The effects of photoperiod and temperature on the induction and termination of reproductive resting stage in the freshwater amphipod *Hyalella azteca* (Saussure)

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Three experiments, one on the induction of reproductive resting stage and two on its termination, were performed to determine the effects of various combinations of temperature and photoperiod on the induction and termination of reproduction in *Hyallella azteca*. These showed that only photoperiod determined whether reproduction was continued or discontinued but that temperature influenced the rate of all changes. The 12 L – 12 D photoperiod terminated reproduction for at least 4 months at temperatures between 12 and 25°C in animals previously reproducing at a 16 L – 8 D photoperiod. The 12 L – 12 D photoperiod also induced reproduction at temperatures between 16 and 26°C in animals previously held in a reproductive resting stage in dim light. In contrast, the 16 L – 8 D photoperiod induced and maintained reproduction consistently, and the 8 L – 16 D photoperiod halted reproduction and maintained a reproductive resting stage consistently. The induction of reproduction occurred faster at higher temperatures.

It is believed that although photoperiod is the main cue in the induction and termination of reproduction, active reproduction takes place when environmental temperatures are 20 to 26°C, since optimum reproduction and growth rates occur in this range. The adaptive advantage and the biogeographic variability of the photoperiodic response are discussed.

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On a étudié les effets de diverses combinaisons de température et de photopériode sur le déclenchement et l'arrêt de la reproduction chez *Hyalella azteca* par trois expériences, une sur l'induction du stade de repos et deux sur la cessation de ce stade. Les résultats démontrent que seule la photopériode détermine la continuation ou l'arrêt de la reproduction; cependant, la température influence la vitesse de tous les changements. Une photopériode de 12 L – 12 O provoque l'arrêt de la reproduction pour au moins 4 mois entre 12 et 25°C chez des animaux se reproduisant à une photopériode de 16 L – 8 O. Une photopériode de 12 L – 12 O réussit aussi à provoquer la reproduction entre 16 et 26°C, chez des animaux maintenus en repos à une lumière diffuse. Par contre, une photopériode de 16 L – 8 O provoque invariablement une phase reproductrice continue; de même, la photopériode de 8 L – 16 O provoque l'arrêt de la reproduction et maintient l'état de repos. L'induction de la reproduction est plus facile à des températures élevées.

Bien que la photopériode soit le principal facteur de contrôle de la phase de reproduction, la reproduction est active à des températures de 20 à 26°C, températures où la reproduction est optimale et les taux de croissance les plus élevés. On discute des avantages adaptatifs et de la variabilité biogéographique des réactions de l'espèce à la photopériode.

[Traduit par le journal]

Introduction

Hyalella azteca is a small amphipod which breeds in many lakes, ponds, and streams in North America during the summer months (Bousfield 1958). It is an excellent animal for population studies because of its high reproductive capacity, manageable size, and detritivorous feeding habits. To define culture conditions for *H. azteca*, it was necessary to define conditions which induced and terminated reproduction. Also, since a reproductive resting stage is probably unique to north temperate populations of *H. azteca*, it was of biogeographic interest to define the physical factors related to it.

Reproduction in *H. azteca* is obligately sexual. During the summer, the females mate and release live young at regular intervals, as often as once every 8 days. At each mating, the female moults, releases the young fertilized at the previous

mating, and is refertilized. The young usually reach maturity after eight moults, which takes at least 30 days in total. Details of mating behavior are described by Kruschwitz (1972) and details of development are described by Geisler (1944). Both reproductive and growth rates depend on temperature (Geisler 1944; Bovée 1950). The reproductive resting stage, which typically occurs during the winter months, involves a complete cessation of egg development and reproductive behavior, not simply a slowing of these functions. It is not known whether a reproductive resting stage occurs only in the female or in both sexes. *Hyalella azteca*'s reproductive resting stage can occur at any time or never at all during the adult life stages.

