1974

A method of detecting reaction to environmental influence by recording circadian activity, using the flagfish Jordanella floridae

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A METHOD OF DETECTING REACTION TO ENVIRONMENTAL INFLUENCE BY RECORDING CIRCADIAN ACTIVITY, USING THE FLAGFISH *Jordanella floridae*

A Thesis

Presented to

The Graduate Faculty

University of the Pacific

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science

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by

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3 March 1974

This thesis, written and submitted by

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Dated 4 march 1974

ACKNOWLEDGMENT

To those who tolerated:

Through a season of life,
within a forest of compassion,
a chance walk along a causeless path.
embraced by tranquil boughs,
healed by herbal teas,
sheltered by tender leaves.
the sage, the innocent, the wild, the subtle,
all of inexpressible beauty.
i call you friends.

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ABSTRACT

This paper investigates a means of detecting the reaction of aquatic vertebrates to environmental change by recording behavioral patterns within the diurnal activity. An apparatus measuring the activity of Jordanella floridae, Goode and Bean, using a photoelectric sensor system is described. Individual sensors monitored specific areas of the test aquarium that had special significance to the fish in the way of a particular behavioral category. An event recorder documented the breakage of light beams, giving continuous and accurately timed data of activity. Two distinct patterns of activity were recognized; a control, or typical, pattern, and a pattern resulting from the administration of a large (10°C) change in environmental temperature. Modifications in the diurnal behavior were consistent with reactions that could be adaptive for the species under similar conditions in nature.

INTRODUCTION

Activity measurements are often used to study the consequences of environmental factors on the biology of animals. Most studies have been concerned with the rhythmic variations of general activity. Bindra (1961) points out that such recorded activity may consist of many different types of movements (posture change, object manipulation, examination of surroundings), that fill up a large portion of the animal's waking hours, and have no "purpose" other than the accomplishment of the immediate individual result. When the movements are examined collectively there may be no particular sequence to the occurrence of the separate acts. In a certain situation, however, they may be integrated and directed toward some specific goal. For example, if the animal is hungry, exploration of the adjacent areas may serve as the initial phase of a feeding behavior. In combination with other individual movements, the result is a very stereotyped behavior pattern that brings about satisfaction of the animal's needs; the occurrence of these types of activity patterns may possibly be used as an indicator of those expressed needs.

Throughout the life of the organism, its biological needs at any given moment will determine the types and amounts of behavior categories shown. For example, under certain environmental conditions reproductive behavior may have priority. As environmental conditions change to a less optimum set of circumstances, a larger proportion of the animal's activity might be devoted to some other behavior, as other more pressing needs arise. This change to a newly appropriate behavior is expressed in the general activity pattern, but may not be evident from, or detectable by, many of the monitoring techniques used.

Behavioral research under field conditions has employed techniques ranging from periodic trapping methods (Barlow, 1958; Williams, 1958), to telemetry (Mech, Heezen, and Siniff, 1966), and radioactive trapping (Frigerio and Eisler, 1968). Due to the variability of field conditions, such studies may give only an approximation of average activity, since strict controls are sometimes not possible.

Study of the animal in the laboratory allows close control over the conditions under which activity is recorded. ~~It should be recognized that all the important factors of~~ the animal's usual habitat cannot be duplicated, and that such studies in the lab can produce their own bias. Capture and transport to the simulated environment, or confinement itself (Kavanau, 1964), may have some effect on the animal's behavior. An acclimation period may resolve some of the trauma, but is dependant upon the species, and there is no certainty that the behavior observed is exactly the same as the animal would have shown in nature.

The desire to quantify a specific aspect of activity often leads to the use of techniques which limit the animal's response to specialized objectives. Richards (1966), and Suter and Rawson (1968) obtained locomotor activity data by counting the revolutions of exercise wheels. Such activity cages can reach a high degree of complexity (Kavanau, 1963), and incorporate concepts such as tilting cages (Kissel, 1963), resonant radio circuits (van Toller and de Sa, 1968), and air capacitance systems (McClelland, 1965). An artificial behavior pattern, such as a conditioned reflex, is often used in conjunction with the above types of apparatus and may further affect the behavior in an unmeasurable way.

The mechanical difficulty of obtaining activity patterns for terrestrial animals is compounded in an aquatic system due to the nature of the medium and the subjects. This fact is reflected in the paucity of data on fish activity as compared to the data on other vertebrates (Farner, 1965). Mertz and Barlow (1966) quantified their observational studies with records produced by a manually operated keyboard. Other methods used in aquatic studies are heat conductance (Heusner and Enright, 1966), photography (Gans, 1966), and photocells (Thines and Delabastita, 1965; and Kleerekoper, *et al.*, 1969).

This study explores a method of recording the activity of small aquatic vertebrates as a function of environmental influence. Behavior patterns that the organism itself exhibits under a particular situation are quantified. The results show a basic shift in the type and extent of behavior for the group of subjects used. Further studies should determine if the nature of the response is characteristic for the entire species. The test does establish that changes in the behavior can be used as as indication of biological reaction to gross changes in surroundings.

MATERIALS AND METHODS

Description of the Apparatus

The apparatus used in this study to monitor activity was similar to designs used by Hall and Armstrong (1969), and Cairns and Waller (1971), utilizing a photoelectric detector system. The adaptation of this type of equipment to the experimental purpose offered continuous recording of activity, low cost, reliability of operation, high resolution of data, and minimum effects on the behavior of the subjects.

The components were purchased from Farmer Electric Products Company, Inc., Tech Circle, Natick, Massachusetts, and were available from stock. The sensors consisted of six low-impedance cadmium sulfide photocells (PC-1L), linked to six 4.5 volt light sources (LS-2C), with adjustable focus. Infrared filters on the lamps allowed use under completely dark conditions. Signals from the photocells were amplified by solid state logic modules (TR3), that could be adjusted for the degree of photoelectric response. The amplified signals were sent to the recording device through non-inductive relays (S-2443). Transformers (F9-136) converted standard 115 volt current to the required voltage for operation of the system. All components were plugged into amphenol sockets mounted in pressboard, and the entire circuitry was placed in a protective aluminum housing. The basic system (Figure 1) could be modified to include features for increased sensitivity of reaction, counting, and waterproofing.

For the sensors to operate at maximum efficiency, each photocell had to remain in close alignment with its respective light source. This was accomplished by constructing plexiglas holding brackets which rested upon the test aquarium (Figure 2). Holes in the vertical arms of the brackets recieved the photocells and light sources, maintaining proper alignment even if the apparatus was accidentally jarred.

With the aligned sensor units in operation, any interruption of the light beam by the subjects caused the dark sensitive logic module to send a signal to the relay. The relay, in turn, completed a circuit to one of the solenoid-actuated pens of an Esterline Angus event recorder (model #A620X). The record chart was advanced by a spring driven system of gears at the rate of three inches per hour. Each pen of the recorder, in its unactuated position, traced a continuous line along the time axis of the record chart. The deflection of a pen made a mark at the particular time that the event occurred, thus producing an accurately timed activity record for each sensor.

The incorporation of the event recorder completed the self-contained nature of the apparatus. Little maintainance was needed, outside of rewinding and inking of the recorder. Any subjective impressions from direct observations, as well as additional notations such as date, feeding, or extraneous disturbances, were made on the chart in progress. All this was done with minimum disturbance to the subjects from experimenters, allowing uninterrupted long-term activity recording.

Experimental Subjects

The flagfish, *Jordanella floridae*, Goode and Bean, gave preliminary clues to being a suitable animal for determining if changes in behavior could be used as an indication of environmental effects. This fish is commonly encountered as a "tropical fish", in the commercial sense. Its small size and ease of maintainance makes it compatible to laboratory studies. It can be kept in a completely bare aquarium, or with just a gravel substrate, and still carry on daily activities throughout the year (Smith, 1973), if adequate water quality and food are available. Observations of Jordanella in nature are difficult and verification of whether such lab behavior is typical of behavior in the wild has not been accomplished. However, Mertz and Barlow (1966) quantified certain behaviors under laboratory conditions and found them to be consistent in pattern, suggesting a fairly constant species-typical behavioral repertoire, as is found in many other cyprinodonts.

Jordanella also demonstrates a certain sensitivity to marginal levels of some environmental conditions, which is reflected in behavioral changes. Water chemistry, particularly hardness, if not within a certain range, causes the fish to be more susceptible to auditory and visual stimuli, resulting in a shy, retiring behavior. The addition of salts brings the level of dissolved solids closer to an optimum, enabling the fish to better tolerate such problems (laboratory observations). Foster, Cairns, and Kaesler (1969) also noted that a build up of metabolic waste products in the water has an increasingly depressive effect on egg laying, which is renewed at previous levels after removal of such substances. The same investigators found a similar effect due to the addition or removal of a soluble detergent.

