EXPLORATORY STUDIES OF LEECH BEHAVIOR

Thesis for the Degree of M. A.
MICHIGAN STATE UNIVERSITY
THOMAS E. HAGAMAN
1974

ABSTRACT

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By

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Eight exploratory experiments were conducted on various aspects of behavior in Haemopis marmoratis. A total of 120 leeches were used in this study. Contrary to some reports, longevity in captivity was found to be short on the average and highly variable. The dying leech was found to show reliable changes in behavior preceeding death. Behavior in 2 artificial habitats was studies. The topography of food capture and ingestion was defined. This species of leech was very active under both light and dark conditions but also showed a slight tendency to avoid light. The introduction of a protein substance failed to elicit activity reliably.

Stanley C. Ratner,
Thesis Chairman

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF ARTS

Department of Psychology

1974

ACKNOWLEDGEMENTS

The author is pleased to acknowledge the advice and encouragement of Dr. Stanley C. Ratner, Dr. Lawrence I. O'Kelly, and Dr. Ralph L. Levine.

I am also indebted to Dr. T. Wayne Porter of the Department of Zoology at Michigan State University who identified some of the leeches used in this study.

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LITERATURE REVIEW

The leeches (phylum Annelida, class Hirudinea) are derived from oligochaetes and are recognized by anterior and posterior suckers with at least the posterior being well developed. Most of the 300 known species live in fresh water. Some species are marine and a few have become adapted to a terrestrial life, living in moist soil or in foliage in tropical climates. Although leeches are rather difficult to measure accurately, they range in size from tiny to 65 cm. long. In addition to having suckers, leeches differ from other annelids in that they lack setae and the coelum is reduced to a set of narrow channels. The leech nervous system is considered to be more specialized than in any other annelid (Barnes, 1963).

The body of the leech is composed (starting at the skin surface) of layers of epidermis, circular muscle, and longitudinal muscle. These layers form a cylinder of muscle enclosing a modified coelum, a fluid filled cavity or cavities functioning as a hydrostatic skeleton since the coelemic fluid is incompressible.

The body segments are linked together by the gut, the vascular system, and the nervous system. Digestion in blood sucking leeches depends on the action of bacteria living in the gut as these annelids lack digestive enzymes. In the vascular system, the blood flows forward on the dorsal side, and back on the ventral side. The nervous system is composed of a solid ventral nerve cord with lateral nerves in each segment. In Haemopis marmoratis the brain lies just behind the 7th segment.

Leeches are hermaphroditic and lay eggs in the spring and summer. Some species will continue to produce eggs for 5 to 6 months. With the exception of the Glossiphonidae, the eggs of all species are surrounded by a horny capsule or cocoon, each of which may contain one or more eggs. The egg cases are then deposited in damp soil or glued to stones, plants, etc. The family Glossiphonidae carries eggs in cases glued to the ventral side of the adults. After these eggs hatch, the young attach themselves with their posterior suckers to the parent. The eggs of Macrobdella are laid in spongy capsules and are usually deposited in mud or turf at the water's edge just above the water level. In Hirudidae sperm transfer is accomplished with an eversible penis.

H. marmoratis, commonly known as the horse leech, frequently lives in drinking troughs and ponds and feeds on the blood of livestock. This animal is also a predator and scavenger. Kopenski (1969) reported finding this species feeding on dead crayfish, dead fish, frog egg masses, other leeches, earthworms, aquatic annelids, snails, salamanders, aquatic insects, and mammals. These leeches frequently leave the water to hunt, and one species is completely terrestrial.

The main blood sucking leeches found in the United States belong to the genus *Macrobdella* although the European medicinal leech *Hirudo medicinalis* is established in parts of New York. Kopenski (1969) referred to *Macrobdella decora* as a "voracious parasite" which has been known to attack fish, frogs, salamanders, turtles, man, and other vertebrates. This leech has been known to kill brook trout. According to Moore (1923) *Macrobdella* were collected for medicinal purposes in New York well into this century. During World War I, there was some commercial interest in this leech as a source of hirudin for use in surgery to prevent blood clotting. Moore concluded his section on the economic history and importance of the leech by pointing out that "leeches can be recommended as a most effective and a satisfactory bait for bass, yellow perch, the larger sunfishes, and similar fishes."

Kopenski (1969) reported the results of a study concerning ecological factors affecting the distribution of the 22 species known to occur in Michigan. Water temperature was not a limiting factor for most species nor was type of bottom. The presence of submerged objects providing shelter was found to be very important. Such factors as pH, the type of local soil, and the chemical nature of the water indirectly affect the distribution of leeches by determining the amount and type of food available. Most leeches were found to prefer lentic (still) to lotic (moving) waters and this was especially true for M. decora which was never found in any lotic water sampled in Kopenski's work.

Leech predators include carnivorous fishes, wading birds, and other leeches. Although no leech parasites are known, Moore (1923) mentioned that medicinal leeches in confinement have a high mortality rate due to infection.

Most authors working with leeches reported that with the exception of some of the fish leeches, these annelids are easily kept in the laboratory. Moore (1959) recommended housing both Haemopis and Macrobdella in covered aquaria with sloping sand bottoms and shallow water. He reported resistance to a wide range of lower temperatures and keeping M. decora in good condition for as long as 14 months without food. The only requirements are clean, pollution free water, and an occasional meal of worms.

Several collection methods have been used with varying degrees of success. Kopenski used 4 methods; water agitation and netting, catching and examining vertebrate hosts, pulling up and examining submerged objects and plants and scooping up debris and spreading it out to dry. When this last method is used the leeches attempt to leave the drying material and may be readily captured. Kopenski reported finding most of his specimens at depths of between

0 and 1.2 m. Moore (1923) recommended placing boards at the edge of the water for the leeches to gather under. Moore also tried (unsuccessfully) to develop traps in an eradication program. One trap, made with boards and enclosing an area 4-m^2, baited with blood, and placed in water 15-cm. deep, did yield a few animals (7 in 3 visits). Moore concluded that the best traps were readily available in the form of small boys.

