ENVIRONMENTAL FACTORS CONTROLLING REPRODUCTION OF THE AMPHIPOD *HYALELLA AZTECA*

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The effects of temperature, photoperiod, and mate stimulation on reproductive intervals were assessed in the laboratory. Field observations of sex ratios and the proportion of females carrying eggs provided comparisons for laboratory studies. Generally, within the ranges studied, higher temperatures, longer photoperiods, and pairing increase reproductive activity. Primarily, temperature regulates the reproductive activities investigated. Photoperiod and male stimulation affect specific reproductive activities.

INTRODUCTION

Temperature and light are often considered major exogenous determinants of the breeding cycles of crustaceans and other animals. (1). Geisler, Clemens, Hynes, and Cooper stressed the importance of temperature in the breeding biology of *Hyalella azteca* and other freshwater amphipod species (2, 3, 4, 5). Although there are indications that photoperiod is important in the control of reproductive activities of crustaceans, the mechanisms are not well understood (6, 7, 8, 9, 10, 11). In some amphipods the absence of the male has been shown to prolong female reproductive cycles by increasing the intermolt period and by delaying oviposition (12, 13).

Temperature, light, and pairing are indicated as possible important exogenous determinants of the breeding activity in amphipods. The present investigation is concerned with the influence of these factors in the field and in the laboratory. I investigated the timing of the onset and cessation of measurable reproductive activity in field populations. The control of reproductive rates within the breeding season was studied in the laboratory. The laboratory inquiry determined the relative influence and interactions of temperature, photoperiod, and mate stimulation on dependent variables related to the reproduction of the amphipod.

Hyalella azteca is a common freshwater amphipod crustacean in North America (2, 14). Breeding females are readily identified by their externally visible, dark, developing ovaries, by the presence of a marsupium, and by eggs in the marsupium.

The reproductive cycle of the female is characterized by a relatively long breeding season with many small broods. Males carry females for extended periods preceding fertilization and copulation takes place shortly after the female molts.

Hyalella is easily collected and cultured, and its reproduction readily studied. Molting and pairing can be readily recorded. Ovarian maturation, oviposition, and egg incubation can be observed through the translucent body.

Sexual dimorphism is exemplified by the enlarged second gnathopods of the male and by the oostegites with fringing setae of the female. The large second gnathopods of the male are used for maneuvering the female into the carrying and copulatory positions and the small first gnathopods are used in carrying the female. In *Hyalella* carrying or amplexus is usually initiated several days before molting and copulation. Eggs are laid in the marsupium of the female where sperm have usually been deposited.

Hyalella is found in permanent freshwater reservoirs clinging to vegetation and burrowing in bottom sediments. Hargrave described it as an important member of the benthos and as a deposit-feeder limited to the upper two centimeters of the sediment (15). *Hyalella* is euryecious and its range is wide, from Canada to Central America. Ecotypic variations of length of amplexus, fecundity, and length of life stages have been described (16, 17).

METHODS

From April 1969 to July 1971, field observations and regular collections were made at Pawnee Lake near Lincoln, Nebraska, and in a small farm pond near Burwell, Nebraska. Field observations included date, time, ambient temperature, and macroscopic algae and plants of the habitat. Population estimates of sex, maturity, and proportion of females carrying eggs or displaying ripening gonads were made.

Females were identified as adult by having one of the following characteristics: at least 21 segments in the first and second antennae, head at least 0.45 mm length, total length of at least 4.0 mm, developing ovaries, eggs in the marsupium, or presence of a marsupium. Ovaries became visible when pigment was deposited in the oocytes. Females in breeding condition were smaller than the minimum size specifications. They were classified as adults because they met the specifications of the reproductive condition.

Males were identified as adult by having one of the following characteristics: first and second antennae of at least 21 segments, head of at least 0.45 mm length, or total length of at least 4.0 mm. These guidelines were based on determinations by Geisler and by Cooper.

Environmental Chamber Experiments

An ISCO Model E-2 environmental chamber was used to control temperature and photoperiod. Water baths were used to vary the water temperature under a particular photoperiodic regime. Observations were made on isolated pairs or individuals kept in plastic partitioned trays which were divided into 150-m*l* compartments.

Daily records were kept on individual amphipods under various conditions of temperature, photoperiod, and isolation. From these data were obtained incidence of: 1) carrying or amplectic behavior; 2) molts with their sexual designation; 3) pigment deposition in the ovary; and 4) eggs, embryos or young in the marsupium.

