OVERWINTERING STRATEGY OF *CLOEON DIPTERUM* (L.) LARVAE

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ABSTRACT

In Sweden, larvae of *Cloeon dipterum* L. are very abundant in ponds that are anoxic for three to four months each winter. Adaptation to this winter environment was investigated. Some comparative studies were carried out on larvae from an English non-anoxic winter environment.

Investigations on the anaerobic metabolism of the Swedish larvae indicate that glycogen is stored in the autumn and used during the winter anaerobiosis. The end product of the anaerobic catabolism of glycogen was lactate, but there were also some indications of a higher energy yielding metabolic pathway, including the simultaneous catabolism of carbohydrate and amino acids.

INTRODUCTION

In Sweden the larvae of *Cloeon dipterum* L. live in high numbers in small ponds, especially where there are no fish since they are very susceptible to fish predation. These ponds are characterized by being small, shallow, receiving allochthonous plant material and having little or no flow-through. These ponds are usually anoxic for three to four months each winter. Two Swedish fish species can survive anoxia, the crucian carp (*Carassius carassius* L.) and the carp (*Cyprinus carpio* L.). However, these fish species only occur in ponds of this kind, in central Sweden, where they have been introduced by man. Thus *Cloeon* largely avoids fish predation - although to do so they must withstand adverse winter conditions.

The susceptibility of the larvae to fish predation was clearly demonstrated when their abundance was compared in four very similar and closely situated ponds. In one of the ponds containing a few crucian carp their abundance was only about one twentieth of that of the other three.

WINTER ENVIRONMENT

The winter environment of a typical pond of this type in central Sweden is usually as follows: in November or at the beginning of December the pond freezes over. This produces pronounced temperature stratification with 0°C at the surface and 4°C at the bottom. Within a few days, due to this stratification and to the oxygen consumption of the mud, the bottom water becomes anoxic. Ten to fourteen days after freeze-up the whole water mass is usually anoxic and contains H_2S in low concentrations. The redox potential of the water is negative and decreases towards the bottom. After about six weeks gas-filled plant material floats up beneath the ice, mainly in eutrophic ponds, and increases the H_2S concentrations there. Towards the end of the winter, usually at the end of March, the maximum concentrations of undissociated H_2S are $\simeq$ 0.5-1.0 mg/L During anoxia the water is grayish in colour due to FeS in suspension.

To all appearances the whole water mass is anoxic except during thaw periods when oxygen rich melt water can percolate through the ice. Due to its low density this oxygen rich water remains in a thin, one or two cm thick, layer just beneath the ice. However, shortly after the end of the thaw period anoxic conditions return. Further details of the winter environment are given by Nagell and Brittain (1977).

OVERWINTERING STRATEGY

Chronologically the overwintering strategy is as follows: the first response, indicative of approaching winter, is that the larvae successively, over a 10 to 20 day period, cease feeding. This happens when the temperature of the ambient water is close to or below 4°C. The larvae start feeding again if the temperature rises to 5°C. Experimentally it has been shown that the factor that ultimately controls the cessation in feeding is the low temperature and not, as might be expected, the decreasing photoperiod during the autumn (Nagell in prep.).

The cessation of feeding and the development of resistence to anoxia, measured as survival after a four day exposure to anoxia at 0.1°C, were investigated with respect to the time of acclimation to 0.1°C. The results showed that these two features were closely related. After ten days of acclimation about 90% of the larvae had ceased feeding and 10% survived exposure to anoxia. After 20 days 100% had ceased feeding and about 70% survived anoxia. With a further ten days of acclimation almost 100% survived (Nagell in prep.). Another group of larvae in otherwise identical conditions but acclimated to 10° C for six weeks did not shown any resistance to anoxia (exposed for 40 hours at 0.1° C) (Nagell and Fagerström 1978). Thus the acclimation to low temperature induces resistance to anoxia. Fully developed, resistance corresponds to a LT_{50} value of about 130 days and some larvae survived as long as 155 days (Nagell 1977a).

In oxygenated water the larvae are negatively phototactic but when the water becomes anoxic they become positively phototactic, moving up to the underside of the ice (Nagell 1977b). This location greatly aids their survival, giving the larvae access to oxygen-rich melt water during thaw periods. In a pond it was shown experimentally that this is very important for survival during the winter. Sixty percent of larvae with access to the percolating water survived for 138 days (December to mid-April), compared with 18% of larvae screened off from this water by a plastic film but otherwise given identical conditions (Nagell 1979). Evidently during overwintering the larvae are facultative anaerobes. In anoxia the larvae crawl and swim when disturbed, although considerably more sluggishly than in aerated water.