In casual laboratory and field observations, it was observed that light intensity, photoperiod, temperature, and possibly food affected the reproductive rates of *H. azteca*. Photoperiod and temperature are believed to control diapause in several marine amphipods (Steele 1967), in many insects (Way and Hopkins 1950; Corbet 1956), and in copepods (Elgmork 1967) and were therefore the main factors studied. The effects of light intensity were examined briefly in one experiment only since it was felt that while low intensities were uncommon in *H. azteca*'s normal breeding season, they could occur under snow in spring and thus influence the induction of reproduction. The effect of food on reproduction was considered to be of secondary importance.

The terms 'photoperiod' and 'daylength' are used inconsistently in the literature and will therefore be defined for the purpose of this paper. 'Photoperiod' here means the relative lengths of alternating periods (in hours) of light (L) and darkness (D), for example, a '16 L – 8 D' photoperiod. 'Daylength' means the duration of light (in hours) in a 24-h day, for example, a '16 L' daylength.

Materials and Methods

Three experiments, one involving the termination of reproduction and two involving its induction, were performed. The experimental designs are outlined in Table 1. All experiments were performed in a two-dimensional, continuous-flow system of 25 tanks, 5 tanks high by 5 tanks wide, or in a subset of this system. Each tank was 40 × 20 × 15 cm, containing 11 cm water. Water was replaced at the rate of 2.2 $\ell\ h^{-1}$ (a 4-h replacement rate) in each tank. Temperature was controlled along each of the five rows of the tank system by adjusting the temperatures in five header tanks, and photoperiod was controlled along each of the five columns with timers.

Two fluorescent cool-white bulbs (Sylvania TS-15-WT-12) were located above the tanks to provide a surface intensity of 55 $\mu E\ m^{-2}\ s^{-1}$ (1 $\mu E\ m^{-2}\ s^{-1}$ = 9.52 ft-c in my laboratory). Tanks were separated from each other by shelves and curtains which allowed only dim light (< 9 $\mu E\ m^{-2}\ s^{-1}$) to escape at the edges. (Previous observations showed that low light intensities of 12 $\mu E\ m^{-2}\ s^{-1}$ prevented reproduction between 20°C and 30°C.) Tanks underwent a maximum diurnal variation of ±1°C about the stated experimental temperatures owing to heating from the lights. In experiment 3 (Table 1), light intensities were reduced to 18 $\mu E\ m^{-2}\ s^{-1}$ in one column of the grid by placing a sheet of $\frac{3}{8}$-in. (9.5-mm) Styrofoam on top of the tanks.

Animals were allowed to acclimate to experimental temperatures at a rate of 2°C per day. Experimental photoperiods were introduced without acclimation after the last tanks were adjusted to the desired temperatures. The mating levels during the temperature acclimation times were not monitored.

Oxygen levels were close to saturation at all times.

Animals used in experiments 1 and 2 were offspring of animals collected from the Rat River, Manitoba (49°19′ N, 96°57′ W), and those used in experiment 3 were offspring of those from Lake 103 at Erickson, Manitoba (Sunde and Barica 1975) (50°30′ N, 100°10′ W). Animals from the 2nd to the 10th laboratory generation were used. The laboratory cultures showed no obvious changes from the field stock except the loss of acanthocephalan parasites which only the field population contained.

Animals were fed either Tetra-Min B (Tropical Fish Food, Tetra-Werke, W. Germany) or a pressed and dried mixture of Tetra-Min B, Cerophyl (dehydrated cereal grass leaves, Cerophyl Laboratories, Kansas City, Kansas) and yeast,[1] *ad libitum* three times a week. All tanks developed periphyton and algal mats, which the animals also ingested.

Each tank contained about 5 mm of sand into which animals could burrow and a Hykro Fish Spawning Mat (Hykro Ltd., Denmark) for shelter.

All counts of mating pairs were made between 0900 hours and 1200 hours. Counts were made three times per week (Monday, Wednesday, and Friday) in experiment 1 and 7 days per week in experiments 2 and 3. Animals were believed to begin mating overnight and then continue to do so for several days. One exception may have been in tanks at 25°C and 26°C, where animals possibly mated within one night and were therefore not always observed mating (first footnote of Table 1).