Moore's practical work also produced some information on the chemical sensing abilities of these animals. In a test to determine poison sensitivity, leeches (species not stated) immediately responded to a solution of 1 part copper sulfate to 5,000,000 parts of water by becoming extremely active in comparison to a control group. The leeches quickly left the contaminated water if they were allowed to. Additional work showed that leeches are also extremely sensitive to calcium chloride, chloroform, and nicotine. Moore also reported a laboratory experiment testing potential baits for trapping. The test substances were allowed to diffuse from a finely drawn out tube into an aquarium containing leeches. He obtained his most active response to beef extract which was quickly traced to its source and imbibed. The composition of the beef extract used was not given and nothing was said about the salt content of the solution. Some leeches also followed a solution of dried blood but no eating was observed. Musk elicited a very weak response but no response was made to urine or to oil of anise.

In addition to chemical sensitivity, leeches respond to vibration, shadows, and light. Barnes (1963) listed 3 types of sense organs; free nerve endings, sensory cells with terminal bristles, and photoreceptors. Eyes are composed of clusters of these photoreceptor cells surrounded by a pigment cup and located on the dorsal surface of the anterior segments. The number of eyes (2-10) is used for classification of leeches. Kopenski (1969) pointed out that the eyes can be located and counted more easily in a preserved specimen by immersing the head portion in a 5% solution of potassium hydroxide. This serves to bleach out the surrounding pigment. Moore (1930) stated that in addition to the eyes there is a circlet of "light perceiving" organs on each segment.

Dales (1963) reported the existence of epidermal sense organs, apparently capable of great distortion, that may be proprioceptors and that are completely unlike anything found in other annelids. Barnes (1963) described "sensory papillae" that occur as small clusters of cells, each bearing a terminal bristle, which projects above the cuticle surface. These may occur in a dorsal row or arranged in a ring around one annulus of each segment. The function of these structures is unknown.

A few fragments of information are known about the general behavior of the leech. Moore finally solved his eradication problem after observing that leeches dig into the mud as winter approaches and that they are usually found at this time of the year at water depths between .5 and 1 m. At 4° C. he found that leeches are "dormant and nearly insensitive" and at -10° C., dead. During the winter he lowered the water level in the infested lake for a period long enough to freeze the exposed mud and the leeches within it.

The behaviors most frequently studied are locomotion and feeding. One observation of predator defense was found. Morgan (1930) mentioned that members of the family Glossiphonidae immediately roll up into a ball when disturbed and drop to the bottom. Locomotion has 2 forms, creeping by alternating suckers in a looping fashion, and swimming by a dorsal/ventral undulation of the body. Elongation occurs when the circular muscles contract and longitudinal muscle contraction results in shortening and flattening the body. Volume does not change during this process. Annelids depend on the coelum for locomotion. Dales (1963) reported a "virtual loss of locomotor ability when an annelid is punctured" or if the fluid is withdrawn. If the muscular cylinder is damaged the circular muscles in the nearest intact segments contract and restore pressure to the system.

Dales also reported that swimming occurs only in the absence of tactile stimulation. Swimming may be stopped by lightly touching the ventral side of the body. Removing the stimulus elicits swimming again. If a leech is suspended from a string and a cover glass is presented to one sucker the glass will be grasped and held until another cover glass is grasped by the other sucker. The first glass will be dropped at this time. This may be continued indefinitely.

Some leeches are predators and some are parasites, but most species use both methods of feeding. When a blood sucking leech finds a skin surface it attaches itself to the skin with the posterior sucker, swings the anterior end about and finally attaches the anterior sucker, bites, and secretes hirudin into the wound. Morgan (1930) stated that the "American medicinal leech" (probably M. decora) takes up to 2-1/2 times its own weight in blood. Extra fluid is drawn off by the kidneys while sucking. A blood meal will "keep" in the gut for nearly a year, explaining why these animals have been kept in aquaria for up to 15 months without any visible harm. Predacious leeches usually grasp their prey (such as an earthworm) between their suckers and swallow it whole.

The problem of prey recognition has received some attention, both formal and informal. Morgan (1930) discussed the highly developed chemical sensitivity of the leech yet she noted that leeches will attach themselves to rubber boots "almost as readily as to legs". Dales (1963) reported that most fresh water leeches will move towards a mechanical disturbance. Ratner (1972) reported that *M. decora* reliably responds to mechanical disturbance in the form of a water current by swimming or at least detaching the anterior end from the substratum. *Hemiclepsis marginata* is attracted by host mucus and *Haemadipsa*, a terrestrial leech, is said to respond to a hand several cm. away. Dales also reported an experiment in which *Hirudo* and *Glossiphonia* were attracted to a test tube containing water at 33-35° C. The tank water temperature was not given and it is not clear that this is a component of feeding.

Ratner (1972) demonstrated habituation to light but the role of light in the behavior of the leech is not clear as there is little information available on sensory psychology and orienting behavior. Smith *et al*. (1971) found that *Hirudo* will orient to light by keeping its dorsal surface towards the light source and that shadows may elicit activity with the leech swimming vaguely upward. There is some evidence for the existence of an activity cycle that may be based on light. Moore (1959) mentioned that members of the family Erpobdellidae and the genus *Haemopis* are nocturnal predators.

There have been three recent attempts to demonstrate some form of behavior modification in the leech. Ratner (1972) found habituation to light onset and to water current. He also reported significant retention of habituation to light onset.

Henderson and Strong (1972) reported classical conditioning in *Macrobdella ditetra* using shock as the UCS and light as the CS. Thompson and Porter (1966) and Thompson (1971) found some evidence for escape learning but none for avoidance learning in *Haemopis grandis*.

EXPERIMENTS

Contrary to both reports in the literature and our expectations, it was discovered at an early stage of this study that leeches are not necessarily easy to keep alive in the laboratory. A second look at the literature produced the same general statements of ease of maintenance but no empirical data on longevity in captivity or the numbers of animals actually kept by workers reporting successful maintenance. The first 5 experiments were conducted in an attempt to get quantitative data on some maintenance problems.

Experiment 1: Longevity

The purpose of this experiment was to determine longevity for leeches in captivity.

