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MAJOR ENVIRONMENTAL FACTORS INDUCING THE TERMINATION OF LARVAL DIAPAUSE IN *CHAOBORUS AMERICANUS* JOHANNSEN (DIPTERA: CULICIDAE) ¹

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Diapause occurs in a great variety of arthropods and characteristically involves a cessation of differentiation. The developmental arrest may either encompass the whole animal as in egg, larval, and pupal diapause, or reside primarily in the germ cells, as in adult reproductive diapause. Arrest is usually accompanied by a metabolically quiescent state, and often involves the production of protective enclosures such as special egg cases, larval hibernacula, or pupal cocoons. However, in temperate climates, some immature aquatic insects overwinter in a state of diapause but appear as active as non-diapausing summer animals. Examples include *Metriocnemus* (Paris and Jenner, 1959), *Chironomus* (Engelmann and Shappirio, 1965), and *Chaoborus*, the subject of the current paper.

Chaoborids are noted for their tracheal air bladders (probably hydrostatic organs, Damant, 1924) and their voracious appetite. Lacking any thoracic appendages, chaoborids capture prey with prehensile antennae and swallow them with minimal mastication. *C. americanus* larvae in the vicinity of Ann Arbor, Michigan, swim beneath the winter ice of shallow ponds and kettle holes. A major question thus arises concerning the environmental factors involved in the maintenance and termination of a developmental arrest in this otherwise active animal.

MATERIALS AND METHODS

Animals in the terminal larval instar were obtained November 11, 1965 and January 22, 1966 from a small stagnant woodland pond in Ann Arbor, Michigan. They were placed in Precision Scientific model 805 incubators at $5 \pm 1^\circ$ C equipped with 40 Watt cool white fluorescent lamps regulated by standard Lumenite timers. Animals from each day's collection were maintained *en masse* on short day (light:dark = 8:16) until used for experimentation. All experiments were run in pond water strained through at least three layers of cotton cloth at $25 \pm 1\frac{1}{2}^\circ$ C with 10 or 20 animals in a 3 oz jar, 25 in a 7 oz jar, or individually in $\frac{1}{2}$ oz flint glass creamers (Table I). Long (light:dark = 17:7) or short (light:dark = 8:16) daylength was provided. Fed experimental animals received an excess supply of *Culex pipiens* larvae.

The resumption of development may first be discerned by the appearance of a

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TABLE I
Summary of food and photoperiod experiments

	n	Ct	Exp	No/Con	Size Con	%P
Long day, fed	51	1/23/66	1/24/66	20,20,10	4 oz	94
	51	1/23/66	2/7/66	25	7 oz	98
	50	1/23/66	2/18/66	1	$\frac{1}{2}$ oz	98
	48	1/23/66	9/4/66	1	$\frac{1}{2}$ oz	92
Long day, starved	50	11/20/65	1/22/66**	20,20,10	4 oz	20
	50	1/23/66	2/7/66***	25	7 oz	50
	49	1/23/66	2/18/66	1	$\frac{1}{2}$ oz	39
	54	1/23/66	8/7/66	1	$\frac{1}{2}$ oz	4
	39	1/23/66	8/31/66	1	$\frac{1}{2}$ oz	11
	46	1/23/66	9/4/66	1	$\frac{1}{2}$ oz	4
Short day, starved	50	11/20/65	1/22/66	20,20,20	4 oz	0
	50	1/23/66	1/23/66	25	7 oz	0
	51	1/23/66	4/4/66	1	$\frac{1}{2}$ oz	6
	42	1/23/66	8/7/66*	1	$\frac{1}{2}$ oz	5
	40	1/23/66	11/1/66	1	$\frac{1}{2}$ oz	5
Short day, fed	50	1/23/66	1/31/66	20,20,10	4 oz	2
	50	1/23/66	2/7/66	25	7 oz	4
	48	1/23/66	4/20/66	1	$\frac{1}{2}$ oz	44
	47	1/23/66	8/7/66*	1	$\frac{1}{2}$ oz	47
	47	1/23/66	11/1/66	1	$\frac{1}{2}$ oz	49