It was anticipated for this study that a change in behavior in the diurnal activity pattern along the above lines could be recorded.

Experimental Design

Two pairs of sexually mature Jordanella were introduced into the test aquarium, which measured 12" x 12" x 24", and contained 15 gallons of aged tap water. The bottom of the tank was covered with #1 grade aquarium gravel to a depth of two inches, and synthetic yarn tufts were anchored near each end of the tank. These tufts were used as substitutes for real plants which tended to fragment and drift between the lights and photocells, creating an erroneous record. The fish showed no adverse reactions to this surrogate vegetation, which is often used by hobbyists as a spawning medium. Substrate filtration of the water provided constant areation and removed suspended sediment that might have impaired sensor operation. A sheet of glass covered the top of the aquarium to prevent water loss and temperature change due to evaporation. An acclimation temperature of 24°C - 26°C was maintained by a thermostatic aquarium heater. Lighting was by means of a 40 watt florescent fixture mounted 24 inches above the aquarium. An electric timer controlled the photoperiod at 14 hours. A secondary set of lights, shielded from directly illuminating the aquarium, were set to turn on a half hour before, and off a half hour after, the main lamp. This provided a transitory period of "twilight", that reduced the shock of a sudden light change.

The entire complex was surrounded by a cloth curtain to preclude visual stimuli from sources outside the aquarium. Observations of activity within the tank were through a screened viewing port (Foster, et al., 1969) placed at one end.

In order to prevent any anticipatory activity of the fish relating to feeding, regular intervals were not observed, though no attempt was made to randomize such times. Placement of food into the aquarium was done as inconspicuously as possible, mainly during the lighted hours, and was noted on the record chart.

Since the apparatus was located in a building where classes were regularly conducted, a fan mounted on a resonator box was used to mask the vibrations of student traffic. This device itself showed no adverse effects on the activity of the fish.

Prior to the onset of the test conditions, the aquarium and apparatus were allowed to operate undisturbed until the fish established what seemed to be a repeated regular routine. Periodic examination of the record chart revealed that a general pattern of activity emerged after a few days of acclimation to the new surroundings. Once the routine was established, observations allowed a determination of the most advantageous positioning of the sensors to incorporate as much of the areas of the tank where behavior was taking place (Figure 3). Each of the six sensors monitored an area that had particular significance in daily activity. The extent and duration of the activity that the fish showed in the proximity of each photocell channel indicated the importance of that particular area in satisfying some aspect of the animal's biology. The number of light beam interruptions made, over a time period, and for each channel, were taken as the "control" activity pattern.

With an alteration of the environmental conditions, any reaction to the changed surroundings would be demonstrated as a change in activity, either over time or between channels. To produce the test condition, the temperature was raised using the aquarium heater. In the interest of eliciting a behavioral change, a large temperature interval was used, raising the acclimation temperature of $24^{o}C$ - $26^{o}C$ to the test temperature of $34^{o}C$ - $36^{o}C$. The recorded activity of the fish under this modified environmental condition was used as the "experimental" pattern. Data was then available for a comparative analysis of the effects of the environmental change.

Method of Analysis

The pen deflections on the record chart, indicating the breakage of a photocell beam, were quantified by accumulating all of the marks in two hour time blocks for each channel. This interval was chosen to reduce the large amount of data for handling in analysis. At times of peak activity the marks often overlapped, producing a solid band on the record. The number of marks present in such cases were estimated by measuring the length of the band and assuming there to be two marks per millimeter. The counts for each time block throughout a 24-hour period were recorded for each photocell for five consecutive days. This procedure was followed for both control and experimental data.

The objectives in analysis were to determine the reliability of each set of activity data, in terms of deviation in activity of one 24-hour period from any other 24-hour period. That is, once the pattern was established, how dependable was it in terms of on-going cycles.

According to the experimental design, if a change in behavior did occur, then the activity would show a consistent pattern under the experimental conditions that was different from the pattern for control conditions. The Winer (1962) reliability test was used to evaluate the extent of the repeatability of pattern.

The Winer test is most used in psychology, where it is a tool for the evaluation of the effectiveness of a test in obtaining consistent results. For example, consider the case of an intelligence test that is given to a group of people several times. The coefficient of reliability is a measure of how accurately the test assesses each person's level of intelligence. The analysis assumes that the conditions under which the test is given, as well as the test itself, remain constant. The purpose of estimating the reliability of the test is in a determination of how the scores of each person differ from one session of the test to the next; a "within person" variance. For each session, the scores may also differ from one person to another; a "between persons" variance. The Winer calculations use much of the power of analysis of variance to obtain the mean square values for the above sources. The reliability coefficient itself involves a ratio between the variance being investigated (within person), and the major variance present in each test (between persons).

$$\text{Reliability Coefficient} = 1 - \frac{\text{mean square within person}}{\text{mean square between persons}}$$

The coefficient, then, is an expression of the variance produced by all of the uncontrolled factors. The smaller the variance in the scores for each person, the greater the reliability of the test. The nature of the coefficient is essentially a percentage measurement and no levels of significance are associated with it. Accuracy of the test, within its limitations, is quite good (see Nunnaly, 1967).

In adapting the above technique to this study, the reliability coefficient was used as a measure of the repeatability of a temporal pattern. This shows the degree of consistency that exists between the organization of replications for control and experimental periods. The data for each period was set up for a two-way analysis of variance for the effects of replication and time (Table I). The source of the variation being investigated was the replicate-time interaction. Other variances present were the total, between time, and between replications. The percentage of the total variance not due to the replicate-time interaction was the estimate of reliability. The use of mean square values adjusted for the differences in the absolute amounts of activity from one replicate to another.

$$\text{Reliability Coefficient} = 1 - \frac{\text{mean square replicate x time}}{\text{mean square total}}$$

The coefficient was then a method of comparing the replicates from a recording period to see if some sort of similar pattern was present in each. A coefficient of 1.0 indicated a perfectly repeatable pattern, and a coefficient of zero meant no repeatability in the patterns. A comparison of the control pattern to the experimental pattern was made by using the mean time block scores for each set of data in the calculation of the variances.

RESULTS

Visual examination of the record charts, disregarding channel distributions, showed that at different times during a 24-hour replication there occurred periods of activity characterized by either heavy or light concentrations of marks. The patterns formed by these groupings were distinct for the control and for the experimental recordings (Figure 4). The reliability coefficient, calculated for the composite activity of the six channels during the control period show a value of 0.77, indicating high pattern repeatability. The experimental pattern showed less repeatability with a score of 0.32.. The lower figure for these patterns suggests a less tightly organized regimen of behavior. When the means of the time-channel blocks for the five replications were calculated, and the control period tested against the data from the experimental period, a reliability coefficient of 0.05 resulted, indicating quite dissimilar temporal organization between the two patterns. Plotting means of the two sets of data revealed qualitative differences in patterns (Figure 5). During the control period the greatest activity was in the lighted hours, while in the experimental period the activity was during the darkened hours. The replications, plotted as percent daily activity, also verified the impression of distinctly repeated patterns (Figure 6).

Although composite data by channel gave distinct control-experimental differences, there were also differences in activity between the channels within a set. Figure 7, A-F, shows these channel activity differences, with respect to pattern and repeatability. Channels of maximum mean activity were different for the control period as compared to those of the experimental period, indicating that changes were taking place not only in the diurnal pattern, but also in the fish's positional priorities in the water column for a particular time of day.

Thus, differences between the two systems can be categorized as spatial as well as temporal. In the control period, most of the activity took place in the near substrate areas. This reflects the males spending time in established territories, and male to male interactions at the boundaries of mutual territories. The former produced records in channels 2 and 6, while the latter produced markings in channel 4. In records from the control period, the upper zones of the aquarium (channels 1, 3, and 5) showed sporadic records relating to feeding and exploratory behavior. There was little or no activity during the unlighted hours, and what there was, was sparce and of low organization. Observations verified that the fish were sleeping in various positions on the substrate during the darkened hours. Under conditions of the experimental period, most of the activity took place within the near surface areas, and during the unlighted hours. Visual observations of the activity revealed intense to and fro swimming motions a few inches below the water's surface; much more intense than any swimming motions seen during the control period. The fish were oriented with their snouts pressed against the glass of the aquarium, and the impression was that they were attempting to swim through the invisible barrier of the glass. Activity of the fish during the lighted hours of this period consisted of hiding in the substrate cover, with short and furtive foraging behavior.