Method

Subjects.--Three groups of leeches were obtained from Mogul Ed. Co., Oshkosh, Wisc. Eight leeches were caught in the Lansing, Michigan area. Ninety-five of the 110 animals studied were *H. marmoratis*. Twelve *M. decora* and 3 turtle leeches (species unknown) were also obtained. Leeches were kept singly or in groups in 1-1 beakers covered with cloth netting or in plastic aquaria 16 cm. by 28 cm. by 12 cm. deep covered with cloth netting or clear, perforated, plexiglass sheets. The animals were kept in a dark room at a temperature of 18-22° C.

Procedure.--Three groups of leeches were established. The first was comprised of 28 leeches from the shipment received from Mogul Ed. Co. in October, 1972. These animals were kept in aged M.S.U. tap water, aged Red Cedar River water, or distilled water. The second group was comprised of 51 leeches from the shipment received in April, 1973. This group was kept in aged tap water. The third group was comprised of 28 leeches from the shipment received in July, 1973. The leeches in the first group were fed, the rest received no food. Longevity records were kept during all phases of the study.

Results and Discussion.--Unequal Ns and in some cases small Ns prevented a thorough controlled test of longevity in the 3 types of water used in Group 1. There did seem to be a slight tendency for leeches to live longer in small aquaria with aged tap water.

Table 1 shows mean and median longevity (lifespan in the laboratory for leeches alive on arrival) for the 3 groups of leeches obtained from Mogul Ed. Co. Figure 1 is a cumulative mortality curve for Group 1. Data for leeches that died from known causes such as escape and dessication are not included. The longevity of leeches captured locally and immediately brought to the lab ranged from 6 to

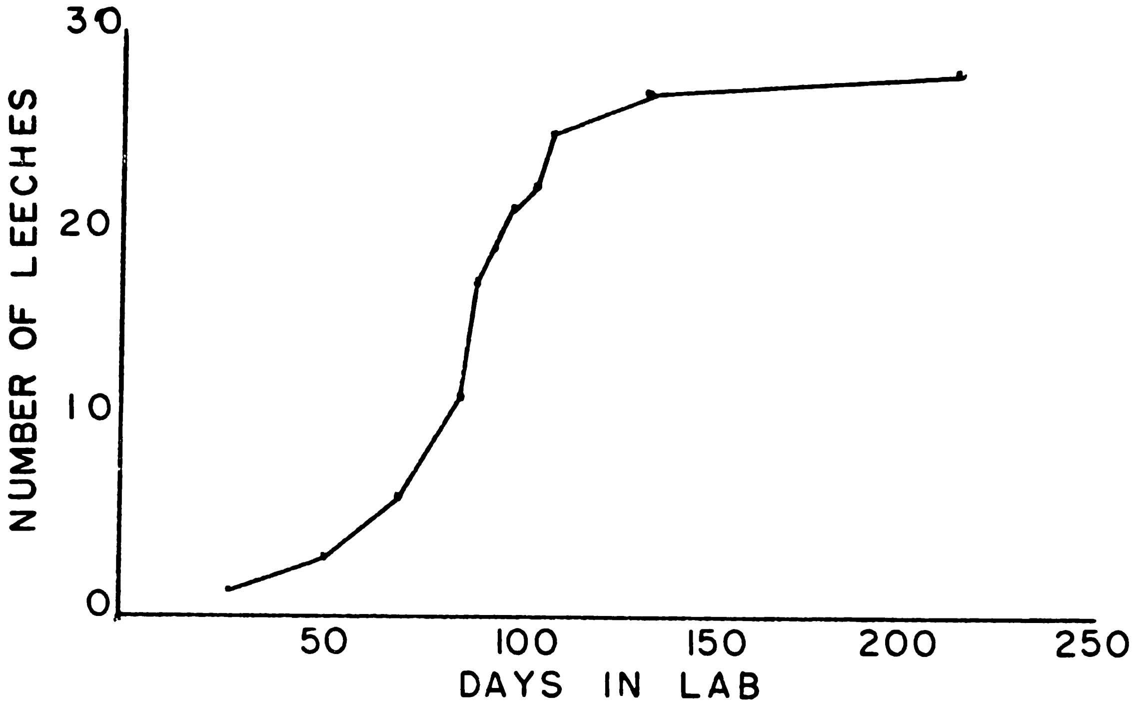

Figure 1.--Cumulative Mortality Curve for Group 1 in which the leeches were fed on days 40-45.

25 days. Figure 1 also shows that the leeches in this group were fed just before they began to die. Feeding probably did not contribute to the longevity of this group.

These data do not agree with reports in the literature that leeches are easy to maintain. Part of the discrepancy may be due to the lack of published empirical data. It is also significant that no reports of reproduction in captivity were found. The failure to meet this standard criterion for successful adaptation to captivity may indicate that leeches do not live in captivity but die slowly. This is obviously important for anyone studying the behavior of captive leeches.

There seems to be large differences between the 3 groups. No statistical tests were conducted on these differences because maintenance conditions were not constant within or between groups. It is possible that all of the leeches received from Mogul Ed. Co. were collected in the preceeding summer and fall. This might explain some of the differences in longevity.

Table 1.--Days Longevity in Captivity for Leeches Received in October 1972 (G-1), April 1973 (G-2), and July 1973 (G-3).

	Group		
	1	2	3
n	28	51	28
mean	90	48	24
median	84	60	14
range	24-216	20-65	1-73

Experiment 2: The Behavior of the Dying Leech

Maintenance and mortality problems were not completely solved during the course of this study. It was necessary to determine what, if any, changes in behavior immediately preceded death and at what point the animals' behavior was conspicuously and reliably altered.

Method

Subjects.--Eleven *H. marmoratis* were used from the group received from Mogul Ed. in July, 1973. Each worm was kept in a 1-l.beaker. Each beaker contained 500 ml. of aged tap water. The temperature ranged from 18° to 20° C. during the experiment.

Apparatus.--A Gossen light meter was used to measure light intensity.

