

## 5. DIAPAUSE IN AQUATIC INSECTS, WITH EMPHASIS ON MOSQUITOES

### 5.1 INTRODUCTION

Diapause is the primary factor synchronizing insect life cycles with seasonal changes in the environment. Diapause is the major factor regulating the timing of growth, development, and reproduction, both before and after the period of dormancy.

Many definitions of diapause may be found in the literature. Tauber et al. (1986) define diapause as “a neurohormonally mediated, dynamic state of low metabolic activity. Associated with this is a reduced morphogenesis, increased resistance to environmental extremes, and altered or reduced behavior activity. Diapause occurs during genetically determined stage(s) of metamorphosis, and its full expression develops in a species-specific manner, usually in response to a number of environmental stimuli that precede unfavourable conditions. Once diapause has begun, metabolic activity is suppressed even if conditions favorable for development prevail.”

Aquatic insects are represented in many orders including the Diptera (Culicidae, Chironomidae, Simuliidae, Ceratopogonidae, Chaoboridae, Dixinae), Coleoptera, (Dytiscidae, Hydrophylidae), and Heteroptera (about 20 families); some orders such as Odonata, Ephemeroptera, and Trichoptera are exclusively aquatic. In most cases only immature stages of insects are true water inhabitants whereas the adults are terrestrial. Hibernation, diapausing stages, and induction and termination of diapause are studied in the mentioned insect groups to varying degrees. Among the aquatic insects, the most voluminous literature is devoted to the mosquitoes because of their medical and veterinary importance, thus the mosquitoes were selected as a model group for this discussion of diapause and controlling environmental factors. Diapause in other aquatic insects will be reviewed briefly at the end of this chapter. Though aquatic insects occur in specific habitats that cool more slowly in autumn, metamorphosis appears to be controlled by cues similar to those used by terrestrial insects. The photoperiod and temperature responses of aquatic insects are as broad as the range found in terrestrial species, though there may be greater flexibility in overwintering instars (Danks 1978).

In mosquitoes the environmental control of diapause has been investigated from the early 1960s coinciding with the beginning of intensive studies in the field of seasonality, diapause, and photoperiodism in insects. The main ideas on this problem were reviewed by Lees (1955), Danilevsky (1961), Tauber et al. (1986), and Danks (1987). Mosquito diapause and its environmental control has been reviewed in monographs by Vinogradova (1969, 2000) and Mitchell (1988).

The study of this topic in mosquitoes was additionally stimulated by the great applied importance of this taxa as active bloodsuckers and vectors of many agents

of medical and veterinary significance such as malaria, filariasis, and many arbovirus infections (yellow fever, Western equine, St. Louis, Japanese, and West Nile encephalitis).

Phenological studies, which ascertain the dates of onset and termination of diapause, the period of reproductive activity, and number of generations provide predictive capabilities that are important for developing control tactics for mosquitoes. Data on the winter survival of mosquito females provide insights on the mechanism of arbovirus circulation and maintenance in nature. The study of diapause and its environmental control in mosquitoes, like other insects, is usually based both on the field observations and laboratory experiments, which require the use of laboratory colonies. It is important to note that only a few mosquito species, not more than 20 stenogamic species, are willing to mate in cages.

The role of data concerning diapause and its regulation may be illustrated by the next examples. Progress with the elimination of malaria in the former USSR in the 1960s was possible only because many years (15–30 years) of observations had been devoted to the patterns of seasonal development of the *Anopheles maculipennis* complex in different climatic zones during autumn (Shipitsina 1957a, b). *Aedes albopictus* is another example. This species is a daytime biting mosquito known as a vector of dengue and haemorrhoidalis fever, and also a potential vector of a number of arboviruses, which it can transmit vertically or transversely, thereby providing a means for arbovirus maintenance and transmission. This native Asian mosquito occurs in China, the Pacific, and on islands of the Indian Ocean. *A. albopictus* has been rapidly spreading across all continents for the last decade: it is now established in North and South Americas, Africa, Oceania, and Europe (Knudsen 1995; Moore 1999). The international shipment of used tire provides *A. albopictus* with an ideal mechanism of dispersal, and tire stocks throughout much of the world constitute an extremely productive ecological niche for this mosquito to inhabit. It can breed in many different habitats within urban and suburban environments, as well as in perirural areas. The great applied importance of this species increases the interests of its biology and especially on the seasonal adaptations, which are responsible for its successful invasion into new regions. Temperate populations of *A. albopictus* overwinter as diapausing eggs, but tropical populations lack the ability to diapause, therefore they will not succeed when introduced into temperate zone.

Currently about 2,500 mosquito species are known worldwide, and most inhabit tropical and subtropical regions, with the rest occurring in the temperate zones. Clearly diapause as an adaptation for hibernation is common in mosquitoes from northern and temperate latitudes. In mosquitoes three of the four known types of diapauses occur: diapause during the egg, larval, and adult stages.

In northern regions including the USA, Canada, Europe, nontropical Asia including Japan, North Africa, and the Middle East, fewer than 300 species have been recorded, but hibernating stages are known only for a small number of these species. For instance, among mosquitoes distributed in North America and the former USSR with neighboring countries (Matheson 1944; Gutsevich et al. 1970), the majority of mosquito species enter an overwintering diapause as eggs (55 species), as adults

(about 30 species), and as larvae (16 species). The seasonality of the rest (about 80 species) has not been studied.

Some trends in the relationship between the diapausing stage and systematic position of mosquitoes are evident. Egg stage diapause is typical for *Ochlerotatus*, *Aedes*, and *Psorophora*; adult diapause occurs mainly in *Anopheles*, *Culex*, and *Culiseta*; larval diapause is encountered in distinct representatives of many genera such as *Anopheles*, *Ochlerotatus*, *Culiseta*, *Mansonia*, *Orthopodomyia*, *Topomyia*, *Tripterooides*, *Armigeres*, *Wyeomyia*, and *Toxorhynchites*.

Only one diapausing stage is typical of most mosquito species, but a few may overwinter in two different stages in different parts of their distribution range. Egg and larval diapauses are encountered in *O. togoi* (Galka & Brust 1987a, b), *O. triseriatus* (Vinogradova 1967), *O. caspius* (Vinogradova 1975; Abdel-Rahman et al. 1985), and *O. geniculatus* (Sims & Munstermann 1983). An interesting case is *O. sierrensis*: in Oregon, USA, a single individual undergoes egg diapause in summer and larval diapause later, in winter (Jordan 1980a, b).

In this chapter the terms “facultative” and “obligate” diapauses are used despite their debatable character and difficulties in knowing for certain which type of diapause is occurring (Tauber et al. 1986; Danks 1987). These terms reflect variations in seasonal cycles of insects ranging from almost complete reliance upon environmental factors (monovoltinism or multivoltinism with environmental determinate facultative diapause) to genetic predominance (multivoltinism without any diapause) at one extreme, and monovoltinism with obligate diapause in each generation at the other extreme.

In this chapter a new classification will be employed for the composite genus *Aedes* with elevation of the subgenus *Ochlerotatus* to generic rank (Reinert 2000; Snow & Ramsdale 2003; Darsie & Ward 2005).

## 5.2 MOSQUITOES (CULICIDAE)

### 5.2.1 Egg Diapause

**5.2.1.1 Diapause and quiescence.** Egg or embryonic diapause occurs in mosquitoes of the genera *Ochlerotatus*, *Aedes*, and *Psorophora*. Neither term is quite correct because diapause in all of these cases occurs in the stage of the pharate first instar larva (embryonic development has been completed but the fully formed first instar larva remains within the chorion of the egg). This is the stage of diapause in *O. dorsalis*, *O. nigromaculis*, *O. squamiger* (Telford 1958), *O. hexodontus* (Beckel 1958), *A. cinereus*, *O. flavescens* (Khelevin 1958b, 1959), *O. triseriatus*, and *O. togoi* (Vinogradova 1969), and is most likely true for other species as well.

Egg diapause is manifested as a long stable arrest of hatching even when environmental conditions are favorable for this process. Diapause is terminated as a result of the reactivation of development. Egg diapause is an adaptation to the seasonality of climatic conditions, an adaptation that promotes successful survival of winter (winter diapause) or summer (aestivation). The winter egg diapause is typical for mosquito species occurring in the temperate zone. In addition to egg diapause in

mosquitoes there is aseasonal quiescence, a state of inactivity induced by unfavorable environmental conditions and which ceases shortly after exposure to adequate hatching stimuli. Aseasonal quiescence is an adaptation to the peculiar conditions of special larval habitats, such as tree and rock holes, or transient ground pools, where water level may be subjected to large and abrupt fluctuation. Such aseasonal quiescence results in an asynchronous hatching of eggs. Usually the first flooding induces hatching of the majority of the eggs, whereas the remainder of the eggs may hatch much later after a subsequent flooding episode. Asynchronous hatching is based on intrapopulation variation in sensitivity of individuals to environmental cues. Such arrest of hatching is a very useful adaptation favoring conservation of populations after the pernicious early-spring freezing or after quick drying of water bodies. Though egg diapause is quite distinct from aseasonal quiescence, in some cases it is very difficult to distinguish these phenomena, and it takes special experiments to do this.

Egg diapause may be obligate or facultative. Obligate diapause is common in monovoltine mosquito species such as *O. canadensis*, *O. hexodontus*, *O. squamiger*, *O. excrucians*, and *O. communis*. It occurs spontaneously in each generation irrespective of environmental conditions and lasts usually for a long period, up to 1 year, from the end of spring – beginning of summer to the spring of the next year. On the contrary, facultative diapause is recorded in the multivoltine species (*A. vexans*, *O. caspius*, *A. cinereus*, etc.). This diapause is controlled by environmental conditions, mainly photoperiod and temperature. Low temperatures are usually responsible for diapause termination in both monovoltine and multivoltine species.

**5.2.1.2 Hatching stimuli.** Practically all eggs of *Ochlerotatus*, *Aedes*, and other genera require a hatching stimulus in addition to submergence in water. Temperature, relative humidity, repeated flooding and drying, mechanic stimuli, especially the concentration of oxygen dissolved in water, and other stimuli may influence hatching (Clements 1963, 1992). The efficiency of these various stimuli depends on the physiological state of the diapausing eggs. Some peculiarities concerning the hatching stimuli for the quiescent and reactivated eggs are briefly considered below.

Temperature influences diapause in a complicated manner. According to field observations in Moscow (Shlenova 1950), in spring the minimum temperature of water, which allows diapausing eggs to hatch varies from 4–6°C (*O. communis*, *O. cataphylla*, *O. intrudens*) to 13–14°C (*O. cyprius*) and to 14–18°C (*O. caspius*, *A. vexans*). Some experimental data show that not only the temperature of water but the temperature conditions before the flooding are very important (Moore & Bickley 1966; Horsfall & Trpis 1967). It has frequently been found that eggs respond more readily to a hatching stimulus if they have previously been held at a high humidity. For instance, this was ascertained in *A. aegypti* (Fig. 5.1) and *O. triseriatus* (Hayes & Morlan 1957).

