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Effects of Photoperiod on the Occurrence of Symbiotic Rotifers, Nematodes and Branchiobdellids of Two Orconectid Species of Crayfishes

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EFFECTS OF PHOTOPERIOD ON THE OCCURRENCE OF SYMBIOTIC ROTIFERS, NEMATODES, AND BRANCHIOBDELLIDS OF TWO ORCONECTID SPECIES OF CRAYFISHES

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INTRODUCTION

Periodicity of light as well as continuous exposure to light or darkness can have various effects on the biological systems of organisms. Subtle to marked changes in behavior, reproduction, growth, development, and physiology of organisms have often been correlated with changes in periods of exposure to light.

One of the most frequently noted effects is the onset of diapause (total cessation of growth or reproduction) in certain invertebrates. Lees (1953a; 1953b) experimentally demonstrated that the red mite, Metatetranychus ulmi, goes into diapause during short periods of light exposure (8 to 12 hours) while longer periods of light bring about continuous growth and development. A similar response was also found in the caterpillars of Lepidoptera (Geyspitz, 1957) and in the Colorado beetle, Leptinotarsa (de Wilde, 1954; Goryshin, 1956).

The mating and ovipositing of various moths are known to be influenced by certain periods of light exposure. Williams (1936) reported the maximum period of activity of certain noctuid moths to be around midnight, with less activity on bright moonlit nights than on dark cloudy nights. Goodwin and Madsen (1964) reported that twilight stimulated the reproductive activity of the moth of the navel orangeworm,

Paramyelois transitella. Miskimen (1966) found that continuous light suppressed the mating and ovipositing of the sugar cane borer, Diatraea saccharalis.

Periods of activity are often influenced by the length of exposure to light or the time of day at which the exposure occurs. Some animals are more active during the hours of darkness, while others are most active during the daylight hours. Rawson (1959), using nocturnal mice, found that continuous light delayed the onset of the activity period. Twelve hours of light during the normal nocturnal activity period also delayed activity. Rawson also maintained mice in continuous darkness for 22 days and found the activity period to begin 50 minutes earlier each day. Although Rawson could delay or speed up the rhythms, the same overall 24 hour rhythm persisted in all of the mice tested.

Meyer and McCormack (1967) performed an experiment in which they used FSH to stimulate ovulation in rats. It was noted that continuous light inhibited the FSH-induced ovulation. In an experiment performed by Negro-Vilar, Dickerman, and Meites (1968) the effects of continuous light on the FSH-releasing factor in rats were observed. Adult female rats exposed to continuous light for 21 days showed more progressive follicle stimulation than the controls, increased vascularity in the uterus, and thicker epithelium in the uterine wall. These conditions were considered to result from a prolonged estrogen cycle, and thus indicated that continuous light increases FSH secretion by the pituitary through

augmentation of FSH-RF secretion from the hypothalamus.

An experiment designed to assay the effects of continuous darkness on ovulation was carried out by Wilson and Abplanalp (1956) at the University of California. Sixty Leghorn hens were divided equally among 3 cages. After 5 weeks of continuous darkness egg production had dropped from 66 to 23 percent. The hens were then returned to a normal photoperiod and egg production returned to its previous rate in less than 4 weeks.

A somewhat more specific influence of photoperiod was revealed by the studies of Wurtman, Axelrod, and Phillips (1963) and Axelrod, Wurtman, and Snyder (1965). The enzyme hydroxyindole-O-methyltransferase (HIOMT), which is highly localized in the pineal gland, was shown to have an activity 3 to 5 times greater in rats maintained in a continuous dark environment than in rats maintained in continuous light.

Continuous light has been demonstrated to have lethal effects on some organisms. White and Perlmutter (1962) found that continuous fluorescent light on the eggs of the brook trout in hatchery tanks produced a high mortality rate. They discovered that shading the hatchery tanks with opaque covers reduced the mortality of the eggs.

In experiments conducted to identify the effects of continuous light on cave ostracods (Candana sp.), it was demonstrated that continuous light of approximately one-twentieth the intensity of normal sunlight was lethal to the experimental organisms (Maguire, 1960).

No studies were found relative to the role of light in the host-symbiont relationship between crayfish and nematodes, rotifers, or branchiobdellids. In view of this, a study was undertaken to determine the effect of various periods of light exposure on the symbiotic nematodes, rotifers, and branchiobdellids of pond and cave crayfishes of this region of Kentucky. The abundance of the pond and cave crayfishes presented an opportunity to investigate the effects of light on the symbionts of hosts from greatly contrasting conditions. Orconectes immunis (Hagen), the pond crayfish, is exposed to a natural seasonal photoperiod, whereas O. pellucidus (Tellkampf) is exposed, in its natural habitat, to continuous darkness.

A preliminary survey of the occurrence of parasites and commensals of O. immunis and O. pellucidus revealed that similar as well as dissimilar symbionts are harbored by these crayfishes. Rotifers are found in the gill chambers of both species of crayfishes. Branchiobdellid worms, on the other hand, occur only on O. pellucidus, and only the pond crayfish, O. immunis, is infected with nematodes. Repeated sampling of the pond and cave crayfish populations confirmed the preliminary results.

The rotifer occurring in the gill chamber of O. immunis and O. pellucidus is a bdelloid form, and its body is typically elongate and cylindrical (Plate I, Fig. 1). The body is divided into 3 main regions: the head, trunk, and foot. Bdelloid rotifers of this type are widespread and have been

reported from practically all bodies of fresh water. They are comparatively prolific in their reproduction and may reproduce asexually (parthenogenesis) as well as sexually (Barnes, 1965). Most are free-living forms, although a few parasitic or epizootic species have been identified. Donner (1956) reported parasitic or epizootic rotifers from the gills of small crustaceans.

The nematodes occurring on the pond crayfish are representative of forms that tend to be more widespread and more inclined toward true parasitism than do the rotifers. Initial observations revealed that the number of worms per crayfish is often quite high; 500 nematodes per crayfish was not unusual. These worms have elongate bodies that taper at both ends, and they average approximately 2 mm in length (Plate I, Fig. 2).

