# THE EFFECTS OF CARBAMATES ON BOBWHITE (COLINUS VIRGINIANUS) ACTIVITY

by

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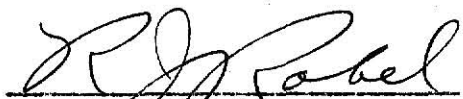

Major Professor

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# INTRODUCTION

For years biologists have observed regular 24-hour rhythms in both captive and wild animals (Hediger, 1950; Calhoun, 1945). Experiments placing animals under constant environmental conditions have demonstrated that these rhythms are not merely reactions to periodically changing environmental stimuli but are based on an endogenous biological clock. Reviews of these experiments have been published by Bruce and Pittendrigh, 1957; Harker, 1958; Cloudsley-Thompson, 1961 and Aschoff, 1963. These free-running rhythms (after Pittendrigh, 1958) have been termed circadian (Halberg, 1959) because they approximate 24 hours in duration. It is now known that these physiological and behavioral rhythms are part of the phylogenetic make-up of an organism and have obvious ecological and biological significance to an organism. (Pittendrigh, 1961; Aschoff, 1964).

Time measurement is of critical importance in the synchronization of physiological and behavioral activities of a living organism to its natural environment. There are times of a day, month or year to which a given biological activity, such as breeding, egg-laying, or molting is either necessarily restricted or is most appropriately undertaken. As Harker (1964) aptly states, "physiological regulation as a whole can be viewed in terms of supplying the right amount of material to the right place, but such regulation would be inefficient if at the right time were not fulfilled." The presence of an endogenous circadian rhythm with approximately the same frequency as environmental

cycles prepares the animal for environmental changes beforehand. This enables the animal to use the most favorable conditions for survival while avoiding those that are harmful. Such temporal organization is necessary to synchronize reproduction cycles and breeding behavior between sexual partners, to provide maximum utilization of feeding times and to limit activity to those times when predators are less active (Aschoff, 1964). Time measurement is found in bees that can be trained to return to feeding sites at specific times (Renner, 1959) and in the celestial navigation of various organisms that can compensate for the changing position of celestial direction givers (Kramer, 1952; and Papi, 1955). The seasonal timing of many plants and animals is regulated by time measurement of photoperiod (Pittendrigh, 1966). Hamner (1963) showed that some sort of time measurement by a circadian clock must be involved in the photoperiodic testicular response of house finches (Carpodacus mexicanus). Menaker (1965) studied the effect of photoperiod on both the activity rhythm and testicular response in house sparrows (Passer domesticus). He found a significant correlation between the effect of an experimental photoperiod on testis weight and the amount of the phase shift which it induces in the onset of the activity rhythm.

Twenty-four hour rhythms of activity are usually characterized by temporal patterns of activity that show a definite phase-relationship to the day-night cycle. Leopold and Eymon (1961) found the morning and evening song of 20 species of songbirds were closely correlated to light intensity. Williams and Stokes (1963) found similar results in the rally call of chukar partridge (Alectoris chukar). The most common pattern is bimodal with most nocturnal animals possessing a major premidnight and a secondary predawn period of activity while

most diurnal animals possess a major prenoon and a secondary predusk period of activity (Aschoff, 1957 and Calhoun, 1945). Aschoff (1966a) has shown that the bimodal activity patterns in three species of finches are endogenously determined; i.e., they do not depend upon a concurrent change in environmental conditions.

Not only the circadian rhythm but also its pattern are fitted to the ecological requirements of an organism (Aschoff, 1966b). Kavanau and Rischer (1968) found the patterns of time, duration and speed of running in small nocturnal mammals in activity wheels are almost duplicated from night to night. They concluded that the biological clock controlling these patterns acts as a program sequencer of behavior. Kavanau (1968) concluded that such patterns enable these animals to "keep close track" of their absolute position and displacement from their nest. Spieth (1958) found different diurnal periods of maximum activity served as an isolating mechanism between two species of drosophila, while Hirth (1963) found that because of different activity periods, due to different thermal tolerances, two species of lizards can occupy similar ecological niches.

Studies on chemical effects on circadian rhythms have been aimed mostly at elucidating the biological mechanism of the clock. To date, however, most studies have only shown how chemicals affect various processes (e.g., spore discharge, running activity) controlled by the clock. Reviews of these studies have been published by Hastings (1960) and Pittendrigh et al. (1973). Wahlstrom (1965) studied the effects of amphetamine sulphate, on the self-selected circadian rhythm of activity and rest in the canary (Serinus canarius), administered at

different times during the activity cycle. He found amphetamine increased the duration of activity in the P.M. series but had no effect during the A.M. series. He concluded the responses to this drug were dependent upon the time of administration. Wahlstrom (1964) found a single dose of pentobarbitol had no effect on the self-selected activity rhythm in the canary while phenylisopropylhydrazine caused a distinct shortening of the circadian period. He also found a single oral dose of resperine decreased the amount of activity and increased the amount of rest in such a manner that the length of the circadian period was unaffected. Palmer and Dowse (1969) added deuterium to the drinking water of African waxbills (Estrilda troglodytes) and found the periods of the activity rhythms were increased as a direct function of the dosage level. The effects of monoamine oxidase inhibitors and barbiturates on canary activity rhythms have also been studied (Wahlstrom, 1965).

There is a paucity of research on how chemicals, especially pesticides, affect free-running activity rhythms, temporal patterns of activity and gross locomotor activity in a vertebrate as complex as a bird. Therefore, the objective of this research was to study how sub-lethal dosages of carbamate insecticides affect these parameters of bobwhite (Colinus virginianus) behavior.

# LITERATURE REVIEW

Pesticide studies during the past two decades have revealed startling new data on the environmental fate and ecological damage of these chemicals. Evidence indicates the greatest cause of ecological damage results from persistent chemicals that accumulate in natural systems (Macek, 1970). This accumulation of biomagnification has resulted in serious toxicological and behavioral damage to birds, fish, mammals and other organisms in the food chain (Stickel, 1968). Coupled with these findings has been the increase in the number of pesticide resistant insect species. It is currently estimated that over 224 pest species, one half of which are harmful agricultural pests, have developed resistance to various insecticides (Brown, 1972). Concern over the environmental effects of persistent, non-biodegradable pesticides has resulted in the development and increased use of less persistent substitutes. One such group of chemicals is the carbamate group.

Carbamates are highly regarded replacements for organochlorines because of their rapid biodegradability, their toxicity to organochlorine resistant pests, their synergistic capabilities and in most cases their low acute toxicity to non-target organisms (Metcalf, 1961; Fukuto et al., 1962 and Carpenter et al., 1961). Carbamates can be used as insecticides, herbicides, nematocides, fungicides and acaricides. They have also been used as temporary immobilizing agents (Schafer et al., 1967) and repellents (West et al., 1969). Certain carbamates are readily synergized by such compounds as piperonyl butoxide (Brattsten and

Metcalf, 1970) sesamex (Eldefrawi and Hoskins, 1961) atrazine (Lichtenstein et al., 1973) and the PCB aroclor 1253 (Plapp, 1972). Many more uses of this chemical group can be found in the literature.

Carbaryl and carbofuran are both broad spectrum carbamate insecticides. Carbaryl exhibits low mammalian and avian toxicity (Tucker and Crabtree, 1970 and Heath et al., 1970) but is extremely toxic to honeybees (Apis spp.) (Shaw, 1959 and Morse, 1961), fish (Cope, 1961) and molluscs (Stewart et al., 1967).

Carbofuran is highly toxic to mammals (Neumeyer et al., 1969), birds (Tucker and Crabtree, 1970) and earthworms (Kring, 1969). Sangha (1972) studied the environmental effects of carbamates in a "model ecosystem" and found carbofuran was highly toxic to several species of snails (Physa spp.) and water fleas (Daphnia spp.).

An organism's susceptability to a chemical depends on a number of factors such as age, sex, environmental conditions and method of application (Durham, 1969). Hudson et al. (1972) reported 1-week old carbofuran treated mallards (Anas platyrhynchos) were less susceptable than 1-month and 6-month old ducks and 1-day old ducklings were the most susceptable. An application of 1 lb/acre of carbaryl for gypsy moth (Porthetria dispar) control resulted in the death of five tree swallow nestlings (Iridoprocne bicolor) within 16 days after spraying (Bednarek and Davidson, 1967). This was the only nest that hatched young during the week of spraying. Sherman and Ross (1969) found no difference in susceptability of Japanese quail (Coturnix japonica) due to sex for a single oral dose of carbofuran, however, in chronic studies of 400 to 800 ppm in diet male quail were more susceptable.

Severe pesticidal kills can disrupt community compositions, alter species distribution and interfere with predator-prey relationships (Ferguson, 1970). Moulding (1973) not only found a 55 percent reduction in numbers but also a reduction in species diversity of forest bird populations in an area sprayed with carbaryl. In an area sprayed at a rate of 1 lb/acre, Kurtz and Studholme (1974) found lower residues of carbaryl in ground feeding birds than in canopy feeders. Barrett (1968) found a sub-dominent house mouse (Mus musculus) population replaced the dominent cotton rat (Sigmodon hispidus) population on a semi-enclosed grassland ecosystem treated at a rate of 2 lbs. of carbaryl/acre.

Lethality to living organisms is only one aspect of the dangers of pesticides. Heath et al. (1970) states . . . "there is little apparent correspondence between a chemical's lethal toxicity and its capacity to induce sub-lethal complications." Sub-lethal exposure to carbaryl has resulted in the massive invasion on the central nervous system in mosquitofish (Gambusia affinis) by a microsporidian parasite (Ferguson, 1972). Sub-lethal dosages of carbamates affect the reproductive performance of organisms in various ways. Shtenberg and Ozhovan (1971) found sub-lethal dosages of carbaryl affected the function of testes and ovaries in rats (Rattus rattus), with a resultant decrease in fertility, a high loss of progeny during the first month of life and a lag in physical development. Collins et al. (1971) also found that carbaryl significantly decreased fertility in female rats and gerbils (Gerbillus gerbillus) while Shilova et al. (1968) reported reproduction in redbacked voles (Clethionomys gloreolus and Clethionomys refocanus) was down 42 percent on a carbaryl treated area. Carbaryl

has been reported to increase the mean duration times of the estrous cycle and its phases in both rats and mice (Rybakova, 1968). Barrett (1968) found carbaryl caused a 4-week delay to cotton rat reproduction. Nir et al. (1966) reported finding degenerated ovaries in white leghorn chickens (Gallus domesticus). Sherman and Ross (1961) reported levels of carbofuran as low as 200 ppm in diet greatly depressed weight-gain, egg production, fertility and hatchability in white leghorn chickens. At high dosages carbaryl has reduced reproduction in bobwhite quail and pheasants (Colchicus phaseolus) (Anonymous, 1961). Reproductive effects have also been reported in aquatic organisms. Exposure of fathead minnows (Pimephales promelas) to concentrations of .68 mg/liter of carbaryl for nine months prevented reproduction and decreased survival (Carlson, 1971).

Carbaryl has been reported to be teratogenic in beagle dogs (Canis familiaris) (Smalley et al., 1968) and white rats (Orlova and Zhalbe, 1968). Robens (1969) reported carbaryl produced abnormalities in guinea pigs (Covia procellus) embryoes only when treated on days 12-16 of gestation and suggests . . . "that time of ingestion is important criteria in determining its reproductive effects." Carbaryl has been found to be teratogenic to chickens, both when injected into eggs (Marliac, 1964 and Dunachie and Fletcher, 1969) and when included in the diet (Ghadiri et al., 1967). Ghadiri and Greenwood (1965) found a direct correlation between the maximum quantity of carbaryl administered and chick hatchability.

Changes in the electrical activity of the brain has been used to indicate an altered functional state of the intact animal. Desi et al. (1974) reported EEG records made after 50 days of treatment

showed that carbaryl at 100 ppm and 200 ppm/day in diet slightly increased slow and fast wave components in rats while arprocarb at dosages of 25 and 12.5 ppm/day in diet decreased all wave components. These researchers concluded the two agents have slightly different modes of action in the nervous system. Santolucito and Morrison (1971) conducted an 18 month low-level carbaryl feeding experiment with Rhesus monkeys (Macoca mulata) and observed no obvious change in behavior, however, they did find a significant reduction in waveform abundance for the 12-18 $H_z$ class.

Carbaryl and carbofuran both produce cholinesterase inhibition (Hayes, 1967 and Metcalf et al., 1968). Carpenter et al. (1961) found carbaryl significantly reduced erythrocyte and brain cholinesterase levels in rats. Rats given carbaryl for three months had reduced plasma and tissue cholinesterase levels while monkey plasma cholinesterase was inhibited in a 6-month study (Serrone et al., 1966). Cholinesterase is a vital component of the neuromuscular system in vertebrates. Neuromuscular effects of carbaryl have been found in swine (Sus scrofa) and chickens (Smalley et al., 1968 and Gaines, 1969).

Behavioral changes represent the final integrated results of a diversity of biochemical and physiological processes (Warner et al., 1966). Evidence relating behavioral alteration to inhibition of brain acetylcholinesterase caused by carbamate poisoning has been reported (Rosecrans et al., 1968). Goldberg et al. (1963) found compound 10854, an N-methyl carbamate, reduced avoidance behavior of rats to footshock by 90 percent with a concomitant 30 percent loss in escape behavior. Goldberg et al. (1965) reported carbaryl injected at 5 mg/kg significantly decreased avoidance behavior of rats to footshock and the response

was dose related. Pfeiffer and Jenny (1957) reported physotigmine was a potent depressant of learned behavior in rats.

Because of obvious physiological and behavioral implications, locomotor activity has been the subject of numerous pharmacological and toxicological studies. Desi et al. (1974) found carbaryl and arprocarb increase the running time and number of errors for rats in a T maze. Rats injected subcutaneously with a 1 percent solution of carbaryl in corn oil at 10 mg/kg exhibited a transient decrease in locomotor activity of 1-2 hours duration (Sideroff and Santolucito, 1972). Singh (1968) found carbaryl antagonized the running wheel performance of caffeine treated rats and Singh and Frazold (1970) found carbaryl alone depressed running wheel activity in female rats.

Perhaps, because of their obvious survival values, most pesticide studies have been concerned with mortality and reproductive effects. However, Warner et al. (1966) believes, . . . "that most, if not all normal processes probably have distinct survival value to organisms in their natural habitats" and that "any deviation from the norm of any process is deleterious." Therefore, in order to thoroughly evaluate the effects of pesticidal contaminants all physiological, behavioral and ecological aspects of an animal should be assayed.

## MATERIALS AND METHODS

### Subjects

Quail used in all experiments were adult males obtained from the Kansas State Quail Farm at Pittsburg, Kansas. The birds were individually housed in polypropylene cages that measured 48x24x13 cm and were equipped with 0.63 cm (1/4 inch) hardware cloth sliding tops and 1.27 cm (1/2 inch) hardware cloth bottoms. The birds were divided into two groups and kept in walk-in environmental chambers prior to and during all experiments. Group I was kept under conditions of 25°C, 65 percent relative humidity and a 14L:10D light regimen while group II was kept under conditions of 20°C, 65 percent relative humidity and 14L:10D light regimen. Birds were kept under these conditions for at least one month prior to being used in experiments.

### Apparatus and Equipment

Activity cages measured 76x35x30 cm and were constructed of 1 cm thick plywood. Each cage was equipped with 1.27 cm mesh hardware cloth sliding top. The floor was spring mounted and constructed of a 0.63 cm diameter steel rod covered with 0.63 cm mesh hardware cloth. A 0.63 cm diameter steel rod was welded across the width of the floor and acted as a fulcrum. Plastic tubing spacers kept the floor centered within the cage frame. A pressure sensitive microswitch was mounted beneath one end of the floor (Fig. 1). Metal trays were used to collect feces and spilled feed.

Fig. 1. Features of activity cage: (A) cage mounting block, (B) springs, (C) cage frame, (D) floor, (E) fulcrum rod, (F) plastic tubing spacers, (G) base, (H) feed and water bottles, (I) microswitch assembly.

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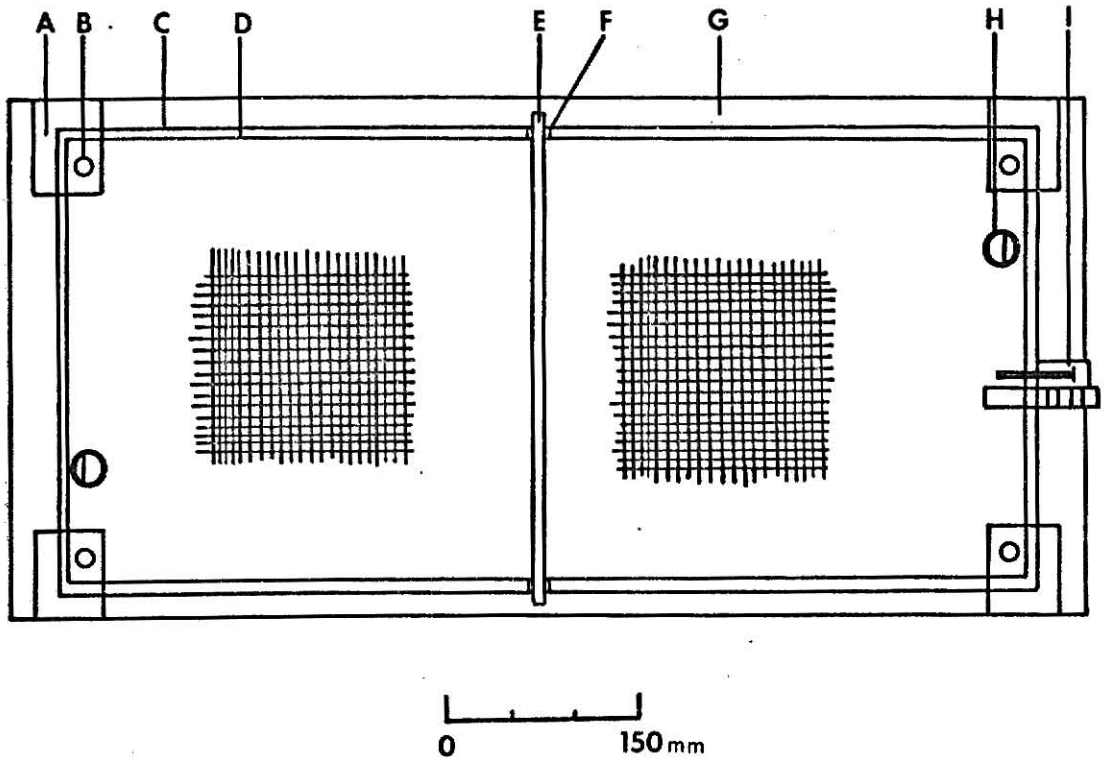


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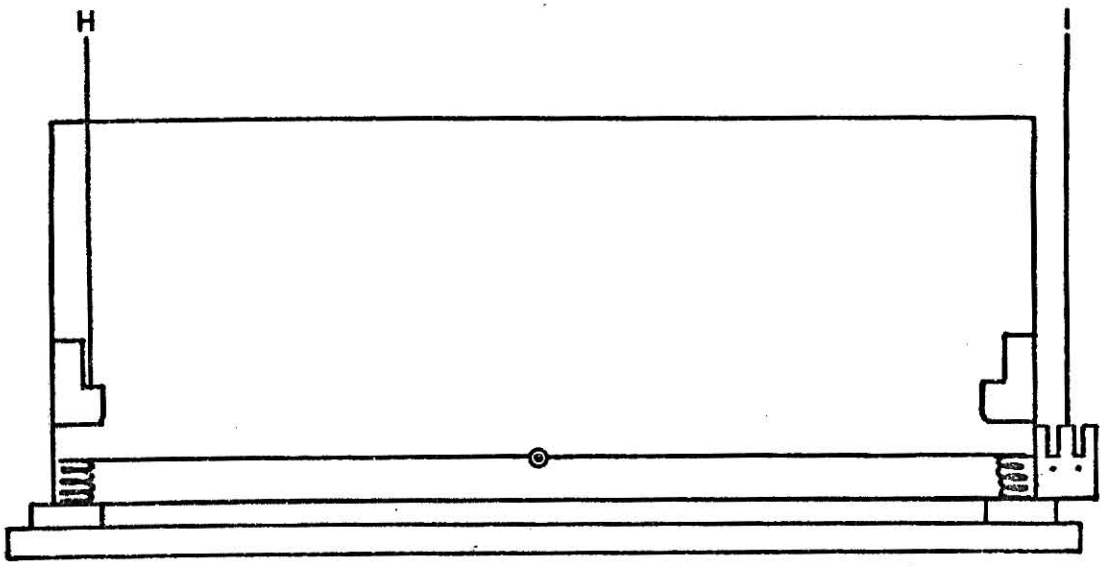

Locomotor activity was recorded on an Esterline Angus 8-channel Minigraph continuous event recorder operating at a chart speed of 2.54 cm/hr (1 inch/hr). Chart paper was graduated into 15-minute intervals. Locomotor activity was defined as the movement of the bird from one end of the cage to the other. A contact was elicited each time the bird crossed over the fulcrum and was recorded simultaneously by the recorder. These recordings appear as vertical marks on the chart paper and fuse together to form a solid band during intense activity (Fig. 2).

Eight cool white flourescent lights were suspended directly above each cage and provided the light source for the continuous bright light and light-dark experiments, while 6-watt lamps were the light source in the continuous dim light experiment. Light readings were taken at the top center of each cage with a Gossen Tri-Lux foot candle illumeter. Light intensity varied by 30 lux, depending upon the bird's position in the cage, during CBL and LD experiments.

During the first two experiments it was found that some birds were mimicking each others activity. Although visually isolated they could still hear each other. To prevent this behavior a random-noise generator operating at 80 dB of white noise was installed in the environmental chamber for the remaining experiments.

Feed

Feed was prepared by the Grain Science and Industry Department at Kansas State University. It was a mixture of grain (P-18) consisting of 46 percent ground sorghum (Sorghum vulgare) grain, 28 percent soybean (Glycine max) oil meal, 15 percent ground corn (Zea mays), 2 percent

Fig. 2. Typical activity recording showing how pen marks fuse together during intense activity periods.

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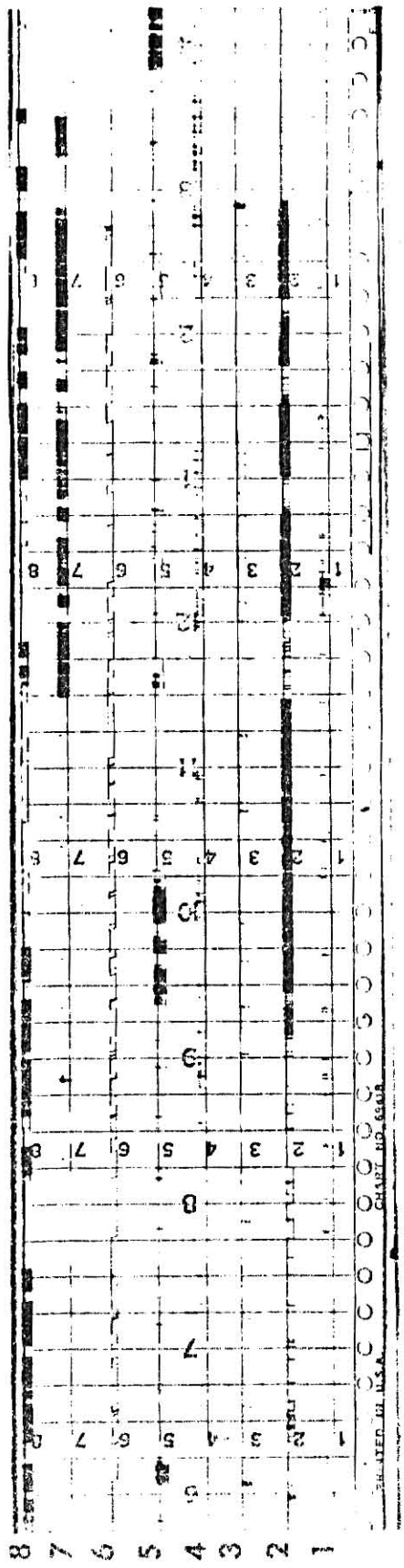
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alfalfa (Medicago sativa) meal, and trace amounts of vitamins, coarse fibre and antibiotics. The feed was sifted through #20 (0.83 mm openings) and #6 (2.10 mm openings) mesh screens in the laboratory to provide uniform particle size and facilitate gross separation of spilled feed from feces. Grit was not provided.