Mechanical influences such as mechanic agitation of eggs (*A. aegypti*) or stroking of eggs with a brush (*O. triseriatus*) may also stimulate hatching (Vinogradova 1969).

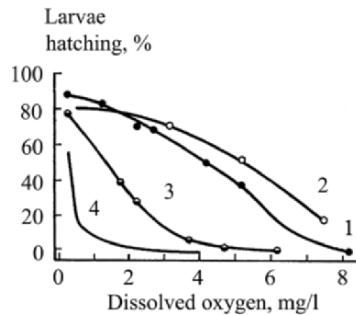


Figure 5.1. Effectiveness of various concentrations of dissolved oxygen on the hatching of larvae of *Aedes aegypti*, *Ochlerotatus nigromaculis*, and *O. sierrensis*. (Modified from Judson 1960 and Judson et al. 1966.) 1 and 3, *A. aegypti*; 2, *O. nigromaculis*; 4, *O. sierrensis*. *A. aegypti* eggs previously held at 90–100% RH (1) or 50% RH (3) and *O. nigromaculis* at 50% RH.

The stimulating effect of water with low dissolved oxygen concentration was established experimentally in many multivoltine species *A. vexans*, *A. aegypti*, *O. triseriatus*, and *Psorophora ferox* (Clements 1963; Vinogradova 1969). As Fig. 5.1 shows, in *A. aegypti* and *O. sierrensis* 0–20% and 80–90% of eggs hatched at 6–8 ppm and 0 ppm, respectively. A more powerful effect is observed if the oxygen tension of water declines gradually. The decrease in dissolved oxygen concentration may be caused by physical (boiling, shaking), chemical, or biological means. The disappearance of oxygen has been demonstrated in ascorbic acid solution, enzyme preparations, and bacterial infusion (Clements 1963). In particular, the ascorbic acid treatment has been successfully used in experimental conditions for the differentiation between quiescent and diapausing eggs of mosquitoes: the diapausing eggs did not hatch in response to this stimulus. Though a reduction in dissolved oxygen is a powerful hatching stimulus in the laboratory, the effect may be modified by other environmental cues in the field.

**5.2.1.3 Viability, drought, and cold hardiness.** The capacity of eggs to survive after long periods of drying is frequently associated with mosquitoes inhabiting temporary water bodies. Cold hardiness favors the occurrence of such species in the North and temperate latitudes that experience low winter temperatures. Both cold hardiness and drought resistance influence the viability and life duration of the eggs. The duration of egg life in species distributed in the temperate zone is longer than in species from more southern regions. For example, in experiments at 0–10°C, some eggs of *O. campestris* and *O. dorsalis* hatched after 2 years; the eggs of *O. squamiger* found in soil remained viable for at least 2 years (Telford 1958). The eggs of *O. dorsalis* survived in a basement for nearly 3 years (Khelevin 1958a). The eggs of *A. vexans* and *O. sticticus*, which were in soil in the field hatched 5 and 7 years later, respectively (Kliwer 1961). In contrast, *A. aegypti* eggs may survive only for 9–15 months (Christophers 1960). In general, egg viability varies considerably depending on their

physiological state (diapause, postdiapause, quiescence), the relative humidity, temperature, water regime, and many other factors.

Special studies on cold resistance in mosquito eggs are not numerous. Such data on this phenomenon may be exemplified by work in *A. albopictus*. Field experiments with the eggs, which overwintered above and below water in outdoor locations, were carried out in Indiana, USA, in 1986–1987 and 1987–1988, when the mean daily temperatures decreased to  $-10^{\circ}\text{C}$  (Hawley et al. 1989). Geographic variation of the ability to overwinter was found: the strains from northern Asia and North America showed higher overwintering survival rates than the strains from tropical Asia, Hawaii, and Brazil. A strain from Indiana survived better than those from Texas, Louisiana, and Florida. Laboratory experiments with eggs of five temperate and tropical strains of *A. albopictus* were also conducted (Hanson & Craig 1994, 1995). They were maintained at both diapause and nondiapause conditions, and exposed to various acclimation regimes ( $+10^{\circ}\text{C}$  and later  $0^{\circ}\text{C}$ ), after they were subjected to various chilling treatment ( $-12^{\circ}\text{C}$ ). Cold acclimation and diapause enhanced cold hardiness of only the temperate strains. Both the temperature and duration of cold acclimation modulated cold hardiness. However, diapause, cold acclimation, and geographic origin did not affect *A. albopictus* egg's supercooling points. All lower lethal temperatures were above  $-13^{\circ}\text{C}$  and all supercooling points were below  $-26^{\circ}\text{C}$ , indicating prefreeze mortality. Eggs of *A. aegypti* and *O. triseriatus* also die at temperatures above their supercooling points. But field observations show that eggs of *Ochlerotatus* and *Aedes* are capable of surviving at temperatures below the freezing point of water, as recorded for eggs of *O. togoi* in southern Primorsky krai, Russia (Shestakov 1961).

*5.2.1.4 Photoperiodic and temperature induction of egg diapause.* The role of photoperiod and temperature in the induction of egg diapause has been studied in more than 10 mosquito species (Table 5.1). As examples, three model species, *O. togoi*, *O. triseriatus*, and *A. albopictus* may be considered. The first of such studies was fulfilled in *A. togoi* (Vinogradova 1965). This rock pool mosquito occurs along the Pacific coast of Asia, Canada, and the USA. *O. togoi* may overwinter in both the egg and the 4th-instar larva (Galka & Brust 1987a, b). In experiments with the strain from Zarubino, Russia ( $43^{\circ}\text{N}$ ,  $132^{\circ}\text{E}$ ) the immature stages, adults, and laid eggs were subjected to long or short photoperiods at different temperatures (Table 5.2). Under long-day conditions 95–100% of eggs hatched during the first 10 days following submersion. By contrast, under short-day conditions the majority of eggs entered diapause, and hatching occurred 70–120 days later. (These diapausing eggs were insensitive to a strong hatching stimulus such as ascorbic acid solution). The variation in response of the progenies of various females to the same environmental stimuli was observed: the hatching response of the various egg rafts changed from 0% to 100% (Table 5.2). This feature has a heritable basis that was experimentally confirmed in *A. aegypti* (Gillett 1955). Special experiments with the inversion of long- and short-day regimes during the life history of the parental generation and of eggs revealed the photoperiodic sensitivity in all these stages of *A. togoi*. Maternal

TABLE 5.1. Effect of photoperiod and temperature on the induction and termination of the egg diapause in mosquitoes

Species	Population	Diapause induction	Diapause termination	References
<i>Aedes albopictus</i>	Shanghai, Yixing Country, China	SD 25°C (eggs) SD 9–15 h; LT; ME		Wang 1966; Yang 1988
<i>Ochlerotatus campestris</i>	Manitoba, Saskatchewan, USA	SD (<14 h), 23°C (eggs)	LD 16 h; LD and SD 30°C	Tauthong and Brust 1977
<i>O. canadensis</i>		SD 9 h, 25°C; CP (between 13 and 14 h), eggs; ME		Pinger and Eldridge 1977
<i>O. sollicitans</i>	North Carolina, USA	SD (6, 10 h), 15°C		Parker 1988
<i>O. togoi</i>	Far East, Russia, Vancouver, Canada	SD 12 h, 15–20° (eggs); 12 h, 20°C (parents); ME	+5°, 0–3°C, 0 h	Vinogradova 1965; Galka and Brust 1987a
<i>O. triseriatus</i>	Ohio, Alabama, USA	SD 15 h, 22–28°C SD 10 h, 15–25° (eggs) SD 18–27°C; CP: between 13–14 h (Ohio), 12–13 h (Alabama)	0 h, LT (3–4°C)	Kappus and Venard 1967; Vinogradova 1969
<i>O. caspius</i>	Bairam-Ali, Turkmenia, South France	SD 11 h, 12°C (eggs); 12 h, 25°C (parents); ME SD 8–10 h, 16–20°C; 12°C (eggs); ME		Vinogradova 1975; Sinegre 1983
<i>O. dorsalis</i>	USA	SD 10 h, 5–20° (eggs); CP: 12.2 h; ME	LD 13, 14 h, 27°C	Mulligan 1980
<i>O. atropalpus</i>	Canada (45°N) Georgia, USA (34°N) El Salvador (14°N)	SD 8 h, 23°C (eggs) SD 10–14 h, 23°C; CP: 13–14 h SD 14.5 h, 22–23°C SD 12 h, 22°C SD 11.5 h, 20–22°C (parents + eggs)	LD, SD, 30°C	Anderson, 1968; Kalpage and Brust 1974; Beach 1978
<i>O. geniculatus</i>	Sussex, England, Sardinia, Italy	SD 10, 14 h, 21°C (eggs)	18 h, 21°C	Sims and Munster-mann 1983
<i>O. hexodontus</i>	Canada	Obligatory diapause	>140 days at –3°C	Beckel 1958
<i>O. mariae</i>	Central Italy	SD 9 h, 12 h (parents); SD <16°C (eggs)		Coluzzi et al. 1975

(Continued)

TABLE 5.1. Effect of photoperiod and temperature on the induction and termination of the egg diapause in mosquitoes—Cont'd

Species	Population	Diapause induction	Diapause termination	References
<i>Aedes vexans</i>	Washington, DC, USA	LD 16 h (parents), 11 h, 16 h, 10°C (eggs); ME SD 12 h (parents); 3 weeks at 18°C		Wilson and Horsfall 1970; McHaffey, 1972
<i>O. taeniorhynchus</i>	North Carolina, USA	SD 6 h, 15°C (42 days) following 14 h, 27°C (5 days)	14 h, 27°C	Parker 1985

SD and LD: short and long day length; resp: the length of photophase, h; LT: low temperature; ME: the maternal effect, the treated stage in brackets; CP: critical day length.

TABLE 5.2. Effect of photoperiod treatment of parents and eggs on the induction of egg diapause in *Ochlerotatus togoi* (Vinogradova 1965)

Photophase, h	Temperature, °C	Number of egg batch	Batches distribution on hatching (%)				Total number of eggs	Diapausing eggs, %
			0	1–10	20–80	100		
18	18	13	0	0	0	13	1583	0
22	15	20	0	0	3	17	2287	4.6
12	20	38	30	3	5	0	1280	96.4
12	18	42	21	11	8	2	2658	86.6
12	15	48	29	11	8	0	2097	89.5

effects modify the responses of eggs themselves, which are sensitive at both early and late stages of embryogenesis (Vinogradova 1965).

In a detailed study of *O. togoi* from Vancouver, Canada, the complicated manner of interaction between different photoperiods (from LD 10:14 to 18:6) and temperatures ranging from 15°C to 30°C was analyzed (Galka & Brust 1987a). It was shown that the eggs were induced to enter diapause as a result of certain photoperiodic and temperature cues that were perceived by stages preceding and including the egg stage.

