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Circadian Rhythm in Dugesia Tigrina and Its Correlation With Regeneration

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CIRCADIAN RHYTHM IN *DUGESIA TIGRINA* AND ITS CORRELATION WITH REGENERATION

by

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TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

Plant and animal rhythms have caught the attention of astute observers throughout history, but it was not until the eighteenth century that research in the area really began. Of the many rhythms we casually observe in the creatures around us, the most familiar is the daily rhythm of activity and rest. Ordinarily, the cues of daylight and darkness help to keep plants and animals synchronized with the environment. But neither honeybees, plants, nor man depend entirely upon such cues--all will show their "time sense" even when isolated from the outside world in a deep mine, cavern, or similar situation. In such cases the organism is said to "free run," that is, a more nearly inherent rhythm appears. This rhythm is approximately 24 hours and is usually called circadian, meaning around a day. This period is found to be a constant feature of plants and animals, leading some researchers to conclude that it is a fundamental attribute of protoplasm.

The literature on biological clocks or circadian rhythms dates back to the early 1950's. Many books such as The Physiological Clock by Professor Erwin Bunning, The Living Clocks by Ritchie Ward,

Biological Rhythms by Reinberg and Ghata, and _Of Time, Tides, and Inner Clocks_ by Henry Still, are available on the subject as it relates to various organisms, including man.

Fiddler crabs, lugworms, and _Drosophila_ have been studied extensively with regard to circadian rhythms. Earthworms are reported by J. L. Cloudsley-Thompson (4) as being polyphasic showing about 4 rest and activity periods in the 24 hour cycle and by J. Arbit (1) in "Diurnal Cycles and Learning in Earthworms" as learning better between 8 and 12 P.M. However, while much has been written about circadian rhythms in these and other organisms and about regeneration in _Dugesia tigrina_, the common fresh water planarian, there is little published information with regard to circadian rhythms in planaria. The purpose of this investigation was: 1. to ascertain the peak/peaks--low/lows in the circadian rhythm of _Dugesia tigrina_ and 2. to determine if such extremes in rhythm have a corresponding effect on the regeneration of worms transected during those periods.

EXPERIMENTAL PROCEDURE

The culture of planaria was purchased from the Connecticut Valley Biological Supply Co., Inc. in Southampton, Massachusetts. The worms were kept in a large battery jar at ordinary room temperature and with a northerly exposure to sunlight. They were fed once a week by placing pieces of raw calf liver in the battery jar for 1-2 hours. The liver was removed from the water which was replaced by fresh pond water.

Prior to beginning the experiments, preliminary observations were made to determine the characteristic behavior of the worms. Two experiments were performed in an effort to determine the high and low point/points in the worms' circadian rhythm. Once determined, the experiment on regeneration was scheduled for these times.

Experiment 1 was calculated to determine if regeneration would be affected by lengthy exposures to light or dark versus exposure to naturally alternating periods of night and day. 1. At 4:35 P.M. on May 28, 6 worms were transected just posterior to their pharynxes. 2. Two specimens were placed in each of 3 evaporating dishes approximately one-half full of pond wa-

ter. The dishes were designated as group 1, group 2, and group 3. 3. Group 1 was kept in constant light as provided by a 40 watt night light. Group 2 was kept in continuous dark in a cabinet. Group 3 was maintained under ordinary conditions in the laboratory. 4. All specimens were observed over the course of the next nine days.

Experiment 2 consisted of the following tests to determine the high and low point/points:

1. Level of Activity. Five planaria were placed in each of two 100 ml graduated cylinders. Observations were made periodically from June 16-July 3, and the vertical level occupied by each worm was noted.

2. Temperature. Five planaria were placed in each of two 15 X 125 mm test tubes. A control tube containing only water was also used. The temperature of the water in each tube was determined by means of a centigrade thermometer when observations were made.

3. Hatching of Capsules. Five test tubes each containing one capsule were observed for hatching.

4. Transverse Fission. Two worms were placed in each of three test tubes and observed periodically, within the same time span cited in Test 1, for the occurrence of transverse fission.

