COMPARATIVE STUDIES OF TWO NORTH AMERICAN MOSQUITO SPECIES, *CULEX RESTUANS* AND *CULEX SALINARIUS*: RESPONSE TO TEMPERATURE AND PHOTOPERIOD IN THE LABORATORY

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ABSTRACT. The effect of temperature on the rate of adult eclosion, blood digestion, and ovarian development and the effect of temperature and photoperiod on ovarian follicle size in unfed females was studied in colonized strains of 2 North American mosquito species: *Culex restuans* Theobald and *Cx. salinarius* Coquillett. At 20° and 25° C the processes studied proceeded at about the same rate. At lower temperatures, however, rates were significantly slower in *Cx. salinarius*. Both the temperature and photoperiod to which females were subjected from the pupal stage until 6–8 days post-eclosion influenced the development of ovaries in *Cx. restuans*: a combination of 15° C and a photophase of 8/24 hours resulted in the ovaries remaining in a diapause condition. In *Cx. salinarius*, photoperiod had no effect on ovarian development and ovaries did not undergo diapause under any treatment combination, although the rate of development was slower at lower temperatures so that 15° C follicles were smaller when measured at 6–8 days post-eclosion. The ecological significance of these findings is discussed.

Culex restuans Theobald and *Cx. salinarius* Coquillett are 2 common Nearctic mosquitoes whose ranges largely overlap. Adults of the former species emerge earlier in the spring and persist longer into the fall. Moreover, the range of *Cx. restuans* extends farther north. In an earlier paper (Eldridge et al. 1972) we described the seasonal geographic distribution of these 2 species and presented data which showed interspecific differences in blood-feeding patterns and ovarian development in response to various combinations of temperature and photoperiod in the laboratory. *Cx. restuans* females showed a marked reduction of blood-feeding and exhibited ovarian diapause (=gonotrophic dissociation of some authors) in response to a combination of short photophase and low temperature. *Cx. salinarius* females showed a significant reduction of blood-feeding under short photophase conditions, but only at the lowest conditioning temperatures (15° C) and did not exhibit ovarian diapause. The results suggested that both species underwent true diapause permitting overwintering in the adult stage, but that qualitative and quantitative differences in their response to environmental conditions contributed to the differences in geographic range and phenology.

This paper is an extension of the earlier study and presents observations on the effect of temperature and photoperiod on ovarian follicle growth in non-blood-fed female mosquitoes of the 2 species as well as the influence of temperature upon rates of adult eclosion, blood digestion, and ovarian development. We sought differences between the species which would be consistent with their differences in seasonal and geographic distribution.

MATERIALS AND METHODS

Cx. salinarius females were obtained from our self-mating laboratory colony established in 1969 from larvae collected in a swamp near Pocomoke City, Maryland. The *Cx. restuans* females were reared from egg rafts collected in rainwater-filled stainless steel pans placed outside our laboratory in Washington, D.C. during the spring months. Egg rafts were constantly present in the pans from April through June. Egg rafts were isolated after collection and maintained in individual vials until specific identification

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of larvae was made. Other than this, larval rearing procedures were the same for both species.

Simulated environments were produced in modified BOD incubators. Details of the light sources and temperature compensation system have been described previously (Eldridge et al. 1972).

In all cases, larvae were reared at a temperature of 27° C and a photoperiod of 16:8 L:D. Separation into experimental groups was made randomly at the time of pupation. Other experimental details are given under the results of the particular experiment involved.