## Treated Feed

Treated feed was prepared by mixing the powdered form of each insecticide with dry P-18 meal, tumbling the mixture for 30 minutes and then pelleting it. Maximum temperature of the feed during the pelleting process was 69°C. The pellets were then put through a corn cutter and crumbled. The feed was then stored in a freezer at -16°C until used. Twenty pounds of treated feed was prepared for each experiment. No tests were undertaken to determine if the insecticide was distributed evenly throughout the feed.

Treatments equivalent to daily dosages of 20 and 100 mg/kg carbaryl and 2 and 10 mg/kg carbofuran were prepared. Daily dosages were calculated on the basis of the mean body weight for each group of birds used in an experiment and a mean daily food consumption of approximately 15 grams (wet weight) per bird (Case, 1973).

## Chemicals

Gratis samples of technical grade carbaryl (1 naphthyl N-methylcarbamate) and carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) were provided by the Union Carbide Chemical Corporation and the Niagara Chemical Division of the F.M.C. Corporation, respectively.

## Experimental Design

Four experiments, of eight birds each, were conducted under continuous bright light (CBL; 300 lux), one experiment, of eight birds, was conducted under continuous dim light (CDL; 10 lux) and two experiments, of four birds each, were conducted under a 14L:10D rectangular light-dark cycle (LD). All experiments were divided into a sequence of four phases; a 14-day acclimation phase, a 35-day pretreatment phase, either a 7- or 14-day treatment phase (depending upon the insecticide) and a 14-day posttreatment phase. The 35-day pretreatment phase served as each bird's control against which data collected during treatment and posttreatment were compared. All birds were maintained under a 14L:10D rectangular light-dark cycle during acclimation. A summary of methods and environmental conditions for each experiment is shown in Table 1.

## Experiment 1

Eight birds were randomly selected from group I, weighed, individually housed in activity cages and then placed in a walk-in environmental chamber. The experiment was conducted under CBL with constant conditions of 25°C and 65 percent relative humidity. Birds were treated at daily dosages equivalent to 20 mg/kg carbaryl in diet for a period of seven days.

## Experiment 2

Eight birds were randomly selected from group I, weighed, individually housed in activity cages and then placed in a walk-in environmental chamber. The experiment was conducted under CBL with

Table 1. Summary of methods and environmental conditions for each of the seven experiments.

| Experiment | Photoperiod | Light Intensity (lux) | Temperature °C at 65% RH | Insecticide | Treatment (days) | Daily Dosages (mg/kg) |
|---|---|---|---|---|---|---|
| 1 | CBL | 300 | 25 | A | 7 | 20 |
| 2 | CBL | 300 | 25 | A | 7 | 100 |
| 3 * | CBL | 300 | 20 | B | 14 | 2 |
| 4 * | CBL | 300 | 20 | B | 14 | 10 |
| 5A* | LD | 300 | 20 | B | 14 | 10 |
| 5B* | LD | 300 | 20 | B | 14 | 10 |
| 6 * | CDL | 10 | 20 | B | 14 | 10 |

* Random-noise at 80 dB.

Insecticide A: Carbaryl; B: Carbofuran.

Photoperiod: CBL: Continuous Bright Light; CDL: Continuous Dim Light; LD: 14L:10D rectangular light-dark cycles.

constant chamber conditions of 25°C and 65 percent relative humidity. Birds were treated at daily dosages equivalent to 100 mg/kg carbaryl in diet for a period of seven days.

Experiment 3

Eight birds were randomly selected from group II, weighed, individually housed in activity cages and then placed in a walk-in environmental chamber. The experiment was conducted under CBL with constant chamber conditions of 20°C and 65 percent relative humidity. Birds were treated at daily dosages equivalent to 2 mg/kg carbofuran in diet for a period of 14 days. A random-noise generator producing 80 dB of white noise operated throughout the experiment.

Experiment 4

Eight birds were randomly selected from group II, weighed, individually housed in activity cages and then placed in a walk-in environmental chamber. The experiment was conducted under CBL with constant chamber conditions of 20°C and 65 percent relative humidity. Birds were treated at daily dosages equivalent to 10 mg/kg carbofuran in diet for a period of 14 days. A random-noise generator producing 80 dB of white noise was operated throughout the experiment.

Experiment 5A

Four birds were randomly selected from group II, weighed, individually housed in activity cages and then placed in a walk-in environmental chamber. The experiment was conducted under a 14L:10D rectangular light-dark cycle with constant chamber conditions of 20°C and 65 percent relative humidity. Birds were treated at daily dosages

equivalent to 10 mg/kg carbofuran in diet for a period of 14 days. A random-noise generator producing 80 dB of white noise operated throughout the experiment.

Experiment 5B

Four birds were randomly selected from group II, weighed, individually housed in activity cages and then placed in a walk-in environmental chamber. The experiment was conducted under a 14L:10D rectangular light-dark cycle with constant chamber conditions of 20°C and 65 percent relative humidity. Birds were treated at daily dosages equivalent to 10 mg/kg carbofuran in diet for a period of 14 days. A random-noise generator producing 80 dB of white noise operated throughout the experiment.

Experiment 6

Eight birds were randomly selected from group II, weighed, individually housed in activity cages and then placed in a walk-in environmental chamber. The experiment was conducted under CDL with constant chamber conditions of 20°C and 65 percent relative humidity. Birds were treated at daily dosages equivalent to 10 mg/kg carbofuran in diet for a period of 14 days. A random-noise generator producing 80 dB of white noise operated throughout the experiment.

Procedure

During acclimation, microswitch position and spring tension were adjusted to provide maximum sensitivity to the bird's movement from one end of the cage to the other. To encourage bird movement, feed and water bottles were placed at opposite ends of the cage.

Activity cages were cleaned every two weeks and at the beginning and end of each experimental phase. During these times food consumption data were collected. Spilled feed was separated from feces by sifting tray contents through #20 and #6 mesh screens. The amount of feed collected from each cage was then subtracted from the total amount given each bird. This provided a gross estimate of food consumption for each bird. Food consumption data were not collected during acclimation. Body weight data were collected at the end of each experimental phase. Birds were fed every other day at different times so as not to condition them to a false rhythm.

A quantitative expression of locomotor activity per hour was determined by converting the number of pen deflections on the chart paper into minutes. A value of four seconds was assigned for every pen deflection. When pen deflections fused to form solid bands, the length of activity time was taken directly from the chart paper. From these data the daily activity for each bird was determined. Daily activity was defined as the total activity for a 7-day period divided by seven. A chi-square goodness of fit test was performed for each group of birds to determine if daily activity data, food consumption data and body weight data were distributed normally. An analysis of variance was used to test for weekly variations in mean daily activity, mean daily food consumption and mean body weight. A probability of $P<0.10$ was considered significant for AOV tests. An LSD test was used to determine significant differences between treatment means. A probability of $P<0.05$ was considered significant for LSD tests. Product-moment and partial correlations between activity, food consumption and body weight

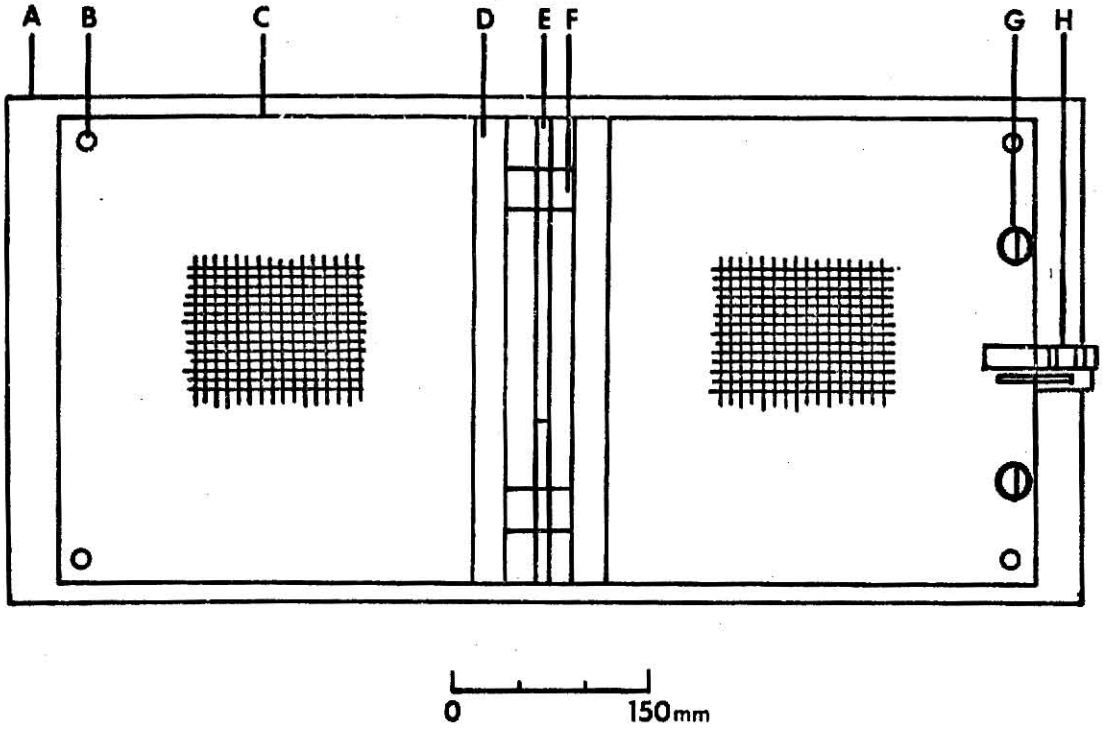
and between changes in these variables were calculated. A probability of $P<0.05$ was considered significant for all correlations. The periodogram analysis as described by Enright (1965) was used to determine the circadian period of free-running activity. The periodogram allows the detection of any natural frequency of periodic phenomena without a-priori knowledge of the exact value of the period (Cauter and Huyberecht, 1973). A periodogram analysis was performed on data collected during six time periods; days 1-7, 8-14, 15-28, 29-35, treatment and post-treatment, for each bird. The periodogram with the greatest amplitude was used to estimate the periodicity of the rhythm. Actograms showing intense activity periods, greater than 45 minutes, were also constructed.

Preliminary Experiments

Two groups of eight birds each were allowed 21-days to self-select either a spatial or temporal preference of light intensity. Birds allowed a spatial selection of light intensity (group I) were individually housed in light-dark boxes constructed of 1 cm thick plywood. Each box measured 76x35x30 cm and was equipped with a 0.63 cm hardware cloth floor and a 1.27 cm hardware cloth sliding top. A 1 cm thick piece of plywood partitioned the box widthways into two equal sections. A piece of black plastic covered one of the sections. An opening 20 cm high by 13 cm wide allowed the bird to move from one section to the other. The box was spring mounted and balanced on a fulcrum. A pressure sensitive microswitch was mounted beneath one end of the box (Fig. 3). The bird's movement from one section to the other elicited a response to an Esterline Angus 8-channel Minigraph continuous event recorder which recorded the bird's position in the

Fig. 3. Features of cage providing birds with a spatial choice of photoperiod: (A) base, (B) springs, (C) cage frame, (D) fulcrum, (E) partition, (F) fulcrum bracket, (G) food and water cups, (H) microswitch assembly.

# TOP VIEW

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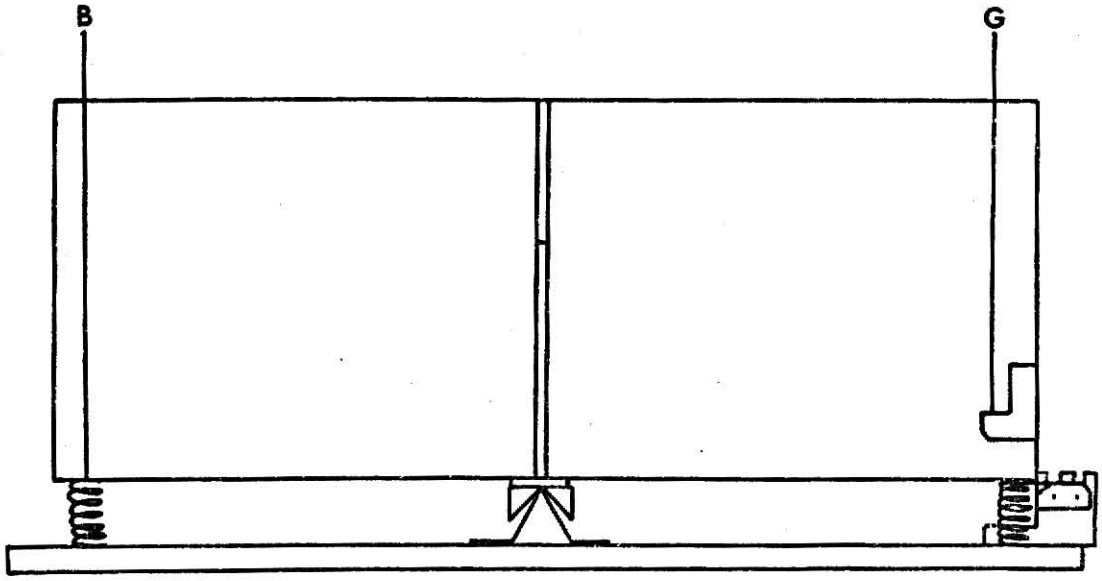


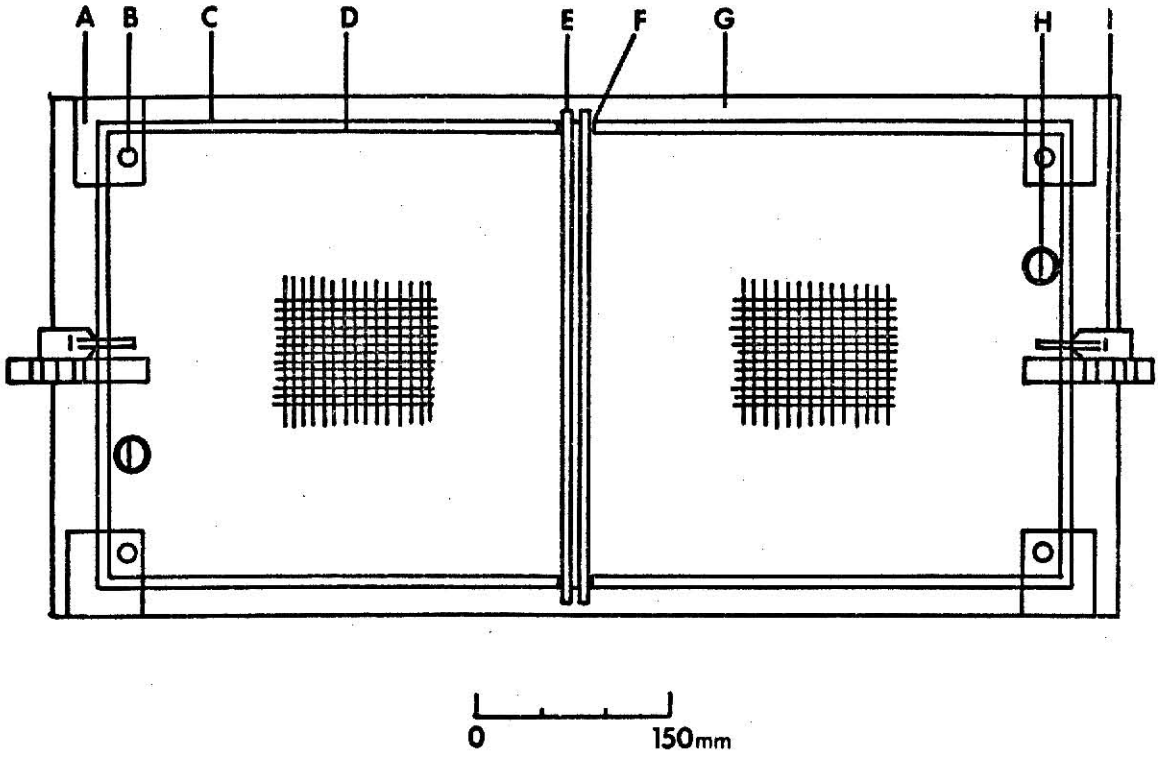
box. Feed and water bottles were placed in the light section of the box. Birds were maintained under chamber conditions of 25°C and 65 percent relative humidity. Light intensities were 300 and 5 lux for the light and dark sections, respectively.

Birds allowed a temporal choice of light intensity (group II) were individually housed in cages constructed of 1 cm thick plywood. Each cage measured 76x35x30 cm and was equipped with a spring mounted split section floor, constructed of a 0.63 cm steel rod covered with 0.63 cm hardware cloth, with microswitches positioned beneath each section (Fig. 4). One microswitch controlled the on-off function of a 30-watt cool white fluorescent light, suspended directly above the cage, while the other microswitch recorded the bird's position in the cage. A 6-watt lamp, also suspended directly above the cage, remained on at all times. The bird's position in the cage determined the light intensity. When on one section of floor the 30-watt fluorescent light was on and light intensity was 300 lux. When on the other section of floor only the 6-watt lamp remained on and light intensity was 10 lux. A black plastic curtain was placed around the entire unit to prevent interference from external light sources. Feed and water bottles were placed in the light section of the cage. Birds were kept under chamber conditions of 25°C and 65 percent relative humidity.

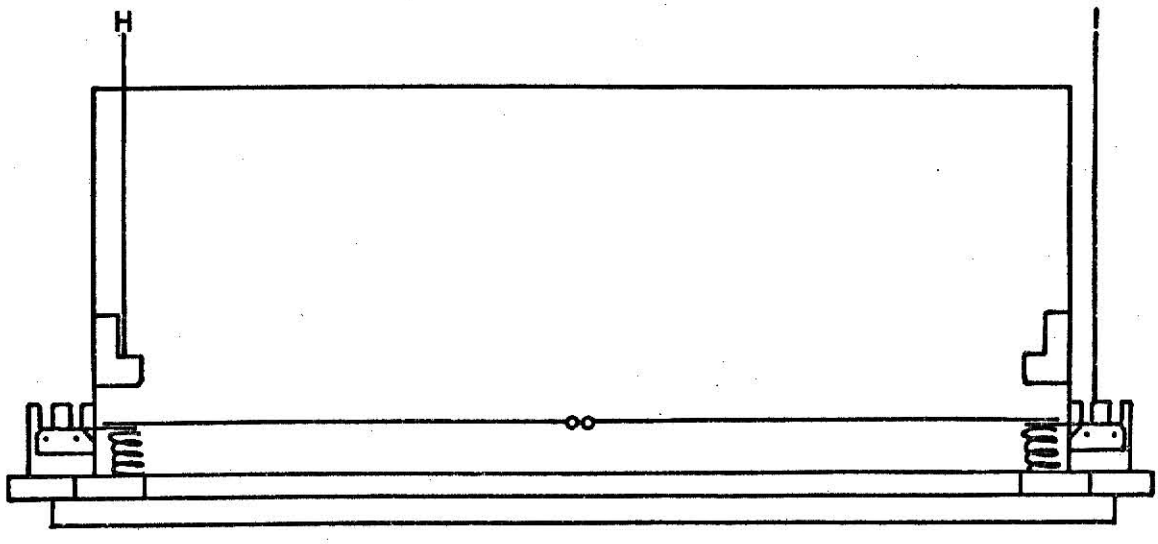
Average period lengths were determined by constructing a trend line, fitted by inspection, through the day to day self-selected onsets for bright conditions for each bird. The slope in the line determined the change in onset times over the 21-day period. This total change (in hours) was divided by 21 days and an average daily

Fig. 4. Features of cage providing birds with a temporal choice of photoperiod: (A) cage mounting block, (B) springs, (C) cage frame, (D) split section floor, (E) fulcrum rods, (F) plastic tubing spacers, (G) base, (H) food and water cups, (I) microswitch assembly.

# TOP VIEW




# FRONT VIEW



shift in onset times determined. This amount was subtracted from 24 hours for phase advancing birds and added to 24 hours for phase delaying birds.

# RESULTS

## Behavior Changes

The only noticeable behavioral changes occurred during treatment to bird no. 6 (experiment 4) and to bird no. 2 (experiment 6). Both birds developed diarrhea by the fourth day of treatment. Fecal matter was greenish white in color and lacked consistency. After ten days of treatment bird no. 2 became practically incapacitated and offered little resistence to hand capture. Symptoms of diarrhea disappeared within three days after the start of posttreatment for both birds. By the eighth day of posttreatment bird no. 2 appeared to have fully recovered its mobility.

## Preliminary Experiments

Only two of 16 birds, (both from group I) established a free-running activity rhythm, when provided with either a spatial or temporal choice of light intensity, during preliminary experiments. The period length was 23.5-hours for both birds. Six of the eight birds in group I never entered the dim section of the cage during the 21-day experiment. Birds in group II reacted differently to dim light conditions. Some birds would remain under dim conditions for a few minutes and then return to bright light conditions, while others would enter the dim light section and then immediately return to bright light conditions. Although data were not quantified, it could be determined from inspection, that the frequency with which birds entered the dim

light conditions decreased with time until no such instances occurred during the last week of the experiment.

Experiment 1 - Carbaryl Low Dosage (20 mg/kg) Continuous Bright Light (300 Lux).

A chi-square goodness of fit test indicated that daily activity data were not normally distributed ($p<0.005$). Therefore, although standard deviations are presented for mean daily activity the variability they represent is not symmetrical about the means. Food consumption and body weight data were normally distributed and therefore standard deviations are symmetrical about the means.

The range and mean (S.D.), in minutes, of daily activity for each bird during each phase of the experiment are shown in Table 2. Variation in a bird's daily activity was considerable as is evident by the range. The range of activity means, from 235-minutes for bird no. 7 to 838-minutes for bird no. 5, also indicates considerable variation exists between birds.

The mean daily activity (S.D.) was 386(195) minutes during pretreatment 344(212) minutes during treatment and 374(214) minutes during posttreatment. Weekly daily activity for each bird is shown in Table 3 and mean daily activity for each week is shown in Figure 5. Changes in mean daily activity during treatment from pretreatment ranged from a 37 percent decrease for bird no. 7 to a 5 percent increase for bird no. 2 (average change was 11 percent; a 42-minute decrease). Changes in mean daily activity during posttreatment from treatment ranged from a 15 percent decrease for bird no. 4 to a 46 percent increase for bird no. 3 (average change was 9 percent; a 27-minute increase).

Fig. 5. Mean daily activity/week of eight carbaryl treated birds (20 mg/kg) under continuous bright light (300 lux).