Procedure.--All leeches were kept in darkness except during the actual observation periods when they were exposed to light ranging in intensity from 645 to 1,184 lux. The leeches were not fed. The beakers were carefully placed on a table and a 15 minute adaptation time was allowed before the light was turned on and observation began. After the 30 minute test period the beakers were returned to a dark chamber.

The ongoing behavior of each leech was sampled daily until all animals had expired. The following behavior classification system was used.

1. Resting on the bottom of the beaker (B).
2. Hanging in a U shape from the side of the beaker (U).
3. Crawling by "looping" (C).
4. Swimming (S).
5. Undulating, 1 sucker attached to the substratum (Un).
6. Thrashing, general, spasmodic, uncoordinated body movements with neither sucker attached and no progress from 1 point to another, frequently upside down (T).

Results and Discussion.--The time of death was not easy to determine. A leech was considered to be dead if it was lying on the bottom of the beaker, usually upside down, and was in the same position for 2 consecutive days. At this time the beaker was shaken and the leech prodded with forceps. If no response occurred the animal was classed as expired. No response ever occurred for animals that met the first criterion. Death was then considered to have occurred on the day previous to the final prodding test.

Two leeches lived for 2 days after the start of the experiment, 1 lived for 3 days, 4 lived for 4 days, 2 lived for 5 days, and 2 lived for 6 days. Figure 2 shows the mean time per 30 minute period spent in each of 4 behaviors. The normal resting position is in a U shape on

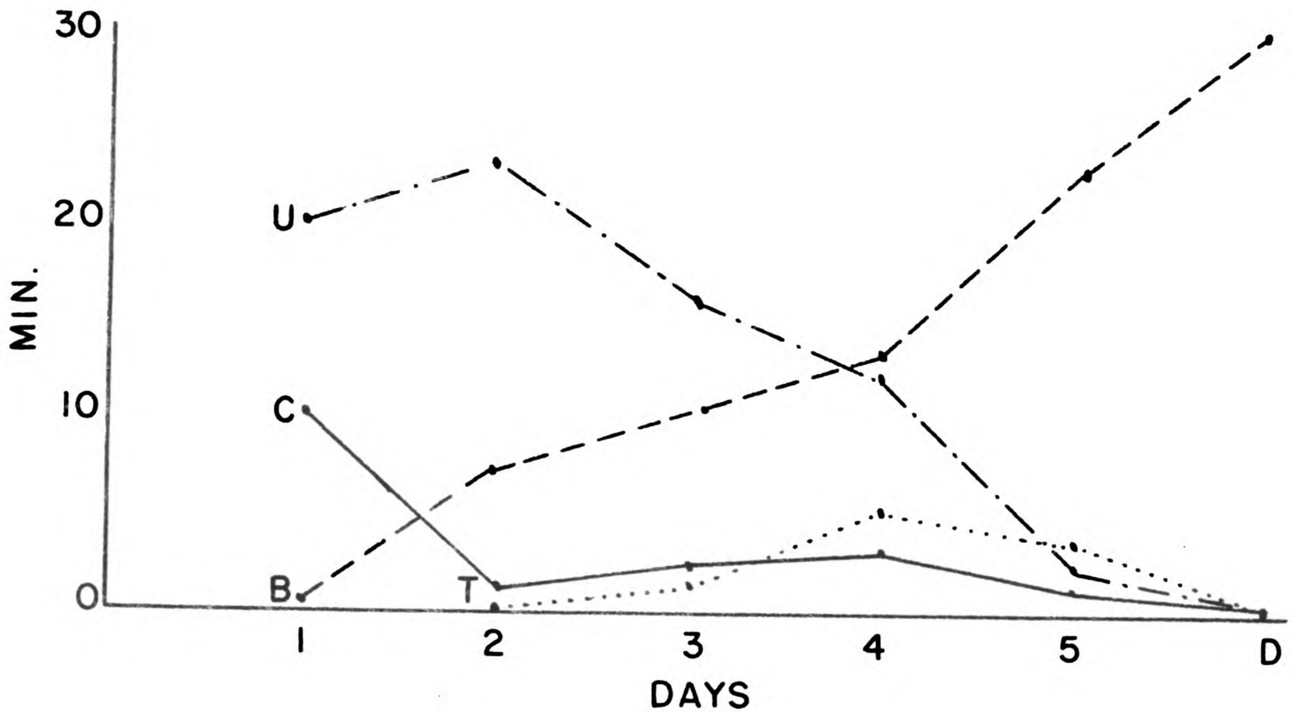

Figure 2.--Behavior changes preceding death.

the beaker wall. The animals gradually spent more and more time on the bottom as they neared death. There was a decline in crawling. The thrashing movements were never seen until the 3rd day prior to death and these movements reliably predicted death within 3 days. Swimming and undulating were not graphed because of their low rate of occurrence (means of less than 1 for each day).

These data agreed with less formal observations of the entire group of 120 leeches used in this study. A criterion of 5 day survival after the conclusion of an experiment for data inclusion was established for the rest of the study.

The results of this experiment do not predict the exact sequence of behaviors that a particular leech would show so no hypothesis concerning the cause of these changes can be given.

Experiment 3: Behavior in a "Natural Habitat"

Two attempts were made to study leech behavior in an artificial habitat in order to gain some insight into the normal behavior of these animals.

Method

Subjects.--Forty-two leeches (*H. marmoratis*) were used. These animals were in the group received in October, 1972 from Mogul Ed. Co. This group had the longest mean longevity (90 days) found in this study.

Apparatus.--A 40-l. aquarium was set up with 8 l. of aged Red Cedar River water and 11 kg. of white silica sand. The sand sloped from a depth of 2 cm. at one end to 10 cm. at the other.

Procedure.--All of the leeches were placed in the tank on arrival and left in the dark. The temperature was kept in 8-22° C. The leeches were observed daily for 14 days. The leeches were not fed during the experiment. On the last day of observation the leeches and sand were carefully removed from the tank and the distribution of the leeches was recorded.