RESULTS

RATE OF ADULT ECLOSION AT DIFFERENT TEMPERATURES. In this experiment, a large number of pupae of both species were collected within 24 hr of pupation. The collection of pupae was made at the mid-point of time of pupation in the larval pans. Although the pupae were a mixture of males and females, the latter predominated. Each batch of pupae was randomly divided into 4 groups of 100

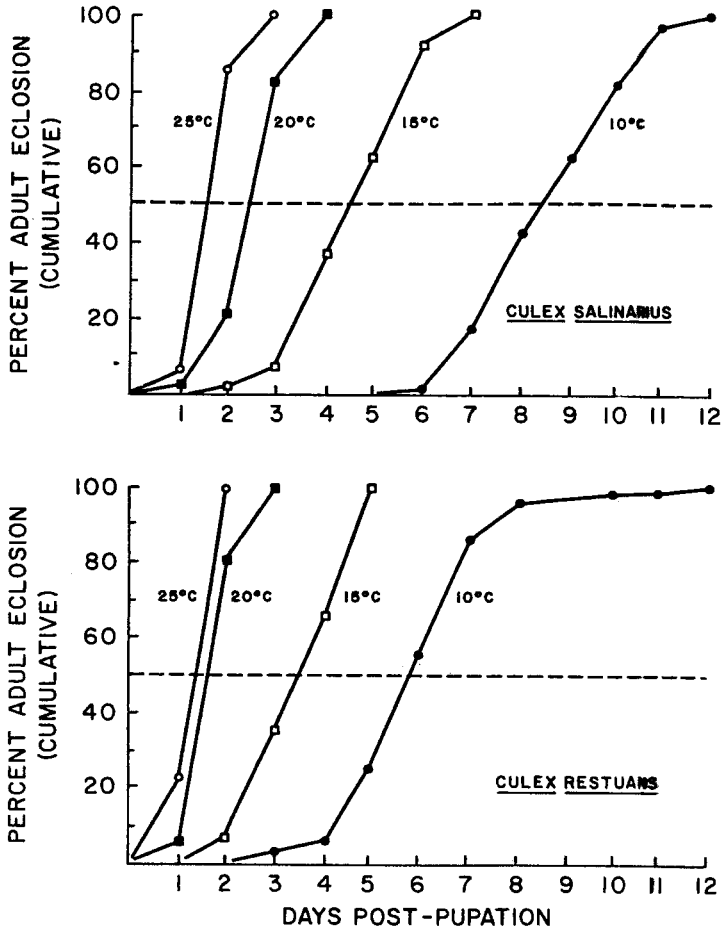


Fig. 1. Rate of adult eclosion for *Culex salinarius* and *Cx. restuans* at indicated temperatures.

pupae each and placed in cages in incubators at 4 different temperatures: 10°, 15°, 20°, and 25° C. Photoperiod was 16:8 L:D throughout the experiment. Emerged adults were removed and recorded daily.

The results are shown in Fig. 1. All pupae eventually underwent ecdysis, even at 10° C. At 25° C, 50% of adult eclosion for both species had occurred by day 2 post-pupation (when pupae were 48–72 hr old). At 20° C, however, 50% of eclosion for *Cx. restuans* had occurred by the same day as at 25° C (day 2), whereas in *Cx. salinarius* it occurred a day later. At 15° C, 50% of eclosion occurred later for both species, but again a full day later in *Cx. salinarius* than in *Cx. restuans*. At 10° C, the median time of eclosion is approximately 9 days for *Cx. salinarius*, 6 days for *Cx. restuans*.

RATE OF BLOOD DIGESTION. A large batch of 3–4 day old females of each species was provided a shaved chick [2] as a blood meal source. Females which took a complete blood meal were randomly divided into 4 groups and placed in cages in incubators at the following temperatures: 10°, 15°, 20°, and 25° C. Photoperiod was 16:8 L:D throughout the experiment. Two to four females were removed from each treatment daily. The status of blood digestion was estimated by the Sella method (Sella 1920). Each female so removed was then dissected and the length of the ovary measured as an indication of the degree of ovarian development (*vide infra*). Rates of blood digestion are shown in Fig. 2. The rates of digestion for both species were similar at both 20° and 25° C. At 10° and 15° however, blood digestion took about 48 hr longer in *Cx. salinarius*.