The conclusions from these experiments were used to manipulate laboratory cultures for about 1 year. During this time, observations in ranges not tested in the experi-

[1]Mixture consisted of 40 g Tetra-Min B, 30 g Cerophyl 1, and 2 g dry yeast, which was moistened to form a paste, squeezed through a syringe into worms, and dried. This food required several days of decaying before the animals ingested it; therefore, it was difficult to monitor whether or not animals were fed sufficiently each day. It was used only for the first experiment performed (experiment 1, Table 1).

TABLE 1. Details of the three experiments performed

Experiment	No. animals per tank	Preexperimental conditions	Time held	Measurements and (or) manipulations	Experimental conditions	Time held	Measurements and (or) manipulations
1. Induction of reproductive resting stage	~100	20 tanks: all at 20°C and a 16 L daylength (reproducing conditions)	4 weeks	1. Animals were shuffled randomly between tanks after 3 weeks 2. Number mating per tank day was measured in weeks 3 and 4	20 tanks: four temperatures (10, 15, 20, 25°C) and five daylengths (4, 8, 12, 16 20 L)	8 weeks 3 weeks	1. Number mating per tank day was measured three times a week (days 2, 4, and 6)* 2. Presence or absence of mating was observed in each tank 3. Final numbers were counted
2. Termination of reproductive resting stage	~100	25 tanks: all at 10°C and an 8 L daylength (resting conditions)	4 weeks	None	1. 25 tanks: five temperatures (10, 12, 14, 16, 18°C) and five daylengths (4, 8, 12, 14, 16 L) 2. The same 25 tanks: five temperatures (16, 18, 20, 22, 24°C) and five daylengths (4, 8, 12, 14, 16 L)	3 weeks	The presence or absence of mating was observed in each tank†
3. Termination of reproductive resting stage	> 750	1. One 20-ℓ tank: 15°C and < 12 μE m^{-2} s^{-1} (resting conditions)	4 months	None	15 tanks: five temperatures (16, 18, 20, 23, 26°C) and two daylengths (8, 16 L) and one dim 16 L daylength at 18 μE m^{-2} s^{-1}	7 weeks	1. Mating pairs were counted and removed daily until the mating peak was over in all tanks. Animals from the bright 16 L and 12 L tanks were saved in identical tanks 2. Remaining animals were counted to determine mortality and returned to the same tanks
	50	2. 15 tanks: same conditions as above	1 week			2 months	3. The presence or absence of mating was observed

*It was very difficult seeing animals mating in 25°C cultures since algal mats formed, animals were small and relatively translucent, and mating was of short duration and therefore not always observed. The final number of animals found in the 25°C tanks at the 16 L and 20 L daylengths was considerably larger than the numbers found at the same daylengths at colder temperatures.

†When this first range of temperatures failed to induce reproduction within 3 weeks, the author thought that these low temperatures would never induce reproduction and raised some of the temperatures. With the second range of temperatures, reproduction occurred very quickly at all temperatures only in tanks with a daylength of 12 L and longer. Although the general nature of the results was apparent, the switch in temperatures made quantitative interpretation of the results difficult. The experiment was partially repeated as experiment 3.

ments were made occasionally. These are presented when they obviously supplement the results of the presented experiments.

Results

Induction of the Reproductive Resting Stage (Experiment 1)

The mating levels of the parent populations in all 25 tanks used in experiment 1 were cyclic and not synchronous with each other both before and after experimental conditions were applied. The sizes of the parent populations also differed among tanks. Animals were shuffled between tanks extensively to eliminate these differences up to 2 weeks before the experimental conditions were applied (Table 1). This mixing did not solve the above problems. The only meaningful measure of reproductive changes was believed to be the ratio of mating levels after 2 weeks to the mating levels before experimental conditions were applied. These are presented in Fig. 1. Preexperimental mating levels were 3 to 10 pairs mating per day.

Mating ceased in all cultures with 12 L and shorter daylengths within 11 weeks, while cultures at the 16 L and 20 L daylengths still underwent regular mating cycles (Fig. 1). Cultures exposed to 4 L daylengths did not enter even one mating cycle after experimental conditions were imposed. Cultures with 8 L and 12 L daylengths took longer to cease mating but did so completely before the end of the 11-week experimental period. Mating levels in the 16 L and 20 L tanks at 26°C must have been considerably higher than measured owing to various complications (first footnote of Table 1). It appeared that at the 16 L and 20 L daylengths, mating levels increased considerably more at 20 and 26°C than in cultures under 20°C (Fig. 1).

