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Photothermal Control of Larval Diapause in the Blowfly, *Calliphora vicina* R.-D. (Diptera, Calliphoridae) from the Lofoten Islands (Northern Norway)

E. B. Vinogradova and S. Ya. Reznik

Zoological Institute, Russian Academy of Sciences, St. Petersburg, 199034 Russia
e-mail: reznik1952@mail.ru

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Abstract—Photothermal control of the rate of development and of larval diapause in the arctic population of *Calliphora vicina* from the Lofoten Islands (Northern Norway) was studied under the laboratory conditions. In the temperature range from 8 to 23°C, the rate of development of the studied population from egg to puparium can be well ($r = 0.95$) approximated by linear regression. The sum of effective temperatures was about 160 degree-days with the lower development threshold of 4.5 ± 0.6°C. The maternal photoperiodic response induced the larval diapause in a significant fraction of individuals only when larvae developed at temperatures of 4–10°C; relatively high proportion of diapausing larvae was recorded even in the progeny of females that were kept under long day conditions. The intensity of the larval diapause was very variable, which was manifested in the separation of diapausing larvae into two fractions differing in the time of pupariation during both spontaneous and induced reactivation.

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Seasonal adaptations of Arctic insects is one of the most important problems of insect ecology (Danks, 2004, 2007) and E. S. Sugonyaev has made a substantial contribution to the studies on this subject (Sugonyaev, 2001, Sugonyaev and Voinovich, 2001, 2006). Species of broad geographic distribution are particularly interesting in this respect because their adaptations are based on intraspecific variation.

The object of our study, the blue blowfly *Calliphora vicina* R.-D. (Diptera, Calliphoridae), is broadly distributed over the temperate and subtropical zones of the Holarctic. This species is considered as optionally synanthropic since it inhabits both human settlements and natural landscapes. *Calliphora vicina* has a variety of practical applications including sport fishery, pollination of plants in greenhouses, determination of the time of death in forensic medicine, and, finally, it is often used as a model for various studies, particularly for the investigations of the mechanisms of the regulation of seasonal cycles (Vinogradova, 1984, 1991).

The seasonal cycles of *C. vicina* development are determined by the adult (reproductive) and larval diapauses. The reproductive diapause is regulated mostly by temperature. The induction of the larval diapause is under maternal control: if females develop under the short day conditions, their progeny show a high inclination for diapause. The temperature is the most important factor directly influencing larvae (diapause is induced by temperatures lower than 16–17°C), whereas the photoperiodic effect plays a minor role (Vinogradova and Zinovjeva, 1972; Vinogradova, 1984, 1991; Nesin and Chernysh, 1999; Vinogradova and Reznik, 2013).

Calliphora vicina is widely distributed over different climatic zones suggesting a high ecological plasticity of seasonal adaptations. Indeed, the experimental analysis of 30 populations from different parts of the range revealed a high latitudinal and altitudinal variation in the thresholds of induction, duration, and intensity of the larval diapause (Vinogradova, 1975, 1980, 1991). A comparative study of diapause in populations distributed near the limits of the geographic range of the species may be particularly interesting.

The northernmost among *C. vicina* populations studied so far originated from Murmansk which is close to the northern limit of the geographical range of this species. At present, we have obtained the possibility to study the photothermal control of diapause in representatives of other arctic population inhabiting the Lofoten Islands (Norway) located to the north of the Polar Circle. *Calliphora* phenology in this region