The photoperiodic induction of diapause was also investigated in *O. triseriatus*, a multivoltine species distributed throughout Central and eastern North America. This species may overwinter either in the egg or larva stage. Egg diapause may be induced in populations from any latitude, but larval diapause occurs mainly among southern populations (Love & Whelchel 1955; Kappus & Venard 1967). When pupae, adults, and laid eggs of the strain from Savannah (Georgia, USA) were kept under LD 12:12 at 15°C, all eggs entered diapause, which persisted for at least



8 months (Vinogradova 1967). Under long-day conditions, an average of 65% of the eggs hatched during the first 30 days. As day length decreased, the percentage of diapause increased at all studied temperatures, from 23–25°C to 14°C, and the critical photoperiod was found to be between LD 12:12 and 13:11. Contrary to *O. togoi*, in *O. triseriatus* no maternal influence on the physiological state of the eggs was recorded.

Egg diapause in two strains from Alabama and Ohio (USA) was also induced by short-day treatment of the egg itself, and the photoperiodic treatment of the parent had no effect on diapause duration (Clay & Venard 1972). Later it was shown experimentally in another strain from the USA (42°N) that only 3–6 short-day cycles (LD 10:14) were required to block the hatching reflex (Shroyer & Craig 1980). In *O. triseriatus* the threshold of light intensity promoting the photoperiodic response was measured and was found to be from 0.0005 to 0.015 footcandles (Danks 1987).

Geographical variation in the photoperiodic response curves was established firstly in two strains (Kappus & Venard 1967) and later in eight strains of *O. triseriatus* from the USA (Fig. 5.2) (Shroyer & Craig, 1983). The critical photoperiod for induction of egg diapause increased 1 h for each increase in latitude of 4.2 over a range of 30–46°N of latitude.

The third example is *A. albopictus*. First the photoperiodic induction of egg diapause was ascertained in an *A. albopictus* strain from Shanghai (31°N) (Wang 1966). The frequency of diapause increased with a decrease of photoperiod. Short-day (LD 8:16) exposure not only induced diapause in 88% of the eggs, but supported this diapause state: during 3rd and 4th months of diapause the hatching of 10% and 15% of eggs, respectively, were observed; the total number of hatching eggs reached 54% only after the 8th month. The study of another northern strain of *A. albopictus* (Yixing County, 31°N) demonstrated that egg diapause could be indirectly induced by a short photoperiod experienced in the parental stage, but only in the range of photoperiods from LD 9:15 to 15:9; furthermore, the eggs with fully formed embryos were also

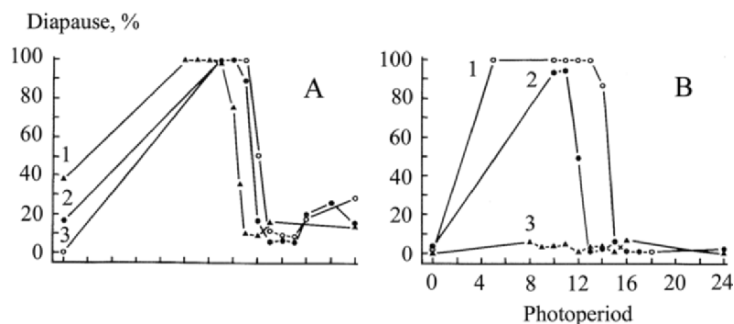


Figure 5.2. Geographical variation in the photoperiodic response curve for induction of egg diapause in the tree-hole mosquito, *Ochlerotatus triseriatus*. (Modified from Shroyer & Craig 1983.) A: 1, Underc-4, Michigan, 46°N; 2, Orono-3, Maine, 45°N; 30°N; 3, Kramer-1, Indiana, 42°N; B: 1, Burdette, Michigan, 42°N; 2, Topsy, Louisiana, 30°N; 3, Vero-4, Florida, 28°N. Eggs were allowed to develop 14 days at LD 16:8 before transfer to experimental photoperiods for an additional 14 days. (Hatching was stimulated by Bacto nutrient broth).

photosensitive (Yang 1988). A maternal effect was also shown in *A. albopictus* from Japan (Mori et al. 1981). Field observations in China (Guandong Province) and Japan (Okinawa Island) demonstrated that adults started to produce diapausing eggs from mid-October to December, when the photoperiod was short (Liu-FuSheng et al. 1990; Toma & Miyagy 1990). In this regard, it is interesting to note the experiments with *A. albopictus*, which directly demonstrated the role of maternally operating photoperiod for induction of egg diapause (Anderson 1968). It is evident (Table 5.3) that induction of diapause is strictly maternal in nature. Males reared under a long photoperiod and mated with females reared under a short photoperiod resulted in diapausing eggs. The sensitive period for light reception was the 4th-instar larva and pupa of the maternal generation.

Numerous experiments and field observations have shown that photoperiod and temperature are the main environmental cues responsible for the induction of egg diapause in mosquitoes, and photoperiod is the major diapause-inducing stimulus. All aedine mosquitoes that have been studied are “long day” insects that enter winter diapause in response to short photoperiod and develop without interruption under long photoperiod. Only one exception has been reported thus far: *O. sierrensis* from Oregon, USA, may undergo an egg diapause induced by long days in summer (Jordan 1980a, b). Temperature–photoperiod interactions in the induction of diapause are species-specific, but in many mosquitoes a definite tendency is observed: when photoperiod length and temperature decrease, the percentage of diapause increases.

Photoperiodic response curves for diapause induction differ not only between various species, but also between geographical strains. The critical photoperiod inducing a 50% response is an important characteristic of photoperiodic curves. For instance, in *A. albopictus* the critical photoperiod for induction varies clinally with latitude, and may be influenced by temperature and larval rearing condition (i.e. diet) (Pumpuni et al. 1992). For this species a statistical model was developed to quantify

TABLE 5.3. Comparative role of photoperiod treatment of females and males in the induction of egg diapause in *Ochlerotatus atropalpus* (Modified from Anderson 1968)

Photoperiod, LD				
Larval, pupal, and pre-mating period				
Male	Female	Mating and post-mating period	Total number of viable eggs	Mean % embryos hatch among replicates
16:8	12:12	12:12	1,463	0.67 a
12:12	16:8	16:8	4,905	97.33 b
16:8	12:12	16:8	1,320	0.50 a
12:12	16:8	12:12	2,222	98.9 b

the role and interaction of photoperiod, temperature, latitude, country of origin (29 strains from Japan and the USA), and elevation of the site of origin, with critical photoperiod (Focks et al. 1994). This model corroborates the idea that North American strains have a temperate origin from Asia and indicates that rapid spread of this mosquito within the USA resulted in founder populations that were only partially adapted in their diapause response to local conditions.

Another pattern of intraspecific variation in diapause response is known for geographically separated populations. This is the occurrence of the number of diel cycles during the photosensitive period. In a southern strain of *O. atropalpus* (14°N) diapause was triggered when nine or more short-day photoperiod cycles occurred during the photosensitive 4th instar and pupa (Beach 1978). Temperatures of 22°C and higher caused part of this population to complete the photosensitive period in less than 9 days, thereby avoiding diapause. In contrast, only four short-day cycles were sufficient to trigger diapause in a strain from 45°N. This population experienced this number of required days at temperatures as high as 28°C. The strain from an intermediate location (34°N) must experience 7 short days, and this requirement was met at temperatures of 24°C or less.

Photoperiodically sensitive stages and the maternal effect on the progeny diapause are closely related phenomena. Usually identification of the sensitive stages requires a suitable bioassay for measuring sensitivity to diapause-inducing factors. Percentage of diapause and the depth of diapause have been useful measures.

Only evaluating the effects of different combinations of photoperiod and temperature during the egg stage and preceding generation may reveal maternal effects on the diapause in progeny. Maternal effects are known now to occur in many insects (Vinogradova 1973; Tauber et al. 1986; Danks 1987). In mosquitoes this phenomenon was shown not only in the above-mentioned *O. togoi* and *A. albopictus*, but also in other species (Table 5.3). In *A. vexans* adults exposed to short-day conditions laid diapausing eggs, only 2–5% of which hatched (Wilson & Horsfall 1970). When all stages of the parental generation of *P. ferox* were subjected to a short photoperiod, there was also marked reduction in the hatch rate of eggs of the subsequent generation (Pinger & Eldridge 1977). Maternal effects were also discovered in two strains of *O. caspius* from Bairam-ali, Turkmenia (Vinogradova 1975) and southern France (Sinégre 1983). In the first strain 56% or 66% of eggs laid by females exposed to LD 12:12 at 25°C entered diapause as compared with 2% or 6% of those laid by females exposed to LD 20:4 (in both experiments eggs themselves were kept for 14 or 93 days at 12°C, LD 12:12). Thus, the maternally operated short photoperiod increased the frequency of egg diapause in progeny. It is notable that an intraspecific variation in the maternal effect was recorded in *A. albopictus*: the northern strain manifested the maternal effect, whereas in the southern strain it was absent (Yang 1988). In the above mentioned cases photoperiods experienced by the maternal organism modified the sensitivity of eggs themselves determining the adequate physiological state of the eggs. However in some mosquitoes developing or fully formed embryos themselves respond to inductive cues. Such species include *O. triseriatus* (Kappus & Venard 1967; Vinogradova 1967;

Shroyer & Craig 1980), *O. campestris* (Tauthong and Brust 1977), *O. canadensis* (Pinger & Eldridge 1977), *O. sollicitans* (Parker 1988), *O. sierrensis* (Jordan 1980a, b), and *O. mariae* (Coluzzi et al. 1975).

In connection with the photoperiodic induction of egg diapause a remarkable change in oviposition behavior has been observed in *O. mariae* (Coluzzi et al. 1975). Experiments showed that females reared under long-day conditions had a strong preference for outside free water for oviposition, whereas those reared under short-day conditions preferred moist surfaces in enclosed positions. In nature overwintering eggs are found in holes and crevices of rock pools. Such photoperiodically induced change of oviposition behavior may have adaptive significance in providing a more constant microclimate for diapausing eggs.

*5.2.1.5 Diapause termination.* Ideas on the environmental cues responsible for the reactivation of diapausing eggs are based on both field and laboratory experiments. A study of diapause development in *Ochlerotatus* mosquitoes was carried out by Khelevin (1958a, b, 1959) in Ivanovo, Russia. The diapausing eggs were placed in the field in November and returned to the laboratory for hatching 1, 2, 3, 4, and 5 months later. As the period of natural chilling increased from 2 to 5 months, the fraction of eggs, which hatched during 20 days, increased gradually in multivoltine *O. caspius* from 9% to 82%, in monovoltine *O. excrucians* from 0% to 74%, in *O. maculatus* from 0% to 63%, and in *O. cyprius* from 0% to 69%. A similar tendency was recorded for eggs of *O. nigromaculis* from California, USA. Among eggs collected in October, 12% hatched after the first flooding; for those collected in November–January, the fraction was 0%; and for those collected in April–May, 52% hatched (Miura & Takahashi 1973). All these data demonstrate the gradual reactivation of the diapausing eggs during hibernation at low winter temperatures in the field.