5. Reaction to Light. The same worms used in the transverse fission test were exposed to either bright sunlight or as was usually the case, to the light of a

large flashlight.

6. pH Determination. One control test tube with only water and an experimental tube containing 5 planaria were used. One drop of brom thymol blue (.2 gm/100 ml) was placed in each tube and the color change noted. This test was run from June 20-July 3. Later, the pH of the water was tested over three hour intervals until a total of 8 readings representing a 24 hour period were obtained.

Experiment 3 the main one of this investigation was to determine whether there is a correlation between the extremes of circadian rhythm and the rate of regeneration. A minimum of 2 worms were transected, usually just posterior to the pharynxes, during those periods interpreted as being peaks and lows in the planarian's circadian rhythm. Frequent observations were made with a stereomicroscope in order to determine progress in regeneration.

RESULTS AND DISCUSSION

The planaria were generally quiescent during the daylight hours. They tended to congregate in one or two clusters, usually with an east-west orientation. Within one day after receipt of the culture, approximately 10 posterior halves were visible, presumably indicating transverse fission. Ten days later two capsules were discovered; in another two weeks five more had appeared.

The results of experiment 1 are shown in Table 1. By reference to this table it can be seen that in the light group regeneration proceeded slightly more rapidly. It was hoped that the results of this experiment would aid in determining quickly the peak/peaks and low/lows of the planarian's daily rhythm. As it turned out, the results are not very conclusive since too few worms were utilized for that experiment.

TABLE 1

REGENERATION IN CONTINUOUS LIGHT VERSUS CONTINUOUS DARK VERSUS DAYTIME/NIGHTTIME CONDITIONS

DATE	GROUP	OBSERVATION
	Light	all 4 pieces quiescent
May 29	Dark	two anterior ends moved vigorously
	Normal	one anterior half mobile
	Light	five subjects observed; specimen derived from pharynx shaped same at both ends, but does move in one direction; pharynxes lacking on bodies derived from posterior parts; auricles just detectable; presence of eyespots uncertain
June 2	Dark	posterior ends have regenerated heads; auricles barely evident; no eyespots or pharynxes in evidence
	Normal	two anterior ends very active, one especially seems almost totally regenerated; two posterior ends quiescent until disturbed; head end is distinguishable; auricles just detectable as are eyespots, but pigment in the latter is barely discernable; pharynxes unnoticeable
	Light	two with pharynx and eyespots; one with pharynx; two with eyespots
June 6	Dark	three with pharynxes; two with small eyes
	Normal	two with minute eyes; no pharynxes

Table 2 summarizes the results of experiment 2. As can be noted in Table 2A, neither transverse fission, capsule hatching, water temperature, nor pH determination are of any value in ascertaining the extremes of the planarian's daily rhythm. The first two processes failed to occur. A centigrade thermometer was not sufficiently sensitive to detect the amount of heat produced by a small number of worms.

TABLE 2A

DETERMINATION OF PERIOD/PERIODS OF MAXIMUM/MINIMUM ACTIVITY IN CIRCADIAN RHYTHM

TIME HOUR	TIME DATE	RESPONSE TO LIGHT	TRANSVERSE FISSION	CAPSULE HATCHING	TEMPERATURE DEGREES C.	pH DETERMINATION
A.M.						
12-1	June 21	+	-	-	29	-
	June 26	+	-	-	26	+
1-2	June 26	+	-	-	26	-
	July 11	-	-	-	$28\frac{1}{2}$	. . .[a]
2-3	June 22	+	-	-	29	+
	July 2	+	-	-	28	-
3-4	June 17	+	-	-	28	. . .
	July 1	+	-	-	28	+
4-5	June 23	+	-	-	28	-
	June 28	+	-	-	25	-