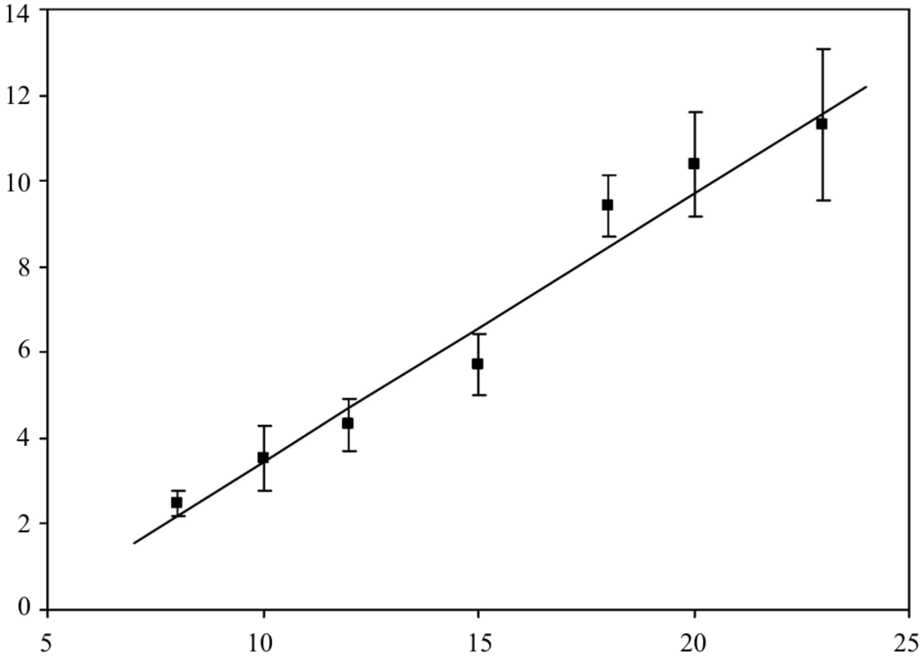

Fig. 1. The rate of development of larvae from the Lofoten population of *Calliphora vicina*. The horizontal axis, temperature (°C). The vertical axis, the rate of development from egg to puparium (%). Means, SD, and regression ($Y = 0.625\ X - 2.8$, $r = 0.95$, $n = 69$) are shown.

has been well studied (Aak et al., 2011a, 2011b). The climates of Murmansk and of the Lofoten Islands are substantially different and thus the comparative study of these populations would clarify the peculiarities of *C. vicina* seasonal development.

MATERIALS AND METHODS

In our study we used individuals from the third and fourth generations of a laboratory strain of *C. vicina* which originated from the flies collected at the Lofoten Islands (Northern Norway). The experiments were conducted in the thermostatic chambers of the Laboratory of Experimental Entomology of the Zoological Institute RAS. The flies were kept in gauze cages (25 × 15 × 25 cm), they were regularly given water, protein food (pig kidneys), and carbohydrate food (sugar). The larvae were placed in 0.5-liter jars with humid sawdust and were fed on pig kidneys which were provided as needed. After the termination of feeding and the emptying of the gut, the larvae were transferred into clean sawdust. In the first series of experiments, we investigated the influence of different temperatures (from 12 to 23°C) on the rate of development of larvae originated from the flies which developed at 20°C and long (18 h) day. In the second series of experiments, the flies were kept at 20°C and at day lengths of 12, 14, 16, 18, 20, or 22 h.

The eggs laid during 6 h by several females were kept at 12°C in the dark until larval eclosion; the eclosed larvae of the first instar were distributed among different thermal regimes that are described below, together with the results of the corresponding experiments. A cohort of larvae eclosed from one egg batch laid by a group of females was considered as a replicate and at least 3 replicates of each treatment (photothermal regime) of each experiment were conducted. When larval feeding was terminated, the number of new puparia was recorded separately for each replicate of each treatment; at the end of the experiment, the living larvae that had not yet pupariated were also counted. In total, about 25000 larvae were used in the experiment. Statistical analysis of the data was conducted with SYSTAT software.