Reliability coefficients and smoothed graphs were produced for all the channels, but only those most representative of the observed changes are presented (Figures 8 and 9). The near substrate area of channel 6 was typified by patterns of high consistency of organization (R.C. = 0.72) during the control period, and by more variable patterns during the experimental period (R.C. = 0.01). Conversely, the reliability of patterns for the surface area monitored by channel 3 increased from control to experimental periods (R.C. from 0.35 to 0.44, respectively).

With respect to the behavioral categories of the channels, there is indication that a change in the type of activity took place. The control pattern was diurnal behavior, primarily territorial and other aspects of reproduction. Under the stress of the temperature change, this pattern of activity became replaced by nocturnal behavior, primarily emigration from the stress area. A summary of the activity patterns, and the extent and significance of the changes in activity are presented in Table II.

DISCUSSION

When an organism is confronted with an environmental hardship, there are certain adaptive morphological and physiological responses shown that are characteristic of the species of animal. Behavioral responses, while undoubtedly also species specific, are not as easy to visualize and measure as those of morphology and physiology. More often the behavior is a flexible complex of "established" patterns present in the animal's behavioral repertoire. A combination of behavioral responses appropriate for one set of conditions may be altered forming a different combination suitable to a different situation. These behavioral patterns are usually directed towards the needs of the individual, such as food supply, proper temperature, humidity, etc., and have threshold levels well below that which would prove lethal to the animal; these levels may also be below that able to be demonstrated by any morphological or physiological changes.

Once a response to environmental conditions is observed, it is necessary to know the context of the behavior as well as the overall life style of the animal, in order to make any sense of it. Several different species of fish, if exposed to an identical negative stimulus, will react in very different ways (Warner, Peterson, and Borgman, 1965). A pelagic species might flee to open waters, while a benthic species will remain motionless near the bottom or attempt to hide beneath some object. Other reactions are possible and do occur, depending upon the individual behavioral/environmental adaptations.

In this study, the control pattern represented a complex of behavior patterns, including feeding, various maintainance movements, territoriality, and courtship and mating. These activities are associated with near optimum conditions. In nature, *Jordanella* is typically very territorial, choosing open areas among vegetation for its nesting sites. When threatened, the fish enters the dense surrounding plants to hide. A further aspect of its behavior is noticed from recording its circadian activity. When conditions are introduced that create a non-ideal or stress situation, the fish not only hides during the day, but attempts to emigrate from the area during the cover of darkness.

The common theme of adaptation of fishes related to *Jordanella* is survival in marginal niche environments. The cyprinodont habitat is typically at the water's edge, in backwater sloughs, or tidal flats. Often, when conditions are shifting with the tides, optimum living factors may be represented by a point that moves with reference to some landmark. If the fish remains too close inshore, it might be trapped by shallow water. If too far from the shore edge, it might be vulnerable to predation. As such, the cyprinodonts are very sensitive to disturbances in local water conditions, utilizing emigration as an adaptive response to such problems.

It should be recognized from the previous discussion that the conditions which elicit such emigration behavior are not so extreme as the test conditions used in this study. Since changes within a population are under way well before mass mortality occurs, there is probably great sensitivity to sublethal influences. Brett (1958) categorizes environmental effects below the lethal level according to the increasing amount of energy expended by a population to maintain physiological constancy. It might be expected that even at minimum levels of some stress factor needed to elicit a response, impairment of ability to compete with other members of the community is present. The very fact that an organism is responding in a manner such as the emigration behavior of *Jordanella* is information that the animal is encountering conditions that will prove detrimental in its efforts to survive. Once gross levels of behavioral response to environmental contingencies are determined, the door is open for increasingly sensitive determinations of threshold levels of a particular species to some environmental problem.

SUMMARY

Behavior, expressed as activity patterns, can be a sensitive indicator of the reaction of an organism to environmental change. In attempting to resolve the effects of environmental variables, many previous studies have encountered difficulties because of the composite nature of behavior patterns within daily activity. This study investigated the use of changes of behavior within circadian activity that an organism itself exhibits under laboratory conditions as a measure of environmental influence.

The activity patterns of two pairs of cyprinodontid fish, Jordanella floridae, were monitored by a series of photoelectric sensors, in conjunction with an event recorder. The apparatus provided a record giving information as to the extent (time sequence), and nature (behavioral priorities) of the fish's activities. A "control" activity pattern under an initial temperature was recorded, followed by recording of an "experimental" pattern at a modified temperature. In order to develope clearly different patterns, a large temperature change of 10°C was imposed.

The Winer reliability coefficient was adapted to analyze the data for repeatability of the activity pattern from one 24-hour period to the next.

Subjective observations, verified by graphs and the reliability coefficient revealed definite shifts in behavior:

1) Diurnal activity during the control period was replaced by nocturnal activity under experimental conditions,

2) and reproductive behavior elicited by the control conditions was replaced by an escape reaction due to the effects of the test condition.

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TABLE I: DATA SET UP FOR CALCULATION OF MEAN SQUARE VALUES

CONTROL

Time Block	Replication Counts				
	I	II	III	IV	V
Mdnt-0200	51	96	115	22	9
0200-0400	51	11	90	100	12
0400-0600	44	58	178	163	4
0600-0800	118	120	328	288	124
0800-1000	290	411	458	581	635
1000-1200	252	353	277	694	454
1200-1400	447	383	309	579	442
1400-1600	333	366	582	532	566
1600-1800	349	232	384	602	345
1800-2000	336	235	489	596	621
2000-2200	280	337	137	630	312
2200-Mdnt	9	141	27	67	114

EXPERIMENTAL

Time Block	Replication Counts				
	I	II	III	IV	V
Mdnt-0200	245	242	668	54	80
0200-0400	245	463	643	98	150
0400-0600	222	368	649	74	27
0600-0800	186	373	198	44	74
0800-1000	3	168	12	56	14
1000-1200	69	131	1	33	61
1200-1400	15	108	111	134	73
1400-1600	11	140	14	160	55
1600-1800	301	193	325	197	158
1800-2000	746	92	330	186	339
2000-2200	558	56	294	244	100
2200-Mdnt	677	58	603	236	382

TABLE II: SUMMARY OF ACTIVITY CHANGES

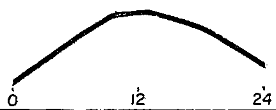
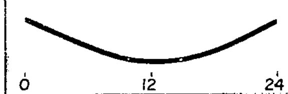
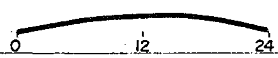
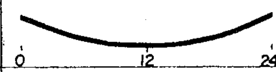
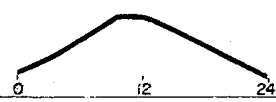
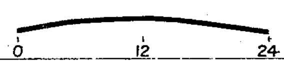
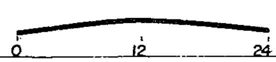
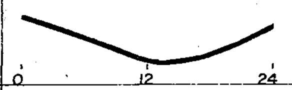
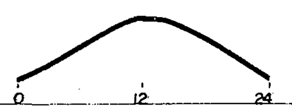
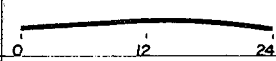
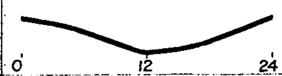
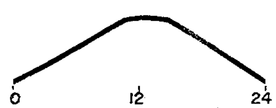
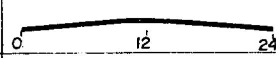
SECTOR OF AQUARIUM WHERE ACTIVITY RECORDED	SMOOTHED CURVE COMPARISON OF MEAN ACTIVITY PATTERN (O RELIABILITY COEFFICIENT): CONTROL	SMOOTHED CURVE COMPARISON OF MEAN ACTIVITY PATTERN (O RELIABILITY COEFFICIENT): EXPERIMENTAL	BIOLOGICAL SIGNIFICANCE OF ACTIVITY TO *Jordanella*: CONTROL	BIOLOGICAL SIGNIFICANCE OF ACTIVITY TO *Jordanella*: EXPERIMENTAL
COMPOSITE OF SIX PHOTOCELLS	 O 0.77	 O 0.32	DIURNALLY ACTIVE, NOCTURNALLY INACTIVE	DIURNALLY INACTIVE, NOCTURNALLY ACTIVE
PHOTOCELL 1	 O 0.24	 O 0.23	FEEDING, EXPLORATORY BEHAVIOR	ESCAPE BEHAVIOR
PHOTOCELL 2	 O 0.49	 O 0.17	REPRODUCTIVE BEHAVIOR	CONCEALMENT, FORAGING
PHOTOCELL 3	 O 0.35	 O 0.44	FEEDING, EXPLORATION	ESCAPE
PHOTOCELL 4	 O 0.79	 O 0.40	MALE TO MALE TERRITORIAL CONFLICTS	HIDING, FORAGING
PHOTOCELL 5	 O 0.53	 O 0.32	FEEDING, EXPLORATION	ESCAPE
PHOTOCELL 6	 O 0.72	 O 0.01	REPRODUCTION	HIDING, FORAGING
	hours	hours		

Figure 1. Schematic Diagram of Photoelectric Activity Recording Apparatus.