All experiments run at 25°C using *Culex pipiens* for food. Short day, 8:16; long day, 17:7; n, sample size; Ct, date caught; Exp, date placed on experimental conditions; No/Con, number of animals in each container; Size Con, size of containers used in fluid ounces; %P, per cent pupating. All experiments were run until all animals either pupated or died except *, **, and *** which were terminated after 26, 34, and 38 days, respectively.

third pair of air sacs on the anterioventral portion of the animal. These sacs become the exterior air sacs of the pupa and appear about eight hours prior to the pupal moult. Pupation itself was used as an indicator of development since: (1) the internal sacs were not always readily identifiable *en masse* or in large numbers of individual containers, (2) there is a striking transformation at pupation due to change in external form and change from a horizontal to vertical orientation, (3) very few larvae in which the pupal air sacs appeared failed to pupate, and (4) ecdysis and orientation change at pupation are almost instantaneous.

EXPERIMENTAL RESULTS

(1) Long-term maintenance of stocks

Animals maintained in the short day, low temperature, starved stocks would eat if fed; but, no larvae, fed or starved, ever developed under these conditions. Animals appeared to remain healthy from November 1965 until September–October 1966 when they began to die in large numbers. Some animals, however, were still alive through December 1966.

(2) *Effect of warming*

Sample populations of 40–51 animals were removed from the stocks and placed under short day conditions without food at 25° C. In all cases, no more than 6% development was observed (Fig. 1b).

(3) *Effect of food and long day*

Sample populations of 48–51 animals were removed from the stocks, placed under long day conditions at 25° C, and fed. 92–98% of these animals developed in each case (Fig. 1a).

(4) *Effect of food alone or long day alone*

When sample populations of 39–54 animals were removed from the stocks and placed on long day but without food, a wide spread of 4–50% development resulted. This distribution fell into two distinct groups: one set of 20–50% pupating popula-

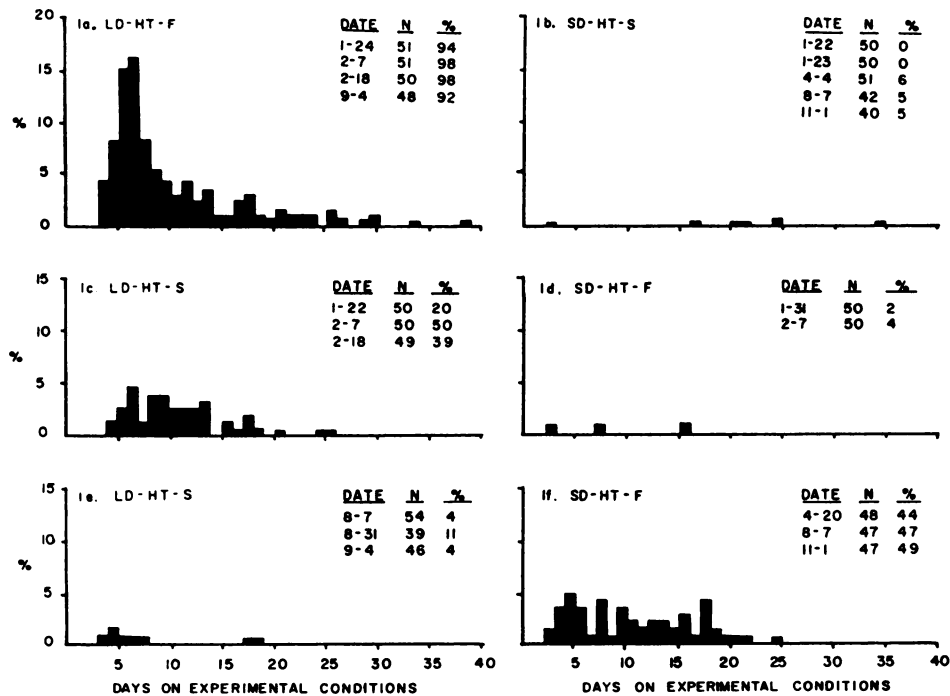


FIGURE 1. Pupation of *Chaoborus* under different combinations of light and photoperiod. Ordinate: average per cent of animals pupating that day; abscissa: number of days on experimental conditions; LD, long day; SD, short day; HT, 25° C; F, fed; S, starved; Date, date in 1966 that the experiment was initiated; N, sample size of replicate; %, total per cent pupation of that replicate.