RESULTS

To evaluate the effect of temperature on the rate of larval development, we have used the results of all the experiments conducted at temperatures from 8 to 23°C, independently of the photoperiodic conditions of the rearing of the maternal generation. Only the data for the active (non-diapausing) fraction of the larvae were used, the mean for a replicate being considered as a unit of statistical analysis. As seen in Fig. 1, in the studied range of temperatures, the rate of development

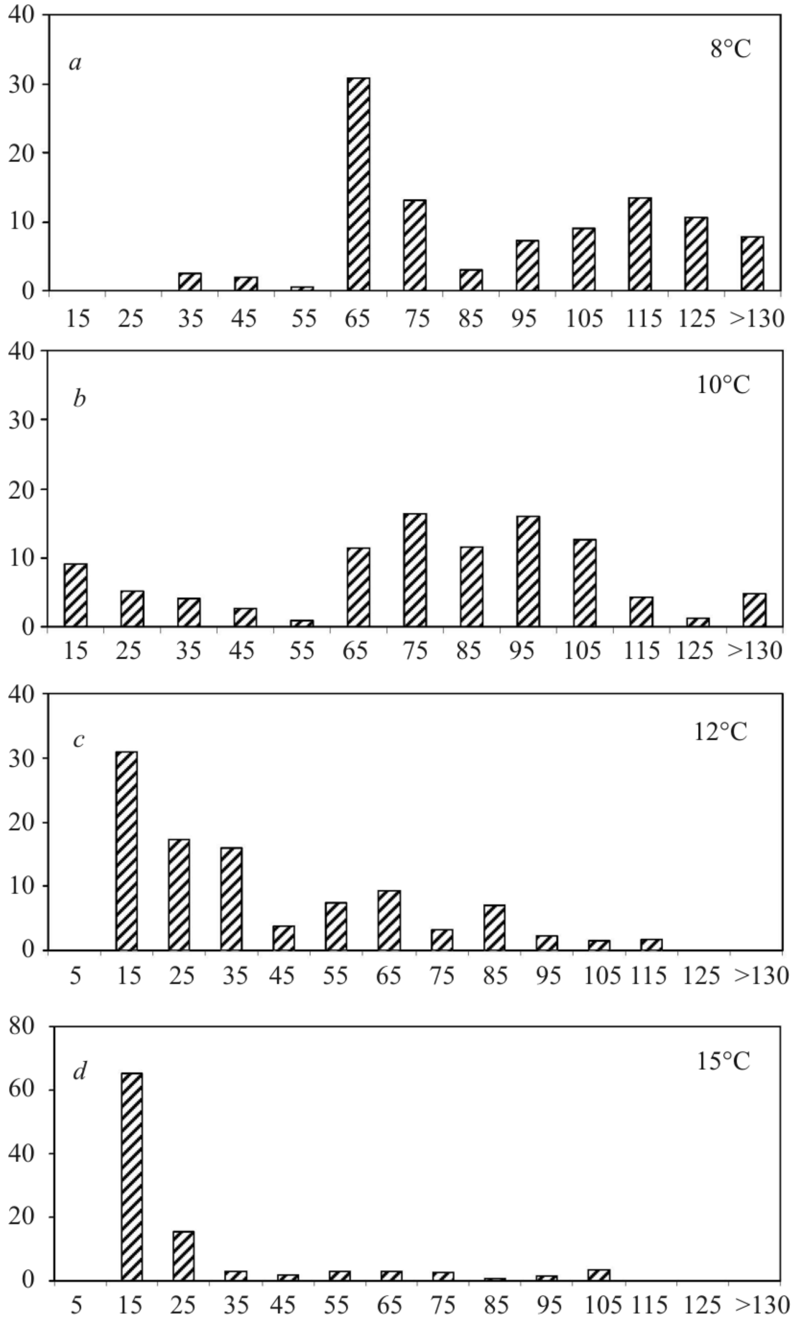

Fig. 2. The dynamics of pupariation of larvae from the Lofoten population of *Calliphora vicina* under different thermal regimes of development. The horizontal axis, time (days, means for intervals). The vertical axis, the proportion of individuals which pupariated during a given interval (%). Thermal regimes are indicated in the graphs.

from egg to puparium can be rather closely (r = 0.95, n = 69) approximated by linear regression; the thermolability coefficient was 0.625 ± 0.024, the lower thermal threshold of development was 4.5 ± 0.6°C, the sum of effective temperatures was about 160 degree-days.