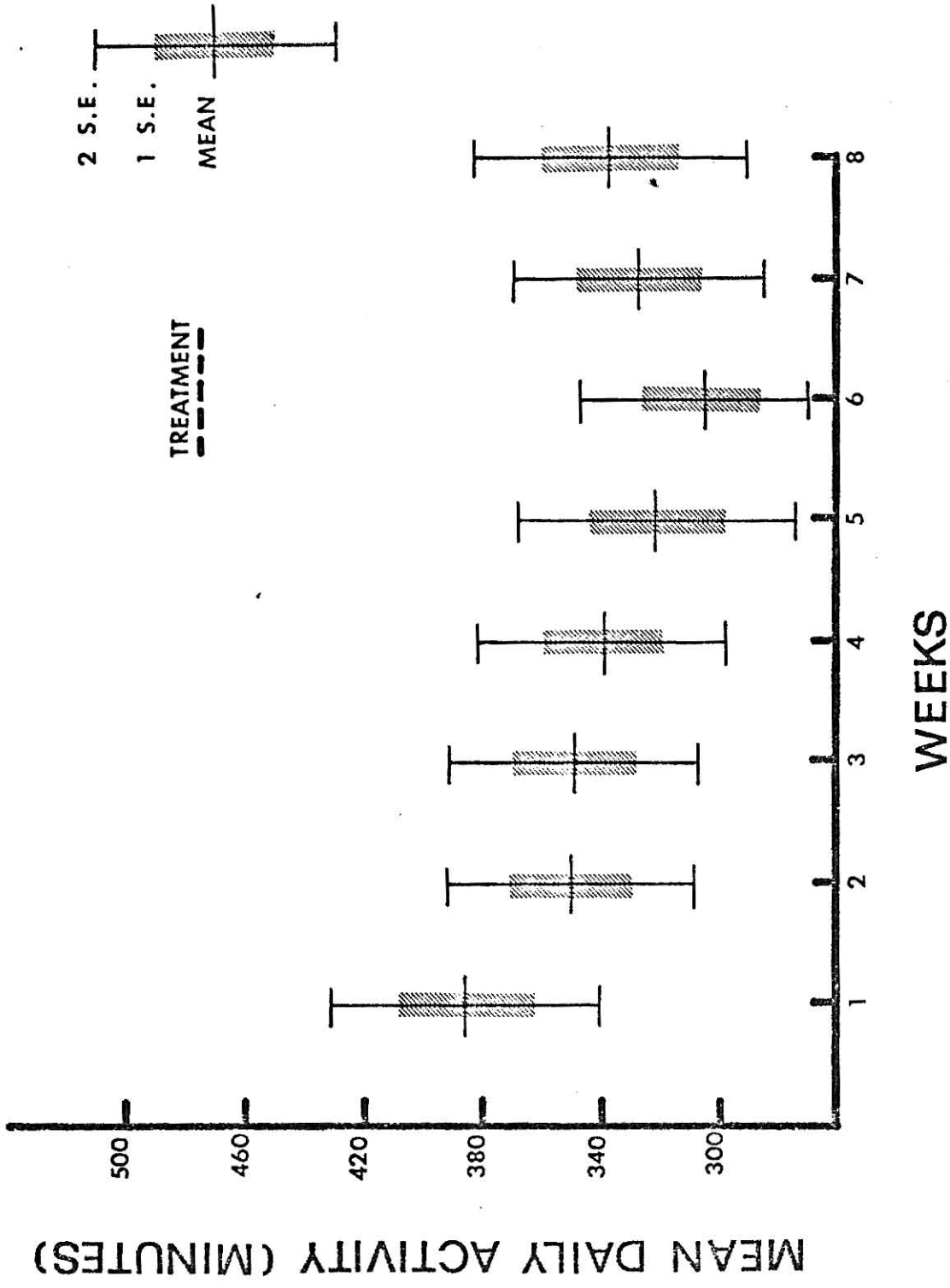

2 S.E.
1 S.E.
MEAN
TREATMENT
MEAN DAILY ACTIVITY (MINUTES)
500
460
420
380
340
300
1
2
3
4
5
6
7
8
WEEKS

Table 2. Daily activity of eight carbaryl treated birds (20 mg/kg) during pretreatment, treatment and posttreatment phases under continuous bright light (300 lux).

| BIRD | MINUTES OF DAILY ACTIVITY | | | | | |
|---|---|---|---|---|---|---|
| | PRETREATMENT | | TREATMENT | | POSTTREATMENT | |
| | Range | Mean (S.D.)* | Range | Mean (S.D.)** | Range | Mean (S.D.)*** |
| 1 | 116-563 | 289 (112) | 188-492 | 298 (116) | 221-515 | 343 ( 87) |
| 2 | 117-557 | 363 ( 96) | 252-565 | 381 (102) | 224-376 | 325 ( 44) |
| 3 | 43-680 | 284 (181) | 73-432 | 228 (124) | 177-431 | 332 ( 69) |
| 4 | 94-519 | 313 ( 89) | 167-276 | 203 ( 38) | 116-285 | 173 ( 47) |
| 5 | 763-985 | 838 ( 62) | 702-969 | 832 ( 94) | 743-944 | 845 ( 61) |
| 6 | 126-709 | 313 (118) | 271-376 | 324 ( 37) | 163-889 | 389 (189) |
| 7 | 104-717 | 235 (167) | 119-181 | 147 ( 22) | 102-195 | 151 ( 36) |
| 8 | 162-811 | 444 (122) | 249-437 | 340 ( 76) | 210-606 | 407 (119) |

* Number of days = 35
** Number of days = 7
*** Number of days = 14

Table 3. Weekly mean daily activity (minutes) of eight carbaryl treated birds (20 mg/kg) under continuous bright light (300 lux).

| PHASE | WEEK | BIRD 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| PRETREATMENT | 1 | 377 | 369 | * | 237 | 849 | 310 | 410 | 430 |
| | 2 | 249 | 348 | 362 | 333 | 839 | 331 | 265 | 395 |
| | 3 | 224 | 349 | 324 | 440 | 833 | 309 | 155 | 479 |
| | 4 | 264 | 365 | 265 | 294 | 820 | 309 | 170 | 472 |
| | 5 | 333 | 386 | 185 | 261 | 850 | 307 | 174 | * |
| TREATMENT | 6 | 298 | 381 | 228 | 203 | 832 | 324 | 147 | 340 |
| POSTTREATMENT | 7 | 312 | 325 | 307 | 194 | 839 | 376 | 160 | 433 |
| | 8 | 374 | * | 356 | 152 | 851 | 402 | 141 | 381 |

* Missing data due to equipment failure.

An analysis of variance disclosed no significant weekly variation in mean daily activity for the group (Table 18, Appendix).

Mean daily food consumption (±S.D.) was 12.9(±1.3) grams during pretreatment, 12.9(±2.2) grams during treatment and 12.4(±2.2) grams during posttreatment (Table 19, Appendix). The changes in mean daily food consumption during treatment from pretreatment ranged from a 23 percent decrease for birds no. 3 and 5 to a 10 percent increase for bird no. 2 (average change was 0.7 percent; a 0.09-gram decrease). Changes in mean daily food consumption during posttreatment from treatment ranged from a 14 percent decrease for bird no. 5 to a 17 percent increase for bird no. 3 (average change was 4 percent; a 0.5-gram decrease). An analysis of variance disclosed no significant differences between collections in mean daily food consumption for the group (Table 20, Appendix).

Mean body weight (±S.D.) was 177.5(±10.1) grams at the end of acclimation, 178.2(±8.0) grams at the end of pretreatment, 178.2(±8.9) grams at the end of treatment and 178.0(±8.6) grams at the end of posttreatment (Table 21, Appendix). Changes in body weight during pretreatment from acclimation ranged from a 1.6 percent decrease for bird no. 7 to a 2.7 percent increase for bird no. 3 (average change was 0.4 percent; a 0.7-gram increase). Changes in body weight during treatment from pretreatment ranged from a 1.6 percent decrease for bird no. 8 to a 0.7 percent increase for bird no. 5 (average change was 0.0 percent). Changes in body weight during posttreatment from treatment ranged from a 1.3 percent decrease for bird no. 5 to a 0.8 percent gain for birds no. 2 and 8 (average change was 0.1 percent; a 0.2-gram decrease).

An analysis of variance disclosed no significant variation between phases for the group (Table 22, Appendix).

No significant correlations were found between activity and body weight, or body weight and food consumption during any phase. Activity and food consumption were significantly correlated during all three phases ($r = 0.8862$, 0.9160 and 0.8410 for the pretreatment, treatment and posttreatment phases, respectively). No significant correlations were found between changes in activity, food consumption and body weight during any phase.

Experiment 2 - Carbaryl High Dosage (100 mg/kg) Continuous Bright Light (300 Lux).

A chi-square goodness of fit test indicated that daily activity data were not normally distributed ($P<0.005$). Therefore, although standard deviations are presented for mean daily activity the variability they represent is not symmetrical about the means. Food consumption and body weight data were normally distributed, therefore, standard deviations for these variables are presented as being symmetrical about the means.

The range and mean (S.D.), in minutes, of daily activity for each bird during each phase of the experiment are shown in Table 4. Variation in a bird's daily activity was considerable as is evident by the range. The range of activity means, from 188-minutes for bird no. 4 to 685-minutes for bird no. 6, also indicates considerable variation exists between birds.

The mean daily activity (S.D.) was 481(176) minutes during pretreatment, 466(162) minutes during treatment and 482(177) minutes during posttreatment. Weekly daily activity for each bird is shown

in Table 5 and mean daily activity for each week is shown in Figure 6. Changes in mean daily activity during treatment from pretreatment ranged from a 29 percent increase for bird no. 1 to a 22 percent decrease for bird no. 3 (average change was 3 percent; a 14-minute decrease). The changes in mean daily activity during posttreatment from treatment ranged from a 46 percent increase for bird no. 3 to a 16 percent decrease for bird no. 6 (average change was 3 percent; a 14-minute increase). An analysis of variance disclosed no significant weekly variation in mean daily activity for the group (Table 23, Appendix).

Mean daily food consumption (±S.D.) was 12.2(±1.7) grams during pretreatment, 12.2(±2.0) grams during treatment and 12.9(±2.3) grams during posttreatment (Table 24, Appendix). The changes in mean daily food consumption during treatment from pretreatment ranged from a 9 percent increase for bird no. 2 to a 13 percent decrease for bird no. 4 (average change was 0.0 percent). Changes in mean daily food consumption during posttreatment from treatment ranged from a 0.7 percent decrease for bird no. 7 to a 16.5 percent increase for bird no. 1 (average change was 6 percent; a 0.7-gram increase). An analysis of variance disclosed no significant differences between collections in mean daily food consumption for the group (Table 25, Appendix).

Mean body weight (±S.D.) was 185.2(±11.0) grams at the end of acclimation, 185.9(±10.5) grams at the end of pretreatment, 187.6(±10.8) grams at the end of the treatment, and 189.4(±12.9) grams at the end of posttreatment (Table 26, Appendix). Changes in body weight during pretreatment from acclimation ranged from a 0.8 percent decrease for bird no. 7 to a 1.7 percent increase for bird no. 4 (average change was 0.4 percent, a 0.7-gram decrease). The changes in body weight during treatment from

Fig. 6. Mean daily activity/week of eight carbaryl treated birds (100 mg/kg) under continuous bright light (300 lux).

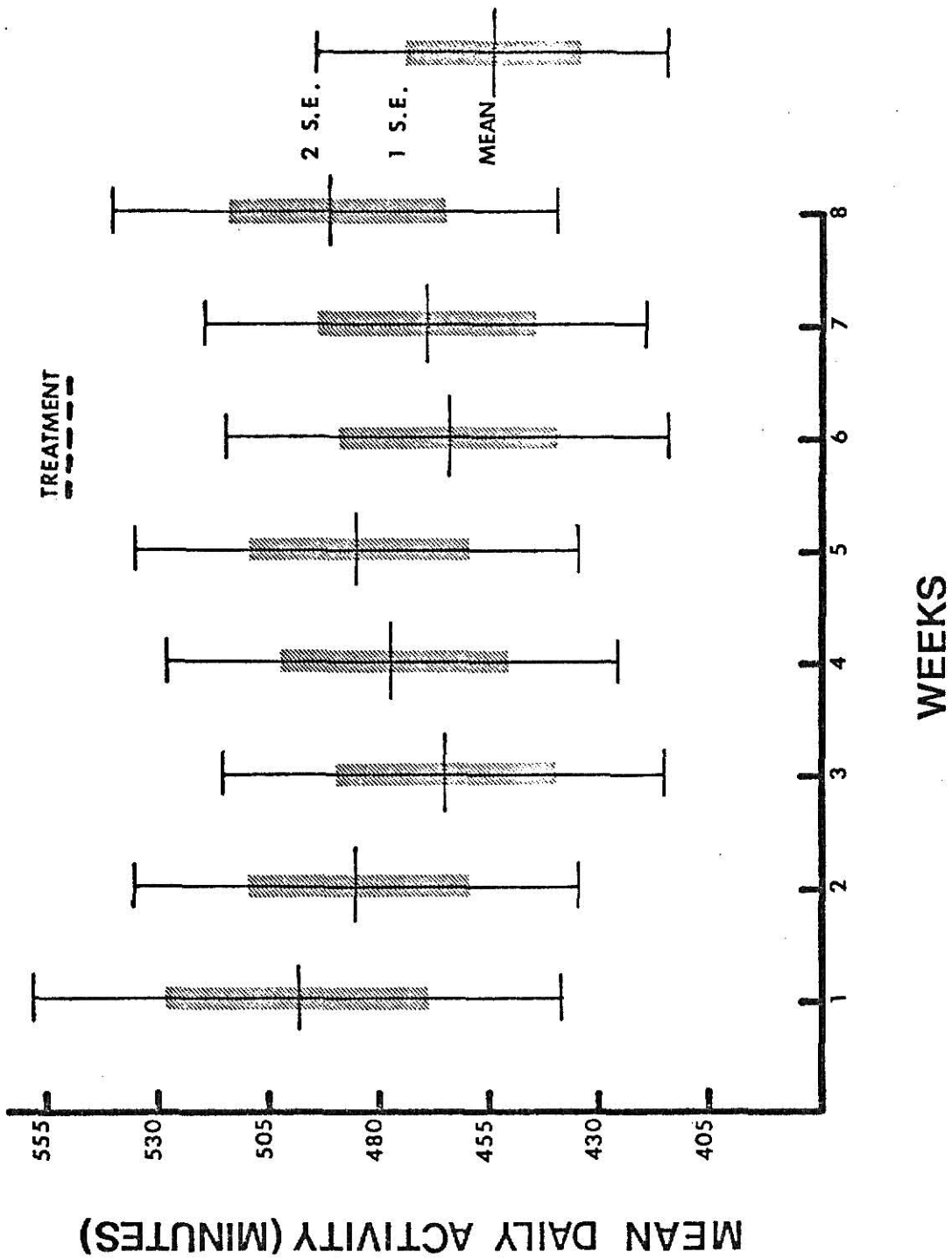

MEAN DAILY ACTIVITY (MINUTES)
555
530
505
480
455
430
405
1
2
3
4
5
6
7
8
WEEKS
TREATMENT
2 S.E.
1 S.E.
MEAN

Table 4. Daily activity of eight carbaryl treated birds (100 mg/kg) during pretreatment, treatment and posttreatment phases under continuous bright light (300 lux).

| BIRD | MINUTES OF DAILY ACTIVITY | | | | | |
|---|---|---|---|---|---|---|
| | PRETREATMENT | | TREATMENT | | POSTTREATMENT | |
| | Range | Mean (S.D.)* | Range | Mean (S.D.)** | Range | Mean (S.D.)*** |
| 1 | 235-558 | 389 ( 75) | 433-593 | 501 ( 62) | 381-693 | 533 ( 76) |
| 2 | 477-866 | 677 ( 91) | 532-954 | 666 (143) | 693-913 | 795 ( 59) |
| 3 | 49-674 | 318 (108) | 163-341 | 248 ( 66) | 137-491 | 361 (113) |
| 4 | 87-426 | 188 ( 75) | 113-326 | 195 ( 81) | 108-326 | 184 ( 62) |
| 5 | 217-767 | 544 (166) | 298-782 | 532 (171) | 253-679 | 458 (147) |
| 6 | 511-882 | 685 ( 94) | 410-727 | 567 (107) | 334-676 | 474 ( 97) |
| 7 | 326-687 | 492 ( 96) | 357-694 | 472 (121) | 395-766 | 507 (105) |
| 8 | 175-904 | 547 (164) | 314-687 | 545 (125) | 289-681 | 541 (107) |

* Number of days = 35
** Number of days = 7
*** Number of days = 14

Table 5. Weekly mean daily activity (minutes) of eight carbaryl treated birds (100 mg/kg) under continuous bright light (300 lux).

| PHASE | | BIRD | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | WEEK | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| PRETREATMENT | 1 | 429 | 661 | 408 | 154 | 377 | 743 | 474 | 664 |
| | 2 | 400 | 627 | 364 | 152 | 605 | 733 | 491 | 521 |
| | 3 | 365 | 703 | 266 | 200 | 588 | 617 | 461 | 531 |
| | 4 | 364 | 670 | 193 | 215 | 622 | 705 | 534 | 523 |
| | 5 | 389 | 724 | 359 | 219 | 577 | 626 | 502 | 498 |
| TREATMENT | 6 | 501 | 666 | 248 | 195 | 532 | 567 | 472 | 545 |
| POSTTREATMENT | 7 | 491 | 806 | 308 | 197 | 533 | 411 | 469 | 550 |
| | 8 | 575 | 784 | 414 | 171 | 382 | 537 | 544 | 531 |

pretreatment ranged from a 0.9 percent decrease for bird no. 1 to a 2.6 percent increase for bird no. 5 (average change was 0.9 percent; a 1.7-gram increase). Changes in body weight during posttreatment from treatment ranged from a 4.8 percent decrease for bird no. 3 to a 4.3 percent increase for bird no. 6 (average change was 0.9 percent; a 1.8-gram increase). An analysis of variance disclosed no significant variation between phases for the group (Table 27, Appendix).

No significant correlations were found between body weight and food consumption; or body weight and activity during any phase. Activity and food consumption were significantly correlated during pretreatment ($r = 0.8181$) and treatment ($r = 0.7834$) but not during posttreatment ($r = 0.4296$).

No significant correlations were found between changes in activity and changes in food consumption during any phase. However, there was a significant negative correlation between changes in activity and changes in body weight during posttreatment ($r = -0.7704$).

Experiment 3 *Carbofuran* Low Dosage (2 mg/kg) Continuous Bright Light (300 Lux).

A chi-square goodness of fit test indicated that daily activity data were not normally distributed ($P<0.005$). Therefore, although standard deviations are presented for mean daily activity the variability they represent is not symmetrical about the means. Food consumption and body weight data were normally distributed, therefore, standard deviations for these variables are presented as being symmetrical about the means.

The range and mean (S.D.), in minutes, of daily activity for each bird during each phase of the experiment are shown in Table 6.

Variation in a bird's daily activity was considerable as is evident by the range. The range of activity means, from 190-minutes for bird no. 1 to 673-minutes for bird no. 7, also indicates considerable variation exists between birds.

Mean daily activity (S.D.) was 422(159) minutes during pretreatment, 417(187) minutes during treatment and 410(160) minutes during posttreatment. Weekly daily activity for each bird is shown in Table 7 and mean daily activity for each week is shown in Figure 7. Changes in mean daily activity during treatment from pretreatment ranged from a 27 percent decrease for bird no. 1 to a 27 percent increase for bird no. 3 (average change was 1 percent; a 5-minute decrease). Changes in mean daily activity during posttreatment from treatment ranged from a 15 percent decrease for bird no. 7 to a 14 percent increase for bird no. 2 (average change was 1.6 percent; a 7-minute decrease). An analysis of variance disclosed no significant weekly variation in mean daily activity for the group (Table 28, Appendix). No apparent trends in pretreatment or posttreatment activity were observed, however, seven out of eight birds had a higher mean daily activity during the second week of treatment than the first.

Mean daily food consumption (±S.D.) was 16.4(±2.1) grams during pretreatment, 14.9(±1.8) grams during treatment and 16.1(±2.3) grams during posttreatment (Table 29, Appendix). An analysis of variance disclosed significant ($P<0.10$) differences in mean daily food consumption between collections (Table 30, Appendix). An LSD separation of means disclosed that food consumption during treatment was significantly less than food consumption during pretreatment and posttreatment. All

Fig. 7. Mean daily activity/week of eight carbofuran treated birds (2 mg/kg) under continuous bright light (300 lux).