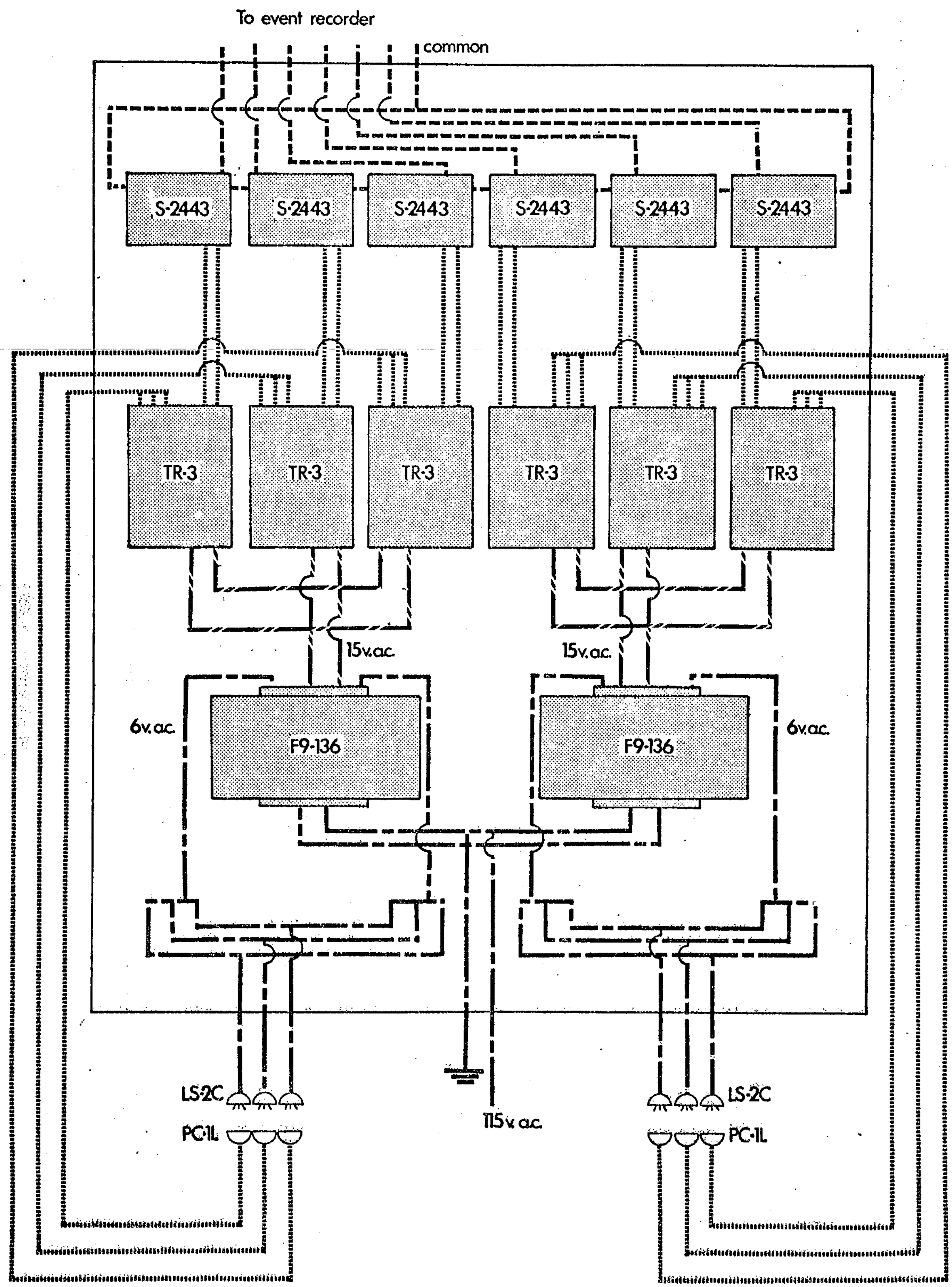

To event recorder
common
S-2443
S-2443
S-2443
S-2443
S-2443
S-2443
TR-3
TR-3
TR-3
TR-3
TR-3
TR-3
15v.a.c.
15v.a.c.
6v.a.c.
F9-136
F9-136
6v.a.c.
LS-2C
PC-1L
115v.a.c.
LS-2C
PC-1L

Figure 2. Apparatus Used to Monitor the Influence of Temperature Change on Activity of *Jordanella floridae*. The Drawing Shows only Four of the Six Photocell Units.

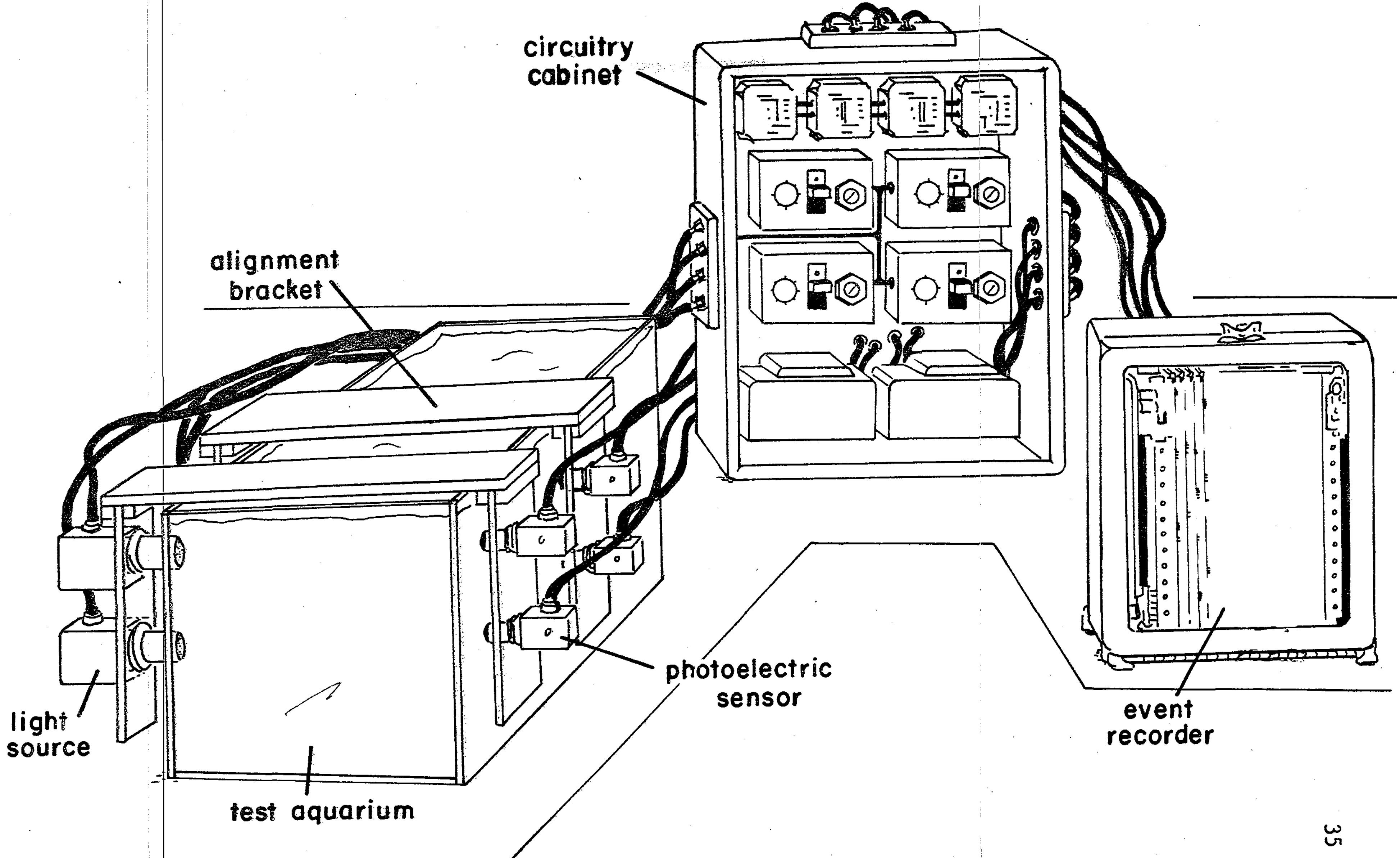

circuitry cabinet
alignment bracket
light source
test aquarium
photoelectric sensor
event recorder

Figure 3. Side View of the Test Aquarium with the Placement of the Photoelectric Sensors Superimposed. Dotted Lines Are Meant only to Approximate the Areas of Behavior Monitored by the Different Channels during the Control Period.