It should be noted that the above data concern only the active (non-diapausing) fraction of the larvae. At temperatures of 18–23°C, this fraction constituted 100% of the studied sample: all the individuals pupariated almost synchronously during 15–20 days after oviposition (Figs. 2*e*–2*g*). At 15°C most of the larvae (about 80%) also developed actively and pupariated during 30 days, but the development of the rest of the larvae was delayed for up to 100 days (Fig. 2*d*). At 12°C, the proportion of actively devel-

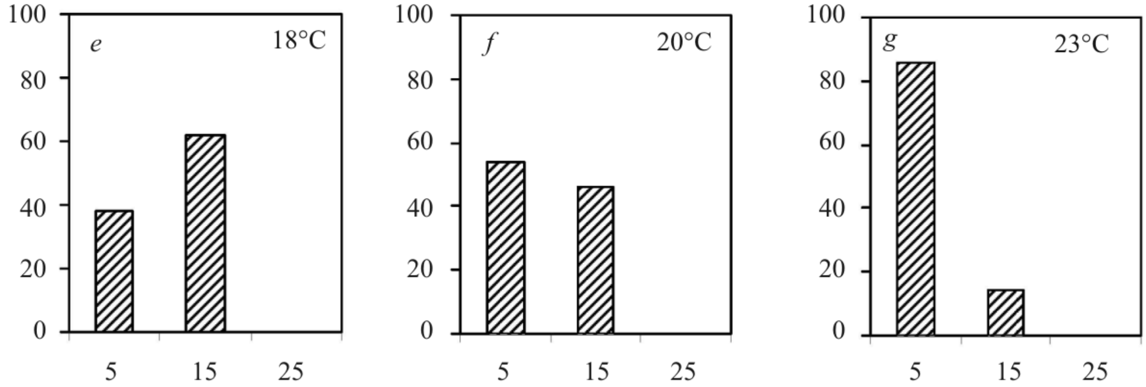

Fig. 2. (Contd.)

oped individuals decreased to 64%, whereas the delay in development increased (a distinct border between the pupariation of the active and diapausing fractions falling on 40–50 days after oviposition is clearly seen in Fig. 2*c*). Further decrease in the temperature to 10 and 8°C (Figs. 2*a*, 2*b*) increased this tendency: the proportion of actively developed individuals decreased to 22 and 4%, correspondingly, whereas the pupariation of most larvae was markedly delayed (at these temperatures, the distinct border between the pupariation of the active and diapausing individuals falls on 50–60 days after oviposition). Moreover, as seen in Figs. 2*a*–2*c,* at temperatures of 8–12°C diapausing larvae also seem to be separated into two fractions differing in the intensity of diapause which is manifested by the existence of not two but three "waves" of pupariation. The first wave, as it was noted above, corresponds to the actively developed individuals (their proportion increased with the temperature), whereas the second and third waves (the border between them falls on 70–90 days after larval eclosion) probably correspond to the two fractions of diapausing larvae differing in the intensity of diapause.

The difference in diapause intensity is also suggested by the experiments with the induced and spontaneous reactivation of larvae. In the first experiment (Fig. 3) the females which developed under the short (14 h) day conditions laid eggs; the eclosed larvae which developed at 8°C in the dark and at the age of 37 days were transferred to higher temperatures. The sharp increase of temperature up to 20 and 25°C caused a mass reactivation of all diapausing larvae during 11 and 5 days, correspondingly (Figs. 3*c,* 3*d*), but at a temperature of 12°C two fractions of diapausing larvae having a border on approximately the 18th day after the increase in temperature can be seen (Fig. 3*a*).