TABLE 2A--*Continued*

TIME HOUR	DATE	RESPONSE TO LIGHT	TRANSVERSE FISSION	CAPSULE HATCHING	TEMPERATURE DEGREES C.	pH DETER-MINATION
A.M.						
5-6	June 24	+	-	-	28½	+
	July 1	+	-	-	27½	. . .
6-7	June 24	-	-	-	28	+
	July 1	-	-	-	27	-
7-8	June 19	. . .	-	-	29½	. . .
	June 28	-	-	-	27	. . .
	July 1	-	-	-	27	. . .
8-9	June 20	-	-	-	28	. . .
	June 21	+	-	-	28½	+
	June 26	. . .	-	-	. . .	. . .
	June 28	-	-	-	28	-

TABLE 2A--Continued

TIME HOUR	TIME DATE	RESPONSE TO LIGHT	TRANSVERSE FISSION	CAPSULE HATCHING	TEMPERATURE DEGREES C.	pH DETER-MINATION
A.M.						
9-10	June 20	-	-	-	28½	. . .
	June 22	-	-	-	29	-
	June 27	-	-	-	. . .	. . .
10-11	June 20	-	-	-	29	-
	July 1	-	-	-	28½	. . .
11-12	June 19	-	-	-	29½	. . .
	July 1	-	-	-	29	. . .
P.M.						
12-1	June 23	-	-	-	30	-
	June 28	-	-	-	28	-
1-2	June 23	+	-	-	31	-

TABLE 2A--Continued

TIME HOUR	TIME DATE	RESPONSE TO LIGHT	TRANSVERSE FISSION	CAPSULE HATCHING	TEMPERATURE DEGREES C.	pH DETERMINATION
P.M.						
1-2	June 25	+	-	-	. . .	-
	July 3	-	-	-	30	-
2-3	June 18	. . .	-	-	30½	. . .
	June 21	+	-	-	31	+
	June 22	-	-	-	30½	-
3-4	June 23	-	-	-	32	-
	July 1	-	-	-	30½	-
4-5	June 22	-	-	-	31½	-
	July 3	-	-	-	31	. . .
5-6	June 23	+	-	-	33	-
	July 3	-	-	-	31	. . .

TABLE 2A--Continued

TIME HOUR	DATE	RESPONSE TO LIGHT	TRANSVERSE FISSION	CAPSULE HATCHING	TEMPERATURE DEGREES C.	pH DETER-MINATION
P.M.						
6-7	June 20	-	-	-	34	-
	June 23	+	-	-	36½	-
7-8	June 16	. . .	-	-	29	. . .
	June 23	-	-	-	33	-
8-9	June 23	-	-	-	32	-
	July 2	-	-	-	30½	. . .
9-10	June 23	+	-	-	31½	-
	June 27	+	-	-	. . .	. . .
10-11	June 16	+	-	-	30	. . .
	June 18	+	-	-	30	. . .
	June 21	+	-	-	29½	+

TABLE 2A--Continued

TIME HOUR	DATE	RESPONSE TO LIGHT	TRANSVERSE FISSION	CAPSULE HATCHING	TEMPERATURE DEGREES C.	pH DETER-MINATION
P.M.						
11-12	June 16	+	-	-	30	. . .
	July 1	+	-	-	29	+

[a]No reading taken.

Gross vertical movement as indicated by the level of activity test appears to be the most reliable factor for determining extremes of circadian rhythms. This test coupled with the planarias' response to light provided the basis for selecting the peaks and lows of the worms' daily rhythm. The worm's reaction to light is interpreted as + if the animal exhibited any movement; it is considered as - if no movement occurred. In brief, reaction to light as recorded in Table 2A is positive from 12 midnight to 6 A.M. and generally negative until between 1-3 P.M. when 3 out of 5 readings are positive. From 3 P.M. until 9 P.M. responses to light are generally negative, but positive again from 9 P.M. until 12 midnight. Gross vertical movement as indicated by the level of activity test and recorded in Table 2B is high between 12 midnight and 9 A.M. and between 9 P.M. and 12 midnight with gross vertical movement greatest between 11 P.M. and 2 A.M. From 9 A.M. to 9 P.M. gross vertical movement is low, but especially so between 11 A.M. and 6 P.M.