Surface Channels 1, 3, and 5: Activity Related to Feeding and Exploration.

Substrate Channels 2 and 6: Areas within the Proximities of Spawning Mops, Where Male Fishes Established Territories.

Substrate Channel 4: Area between Territories Where Activity Composed of Disputes between Males

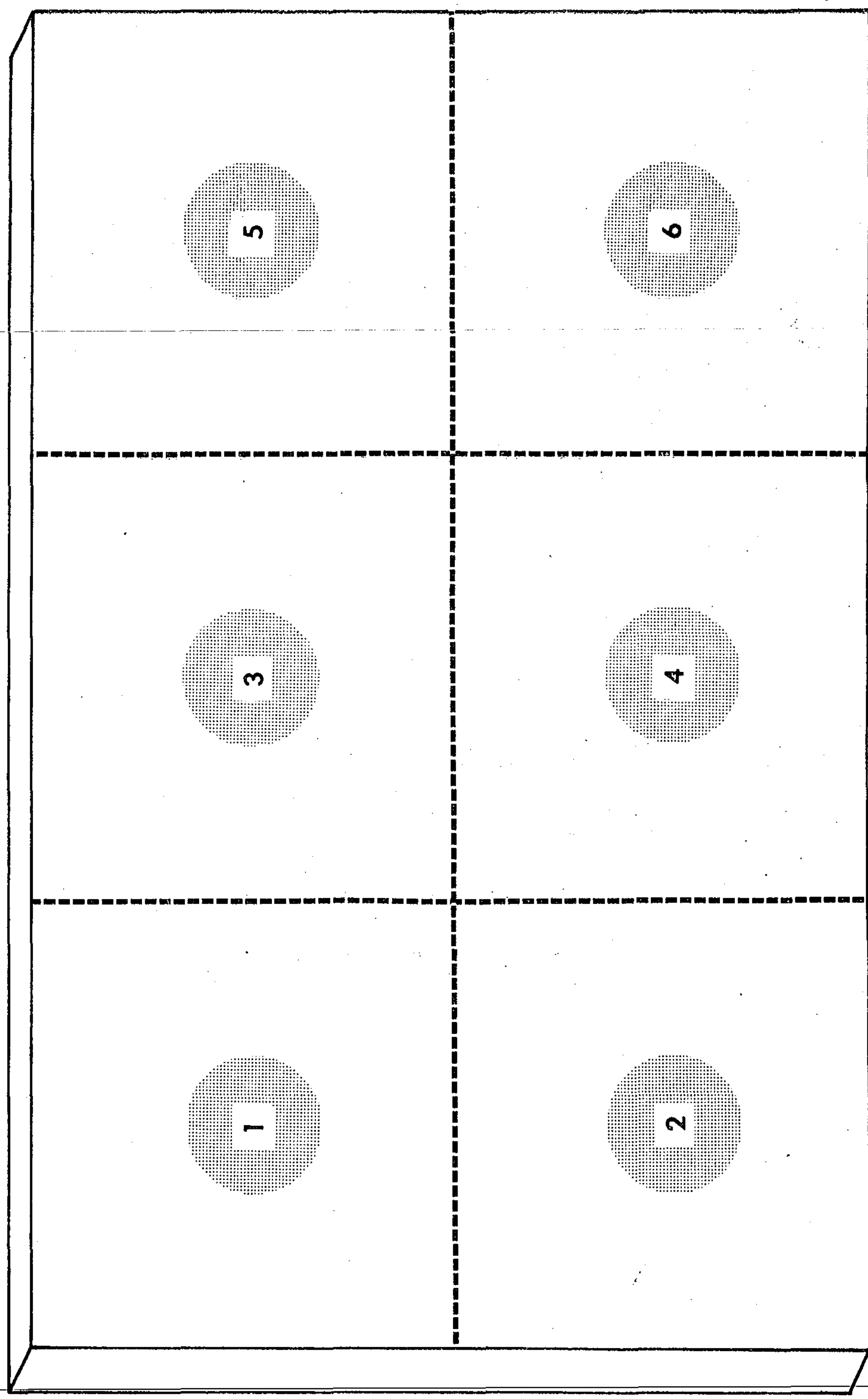

5
6
3
4
1
2

Figure 4. Esterline Angus Event Recorder Charts of Control and Experimental Replications, Showing Differences in Patterns of Activity

░░░ Darkened Hours

☐ Lighted Hours

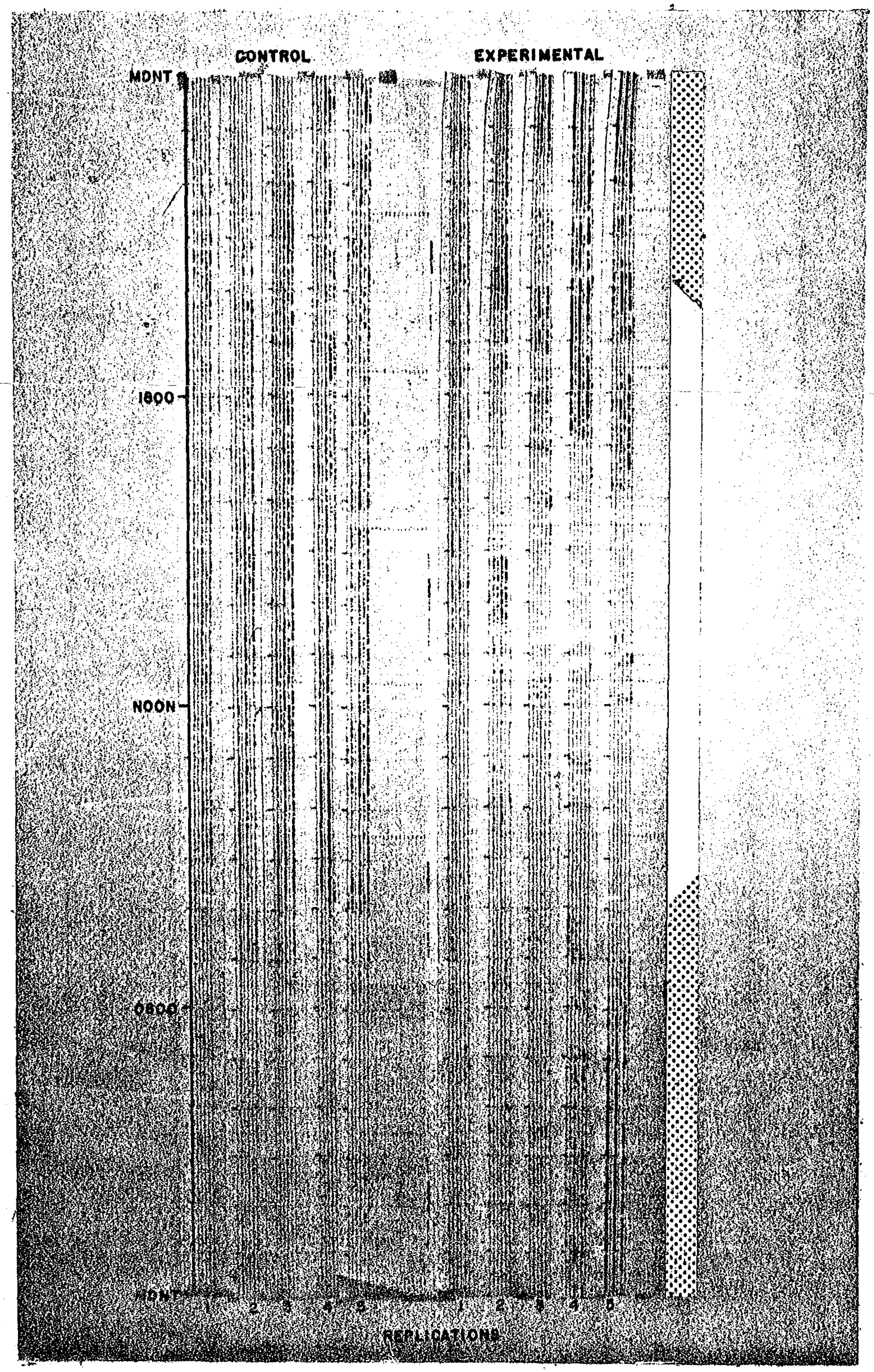

CONTROL
EXPERIMENTAL
MDNT
1800
NOON
0600
MDNT
1
2
3
4
5
1
2
3
4
5
REPLICATIONS

Figure 5. Mean Daily Activity Patterns Resulting from Composite Data of Six Photocell Channels.

