.1
K ⁺	6.7
HCO ₃ ⁻	63.1
SO ₄ ²⁻	37.7
Cl ⁻	12.8

Conductivity (μS at 20°C) = 220.

termination of the number of dead and alive specimens, because most larvae were immobile immediately after an experiment. After the recovery period, however, alive specimens were active whereas dead ones showed signs of decomposition.

3. Results

Survival experiments

For *N. cinerea*, acclimated to 2°C (Fig. 2), mortality increases from 40% at 0°C to 100% at approximately 9°C. When acclimated to 10°C (Fig. 3), the same species exhibits a v-shaped curve, the mortality being 100% at both 0°C and 9°C.

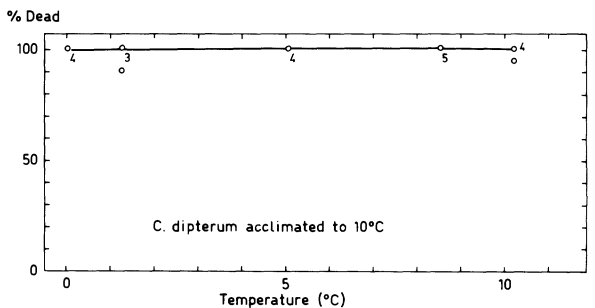
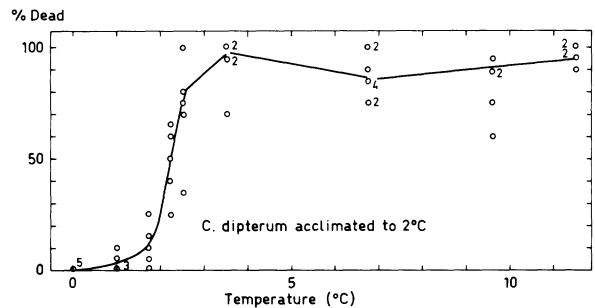
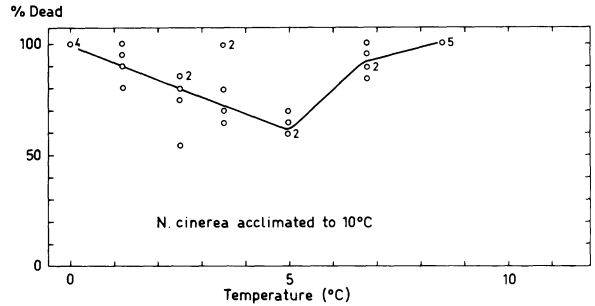
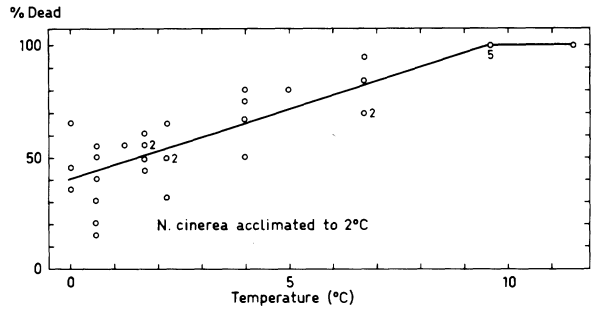
C. dipterum, acclimated to 2°C (Fig. 4), shows an almost complete mortality in the temperature interval ~3.5°C–10°C. Below this interval the resistance to anoxia increases rapidly with decreasing temperature; at 0°C the survival is complete. In contrast, for larvae acclimated to 10°C (Fig. 5) the mortality is almost complete over the whole temperature interval.

Acclimation time experiment

All (80) larvae of *C. dipterum*, acclimated to 10°C for 6 wk and to 2°C for 6 d, died when exposed to anoxia for 40 h at 2°C. Thus, 6 d of acclimation to 2°C was insufficient to induce a resistance to anoxia at low temperatures similar to that attained by larvae acclimated to 2°C for 6 wk.

Time-course experiment

Fifty percent of the larvae of *N. cinerea* became unconscious after about 10 min exposure to anoxia, whereas the corresponding time for *C. dipterum* was ca. 60 min (Fig. 6). Initially *C. dipterum* were actively crawling and swimming, in contrast to *N. cinerea*. The time-course of recovery differs only slightly between the two species initially but at the end of the recovery period the survival of *N. cinerea* is higher than that of *C. dipterum*. Thus, *N. cinerea* survives 7 h of anoxia better than *C. dipterum* but *C. dipterum* is able to remain active for a considerably longer initial time than *N. cinerea*.