The chief means of reproduction of nematodes is through formation and union of sex cells (Barnes, 1965). Pennak (1953) reported that the period of time required for a fertilized egg to hatch varied from a few hours to a few weeks, depending on the environmental conditions.

The branchiobdellids identified on O. pellucidus are unlike many species of rotifers and nematodes in that these oligochaetes are never found in a "true" free-living state, always being associated with some freshwater crustacean (Holt, 1968). Until recently branchiobdellids were believed to exist only on crayfishes. However, Holt (1963) found an isopod which was infected with the branchiobdellid, Cambrinicola aliena. The branchiobdellid of O. pellucidus has a

short rotund body that consists of 15 segments (Plate I, Fig. 3). Although the name, branchiobdellid, suggests these worms occur only on the gills, they are reported by Hobbs, Holt, and Walton (1967) to occur on the inner surface of the gill chamber and the anterior ventral side of the crayfish as well as the gills.

Even though branchiobdellids are hermaphroditic, cross fertilization is the major means of reproduction. After copulation the eggs are deposited in cocoons, and the cocoons are fixed to the surface of the host and will hatch after 3 days to 10 weeks, depending not only on the species but the environmental conditions (Barnes, 1965).

In spite of the apparent obligatory relationship that branchiobdellids have with crayfish, there is disagreement as to the nature of the host-symbiont association. Goodnight (1940) reports that few, if any, branchiobdellids are harmful to their hosts, while Holt (1963) asserts that at least one species (Cambrinicola branchiophila) parasitizes its host by clipping off gill filaments and sucking blood from the incisions.

MATERIALS AND METHODS

Representative specimens of O. immunis and O. pellucidus were brought to the laboratory in order to investigate the influence of light on the occurrence of the symbionts encountered during the preliminary field study. After one week of acclimating the crayfishes they were distributed among environmental control chambers that permitted exposure of the hosts and symbionts to 1) continuous light (C.L.), 2) a daily cycle of 12 hours of light and 12 hours of darkness (12L-12D) and 3) continuous darkness (C.D.).

"Light-tight" chambers like the one pictured in Plate II, Figure 4 were used to attain the desired laboratory conditions. Three chambers (one representing each of the above numbered conditions) were supplied with crayfishes, and these were maintained for a period of 16 weeks. Each environmental control chamber was divided into 6 sub-units (a, b, c, d, e, and f of Plate II, Fig. 5), and each sub-unit housed a 13-1/2" X 18-1/2" X 5" pan containing four 5-1/2" X 7-1/2" X 3-1/2" transparent refrigerator boxes (Plate II, Fig. 6).

A temperature of 14°C (plus or minus one degree) was maintained in the chambers by recirculating refrigerated water through the pans of the sub-units. Water in the pans

was used for cooling purposes only and did not come into contact with the crayfishes. All crayfishes were kept in the smaller plastic refrigerator boxes, and the water in these boxes was pond water or cave water depending upon the species of crayfish present. The water in which the crayfishes were maintained was changed weekly. Replacement water (from the same source as the respective groups of crayfish) was boiled, aerated, and cooled to 14°C before being used. All crayfishes were fed finely ground lean beef or lettuce once a week.

On August 27, 1968, 72 pond crayfish (O. immunis) and 72 cave crayfish (O. pellucidus) were equally distributed among the plastic refrigerator boxes of one-half of the sub-units in the 3 control chambers. The crayfishes were so divided that 4 host specimens were placed in each of the refrigerator boxes. Pond crayfish and cave crayfish were maintained in separate refrigerator boxes. The remaining unoccupied boxes of one-half of the sub-units in the control chambers were filled in the same manner on September 3, 1968. From the onset of each replication specimens were sacrificed and examined every 2 weeks to determine the state of the symbiont population. On this schedule, 2 crayfish of each species were removed from each of the 3 experimental conditions (C.L., 12L-12D, and C.D.), and the number of nematodes, rotifers, and branchiobdellids was determined for the individual host animals.

Crayfish were prepared for examination by removing and discarding the tail section and appendages. The remaining

cephalothorax was placed in a black bottom petrie dish with sufficient water to cover the contents. The cephalothorax was examined externally for branchiobdellids. Following this, the gill chamber was opened and the gills were removed to facilitate counting of the rotifers and nematodes.

While the number of branchiobdellids present on individual crayfish was sufficiently small that a direct count was possible, an aliquot method was necessary due to the numbers of nematodes and rotifers. After the gills had been extracted from the cephalothorax, the contents of the petrie dish were flushed into a 300 ml beaker. Enough water was added to the beaker to bring the total volume to 80 ml. Contents of the beaker were stirred for 30 seconds and while continuing to stir, a 20 ml aliquot was poured back into a petrie dish. The aliquot was examined with the aid of a dissecting scope, and a count of the nematodes and rotifers was conducted. To obtain an estimate of the total numbers of nematodes and rotifers present, each of the counts obtained from the aliquot was multiplied by 4. Crayfish that were sacrificed and examined were selected in such fashion that the contents of an individual refrigerator box were depleted before removing crayfish from any other box of the same pan. This procedure insured that at least one container in all of the sub-units would remain undisturbed by the periodic sampling procedure. At the end of the 16 week period, all the remaining crayfishes were sacrificed and examined for symbionts.

During the course of the experiment, 3 collections of O. immunis and O. pellucidus were made from the field in order to monitor the level of the symbiont infection in the pond and cave habitats for comparison with the laboratory results. The collections were made prior to the first replication, midway through the experiment, and when the study was terminated.