Results and Discussion.--After 24 hours in darkness 15 of the worms had buried themselves in the sand. After 48 hours 27 leeches were buried or partially buried in the sand. The rest were either hanging in the corners above or below the water line (13) or hanging in a U shape on the wall (2). This distribution was typical for the remainder of the experiment. Many holes were visible in the sand.

On the 14th day the leeches and sand were removed. Table 2 shows the distribution of the leeches on the walls and in the sand at 4 depths. The leeches buried in the sand were frequently found in intertwined balls of 3 to 5 animals each. Some mucus was obviously present around resting animals but no traces were found lining the tunnels leading down to the aggregations.

Table 2.--Distribution of Leeches in Aquarium on Last Day of Experiment.

Location	Number
In corners above water	10
On walls above water	2
On top of sand	2
0-2.5 cm. below surface of sand	1
2.5-5 cm. below surface of sand	26
5-7.5 cm. below surface of sand	1
7.5-10 cm. below surface of sand	0

A second attempt at creating an artificial habitat involved building an "ant farm" for leeches. This procedure was used to determine the distribution of leeches in a substratum with greater depth than in the previous habitat experiment. The structure was designed so leeches could be observed below the surface of the sand without disturbing them.

Method

Subjects.--Four *H. marmoratis* were used from the group received from Mogul Ed. Co. in October, 1972.

Apparatus.--A glass structure similar to commercially available toy "ant farms" was built. This unit was 50 cm. high, 40 cm. wide, 2 cm. deep, and had an enclosed shelf at the top (12 cm. by 40 cm.) that held a petri dish full of aged tap water. The unit was filled with silica sand and placed in a 40-l. aquarium. The shelf was filled with sand to a depth of 2 cm. The water level in the aquarium could be varied to control the moisture content of the sand filled case. During all phases of this experiment the sand was kept saturated with water. A leech could obtain moisture by burrowing into the sand or crawling into the petri dish.

Procedure.--The room temperature was kept between 18° and 22° C. Leeches were placed individually in the shelf area and observed. The leeches were not fed during the experiment.

Results and Discussion.--All subjects failed to orient to moisture even if initially placed in the dish or in a water filled depression in the sand. All of the leeches eventually died, apparently of dessication. These results were not expected and cannot be explained at the present time. The leeches used were healthy, active, and of the same species and group used in the previous experiment. The leeches did tend to move into the corners of the shelf.

Perhaps netting and placing them in the habitat elicited a predator defense reaction of movement away from the area of initial placement and into an area of thigmotactic stimulation.

Experiment 4: Feeding

It has been reported that *H. marmoratis* feed on worms in the wild and that worms are a suitable food for captive leeches. This was tested in the laboratory and the animals were observed in an attempt to define the behavioral topography of worm capture and ingestion.

Method

Subject.--Twenty-seven *H. marmoratis* from the group received from Mogul Ed. Co. in October, 1972 were used. The animals were maintained individually in 1-l. beakers. Each beaker contained 500 ml. of aged Red Cedar River water. The worms were kept in a dark room in which the temperature ranged from 18° to 20 ° C.

Procedure.--The experiment was conducted in red light. A piece of earthworm (*Eisenia foetida*) approximately one-third the length of the leech to be tested was dropped into each leech's beaker. The times elapsed before capture and complete ingestion were recorded as well as notes on general behavior. The animals were observed for 30 minutes and then left undisturbed for 24 hours in darkness, after which they were checked for worm ingestion.

Results and Discussion.--Three leeches failed to consume their worms during the 24 hour period. At least 1 leech caught his worm and later released it. Five leeches consumed their worms at some time after the 30 minute observation period. For the 19 animals that caught their worms during the observation period the mean time to capture was 10 minutes (mode = 5 min.). The mean time to ingest after capture was 5 minutes (mode = 5 min.).

The topography of worm catching behavior was fairly consistent. The preconsummatory components elicited by dropping the cut worm into the beaker consisted of actively crossing the beaker from one side to the other and "searching movements" with the anterior end while the posterior sucker remained attached to the substratum. The components of this stage seemed to be the same if the leech was aroused and began searching immediately or if the leech did not respond for several minutes. Once the leech responded to the presence of the worm by becoming active capture always occurred in less than 5 minutes. Time to become active after the worm was placed in the beaker ranged from a few seconds to over 30 minutes in the 5 cases where the leeches became aroused, captured and ingested their worms after the 30 minute observation period. When the anterior end of the leech touched the worm the posterior sucker was quickly brought into position to hold the worm clasped between the 2 suckers. The consummatory sequence consisted of worm ingestion, apparently aided by "stuffing movements" of the posterior end. Occasionally, if the worm was first grasped by its middle, it was ingested doubled. The worm was swallowed whole. Occasionally, after the process was about half complete, the leech released the posterior sucker, straightened out, and began a succession of sine wave undulations while ingesting the remainder of the worm.

Post-consummatory behavior consisted of more "searching movements" with the anterior end and tapping the substratum while the posterior sucker was attached to the substratum. The animal might then crawl briefly and then rest, usually still on the bottom for a variable length of time before resuming its U resting position on the beaker wall.

The response of _H. marmoratis_ to fresh, raw pork liver was also briefly studied. It was hoped that liver could be used as bait in a leech trop. Leeches in small aquaria located and appeared to feed on liver. This behavior was filmed. Unfortunately, all attempts to attract and trap wild leeches failed although a few were caught as a result of more or less random searching.

Feeding did not have a clear effect on longevity.

Experiment 5: Response to Light

An experiment was conducted in an attempt to elicit reliable light escape behavior in an alley as a preliminary to a planned study of escape learning. Attempts have been made to establish learning based on light escape and avoidance but no quantitative data showing reliable light escape in *H. marmoratis* was found.

Method

Subject.--Twelve *H. marmoratis* from the group received in April, 1973 were used. They were maintained individually in 1-l beakers in darkness at 18° to 22° C. Each beaker contained 500 ml. of aged tap water.

Apparatus.--A Gossen light meter was used to measure light intensity.