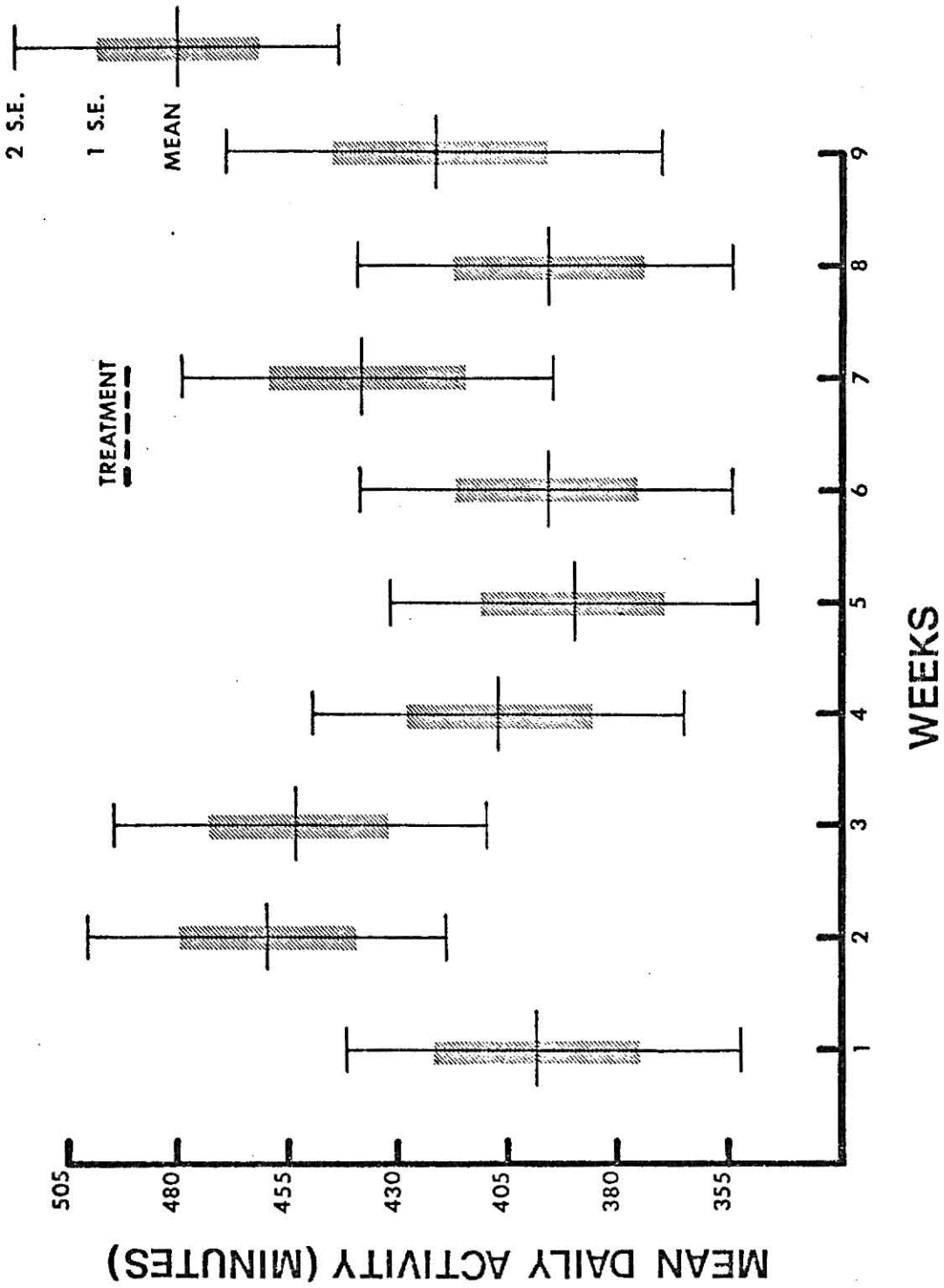

2 S.E.
1 S.E.
MEAN
TREATMENT
505
480
455
430
405
380
355
MEAN DAILY ACTIVITY (MINUTES)
1
2
3
4
5
6
7
8
9
WEEKS

Table 6. Daily activity of eight carbofuran treated birds (2 mg/kg) during pretreatment, treatment and posttreatment phases under continuous bright light (300 lux).

| BIRD | MINUTES OF DAILY ACTIVITY | | | | | |
|---|---|---|---|---|---|---|
| | PRETREATMENT | | TREATMENT | | POSTTREATMENT | |
| | Range | Mean (S.D.)* | Range | Mean (S.D.)** | Range | Mean (S.D.)** |
| 1 | 50- 581 | 190 (116) | 57-202 | 139 ( 46) | 65- 446 | 159 ( 98) |
| 2 | 244-1087 | 487 (174) | 296-548 | 394 ( 74) | 287- 587 | 389 ( 88) |
| 3 | 129- 374 | 220 ( 58) | 186-385 | 280 ( 62) | 159- 379 | 252 ( 57) |
| 4 | 103- 592 | 377 (116) | 295-484 | 374 ( 49) | 254- 513 | 374 ( 77) |
| 5 | 240- 708 | 498 (115) | 415-589 | 485 ( 72) | 391- 774 | 543 (142) |
| 6 | 194-1326 | 507 (192) | 232-646 | 410 (137) | 278- 598 | 431 ( 75) |
| 7 | 287-1175 | 673 (190) | 591-936 | 789 ( 93) | 532-1006 | 671 (110) |
| 8 | 290- 594 | 425 ( 81) | 415-525 | 468 ( 38) | 346- 593 | 458 ( 68) |

* Number of days = 35
** Number of days = 14

Table 7. Weekly mean daily activity (minutes) of eight carbofuran treated birds (2 mg/kg) under continuous bright light (300 lux).

| PHASE | | BIRD | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | WEEK | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| PRETREATMENT | 1 | 217 | 435 | 169 | 301 | 416 | 512 | 670 | 460 |
| | 2 | 251 | 509 | 206 | 371 | 610 | 581 | 726 | 434 |
| | 3 | 237 | 684 | 221 | 330 | 447 | 524 | 737 | 445 |
| | 4 | 132 | 448 | 273 | 441 | 505 | 451 | 620 | 391 |
| | 5 | 112 | 357 | 233 | 440 | 511 | 465 | 612 | 397 |
| TREATMENT | 6 | 125 | 370 | 253 | 351 | 460 | 368 | 772 | 473 |
| | 7 | 153 | 418 | 306 | 396 | 509 | 451 | 806 | 463 |
| POSTTREATMENT | 8 | 187 | 383 | 218 | 402 | 450 | 412 | 658 | 461 |
| | 9 | 130 | 395 | 286 | 346 | 636 | 449 | 684 | 454 |

birds showed a decrease in food consumption during treatment and an increase during posttreatment. Changes in mean daily food consumption during treatment from pretreatment ranged from a 4.5 percent decrease for bird no. 4 to a 17 percent decrease for bird no. 2 (average change was 9.0 percent; a 1.5-gram decrease). Changes in food consumption during posttreatment from treatment ranged from a 0.1 percent increase for bird no. 1 to a 14 percent increase for bird no. 7 (average change was 8.0 percent; a 1.2-gram increase).

Mean body weight (±S.D.) was 195.2(±10.6) grams at the end of acclimation, 197.4(±10.2) grams at the end of pretreatment, 198.0(±9.6) grams at the end of treatment and 199.1(±9.3) grams at the end of post-treatment (Table 31, Appendix). Changes in pretreatment body weight from acclimation ranged from a 0.4 percent decrease for bird no. 3 to a 3.3 percent increase for bird no. 1 (average change was 1 percent; a 1.9-gram increase). Changes in body weight during treatment from pretreatment ranged from a 4.5 percent decrease for bird no. 7 to a 4 percent increase for bird no. 2 (average change was 0.3 percent; a 0.6-gram increase). Changes in body weight during posttreatment from treatment ranged from a 1 percent decrease for birds no. 1 and 5 to a 2 percent increase for bird no. 6 (average change was 0.5 percent; a 1.1-gram increase). An analysis of variance disclosed no significant variation between phases for the group (Table 32, Appendix).

Body weight and food consumption were significantly correlated during posttreatment ($r = 0.8206$). There was a highly significant correlation ($P<0.001$) between activity and food consumption during all three phases ($r = 0.8990$, 0.8825 and 0.9649 for pretreatment, treatment

and posttreatment respectively). A partial correlation analysis disclosed a highly significant ($P<0.001$) negative correlation existed between activity and body weight during pretreatment ($r = -0.8505$) and post-treatment ($r = -0.8336$).

No significant correlations were found between changes in activity and changes in body weight during any phase. However, a significant negative correlation existed between changes in activity and changes in food consumption during posttreatment ($r = -0.7553$) while changes in food consumption and changes in body weight were significantly correlated during posttreatment ($r = 0.7543$).

Experiment 4 - Carbofuran High Dosage (10 mg/kg) Continuous Bright Light (300 Lux).

A chi-square goodness of fit test indicated that daily activity data were not normally distributed ($P<0.005$). Therefore, although standard deviations are presented for mean daily activity the variability they represent is not symmetrical about the means. Food consumption and body weight data were normally distributed, therefore, standard deviations for these variables are presented as being symmetrical about the means.

The range and mean (S.D.), in minutes, of daily activity for each bird during each phase of the experiment are shown in Table 8. Variation in a bird's daily activity was considerable as is evident by the range. The range of activity means, from 345-minutes for bird no. 6 to 710-minutes for bird no. 3, also indicates considerable variation exists between birds.

Mean daily activity (S.D.) was 452(130) minutes during pretreatment, 431(149) minutes during treatment and 402(101) minutes during

posttreatment. Weekly mean daily activity for each bird is shown in Table 9, and mean daily activity for each week is shown in Figure 8. Changes in mean daily activity during treatment from pretreatment ranged from a 41 percent decrease for bird no. 1 to a 30 percent increase for bird no. 6 (average change was 5 percent; a 21-minute decrease). Changes in mean daily activity during posttreatment from treatment ranged from a 35 percent decrease for bird no. 6 to a 63 percent increase for bird no. 1 (average change was 6.7 percent; a 29-minute decrease). An analysis of variance disclosed significant weekly variation in mean daily activity for the group (Table 33, Appendix). An LSD separation of means disclosed activity during the second week of treatment was significantly less than activity during the first three weeks of pretreatment and the first week of treatment. Activity during the first week of posttreatment was significantly less than activity during the first two weeks of pretreatment and the first week of treatment.

Mean daily food consumption (±S.D.) was 15.8(±1.2) grams during pretreatment, 9.8(±2.4) grams during treatment and 17.6(±1.9) grams during posttreatment (Table 34, Appendix). Changes in mean daily food consumption during treatment from pretreatment ranged from a 19 percent decrease for bird no. 2 to 68 percent decrease for bird no. 6 (average change was 38 percent; a 6-gram decrease). Changes in mean daily food consumption during posttreatment from treatment ranged from a 25 percent increase for bird no. 2 to a 246 percent increase for bird no. 6 (average change was 80 percent; a 7.8-gram increase). An analysis of variance disclosed highly significant ($P<0.05$) differences in mean daily food consumption between collection times (Table 35, Appendix). An LSD separation of means disclosed that food consumption during treatment was significantly less than any other time during the experiment.

Fig. 8. Mean daily activity/week of eight carbofuran treated birds (10 mg/kg) under continuous bright light (300 lux).

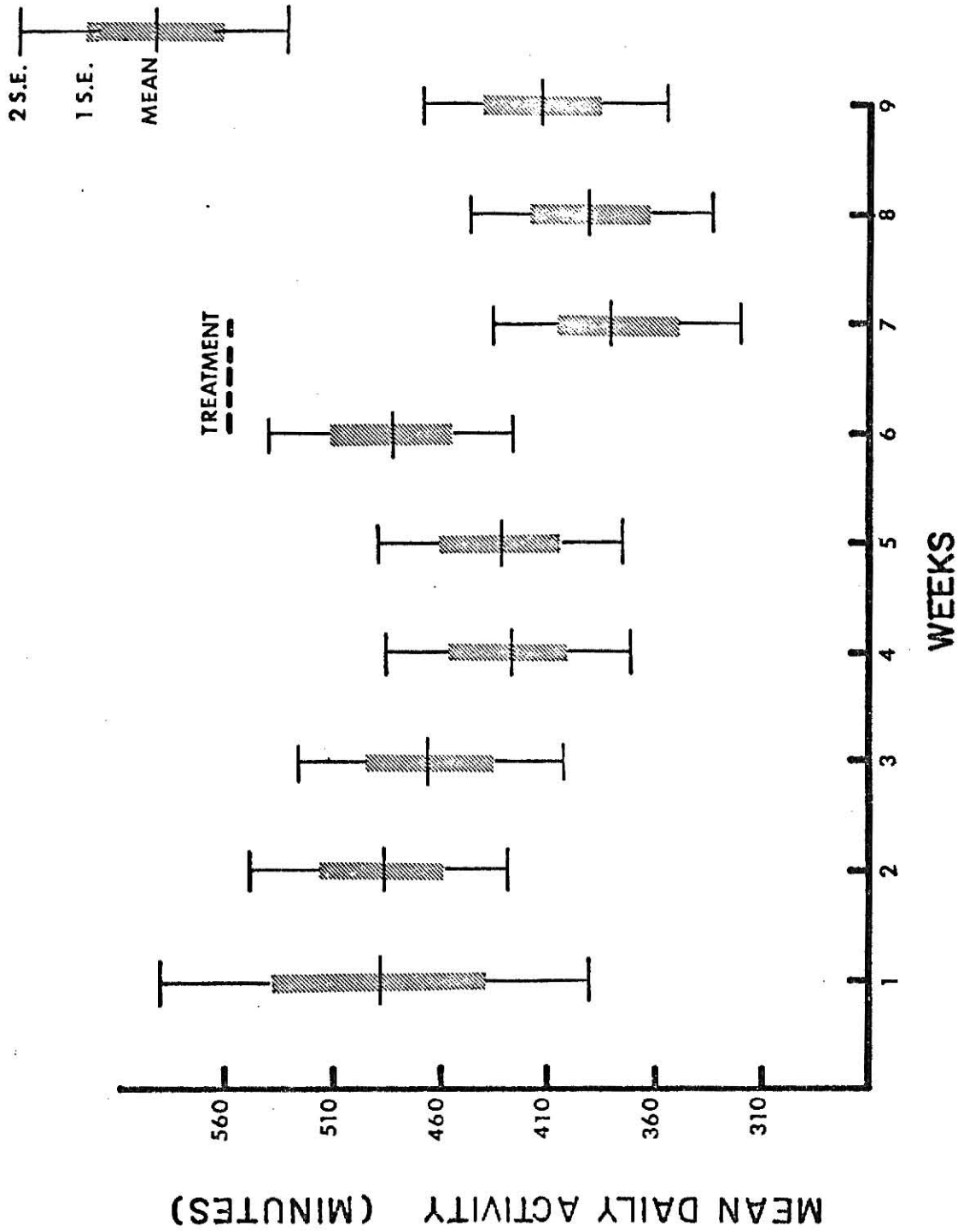

2 S.E.
1 S.E.
MEAN
TREATMENT
MEAN DAILY ACTIVITY (MINUTES)
560
510
460
410
360
310
1
2
3
4
5
6
7
8
9
WEEKS

Table 8. Daily activity of eight carbofuran treated birds (10 mg/kg) during pretreatment, treatment and posttreatment phases under continuous bright light (300 lux).

| BIRD | MINUTES OF DAILY ACTIVITY | | | | | |
|---|---|---|---|---|---|---|
| | PRETREATMENT | | TREATMENT | | POSTTREATMENT | |
| | Range | Mean (S.D.)* | Range | Mean (S.D.)** | Range | Mean (S.D.)** |
| 1 | 145-776 | 369 (192) | 93- 358 | 216 ( 92) | 179- 576 | 351 (129) |
| 2 | 228-597 | 430 ( 85) | 199- 532 | 423 ( 78) | 195- 578 | 441 (110) |
| 3 | 532-864 | 710 ( 90) | 289- 871 | 652 (149) | 334- 597 | 494 ( 81) |
| 4 | 137-950 | 582 (259) | 201-1144 | 594 (315) | 56- 890 | 571 (214) |
| 5 | 175-535 | 395 (107) | 164- 378 | 256 ( 74) | 158- 404 | 293 ( 82) |
| 6 | 63-607 | 345 (156) | 84- 821 | 447 (232) | 74- 560 | 290 (130) |
| 7 | 233-745 | 372 (135) | 184- 618 | 406 (117) | 124-1063 | 442 (240) |
| 8 | 101-645 | 451 (112) | 99- 675 | 444 (160) | 198- 548 | 336 ( 97) |

* Number of days = 35
** Number of days = 14

Table 9. Weekly mean daily activity (minutes) of eight carbofuran treated birds (10 mg/kg) under continuous bright light (300 lux).

| PHASE | WEEK | BIRD 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| PRETREATMENT | 1 | 435 | 450 | 635 | * | * | 401 | * | 510 |
| | 2 | 531 | 444 | 702 | 540 | 416 | 367 | * | 419 |
| | 3 | 440 | 342 | 789 | 716 | 346 | 252 | * | 392 |
| | 4 | 257 | 435 | 734 | 595 | 320 | 273 | 345 | 464 |
| | 5 | 184 | 479 | 692 | 477 | 338 | 430 | 399 | 472 |
| TREATMENT | 6 | 279 | 456 | 693 | 684 | 264 | 540 | 392 | 562 |
| | 7 | 152 | 391 | 610 | 516 | 248 | 356 | 420 | 326 |
| POSTTREATMENT | 8 | 322 | 406 | 461 | 589 | 289 | 289 | 434 | 331 |
| | 9 | 379 | 475 | 526 | 552 | 297 | 290 | 449 | 341 |

* Missing data due to equipment failure.

Mean body weight (±S.D.) was 196.4 (±12.6) grams at the end of acclimation, 197.3(±12.8) grams at the end of pretreatment, 178.1(±7.7) grams at the end of treatment and 192.7(±11.1) grams at the end of posttreatment (Table 36, Appendix). Changes in body weight during pretreatment from acclimation ranged from a 0.4 percent increase for bird no. 1 to a 2.2 percent increase for bird no. 4 (average change was 0.5 percent; a 0.9-gram increase). Changes in body weight during treatment from pretreatment ranged from a 4.3 percent decrease for bird no. 2 to an 18 percent decrease for bird no. 6 (average change was 9.7 percent; a 19.2-gram decrease). Changes in posttreatment body weight from treatment ranged from a 0.9 percent increase for bird no. 2 to an 18.9 percent increase for bird no. 6 (average change was 8.2 percent; a 14.6-gram increase). An analysis of variance disclosed significant differences in mean body weight between phases (Table 37, Appendix). An LSD separation of means disclosed body weight at the end of treatment was significantly less than any other phase.

No significant correlations were found between any of the variables during any phase of the experiment. However, changes in food consumption and changes in activity were significantly correlated during treatment ($r = 0.8035$). There was a significant negative correlation between changes in activity and changes in body weight during treatment ($r = -0.8662$). Changes in body weight and changes in food consumption were significantly correlated during treatment ($r = 0.9249$) and posttreatment ($r = 0.7211$).

Experiment 5A - Carbofuran High Dosage (10 mg/kg) Light-Dark Photoperiod (14L:10D).

A chi-square goodness of fit test indicated that daily activity data were not normally distributed ($P<0.005$). Therefore, although standard deviations are presented for mean daily activity the variability they represent is not symmetrical about the means. Food consumption and body weight data were normally distributed, therefore, standard deviations for these variables are presented as being symmetrical about the means.

The range and mean (S.D.), in minutes, of daily activity for each bird during each phase of the experiment are shown in Table 10. Variation in a bird's daily activity was considerable as is evident by the range. The range of activity means, from 368-minutes for bird no. 4 to 545-minutes for bird no. 1, also indicates considerable variation exists between birds.

At approximately 6:15 P.M., during the thirteenth day of treatment, a mechanical failure caused chamber temperature to rise at a rate of 2.5°C/hr. All birds subsequently died of hyperthermia during the dark period which started at 6:00 P.M. The birds showed sporadic bursts of intense activity during the dark period up until 2:15 A.M. at which time a marked increase in activity occurred. All activity stopped at 3:30 A.M. It was assumed death occurred at this time. Chamber temperatures were 40°C and 43°C at 2:15 A.M. and 3:30 A.M., respectively.

Mean daily activity (S.D.) was 450(74) and 410(47) minutes for pretreatment and the first 13-days of the 14-day treatment phase, respectively. Weekly daily activity for each bird is shown in Table 11

and mean daily activity for each week is shown in Figure 9. Changes in mean daily activity during treatment from pretreatment ranged from a 5 percent increase for bird no. 4 to a 20 percent decrease for bird no. 2 (average change was 9 percent; a 40-minute decrease). An analysis of variance disclosed no significant weekly differences in mean daily activity for the group (Table 38, Appendix).

Mean daily food consumption (±S.D.) was 15.3(±0.9) and 11.3 (±0.8) grams for the pretreatment and first 13 days of the 14-day treatment phase, respectively (Table 39, Appendix). Changes in mean daily food consumption during treatment from pretreatment ranged from a 20 percent decrease for bird no. 4 to a 32 percent decrease for bird no. 1 (average change was 26 percent; a 4-gram decrease). An analysis of variance disclosed highly significant differences in food consumption between collections ($P<0.05$) (Table 40, Appendix). An L S D separation of means disclosed treatment food consumption was significantly less than pretreatment food consumption.

Mean body weight (±S.D.) was 178.0(±13.6) grams at the end of acclimation, 179.5(±12.9) grams at the end of pretreatment and 170.9(±9.7) grams at the end of treatment (Table 41, Appendix). There were no changes in mean body weight during pretreatment from acclimation greater than 1.5 percent. Changes in body weight during treatment from pretreatment ranged from a 1.6 percent increase for bird no. 2 to a 10.8 percent decrease for bird no. 3 (average change was 4.8 percent; an 8.6-gram decrease). An analysis of variance disclosed no significant differences in mean body weight between phases (Table 42, Appendix).

Fig. 9. Mean daily activity/week of four carbofuran treated birds (10 mg/kg) under a 14L:10D rectangular light-dark cycle.

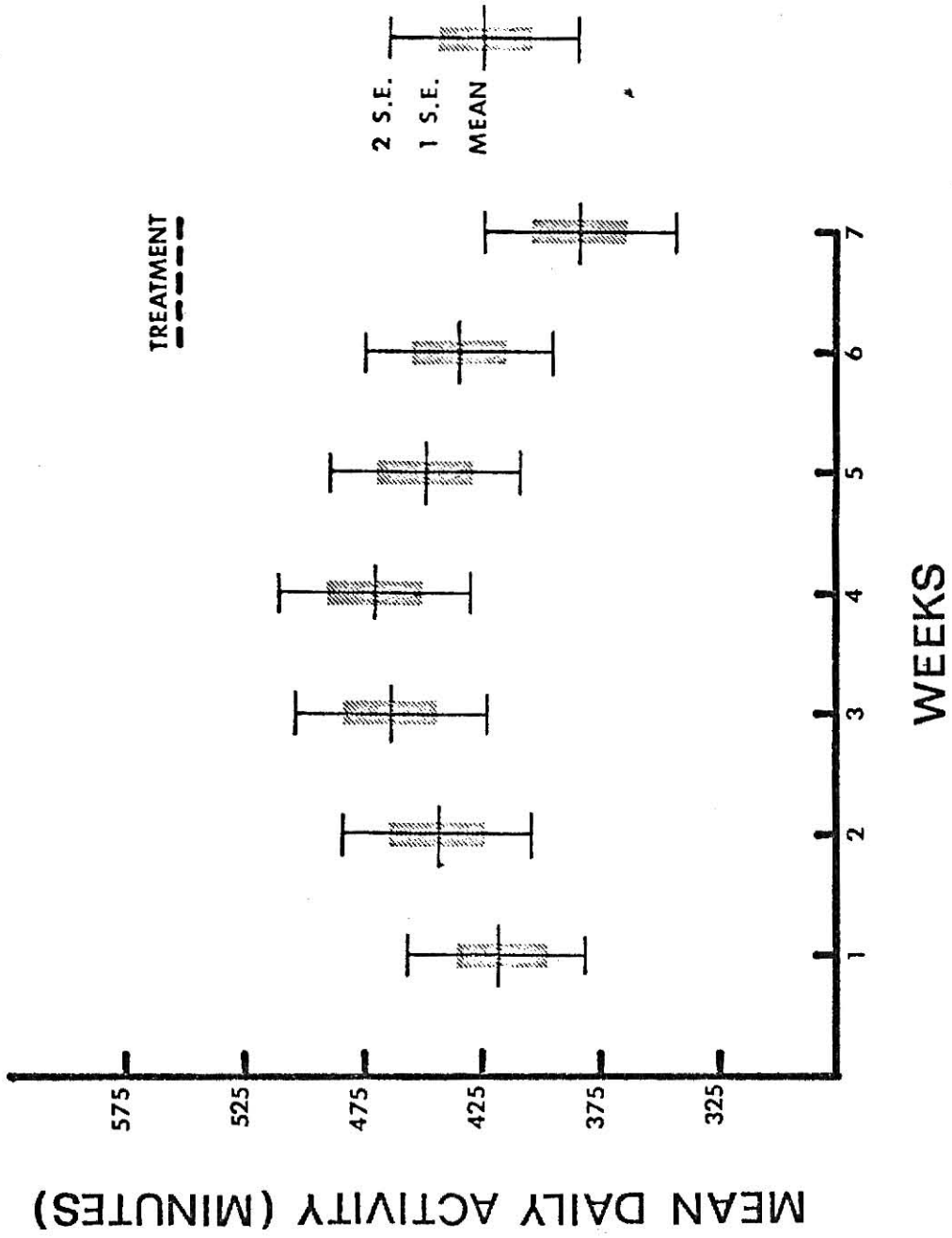

MEAN DAILY ACTIVITY (MINUTES)
575
525
475
425
375
325
1
2
3
4
5
6
7
WEEKS
TREATMENT
2 S.E.
1 S.E.
MEAN

Table 10. Daily activity of four carbofuran treated birds (10 mg/kg) during pretreatment, treatment and posttreatment phases under a 14L:10D light regimen.

| BIRD | MINUTES OF DAILY ACTIVITY | | | | | |
|---|---|---|---|---|---|---|
| | PRETREATMENT | | TREATMENT | | POSTTREATMENT | |
| | Range | Mean (S.D.)* | Range | Mean (S.D.)** | Range | Mean (S.D.)** |
| 1 | 337-696 | 545 ( 85) | 399-601 | 477 (62) | ... | ... |
| 2 | 303-583 | 457 ( 75) | 290-449 | 368 (54) | ... | ... |
| 3 | 262-632 | 426 (106) | 310-546 | 410 (76) | ... | ... |
| 4 | 102-596 | 368 (152) | 279-497 | 386 (56) | ... | ... |

* Number of days = 35
** Number of days = 14
... Missing Data

Table 11. Weekly mean daily activity (minutes) of four carbofuran treated birds (10 mg/kg) under 14L:10D light regimen.

| PHASE | WEEK | BIRD 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| PRETREATMENT | 1 | 523 | 414 | 342 | 397 |
| | 2 | 521 | 497 | 427 | 331 |
| | 3 | 573 | 456 | 497 | 329 |
| | 4 | 570 | 459 | 441 | 418 |
| | 5 | 540 | 444 | 434 | 360 |
| TREATMENT | 6 | 485 | 395 | 469 | 392 |
| | 7 ** | 466 | 341 | 350 | 379 |
| POSTTREATMENT | 8 | * | * | * | * |
| | 9 | * | * | * | * |

* Missing data due to death of 4 birds.
** Based of 6 days.