Statistical Analysis

In order to more easily determine significant responses, the results of the experiment were analyzed statistically. In a split-plot design the main plots were the 3 light conditions and the sub-plots were the lengths of time the organisms were exposed to the light conditions. This design was employed to determine whether there were any responses to light periodicity or length of exposure in the number of symbionts present on the crayfish during the 16 weeks of the experiment. In addition, the split-plot design would indicate if there were any interaction between these two variables. A randomized complete block design with single degree of freedom comparisons was used to determine whether variations in the number of symbionts on the hosts during the course of the experiment were linear or quadratic. Linear correlations were employed to determine if changes in the number of symbionts present on each crayfish during the course of the experiment were significantly affected by the length of time exposed to the light conditions. An r value was determined for each symbiont in each of the 3 light conditions.

RESULTS AND OBSERVATIONS

Field Observations

The population level of O. immunis in the pond environment was approximately 50 times as dense as the population level of O. pellucidus in the cave situation. Representatives of each species were taken from their natural environment 3 times during the course of the experiment and examined to determine the numbers of rotifers, nematodes, and branchiobdellids present. Each pond crayfish harbored an average of 191 rotifers and 582 nematodes at the outset of the experiment. There were no branchiobdellids found on O. immunis at any time during the course of the experiment. During this same collecting period the cave crayfish averaged 22 rotifers and 4.5 branchiobdellids per host, with no nematodes observed. The counts made at the mid-point of the experiment showed pond crayfish to average 166 rotifers and 790 nematodes per host. O. pellucidus averaged 20 rotifers and 3 branchiobdellids on each crayfish at this time. Seventeen weeks after the onset of the experiment, O. immunis averaged 167 rotifers and 661 nematodes on each crayfish, while cave crayfish taken from their natural environment harbored an average of 21 rotifers and one branchiobdellid per host.

Laboratory Results

The number of rotifers present on each O. immunis and each O. pellucidus at the examination intervals is given in Tables 1 and 2 respectively. The population of rotifers was much more dense on the pond crayfish than on the cave crayfish. It is evident from these tables that a considerable variation in the number of symbionts occurred between the replications of each 2-week sampling period.

The number of nematodes present on each O. immunis when it was sacrificed is presented in Table 3. The number of symbionts on each host is given according to light conditions, replications, and length of time the host and symbiont had been exposed to each of the light conditions. Again, as with the rotifers, it was noted that a large variation in the numbers of nematodes occurred between replications of corresponding exposure periods. In addition to this variation, Table 3 indicates that the number of nematodes gradually decreased in all the light conditions during the course of the 16 week experiment.

The number of branchiobdellids present on each O. pellucidus as it was sacrificed is given in Table 4. The number of symbionts is shown according to light conditions, replications, and the length of time the host and symbiont had been exposed to the respective light conditions. The branchiobdellids had completely disappeared after the tenth week of exposure in both the C.L. and 12L-12D conditions. Only the C.D. condition had any branchiobdellids present after 10 weeks of exposure.

TABLE 1

NUMBER OF ROTIFERS PRESENT ON EACH O. IMMUNIS SACRIFICED AT INTERVALS OF TWO WEEKS

No. of weeks exposed		Continuous light	12L-12D	Continuous darkness
2	rep I	29	12	10
	rep II	58	48	8
4	rep I	72	40	38
	rep II	40	62	28
6	rep I	68	18	62
	rep II	26	70	18
8	rep I	63	20	42
	rep II	108	108	148
10	rep I	34	14	140
	rep II	40	60	20
12	rep I	26	84	42
	rep II	116	118	36
14	rep I	90	38	150
	rep II	138	178	52
16	rep I	70	40	63
	rep II	152	108	91

TABLE 2

NUMBER OF ROTIFERS PRESENT ON EACH O. PELLUCIDUS SACRIFICED AT INTERVALS OF TWO WEEKS

No. of weeks exposed		Continuous light	12L-12D	Continuous darkness
2	rep I	121	298	118
	rep II	244	184	74
4	rep I	164	290	152
	rep II	74	64	22
6	rep I	190	142	164
	rep II	102	64	108
8	rep I	112	80	114
	rep II	38	146	98
10	rep I	34	82	176
	rep II	416	104	86
12	rep I	296	174	66
	rep II	526	148	148
14	rep I	278	212	40
	rep II	340	20	144
16	rep I	58	246	59
	rep II	95	59	150

TABLE 3

NUMBER OF NEMATODES PRESENT ON EACH O. IMMUNIS SACRIFICED AT INTERVALS OF TWO WEEKS

. of weeks exposed	Continuous light	12L-12D	Continuous darkness
rep I	582	1085	936
rep II	816	780	726
rep I	636	922	928
rep II	802	564	596
rep I	950	746	958
rep II	558	730	512
rep I	790	810	676
rep II	580	616	540
rep I	730	654	584
rep II	1340	546	694
rep I	714	884	462
rep II	650	662	530
rep I	436	884	470
rep II	464	328	222
rep I	379	672	401
rep II	348	354	306

TABLE 4

NUMBER OF BRANCHIOBDELLIDS PRESENT ON EACH O. PELLUCIDUS SACRIFICED AT INTERVALS OF TWO WEEKS

No. of weeks exposed		Continuous light	12L-12D	Continuous darkness
2	rep I	1.5	0.5	1.5
	rep II	1.5	0.5	1.5
4	rep I	1.0	6.0	8.0
	rep II	1.0	0	1.0
6	rep I	0	0	4.0
	rep II	1.0	0	1.0
8	rep I	0.5	8.0	0.5
	rep II	1.0	0	1.5
10	rep I	0	0	1.5
	rep II	0	1.0	4.0
12	rep I	0	0	0
	rep II	0	0	0
14	rep I	0	0	1.0
	rep II	0	0	0
16	rep I	0	0	0
	rep II	0	0	0.7

In order to facilitate a comparison of the fluctuation in the numbers of symbionts among the light conditions, graphs were prepared. The average numbers of rotifers and branchiobdellids present on each cave crayfish at each of the examination intervals are indicated in Figures 7 and 8 respectively. The average numbers of nematodes and rotifers present on each O. immunis at each of the 2 week intervals are given in Figures 9 and 10. Number of organisms is represented by the Y axis and length of time the organism has been exposed to each light condition is indicated on the X axis. The average number of symbionts per host was determined by averaging the corresponding replications for each examination interval.