A white plastic cutlery tray was used that had 3 alleys 5 cm. by 24 cm. by 4.5 cm. deep. The alleys were covered with a sheet of clear glass, half of which was covered with black plastic. A 25 watt, frosted, incandescent bulb provided a light intensity of 270 lux at the water surface. The shaded half of the alley was constant for each trial. No heat filter was used but both room and water temperature were monitored. The water temperature never varied more than the room temperature (+ or -1° C.) during any trial.

Procedure.--At the beginning of the trial the light was turned on over the alleys and leeches were netted and removed from their beakers and transferred to each of the

3 alleys (4 leeches per alley). No adaptation period was allowed. The number of leeches that were more than 1/2 in the lighted portion of each alley was recorded once per minute for 30 minutes. The leeches were not fed during the experiment.

Results and Discussion.--The leeches tended to avoid lighted areas and were in the dark on 78% of the observations. When the frequency distribution of animals found in the light and in the dark was compared with a random distribution (dark and light having equal numbers of leeches) the difference was significant at the .01 level (Chi^2=9.6). However, the leeches did not escape from light and stay in the shaded area but moved between the light and shade throughout the 30 minute observation period. There was no tendency for the subjects to spend more or less time in the shade as a function of time during the period. This form of behavior would complicate any study of learning in this type of apparatus so no additional work was done in this area.

Experiment 6: Behavior Under A Light Cycle

This experiment was conducted to gain more information about the role of light in leech behavior. The results of Experiment 6 indicated that leeches will remain active in the light over a short period of time. Some workers have suggested that at least some leeches are primarily nocturnal yet most human contact with leeches occurs in the daytime. This experiment placed leeches under a light-dark cycle to determine if any activity cycles could be found that correlated with a light cycle.

Method

Subjects.--Eleven *H. marmoratis* from the group received from Mogul Ed. Co. in July, 1973 were used.

Apparatus.--Eleven 4-l.covered clear plastic aquaria served as test tanks. A timer was used to establish the light-dark cycle. A minolta time lapse Super-8 camera system was used for data recording. A Gossen light meter was used to measure light intensity.

Procedure.--The subjects were placed individually in the 11 tanks. Each tank was set up with silica sand to a depth of 2 cm., and a small metal box for shelter. The light cycle was 16 hour at 215 lux of fluorescent light and 8 hour at 5 lux. A 1 week adaptation period under the cycle was allowed before any data was collected. The temperature was kept between 18° and 22° C. The leeches were not fed during the experiment.

The following behavior classification system was used.

1. Resting: U shape.
2. Resting: on bottom.
3. Crawling.
4. Swimming.

Section A. Habituation Trials to Electronic Flash.

Response and habituation to light from the electronic flash unit needed for data recording during the dark portion of the light cycle was studied. This was done on the 8th day of adaptation during the 2nd hour of darkness. Data was manually recorded under red light according to the system outlined above once per minute for each subject for 40 minutes. For the first 20 minutes no external stimulus was given. During the next 20 minutes the flash was fired once per minute. Activity data was examined to determine response to the flash.

Results.--The mean number of minutes of activity per animal under the red light condition was 2 (median =0). The mean number of minutes of activity under the flash condition was 3.2 (median = .5). This difference was not considered to be a major disruption of normal behavior.

Section B. Response to a Light Cycle.

On the 9th day a 24 hour data collection run was made. The camera was set to take one frame per minute. On the 16th day another 24 hour data collection run was made.

Results and Discussion.--Figure 3 shows activity data (crawling + swimming) for the first run and Figure 4 shows the activity data for the 2nd run. In both cases the vertical axis is the number of minutes active in each 30 minute period. The horizontal axis is the 48 half hour periods. Periods 1-30 were in light and 31-48 were in darkness.

There was no tendency for individual activity scores to be correlated between the two data collection trials. The mean correlation, period by period for all subjects between run 1 and run 2 was .08. There were no reliable period to period changes and no regular cycles. There was no reliable preferred resting position (hanging in a U on the wall or resting onthe bottom) for individuals.

The average leech in this experiment spent 37% of the time in a U shape on a wall, 33% of the time lying on the bottom or in the small shelter, and 30% of the time crawling or swimming. Activity did not correlate with the light-dark cycle.

Experiment 7: The Effects of Light Intensity on Resting

Previous experiments have suggested that *H. marmoratis* is equally active under light and dark conditions, but that light is avoided to a certain extent. A final experiment was conducted investigating the effect of light intensity on amount of activity.