There was a highly significant ($P<0.001$) correlation between body weight and food consumption during pretreatment ($r = 0.9900$). No significant correlations existed between activity and body weight or activity and food consumption during any phase of the experiment. No significant correlations were found between changes in any of the variables during any phase.

Experiment 5B - Carbofuran High Dosage (10 mg/kg) Light-Dark Photoperiod (14L:10D).

A chi-square goodness of fit test indicated that daily activity data were not normally distributed ($P<0.005$). Therefore, although standard deviations are presented for mean daily activity the variability they represent is not symmetrical about the means. Food consumption and body weight data were normally distributed, therefore, standard deviations for these variables are presented as being symmetrical about the means.

The range and mean (S.D.), in minutes, of daily activity for each bird during each phase of the experiment are shown in Table 12. Variation in a bird's daily activity was considerable as is evident by the range. The range of activity means, from 235-minutes for bird no. 4 to 638-minutes for bird no. 1, also indicates considerable variation exists between birds.

Mean daily activity (S.D.) was 504(175) minutes during pretreatment, 320(138) minutes during treatment and 488(167) minutes during posttreatment. Weekly daily activity for each bird is shown in Table 13 and mean daily activity for each week is shown in Figure 10. Changes in mean daily activity during treatment from pretreatment ranged from a 29 percent decrease for bird no. 3 to a 45 percent decrease

Fig. 10. Mean daily activity/week of four carbofuran treated birds (10 mg/kg) under a 14L:10D rectangular light-dark cycle.

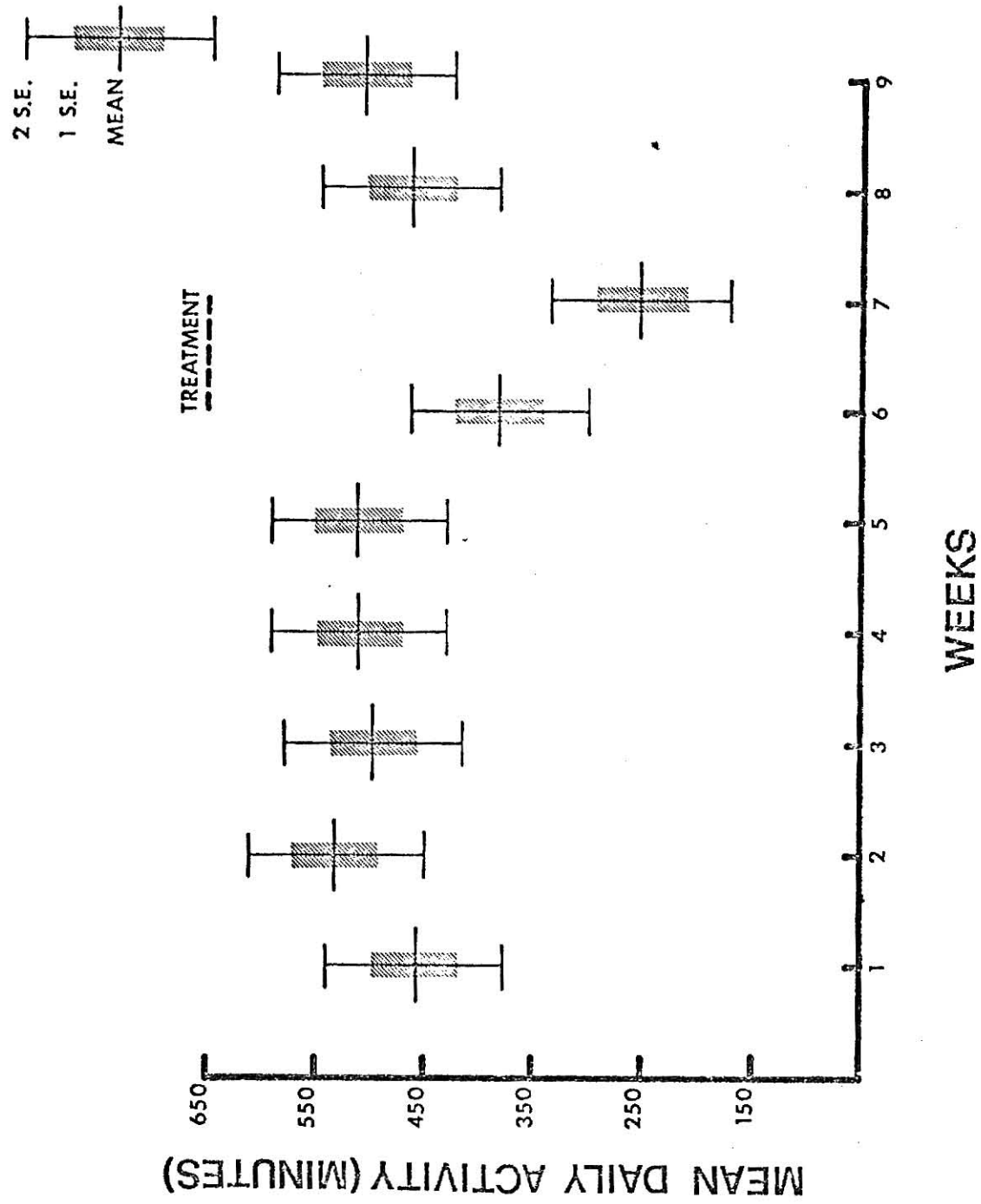

2 S.E.
1 S.E.
MEAN
TREATMENT
650
550
450
350
250
150
1
2
3
4
5
6
7
8
9
MEAN DAILY ACTIVITY (MINUTES)
WEEKS

Table 12. Daily activity of four carbofuran treated birds (10 mg/kg) during pretreatment, treatment and posttreatment phases under a 14L:10D light regimen.

| BIRD | MINUTES OF DAILY ACTIVITY | | | | | |
|---|---|---|---|---|---|---|
| | PRETREATMENT | | TREATMENT | | POSTTREATMENT | |
| | Range | Mean (S.D.)* | Range | Mean (S.D.)** | Range | Mean (S.D.)** |
| 1 | 503-832 | 638 (133) | 142-551 | 352 (133) | 216-631 | 444 (121) |
| 2 | 352-821 | 544 (106) | 110-678 | 368 (178) | 556-722 | 644 ( 59) |
| 3 | 219-825 | 599 (160) | 277-658 | 423 (106) | 409-738 | 603 ( 95) |
| 4 | 124-325 | 235 ( 47) | 89-200 | 140 ( 31) | 199-385 | 263 ( 53) |

* Number of days = 35
** Number of days = 14

Table 13. Weekly mean daily activity (minutes) of four carbofuran treated birds (10 mg/kg) under 14L:10D light regimen.

| PHASE | WEEK | BIRD 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| PRETREATMENT | 1 | 655 | 547 | 417 | 218 |
| | 2 | 653 | 550 | 690 | 248 |
| | 3 | 686 | 480 | 577 | 254 |
| | 4 | 668 | 549 | 600 | 229 |
| | 5 | 530 | 592 | 711 | 226 |
| TREATMENT | 6 | 422 | 464 | 499 | 153 |
| | 7 | 282 | 271 | 347 | 127 |
| POSTTREATMENT | 8 | 399 | 647 | 531 | 290 |
| | 9 | 490 | 640 | 674 | 236 |

for bird no. 1 (average change was 37 percent; a 184-minute decrease). Changes in mean daily activity during posttreatment from treatment ranged from a 14 percent increase for bird no. 1 to a 68 percent increase for bird no. 4 (average change was 55 percent; a 157-minute increase). An analysis of variance disclosed significant weekly differences in mean daily activity (Table 43, Appendix). An LSD separation of means disclosed mean daily activity during the first week of treatment was significantly less than weeks 2, 4, 5 and 9 and significantly greater than the second week of treatment. Activity during the second week of treatment was significantly less than every other week.

Mean daily food consumption (±S.D.) was 14.5(±1.7) grams for pretreatment, 9.1(±1.1) grams for treatment and 15.6(±2.4) grams for posttreatment (Table 44, Appendix). Changes in mean daily food consumption during treatment from pretreatment ranged from a 22 percent decrease for bird no. 4 to a 50 percent decrease for bird no. 2 (average change was 37 percent; a 5.4-gram decrease). Changes in mean daily food consumption during posttreatment from treatment ranged from a 33 percent increase for bird no. 4 to a 123.5 percent increase for bird no. 2 (average change was 71 percent; a 6.5-gram increase). An analysis of variance disclosed significant differences in mean daily food consumption between collections (Table 45, Appendix). An LSD separation of means disclosed treatment food consumption was significantly less than pretreatment and posttreatment.

Mean body weight (±S.D.) and 168.0 (±12.2) grams at the end of acclimation, 165.8 (±13.9) grams at the end of pretreatment, 164.7 (±12.6) grams at the end of treatment and 167.4(±14.3) grams at the

end of posttreatment (Table 46, Appendix). Changes in body weight during pretreatment from acclimation ranged from a 3.3 percent decrease for bird no. 3 to a 0.5 percent increase for bird no. 1 (average change was 1.3 percent; a 2-gram decrease). Changes in body weight during treatment from pretreatment ranged from a 2.3 percent increase for bird no. 4 to a 3.3 percent decrease for bird no. 1 (average change was 0.7 percent or 1.2-gram decrease). Changes in body weight during post-treatment from treatment ranged from a 1.6 percent decrease for bird no. 4 to a 3.5 percent increase for bird no. 1 (average change was 1.6 percent; a 1.7-gram decrease). An analysis of variance failed to disclose any significant differences in mean body weight between phases (Table 47, Appendix).

No significant correlations were found between body weight and food consumption, or body weight and activity during any phase. However, there was a significant correlation between activity and food consumption during posttreatment ($r = 0.9910$). No significant correlations were found between changes in any of the variables during any phase.

Experiment 6 - Carbofuran High Dosage (10 mg/kg) Continuous Dim Light (10 Lux).

A chi-square goodness of fit test indicated that daily activity data were not normally distributed ($P<0.005$). Therefore, although standard deviations are presented for mean daily activity the variability they represent is not symmetrical about the means. Food consumption and body weight data were normally distributed, therefore, standard deviations for these variables are presented as being symmetrical

about the means.

The range and mean (S.D.), in minutes, of daily activity for each bird during each phase of the experiment are shown in Table 14. Variation in a bird's daily activity was considerable as is evident by the range. The range of activity means, from 56-minutes for bird no. 8 to 697-minutes for bird no. 7, also indicates considerable variation exists between birds.

The mean daily activity (S.D.) was 369(219) minutes during pretreatment, 311(244) minutes during treatment and 207(255) minutes during posttreatment. Weekly daily activity for each bird is shown in Table 15 and mean daily activity for each week is shown in Figure 11. An analysis of variance disclosed significant weekly differences in mean daily activity (Table 48, Appendix). An L S D separation of means disclosed mean daily activity during weeks eight and nine was significantly less than week one. Changes in daily activity during treatment from pretreatment ranged from an 86 percent decrease for bird no. 5 to a 39 percent increase for bird no. 1 (average change was 15.7 percent; a 58-minute decrease). Changes in mean daily activity during posttreatment from treatment ranged from a 94 percent decrease for bird no. 1 to a 232 percent increase for bird no. 8 (average change was 33.4 percent; a 104-minute decrease).

Mean daily food consumption (±S.D.) was 13.8(±1.4) grams during pretreatment, 8.6(±3.2) grams during treatment and 16.8(±2.8) grams during posttreatment (Table 49, Appendix). Changes in mean daily food consumption during treatment from pretreatment ranged from a 4 percent decrease for bird no. 3 to 70 percent decrease for bird no. 2 (average

Fig. 11. Mean daily activity/week of eight carbofuran treated birds (10 mg/kg) under continuous dim light (10 lux).

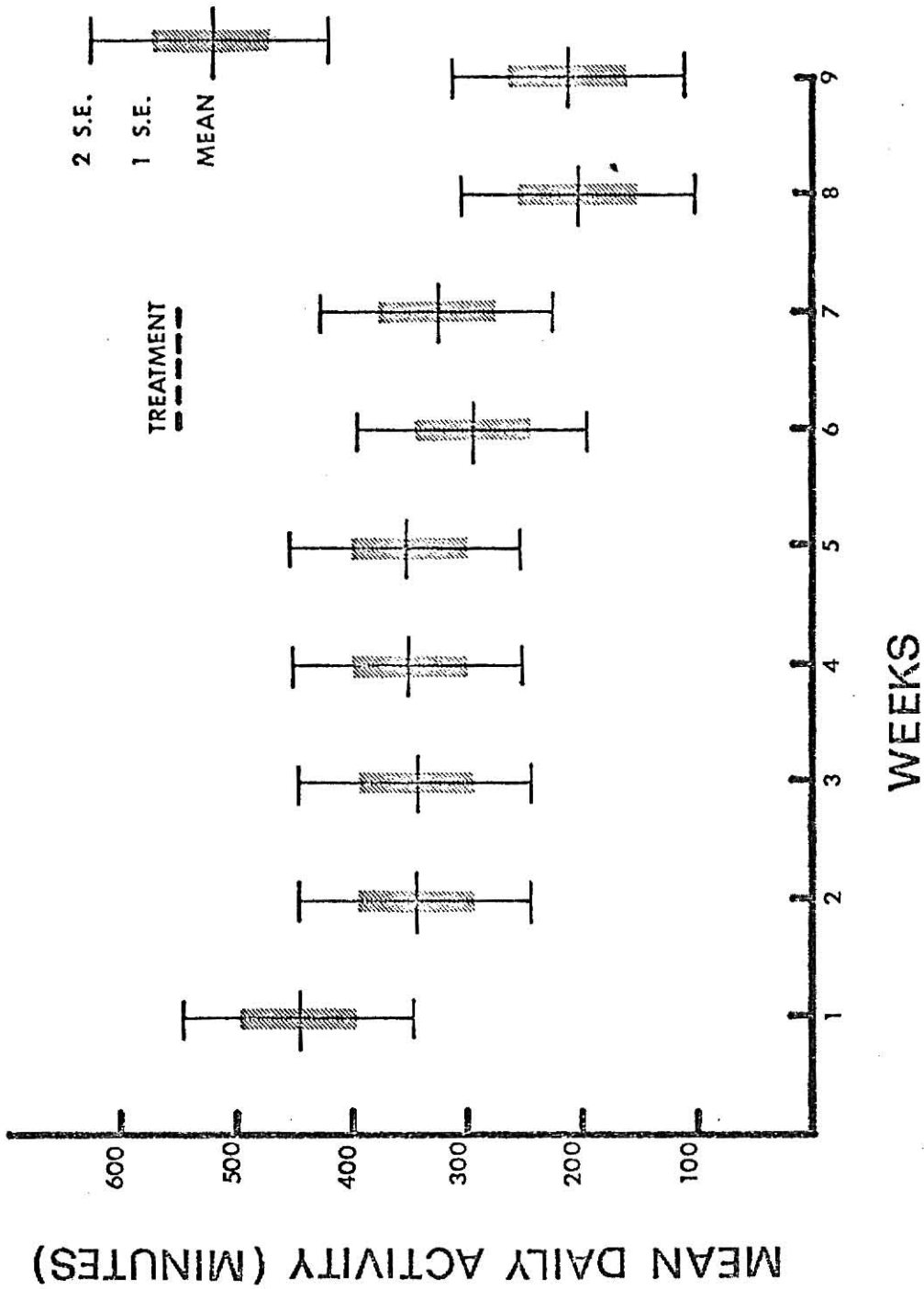

2 S.E.
1 S.E.
MEAN
TREATMENT
600
500
400
300
200
100
1
2
3
4
5
6
7
8
9
WEEKS
MEAN DAILY ACTIVITY (MINUTES)

Table 14. Daily activity of eight carbofuran treated birds (10 mg/kg) during pretreatment, treatment and posttreatment phases under continuous dim light (10 lux).

| BIRD | MINUTES OF DAILY ACTIVITY | | | | | |
|---|---|---|---|---|---|---|
| | PRETREATMENT | | TREATMENT | | POSTTREATMENT | |
| | Range | Mean (S.D.)* | Range | Mean (S.D.)** | Range | Mean (S.D.)** |
| 1 | 146-629 | 321 (133) | 169- 724 | 445 (186) | 6- 47 | 29 ( 13) |
| 2 | 448-936 | 631 (112) | 77-1025 | 478 (318) | 3- 353 | 102 (120) |
| 3 | 101-453 | 297 ( 87) | 138- 495 | 339 ( 83) | 144- 498 | 346 (105) |
| 4 | 124-675 | 325 (102) | 169- 696 | 425 (140) | 32- 302 | 124 ( 79) |
| 5 | 13-682 | 345 (175) | 2- 211 | 49 ( 60) | 9- 564 | 126 (162) |
| 6 | 19-657 | 277 (214) | 1- 636 | 107 (197) | 9- 258 | 50 ( 88) |
| 7 | 162-987 | 697 (215) | 326-1000 | 628 (204) | 514-1029 | 810 (138) |
| 8 | 8-212 | 56 ( 46) | 3- 82 | 21 ( 21) | 12- 119 | 70 ( 33) |

* Number of days = 35
** Number of days = 14

Table 15. Weekly mean daily activity (minutes) of eight carbofuran treated birds (10 mg/kg) under continuous dim light (10 lux).

| PHASE | | BIRD | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | WEEK | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| PRETREATMENT | 1 | 515 | 531 | 306 | 379 | 374 | 490 | 889 | 89 |
| | 2 | 283 | 650 | 310 | 294 | 298 | 359 | 541 | 33 |
| | 3 | 225 | 701 | 283 | 321 | 278 | 405 | 501 | 46 |
| | 4 | 293 | 649 | 313 | 315 | 381 | 72 | 738 | 54 |
| | 5 | 291 | 623 | 272 | 317 | 394 | 59 | 814 | 57 |
| TREATMENT | 6 | 315 | 670 | 331 | 348 | 77 | 165 | 459 | 9 |
| | 7 | 575 | 286 | 346 | 501 | 20 | 49 | 796 | 32 |
| POSTTREATMENT | 8 | 23 | 48 | 357 | 109 | 155 | 88 | 794 | 52 |
| | 9 | 34 | 156 | 334 | 139 | 96 | 12 | 825 | 87 |

change was 37.6 percent; a 5.2-gram decrease). Changes during post-treatment from treatment ranged from a 30.6 percent increase for bird no. 3 to 405 percent increase for bird no. 2 (average change was 95 percent; an 8.2-gram increase). An analysis of variance disclosed significant differences between collections in mean daily food consumption (Table 50, Appendix). An L S D separation of means disclosed mean daily food consumption during treatment was significantly less than pretreatment or posttreatment.

Mean body weight (±S.D.) was 175.2(±12.3) grams at the end of acclimation, 178.5(±11.4) grams at the end of pretreatment, 163.8 (±18.8) at the end of treatment and 184.7(±13.2) grams at the end of posttreatment (Table 51, Appendix). Changes in mean body weight during pretreatment from acclimation ranged from a 3.4 percent decrease for bird no. 2 to 5.6 percent increase for bird no. 6 (average change was 1.9 percent; a 3.3-gram increase). Changes in body weight during treatment from pretreatment ranged from a 1.4 percent decrease for bird no. 7 to a 32.2 percent decrease for bird no. 2 (average change was 8.0 percent; a 14.7-gram decrease). Changes in body weight during posttreatment from treatment ranged from a 7 percent loss for bird no. 8 to 55.5 percent gain for bird no. 2 (average change was 12.8 percent; a 20.8-gram increase). An analysis of variance disclosed significant differences in mean body weight at the end of each phase (Table 52, Appendix). An L S D separation of means disclosed that mean body weight at the end of treatment was significantly less than body weight at the end of pretreatment and posttreatment.

There was a significant negative correlation between activity and body weight during treatment and posttreatment ($r = -0.8255$ and $-0.7662$, respectively). Body weight and food consumption were signifi-

cantly correlated during treatment and posttreatment (r = 0.7828 and 0.7749, respectively). No significant correlations were found between activity and food consumption during any phase.

There was a highly significant (P<0.001) correlation between changes in activity and changes in food consumption during treatment (r = 0.8771). There was a highly significant correlation (P<0.001) between changes in body weight and changes in food consumption during treatment and posttreatment (r = 0.8850 and 0.8960, respectively). There was a significant negative correlation between activity and body weight during posttreatment (r = -0.8077).

The establishment of a free-running rhythm under CBL.

Six of 32 birds established a free-running activity rhythm for at least seven periods during the pretreatment phase. The length and time periods within which each bird established a free-running activity rhythm during CBL experiments are shown in Table 16. Period lengths during pretreatment ranged from 23.4-hours for bird no. 7 (experiment 4) to 25.5-hours for bird no. 8 (experiment 1). Only bird no. 8 maintained a free-running rhythm throughout the 56-day experiment. The response of this bird to constant bright illumination is seen in Fig. 12. Period length of the rhythm gradually changed from 25.5-hours for days 1-7 to 23.9-hours for seven days prior to treatment. All birds had random activity distributed throughout their periods.

Effects of treatment on period length.

Only birds no. 7 and 8 exhibited a rhythm during the seven periods just prior to treatment. In both cases period length during treatment increased from 23.4 to 25.6 and 23.9 to 26.3 hours for birds no. 7 and 8, respectively. Only bird no. 8 exhibited a free-running rhythm during posttreatment. The periodicity of the rhythm was 23.9 hours.

The establishment of a circadian rhythm under CDL.

Seven out of eight birds established a free-running rhythm of rest

Fig. 12. Free-running activity rhythm for bird no. 8 (experiment 1) under continuous bright light (300 lux). Each horizontal line from 0 to 24 represents intense activity periods (greater than 45 minutes), reproduced from activity charts, for one day. Records from successive days are mounted beneath each other. The entire record is reproduced and displaced upwards one day. (A) represents beginning of treatment, (B) represents end of treatment. Arrows indicate changes in periodicity.