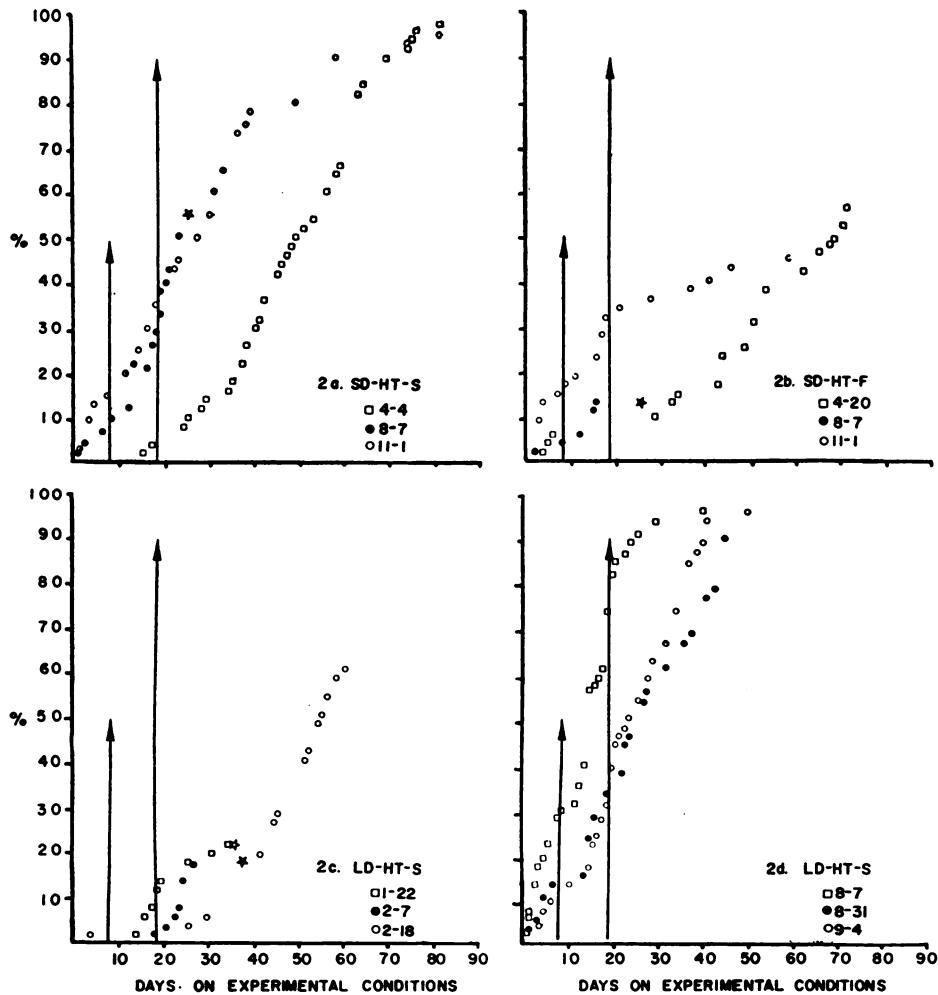


FIGURE 2. Death distribution of *Chaoborus* under different light and food combinations. Vertical arrows indicate average 50% (shorter) and 90% (taller) development under long day fed conditions. A star indicates that the experiment was not carried to completion but was terminated on that day. Ordinate, cumulative per cent dying; abscissa, number of days on experimental conditions. Other abbreviations as in Figure 1.

tions in January and February 1966 (Fig. 1c), the other set of 4-11% pupating populations in August and September 1966 (Fig. 1e).