Design of environmental chamber experiments

Model I (fixed effects nested design) analysis-of-variance statistical tests with a cross-classification experimental design were used to determine the effects of temperature, photoperiod, sex, or paired status on dependent variables. Isolated females and male and female pairs were kept under six environmental conditions. An environmental chamber was set to maintain three constant temperatures (20±1 C, 24± 1 C, 27± 1 C) and two 24-hour photoperiod cycles (8:16 light: dark and 16:8 light: dark).

The dependent variables were: interval between molts, interval between ovipositions, molt-to-oviposition interval, carrying time, egg incubation time, and ovary development time. These intervals were expressed in days. Ovary development time was operationally defined as the period between first visible deposition of pigment in the ovaries and oviposition. Egg incubation time was estimated from oviposition until the last young left the marsupium or until the incubating female molted.

The dependent variables were analyzed in 3 × 2 × 2 and 3 × 2 factorial designs. The 3 × 2 × 2 factorial design represented 3 fixed-temperature variables (20±1 C, 24±1 C, 27±1 C), 2 fixed-photoperiod variables (8:16 light:dark and 16:8 light:dark), and 2 fixed-classification variables. The classification variables were changed according to the experiment. Sometimes they were the isolated or paired status of the female and sometimes they were the sexual classification (male or female).

Experiments 1 and 2 tested the effects of photoperiod, temperature, and sexual classification on the molting interval in days. The factorial design was 3 × 2 × 2 (3 temperatures, 2 photoperiods, and 2 sexes). Paired females were used in experiment number 1 and isolated females in reproductive condition were used in the second experiment. The independent variables for both experiments were temperature, photoperiods, and sexual classification. The treatment combinations for the independent variables were 12. The number of observations for each combination was 6 and the total observations for each of the experiments was 72.

Experiments 3, 4, and 5 tested the effects of photoperiod. Both males and females were in the carrying or amplexed condition before being separated and placed under experimental conditions. All experiments using analysis of variance were carried out

early in the reproductive season (May and June), using recently collected males and females in reproductive condition.

RESULTS AND DISCUSSION

Field Studies

There were no apparent differences in spatial distributions according to size and age. Animals were widely distributed but were mostly found in shallow areas of permanent fresh-water reservoirs. During the winter the populations occurred in deeper water and collections were made at approximately 1.5 m.

Females predominated in 17 of 21 collections from 6 locations. A chi-square test on all males and females counted indicated that there were significantly more females (458) than males (304). The statistical significance was at the .01 level of confidence (chi-square = 31.1 d.f. = 1).

Breeding Season

Animals in reproductive condition were found in Nebraska reservoirs from mid-April to late September, 1970. A third of the Pawnee Lake population was in breeding condition April 14 when 13 C ambient temperature and a day length of 13 hr and 13 min were recorded. By May 3, 1970, when a water temperature of 15 C was recorded, 100% of the females showed developing ovaries and 92% carried eggs in the marsupium.

In the April 16, 1970 Burwell Pond collection, 92% of the females had developing ovaries and 87% of the females had new eggs in the marsupium. The ambient temperature was 15 C and the daylength was 13 hr and 24 min.

The spring onset of reproduction appeared to be highly synchronized, i.e., a large percentage of females were found with developing ovaries but no eggs, with eggs only, or with eggs and embryos at the same maturation stages. The June 11, 1970, Pawnee Lake collection indicated a high percentage of females with developing ovaries but a low percentage carrying eggs. The majority of the amphipods that comprised this collection was apparently the first cohort of the new season to be produced and become sexually mature.

In both the Pawnee Lake and Burwell Pond populations maximum percentages of females with ovarian development occurred in the spring and late summer. The spring maximum was greater and occurred at lower temperatures than in the fall.

Ovarian development ceased by the end of September, 1970. Ovaries could be seen in only 44% of the Pawnee females collected September 11, when the ambient temperature was 22 C and the daylength 12 hr and 42 min. By September 22, when the temperature was 19 C and the daylength 12 hr and 40 min, ovaries were visible in only 25% of the females. By September 29 no females with ovaries were visible, although 18% of them still carried eggs. Breeding ceased under higher temperatures and shorter photoperiods than when it was initiated. Similar results were recorded in the laboratory in an experiment carried out at 16 C and a 16-hr day length. Different results were obtained in the spring and fall. The 24 amphipods collected in reproductive condition during late August stopped breeding approximately two weeks before the field populations. The 24 females collected in early February commenced reproducing immediately. Nonbreeding populations subjected to optimal temperatures and a 16-hr day length never failed to commence breeding and continued to breed year round in the laboratory. It is possible that minimal temperatures critical for monitoring reproduction increase as the breeding season progresses.