Dashed Line, Control Pattern

Solid Line, Experimental Pattern

Shaded Areas Represent Unlighted Hours

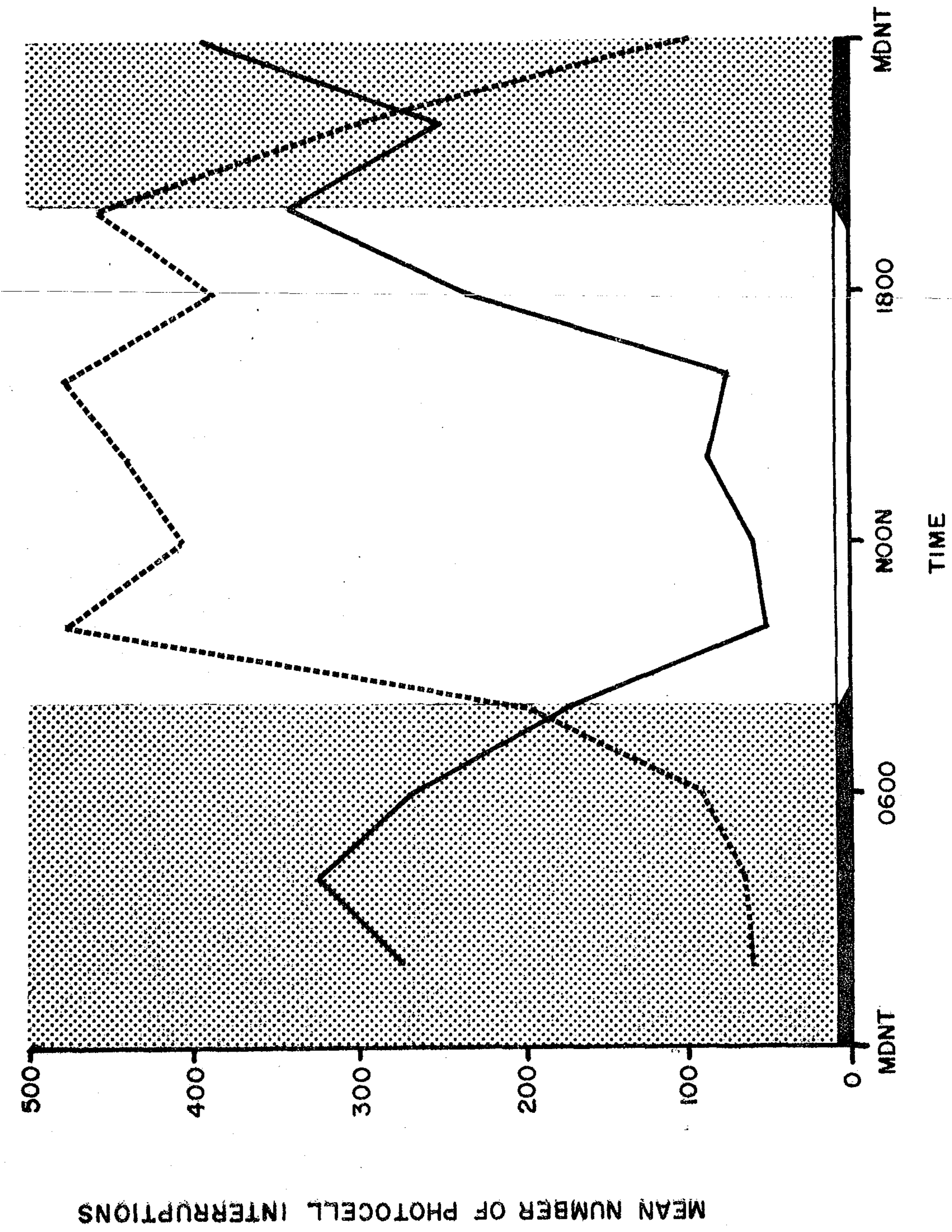

MEAN NUMBER OF PHOTOCELL INTERRUPTIONS
500
400
300
200
100
0
MDNT
0600
NOON
1800
MDNT
TIME

Figure 6. Daily Activity throughout Control and Experimental Replications for Composite Data of Six Photocell Channels. Shaded Areas Represent Periods of Darkness