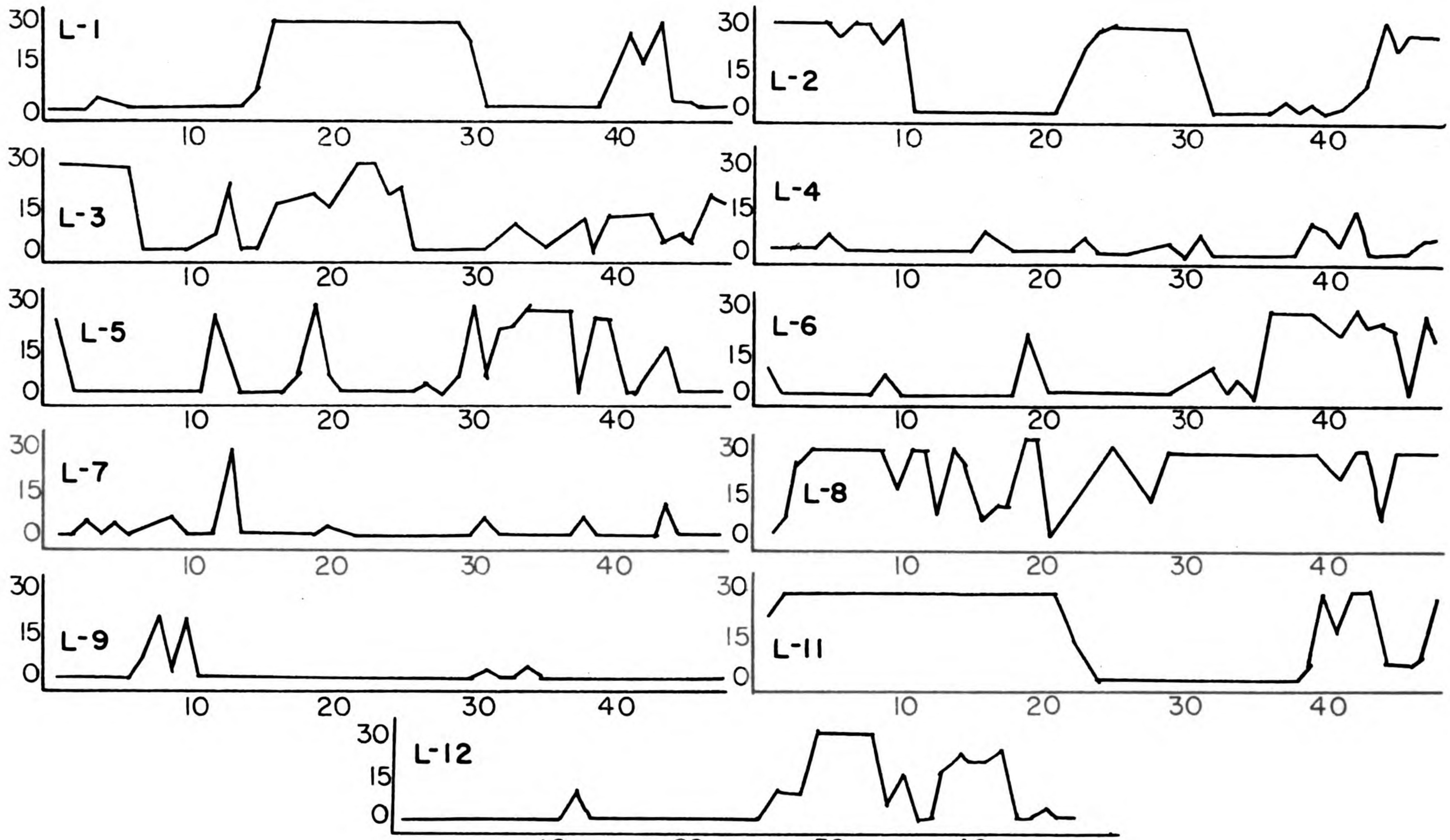

Figure 3.--Time Spent in Activity (first run).

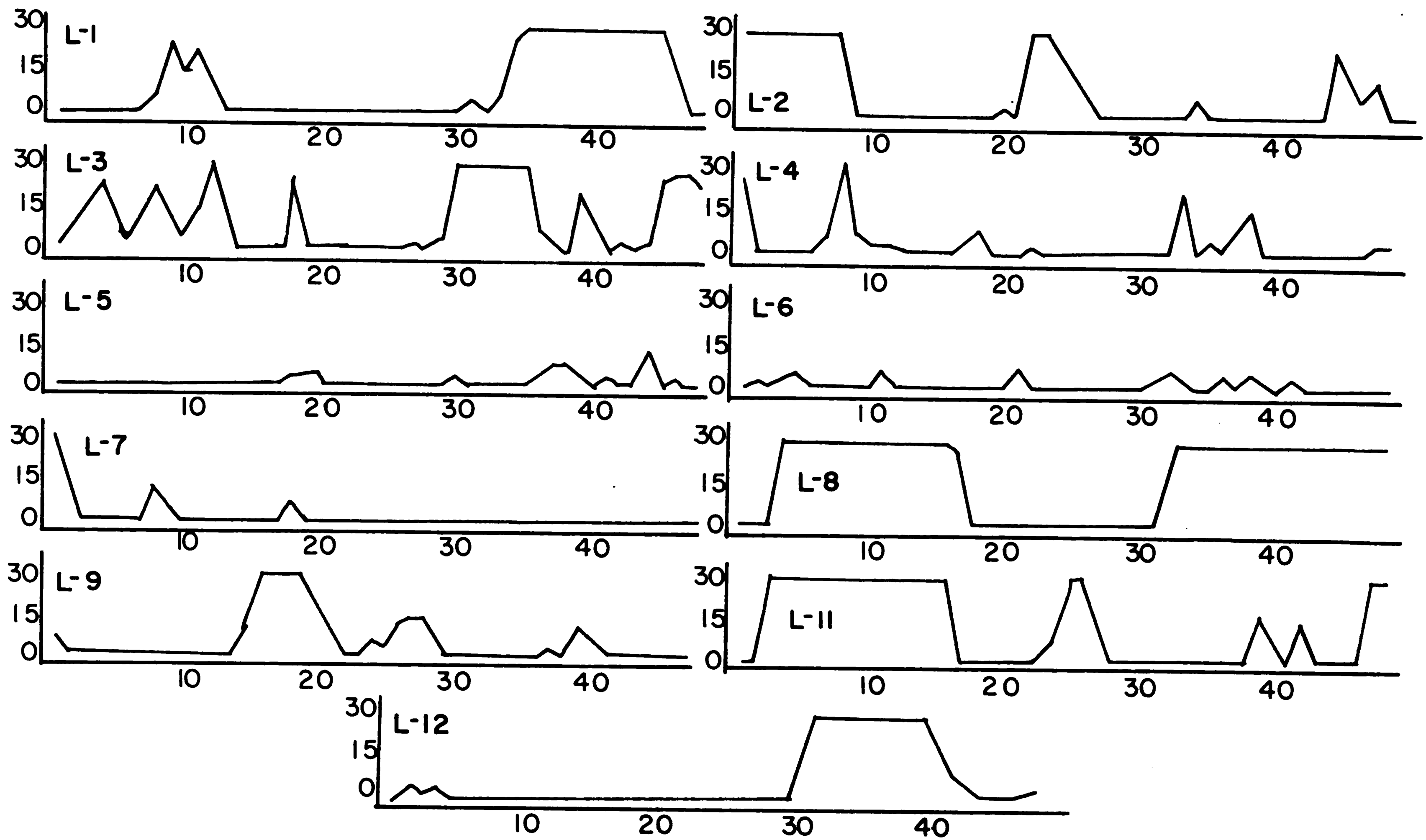

Figure 4.--Time Spent in Activity (second run).