The role of high temperatures in the termination of egg diapause was shown for two mosquito species from Canada. When the diapausing eggs of a monovoltine strain of *O. campestris* were placed at 30°C for 10 days, 59% of the eggs hatched (Tauthong & Brust 1977). In *O. atropalpus* a high percentage of diapausing eggs hatched after only 5 days at 30°C, provided they were 60–90 days old (Kalpage & Brust 1974). High temperature acted independently of photoperiod and eggs hatched in short as well as long photoperiods. In contrast, the winter eggs of *O. caspius* could not be induced to hatch at 25°C (Sinegre 1983).

The rate of reactivation at temperatures from 18°C to 25°C differed in various species. Of seven species studied (*A. cinereus*, *O. flavescens*, *A. vexans*, *O. hexodontus*, *O. triseriatus*, *O. togoi*, and *O. dorsalis*) only the latter two responded to 1 month exposure of temperatures from 15°C to 20°C, showing a 42% and 5% incidence of hatching, respectively (Khelevin 1958a, b; Vinogradova 1967). Only single larvae of *O. communis*, *O. cataphylla*, and *O. cantans* from Moscow, Russia, hatched at 24°C (Yakubovich 1975).

Low temperatures appear to be most effective for the termination of egg diapause. For instance, the chilling of diapausing eggs of *O. togoi* at 5°C for 1, 2, and

3 months induced hatching in 26%, 65%, and 99% of eggs, respectively during 30 days, whereas chilling at 0°C to -3°C induced hatching in only 20–26% of the eggs (Vinogradova 1969). A similar correlation between the chilling duration at -3°C and percentage of reactivated eggs was also recorded in the monovoltine species *O. squamiger* (Beckel 1958). Long-term cooling was effective for *O. triseriatus* from Indiana, USA: nearly all eggs had broken diapause after 180 days at +4°C, LD 10:14 (Shroyer & Craig 1983). In *O. communis*, *O. cataphylla*, and *O. cantans* 88%, 72%, and 54% of eggs, respectively, hatched after 1 year exposure to +3–5°C (Yakubovich 1975).

Baker (1935) was the first to demonstrate the photoperiodic reactivation of egg diapause in mosquitoes by comparing the hatching in *O. triseriatus* under LD 16:8 and 10:24. Later, this phenomenon in this species was studied in detail (Vinogradova 1967; Shroyer & Craig 1983). It was shown that diapausing eggs, which were kept under short day for 2 or 5 months, responded to long-day treatment in different manners. In the first group 56% of the eggs hatched within 1 month, but in the second group no larvae appeared in this period. Probably, this is connected to the increase in diapause intensity with increase of egg age. The termination of diapause increased when a long-day treatment was preceded by exposure to a low temperature of +4°C.

It is interesting that eggs of *O. triseriatus*, which terminated diapause as a result of long chilling (40–90 days), continued to preserve their sensitivity to photoperiod: the short-day treatment inhibited hatching (not more than 13% of eggs hatched) and the long-day treatment had a stimulating effect (58% of eggs hatched) (Vinogradova 1969). Probably this phenomenon is adaptive in nature, where short photoperiod may duplicate the role of low temperatures, thus preventing the premature hatching of larvae from reactivated eggs in late autumn to early winter during the temporary increases of temperature. The reactivation of diapausing eggs under long photoperiod was also shown in *O. caspius* (Sinigre 1983), *O. geniculatus* (Sims & Munstermann 1983), and *O. atropalpus* (Kalpage & Brust 1974). In *O. dorsalis* diapause was terminated only under a combination of long photoperiod and high temperature (Mulligan 1980). In *O. atropalpus* eggs with their anterior end hidden from the light did not respond to long photoperiod.

Thus, in nature, winter chilling is the key factor in the termination of egg diapause in aedine mosquitoes. The role of photoperiodism in this process is open to question. In nature a portion of eggs terminate diapause in autumn, and only temperatures and probably short day length inhibit their hatching. The fraction of reactivated eggs, as well as the rate of their hatching simultaneously, increases as the duration of chilling increases. The range of temperatures effective for diapause termination may be different in various mosquito species.

Besides temperature and photoperiod, some additional cues may act during winter hibernation to terminate egg diapause. Mosquitoes are known to lay eggs at various sites: in moist soil in locations subject to flooding, above the water level in tree and rock holes, in permanent and transient water bodies, etc., where they may hibernate in dry or moist states, and may be flooded periodically and frozen. All these hydrological conditions may also affect diapause development.

### 5.2.2 Larval Diapause

*5.2.2.1 Syndrome of larval diapause.* The main characteristic of larval diapause in mosquitoes is a strong arrest of development, most often in the 3rd- or 4th-instars; certain environmental factors are required for the termination of such developmental delays. A similar retardation of larval development may be induced directly by low temperature; however, in this case development quickly resumes in response to an increase in temperature.

Diapausing larvae are often characterized by reduced locomotor and feeding activity, by accumulation of fat body reserves and sometimes by alteration of cold hardiness. Locomotor activity depends on the water temperature. For instance, in northern regions of Russia at water temperatures of 0°C, diapausing larvae of *A. claviger* sink to the bottom of the pool, where they may remain for a long time. In more southern regions they occur in the upper water layers and continue to feed (Vinogradova 1969).

A reduced rate of feeding was recorded for diapausing larvae of the predacious mosquito, *T. rutilus* (Trimble 1983; Louinibos et al. 1998). Short day length and low temperature significantly reduced prey (*A. aegypti*) consumption rates. At low frequency diapausing larvae killed prey without consumption. Termination of diapause by transfer of 4th-instars to long day length was accompanied by an increase in the prey consumption rate prior to pupation.

Accumulation of fat body reserves supports the energy demands of the mosquito during diapause when feeding is reduced or terminated. In Uzbekistan, during October–November, hibernating larvae of *A. pulcherrimus* were observed to have well developed fat bodies. In experiments at 15°C and under short days the diapausing larvae of *A. plumbeus*, *A. claviger*, and *O. triseriatus* also had large fat storage (Vinogradova 1969).

There are only fragmentary and frequently contradictory data on the cold hardiness of diapausing mosquito larvae. In Canada *Wyeomyia smithii* is known to pass winter in the frozen ice cores of the leaves of *Sarracenia purpurea* (Evans & Brust 1972). However, the diapausing larvae are unable to withstand extended periods of sub-zero temperature under laboratory conditions: at –5°C 60% mortality occurred after 8 weeks. In the field, where ground temperatures averaged –3.7°C during the five coldest months, larval mortality averaged 45% after 4 winter months. In Shandong Province, China, *Armigeres subalbatus* larvae entered diapause when temperature dropped to 16°C and below at the end of October. The survival ratio was 90% after 12 h at –5°C, but none survived 60 h at this low temperature (Zhang et al. 1992).

There are also some data concerning the tree-hole mosquito, *A. barberi* from North America (Copeland & Craig 1989). Diapause is induced by photoperiod (LD 14.7:9.3) mainly in the 2nd-instar (75%) and also in the 3rd-instar: the first group of larvae survived –15°C for 24 h better than the second group. Larvae were more likely to survive at –15° in water from tree-holes, the site in which they are commonly found in nature. The capacity of the hibernating larvae to survive after full freezing of water in the field was recorded in *A. pulcherrimus*, *A. plumbeus*, *A. claviger*, *W. smithii*, and *Orthopodomyia alba* (Roubaud & Colas-Belcour 1933; Avdeeva & Nikipforova 1941; Horsfall 1955).

5.2.2.2 *Photoperiodic and temperature induction and termination of larval diapause.* By now the photoperiodic and temperature induction and termination of larval diapause is studied in more than 10 mosquito species (Table 5.4). Some of these species – *A. plumbeus*, *O. triseriatus*, and *W. smithii* may be considered as typical examples.

The tree-hole malaria mosquito, *A. plumbeus* is distributed in forests in Europe and Asia (Transcaucasia, Turkmenia, North Iran, Tadjikistan) (Gutsevich et al. 1970). In this species, eggs, as well as 3rd- and 4th-instar larvae, may overwinter. Experiments on the strain from Crimea, Ukraine, have been performed (Vinogradova 1962). Long-day photoperiod was found to promote rapid growth and pupation at 16–20°C in 70 days, whereas short-day photoperiod greatly slows down the development of the 3rd-instar and induces diapause in the 4th-instar larvae; solitary pupae appear up to 90 days later, but for 1 year the number does not exceed 4% (Fig. 5.3).

TABLE 5.4. Effect of photoperiod and temperature on the induction and termination of the larval diapause in mosquitoes

Species	Population	Stage	Diapause		References
			Induction	Termination	
<i>Anopheles claviger</i>	St. Petersburg, Russia	4th-instar	SD 12 h, 15–18°C	3–4°C followed LD 17 h, 14°C	Vinogradova 1963
<i>A. plumbeus</i>	Crimea, Ukraine	4th-instar	SD 9 h, 15°C; CP: 13 h, 15°C	LD 15°C; LD and SD 20°C	Vinogradova 1962
<i>Armigeres subalbatus</i>	Nagasaki, Japan	4th-instar	SD, LD (in field)	LD	Chiba 1968; Oda et al. 1978
<i>Ochlerotatus hendersoni</i>	Manitoba, USA	Larva	SD 8 h, 20°C		Gallaway 1985
<i>O. sierrensis</i>	Oregon, California	4th-instar	SD 16°C (embryogenesis at 16 h, 24°C)		Jordan and Bradshaw 1978
	California, USA	4th-instar	SD	HT, LD	Ahmadi et al. 1985
<i>O. togoi</i>	Vancouver, Canada, Japan	4th-instar 4th-instar	SD 12 h and less; 16°C and less; CP: SD 10.9 h, 16°C 10 h, 15°C or 21°C	>12 h, 16°C 16 h, 25°C or 30°C	Mori et al. 1985; Galka and Brust 1987b
<i>O. triseriatus</i>	Manitoba, Canada	4th-instar	SD 8 h, 20°C	LD 15°C and 20°C	Love and Whelchel 1955;
	Georgia	4th-instar	SD 15°C and 20°C		Vinogradova 1967; Gallaway 1985
	Georgia, USA	4th-instar	20°C SD 27°C	24 h, 29°C	

Designations are the same as in Table 5.1.

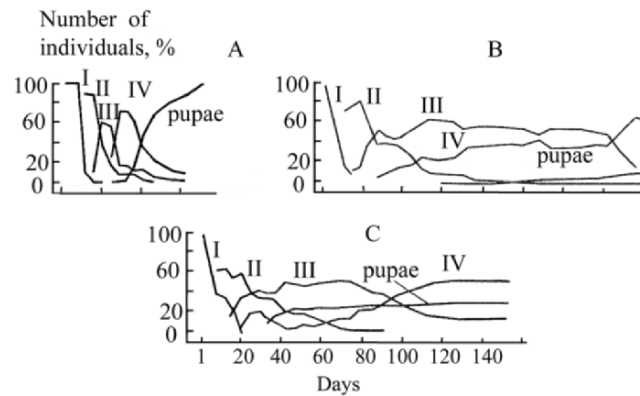


Figure 5.3. Effect of photoperiod on the larval development in *Anopheles plumbeu*. (After Vinogradova 1969.) A, LD 16:8, 16°C; B, LD 9:15, 16°C; C, LD 9:15, 20°C.