TABLE 2B

DISTRIBUTION OF WORMS ALONG HEIGHT OF GRADUATED CYLINDER AS DETERMINED OVER CONSECUTIVE AND NON-CONSECUTIVE HOURLY INTERVALS

A.M.	12-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12
91-100	3	1		1		1						
81-90	2,3	2,1	2		1	1	1	1	1			
71-80	1	4,1	½					1			1	
61-70				1	½			2			½	½
51-60							1		1			
41-50	1		2		2			1			½	
31-40	1	1,1		1								
21-30	1			2		1,1	1,2					
11-20	2		1	2		1	2	1 2 1½	1,1	1	2	
6-10		1		1		1,1	1		1 1,2			1
3-5	2	1,4	3,1	5,3	2,4	3,1	4,2	1,2	1 1,1		1	
0-2	1,1	2,2	3½ 7	3,1	7,4	4,5	5,2	5½ 10 6,5	8½ 6 8,5	10 9	5,8½	9,9½

TABLE 2B--Continued

P.M.	12-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12
91-100		½		½,½						1,1	1	4½
81-90	1	½			½	½			1,1		1,3	
71-80				½						1		3
61-70												1
51-60								1	½		1	
41-50								1		½	1	
31-40			1		½		1					1
21-30										3	1	
11-20						1					1	
6-10						1	1	1	1	1 1,1		2
3-5			1				2	2		3,2	3	5
0-2	8,10	9½ 9½ 10	9 9,8	8 9½	9½ 10	8 9½	6,8	6,9	8 8½	5 6,4	4,3	3,1

The pH determination over one hour intervals shows no detectable change as is seen in Table 2A. This is true even during times the other tests strongly indicate as periods of high activity. In an effort to obtain more significant results the test was repeated using three hour intervals. Table 2C records the results of this series of readings. There were changes in pH which lend support to what the other tests indicated as highs.

TABLE 2C

pH DETERMINATION OVER THREE HOUR INTERVALS DURING A TWENTY-FOUR HOUR SPAN

TIME	RESULT
12:45 A.M.-3:45	+
3:45-6:45	-
6:45-9:45	+
9:45-12:45 P.M.	-
12:45-3:45	-
3:45-6:45	-
6:45-9:45	+
9:45-1:00 A.M.	+

Table 2B reflects the frequency of posterior halves of worms occupying upper levels of the cylinders. With the possibility that the internal clock is in the brain (3), it is likely that decapitated worms would not respond as expected during what would ordinarily

be periods of highs or lows. Note: the halves on the bottoms of the cylinders could not be distinguished from whole worms.

A peak then is interpreted as that time when high gross vertical movement is coupled with a positive reaction to light. A low is interpreted as that time when low or zero gross vertical movement is associated with a negative response to light. The high point in the cycle is fairly obvious. The identification of low points is complicated by the frequent appearance of posterior halves at upper levels of the graduated cylinders.

After transection of the worms in experiment 3, anterior ends were observed for regeneration of new tails and posterior ends for evidence of regeneration, especially of eyespots, pharynxes, and eventually auricles. Auricle regeneration occurred last, probably due to the fact stated by some authors that speed of regeneration in the planarian lessens as one proceeds laterally from the mid-line of its body.

The results of the regeneration phase of this investigation can be seen in Table 3. In general, anterior halves of worms transected during the 12-1 A.M. period precedes that of those transected during either the 9-10 A.M. or the 4-5 P.M. periods. Specific examples of this given in the table are observation-transection dates as follows: July 5-July 3, July 6-July 4,