RATE OF OVARIAN DEVELOPMENT. As mentioned above, the same bloodfed females removed for the purpose of estimating rate of blood digestion were also dissected to determine the degree of ovarian development. The results of these dissections are shown in Fig. 3. The overall pattern seen in the previous experiments is evident. Ovarian development proceeded at ca. the same rate in both species at 25° C, and it proceeded more slowly at lower temperatures in *Cx. salinarius*. The lower the temperature, the greater was the disparity in rates. The response to temperature in *Cx. restuans* was graded, however, whereas in *Cx. salinarius* there was a distinct break between rates at 20° C and 15° C.

OVARIAN FOLLICLE SIZE IN NON-BLOOD-FED FEMALES HELD UNDER VARIOUS COMBINATIONS OF TEMPERATURE AND PHOTOPERIOD. A large group of female pupae of each species was divided at random into 4 groups and placed in incubators maintained under the following conditions: 25° C/16:8 L:D; 25° C/8:16 L:D; 15° C/16:8 L:D; and 15° C/8:16 L:D. After emergence, females were provided only 5% sucrose. After 6–8 days of adult life, 15 or more females were removed from each treatment and dissected in physiological saline. Both ovaries of each female were examined and 10–15 ovarioles of each measured using an ocular micrometer with a compound microscope. A mean value for each female was recorded. The stage of development for the ovarioles examined was also recorded, using the system of Christophers', as modified by Kawai (1969). Percentages of females from each treatment having ovarioles containing follicles in various measurement classes and in various stages of development are shown in Figs. 4 and 5. The results are presented quantitatively for comparison, but it is more convenient to discuss the development in terms of the arbitrarily defined stages of Christophers/Kawai. In *Cx. pipiens pallens* ovaries of gonoactive females develop to stage Ib before a blood meal, not past stage N in diapausing females (Oda 1971).

[2] In conducting the research described in this report, the investigators adhered to the Guide for the Care and Use of Laboratory Animals DHEW Publication No. (NIH) 73–23, as prepared by the Institute of Laboratory Animal Resources, National Research Council.