All immature stages are sensitive to photoperiod, but the key factor is the photoperiodic regime experienced in late larval instars. Continuous photoperiodic sensitivity promotes the effectiveness of photoperiodic reactivation. Under long-day conditions at 15°C all 4th-instar larvae resume development and pupate within 30 days, whereas under short-day conditions (LD 12:12) no reactivation is observed and during 6–7 months one half of the diapausing larvae perish; under LD 13:11 only a portion of the larvae may terminate diapause. At 15°C diapausing larvae may survive up to 13 months, but only 4% pupate within this time frame. The increase of temperature (20–25°C) stimulates pupation, though its rate under some photoperiods is lower when compared with the pupation incidence at 15°C. A similar effect of photoperiod and temperature on the induction, maintenance, and termination of larval diapause was revealed in the spring malaria mosquito, *A. claviger* from Luga (60°N), Russia (Vinogradova 1963).

The nonbiting pitcher-plant mosquito, *W. smithii* inhabits water-filled leaves of *S. purpurea*. This plant is found from the Gulf of Mexico to Canada. The mosquito range follows that of its host (30–55°N). Larvae of *W. smithii* normally diapause as 3rd-instars, but observations in Massachusetts revealed that the 4th-instar diapause phenotype is abundant in the spring after termination of 3rd-instar diapause and in autumn, when a new overwintering generation of 3rd-instar larvae accumulated in the pitcher-plant habitat (Farkas & Brust 1986; Louinibos & Bradshaw 1975). However, 4th-instar larvae did not survive the winter. 4th-instar diapause could be induced in diapausing 3rd-instar larvae by brief exposure to long-day photoperiods followed by short-day or by a long-term exposure in short days at 25°C. Continuous exposure to long days readily terminated 4th-instar diapause.

The 3rd-instar diapause of *W. smithii* is initiated, maintained, and terminated by photoperiod (Bradshaw & Louinibos 1972; Bradshaw & Phillips 1980). The photoperiodic cues are monitored by early instar larvae. Development is also limited by



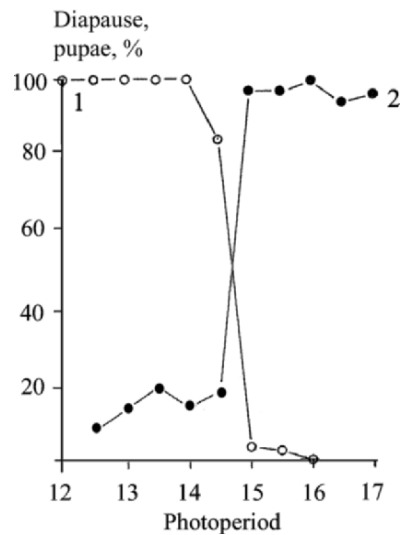


Figure 5.4. Effect of photoperiod on the induction and termination of the 3rd-instar diapause in pitcher-plant mosquito, *Wyeomyia smithii*. (Modified from Bradshaw & Louinibos 1972.) The photoperiodic response curve for induction of diapause (on the left) and termination of diapause (on the right). The induction of diapause was studied in groups of the 1st-instar larvae which were reared at 21°C; the termination of diapause (pupation) was studied in the 4th-instar larvae of 25 days old at 21°C.

temperatures below 15°C. Long days avert or terminate, and short days promote or maintain diapause (Bradshaw & Louinibos 1972). Approximately 3 long days are required for the median number of larvae to terminate diapause, although they do not molt to 4th-instar for another 6.5 days. The critical day length is identical for both the initiation and termination of diapause (Fig. 5.4).

The interaction between photoperiod, temperature, and chilling was analyzed in diapausing larvae of *T. rutilus* (Bradshaw & Holzapfel 1977). Chilling of dormant larvae promoted a response to progressively shorter day lengths, thus reducing the critical photoperiod. Chilling also accelerated the response to long day lengths, thereby reducing the depth of diapause; after a prolonged period of exposure to cold, the larvae sometimes eventually terminated diapause directly, and subsequent development was independent of photoperiod. The best chilling temperature for producing these effects was between 4°C and 16°C (probably ~7°C).

In connection with photoperiodism the photic environment of *W. smithii* was studied (Bradshaw & Philips 1980). Both in dawn and in dusk diapausing larvae were photoperiodically most sensitive to blue light (390–450 nm) with a shoulder in response in the blue-green and green (480–540 nm) regions of the spectrum. The photic environment of *W. smithii* during twilight is rich in yellow-green light, but sufficient light is available at 390–540 nm to trigger a photoperiodic response early during morning civil twilight and to sustain the response until late in evening civil twilight.

In *O. triseriatus* the threshold of light intensity, which may be perceived by larva is very low, about 0.012 lux, and the effective region of the spectrum is 320–720 nm (Wright 1967). In *T. rutilus* it is even less than 0.002 lux (Jenner & McCrary 1964).

In *W. smithii* larval food is an additional environmental cue, which was most marked in the larval part of the life cycle (Roughgarden et al. 1975). Each larva was programmed for pupation or diapause when in the 3rd-instar at 20–30 days of age: the food level widely modified the fraction of the cohort entering diapause (from 15% to 100%) compared with an expected figure of 56% from photoperiod alone.

Thus, the most common stage for a larval diapause in mosquitoes is the 3rd- and 4th-instars. This diapause is most commonly induced by short-day photoperiods experienced during larval development; long-day photoperiods promote continuous development resulting in pupation. No maternal effects have been linked to larval diapause. The photoperiodic effect is observed usually within a certain temperature range; high temperatures decrease the frequency of diapause, while low temperatures increase it. Typical photoperiod–temperature interaction for larval diapause induction may be illustrated by data in *O. togoi* (Galka and Brust 1987b). As Fig. 5.5 shows, larval diapause occurs when immature individuals are maintained at 16°C and lower, at photoperiods shorter than LD 12:12.

There are some trends concerning the geographical variation in larval diapause and the environmental cues controlling it. These include the frequency of diapause over a range of distribution, the intensity of diapause and the critical photoperiod inducing 50% response. The spring malaria mosquito, *A. claviger*, occurs in Europe, North Africa, and Asia (West Siberia, Middle Asia, Minor Asia) (Gutsevich et al. 1970). In the northern part of its range mainly 3rd- and 4th-instar larvae overwinter, whereas in southern and western parts all instars may be observed in winter. Only the 4th-instar larva undergoes diapause. The retarded development of other instars is induced directly by low temperatures and resumes in response to its increase

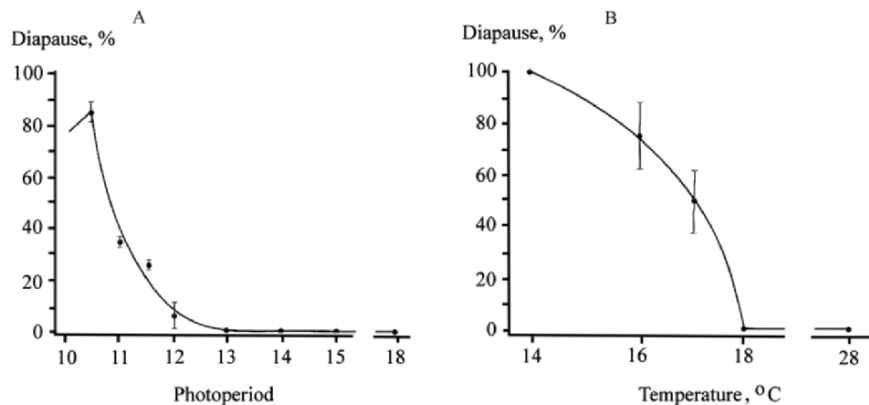


Figure 5.5. Effect of photoperiod (A) and temperature (B) on the larval development in *Ochlerotatus togoi* (Modified from Galka & Brust 1987a, b.) A, 16°C; B, LD 10:14.

(Vinogradova 1969). Duration of the 4th-instar hibernation varies over the area of distribution from 6–7 months in the northern regions of Russia to 2–3 months in Uzbekistan and Georgia. From north to south both the diapause intensity and incidence of diapause decrease, and in the southern regions such as Italy, Syria, Morocco, and southern Turkmenia no diapause occurs.

In the tree-hole mosquito, *O. sierrensis*, a widespread species on the Pacific slope that ranges from British Columbia, Idaho, and Montana to southern California, five populations from the USA, from a range of over 10 degrees latitude (33–44°N) were studied (Jordan 1980a, b; Jordan & Bradshaw 1978). Short days elicited 100% of diapause among the 4th-instar larvae from central Oregon and northern California, but the incidence of diapause decreased with latitude so that in southern California only 35% of the sample populations entered diapause. Among that portion of the population capable of responding to photoperiod, the critical photoperiod increased by 1 h for each 4–8 degree increase in latitude. A second tree-hole mosquito, *O. geniculatus*, occurring in Europe, Africa, and Asia Minor, has both an egg and larval diapause. Two strains of this species were investigated (Sims & Munstermann 1983). Larvae of the English strain have a longer critical photoperiod for diapause induction and stronger diapause intensity as compared with those from Sardinia, Italy.

In a third species, *W. smithii* photoperiodic response was studied for 22 populations collected at different latitudes, longitudes, and altitudes in North America (Hopkins & Bradshaw 1976). It was established that the growing season (the mean number of freeze-free days), which closely correlated with latitude and altitude but not with longitude, was an excellent predictor of critical photoperiod, and that an increase in latitude of 1 degree was equivalent to an increase in altitude of 122 m. The subsequent experiments with exotic light and dark cycles of varying period supported the conclusion that photoperiodic time measurements regulating larval diapause in *W. smithii* vary in a close relationship with latitude. The critical photoperiod mediating the maintenance and termination of diapause was found to be positively correlated with latitude among populations from southern (30–31°N), intermediate (40°N), and northern (46–49°N) latitudes in the USA and Canada.

The geographic variation of larval diapause in *O. triseriatus* from the USA was analyzed using experimental data for eight local populations distributed from 30–40°N and from 30–1,100 m of altitude (Holzapfel & Bradshaw 1981). Photoperiod was shown to have a significant effect not only on the initiation and maintenance of diapause but also on the rate of postdiapause development, both directly and by modifying response to temperature. The response to temperature was mainly a function of photoperiod, and the Q<sub>10</sub> for rate of completion of the 4th-instar was proportional to photoperiod. The critical photoperiod for the induction and maintenance of larval diapause, and for rate of development is shorter than the critical photoperiod controlling egg diapause (Fig. 5.6). This was also confirmed by Sims (1982), who studied *O. triseriatus* from other locations in the USA from 26°N to 46°N.