Figs 2–5. Survival of larvae exposed to 40 h anoxia, as a function of temperature. Each circle represents one test chamber with 20 specimens; where more than one test chamber coincide the number of such coinciding chambers is given. We have drawn the curves by hand since we are unaware of any well-supported hypotheses as to expected curve-forms that could be used as a basis for a formal statistical curve-fitting.

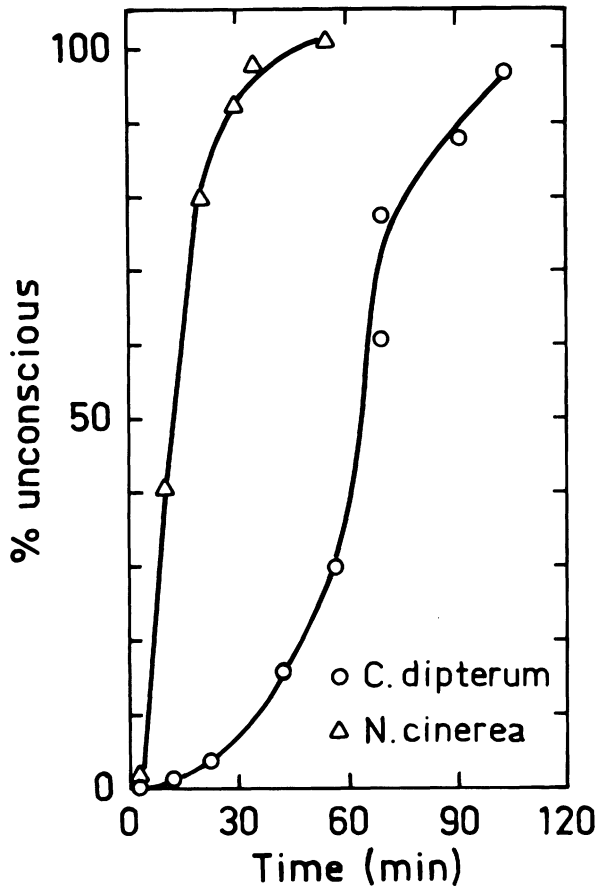


Fig. 6. Time course according to which the larvae become unconscious when exposed to anoxia at 10°C. Larvae acclimated for 6 weeks to 10°C, 100 of each species were used.

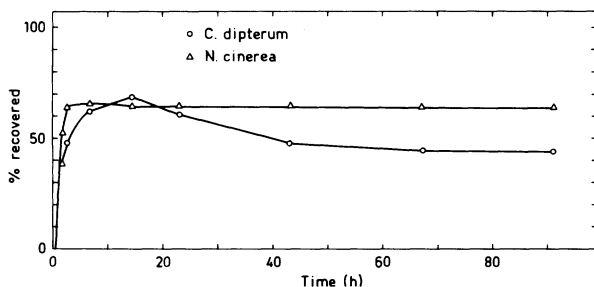


Fig. 7. Time course of recovery of larvae when transferred to aerated water after having been exposed to anoxia for 7 h at 10°C. Some larvae of *C. dipterum* were alive in the beginning of the recovery but died subsequently. This explains the decline of the curve. The end-points of the curves correspond to 64% and 44%, respectively. The specimens are the same as in Fig. 6.

4. Discussion

The results given in Figs 2–5 accord with the preliminary observations: when acclimated to late spring temperatures (10°C), *N. cinerea* is the more resistant to anoxia, while the opposite is true when the larvae are acclimated to late autumn temperatures (2°C). The latter difference is further confirmed by Nagell (1977a), showing that larvae of *C. dipterum* acclimated to 1°C survived to 50% in anoxia at 0°C for about 130 d. In contrast, out of 100 equally treated larvae of *N. cinerea* none survived a 6-d exposure.