Seasonal appearance of ovigerous hairs

Overwintering females did not possess a marsupium or the hooked ovigerous hairs which line the oostegites. There were small oostegites on the overwintering adult females. The fall disappearance of ovigerous hairs closely follows the cessation of breeding. Toward the end of September almost all of the females had ovigerous hairs, but by the middle of October no females with this characteristic were found.

There are two possible hypotheses for the absence of hairs in overwintering females: 1) hairs were lost following the reproductive season, 2) immature individuals became adults after the breeding season ended and never developed ovigerous hairs while older females died off. The first of these possibilities seems likely because larger females without ovigerous hairs (females with 26 antennal segments in the two an-

tennae, 0.6 mm head length and 5.6 mm total length) were found at the end of the breeding season. The second possibility cannot be excluded because the population was mostly immature at the end of the reproductive season. Overwintering populations were composed mostly of young adults. The spring appearance of ovigerous hairs coincided with the molt preceding the first egg-laying.

Environmental Chamber Experiments

Differences in reproductive rates occurred under differing environmental conditions during the breeding season (Tables 1 and 2). Generally, increases in temperature, longer photoperiods, and pairing increased reproductive activity. An integrated pattern emerged from the F tests used to assess the significance of the independent variables. Temperature widely affected reproductive activity, while photoperiod and pairing were more specific in the reproductive activities affected. Physical environmental factors appear to act simultaneously with behavioral agents to influence reproduction.

Temperature

Temperature was found to produce the greatest influence on reproductive rates. It significantly affected all the dependent variables tested except the interval between molting and oviposition. For the interval between oviposition and the molting interval, temperature effects were significant at the .01 level of confidence. Its effect on the oogenesis interval was at the .05 level of confidence. Higher temperatures shortened the intervals tested and increased reproductive rates. Increases in temperature reduce the amounts of time required for ovarian maturation, egg incubation, and carrying of the female by the male. Higher temperatures significantly shorten intervals between ovipositions, two moltings, and molting and oviposition. A widespread temperature effect was expected on the basis of previous studies (3, 4, 5, 18).

Photoperiod

Photoperiod was found to affect significantly the dependent variables related to egg production and ovarian maturation: oogenesis interval and interval between ovipositions. These effects were found significant at the .05 level of confidence (Tables 1 and 2). Longer photoperiods shortened intervals or increased reproductive rates.

Since molting was linked with egg-laying, it might be expected that if photoperiod were influencing ovarian maturation and egglaying, it would also influence molting. The fact that daylength did not significantly affect molting may be attributed to the fact that some molting occurred without a following oviposition. Possibly these moltings were not as sensitive to

TABLE 1. *Mean reproductive time intervals (expressed in days) under different environmental conditions*

	Photoperiod and Temperature					
	8-h day			16-h day		
	20 C	24 C	27 C	20 C	24 C	27 C
Molting-to-oviposition interval						
Paired females[a]	1.1	1.2	0.6	0.5	0.7	0.5
Isolated females	0.9	2.8	1.2	0.8	1.3	1.9
Oviposition interval						
Paired females[a]	15.00	10.00	7.75	12.75	9.00	7.50
Isolated females	16.25	11.75	9.50	13.50	9.75	11.25
Carrying time	3.10	2.40	1.40	3.50	2.00	1.80
Ovarian maturation time	11.60	8.00	6.00	10.60	6.60	5.20
Egg incubation time	10.00	6.10	4.80	18.20	5.90	5.10
Molting intervals						
Paired females[a]	14.50	9.50	8.33	13.50	8.83	7.83
Males	21.00	15.00	13.50	17.17	11.33	17.17
Molting intervals						
Isolated females	15.67	9.50	9.67	14.00	9.17	10.33
Males	21.00	19.33	13.50	17.17	11.33	17.17
Molting intervals						
Paired females[a]	14.60	9.10	8.20	13.00	9.00	8.20
Isolated females	15.40	9.80	10.40	13.70	9.10	10.60

[a]Male available in the same container.

photoperiod as those occurring before ovipositions.