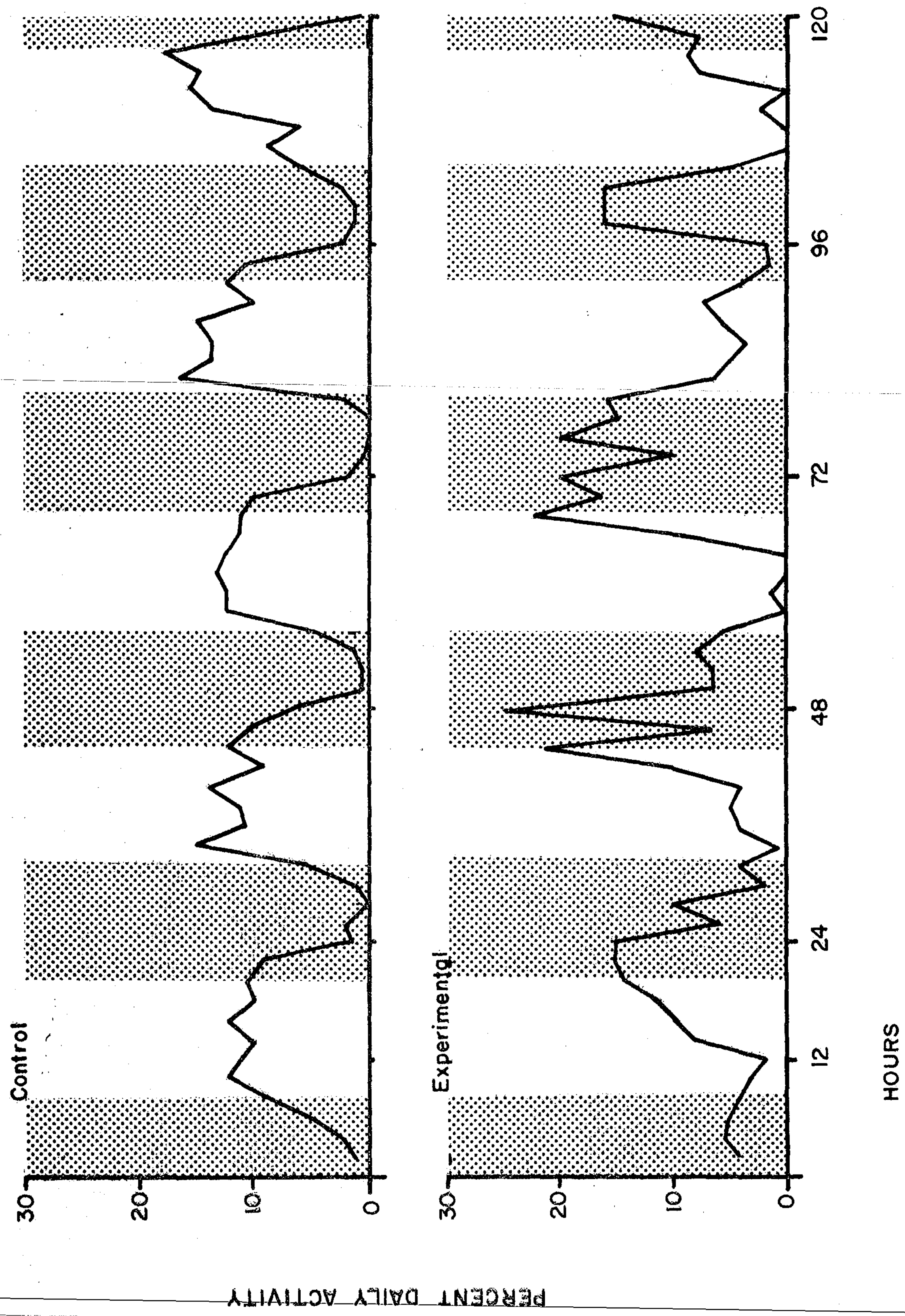
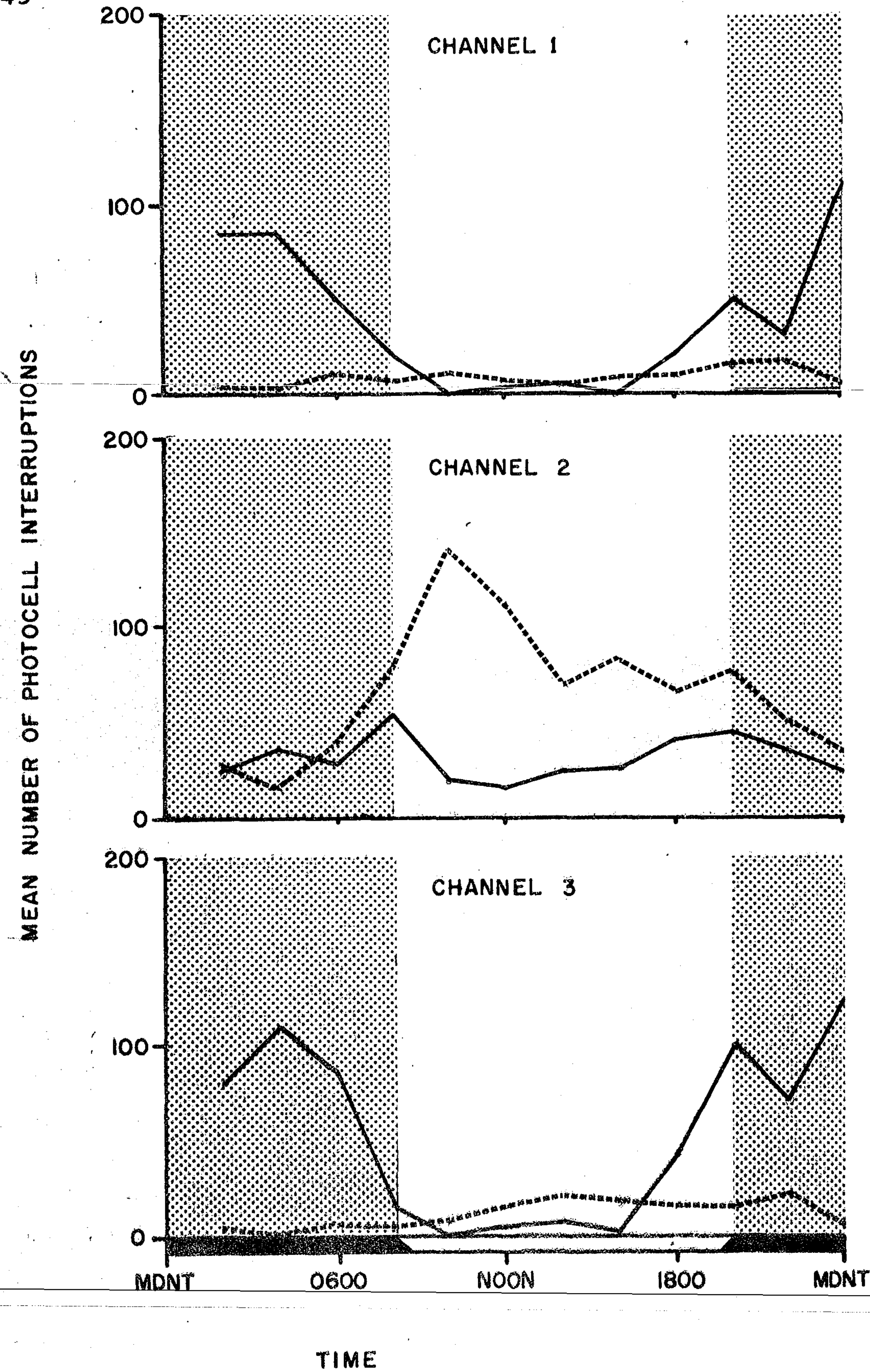

Control
Experimental
PERCENT DAILY ACTIVITY
HOURS
0
10
20
30
12
24
48
72
96
120

Figure 7, A-F. Mean Daily Activity Patterns Resulting in Each of the Individual Photocell Channels

Dashed Lines, Control Patterns

Solid Lines, Experimental Patterns

Shaded Areas Represent Darkened Hours


CHANNEL 1
CHANNEL 2
CHANNEL 3
200
100
0
MEAN NUMBER OF PHOTOCELL INTERRUPTIONS
MDNT
0600
NOON
1800
MDNT
TIME

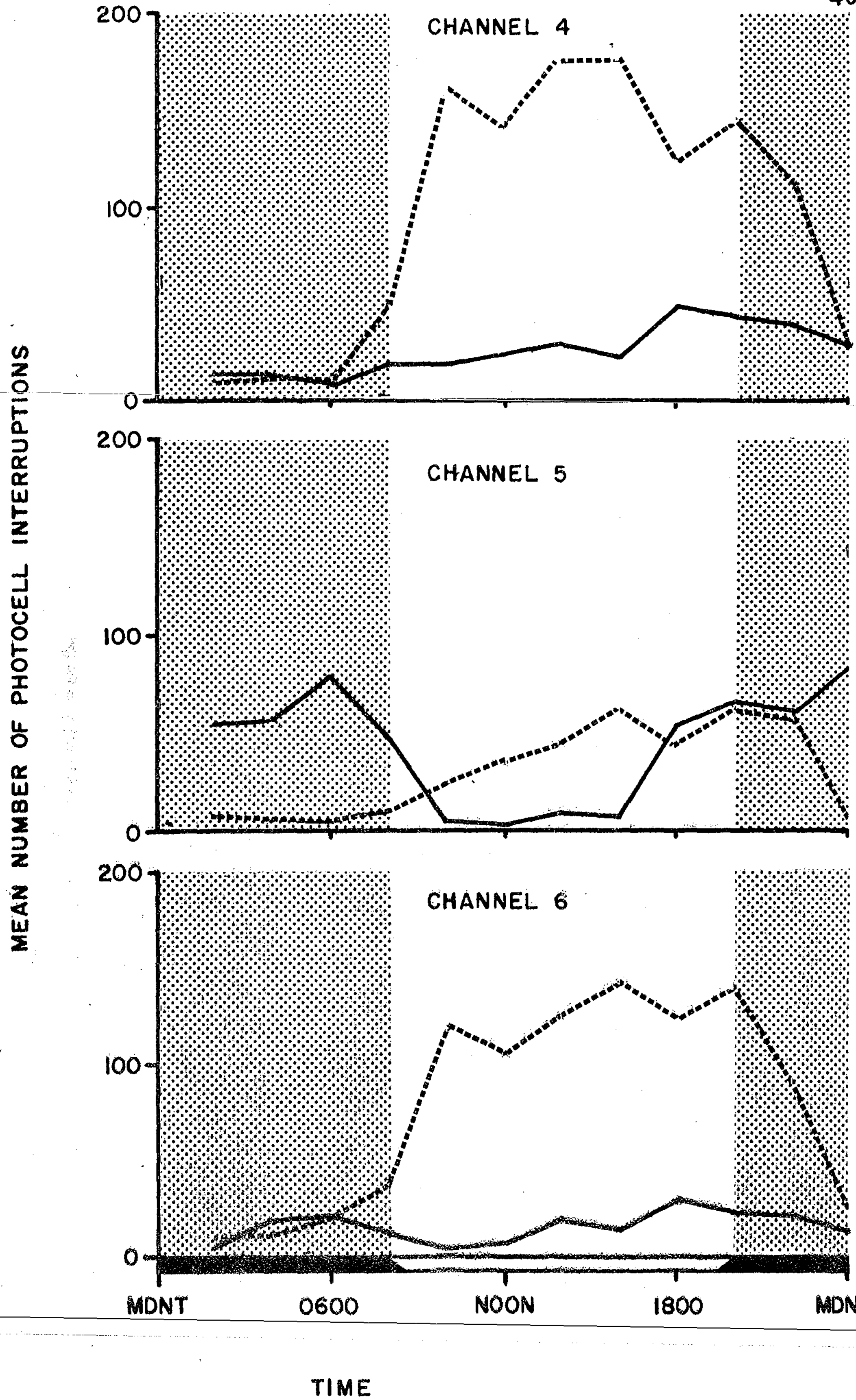

CHANNEL 4
CHANNEL 5
CHANNEL 6
200
100
0
MEAN NUMBER OF PHOTOCELL INTERRUPTIONS
MDNT
0600
NOON
1800
MDNT
TIME

Figure 8. Daily Activity Patterns Throughout Control and Experimental Replications Resulting from Activity in the Near-Substrate Channel 6. Shaded Areas Represent Darkened Hours

