

Combined effects of photoperiod and temperature on the diapause of an intertidal chironomid

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Summary. Breeding experiments with a temperate zone population of the marine insect *Clunio marinus* (stock Helgoland–North Sea from 54° N) demonstrated an onset of larval diapause under combined short day and low temperature conditions only (examined LD 8:16 with 7 or 10° C resp.). Long photoperiods (LD 16:8) at 7–10° C or higher temperatures in shorter photoperiods completely prevented any dormancy response. The diapausing larvae were still locomotory active and fed in their benthic environment; their metamorphosis, however, stopped during a specific stage of the early imaginal disc formation in the last instar. In the experiments, the diapause was maintained up to 5.5 months and longer. In nature, the termination may be mainly controlled by temperature rises during low tide and fine weather under long day conditions, at Helgoland generally in late April or early May. The dormancy response can be classified as a larval oligopause (sensu H.J. Müller) of a potentially multivoltine species. It is supposed that this kind of diapause control is more widely distributed in aquatic chironomids.

In northern temperature zones, the seasonality of insect life cycles is mostly controlled by photoperiodic conditions inducing some state of diapause which is a neuro-endocrine controlled arrest of development during winter or even an unfavourable summer (Danilevskii 1965, Beck 1980, Müller 1970, Saunders 1977, Tauber and Tauber 1976). During recent years, it has become increasingly evident that the seasonal temperature conditions influence not only the induction of the diapause but also its maintenance as well as its termination (Tauber and Tauber 1982). The following experiments with the aquatic chironomid *Clunio marinus* Hal. demonstrate the incidence of a larval diapause and its combined control by short photoperiods and low temperatures. This midge inhabits the midlittoral and upper sublittoral of the intertidal zone along rocky European seashores. According to field observations, the life-cycle is potentially multivoltine with one overwintering generation and 1–2 summer generations (Neumann 1976).

Methods

The breeding conditions (sea water with diatoms, blue-green algae and fine nettle powder with some sand and cellulose fibres in 21 basins, all temperature conditions

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$\pm 0.5^\circ\text{C}$) have been described in detail elsewhere (Neumann 1966). The cultures of the larvae were composed of all age groups (4 larval instars) so that emergence and reproduction of the adults, as well as growth of the progeny, occurred continuously over a period of months under standard laboratory conditions (LD 12:12, 18° C). In order to simulate the developmental conditions in nature as far as possible, the cultures were conditioned to lunar-semimonthly (syn. semilunar) rhythms of eclosion by the combination of a 24 h light-dark cycle (LD, 500–1,000 lux from fluorescent light tubes) with an artificial tidal cycle of mechanical water disturbances (12.413 h period with 8 h of bottom vibrations and underwater sound of 50–200 cps – about 20 dB above background noise level). As a result of the different periods, both cycles shifted from day to day to different phase relationships, but each of the resulting phase relationships recurred on every 15th day. It is evident that one or only a few of the 15 phase relationships represent the lunar-semimonthly time cue of the eclosion rhythm (Neumann 1978). Throughout the experiments, the numbers of midges which emerged were counted every day.

The eclosion patterns, as plotted in Fig. 1, additionally reflect the pupations that had occurred in each specimen a few days previously. According to detailed experiments with semilunar-periodically synchronized cultures in the range 8–23° C, the pupations are induced on days 5–8 of the semimonthly zeitgeber cycle only (definition of day 1: onset of the tidal water disturbances at 0.00 and 12.25 h, day 2: about 49 min later at each time-point and so on). With reference to the duration of the pupal development which is temperature-dependent (males: 5 days at 19° C, 11 d or more at 8° C), the peaks of the semilunar eclosion rhythms are delayed from day 11 (median at 19° C, LD 16:18) to day 2 of the following cycle (8° C) (Krüger and Neumann 1983). Correlating to these temperature influences as well as to small additional effects of different photoperiods (Neumann and Heimbach 1979), different phase relationships were apparent between the days of the zeitgeber cycles and the peaks of midges which emerged in the experiments of Fig. 1. However, as far as the incidence of diapause or non-diapause is concerned, there is no need for these different phase relationships to be evaluated in detail.