For all the experiments there were three significant interactions between the independent variables tested. Two of these were the effect that combining longer photoperiods and higher temperatures had on shortening the intervals between ovipositions and between molts. These nonsymmetrical photoperiods and temperature interactions were significant at the .05 level of confidence. They were not consistent with other reproductive activity patterns which emerged from these experiments and could be due to chance.

Paired status of the female

When males and females are paired the intervals between moltings, ovipositions, and molting to oviposition are significantly

TABLE 2. *Summary of analysis of variance: effects of temppperature, photoperiod, isolation, and sex on reproductive variables*

Source	SS	df	MS	F	SS	df	MS	F	SS	df	MS	F
	Molting intervals				Molting-oviposition interval				Oviposition interval			
Paired status	39.67	1	39.67	12.16**	15.41	1	15.41	8.91**	33.33	1	33.33	10.39*
Photoperiod	12.67	1	12.67	3.89	3.67	1	3.67	2.13	14.08	1	14.08	4.39*
Temperature	633.95	2	316.97	97.17**	9.45	2	4.72	2.73	257.17	2	128.58	40.08**
Pair X photo	0.21	1	0.21	0.06	0.07	1	0.07	0.04	0.08	1	0.08	0.03
Pair X temp	20.45	2	10.22	3.13*	6.72	2	3.36	1.94	7.17	2	3.58	1.17
Photo X temp	16.25	2	8.12	2.49	8.45	2	4.23	2.44	22.17	2	11.08	3.45*
Residual	0.81	2	0.41	0.12	4.65	2	2.32	1.34	5.16	2	2.58	0.80
Error	352.30	108	3.26		186.70	108	1.73		115.50	36	3.20	
	Carrying time				Ovarian maturation time				Egg incubation time			
Photoperiod	0.27	1	0.27	0.20	8.53	1	8.53	7.76*	114.82	1	114.82	1.08
Temperature	29.73	2	14.87	10.88**	158.60	2	79.30	72.09**	1002.90	2	501.45	4.70*
Photo X temp	2.13	2	1.07	0.78	0.47	2	0.23	0.21	221.97	2	110.99	1.04
Error	73.80	54	1.37		26.40	24	1.10		5759.84	54	106.66	
	Molting intervals (paired ♀s & ♂s)				Molting intervals (isolated ♀s & ♂s)							
Sex	533.55	1	533.55	53.18**	485.68	1	485.68	23.24**				
Photoperiod	18.00	1	18.00	1.79	45.13	1	45.13	2.15				
Temperature	420.36	2	210.18	20.95**	319.37	2	159.68	7.64**				
Sex X photo	1.39	1	1.39	0.14	23.35	1	23.35	1.12				
Sex X temp	32.86	2	16.43	1.64	9.36	2	4.68	0.22				
Photo X temp	60.25	2	30.13	3.00	132.58	2	66.29	3.17*				
Residual	50.19	2	25.10	2.50	85.36	2	42.68	2.04				
Error	602.00	60	10.03		1254.16	60	20.90					

shortened. The paired status of the female was the only significant effect found on the molting oviposition interval. It was significant at the .01 level of confidence. Isolated females took longer to oviposit. These results might be interpreted in two ways: females have the adaptive trait of delaying oviposition for a limited period until a male is present, or the female is primed by the presence of the male. Delayed oviposition by unpaired females has been suggested for other species of amphipods (13).

Possibly associated with delayed oviposition, the paired status of the female was found to affect significantly the intervals between oviposition at the .05 level of confidence. Isolated females had larger intervals between ovipositions (Table 1).

Molting intervals were also found to depend on the paired or isolated status of the female. Isolated females had larger intervals between moltings. Kinne has recorded a similar phenomenon of progressive lengthening of the molting interval in isolated female *Gammarus* (19).

Sexual classification

Significant differences between male and female molting intervals appeared when either paired or isolated females were used with males as classification variables (Table 2). This difference has been previously observed in *Hyalella* and other amphipod species (2, 4, 18, 20). However, no statistically significant difference was found between molting frequencies of the sexes when kept at 5 C and in the dark. Of 22 molts during this period, 10 were female, 12 were male. This may indicate that sexual differences in molting accompany reproductive activity and the breeding season.

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