TABLE 3

PROGRESS OF REGENERATION IN PLANARIA TRANSECTED DURING EXTREMES OF CIRCADIAN RHYTHM

TRANSECTION DATES	July 5	July 6	July 7	July 8
July 3/4:50 P.M. (low) 6 worms cut	8:00 A.M.- no change; 3:15 P.M.- posts. have protrusions ants.rounded	11:35 A.M.- 2 ants. have protrusions; 2-3 have small eyes	1:40 P.M.- ants.-5 have small tails; posts.-small eyes,auricles & pharynxes	4:20 P.M.- ants.-3 whole,2 lack tail; all posts. whole
July 4/12:45 A.M. (high) 5 worms cut	3:15 P.M.- ants. & posts.have protrusions	11:35 A.M.- all ants.- small point at rear; posts. all rounded	1:00 P.M.- ants.-all complete; posts.-all small eyes; 4 small auricles	4:50 P.M. ants.-1 deformed rear & 1 blunt; posts.-6 whole
July 10/9:10 A.M. (low) 5 worms cut				
July 10/4:40 P.M. (low) 5 worms cut				
July 12/12:15 A.M. (high) 4 worms cut				
July 12/1:45 A.M. (high) 4 worms cut				

TABLE 3--Continued

July 11	July 13	July 14	July 16	July 17
8:00 P.M.- 7 complete				
8:00 P.M.- 1 entire; 1 has deformed tail				
8:35 P.M.- ants.-3 pin-tails, 1 fine, 1 blunt; posts. 2 some regen. 2 none	8:50 A.M.- ants.-5 fine except 1 tail posts.-3 have eyes & pharynx but no auricles			11:15 A.M.- ants.- 2 complete, 1 deformed;
8:20 P.M.- ants.-5 fine; posts. 1 has some regeneration	9:15 A.M.- ants.-3 fine. 2 odd tails; 1 post.-small eyes, no auricles			11:15 A.M.- ants.-3 complete; posts.- 2 complete
	12:25 P.M.- ants.-1 has some regeneration; posts.- 3 have some regeneration	11:00 P.M.- ants.-3 fine posts.-3 have eyes	1:15 P.M.- ants.-4 fine; 2 others?- have eyes & pharynx, no auricles	11:35 A.M.- ants.-2 complete; posts.- 2 complete
	12:45 P.M.- ants.-1 pin-tail & 2 cigar tail; posts.- 4 have some regeneration	11:15 P.M.- ants.-2 fine except 1 odd tail; posts.-2 have eyes, pharynx uncertain	1:40 P.M.- ants.-1 fine 1 has odd tail; posts.- 2 entire but 1 lacks auricles; 1 odd	11:35 A.M.- ants.-4 complete; posts.- 2 complete but have minute auricles

July 7-July 4, and July 13-July 10. Eyespots on the regenerated heads of posterior halves usually appear by the third day after transection regardless of the hour of day the worms were cut. Several examples of this can be seen in Table 3.

Very near the end of the experiments determining extremes of circadian rhythm, it was discovered that lights in the room where the worms were kept had probably been briefly turned on every night. In Drosophila a single brief exposure to light at any time from the earliest larval stage until a day or so before emergence from their pupal cases is enough to synchronize the flies' clocks. The flies therefore will emerge about the same time of day as the light exposure, for they appear to interpret this time as dawn. Could the accidental exposure to light experienced by the worms have appreciably affected the results of this investigation?

CONCLUSIONS

The following conclusions can be tentatively drawn from the preliminary experiments described in this paper:

1. a) The peak hour in the circadian rhythm of planaria is between 12 and 1 A.M.

b) There is more than one low in the circadian rhythm of planaria--the most likely being from 9 A.M. until 10 A.M., and from 4 P.M. until 5 P.M.

2. a) The anterior ends of planaria cut during the peak hour in their circadian rhythm show complete regeneration more rapidly than those cut during a low.

b) According to the data accumulated in this investigation, there is no apparent difference in the time required for complete regeneration of posterior ends, regardless of when they were transected.

It is possible that a correlation between regeneration in a planarian and extremes in its circadian rhythm could have significance for higher animals, even man. Gay Gaer Luce (5) in Body Time cites studies with rats and their responses to surgery, medication, etc., as influenced by the various phases of their circadian rhythm. Luce implies medical practioners should delve

more extensively into possible similar relationships as far as man is concerned. And Dr. G. P. Wells (2), zoölogist of the University of London, reported to the Smithsonian Institute that "Studies of a worm may throw light on the internal rhythms of other animals, such as man's heart beat."

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