Results and discussion

Neither short photoperiods at high water temperatures (Fig. 1A) nor low water temperatures with long photoperi-

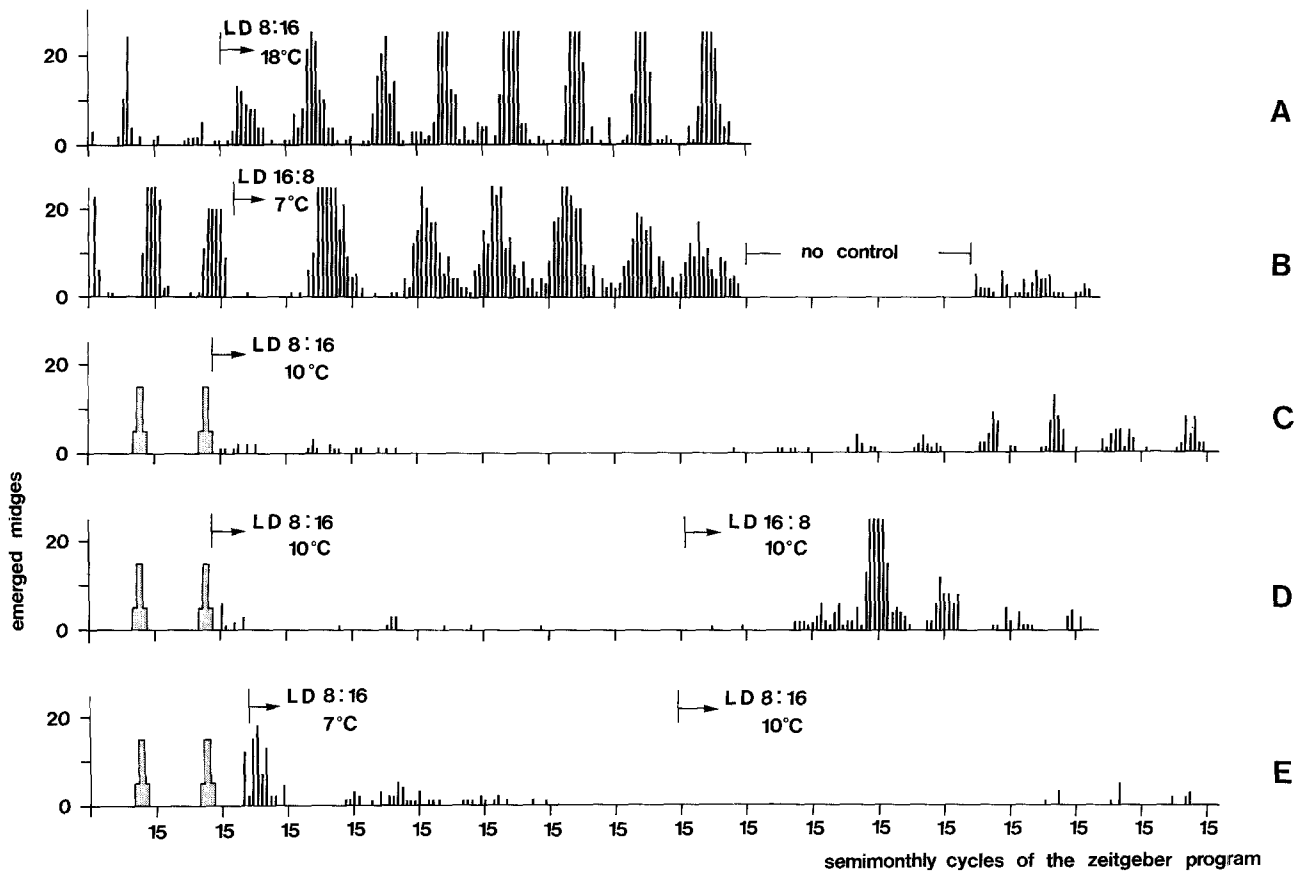


Fig. 1A–E. *Clunio marinus* (Helgoland stock). Five experiments describing the influences of photoperiod and low temperature on the semilunar-periodic emergence pattern of laboratory populations. The periodicity was evoked by semimonthly cycles (15 days each) of a zeitgeber programme (for details, see text). The pretreatment during the first two semimonthly cycles plotted was LD 12:12 and 15°C in A, LD 16:8 and 11°C in B, and in C–E LD 16:8 and 15°C (without controlling during this first month; the polygons represent the approximate days of emergence according to replicate experiments). All the changes in the conditions are marked. The numbers of midges which emerged per day differed between the five experiments. However, the data were plotted on the same scale in order to represent the results up to 25 specimens per day exactly (most of the long columns stay for considerably higher numbers; the maximum was 85 on one day in D)

od (Fig. 1B) can induce any developmental arrest in the larvae. A drop in temperature from 11 to 7°C in experiment B caused only a small retardation of the eclosion days with a short transient gap in the semilunar rhythm. It is the combination of a short day and low temperatures that evokes the larval diapause (Fig. 1C–D). After these changes, only a few specimens were still able to complete their development and to emerge during the following weeks (C, D). A higher number of adults appeared during the first days in the special case of the start of the ‘winter’ programme coinciding with the beginning of an emergence peak and when corresponding numbers of pupae were already existent within the cultures (E). However, the developmental arrest of the bulk of the larval populations lasted for at least about 4 months in C, and up to 5.5 months in E before the emergence rhythm was gradually reinitiated. By a return to long day conditions after 3.5 months, an earlier termination of the diapause was obtained (D). From the pronounced first emergence period in LD 16:8 (the 4 days of the maximum represent 203 specimens) this experiment also demonstrates that high numbers of dormant larvae were really existent in the cultures.