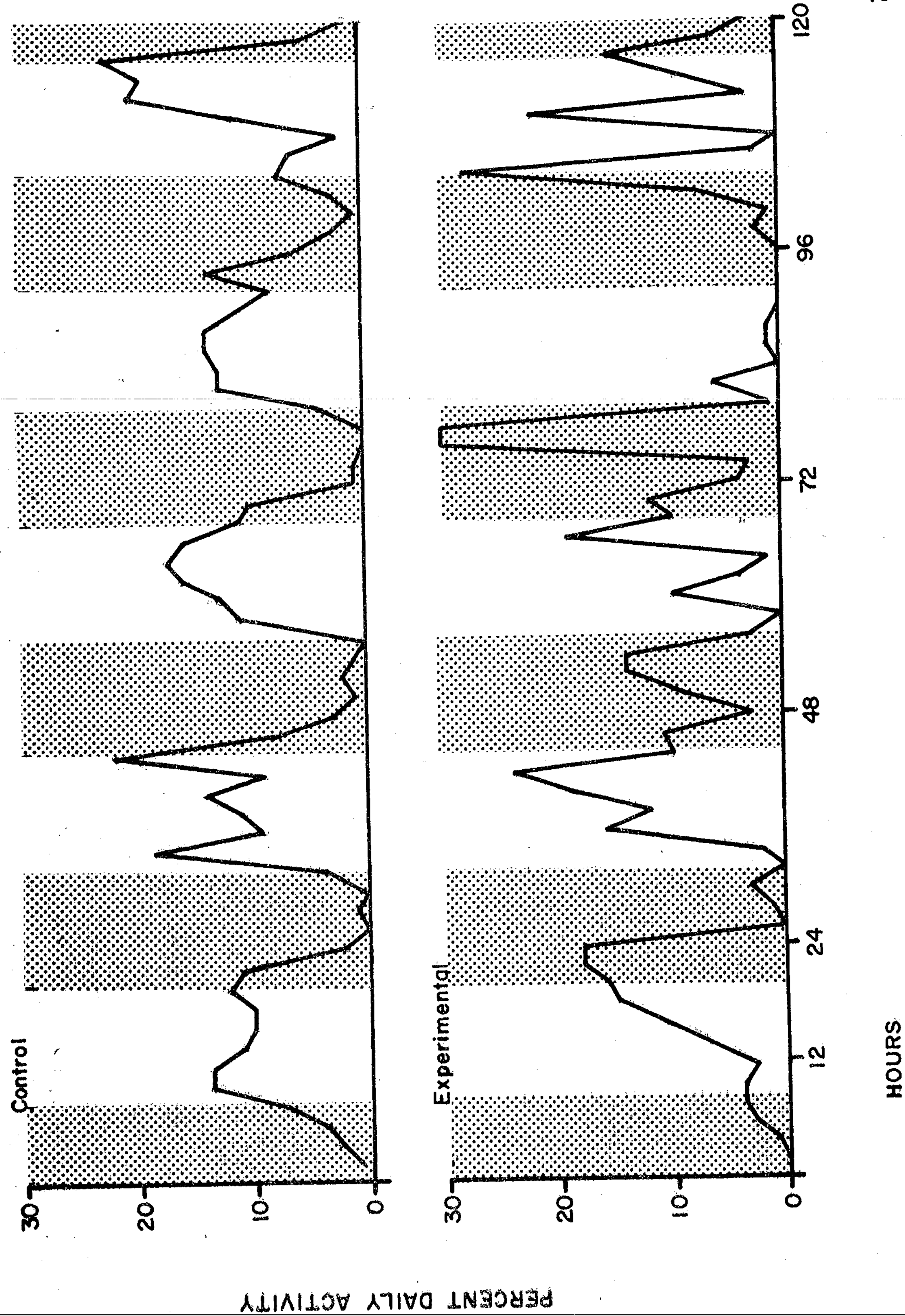
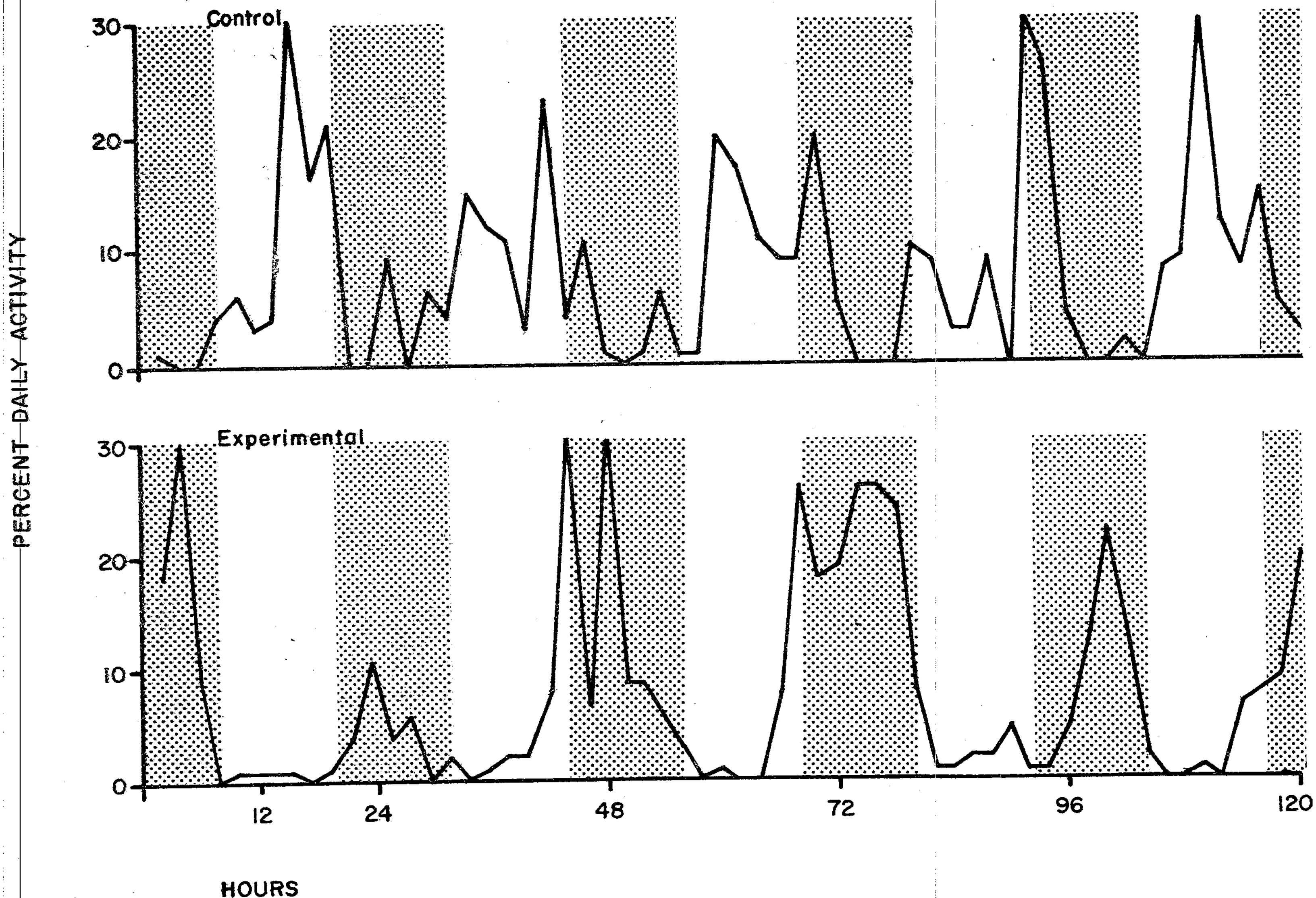

Control
Experimental
PERCENT DAILY ACTIVITY
0
10
20
30
HOURS
12
24
48
72
96
120

Figure 9. Daily Activity Patterns Throughout Control and Experimental Replications Resulting from Activity in the Surface Channel 3. Shaded Areas Represent Unlighted Periods


PERCENT DAILY ACTIVITY
Control
Experimental
30
20
10
0
12
24
48
72
96
120
HOURS