According to Holzapfel and Bradshaw (1981), the critical photoperiod for rate of larval development increases by 1 h for each increase in latitude of 2.06° or in altitude of 549 m. The adaptive significance of larval diapause may relate to the

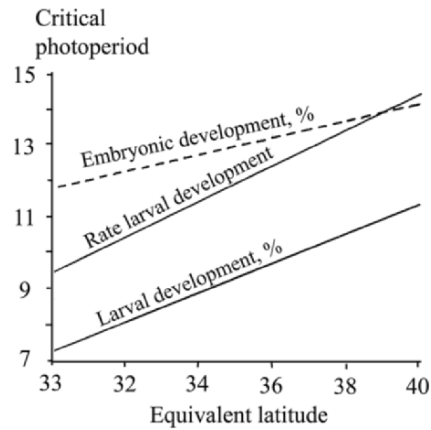


Figure 5.6. Effect of latitude on the critical photoperiod for the induction of larval and egg diapauses and for the rate of postdiapause development in the tree-hole mosquito, *Ochlerotatus triseriatus* (Modified from Holzapfel & Bradshaw 1981.)

modification of late winter and spring development rather than overwintering. In southern populations having incomplete egg diapause, progressively milder winter conditions favor larval diapause and continuous development. Larval diapause is mainly a backup or fail-save system for egg diapause. All authors suggest that egg diapause is the primary state in which *O. triseriatus* enters winter. During winter diapause eggs undergo chilling and may terminate diapause. Developments of larvae, which hatch during winter or spring, then become dependent on temperature and photoperiod. It has been proposed that egg and larval diapauses are not discrete, adaptive developmental strategies, but are part of an integrated finely tuned developmental continuum. Therefore it is suspected that polygenic control of diapause and development with diverse pleiotropy underlies the initiation, maintenance and termination of both egg and larval diapauses (Holzapfel & Bradshaw 1981).

In certain mosquito species sex-related differences in diapause were established. Among eight populations of *O. triseriatus* from the USA from 26°N to 46°N female larvae had a stronger diapause than male larvae (Sims 1982). The differences between the male and female intensities of diapause were most pronounced in four southernmost populations. Similar data are known for larval diapause in a population of *O. geniculatus* (Sims & Munstermann 1983). English males were less likely to enter either diapause state and had a less intense diapause than females. In a laboratory strain of *Culiseta melanura* the larval diapause is induced by short day (LD 9:15) at 15°C. After transfer of these diapausing larvae at 5 weeks of age to 23°C, long day (LD 18:6) males began to pupate on days 11–13 post transfer and were succeeded by females on days 40–44 post transfer. The waves of pupation were separated by a prolonged delay with no overlap between the two. In this case the ability to enter diapause was preserved after 18 years (400 generations) of

continuous laboratory rearing in standard conditions (27–29°C and LD 16:8) without larval diapause.

### 5.2.3 Adult Diapause

**5.2.3.1 Syndrome of adult diapause.** Adult mosquitoes overwinter in various natural (caves, hollows, holes, burrows, etc.) and artificial (cellars, vegetable store-houses, empty sheds, unheated basements, catacombs, bunkers, etc.) shelters. For instance, in Henan Province, China, hibernating females of *Culex pipiens pallens* preferred warm (>5°C), moist (RH > 60%), and dim (illumination no more than 5 lux) shelters away from the wind (<0.25 m/sec) (Su-TianYun et al. 1994).

In mosquitoes only females undergo an adult or reproductive (ovarian) diapause. Usually nulliparous inseminated females enter hibernation as has been shown for *C. pipiens pipiens* (Oda & Kuhlow 1974; Vinogradova 2000), *C. pipiens pallens*, *C. inornata* (Hudson 1979), *C. tarsalis* (Reisen et al. 1986a), *A. earlei*, *C. territans* (Hudson 1978), and *C. peus* (Skultab & Eldridge 1985). Exceptions occur rarely; for instance, in England only 5–9% of hibernating females of *C. pipiens pipiens* were unfertilized (Onyeka & Boreham 1987).

In mosquitoes the adult diapause syndrome involves a set of important characteristics, such as arrest of ovary development, reduced avidity, metabolic changes leading to progressive accumulation of fat body reserves, and an altered behavioral pattern.

Mosquitoes, like other blood-sucking Diptera, which periodically ingest blood, are characterized by a strong correlation between blood-digestion and ovarian development. This is a main link in the gonotrophic cycle, which includes host-seeking, blood-feeding with subsequent blood digestion and ovary development, and oviposition (Swellengrebel 1929; Beklemishev 1940; Clements 1963; Washino 1977). Gonotrophic concordance is typical of the summer gonoactive females in which one blood meal is necessary and sufficient for maturation of one batch of eggs. On the contrary, gonotrophic dissociation (failure of ovarian follicles to mature beyond the resting stage following a full blood-meal) is interpreted as an expression of facultative diapause. This term was originally used to denote the cessation of egg production despite the continued taking of blood meals by overwintering anopheline females, but later it was also used similarly for other mosquito genera. The process of egg maturation in mosquitoes was divided into several developmental stages, which are frequently used to evaluate the state of the ovary (Christophers 1911; Mer 1936). The follicle length or follicle to germarium length ratio (F:G) is another criterion for characterizing the ovarian state in mosquitoes before the first blood meal. The relationship between the two above-mentioned classifications was determined in *C. peus* (Skultab and Eldridge 1985). The mean length of the primary follicle and F:G ratio appear to increase from 0.060 mm (1.4:1) to 0.117 mm (2.4:1), respectively, when the follicles develop from stage N to stage IIb (the resting stage).

The main characteristic of reproductive diapause is the inactive state of the ovaries. In females of *C. pipiens pipiens*, *C. pipiens pallens*, and *C. peus*, *C. restuans*, which enter diapause after adult emergence, the primary follicles are usually small (0.05–0.06 mm) and the F:G ratio is less than 2:1 (Spielman & Wong 1973; Oda &

Kuhlow 1974; Skultab & Eldridge 1985; Madder et al. 1983; Wang et al. 1984). However, during hibernation the primary follicles may develop up to stage II as was shown in *C. pipiens pipiens* (Kupriyanova 1968; Oda & Kuhlow 1973).

Mosquito females that enter diapause may have hypertrophied fat body, which contains huge lipid reserves (Vinogradova 1969; Clements 1992). About 23 to 43 fatty acids were found in the fat body of diapausing *C. tritaeniorhynchus*, but only seven of them were predominant (Xue-RuiDe et al. 1991). In autumnal females of *C. pipiens pipiens* (St. Petersburg, Russia) fat deposits formed as much as 67% of the dry weight and 37% of the live weight of a mosquito, while in spring it dropped to 49% and 22%, respectively (Vinogradova 1969). In southern England, in October the mean quantity of fat in overwintering mosquitoes was found to be 1.06 mg/mosquito and in March it was 0.2 mg/mosquito (Onyeka & Boreham 1987). In some species, e.g. *C. tarsalis*, the hibernating females synthesize lipid from plant juices, which they consume in autumn. Thus, the females ingest fructose when entering and terminating diapause. In another species, e.g. *A. freeborni* in California, USA, females develop extensive lipid reserves whether fed sugar alone or blood alone (Reisen et al. 1986b; Clements 1992).

In the majority of mosquito species the reproductive diapause is followed by an abrupt decrease in avidity and a cessation of blood feeding (*C. pipiens pipiens*, *C. tarsalis*, *C. restuans*, *A. hyrcanus*, *A. messeae*, and probably *C. modestus*, *C. apicalis*, and *C. bitaeniorhynchus*). Only few mosquitoes take a blood meal periodically during hibernation; e.g. *A. superpictus*, *A. sacharovi*, and *A. atroparvus* (Vinogradova 1969). These mosquitoes usually overwinter in warm hibernation shelters, such as cattle sheds, where they may take occasional blood meals resulting in gonotrophic dissociation. However, the capacity to winter bloodsucking may vary considerably both within the species area of distribution and in the same place in connection with different weather conditions.

The behavior patterns of diapausing female mosquitoes include: negative phototaxis forcing them to migrate into hibernation shelters, a reduced locomotor activity, whose degree depends on temperature and illumination, and modified host-seeking behavior.

As to locomotor activity in the hibernacula, *C. pipiens pipiens* females, for example, still retained mobility at temperatures above zero, but at  $-3^{\circ}\text{C}$  they acquired the characteristic "hibernational" pose and would not react to external stimuli (Vinogradova 2000). It was shown that ~50% of the diapausing individuals of this species changed their location every 6 days, searching for more favorable temperature conditions in the hibernacula (Onyeka & Boreham 1987).

One further component of the mosquito hibernation behavior concerns host-seeking activity. Different aspects of this behavior have been considered in *C. pipiens pipiens* (Mitchell 1983; Bowen 1990, 1991, 1995). It was established that host-seeking behavior of diapausing females was depressed during the whole reproductive diapause, while in nondiapausing individuals it was absent only within 4 days after emergence. Changes in host-seeking behavior are known to correlate with the responsiveness of mosquito peripheral receptors sensitive to lactic acid, which is

one of the host-seeking attractants. Electrophysiological experiments established that in diapausing mosquitoes the state of some parts of the sensory system may change so that the peripheral receptors lose their sensitivity to lactic acid. But following diapause termination both this sensitivity and host-seeking behavior are commonly restored. In postdiapausing females some highly sensitive lactic acid-excited cells in the antennal basiconic sensilla of A3 type have been identified.

*5.2.3.2 Photoperiod and temperature induction of adult diapause.* Almost all of the mentioned characters of the reproductive diapause of mosquitoes appear to be controlled by photoperiod and temperature. The effect of photoperiod on blood-feeding activity is typical for those species, which do not take a blood meal during hibernation. In experiments with *C. tritaeniorhynchus* from Japan (Eldridge 1963), an average 92% of females fed on blood at 28°C when they were reared under long-day conditions (LD 14:10), but not more than 11% of females fed on blood under short day exposure (LD 8:16). Low blood-feeding activity increased after transfer of mosquitoes to long-day conditions, depending on the number of long-day cycles received (Fig. 5.7). Similar results were obtained in *C. pipiens pipiens* from St. Petersburg: under LD 24:0 and 12:12 50% and 2% of the females fed, respectively (Danilevsky & Glinyayaya 1958). Likewise, *A. hyrcanus* (Vinogradova 1969) and *C. pipiens pallens* (Hosoi 1954) responded the same way.