A similar segregation of larvae into two fractions differing in diapause intensity was also observed in the second experiment, when larvae developed at a constant low temperature of 2–4°C. Under these conditions, all the larvae evidently entered diapause and then spontaneous reactivation occurred: about a half of individuals pupariated during 180 days after oviposition, while the pupariation of the second fraction was extended to up to 300 days (Fig. 4). It is interesting that even at such a low temperature, in some individuals further pupal development occurs and sporadic adults emerged even 7 months after larval eclosion (it should be borne in mind that the lower thermal threshold of development calculated based on the regression equation was 4.5°C).

In the last experiment (Fig. 5) we investigated the joint effect of photoperiodic conditions of development of parental generation and the temperature conditions of development of their progeny on the induction of diapause. It should be noted that at 12°C the proportion of diapausing larvae was low (about 30%) although it is this temperature that is commonly used for testing the tendency for diapause in different populations of *C. vicina*. Therefore, in the further tests, the larvae developed at low temperatures of 8 and 10°C. The patterns of the two photoperiodic responses were very similar (Fig. 5*a*), although the lower temperature resulted, as expected, in a higher proportion of diapausing individuals. In general, the maternal photoperiodic response of *C. vicina* is characterized by a relatively high proportion of diapausing progeny that was recorded even in females kept under the long (20 h) light day which at the given latitude occurs in June