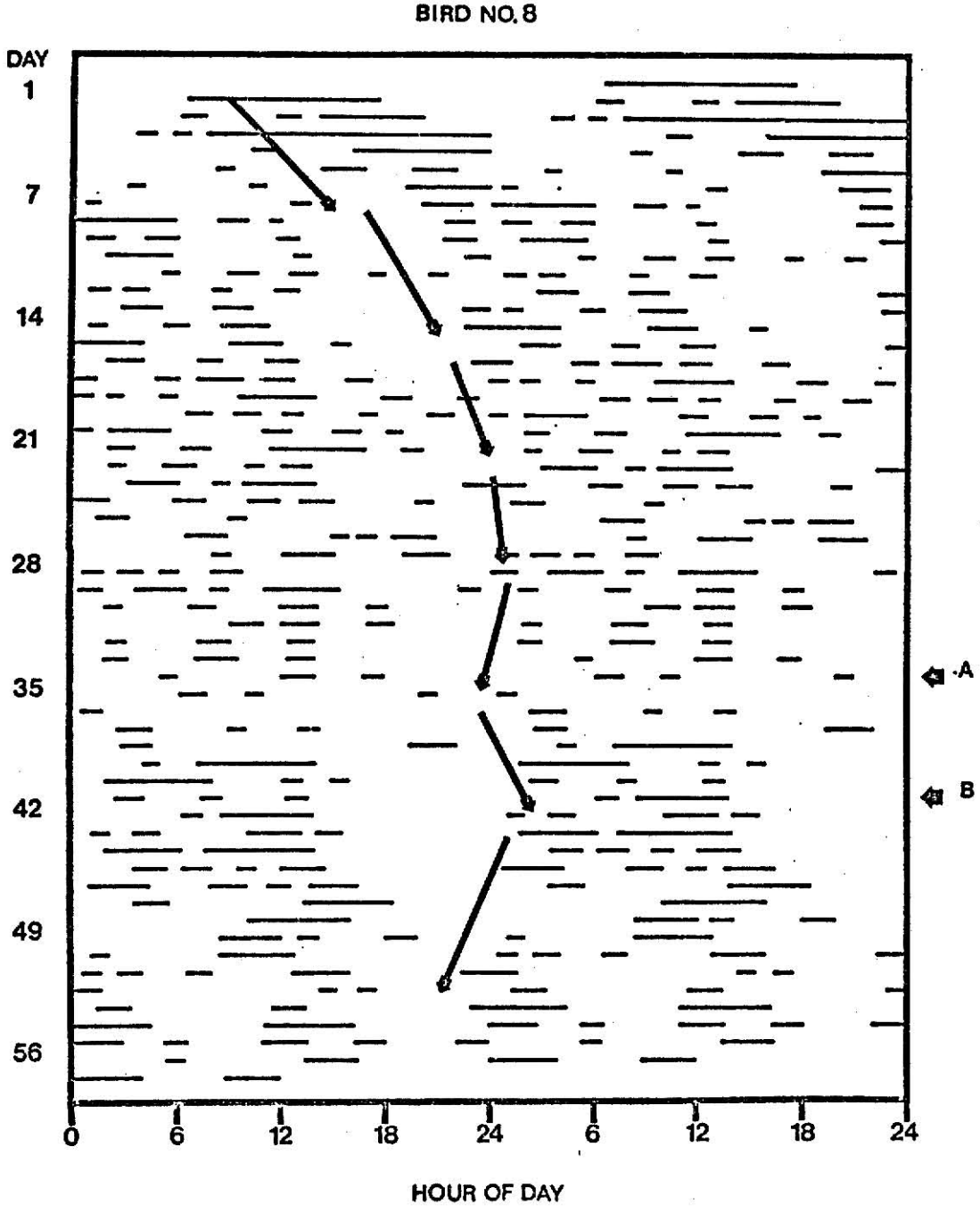

BIRD NO. 8
DAY
1
7
14
21
28
35
42
49
56
A
B
0
6
12
18
24
6
12
18
24
HOUR OF DAY

Table 16. Length of free-running activity periods (hours), during six different times, under CBL conditions.

| BIRD NO. | EXP. NO. | DAY 1-7 | DAY 8-14 | DAY 15-28 | DAY 28-35 | TREATMENT | POST-TREATMENT |
|---|---|---|---|---|---|---|---|
| 8 | 1 | 25.5 | 25.4 | 24.4 | 23.9 | 26.3 | 23.9 |
| 2 | 2 | 24.5 | ... | ... | ... | ... | ... |
| 5 | 2 | 25.0 | ... | ... | ... | ... | ... |
| 2 | 4 | 25.0 | ... | ... | ... | ... | ... |
| 6 | 4 | 25.4 | ... | ... | ... | ... | ... |
| 7 | 4 | * | * | * | 23.4 | 25.6 | ... |

* Missing data due to equipment failure.

and activity for at least seven periods during the CDL experiment (Table 17). Period lengths during pretreatment ranged from 23.9-hours for bird no. 8 to 25.9-hours for bird no. 1. Abrupt changes in free-running periodicity occurred at various times during the experiment. Bird no. 1 established an initial 25.9-hour rhythm during days 1-7, however, the periodicity abruptly changed to 21.9-hours during days 8-14 and to 24.1-hours during days 15-28 (Fig. 13). Bird no. 4 established an initial 24.6-hour rhythm during days 1-7 that changed to 25.2-hours during days 8-14. During days 15-28, a third change occurred and the period of the rhythm decreased to 23.5-hours. During days 29-35, a fourth change occurred and the period increased to 23.9-hours (Fig. 14). Bird no. 2 established a free-running rhythm with a period of 25.7-hours during days 1-7. The period changed to 24.3-hours for days 8-14, then changed to 23.5-hours durirg days 15-28; where it reached a steady state until treatment (Fig. 15). Bird no. 5 established a free-running rhythm with a period length of 25.3-hours that reamined unchanged throughout pretreatment (Fig. 16). All birds had random activity distributed throughout their periods.

Effects of treatment on period length.

Only four of eight birds, nos. 1, 2, 4 and 5 exhibited a free-running activity rhythm during the seven periods just prior to treatment. Of these four, only birds no. 2 and 4 exhibited a free-running rhythm during treatment. Bird no. 2 increased its period length from 23.5-hours to 23.8 while bird no. 4 showed no change in period length.

Birds no. 3 and 7 were arrhythmic during pretreatment. However, during treatment, both birds established free-running activity rhythms with periods of 24.8-hours and 23.1-hours for birds no. 3 and 7,

Fig. 13. Free-running activity rhythm for bird no. 1 under continuous dim light (10 lux). Data presented as explained in Fig. 12. (A) represents beginning of treatment, (B) represents end of treatment. Arrows indicate changes in periodicity.

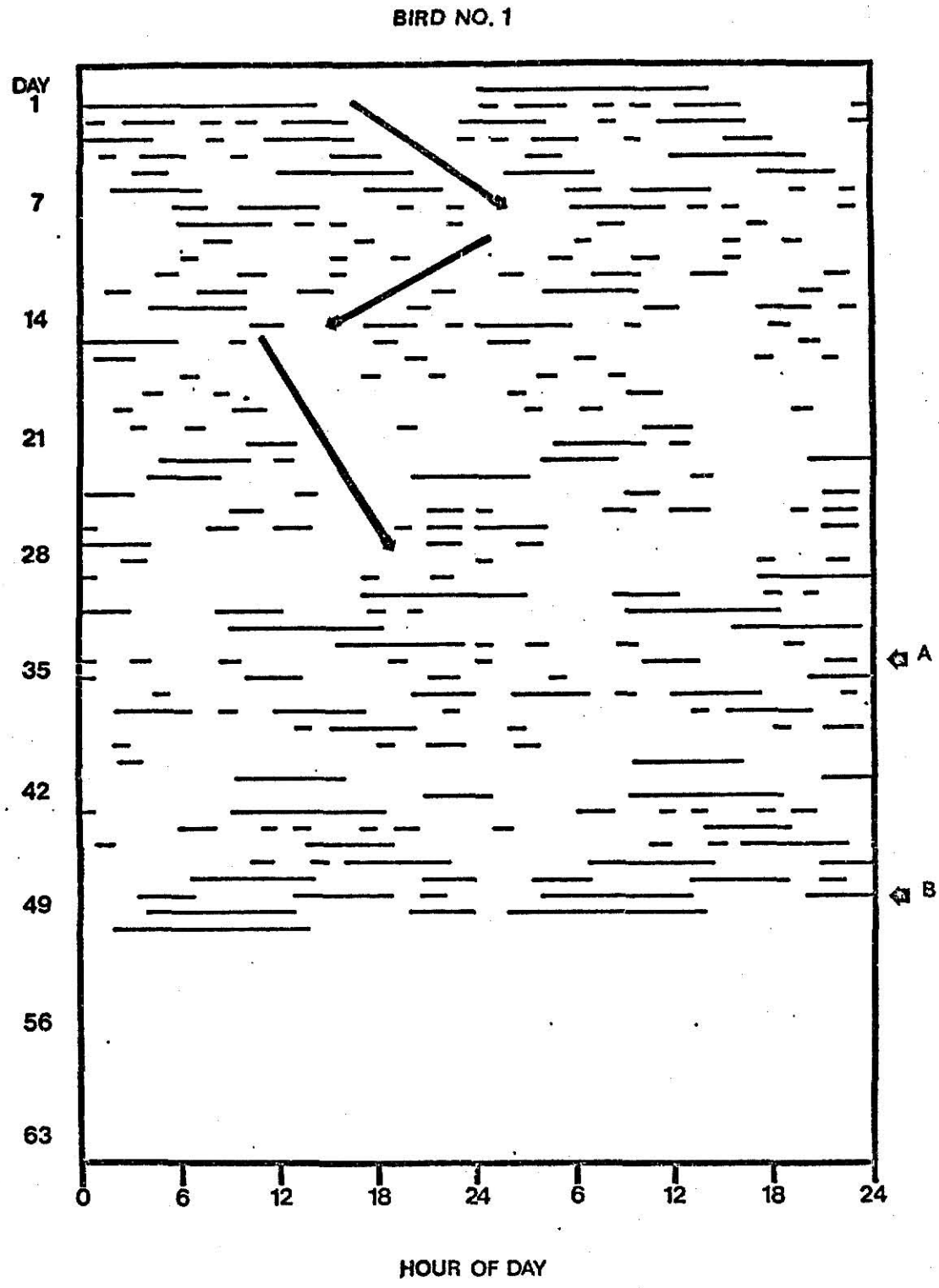

BIRD NO. 1
DAY
1
7
14
21
28
35
42
49
56
63
A
B
0
6
12
18
24
6
12
18
24
HOUR OF DAY

Fig. 14. Free-running activity rhythm for bird no. 4 under continuous dim light (10 lux). Data presented as explained in Fig. 12. (A) represents beginning of treatment, (B) represents end of treatment. Arrows indicate changes in periodicity.

BIRD NO. 4

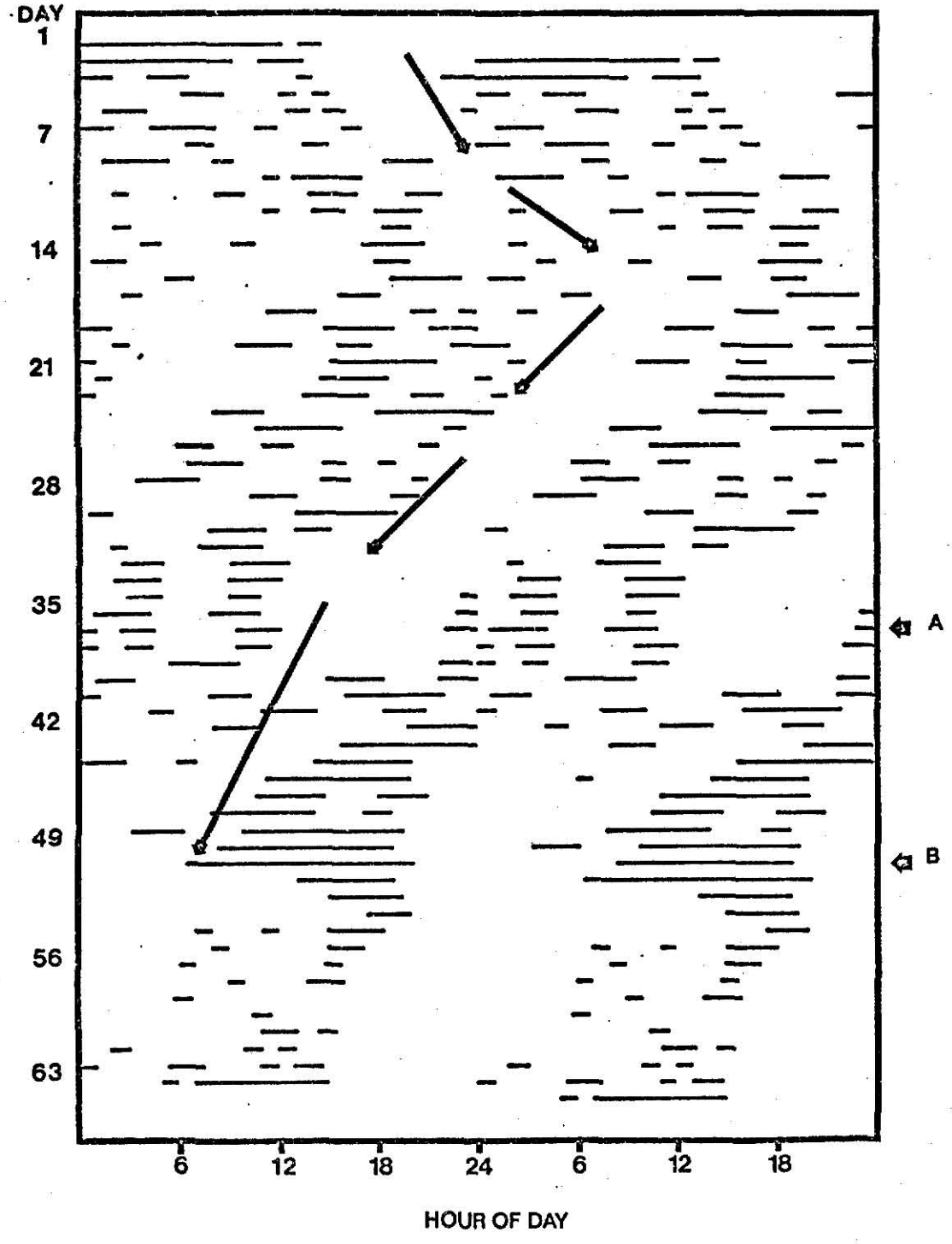


Fig. 15. Free-running activity rhythm for bird no. 2 under continuous dim light (10 lux). Data presented as explained in Fig. 12. (A) represents beginning of treatment, (B) represents end of treatment. Arrows indicate changes in periodicity.

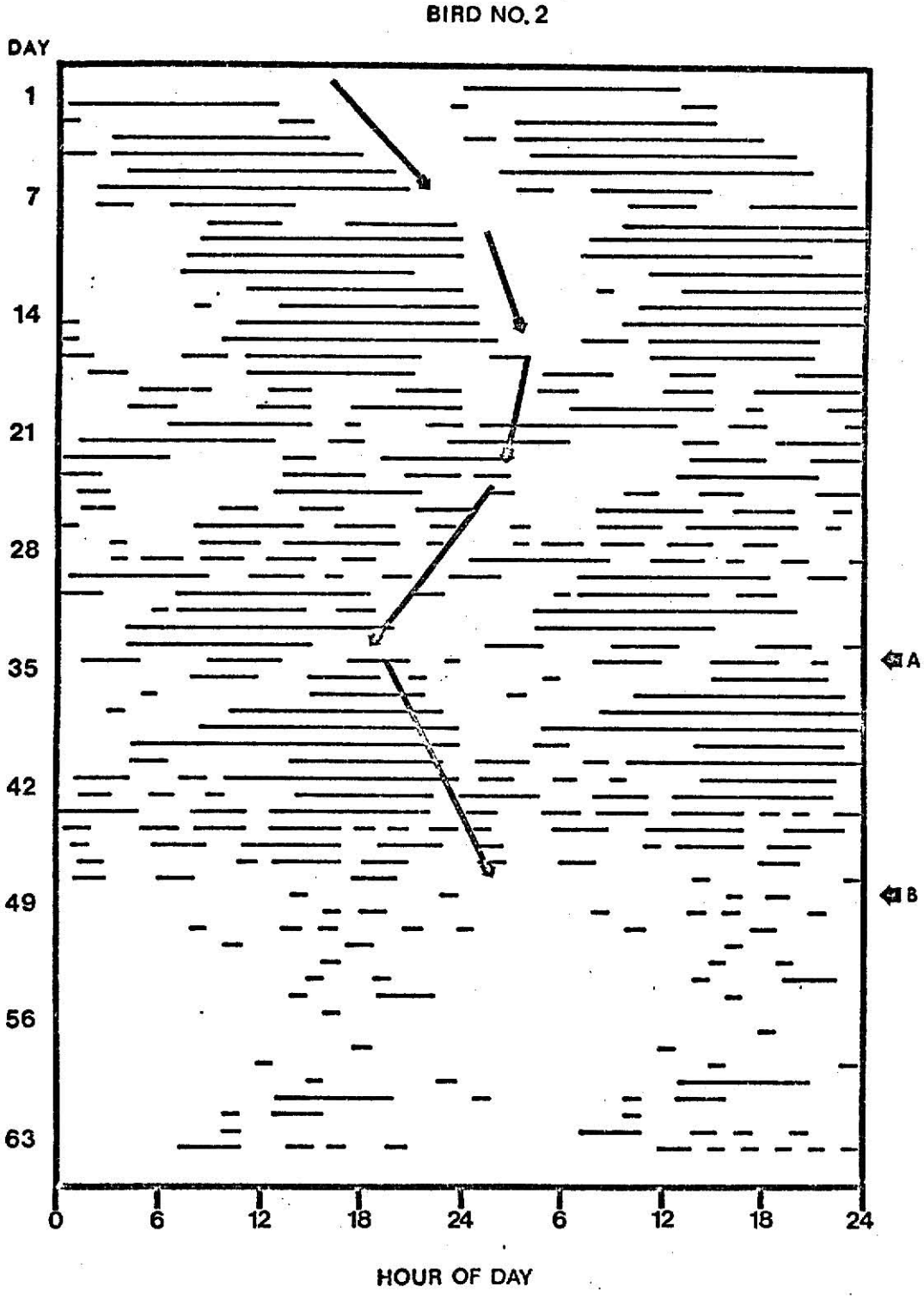

BIRD NO. 2
DAY
1
7
14
21
28
35
42
49
56
63
A
B
0
6
12
18
24
6
12
18
24
HOUR OF DAY

Fig. 16. Free-running activity rhythm for bird no. 5 under continuous dim light (10 lux). Data presented as explained in Fig. 12. (A) represents beginning of treatment, (B) represents end of treatment.

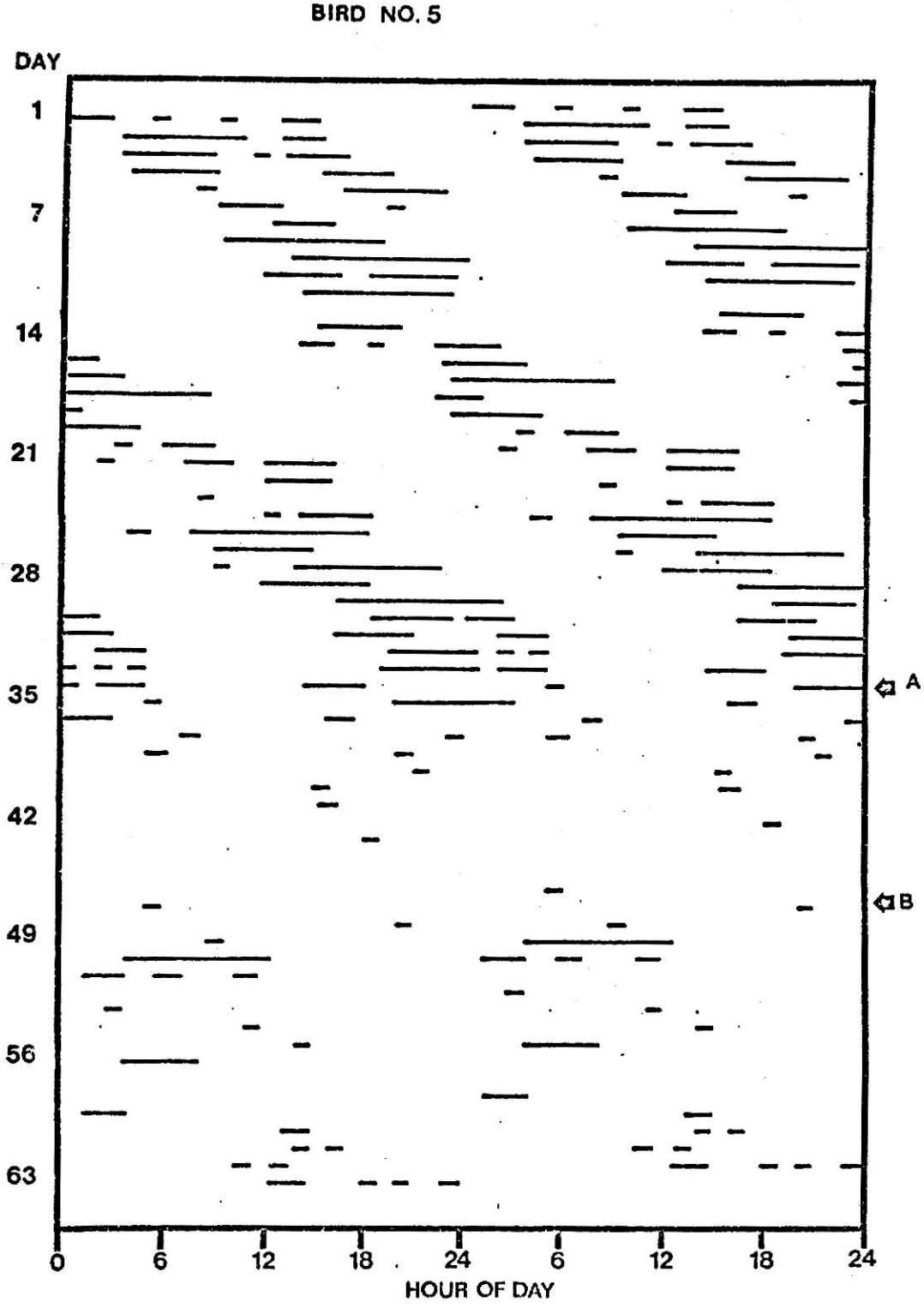

BIRD NO. 5
DAY
1
7
14
21
28
35
42
49
56
63
A
B
0
6
12
18
24
6
12
18
24
HOUR OF DAY

Table 17. Length of free-running activity periods (hours) during six different times, under CDL conditions.

| BIRD NO. | DAY 1-7 | DAY 8-14 | DAY 15-28 | DAY 29-35 | TREATMENT | POST-TREATMENT |
|---|---|---|---|---|---|---|
| 1 | 25.9 | 21.9 | 24.1 | 24.3 | ... | ... |
| 2 | 25.7 | 24.3 | 23.5 | 23.5 | 23.8 | ... |
| 3 | 24.5 | ... | ... | ... | 24.8 | ... |
| 4 | 24.6 | 25.2 | 23.5 | 23.9 | 23.9 | ... |
| 5 | 25.3 | 25.3 | 25.3 | 25.3 | ... | ... |
| 6 | ... | ... | ... | ... | ... | ... |
| 7 | 25.7 | ... | ... | ... | 23.1 | ... |
| 8 | 23.9 | ... | ... | ... | ... | ... |

respectively. This abrupt change can be seen to some extent in Fig. 17 and to a greater extent in Fig. 18. No birds demonstrated a free-running rhythm during posttreatment.