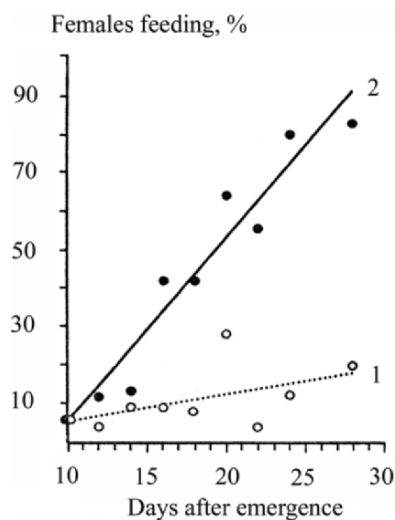


Figure 5.7. The activity of blood-feeding of females in *Culex tritaeniorhynchus*. (After Eldridge 1963.) 1, females were kept at 28°C and LD 8:16 from the time of pupation; 2, females were kept at 28°C and LD 8:16 from the time of pupation to 10 days after adult emergence and were transferred to LD 14:10.

Photoperiod and temperature may have effects both on the follicle size in unfed females and on ovarian development in blood-fed females. Thus, in *C. peus* a low temperature of 15°C caused retardation of follicle growth at the prereesting stage or earlier (stage N, 0.06 mm follicle length) regardless of photoperiod in non-blood-fed females. Only under the influence of both low temperature and short photoperiod did ovaries remain in this condition for as long as 21 days (Fig. 5.8). At 25°C follicles of both photoperiod groups developed well beyond the prereesting stage. The critical photoperiod was LD 13:11 (Skultab & Eldridge 1985). Similar results were observed earlier for *C. pipiens pipiens* from the USA (Eldridge 1968; Sanburg & Larsen 1973). At 22°C and short photoperiods (LD 12:12 and less) the follicle length was small (0.054 mm), but at long photoperiods (LD 13:11 and more) it was longer (0.070 mm). In *C. restuans* the females maintained at 15°C and long- or short-day conditions differed also in their follicle length and stage of follicles (Eldridge et al. 1976). Females of *C. tarsalis* responded to short photoperiod (LD 8:16) at 22°C by minimal ovary length and by increased size of the fat body (Harwood & Halfill, 1964)

The photoperiodic response curve for induction of winter reproductive diapause in blood-fed females was established for *C. pipiens pipiens* from St. Petersburg (60°N), Russia (Vinogradova 1961). At 23°C the incidence of diapausing individuals with gonotrophic dissociation was only 3–6% under long-day conditions (LD 20:4, 18:6) and increased to 86% under short-day treatment (LD 12:12). In a more southern strain from Azerbaijan, Russia (40°N), at 24°C the photoperiodic response was weak and the incidence of diapause was low; only 11% of the females entered diapauses at LD 12:12. Effects of both photoperiod and temperature on ovarian development in blood-fed mosquitoes were investigated in detail in *C. pipiens pipiens* from Indiana, USA (Eldridge 1968). As Fig. 5.9 shows, at 20°C and 25°C almost all

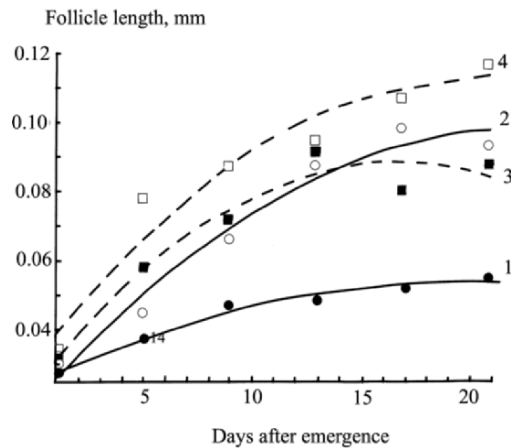


Figure 5.8. Effect of photoperiod and temperature on the ovarian follicle size of *Culex peus* from Oregon, USA (Modified from Skultab & Eldridge 1985.) 1, LD 8:16, 15°C; 2, LD 16:8, 15°C; 3, LD 8:16, 25°C; 4, LD 16:8, 25°C.



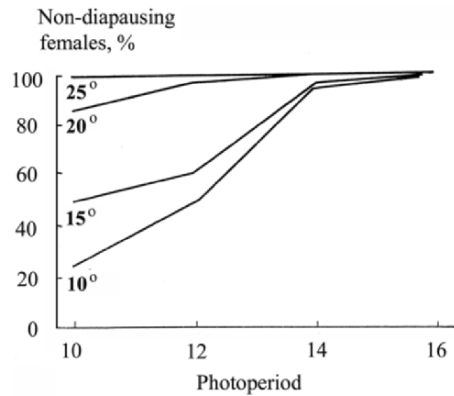


Figure 5.9. Photoperiodic response curve for the induction of the adult diapause in *Culex pipiens* from Indiana, USA. (After Eldridge 1966.)

individuals had matured ovaries, irrespective of photoperiod length. Gonotrophic dissociation occurred in response to the same conditions that inhibited blood-feeding, i.e. a combination of low temperature and short photoperiod.

Besides photoperiod and temperature, larval crowding can also influence the incidence of adult diapause in *C. pipiens pipiens*, as reported for a Canadian strain from southern Ontario (Madder et al. 1983). The combined effect of two photoperiods, three temperatures, and four larval population densities (50, 100, 250, and 500 larvae per 700 ml water) was investigated to show that, with a decrease in temperature and day length, and rising larval density, the incidence of diapausing females increased.

The photoperiodic sensitivity during ontogeny in *C. pipiens pipiens* was studied using different combinations of temperature and photoperiod: larvae were reared at 25°C, pupae were held at 15°C, and adults at 10°C (Eldridge 1965). Ovarian development was reduced by ~50% in mosquitoes subjected to a short photoperiod (LD 12:12) during at least two of the three developmental stages, regardless of the order of treatment. Conversely, when two or more stages were subjected to a long photoperiod (LD 16:8) ovarian development occurred in no less than 83% of the cases. Sanburg and Larsen (1973) confirmed that in determination of the adult diapause the two last stages (pupa and adult) were most important. In another species, *C. restuans* from Washington, USA, both temperature and photoperiod, to which females were subjected during the pupal stage, and for up 6–8 days afterwards influenced the development of the ovaries; the combination of 15°C and LD 8:16 resulted in the ovaries remaining in a diapause state (Eldridge et al. 1976).

Interesting data on the induction of diapause by a decrease in day length were obtained for *C. inornata* (Hudson 1977). Females were reared and maintained at constant LD 16:8 and 20°C until larval–pupal ecdysis and then were transferred to regimes of LD 16:8 or LD 12:12 at 10, 15, or 20°C. At 14–15 days after adult emergence, follicles of the females at short day were as small as those of diapausing

females. The proportion of females 7–8 days after emergence that fed on a man and the proportion of blood-fed females that matured eggs, increased both with day length and temperature, the lowest rates being seen in females at short day length and 10°C (20% fed and none matured eggs) and the highest in females at long day length and 15°C (72% fed and 96% matured eggs). Thus, in this species the final regime determined both the blood-feeding activity and ovarian development.

The photoperiodic response was also studied in some anopheline mosquito species (Vinogradova 1958, 1960). The malaria mosquito, *A. messeae* from St. Petersburg, Russia, is a typical “long-day” insect. Only 11% of the females that were subjected to a long photoperiod (LD 22:2) entered diapause, while short photoperiods (LD 15:9 or less) induced diapause in nearly all individuals. A comparison of the photoperiodic response of two strains from St. Petersburg (60°N) and Astrakhan’ (47°N) confirmed the geographical variation of the critical day length in this species. *A. hyrcanus* (probably *A. hyrcanus* complex), a group widely distributed in southern Europe and Asia (to 50°N) undergoes adult diapause only in the northern and middle parts of the area – Central Asia, China, Japan. The photoperiodic induction of diapause was experimentally established in two strains (Vinogradova 1969). In the strain from Astrakhan, at 23°C under long day length (LD 18:6, 16:8, 15:9), no diapause was observed, but under short day length (LD 14:10, 13:11, 12:12) 31%, 46%, and 68% of females, respectively, entered diapause. In another strain from southern Tajikistan (37°N) only 37–56% of the females underwent diapause under short day length.

The hypertrophied fat body, which is typical for diapause of mosquitoes, may have different origins. Diapausing females may accumulate their fat reserves both through sugar or blood-meal and through the utilization of larval reserves (Vinogradova 1969). It was shown experimentally that in *C. bergrothi*, *C. alaskaensis*, and *Allotheobaldia longiareolata* the extent of fat body in females of 10–12 h age was dependent on the photoperiod and temperature conditions during their larval development. Low temperature and short day exposure during immature stages promoted the accumulation of adult fat reserves. This effect was more highly expressed under daily variations of temperature from 6°C to 29°C as compared to a constant temperature of 18°C. The role of carbohydrate feeding by adults on fat body formation has been extensively examined in *C. pipiens pipiens* (Mitchell & Briegel 1989). Sugar-fed mosquitoes accumulated significantly more lipids under short-day conditions (LD 9:15) than under long-day (LD 15:9) conditions, the values being 11.4 and 6.64 cal., respectively. The extent of the fat reserves also depended on the temperature at which the mosquitoes were maintained: the fat reserves of females fed on sugar at 25°C and 15°C were 1.610 mg (33%) and 2.09 mg (37%), respectively (Tekle 1960).

A photoperiodic effect also is apparent in mosquito species, which periodically take a blood meal during diapause. It was established experimentally that in *A. atroparvus*, *A. messeae*, *A. superpictus*, and *A. hyrcanus* the percentage of the blood-fed females with hypertrophied fat body was greater in short-day conditions than in long-day conditions (Vinogradova 1969). It is interesting to record that a

similar situation was observed in southern populations of *C. inornata* where females aestivate during the hot summer month and general activity occurs primarily in autumn and winter. In California, USA, at 33–34°C the blood- and sugar-feeding females developed increasingly hypertrophied fat bodies between April and June before ceasing activity. It has been shown experimentally that both parous and nulliparous females synthesized very substantial lipid reserves when reared from egg to adult under long photoperiod (Barnard & Mulla 1977).

*5.2.3.3 Adult diapause termination.* The termination of adult diapause in mosquitoes has not been adequately studied. The majority of data concerns *C. pipiens pipiens*. Experimental evidence for the photoperiodic stimulation of blood-feeding in field hibernating mosquitoes in England was obtained long ago (Tate & Vincent 1936). These females were subjected to two light regimes at 17–20°C: under continuous illumination and in the dark 70–85% and 13% of mosquitoes took blood meals, respectively. Diapausing females of two other populations, from Boston, USA, and from Hamburg, Germany, exposed to a long photoperiod also renewed ovarian development (Spielman & Wong 1973; Oda & Kuhlow 1974). Experiments with an English population testify to a dynamic character of diapause (Onyeka & Boreham 1987). The minimum photoperiodic exposure (LD 24:0, 21°C), which restored blood-feeding activity in diapausing mosquitoes decreased as the hibernation period increased. This exposure was 12 days in August–October (26% females took a blood meal), 6 days in February–March (25–47%), and 3 days in April (53%). A similar picture was observed for diapausing mosquitoes near Moscow (Kupriyanova 1968). The most intensive diapause occurred in August–November, when in the laboratory females fed on blood reluctantly, and after the first blood-feeding only 36% of the females had mature ovaries; in January all blood-fed individuals became gravid, i.e. during field hibernation diapause ends gradually and spontaneously during early or late winter.