The nematodes harbored by O. immunis showed a steady decrease in numbers in all light conditions as the length of exposure continued. The rotifers of this host tended to maintain the same level of infection in each of the light conditions throughout the 16 weeks of exposure. On the other hand, the rotifers harbored by O. pellucidus increased in all light conditions during the 16 weeks of exposure. The graph emphasizes the earlier stated fact that the branchiobdellids disappeared after 10 weeks in all but the C.D. condition.

Statistical Analysis

In the split-plot design analysis the light conditions were the main plots. Lengths of time the symbionts were exposed to each light condition were the sub-plots. These indicated that there were significant changes in the number

Figure 7. Average number of rotifers occurring on *O. pellucidus*.

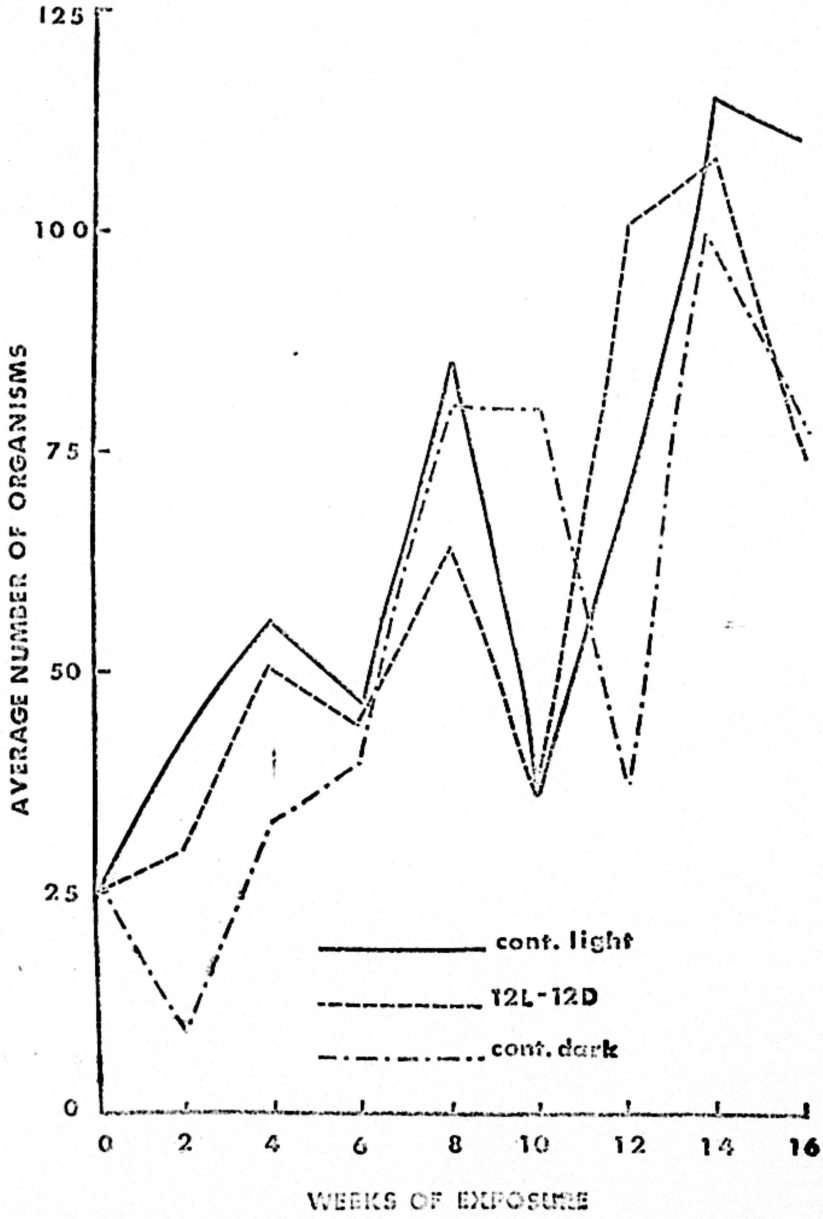

125
100
75
50
25
0
AVERAGE NUMBER OF ORGANISMS
cont. light
12L-12D
cont. dark
0
2
4
6
8
10
12
14
16
WEEKS OF EXPOSURE

Figure 8. Average number of branchiobdellids on *O. pellucidus*.

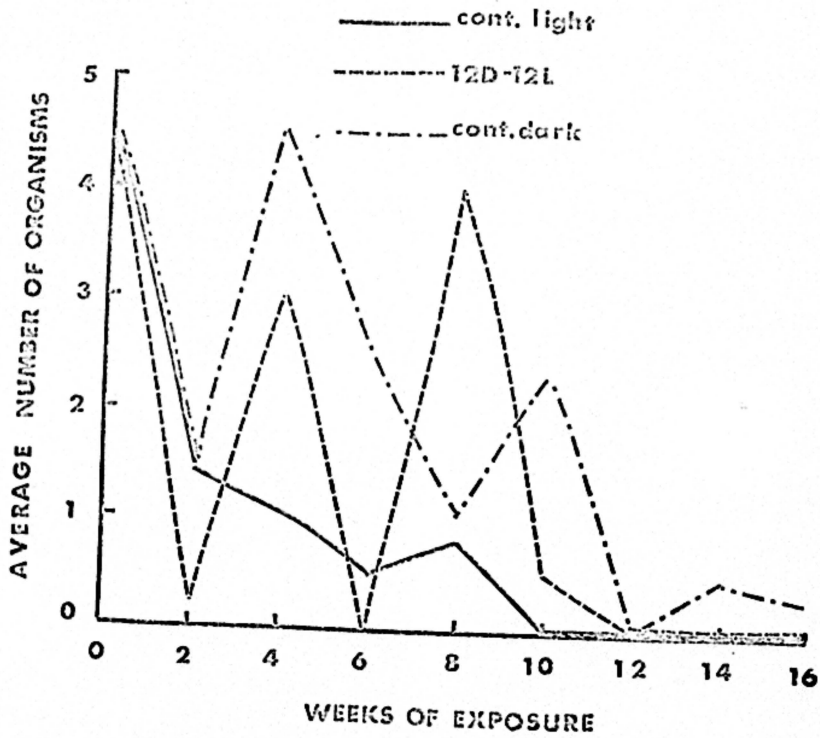

cont. light
12D-12L
cont. dark
AVERAGE NUMBER OF ORGANISMS
0
1
2
3
4
5
0
2
4
6
8
10
12
14
16
WEEKS OF EXPOSURE

Figure 9. Average number of nematodes on O. immunis.