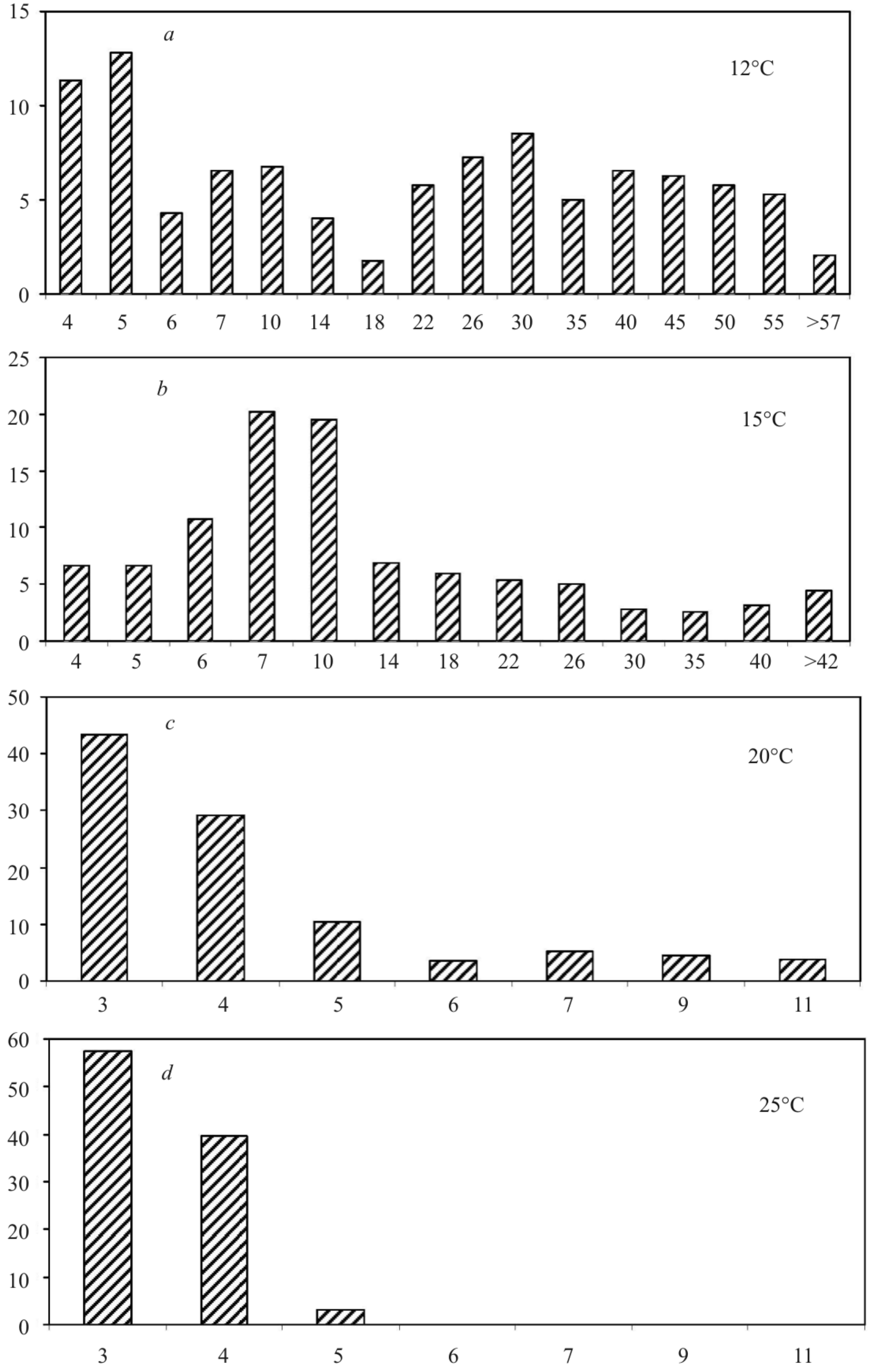

Fig. 3. The dynamics of the induced reactivation of larvae from the Lofoten population of *Calliphora vicina* at temperatures of 12, 15, 20, and 25°C. Explanations as in Fig. 2.

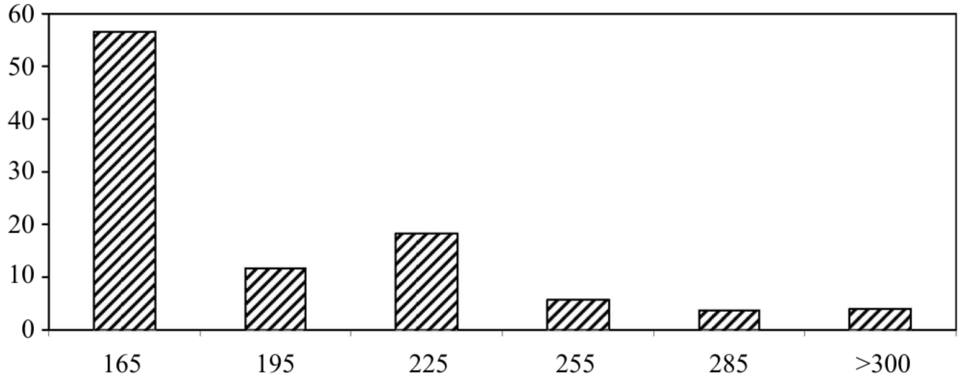

Fig. 4. The dynamics of the spontaneous reactivation of larvae from the Lofoten population of *Calliphora vicina*. Explanations as in Fig. 2.

and in August. These results testify to the weakening of the maternal photoperiodic response and to the corresponding increase in the role of the direct thermal effect on developing larvae.

DISCUSSION

The Lofoten Islands are on the northern border of the geographic range of *C. vicina*. This archipelago is located to the north of the Polar Circle (67–68°) but it is in the sphere of influence of the warm Gulf Stream which results in the Atlantic climate with a warm winter and cool summer. In January, the temperature varies from 0 to 4°C and in July, from 8 to 16°C. The polar night and polar day periods are relatively short.