In *C. tarsalis* diapause termination is a function of increased day length (Mitchell 1981). Exposure to a long day (LD 15:9) results in diapause termination in essentially all mosquitoes by day 7 at 25°C, and the host-seeking behavior is restored in such females. In another mosquito, *C. inornata*, females entering adult diapause in Canada during September–October had small ovarian follicles and did not take a blood-meal in nature (Hudson 1979). In some females that were kept for 7 days at 20°C and LD 16:8 follicle growth occurred and blood-feeding was followed by egg maturation. When females were kept for 2–3 months at 15°C and short day lengths, follicle development also occurred and egg maturation was observed in all blood-fed females.

Thus, during hibernation diapausing mosquitoes are undergoing what is commonly called “diapause development,” or reactivation. Though adult diapause can be terminated by subjecting them to long photoperiod and high temperature, in nature individuals usually overwinter in dark shelters, where photoperiodic reactivation hardly occurs, therefore low temperature reactivation is likely to be more relevant.

## 5.3 OTHER GROUPS OF AQUATIC INSECTS

5.3.1 Chironomids (*Chironomidae*)

Chironomidae are an important part of the benthos and provide food for many fish species. In chironomids the diapausing stage(s) are species-specific as well and even in the same genus diapause may occur in the egg stage or in instars 2, 3, or 4, depending on the species (Danks 1971; Goddeeris et al. 2001). In the voluminous genus *Chironomus* diapause most often takes place in mature larvae of the 3rd- or 4th-instars. Diapausing larvae display important differences in their metabolism. As was shown in *Chironomus plumosus*, the oxygen consumption of the diapausing larvae drops ~30% as compared to that observed in nondiapausing larvae (Adamek & Fischer 1985). In this species the diapausing larvae are still active and take up food (Ineichen et al. 1979), whereas inactive diapausing larvae not feeding and completely enclosed in characteristic cocoons have been observed in many other species (Danks 1971). Freezing tolerance is widely distributed in the genera of most subfamilies except Tanypodinae, and is probably widespread in the Orthocladinae. It was found in some temperate and all arctic larvae and was temperature- and time-dependent. Chironomidae can probably be considered as preadapted to a rigorous winter environment (Danks 1971). For the first time in *C. tentans* it was experimentally established that short photoperiod induced and maintained the larval diapause, while its termination was favored by long day (Engelmann & Shapirrio 1965). The photoperiodic induction of diapause was later described in *C. plumosus* and *C. nuditaris* (Fischer 1974). The larvae of *Clunio marina* and *C. decorus* enter diapause under short photoperiod and low temperature (15°C), whereas for *C. staegeri* and *Endochironomus nigricans* short photoperiod is effective both at 15°C and 20°C (Danks 1978; Neumann & Kruger 1985). *C. plumosus* develops continuously at 15°C and long day, and enters diapause under short day; the duration of diapause may vary from one to several months (Ineichen et al. 1979). In *C. riparius* both 3rd- and 4th-instars may enter diapause in response to short-day and temperature (15°C and lower) induction. Geographical variation in the duration of diapause in 4th-instar of this species is known: the diapause of a Belgian strain was shorter (by at least several weeks) as compared to a synantropic strain from St. Petersburg basements, Russia, (3–9 months) (Goddeeris et al. 2001; Vinogradova & Petrova 2004).

Shilova (1976) divided chironomids from Borok, Russia, into three groups depending on the environmental cues responsible for their diapause:

1. Diapause is induced by short photoperiod and comes to end after long-day treatment (*C. plumosus*, *Polypedium nubeculosum*, *C. tentans*, *Stictochironomus crassiforceps*).
2. Diapause is initiated by short photoperiod and terminates in response to long day, low temperature, or freezing (*C. pulicornis*, *Psectrotanypus varius*).
3. The induction of diapause is the same, but the reactivation occurs only after freezing (*Procladius choreus*, *Anatopynia plumipes*).

These examples show that in principle the environmental control of larval diapause in chironomids is similar to that of mosquitoes.

### 5.3.2 Biting Midges (*Ceratopogonidae*)

Diapause of ceratopogonids was actively studied in the USSR (Isaev 1975, 1976a, b, 1978, 1997; Glukhova 1989). Hibernation during the stage of the 3rd- to 4th-instar larva is typical for the blood-sucking midges in the genus *Culicoides* in the temperate zone. Such a diapause was recorded for 17 species from the Ivanovo region. In the field the inclination for larval diapause increased from the first to the second generation: for instance, in *C. circumscriptus*, *C. pulicaris*, *C. salinarius*, and *C. nubeculosus* the mean incidence of diapausing larvae was 0% and 100%, respectively. The cold reactivation of diapausing larvae occurred gradually during the winter. In 10 among 25 species studied, the tendency to monovoltinism dominated, the remaining species were multivoltine. In experiments with *C. odibilis* a role for photoperiodism and temperature in the induction and termination of larval diapause was shown.

Besides larval diapause some species have an obligate or facultative egg diapause (Isaev 1960, 1976b). In *C. punctatus* the termination of egg diapause was elicited by both high and low temperatures, and the degree of the synchronous hatching of larvae increased as the temperature exposure increased. Differences in the inclination to diapause were recorded in two geographical populations of *C. punctatus* from Ivanovo and the Far East.

In 20 species of nonblood-feeding midges in the genera *Spheromyia*, *Probezzia*, *Mallohelea*, *Palpomyia*, *Bezzia*, *Phaenobezzia*, *Alluaudomyia*, *Stilobezzia*, *Dasyhelea*, and *Forcipomyia* a 4th-instar larval diapause has been observed: larvae collected in July–August did not pupate in laboratory conditions (16–20°C and natural day length) during 8–10 months. In all studied species the 1st–2nd-instar larvae also may be observed in winter but their development is delayed exclusively by low winter temperature. The reactivation of diapausing larvae occurs in winter, and diapausing 4th-instar larvae collected in the field in February–March pupate in the laboratory usually 2–3 months after, whereas the 1st–2nd instars develop without delay. Intra- as well as interpopulation variation in the incidence of diapause and its duration appears to be typical for ceratopogonids.

### 5.3.3 Dragonflies (*Odonata*)

In Odonata both egg and larval diapauses occur in different instars (Corbet, 1980). Embryonic diapause occurs in certain temperate species, notably *Aeshna*, *Sympetrum*, and *Lestes*. For instance, *Lestes congener* oviposits in dry stems, the eggs undergo a bit of embryogenesis in autumn and then enter diapause in winter, at which time they are resistant to both low temperature and desiccation. Hatching of larvae is observed only after wetting and exposure to temperatures of 5°C and higher (Sawchin & Gillott, 1974). Such a response may be augmented in some other species of *Lestes* by sensitivity to photoperiod. Larval diapause is the most common diapausing stage for dragonflies in the temperate zone. Larval growth rate is controlled by the interaction of responses to temperature and photoperiod such that morphological development within and between certain instars is arrested or accelerated at different times of year (Corbet, 1980). A relatively simple example of the mechanism of

environmental regulation is provided by *L. eurinus* from North Carolina, USA, where the populations overwinter in three larval instars preceding the final one (Lutz, 1968). Over a wide temperature range larvae of these instars develop more rapidly under summer than under winter photoperiods. Such a response magnifies the seasonal change in growth rate due to temperature. More complex responses to temperature and photoperiod exist among certain other species from North Carolina, South Ontario, Sweden, and England. Their common feature is that one or more late instars became unresponsive to a long photoperiod stimulus in late summer or early autumn and thus enter diapause. The larvae of some dragonflies may also diapause in a dried (anhydrobiotic) state (Van Damme & Dumont, 1999). In Brazil, one larva of *Pantala flavescens* survived drought at least a few months and after flooding successfully completed metamorphosis. It is argued that early larval tolerance to drought may be common in *Pantala* contributing its success in semiarid environments; possible other species in which a similar phenomenon occurs are also listed by Van Damme and Dumont (1999).

In *Enallagma hageni* it has been shown experimentally (Ingram 1975) that termination of diapause can be caused by exposure to a low temperature, regardless of photoperiod, or to short photoperiod at a permissive temperature. A critical element in the seasonal regulation of many dragonflies at higher latitudes is the annual reversal of response to photoperiod among one or more late instars at, or sometimes before, the autumnal equinox. This reversal can induce the population to molt synchronously at that time and can also establish a latent sensitivity to spring photoperiod (Lutz 1974). In European *Leucorrhinia dubia*, which spends its last winter mainly in the final instar, analogous differential responses to photoperiod operate within the final instar and thus enhance the responses to photoperiod and the degree to which each of several developmental phases is synchronized within the larval population (Norling 1976). Such responses prevent autumnal emergence and reduce temporal variation among overwintering larvae that are due to emerge the next summer.

#### 5.3.4 Heteroptera

Heteroptera, divided into Gerromorpha and Nepamorpha, include approximately 20 families. Gerromorpha are semiaquatic whereas Nepamorpha are aquatic. Their overwintering stages and the environmental cues regulating diapause have been inadequately studied. Among the species studied, both adult and embryonic diapause has been recorded. Adult diapause has been reported more frequently than embryonic diapause. Both obligate (*Pelocoris femoratus*, *Iliocoris cimicoides*) and facultative diapauses are known. It was shown experimentally that environmental control of adult diapause is similar to that of mosquitoes and other insects. Adult and certain preceding stages are sensitive to photoperiod in *Notonecta undulata* (Vanderlin & Streams 1977) and *Gerris odontogaster* (Vepsalainen 1978). Short photoperiod induction of adult diapause is correlated with wing length and alary dimorphism in *Aquarius paludum* (Harada & Numata 1993) and *Gerris odontogaster* (Vepsalainen 1971). In the latter species, a gradually changing photoperiod induced the appearance of diapausing macropters. Therefore in South Finland the 2nd generations was predominantly macropterous (Vepsalainen 1978).

### 5.3.5 *Ephemeroptera*

Immature stages of these insects inhabit the littoral zone and develop over a long time, sometimes up to 3 years. Data on their diapause is fragmentary. Probably both embryonic and larval diapauses occur. Embryonic diapause was shown in *Ephemerella ignata*, and it was terminated within 12 months at temperatures from 1°C to 16°C (Bohle 1972). In *Baetis rhodani* and *B. vernus* embryonic diapause began at 0°C, as well as 20°C, and continued for 1 and 12 months, respectively (Bohle 1969). Larval hibernation was recorded in *Cloeon dipterum* in Sweden and England (Nagell 1981). Low temperature, but not decreasing photoperiod, was decisive for the final induction of its hibernation. In Sweden this occurs at 4–5°C. Swedish larvae initiated hibernation more rapidly and were more resistant to starvation than English larvae of the same species.

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