Activity patterns under continuous bright light.

It was hoped that data on activity patterns could be collected during the acclimation phase of each experiment. However, due to equipment problems and difficulty in the positioning of microswitch assemblies only data for experiment 2 and 6 were collected.

Activity patterns for birds no. 1, 3, 5 and 6 (experiment 2), during the last five days of acclimation and the first five days of continuous bright light are shown in Figure 19. Under rectangular light-dark cycles birds no. 5 and 6 show a bimodal activity pattern, with major morning and evening peaks coinciding with light on - light off stimuli, while birds no. 1 and 3 show a multi-peak activity pattern with morning and evening peaks coinciding with light on - light off stimuli. No clear activity patterns were apparent for any of the birds under continuous bright light.

Activity patterns under continuous dim light.

Activity patterns for birds no. 5, 6, 7 and 8 during the last five days of acclimation and first five days of continuous dim light are shown in Figure 20. Under rectangular light-dark cycles birds no. 5 and 6 show a conspicuous bimodal activity pattern with major morning and evening peaks coinciding with light on - light off stimuli. Bird no. 8 shows a multi-peak activity pattern with major morning and evening peaks coinciding with light on - light off stimuli, while bird no. 7

Fig. 17. Free-running activity rhythm for bird no. 3 under continuous dim light (10 lux). Data presented as explained in Fig. 12. (A) represents beginning of treatment, (B) represents end of treatment.

BIRD NO. 3

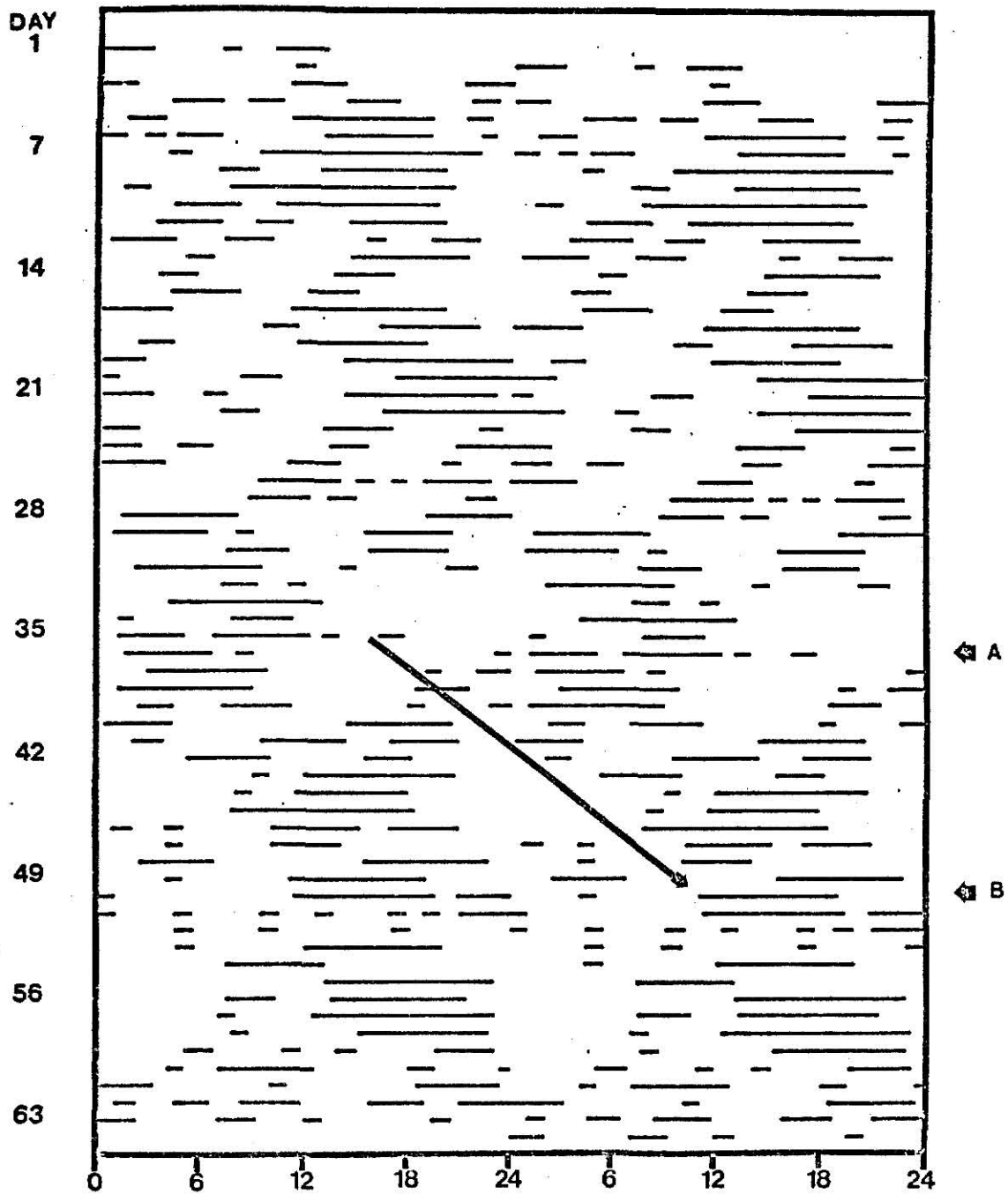


Fig. 18. Free-running activity rhythm for bird no. 7 under continuous dim light (10 lux). Data presented as explained in Fig. 12. (A) represents beginning of treatment, (B) represents end of treatment.

BIRD NO. 7

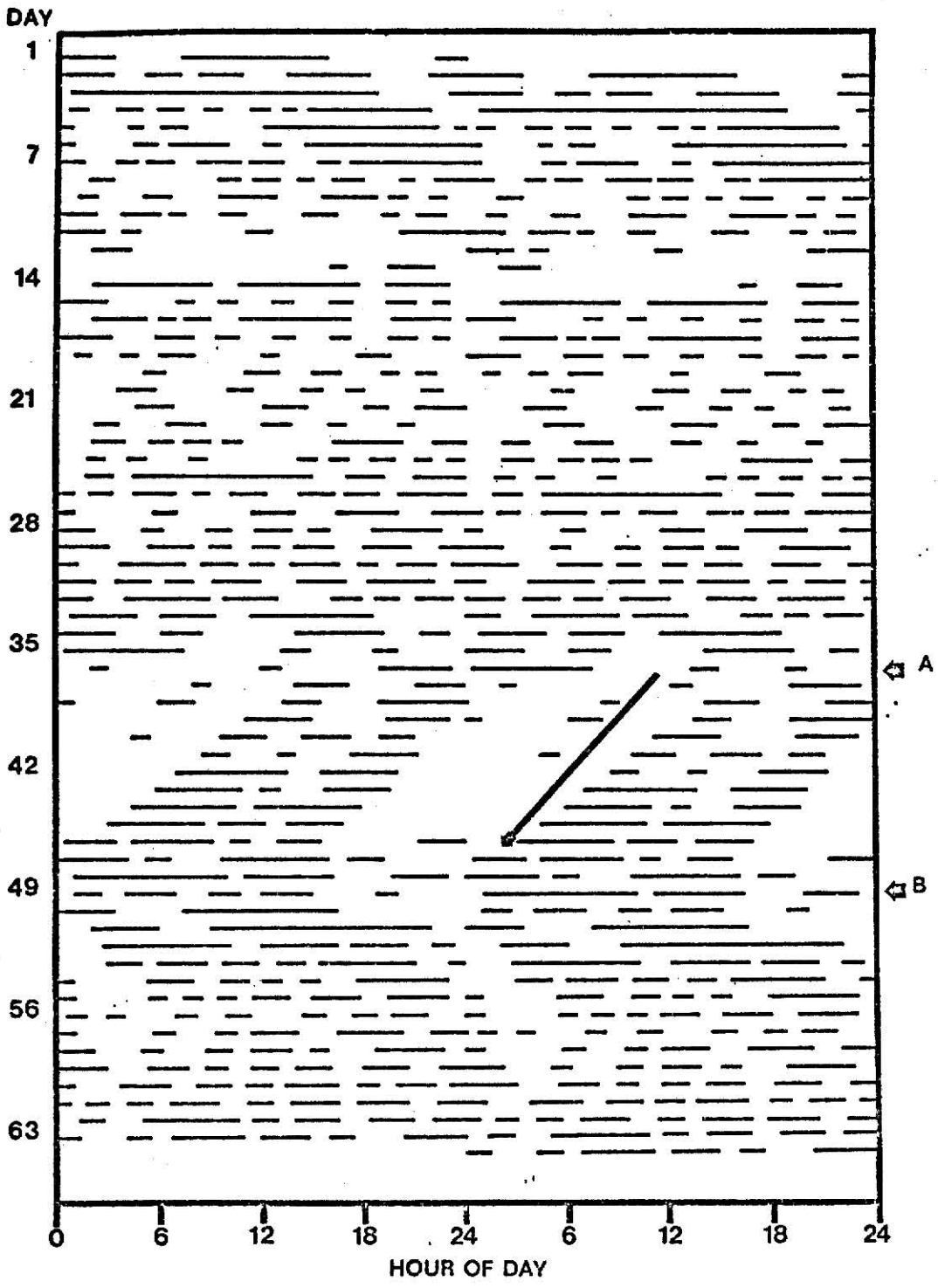


Fig. 19. Continuous activity patterns of birds no. 1,3,5 and 6 (experiment 2) kept first under rectangular light-dark cycles during the last five days of acclimation and then during the first five days under continuous bright illumination (300 lux). Shaded areas represent dark periods.

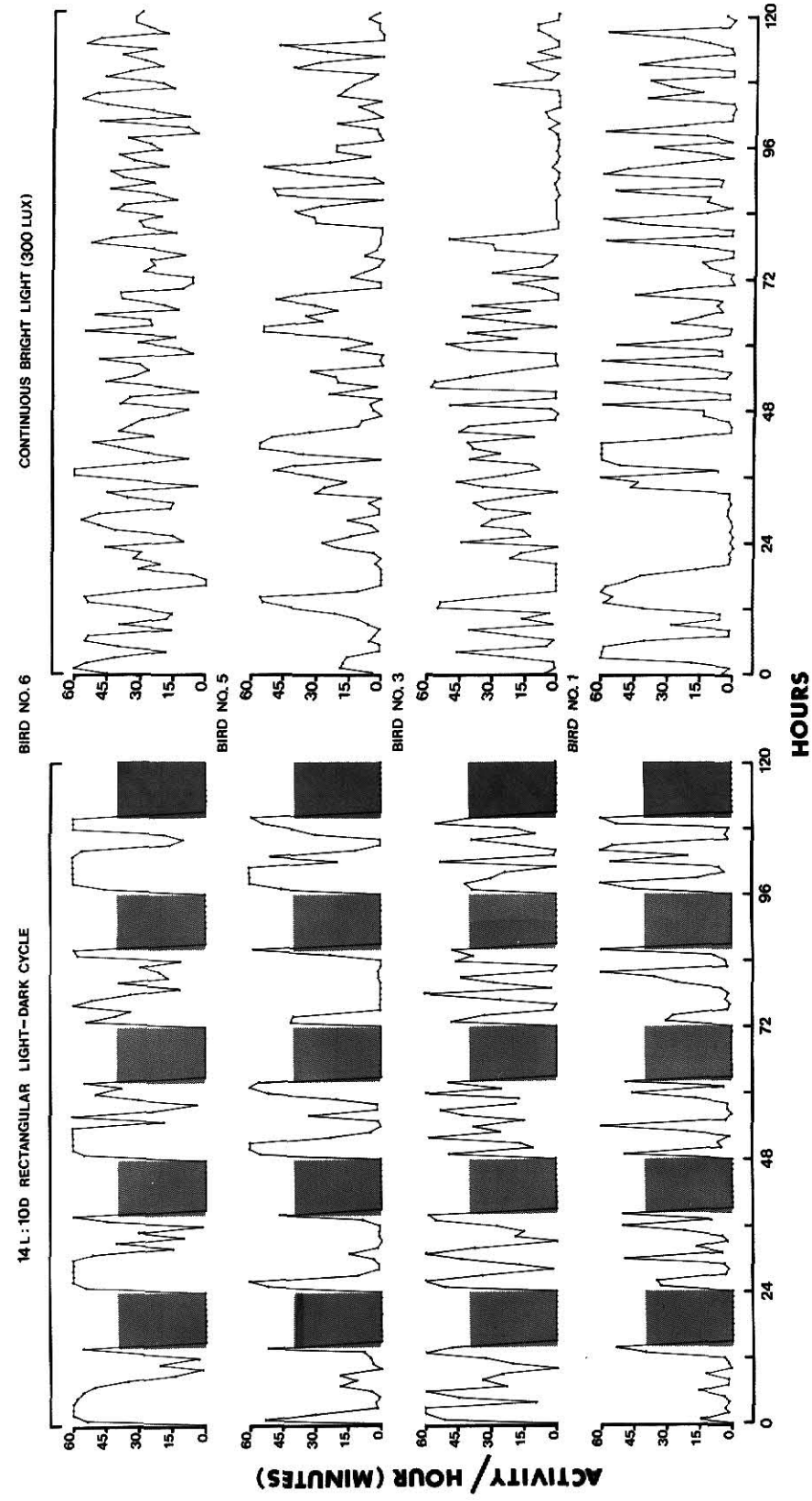
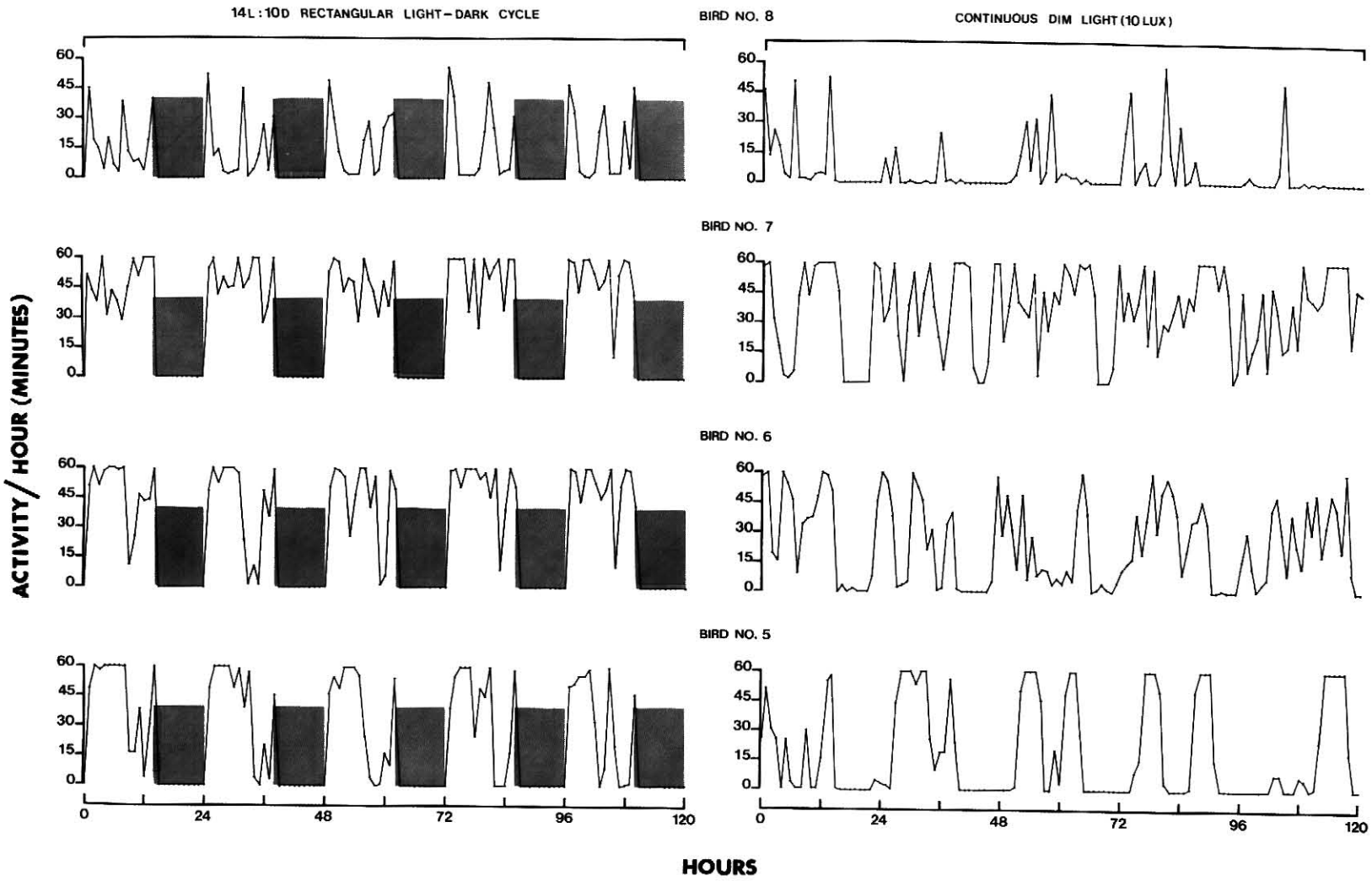

14 L : 10D RECTANGULAR LIGHT–DARK CYCLE
CONTINUOUS BRIGHT LIGHT (300 LUX)
BIRD NO. 6
BIRD NO. 5
BIRD NO. 3
BIRD NO. 1
ACTIVITY / HOUR (MINUTES)
HOURS
0
15
30
45
60
24
48
72
96
120

Fig. 20. Continuous activity patterns of birds no. 5,6,7 and 8 kept first under rectangular light-dark cycles during the last five days of acclimation and then during the first five days under continuous dim illumination (10 lux). Shaded areas represent dark periods.


14L:10D RECTANGULAR LIGHT-DARK CYCLE
CONTINUOUS DIM LIGHT (10 LUX)
BIRD NO. 8
BIRD NO. 7
BIRD NO. 6
BIRD NO. 5
ACTIVITY/HOUR (MINUTES)
60
45
30
15
0
0
24
48
72
96
120
HOURS

shows no clear activity pattern. Activity patterns, under continuous dim light, for birds no. 5 and 8 were similar to those under light-dark conditions, however, no discernable activity pattern for bird no. 7 was apparent.

## Activity patterns under light-dark photoperiods

### Experiment 5A

During pretreatment all four birds exhibited a bimodal activity pattern with major morning and evening peaks coinciding with light on - light off stimuli (Fig. 21). The pattern and timing of major activity peaks during treatment remained unchanged from that during pretreatment. A reduction in peak amplitude was found for all birds during treatment. Amplitude reduction ranged from a 10 percent decrease for bird no. 4 to a 25 percent decrease for bird no. 2.

### Experiment 5B

During pretreatment all four birds exhibited a bimodal activity pattern with major morning and evening peaks coinciding with light on - light off stimuli (Fig. 22). The pattern and timing of major activity peaks for birds no. 1, 3 and 4 during treatment remained unchanged from pretreatment with only the amplitude differing. There was no change in the pattern of activity for bird no. 2, however, the timing of major activity peaks occurred one hour earlier during treatment than pretreatment. In all cases peak amplitude during treatment was less than pretreatment ranging from a 29 percent decrease for bird no. 3 to a 50 percent decrease for bird no. 4. During posttreatment the timing of major activity peaks for all birds coincided with peak timing during

Fig. 21. Activity patterns of four bobwhite kept under a 14L:10D rectangular light-dark cycle. Average hourly activity during pretreatment based on 35 days. Average hourly activity during treatment based on 13 days. Shaded areas represent dark periods.

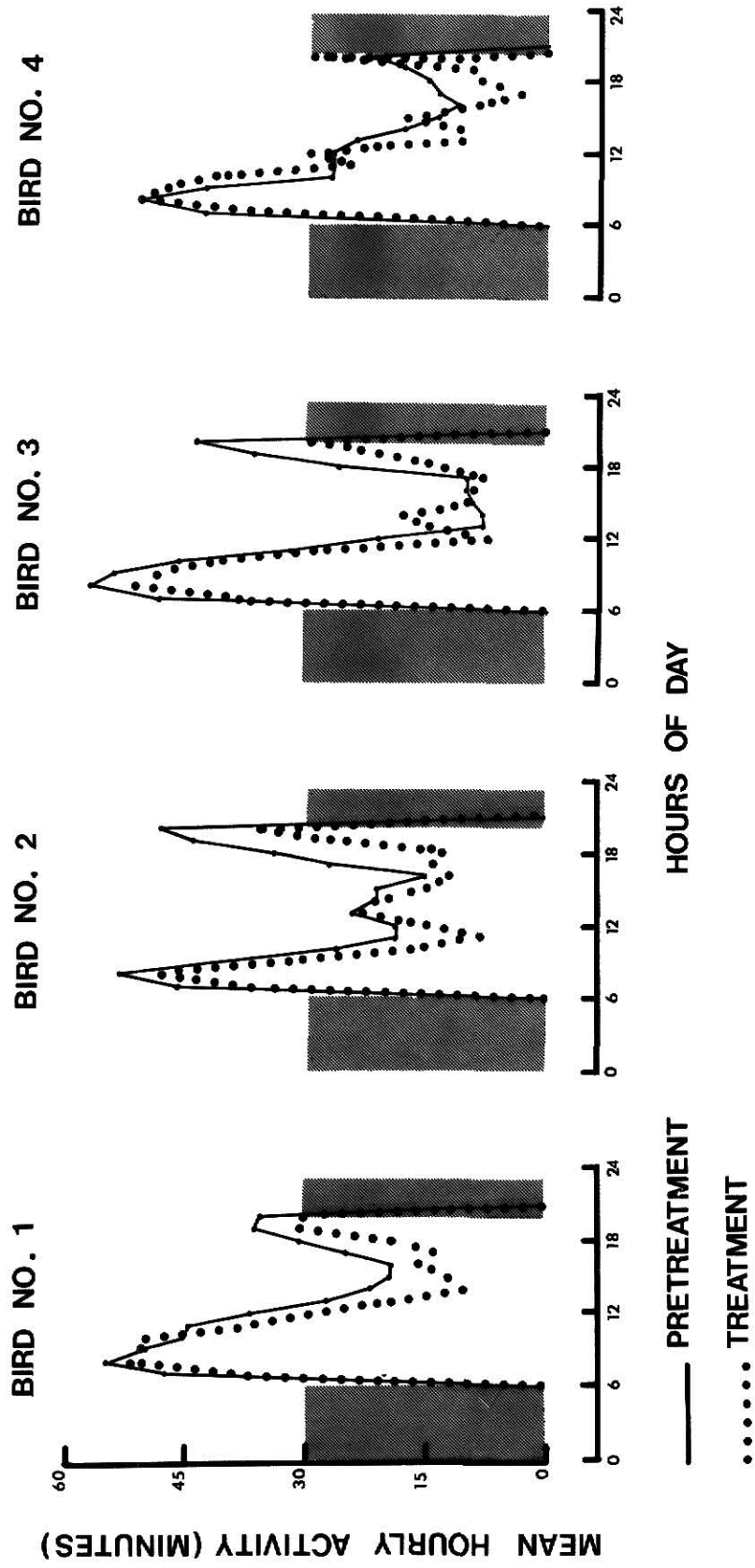
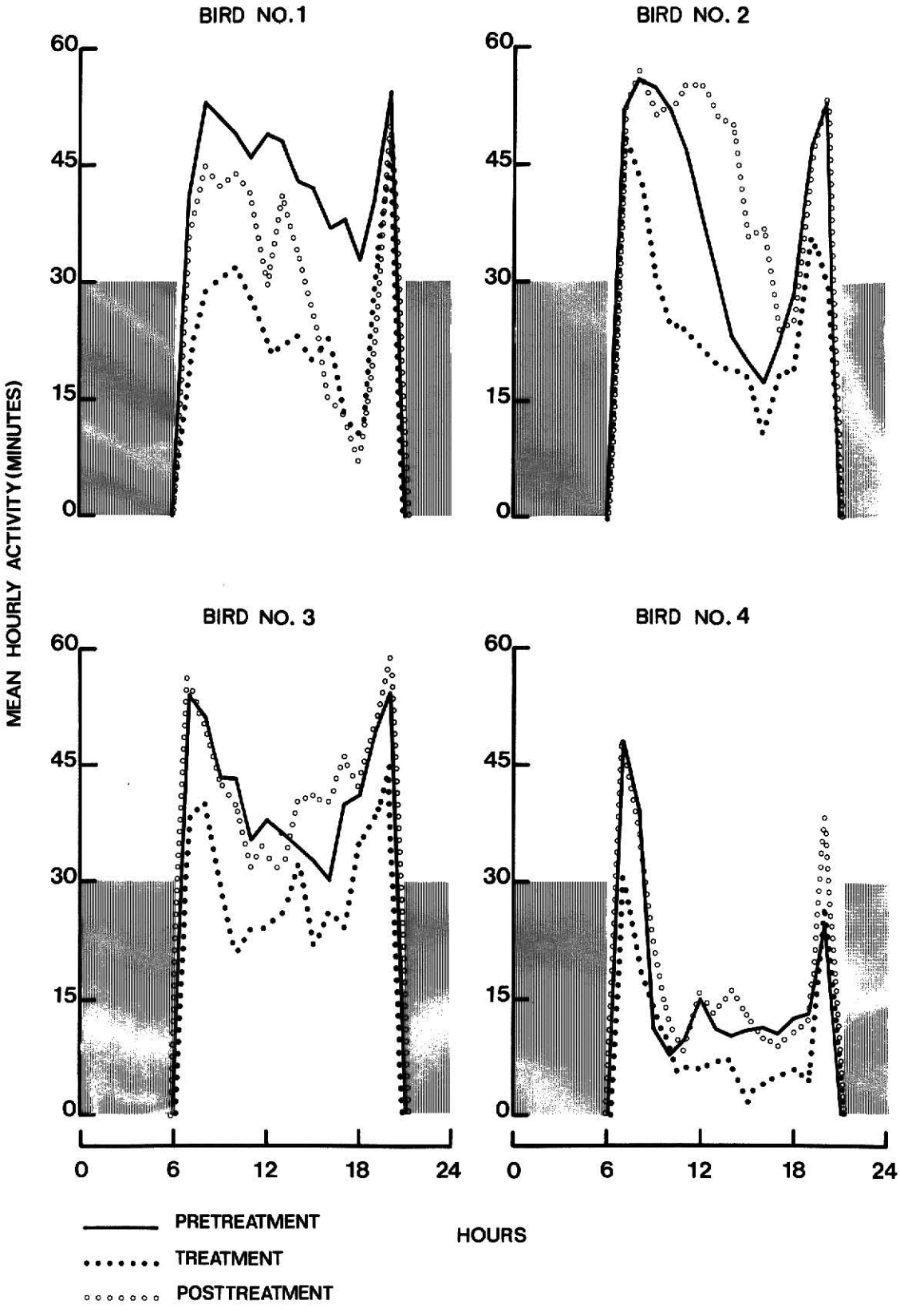