The seasonal cycle of development of *C. vicina* is closely correlated with the traditional local business, catching and drying of cod fish that spawn there in March–April. The fly larvae damage the drying fish causing a great economic loss (1–2 million euros a year) and therefore the fly phenology has been thoroughly studied (Aak et al., 2011a, 2011b). It was demonstrated that larvae, puparia, and adults of *C. vicina* could overwinter on the Lofoten Islands and thus flies could be found over the whole season. A small spring peak of activity is caused by flies that overwintered at the adult stage and individuals emerged from overwintered puparia. These flies lay eggs on raw fish; larval development is slow because of low temperature (average temperatures of April–June vary from 2 to 10°C). In June the larvae terminate feeding and move from fish into soil; adult flies emerge in June (the average temperature is 13.6°C). In warm summers, when the temperature exceeds the local one at least by 2°C, the flies have time to mature and to lay eggs in the same season, i.e., to produce the second generation. Otherwise, they enter reproductive diapause, overwinter, and reproduce the next spring. The above-cited authors believe that most of the progeny that was produced during the first summer and autumn develop without larval diapause, pupariate in winter, and adults emerge in the next spring. However, our experiments showed that if larvae of *C. vicina* from the Lofoten Islands develop at a temperature of 8–10°C and lower, they are able to enter diapause of various intensities. Spontaneous reactivation of such larvae occurs during 2–5 months after oviposition but at higher temperatures it may be accelerated.

It is particularly interesting to compare photothermal responses controlling larval diapause in *C. vicina* from the Lofoten Islands and in the earlier studied (Vonigradova, 1991) population from Murmansk (69° N, 33° E). These regions are located at the same latitude, but their climates are substantially different, particularly in frost duration and severity. The average air temperatures of April–October are rather similar in the two regions and vary, depending on the month, from 2 to 12.6°C, but the autumn and winter temperatures are markedly different. In Murmansk, the frost period with average monthly temperatures from –3 to –11°C lasted 6 months, whereas on the Lofoten Islands on average monthly temperatures during this period are above zero and vary from 0.2 to 7.1°C. Photoperiodic responses of *C. vicina* populations from Murmansk and from the Lofoten Islands are very similar and may be characterized by a high inclination to diapause which is observed even in the progeny of