In order to study adaptation to winter conditions in a region where the period of ice-cover is so short that no anoxia occurs, larvae from the English Lake District were investigated. It was found that these larvae did not cease feeding as quickly as the Swedish larvae. When acclimated to 0.1°C it took 42 days before close to 100% ceased feeding. The English larvae continued to feed unaffectedly at temperatures as low as 2.5 to 1.1°C. It was evident that the English larvae were not adapted to rapidly changing conditions. Continuation of feeding is probably of value for them since risk of anoxia is very low and eating at low temperatures permits the larvae to grow during a greater part of the winter. This probably shortens the development time in the spring, thereby increasing the possibility of two generations per year. This happens only exceptionally in central Sweden, but is not uncommon in Britain (Macan 1979).

In spite of a six week long acclimation to 0.1° C and a decreasing photoperiod the English larvae were not found to be very resistant to anoxia. Of 144 larvae tested at 0.1° C, 134 did not survive the first 24 hours. However, one larva survived three days, three four to five days, two seven to eight days and one as long as 37 days. Thus there are individuals with a certain resistance. This will be important in the case of an unusually cold winter with a period of anoxia. The presence of this character within the population may be taken as an indication that winter anoxia occurs occasionally in the pond.

Recently the species *Cloeon dipterum* L. has been systematically revised (Sowa 1975). According to Dr. Sowa, who kindly examined some experimental larvae and imagines from both localities, the Swedish material belonged to *C. inscriptum* Bengtsson and the English to *C. cognatum* Stephens, two very closely related species. The differences found in the present investigation might be taken as a further reason for their separation.

ANAEROBIC METABOLISM

Investigations are in progress to elucidate the anaerobic pathways in the larvae. It has been found that glycogen is stored during the autumn and used during winter anaerobiosis. From September to December, glycogen increased more than four times reducing slightly below the September value (11.2 mg/g wet wt.) during the overwintering. However under aerobiosis, triglycerides were used and the glycogen reserve was only reduced by 11.5%. This is somewhat surprising as carbohydrates are usually consumed first during starvation (Wigglesworth 1965). However, the larvae are in a diapause and there is probably a mechanism to retard the consumption of glycogen in order to save it for use during anoxia.

After 73 days of anoxia at 0.1°C, lactate concentration in the haemolymph had increased three fold. This indicates that the end product of glycogen degradation is lactate, a metabolic pathway that is not very effective in yielding ATP. Thus many facultatively anaerobic invertebrates probably do not catabolize carbohydrates solely in this simple way. Hochachka *et al.* (1973) have suggested more effective pathways such as simultaneous catabolism of carbohydrate and amino acids, by which the ATP yield can be increased above that of the lactic acid pathway without seriously reducing the cell. In *Cloeon* larvae there are some indications that such a pathway is also present. In the haemolymph of the 73 day anoxia larvae there was an increase in alanin (55%) and a decrease in glutamate (65%) and aspartate (15%) which coincide with some of the suggestions made by Hochachka *et al.* (1973).

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RESUME

Les larves du *Cloeon dipterum* foisonnent dans les mares suédoises qui sont très pauvres en oxygène pendant trois ou quatre mois chaque hiver. Des recherches furent entreprises sur la façon dont l'insecte s'adapte à ce milieu, en prenant comme point de comparaison des études effectuées sur des larves en Angleterre dans un milieu hivernal qui n'est pas privé d'oxygène. Poursuivant l'étude du métabolisme anaérobique des larves suédoises, on constate qu'elles emmagasinent glycogène pendant l'automne et l'utilisent pendant l'anáerobiose d'hiver. Le produit final du catabolisme anaérobique du glycogène est le lactate et l'on a pu constater également à certains indices l'existence d'un métabolisme produisant une plus grande quantité d'énergie, y compris le catabolisme simultané de glucides et d'amino-acides.

ZUSSAMENFASSUNG

In Schweden treten Larven von *Cloeon dipterum* sehr zahlreich in Teichen auf, die im Winter drei bis vier Monate keinen Sauerstoff enthalten. Die Anpassung an diese winterliche Umwelt wurde in der vorliegenden Arbeit untersucht. Auch einige komparative Studien mit englischen Larven aus einer Winterlandschaft, die sauerstoffhaltig ist, wurde vorgenommen. Untersuchungen am anaerobischen Metabolismus der schwedischen Larven weisen nach, daß im Winter Glykogen gespeichert und während der Winteranaerobiose genutzt wird. Als Endprodukt des anaerobischen Katabolismus ergab sich Laktat, jedoch waren auch einige Anzeichen eines stärker energiespendenden Prozesses zu bemerken, der den gleichzeitigen Katabolismus von Kohlehydraten und Aminosäuren umfaßt.

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