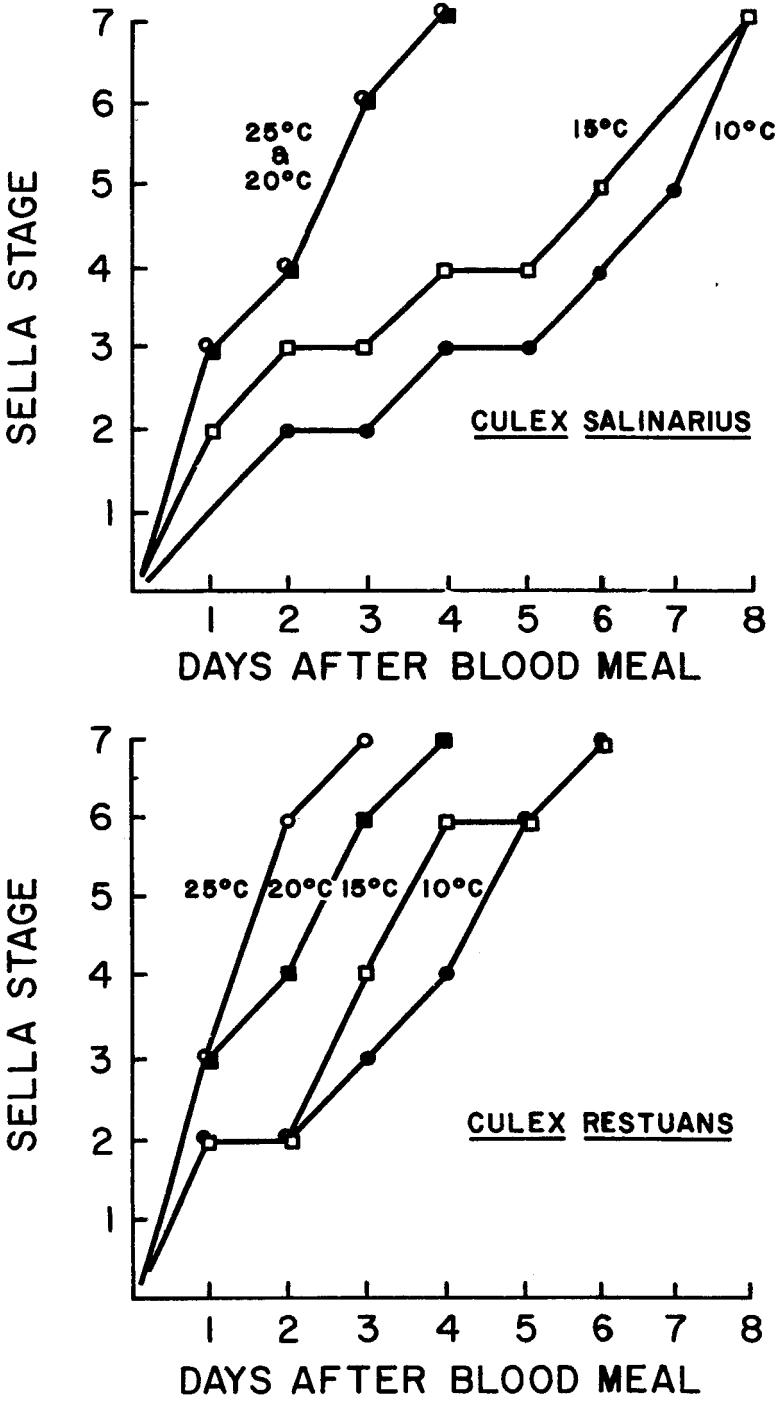
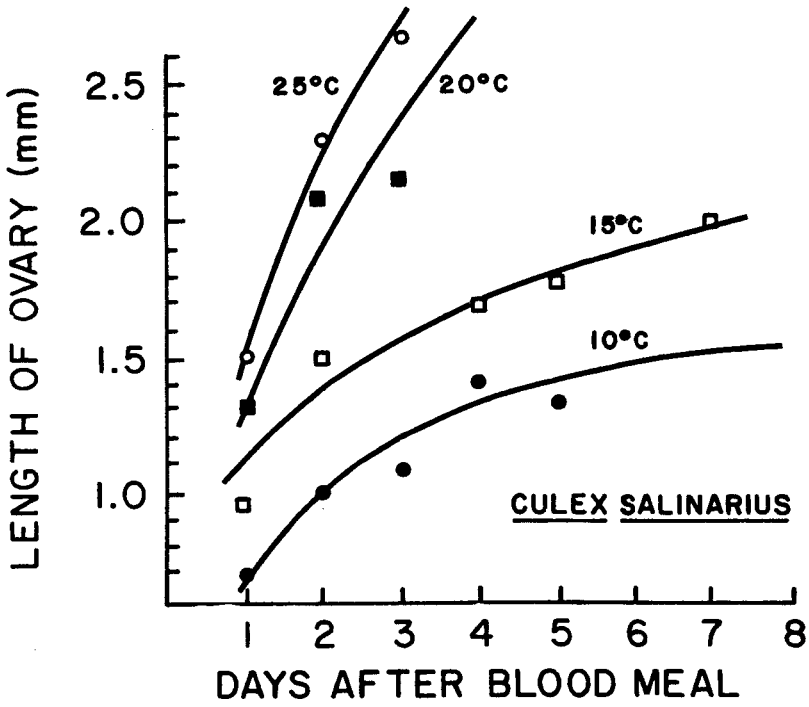


Fig. 2. Rate of blood digestion for *Culex salinarius* and *Cx. restuans* at indicated temperatures.