The results clearly demonstrate that the absence of emergence during winter months at the location Helgoland (October to May, Krüger and Neumann 1983) is not only

some state of quiescence or ‘cold torpor’, as primarily supposed by the senior author (1966), but a clear photoperiodic, and therefore hormone-controlled, diapause response. However, under laboratory conditions, the diapausing state of the *Clunio* larvae can be overridden by temperatures above 10°C. Similar results have been established in several long-day species from terrestrial environments (compare Saunders 1977, p 95ff). However, from an ecological point of view it is noteworthy that the temperature threshold for switching from non-diapause to diapause under potentially inducing short photoperiods is adjusted to such a low value in an aquatic insect such as *Clunio*.

As in various other aquatic chironomids, the *Clunio* larvae are still locomotory active in diapause, even at winter temperatures of the sea water (0–8°C). They may even feed and grow very slowly. However, the diapausing larvae do not pass a specific stage of imaginal disc formation during the last and 4th larval instar (LIV) (Krüger and Neumann 1983). The critical stage is about phase 4 of the LIV according to the description of the imaginal disc differentiation by Wülker and Götz (1968).

It can be supposed that the length of the diapause is mainly modified by low temperature conditions (compare Fig. 1C and E). Thus, the maintenance of the arrest in metamorphosis may be even longer at temperatures below

7° C. When the diapause was terminated in nature and growth, as well as imaginal disc formation in the 4th instar, were continued (at Helgoland after a cold winter about the end of April; comp. Fig. 15 in Krüger and Neumann 1983), the day-length had been in the range of 15.3–16.7 h (values of 15th April and 1st May including civil twilight, Beck 1965) and the mean water temperature gradually increased from about 5° C. However, the termination may be favoured in the species by temperature pulses during low water on days of fine weather (e.g., 13–18° C in the exposed habitat at the start of May 1979; comp. Figs. 3 and 4 in Krüger and Neumann 1983). Summarizing, it may be concluded in the case of the temperate zone population of *Clunio*-Helgoland that temperature is the deciding environmental factor firstly for the realization of the photoperiodically controlled diapause induction, and secondly for the diapause termination during long day conditions in late spring. This kind of diapause control renders a reliable, but flexible response in relation to specific temperature conditions of an aquatic environment during the winter half-year.

According to the classifications of Müller (1970), this diapause of *Clunio* may be defined as an oligopause because it is a facultative response which can be reversed by one of its inducing factors, photoperiod or temperature.

In other chironomids of temperate latitudes and aquatic biotops, similar as well as stronger photoperiodic influences have been shown. In *Chironomus nuditarsis* from a deep and, during summer, stratified lake, the diapause was only induced by short days and temperatures of 10° C (Fischer 1974). In a *Clunio* species from a permanently submersed marine habitat, a long-lasting oligopause was evoked in a LD 8:16 at 15° C (Endraß 1976; *Clunio balticus* n.sp. Heimbach 1978). In *Ch. tentans* and in *Ch. plumosus* from shallow as well as deep dam reservoirs, the diapausing short day response was even manifest at the higher temperature of 20° C (Engelmann and Shapirio 1965, Fischer 1974). It is interesting to note that in the fresh-water chironomids the diapause blocked the metamorphosis at the imaginal disc stages in the same range as in *Clunio* (here stage 4–6, Ineichen et al. 1979). Temperature effects have also been discovered in a pitcher-plant mosquito (Culicidae) in which thermophases of some hours can avert the larval diapause in short day conditions when the higher temperature coincides with the dark period (Bradshaw 1980). Hence, one may suppose that temperature effects on the onset or termination of larval diapause are more widely distributed in aquatic insects.

An open question remains as to whether the diapause response of *Clunio* and other chironomids are restricted to the IVth larval instar and its physiological switching point in the metamorphosis of imaginal discs only. If this is true, the retardation of growth and larval ecdysis at earlier larval stages might be classified as some mode of temperature-dependent quiescence. In agreement with this interpretation, a continuous shifting of the age structure of short-day larval populations to the 4th instar has been observed in *Ch. plumosus* (Fischer 1974) and *C. balticus* (Endraß 1976). At least in these cases, both the reactions of a slowing down of growth on larval instars I–III (but gradual development until LIV) and of diapause induction at the IVth instar, will unify the age group structure of field

populations and result in a well synchronized reproduction period of a local population during the following spring.

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