Likewise, sample populations of 47-50 animals fed but kept on short day also showed a wide spread of 2-49% development with distributions falling into two distinct groups. In this case, however, low percentage pupating populations were observed in January and February (Fig. 1d) and intermediate percentage pupating populations in April, August, and November (Fig. 1f).

(5) *Induction time*

The rapid response of animals on long day with food prompted the question of how many long day cycles with food were necessary to induce development. Consequently, two experiments were run, one in April and one in August. In each case, animals were removed from the stocks and placed on long day with food at 25° C for 1, 2, 3, or 4 days. The food was then removed and animals were placed on short day at 25° C and observed until all animals either pupated or died. Fed and starved controls on short day were run as well. One long day cycle with food was found to induce development in 30–35% of the animals. Two or more cycles induced 42–65% development but a wider scatter was observed (Fig. 3). The continuously fed controls on short day showed 44 and 48% development but reached this level very gradually after prolonged feeding for two weeks.

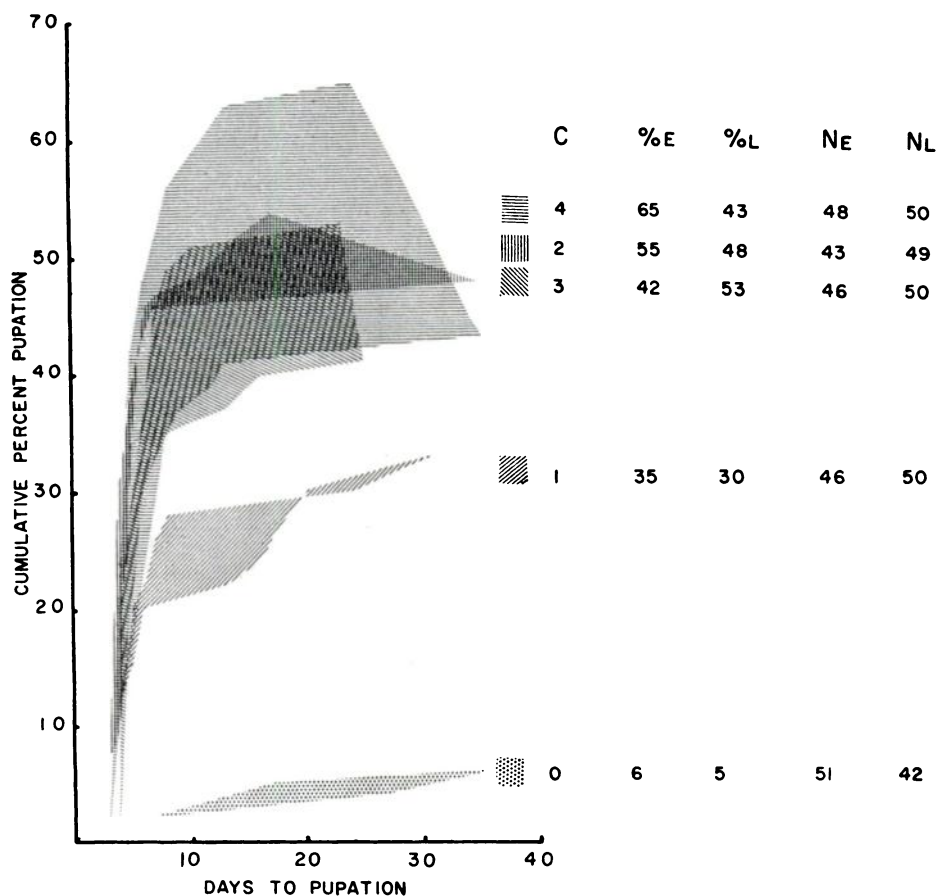


FIGURE 3. Induction time, showing ranges of duplicate experiments. C, number of cycles long day fed before short day starved; %E and %L, total per cent pupation in April and August experiments, respectively; Ne and Nl, sample size of April and August experiments, respectively.