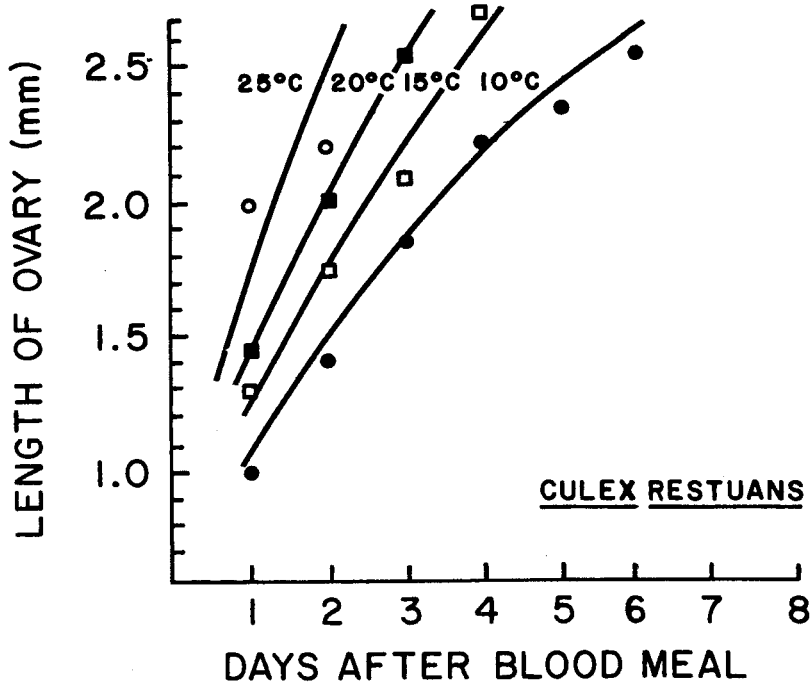


Fig. 3. Rate of ovarian development for *Culex salinarius* and *Cx. restuans* at indicated temperatures.

In females of *Cx. restuans* maintained under conditions of short photophase and low temperature (15° C/8:16 L:D), most ovaries remained in a diapause state (stage N). At low temperature and long photophase, however (15° C/16:8 L:D) follicles of most ovaries developed to the resting stage from which full ovarian development will occur after a blood meal (stage Ib). At 25° *Cx. restuans* follicles devel-