The superior ability of *C. dipterum* to sustain anoxia after a long-term acclimation to temperatures around 1° to 2°C is most likely based on an anaerobic metabolism, which *N. cinerea* either lacks or has to a considerably lower degree. The induction of this anaerobic metabolism in *C. dipterum* evidently requires temperatures close to 0°C. Also, the process of induction takes more than 6 d. However, larvae capable of using an anaerobic metabolism do not lose their aerobic metabolism. When having access to oxygen they utilize it; they are facultative anaerobes (Nagell 1978).

Common to Figs 2–5 is that the curves probably reflect two functions of temperature. One is the rate of cellular metabolism, and hence the rate at which e.g. toxic metabolites accumulate or the cells become chemically reduced. The survival for a given period of exposure increases with decreasing temperature. The other function is a stress on the animals imposed by an experimental temperature different from that of acclimation. Survival should be expected to decrease with increasing such difference.

When the larvae are acclimated to low temperatures (Figs 2, 4) both functions should lead to increased mortality with increasing temperature. The ecologically significant part of the curves is where the experimental temperature corresponds to winter conditions. Then the experimental temperature is close to that of acclimation and the influence of the temperature stress function should be small. At these temperatures the superior survival of *C. dipterum* is likely to reflect a superior ability to sustain anoxia and not a superior ability to sustain temperature stress. When the acclimation temperature is 10°C (Figs 3, 5), on the other hand, the two functions of temperature must be expected to counteract. This is a possible explanation to the v-shaped curve in Fig. 3.

The survival of *N. cinerea* and *C. dipterum* at low oxygen concentrations has been studied by Benedetto (1970) and Kamler (1971) using larvae that probably can be considered to correspond to our larvae acclimated to 10°C. For *N. cinerea* the median lethal time was 60 h (7.5°C; 4.6 mg O₂ l⁻¹) and the corresponding value for *C. dipterum* was 400 h (5.5°C; 1.9 mg O₂ l⁻¹). These results seem to contradict our results for larvae acclimated to 10°C. However, some oxygen was present in the above experiments whereas in ours there was

complete anoxia. Larvae of *C. dipterum* have 6 movable lamellar gills that considerably increase the capacity of this species to extract oxygen from the water. Larvae of *N. cinerea* do not have any gills. This difference should, at moderately low oxygen concentrations, be advantageous for *C. dipterum* and explains the results of Kamler and Benedetto. At very low oxygen concentrations, the oxygen extracting capacity of the gills of *C. dipterum* is probably not sufficient to meet its oxygen demand. Under such conditions, the cellular resistance to oxygen deficit is the major factor determining survival and, according to our results, *N. cinerea* is then superior to *C. dipterum*.

To what extent can the characteristics of the two species established in this investigation be related to the conditions in their natural habitats? Under winter conditions a great resistance to anoxia of *C. dipterum* is a prerequisite for survival in most small ponds in Scandinavia, since these are anoxic for at least 2 or 3 months every winter. For *N. cinerea* it does not appear necessary to sustain anoxia to this great extent. In its normal habitats, i.e. running waters, the oxygen conditions are usually good during the winter. Sometimes, however, larvae of this species also inhabit stagnant waters (Brinck 1949), but then, as observed by us, they do not survive the winter if anoxic conditions occur. Their appearance in our small ponds during the autumn can probably be attributed to egg-laying females from a little brook in the vicinity.

The observed capacity of *C. dipterum* to remain active for a while after a sudden exposure to anoxia (Fig. 6) is probably also of ecological significance. It should give the larvae a respite to swim about in search for better oxygen conditions. This hypothesis is supported by the observation that the larvae were, in fact, activated when exposed to anoxic water. However, the advantage of such activity should increase considerably if the motion were directed towards regions where oxygen is likely to be encountered, i.e. the water surface. In support of the existence of such a mechanism, a positive photo-tactic reaction in anoxia was demonstrated for *C. dipterum* (Nagell 1977b).

N. cinerea, on the other hand, is a poor swimmer and if anoxic conditions or, more likely, low oxygen concentrations should occur, e.g. during summer nights, an immobilisation can be advantageous over an increased activity. Creeping about in running water does not necessarily increase the probability of encountering oxygen-rich water, since the water is continuously mixed by the current. Instead, immobilisation should be advantageous since it implies a lower energy expenditure which should prolong the time the larvae can sustain anoxia.

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