In other laboratory cultures, a 12 L – 12 D photoperiod maintained the reproductive resting stage for 4 months at several temperatures up to 26°C. After this, reproduction conditions were imposed by appropriate long daylengths, hence the possible duration of the reproductive resting stage could not be determined.

Termination of the Reproductive Resting Stage (Experiments 2 and 3)

In experiment 2 (Table 1), cultures at 12 L, 14 L, and 16 L daylengths began reproducing at all temperatures tested, while cultures at the 4 L and 8 L daylengths did not do so. The rates of the positive responses were obtained from

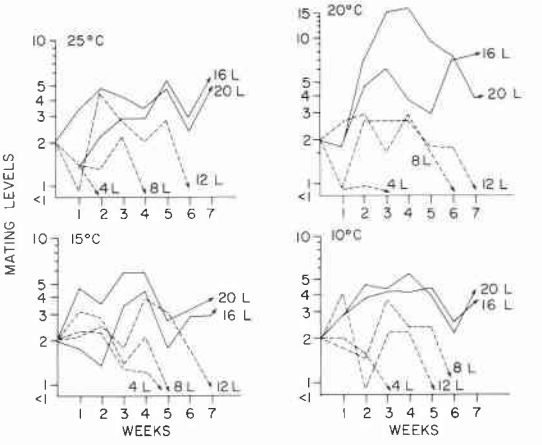

FIG. 1. Mating levels at various combinations of photoperiod and temperature during 7 weeks. Mating level = Number of animals observed mating in a week/number of animals observed mating per week before experimental conditions were applied. Three observations were made in each tank every week. L = hours of light.

experiment 3 (Table 1) because of complications noted in the second footnote of Table 1.

Both light and temperature affected the rate at which reproduction began (Fig. 2). In this figure, the results at 20, 23, and 26°C were combined since they were very similar at all three

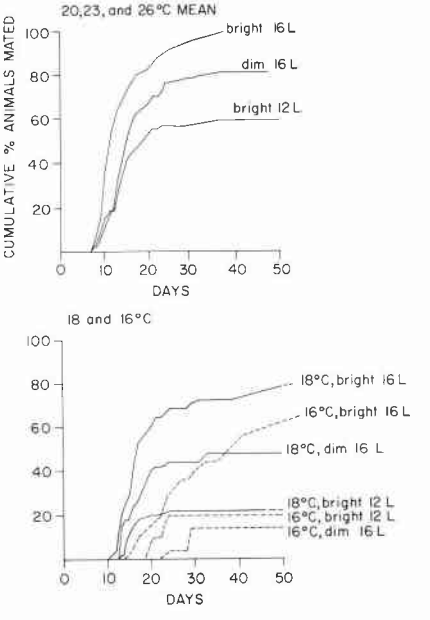

FIG. 2. Cumulative percentage of animals observed mating (and removed from cultures) under various combinations of temperature, light intensity, and photoperiod. L = hours of light.

temperatures. Mating was induced completely only in the bright 16 L – 8 D photoperiod tanks; it was induced to a limited extent in the dim 16 L – 8 D and bright 12 L – 12 D photoperiod tanks. After the first 7 weeks of the experiment, after which only qualitative observations were made, it was observed that mating continued only in the bright 16 L – 8 D tanks. Temperature influenced the rate of the induction of reproduction but not its occurrence.

Other laboratory cultures showed that the reproductive resting stage can be terminated by a 16 L daylength at 10°C in about 10 weeks. Diapause could not be terminated at lower temperatures; animals were completely immobile.

Discussion

In most temperate latitudes, photoperiod is a more reliable phenomenon than temperature. Therefore, any species with a photoperiodic response has an ensured reproductive season and a resting season even if temperatures are highly abnormal. Temperature is still important in modifying this response: if spring is warm, the reproductive season starts earlier than it would in a cool spring. In the fall, the reproductive resting stage is induced later at higher temperatures than at low ones, but it is always eventually induced. This ensures that females are gravid only during propitious times of the year.