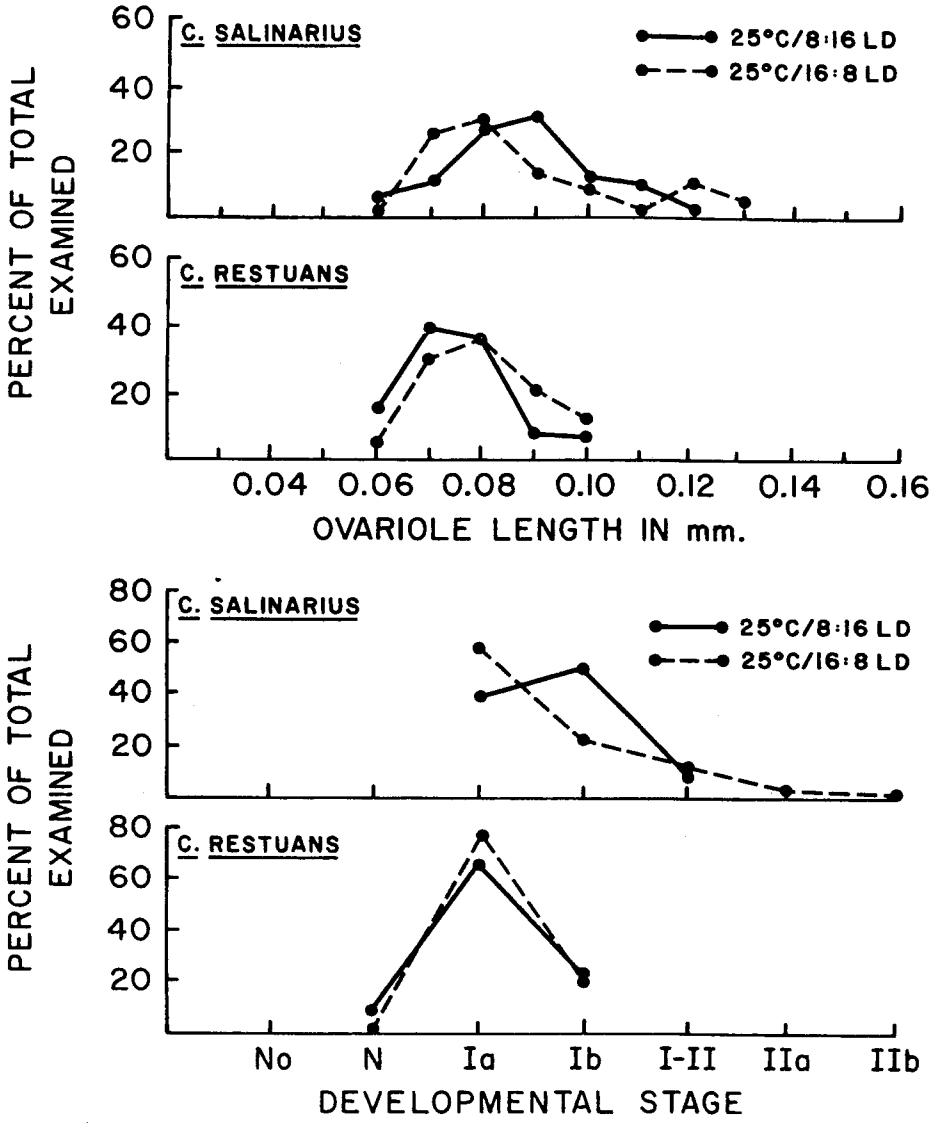


Fig. 4. Ovarian follicle size (above) and developmental stage (below) for unengorged *Culex salinarius* and *Cx. restuans* held under the indicated temperature and photoperiods.

oped equally under both photoperiod regimes, but did not attain as large a size, on the average, as did long photophase follicles at 15° C. Furthermore, more females maintained at 15° C/16:8 L:D had follicles reaching stage Ib than did any of the females held at 25° C at either photoperiod.