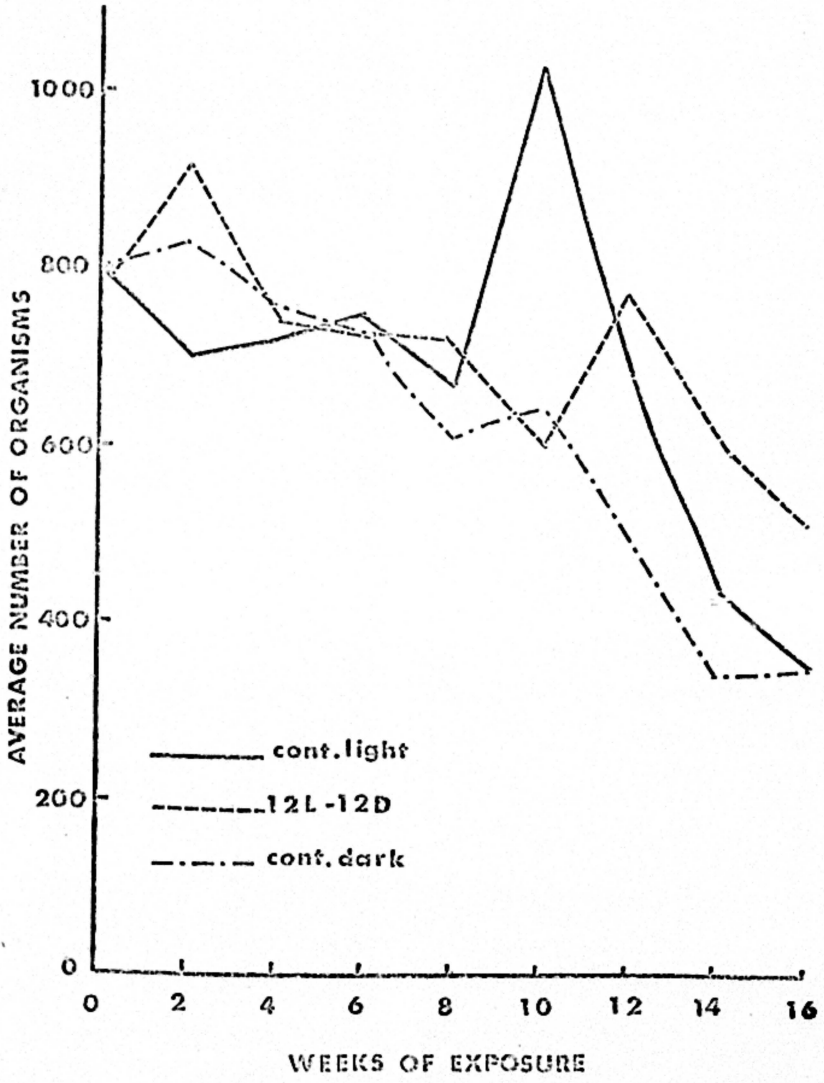
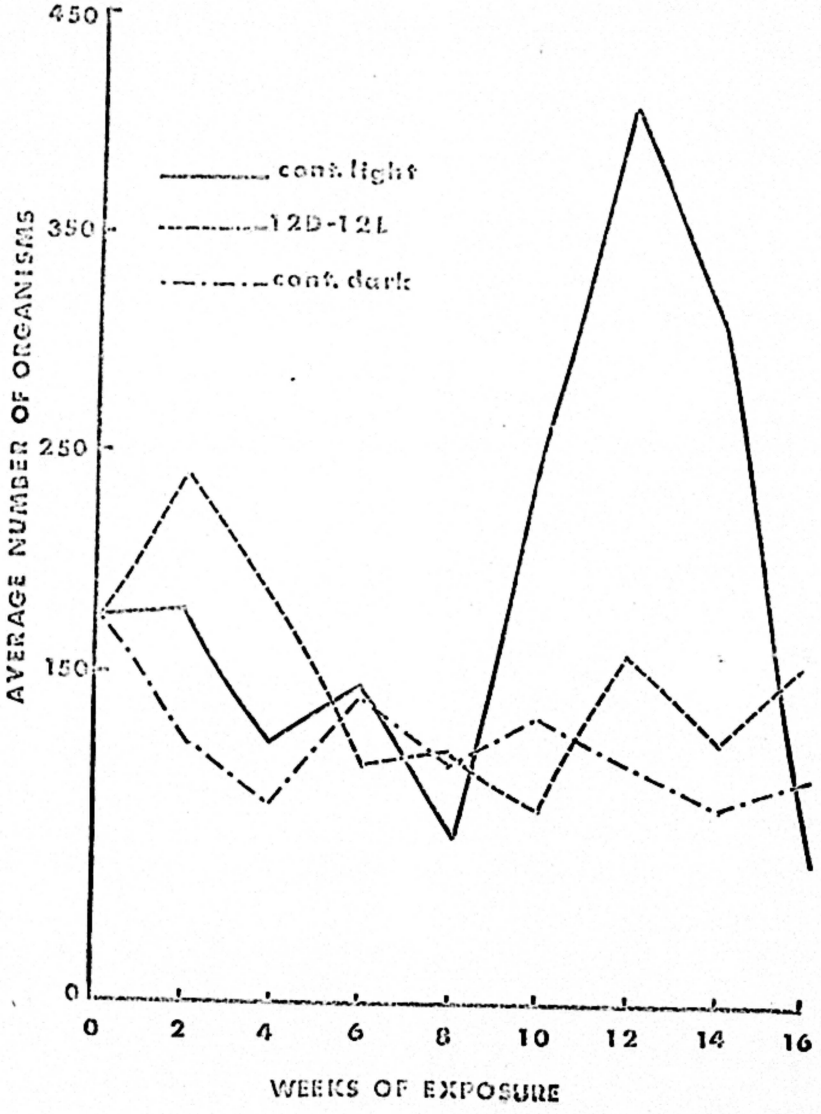

AVERAGE NUMBER OF ORGANISMS
1000
800
600
400
200
0
cont. light
12L-12D
cont. dark
0
2
4
6
8
10
12
14
16
WEEKS OF EXPOSURE

Figure 10. Average number of rotifers on *O. immunis*.