Method

Subjects.--Seven *H. marmoratis* were used from the group received from Mogul Ed. Co. in July, 1973. All leeches were housed individually in darkness in the plastic aquaria used in Experiment 6. The temperature ranged from 18° to 22° C.

Apparatus.--A red darkroom bulb was used to obtain a base rate of responding. A fluorescent lamp was used as a light source. Light intensity was varied by changing the lamp to leech distance. A Gossen light meter was used to measure light intensity.

Procedure.--The leeches were tested individually in their home tanks. The tanks were not moved during the experiment. The leeches were not fed during the experiment. There were 4 light intensity conditions; red light, 2 lux, 650 lux, and 1,200 lux, and 2 pretest treatment conditions, storage in darkness and exposure to room illumination (280 lux) for 2 hour prior to the experimental session. Pilot observations had suggested that leeches do not respond to red light.

An interval sampling technique was used for data recording with each leech's behavior being observed and recorded once per minute during each 30 minute trial. No more than a 1 minute adaptation time to the light was allowed. Each subject was used once in each of the 8 conditions. Running order was randomized. The following behavior classification system was used.

1. Resting: U shape.
2. Resting: on bottom.
3. Crawling.
4. Swimming.
5. Undulating.

Results and Discussion.--The mean number of minutes active (crawling + swimming + undulating) for all subjects with no pre-exposure to light was 1 (standard deviation = 3) under red light, 6 (standard deviation = 10) under 2 lux, 11 (standard deviation = 12) under 650 lux , and 9 (standard deviation = 11) under 1,200 lux. A repeated measures analysis of variance (Winer, 1971) was performed. The difference in activity under the 4 light conditions was significant at the .75 level (F=1.6). There was no evidence for a change in activity during the 30 minute trial as would be expected if habituation to light resulted in a reduction in activity. The leeches were active during a mean of 27 minutes in the no pre-exposure condition and 32 minutes in the light pre-exposure condition. There was a significant difference at the .05 level between total activity in red light and mean total activity for the 3 light conditions (t = 3.1 with 6 degrees of freedom).

This experiment suggests that fluorescent light does disturb resting behavior by eliciting a variable amount of activity. Red light does not seem to have this effect on resting. These leeches did not show any tendency to be more active under a more intense light.

One conclusion that can be drawn from this experiment and the others investigating the role of light in leech behavior is that activity is probably not a good dependent variable for use in the study of this invertebrate. It is also possible that light is simply not very important to a leech.

Experiment 8: Response to a Protein Substance

Leeches in Experiment 5 easily located worms. One can ask many questions about how this was accomplished. This experiment was an attempt to elicit increased activity (equivalent to the beaker-crossing and searching movements found in Experiment 5) by introducing a protein substance into aquaria containing leeches.

Method

Subjects.--Ten *H. marmoratis* from the group received from Mogul Ed. Co. in July, 1973 were used. They were kept in darkness in plastic aquaria as used in Experiments 7 and 8.

Apparatus.--A 1-dkl. plastic aquarium was used as a test tank. A pipette was used to introduce measured amounts of test substances into the tank. A Minolta Super-8 camera system was used for data recording.

Procedure.--One hour prior to a test the leech to be tested was placed in the test tank which contained 2 l. of aged tap water. At this time a light was turned on that provided an intensity of 280 lux at the tank water surface. This light remained on during each trial. After the 1 hour adaptation period 5 cc. of test solution was carefully added by means of a clean pipette to the tank water at a surface point 10 cm. away from the leech.

The test substances were prepared as follows:

Beef extract: 1 cube Cellu low sodium bouillon (10 mg. Na per cube) in 250 ml. of aged tap water.

Milk: whole, full strength.

Water: aged tap water.

Five drops of red food coloring were added to each 250 ml. of solution to aid visibility. Each leech was tested once with each substance. Ongoing behavior was filmed (1 frame every 4 seconds) for 15 minutes prior to the introduction of the test substance and for 25 minutes after the introduction. The subject was then returned to its home tank and the test tank cleaned and filled with aged tap water for the next subject.

Each frame of film was scored as showing activity or resting.

Results and Discussion.--There was no significant increase in activity after the introduction of any of the test substances. There was some indication, based on watching the data films that 2 of the 10 leeches did respond strongly to the milk by showing the same type of anterior end waving and searching movements seen in Experiment 5.

Activity may not be a good index of food detection. The liquids diffused completely in about 20 seconds. If a leech orients to food by moving about to increase taste stimulation, it is conceivable that an animal would respond to a completely homogeneous distribution of food by remaining still.

Experiment 5 did show that leeches do locate worms and pork liver, but that arousal time varies. This may have had an effect on this experiment. It is also possible that milk and beef extract may not be effective elicitors of feeding behavior.

SUMMARY

The first 4 experiments were preliminary investigations dealing with the technical difficulties of long-term maintenance of leeches in the laboratory. The technical problem was partially solved by identifying behavior changes that reliably predicted the approaching demise of a leech. These changes were an increase in the time spent on the bottom of the container, a decrease in crawling, and the appearance of a peculiar thrashing movement which seemed to indicate a loss of coordination between the anterior and posterior suckers.

Three experiments were conducted investigating the effect of light on behavior. The sudden onset of light tended to produce a highly variable increase in activity (compared to activity under red light), and leeches in an alley showed a slight tendency to spend more time in a shaded area then in an area exposed to light. However, leeches were equally active in both the dark and light portions of a day-night cycle.

Two experiments on feeding were conducted. A regular sequence of behaviors for worm capture and ingestion was found. An attempt was made to elicit an increase in

activity (searching behavior) by introducing various protein substances into aquaria containing leeches. Most of the subjects did not show any increase in activity.

Activity was used as a dependent variable in 5 of the 8 experiments because crawling and swimming were the most obvious behaviors found in this annelid. This may still be the best available variable but measurement and analysis techniques need to be refined. Occasionally, a stimulus such as a worm or light onset that would sometimes elicit an immediate response would elicit an apparently identical but delayed response. This delay factor was a major contributor to the large variances found in this study.

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