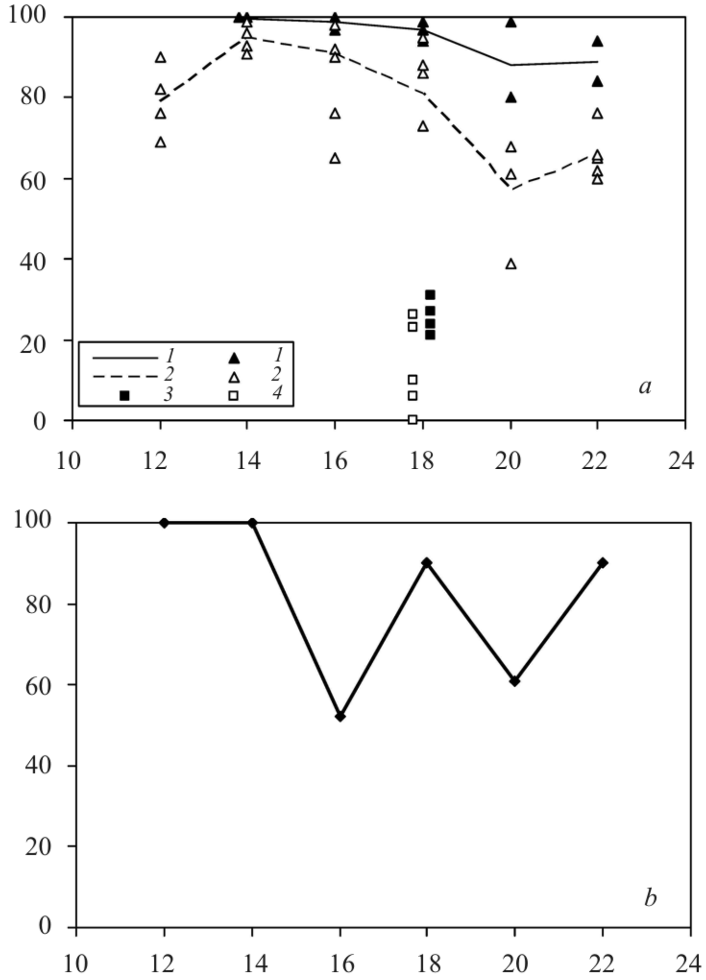

Fig. 5. The proportion of diapausing larvae of *Calliphora vicina* under different thermal regimes of larval development and different photoperiodic conditions of rearing of the maternal females. The horizontal axis, photoperiodic conditions of rearing of the maternal females (day length, h). The vertical axis, the proportion of diapausing larvae in the progeny (%); (*a*) individuals from the Lofoten population; the thermal regimes of larval development: *1*, 8°C (symbols correspond to replicates, line shows the means); *2*, 10°C (symbols correspond to replicates, line shows the means); *3*, 12°C (replicates); *4*, 15°C (replicates); Symbols for temperatures of 12 and 15°C are slightly shifted along the horizontal axis; (*b*) individuals from the Murmansk population (larvae developed at 12°C, data from Vinogradova, 1991).

females kept under long light days (comp. Figs. 5*a* and 5*b*), although the larvae of the Murmansk population developed at 12°C and those of the Lofoten population, at 8 and 10°C. However, comparison of the time required for the termination of diapause (pupariation) after the temperature increase suggests that these populations are significantly different in the intensity of diapause. For example, when the diapausing larvae of *C. vicina* from Murmansk were transferred from 5 to 12°C, 20% of individuals, on average, pupariated during 20 days (Vinogradova, 1991), whereas 47% of *C. vicina* individuals from the Lofoten Islands pupariated during this period (Fig. 3*a*). After the transfer to 20°C, 35% of *C. vicina* larvae from Murmansk pupariated during 10 days and so did almost 100% of *C. vicina* larvae from the Lofoten Islands (Fig. 3*c*).

Geographical variations in seasonal adaptations of insects have long attracted the attention of scientists and therefore they have been thoroughly studied (Danilevsky, 1961; Tauber et al., 1986; Saunders et al., 2002; Saulich and Volkovich, 2004). Analysis of the geographical variations in the inclination for larval diapause and its parameters in 16 populations of *C. vicina*, distributed over a large territory (from 60 to 38°N and from 30 to 69°E) have revealed latitudinal and altitudinal gradients connected with the climate conditions (Vinogradova, 1980, 1986, 1991). It was shown that the intensity of diapause as estimated by the reaction to temperature increase strongly depended on the duration of the frost period. Evidently, it is this regularity which determines the difference in the intensity of larval diapause between the Lofoten and Murmansk populations. Adult diapause may be also explained by a relatively mild winter and by the absence of long-term and severe frosts. In addition, as suggested by the local scientists (Aak et al., 2011a, 2011b), it may be considered as an adaptation to larval feeding on fish which is caught and dried in early spring. Thus, the substantial differences in climate between the two regions located near the northern border of the geographic range of *C. vicina* determine different strategies of the seasonal adaptation of this species.

CONCLUSIONS

(1) Photothermal regulation of the rate of development and of the induction of larval diapause was first experimentally studied in the arctic population of *Calliphora vicina* from Northern Norway (the Lofoten Islands). The rate of development from egg to puparium can be approximated by linear regression. The thermolability coefficient is 0.625 ± 0.024, the lower thermal threshold of development is 4.5 ± 0.6°C, and the sum of effective temperatures is about 160 degree-days.

(2) The maternal photoperiodic response induces larval diapause in a considerable fraction of individuals only when larvae develop at temperatures from 4 to 10°C and a high proportion of diapausing larvae may be found even in the progeny of females which developed under the long day conditions.

(3) The intensity of larval diapause is very variable, which is manifested in the separation of diapausing larvae into two fractions differing in the time of pupariation both during spontaneous reactivation and during reactivation induced by the temperature increase.

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