450
350
250
150
0
cont. light
12D-12L
cont. dark
AVERAGE NUMBER OF ORGANISMS
0
2
4
6
8
10
12
14
16
WEEKS OF EXPOSURE

of rotifers on the cave crayfish at the end of 16 weeks exposure to the 3 light conditions collectively when compared to the numbers present at the onset of the experiment. The F test was significant at the 0.05 level of probability. The changes in the number of nematodes present on the pond crayfish were significant at the 0.005 level of probability. The interaction of lighting conditions and length of time exposed to these conditions was not significantly different.

Linear correlations were computed by using the number of symbionts present versus the length of time they were exposed to each light condition. The r values obtained for each symbiont in each light condition are given in Table 5. Rotifers on the pond crayfish showed no significant change during the course of the experiment in any of the 3 light conditions. However, the rotifers harbored by the cave crayfish displayed a positive change at the 0.05 level in both the C.L. and the 12L-12D conditions. The nematodes found on O. immunis yielded a negative correlation at the 0.01 level in the 12L-12D condition as well as in C.D. Negative correlations at the 0.01 and 0.05 levels of probability occurred respectively for numbers of branchiobdellids in the C.L. and C.D. conditions.

A randomized complete block design with single degree of freedom comparisons determined whether the changes in the numbers of symbionts present on the crayfish during the course of the experiment were linear or quadratic, if in fact they were either. The results are given in Table 6.

TABLE 5

LINEAR CORRELATIONS FOR SYMBIONTS
IN EACH LIGHT CONDITION

Host	Symbiont	Light condition	r value[a]
O. immunis	rotifer	Cont. light	+0.441
		12L-12D	-0.4697
		Cont. darkness	+0.0431
O. pellucidus	rotifer	Cont. light	+0.76*
		12L-12D	+0.743*
		Cont. darkness	+0.657
O. immunis	nematode	Cont. light	-0.5199
		12L-12D	-0.8201**
		Cont. darkness	-0.9712**
O. pellucidus	branchiobdellid	Cont. light	-0.9028**
		12L-12D	-0.3703
		Cont. darkness	-0.7589*

a r value determined from number of symbionts versus length of time exposed to light condition.
* 0.05 level of probability.
** 0.01 level of probability.

TABLE 6

RESPONSE OF SYMBIONTS TO LENGTH OF EXPOSURE TO THE THREE LIGHT CONDITIONS

Host	Symbiont	Light condition	Response linear	Response quadratic
O. immunis	rotifer	Cont. light	ns	ns
		12L-12D	ns	ns
		Cont. darkness	##	ns
O. pellucidus	rotifer	Cont. light	ns	ns
		12L-12D	ns	ns
		Cont. darkness	ns	ns
O. immunis	nematode	Cont. light	#	#
		12L-12D	ns	##
		Cont. darkness	ns	ns
O. pellucidus	branchiobdellid	Cont. light	ns	ns
		12L-12D	ns	ns
		Cont. darkness	ns	ns

ns not significant.
\# 0.05 level of probability.
0.01 level of probability.

DISCUSSION

Numerically, the level of the symbiont infestations on the 2 species of crayfish differed greatly from the outset as well as throughout the period of investigation. Pond crayfish, found initially to be heavily infested with rotifers and nematodes and without branchiobdellids, continued to maintain an overall greater infestation of symbionts than did the cave crayfish. Even though branchiobdellids were not found on the pond crayfish, cocoons of the worm were occasionally identified on this host. The higher population levels of the symbionts on O. immunis reflected the greater density of this host in its natural environment. In the pond, O. immunis averaged approximately 50 crayfish per square meter, while O. pellucidus averaged less than one per square meter in the cave. In spite of the fact the average number of rotifers on cave crayfish increased progressively, it was never as great as the overall occurrence of this symbiont on pond crayfish. The branchiobdellid infestation of cave crayfish averaged less than 5 worms per host at the start of the 16 week period and showed no tendency to increase in number in any of the experimental conditions. By the end of the tenth week the branchiobdellids had, in fact, disappeared from all the cave crayfish in the laboratory except those maintained in the C.D. condition. The decrease in number of worms in the experimental

conditions was paralleled by a decrease in the number of worms in the natural environment. This contrasts with the findings of Brown (1962) who stated that branchiobdellids were not seasonal.

Graphically, the high and low levels of the symbiont populations at each of the sampling intervals were more pronounced throughout the C.L. and 12L-12D conditions than in C.D. with the exception of branchiobdellids harbored by cave crayfish (Figs. 7, 8, 9, and 10). Rotifers found on cave crayfish increased in number during the 16 weeks of the experiment, while other symbionts either decreased or remained relatively stable. The increase of the rotifers occurred in all light conditions, but, according to the computed linear correlations, only the C.L. and 12L-12D conditions were significantly different from no change at all (Table 5). This overall increase indicated that rotifers were favored by the laboratory conditions. Apparently both light and crowding promoted an increase in the number of rotifers. Most important, however, is the fact that crowding alone was not enough to cause the population to increase significantly. Only those rotifers affected by the interaction of crowding and light exhibited a significant response (Table 5). It is obvious that even though the natural environment of the rotifer of O. pellucidus is comparable to the C.D. condition in the laboratory, this is not the optimum living condition for the symbiont. In each of the 3 light conditions rotifers infesting the pond crayfish tended to remain at the same level throughout the course of the experiment. However, there was a large increase in the number of rotifers

in C.L. from the eighth to the twelfth week of exposure, but none of the linear correlations computed for the rotifer infestations of pond crayfish in each of the 3 conditions of lighting revealed a significant difference.