In *Cx. salinarius*, no photoperiod influence on follicular development could

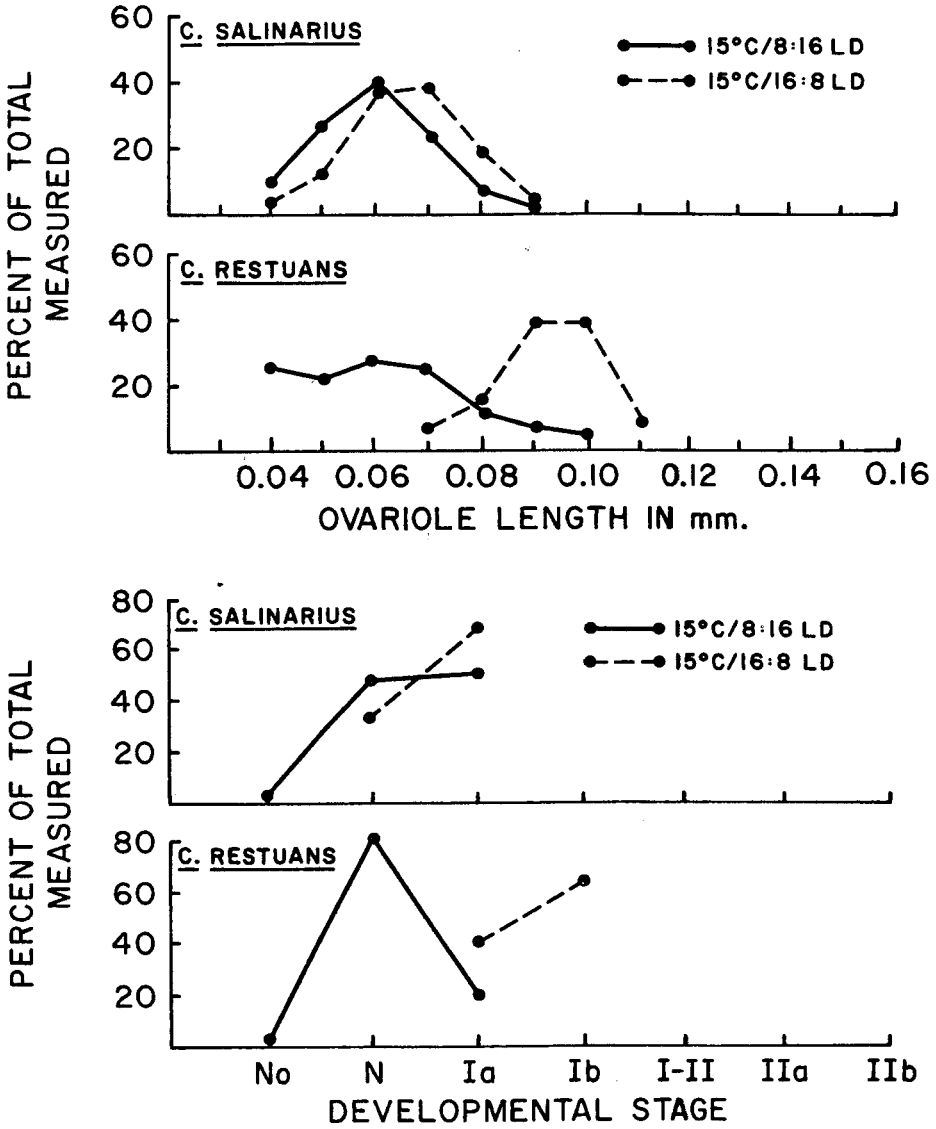


Fig. 5. Ovarian follicle size (above) and developmental stage (below) for unengorged *Culex salinarius* and *Cx. restuans* held under the indicated temperature and photoperiods.

be seen. The influence of temperature, however, was striking. At 15° C, 30–50% of the ovaries of females examined had follicles in the diapause state. Most ovaries had follicles in stage Ia, but none had developed past this point. At 25° C, however, all ovaries had follicles which had developed at least to stage Ia and some which had gone to stage I-II. This temperature influence is also evident in the data shown in Fig. 3.

DISCUSSION

The results presented here and those presented in an earlier study (Eldridge et al. 1972) suggest that the physiological events accompanying overwintering in these 2 species differ considerably, at least in mid-Atlantic U.S. populations. The higher rates observed at low temperatures in the 3 phenomena observed are consistent with the earlier appearance and later persistence of *Cx. restuans*. At the relatively cool spring and fall temperatures, the higher developmental rates in this species give it an advantage in competition with *Cx. salinarius* in common environments. Apparently, mid-Atlantic populations of *Cx. salinarius* do not undergo ovarian diapause in response to autumn photoperiod conditions. The effect of cool temperatures on ovarian development is probably one of retardation rather than arrest, but this needs further investigation. The species does apparently overwinter in the adult stage, however, in the Maryland-Virginia area of the U.S. After intensive searching, we captured a single female *Cx. salinarius* in January of 1973 on Assateague Island, Virginia in a livestock shelter open on one side. This is one of the few instances of this species being collected during the winter months above 35° N latitude.

The differences in physiological response to photoperiod and the more northern range of *Cx. restuans* are consistent with the hypothesis that an ovarian diapause induced by a short photophase is associated with an adult overwintering mechanism which permits survival under very stringent winter conditions. Implicit in this hypothesis would be that the physiological differences would hold even in populations of *Cx. salinarius* at the northern limit of the range of the species, although one would expect some northern extension during the summer months past the point where the females could survive winter conditions. If this is the case, *Cx. restuans* would survive the winter in a dormant state classified by Mansingh (1971) as diapause; *Cx. salinarius,* in oligopause. To test how well these species fit the criteria presented by Mansingh, especially that of "tolerance to adversity," samples of the species should be tested for tolerance to simulated winter conditions in the laboratory.

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