The 12 L – 12 D photoperiod terminated reproduction for an indefinite length of time and induced reproduction only temporarily. This suggests that the actual 12 L daylength, rather than daylength change, is the critical factor in determining the reproductive resting stage.

In southern Manitoba, the 12 L – 12 D photoperiod is usually a 'cue' which precedes desirable reproduction temperatures in the spring and undesirable temperatures in the fall. Daylengths greater than 12 L precede desirable water temperatures by about 3 months so that *H. azteca* is probably physiologically ready to begin reproducing but is held back by low temperatures. In the fall, the 12 L photoperiod generally coincides with temperatures between 10 and 15°C. These temperatures still permit the development and release of the last brood before extremely cold winter conditions set in.

The low light intensity of 18 $\mu E\ m^{-2}\ s^{-1}$ which inhibited mating in *H. azteca* is highly unusual in daytime, even under a heavy amount of ice and snow (L. de March, personal communication). Light intensity therefore appears to be an important consideration only for laboratory culture.

The results of all experiments suggest that 20°C is an important temperature in both induction and termination of the reproductive resting stage, even though photoperiod is of overriding importance. In experiment 1, notably higher reproduction levels occurred at 20 and 25°C than at lower temperatures at all reproduction-maintaining photoperiods. In experiment 3, reproduction was induced at about the same rates at 20, 23, and 26°C but significantly slower at colder temperatures. Other observations to be reported by the author in a following paper also show that 20°C is a temperature important to reproduction: the rates of several processes such as the time between consecutive matings and the time to sexual maturity increase rapidly at temperatures under 20°C. In fact, several authors have concluded on the basis of field observations only that a temperature of 20°C and not photoperiod is the factor controlling the reproductive resting stage (Embody 1911; Geisler 1944; Cooper 1965). This was most likely due to the increased probability of observing mating pairs at temperatures over 20°C. In southern Manitoba, peak mating in the spring also occurs in conjunction with 20°C water temperatures. However, in these experiments where photoperiod and temperature were imposed in unusual combinations, the same populations were shown to respond only to photoperiod.

It is of interest to speculate on the biogeographic variability of the photoperiodic response of *H. azteca*. It is certainly not advantageous for all populations, which range geographically from Guatemala to Inuvik, N.W.T. (about 16° N to 68° N) (P. Stewart, personal communication), to have the same response to photoperiod and temperature. For example, Strong (1972) showed experimentally that his Oregon population did not respond to photoperiod. It is also conceivable that selection for a particular photoperiodic response in climates warmer than southern Manitoba is extremely rapid, perhaps 1 or 2 years. A warm winter or an abnormal amount of food in the winter in a normally resting population could exert heavy selection pressure against animals with a strong photoperiodic response. On the other hand, a very harsh winter in a population without a photo-

periodic response may selectively kill animals that use their energy for reproduction. It is possible that all populations contain animals with potentially abnormal responses since constant north–south genetic exchange takes place through transportation by waterfowl (Niethammer 1953; Rosine 1956; Maguire 1963).

The nature of the photoperiodic response may also depend on the extremes of daylength available at different latitudes. It is possible that many southern populations of *H. azteca* do not respond to photoperiod because photoperiod changes are small. The experiments presented here were performed on animals that experience extreme daylengths of 16 L in summer and 8 L in winter; in the results, 16 L was the daylength always related to reproduction, 8 L was the daylength always related to a reproductive resting stage, while the immediate response to the 12 L daylength was variable and depended on the direction of the change (Figs. 1 and 2). In extreme northern populations, animals may respond to the introduction of light alone rather than to photoperiod.

My populations differed from those of Strong (1972), Cooper (1965), and several other authors in another aspect besides the photoperiodic response: the largest adult sizes were considerably larger than theirs. Relationships between large size, overwintering temperatures, and diapause have often been observed in insects and in copepods and also exist here. These relationships will be discussed in another paper.

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