The nematodes of O. immunis and the branchiobdellids of O. pellucidus decreased in number as the length of exposure to each of the light conditions continued. Collectively, a significant change in the nematode population occurred during the 16 week period. Unlike the rotifers of O. pellucidus, nematodes were not favored by the conditions in the laboratory. This was indicated by the steady decrease in the level of the population during the course of the exposure to the light conditions. The highest negative linear correlation value for the population level occurred in the C.D. condition, and the change of the nematode population in the C.L. condition was not significant (Table 5). After the tenth week of exposure, the branchiobdellids did not occur in any of the light conditions other than C.D. While the branchiobdellids survived only in the C.D. condition, the decrease in number of nematodes was most pronounced in this condition.

With the exception of the branchiobdellids of cave crayfish, C.D. was found to suppress the symbiont populations more than the 12L-12D or C.L. conditions. This was demonstrated by the symbionts of both crayfishes. The increases and decreases in the rotifer populations of cave crayfish in the C.L. and 12L-12D conditions correspond closely. However, in C.D. they were somewhat suppressed. The abrupt changes in populations of the symbionts of O. immunis occurred, without

exception, in the C.L. and 12L-12D conditions.

In determining whether the responses of the symbionts to the light conditions were linear or quadratic it was found that the only significant responses were from O. immunis. This may have been due in large part to the higher number of symbionts of the pond crayfish. The rotifers harbored by O. immunis indicated quadratic responses at the 0.1 level of probability in the 12L-12D and C.D. conditions, but this same symbiont displayed a linear response at the 0.01 level of probability in the C.D. condition. The nematodes of the pond crayfish were characterized by both a linear and a quadratic response at the 0.05 level of probability in the C.L. condition, but only a significant quadratic response is indicated in the 12L-12D condition (Table 6). The predominant response in the conditions involving light is definitely quadratic. This demonstrates statistically the tendency of these symbiont populations to fluctuate sharply.

Apparently the importance of these marked changes in the levels of the populations lies in the reason for these changes. It is probable that reproductive cycles were producing a pulsing effect, thus causing the increases and decreases in the levels of infestation. This explanation is logical in view of the clear 4 week cycle indicated by the population levels of the rotifers harbored by O. pellucidus (Fig. 7). As previously noted, the light period that was most detrimental to the rotifers and nematodes was C.D. Wilson and Abplanalp (1956) found darkness to reduce the ovulation rate of Leghorn hens. A decrease in ovulation rate of the symbionts may be

one explanation for the decrease in number of symbionts on the crayfish maintained in darkness. This suggests that the presence of light affects the reproductive cycles of the rotifers and nematodes harbored by O. immunis and O. pellucidus under these laboratory conditions.

After this study was terminated and the data had been examined it was apparent that certain modifications would have increased the precision as well as the overall validity of the findings. It is likely that the study would have been strengthened by conducting two 8 week experimental periods rather than one period of 16 weeks. In addition, the frequency with which crayfishes were sacrificed as well as the number that were examined should have been increased. A more frequent sampling and examination of greater numbers of host specimens would have helped to reduce the great variation that occurred between replicates. Finally, due to the longer period of time required for reproduction and development, branchiobdellids should not have been considered in the same study with rotifers and nematodes.

CONCLUSIONS

The rotifers which infest the cave crayfish O. pellucidus occur naturally in a subterranean, continuously dark habitat. The population levels of this symbiont, when tested in the laboratory, indicated that the 2 laboratory conditions involving light were more advantageous to an increase in their numbers than was the C.D. condition. Therefore, the natural environment of this rotifer is not necessarily the best for its growth and development.

The fact that the branchiobdellids were absent from all experimental conditions other than C.D. after 10 weeks seemed to point out that this organism was favored by the absence of light. However, conclusions are difficult to draw due to the small number of branchiobdellids present on each O. pellucidus. Statistically the changes in the branchiobdellid population were not clearly significant. These organisms may not have completed a reproductive cycle during the 16 weeks of the study.

The more pronounced fluctuations in the populations of the symbionts other than branchiobdellids and the greater tendency for the rotifers and nematodes to survive in the conditions involving light are noteworthy. The presence of light facilitates a continuance as well as a reproductive cycling of

the rotifer and nematode populations. Rotifers and nematodes of O. immunis and O. pellucidus are positively affected by the presence of light.

SUMMARY

This study was undertaken in order to determine the effects of continuous light, 12L-12D, and continuous darkness on 3 symbionts (rotifers, nematodes, and branchiobdellids) harbored by pond crayfish (O. immunis) and cave crayfish (O. pellucidus). The abundance of these crayfishes offered an excellent opportunity to compare the reactions of symbionts living in a normal photoperiod to those living in the absence of a photoperiod.

Crayfishes from the pond and cave environments were brought into the laboratory and maintained in the aforementioned light conditions at 14°C. All crayfishes were maintained under identical conditions except for the periods of light exposure. Two crayfishes of each species from each light condition were sacrificed every 2 weeks and counts were made of the rotifers, nematodes, and branchiobdellids present.

All of the symbionts, with the exception of the rotifers, decreased in each light condition as the 16 weeks of the experiment progressed. The number of rotifers harbored by O. immunis remained relatively stable in all conditions from the outset to the end of the study. The population level of the rotifers of O. pellucidus, however, showed a general increase in all experimental conditions, but only the increases

in the conditions involving light were statistically significant. This indicated that the natural, continuous dark environment of the rotifer was not necessarily the optimum condition for the organism.