DISCUSSION

C. americanus overwinters in a state of diapause rather than a thermally maintained quiescence. This conclusion follows from the observed lack of a developmental response at 25° C under three conditions: first, short day without food throughout the year (Fig. 1b); second, short day with food in the winter (Fig. 1d); and third, long day without food after prolonged chilling in the laboratory (Fig. 1e). There remains the possibility that animals in all but the long day fed experiments may not have developed due to differential mortality. An examination of the recorded death distributions (Fig. 2), however, indicates that only in one long day starved experiment in August (Fig. 2d) could the absence of development be explained by a high death rate.

Developmental dependence upon long day for a hibernating animal is neither new nor surprising. The interesting aspects of response to the environment in *C. americanus* are the unusually rapid response and the interrelationship between long day and food. A comparison of long day without food with short day fed experiments in winter (Fig. 1c-d) and in spring and summer (Fig. 1e-f) shows that long day is required for any significant development in the winter but not in the spring or summer; conversely, food is required for any significant development only in the spring and summer but not in the winter. There thus appears to be a shift in the major cue relied upon for the resumption of development.

The physiological basis for this observed shift may be the result of adaptation to a temperate environment. Shallow ponds such as those in which *C. americanus* characteristically occur (Cook, 1956) undergo repeated thawing and refreezing in the spring, accompanied by rapid plankton blooms and gradual predator buildups. For an aerial insect overwintering in such an environment, an extremely important consideration is the chance refreezing of the pond which could produce drowning at adult emergence or act as a barrier to oviposition. Hence, it is proposed that they depend upon the most reliable geophysical phenomenon, light, until a time of the year, determined by selection, during which refreezing becomes a lesser danger than consumption by predators or a lack of food. While refreezing may select for delayed termination of diapause, two factors would select for rapid development. First, a prolonged larval stage would be increasingly subject to predation, especially by Hemiptera and Odonata. Second, a prolonged larval stage may decrease the number of summer generations, thereby decreasing the potential of the individual to mix its genotype.

Although light may be the major stimulus in the winter and food in the spring or summer, it should be emphasized that the effect of both food and long day together is not additive but synergistic (Fig. 1). This type of response in most insects may be subject to speculation by anyone familiar with insect endocrine systems; but, *Chaoborus* has a distinctive neurosecretory system. In the larval brain, Füller (1960) found three sets of neurosecretory cells, of which the middle set gave off axons to the ventral abdominal nerve cord in which there were definite neurosecretory tracts, and the posterior set gave off axons to the corpus cardiacum and corpus allatum. Abdominal stimulation at room temperature of animals previously maintained at 5° C produced a general discharge of neurosecretory material from both sets of neurosecretory cells and the corpus cardiacum. The greatest amount of secretion was observed in larvae in which the pupal air sacs were most

highly developed, *i.e.*, immediately prior to pupation. Thus one may suggest that while light is influencing the brain directly in a manner illustrated by Williams (1963), food may be acting via the ventral abdominal ganglia immediately ventral to the gut, with the two stimuli being integrated by the brain. Indeed, preliminary experiments now indicate that food is acting via a neuro-endocrine reflex independent of nutrition.

Chaoborus differs significantly from other "active" diapausing insects such as *Chironomus* and *Metriocnemus* in that the latter feed upon detritus but *Chaoborus* is primarily a carnivore. *Chironomus* maintained at 5° C and short day in the laboratory, for example, terminate diapause in early winter only when exposed to warm temperature and long day (Engelmann and Shappirio, 1965); in late winter without long day but with warm temperature (Shappirio, personal communication); and in mid-summer spontaneously on short day at 5° C (Bradshaw, unpublished observations). Food, however, was present at all times and was contributing an unknown inductive effect. *Chaoborus* has thus provided a unique opportunity to study the food component in "active" diapausing insects.

SUMMARY

1. The termination of larval diapause in *C. americanus* is cued by simultaneous long day and food.
2. One long day cycle with food elicits development in a significant proportion of the population.
3. A shift in the major developmental stimulus from long day during the winter to food after prolonged chilling in the laboratory was observed and is hypothesized to be an adaptation to life in shallow ponds in temperate climatic regions.
4. The action of food may occur via the ventral abdominal nervous system as described by Füller (1960) with ultimate neuro-endocrine control of development residing in the brain.

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