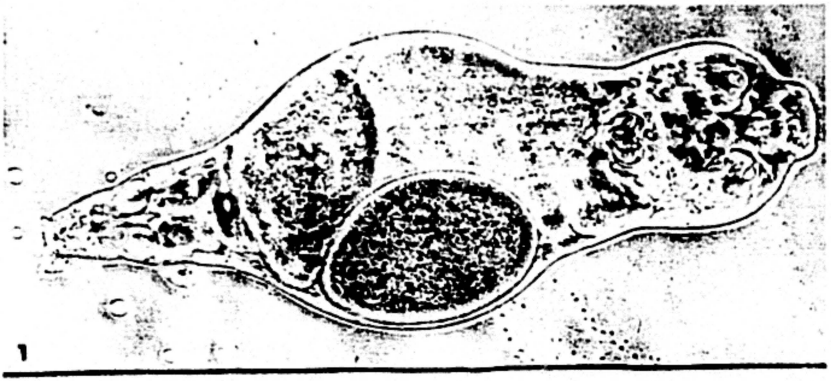
Generally, the more pronounced fluctuations in the population levels of the symbionts other than the branchiobdellids occurred in the continuous light and 12L-12D conditions. This suggested that the reproductive cycles of the rotifers and nematodes found on O. immunis and O. pellucidus were positively affected by the presence of light.

PLATE I

Figure 1. Bdelloid rotifer occurring in the gill chamber of O. immunis and O. pellucidus. Living specimen magnified 200 times.

Figure 2. Nematode harbored by the pond crayfish, O. immunis. Living specimen magnified 100 times.

Figure 3. Branchiobdellid found on O. pellucidus. Living specimen magnified 40 times.



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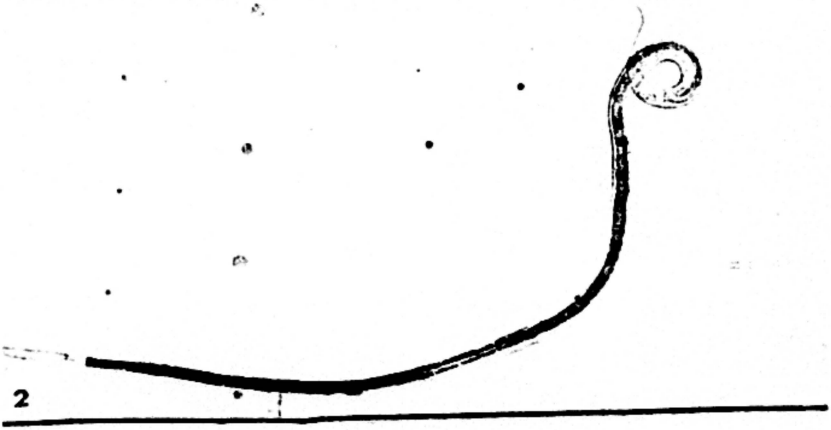

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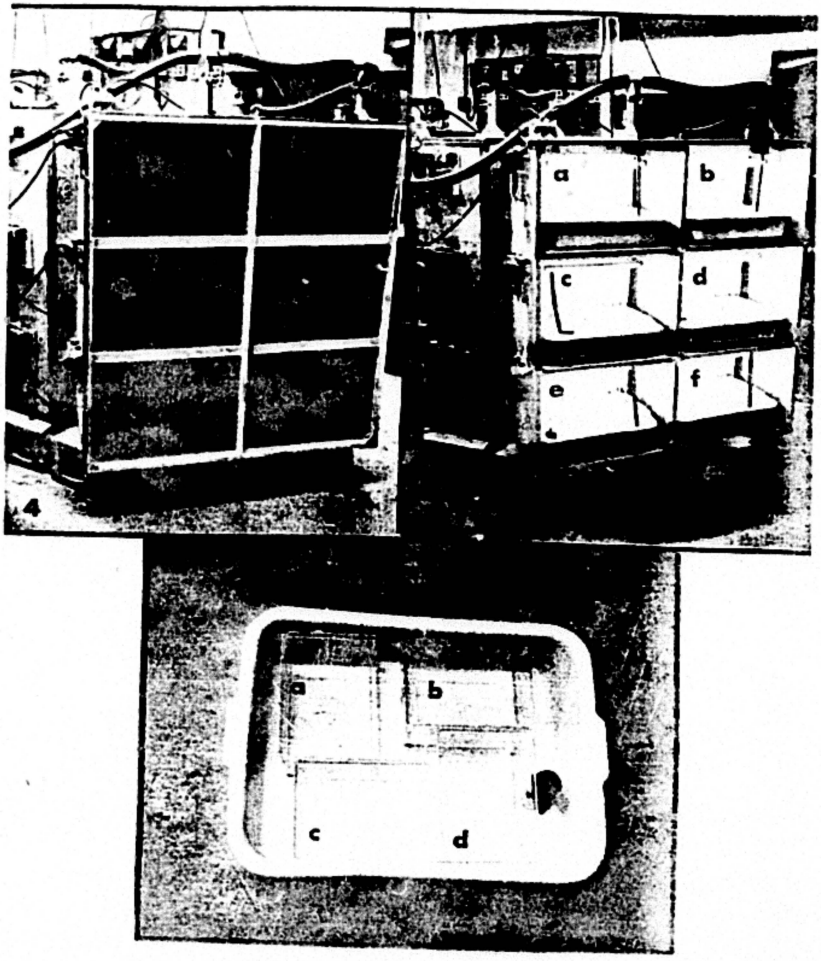
PLATE II

Figure 4. Light-tight chamber similar to the one used to maintain the symbionts in the desired laboratory conditions.

Figure 5. The chambers are divided into 6 sub-units, a, b, c, d, e, and f.

Figure 6. Each sub-unit contains one 13-1/2" X 18-1/2" X 5" pan with four 5-1/2" X 7-1/2" X 3-1/2" transparent refrigerator boxes, a, b, c, and d.


a
b
c
d
e
f
4
a
b
c
d

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