BIRD NO. 1
BIRD NO. 2
BIRD NO. 3
BIRD NO. 4
MEAN HOURLY ACTIVITY (MINUTES)
60
45
30
15
0
0
6
12
18
24
HOURS OF DAY
PRETREATMENT
TREATMENT

Fig. 22. Activity patterns of four bobwhite kept under a 14L:10D rectangular light-dark cycle. Average hourly activity during pretreatment based on 35 days. Average hourly activity during treatment based on 14 days. Average hourly activity during posttreatment based on 14 days. Shaded areas represent dark periods.


BIRD NO. 1
BIRD NO. 2
BIRD NO. 3
BIRD NO. 4
MEAN HOURLY ACTIVITY (MINUTES)
60
45
30
15
0
0
6
12
18
24
HOURS
PRETREATMENT
TREATMENT
POSTTREATMENT

pretreatment. Changes in peak amplitude during posttreatment from treatment ranged from a 32 percent increase for bird no. 1 to a 90 percent increase for bird no. 4. Changes in peak amplitude during posttreatment from pretreatment ranged from a 27 percent decrease for bird no. 1 to a 17 percent increase for bird no. 2.

# DISCUSSION

## Preliminary experiments

The fact that only two of 16 birds (both from group I) established a self-selected activity rhythm during preliminary experiments, when provided with either a spatial or temporal choice of light intensity, does not mean that bobwhite are typically arrhythmic. Such results could simply mean the methods used were not condusive to the establishment of a rhythm. The fact that six birds in group I never even entered the dim section of the cage lends further support to this explanation. The primary reason why birds failed to spatially select for dim light was believed to be cage design. When in the bright section, birds allowed to spatially select for light intensity, could see out above the top of the cage. When in the dim section, however, the birds were visually isolated from outside surroundings. Even when water bottles were placed in the dim section, in an attempt to condition the birds to these conditions, it was found that birds would only enter the dim section for a few minutes at a time and then return to the bright section. It was assumed the birds were drinking during these times. Birds allowed to temporily select light intensity also showed an aversion for dim conditions. When a bird did select for dim conditions it was only for a few minutes at a time. One explanation for this behavior could be that the abrupt change in light intensity startled the birds. Results show the number of these occurrances decreased with time thus indicating the birds learned to avoid these conditions.

Continuous illumination experiments

Although it was possible to distinguish a free-running rhythm in bobwhite by recording activity under constant conditions, with regard to light, such a method has obvious drawbacks. One problem is that it placed the birds in a negative environment (i.e. unnatural). The fact that most birds had activity randomly distributed throughout their periods made it difficult to distinguish clear periods of rest and activity in a bird's rhythm. Cain and Wilson (1972) had similar difficulty in distinguishing definite free-running activity rhythms in white leghorn chickens kept under constant illumination of 130 lux. Kirkpatrick (1957) believes constant light tends to keep bobwhite in a state of wakeful activity. Such an explanation would account for the lack of clear periods of rest and activity for birds in experiments presented here.

Another critical problem is light intensity. Under CBL conditions only six of 32 birds established a free-running rhythm whereas seven of eight birds established a free-running rhythm under CDL conditions. In addition, four of eight birds, under CDL conditions maintained a free-running rhythm throughout pretreatment whereas only one of 32 birds, under CBL conditions, maintained a free-running rhythm throughout pretreatment. Although circadian activity rhythms have been found in starlings (Sturnis vulgaris) kept under light intensities greater than 300 lux (Aschoff, 1960) results presented here indicate 300 lux inhibits the establishment and causes a premature "damping out" of free-running activity rhythms in bobwhite.

Abrupt changes in free-running periods, during pretreatment, together with the fact that only six birds exhibited a free-running

period during the seven days prior to treatment made it difficult to access what effects, if any, treatment had on free-running rhythms. Therefore, discussion of treatment effects is speculative at best.

Results of experiment 6 suggest that free-running activity rhythms were tending to stabilize between days 15-35 of pretreatment. Birds no. 2 and 5 showed no change in periodicity during this time while birds no. 1 and 4 only showed changes of 0.2- and 0.4-hours, respectively (Table 17). The greatest change during pretreatment was 0.5-hours for bird no. 8. Assuming the birds had reached a "steady state" where only small changes (<0.5-hours) in periodicity were occurring, changes greater than 0.5-hours could indicate an abnormal change in periodicity. Results show that no such changes occurred during treatment. An interesting development, during treatment, was the establishment of a free-running rhythm for birds no. 3 and 7. Both of these birds had been arrhythmic during pretreatment. However, explanations for this behavior could not be determined from activity, body weight or food consumption data.

Rectangular light-dark experiments

Results of this study indicate that bobwhite exhibit a typical bimodal activity pattern, with major morning and evening peaks, when kept under rectangular light-dark cycles. The first activity peak may be in response to light-on stimuli; but for the second peak which precedes light-off, there is no concurrent environmental stimulus which can be invoked as the proximate cause. It can be argued, however, that even under a rectangular light-dark cycle both peaks may be in response to the light-on stimuli--the first peak being a direct response and the second peak being a latent response of several hours (Aschoff, 1966b).

If repetitive activity patterns continue in the absence of concurrent external stimulation they are "spontaneous" and by definition endogenous. Activity patterns obtained in experiments presented here, under constant conditions, are inconclusive as to whether locomotor activity patterns of bobwhite are endogenously determined (Figs. 21 and 22). This is partly due to the sample sizes investigated and light intensities used. Bimodal activity patterns have been observed in bobwhite under field conditions (Stoddard, 1936; Bent, 1932 and Fatora and Duever, 1968). If casually related to gradually changing light conditions, as has been suggested, it might be expected that such patterns would disappear under rectangular light-dark cycles. Findings presented here show this is not the case.

Many authors have expounded various explanations for the quiescent periods between activity peaks. Stoddard (1936) suggested bobwhite seek relief from midday heat during the summer. Robinson (1957) suggests the daily activity pattern of bobwhites during the nonbreeding season is controlled by intensity of solar radiation. Although results in experiments presented here fail to provide conclusive proof as to the endogeniety of bimodal locomotor activity patterns of bobwhite, such a possibility should not be ignored when interpreting correlations between a bobwhite's behavior and concurrent environmental stimuli.

Food Consumption

Results of this study indicate that daily dosages of 2 and 10 mg/kg of carbofuran administered in diet significantly reduces food consumption in bobwhite quail. All 32 carbofuran treated birds showed

a decrease in food consumption during treatment. It wasn't known, however, whether this decrease was due to some metabolic effect caused by the insecticide or the palatability of the treated feed. Solomon (1975) found daily dosages equivalent to 2 mg/kg of carbofuran administered orally in corn oil caused an average 2.4 g (dry weight) decrease/bird/day in bobwhite food consumption. This decrease was temporary, however, with food consumption gradually returning to pretreatment levels after eight days. Based on these findings birds in experiment 3 could have shown a 20.4 g (wet weight) decrease in food consumption during the first eight days of treatment, or an average daily decrease of 1.4 g (wet weight)/bird for the 14-day treatment phase. Actual mean daily decrease in food consumption during treatment for experiment 3 was 1.5 g (wet weight)/bird/day. These data suggest that decreases in food consumption during treatment for experiment 3 may have been due to some causes other than the palatability of the treated feed. It is important to note, however, that Solomon's findings were based on acute dosages of 2 mg/kg carbofuran administered orally in corn oil and may not be applicable to chronic feeding studies.

Body Weight

Significant weight losses due to treatment only occurred during experiments no. 4 and 6. Significant decreases in food consumption during treatment were also found in these experiments. It wasn't known, however, whether decreases in food consumption could account for the weight losses. Case (1971) found the existence energy requirements for an adult male bobwhite, under a 15L:10D photoperiod and a temperature of 20°C, was 41.4 kcals/day. Clement (1970) found that

an adult male bobwhite, under a 10L:14D photoperiod and temperature of 20°C, could metabolize only 7.2 kcals/day when fed a diet of ragweed (Ambrosia trifidia), sumac (Rhus glabra) and pin oak (Quercus palustris) and would lose an average of 5.2 g of body weight/day. Using these data it can be shown that for every kcal of energy not metabolized, below that required for existence energy, an adult male bobwhite loses 0.15 g of body weight/day. Average daily decreases in food consumption were 5.7 g (dry weight) and 4.9 g (dry weight)/bird for experiments 4 and 6, respectively. Based on a mean caloric value of 4.35 kcals/g of P-18 feed (Solomon, 1975), this would result in an average reduction in caloric intake of 24.8 and 21.3 kcals/bird/day for experiments 4 and 6, respectively. This means that an average loss in body weight of 3.7 and 3.2 g/bird/day was possible for birds in experiments 4 and 6, respectively. Actual mean daily weight losses were 0.61 and 1.1 g/bird for experiments 4 and 6, respectively. These data suggest that body weight losses for birds treated at 10 mg/kg carbofuran could be more than accounted for by concurrent decreases in food consumption for these birds.

Ecological implications

Results of this study suggest that exposure to carbofuran, and its subsequent effect on locomotor activity, food consumption and body weight, places an adult male bobwhite under considerable stress. Carbofuran is usually applied at the time of year when adult male bobwhites are undergoing a complex sequence of physiological and behavioral stresses associated with courtship, breeding and territorial defense. Physiological and behavioral stress of reproduction have been shown to

be the primary factors influencing late summer and fall mortality in both adult male and female bobwhite (Kabat and Thompson, 1963). Reproduction stress has also been shown to increase a bird's vulnerability to predation, disease and parasites (Brietenbach and Meyer, 1959; and Greely, 1953). Selye (1949) has shown that adaptation to a specific stress such as courtship, molting or breeding lowers a bird's body resistance to stress to which the bird is not adapted. Therefore, exposure to carbofuran during these critical times could possibly create additional stress that would proportionately reduce a bird's resistance, thus increasing its vulnerability to disease, parasites and predation.

This study also indicates that exposure to carbofuran causes a significant reduction in an adult male bobwhite's daily locomotor activity. Therefore, it would be expected that not only the total amount of activity time but also the amount of time spent performing any one activity such as feeding, courtship, breeding, and territorial defense would have to be reduced. It would also follow that the bird would budget its activity time for those activities directly associated with survival. It most of the bird's activity time has to be spent on those activities directly related to survival less time can be spent on other activities such as courtship, breeding and territorial defense. Failure to preform such activities could seriously affect reproduction. Orians (1965) believes that even subtle changes in time budgeting can affect an individual's reproductive success and thus have evolutionary implications.

During the early spring and summer months bobwhite usually feed in the early morning and late afternoon (Stoddard, 1936). Early morning

feeding activity replenishes reduced energy stores from the previous night while late afternoon feeding enables the birds to build up energy stores to last through the night. Results obtained during this study, under light-dark photoperiods, show that carbofuran causes a reduction in average hourly activity during major morning and evening periods. Such a reduction could cause the birds to either feed less or feed at less optimum times of the day. If adaptation has resulted in the timing of feeding activities and other activites to occur at optimum times of the day such an effect could have serious ecological consequences.

Results of this study also indicate that ingestion of 2 or 10 mg/kg carbofuran in diet significantly reduces food consumption in male bobwhite. Haegele and Tucker (1974) found reduced food consumption in female Coturnix quail, treated with carbaryl, resulted in short-term egg shell thinning. The timing of seasonal physiological processes has also been shown to be affected by limited food intake. Breitenbach et al. (1963) found limited food intake during the early breeding season resulted in a 1-month delay in the onset of egg-laying and retarded the rate of molt in hen pheasants. Such a delay in the timing of a physiological process such as egg-laying or molting could also delay the recuperation period beyond the season when environmental conditions are optimum. Kabat and Thompson (1963) found late nesting bobwhite were unable to reach prewinter body weights and subsequently had a higher mortality than early nesters. Bobwhite typically lay a second clutch if the first is either destroyed or disserted. A delay in the laying of the first clutch could prohibit the bird from laying a second clutch and therefore have an effect on population levels.

## SUMMARY AND CONCLUSIONS

A series of seven experiments conducted under different temperatures, photoperiods and illumination intensities were undertaken to study the effects of carbaryl and carbofuran on various aspects of bobwhite quail activity. Variables tested included locomotor activity, free-running activity rhythms and diurnal activity patterns. Food consumption and body weight data were also collected. In addition, data on daily activity during the last five days of light-dark conditions and first five days under constant illumination were collected to determine if quail activity patterns are spontaneous, i.e. not concurrent on changing environmental conditions.

Collected data support the following conclusions:

1. Ingestion of carbofuran at 10 mg/kg of body weight in diet caused a significant decrease in bobwhite quail activity and food consumption under all photoperiods.

2. Ingestion of carbofuran at 10 mg/kg body weight in diet caused a significant decrease in bobwhite quail body weight under continuous illumination experiments.

3. Carbofuran at 2 mg/kg body weight in diet caused a significant decrease in bobwhite quail food consumption under continuous bright light (300 lux).

4. Carbaryl at 20 or 100 mg/kg body weight in diet caused no significant changes in bobwhite quail activity, food consumption or body weight.

5. Under rectangular light-dark cycles bobwhite quail exhibit a conspicuous bimodal activity pattern. However, due to small sample sizes, results obtained under constant illumination are inconclusive as to whether these patterns are endogenously determined.

6. Carbofuran at 10 mg/kg body weight had no effect on the timing of major activity peaks under a rectangular light-dark cycle.

7. Bobwhite quail exhibit a free-running activity rhythm under constant conditions, the establishment and persistence of which depend upon light intensity.

8. The effects of treatment on circadian periodicity are inconclusive due to sample size and spontaneous or induced changes in periodicity during pretreatment.

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# APPENDIX

Table 18. Analysis of variance of activity of eight carbaryl treated birds (20 mg/kg) under continuous bright light (300 lux).

ANALYSIS OF VARIANCE

| SOURCE | D.F. | M.S. | F-RATIO | PROB. |
|---|---|---|---|---|
| BIRD | 7 | 309966.0 | 83.24 | 0.00 |
| WEEK | 7 | 3324.3 | 0.89 | 0.52 |
| RESIDUAL | 46* | 3723.7 | | |
| TOTAL | 60* | | | |

* Missing data due to equipment failure. Should be 49 and 63 for residual and total, respectively.

Table 19. Daily food consumption and mean (±S.D.) of eight carbaryl treated birds (20 mg/kg) under continuous bright light (300 lux).

MEAN DAILY FOOD INTAKE (GRAMS)

| PHASE | BIRD 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | MEAN (±S.D.) |
|---|---|---|---|---|---|---|---|---|---|
| PRETREATMENT | 12.8 | 13.2 | 12.3 | 12.0 | 15.2 | 11.8 | 11.7 | 14.3 | 12.9 (±1.2 ) |
| TREATMENT | 11.7 | 14.5 | 9.5 | 10.7 | 18.7 | 12.8 | 10.5 | 14.9 | 12.9 (±2.2 ) |
| POSTTREATMENT | 12.5 | 13.7 | 11.1 | 9.4 | 16.1 | 11.2 | 10.7 | 14.2 | 12.4 (±2.1 ) |

Table 20. Analysis of variance of food consumption of eight carbaryl treated birds (20 mg/kg) under continuous bright light (300 lux).

ANALYSIS OF VARIANCE

| SOURCE | D.F. | M.S. | F-RATIO | PROB. |
|---|---|---|---|---|
| BIRD | 7 | 21.79 | 32.80 | 0.00 |
| COLLECTIONS | 4 | 1.03 | 1.56 | 0.21 |
| RESIDUAL | 28 | 0.66 | | |
| TOTAL | 39 | | | |

Table 21. Body weight and mean (±S.D.) of eight carbaryl treated birds (20 mg/kg) under continuous bright light (300 lux) at the end of each phase.

BODY WEIGHT (GRAMS)

| PHASE | BIRD 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | MEAN (±S.D.) |
|---|---|---|---|---|---|---|---|---|---|
| ACCLIMATION | 175.3 | 186.5 | 174.5 | 170.9 | 180.4 | 179.0 | 193.8 | 160.0 | 177.5 (±10.1) |
| PRETREATMENT | 177.5 | 184.3 | 179.2 | 172.5 | 179.8 | 178.6 | 190.7 | 163.4 | 178.2 (± 8.0) |
| TREATMENT | 177.2 | 184.8 | 178.9 | 173.5 | 181.0 | 177.7 | 191.6 | 160.8 | 178.2 (± 8.9) |
| POSTTREATMENT | 176.2 | 186.2 | 177.2 | 172.9 | 178.7 | 178.0 | 192.9 | 162.1 | 178.0 (± 8.6) |

Table 22. Analysis of variance of body weight of eight carbaryl treated birds (20 mg/kg) under continuous bright light (300 lux).

ANALYSIS OF VARIANCE

| SOURCE | D.F. | M.S. | F-RATIO | PROB. |
|---|---|---|---|---|
| BIRD | 7 | 317.38 | 157.05 | 0.00 |
| PHASE | 3 | 0.61 | 0.30 | 0.82 |
| RESIDUAL | 21 | 2.02 | | |
| TOTAL | 31 | | | |

Table 23. Analysis of variance of activity of eight carbaryl treated birds (100 mg/kg) under continuous bright light (300 lux).

ANALYSIS OF VARIANCE

| SOURCE | D.F. | M.S. | F-RATIO | PROB. |
|---|---|---|---|---|
| BIRD | 7 | 215782.1 | 36.63 | 0.00 |
| WEEK | 7 | 906.3 | 0.15 | 0.99 |
| RESIDUAL | 49 | 5890.4 | | |
| TOTAL | 63 | | | |

Table 24. Daily food consumption and mean (±S.D.) of eight carbaryl treated birds (100 mg/kg) under continuous bright light (300 lux).

MEAN DAILY FOOD INTAKE (GRAMS)

| PHASE | BIRD 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | MEAN (±S.D.) |
|---|---|---|---|---|---|---|---|---|---|
| PRETREATMENT | 11.5 | 12.8 | 9.3 | 10.8 | 14.5 | 13.9 | 12.4 | 12.1 | 12.2 (±1.7) |
| TREATMENT | 10.9 | 13.9 | 8.9 | 9.4 | 15.5 | 14.3 | 13.5 | 11.4 | 12.2 (±2.0) |
| POSTTREATMENT | 12.7 | 14.1 | 9.5 | 10.3 | 17.9 | 13.7 | 13.4 | 11.7 | 12.9 (±2.3) |

Table 25. Analysis of variance of food consumption of eight carbaryl treated birds (100 mg/kg) under continuous bright light (300 lux).

ANALYSIS OF VARIANCE

| SOURCE | D.F. | M.S. | F-RATIO | PROB. |
|---|---|---|---|---|
| BIRD | 7 | 19.19 | 11.22 | 0.00 |
| COLLECTIONS | 4 | 0.98 | 0.57 | 0.68 |
| RESIDUAL | 28 | 1.71 | | |
| TOTAL | 39 | | | |

Table 26. Body weight and mean (±S.D.) of eight carbaryl treated birds (100 mg/kg) under continuous bright light (300 lux) at the end of each phase.

BODY WEIGHT (GRAMS)

| PHASE | BIRD 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | MEAN (±S.D.) |
|---|---|---|---|---|---|---|---|---|---|
| ACCLIMATION | 189.5 | 179.0 | 181.1 | 188.2 | 192.3 | 170.8 | 205.5 | 175.0 | 185.2 (±11.0) |
| PRETREATMENT | 188.6 | 180.5 | 180.8 | 191.4 | 194.4 | 172.4 | 203.8 | 176.0 | 185.9 (±10.5) |
| TREATMENT | 186.9 | 182.4 | 180.0 | 194.0 | 199.5 | 176.2 | 204.8 | 176.7 | 187.6 (±10.8) |
| POSTTREATMENT | 186.3 | 181.6 | 171.3 | 200.3 | 200.2 | 183.8 | 210.4 | 181.5 | 189.4 (±12.9) |

Table 27. Analysis of variance of body weight of eight carbaryl treated birds (100 mg/kg) under continuous bright light (300 lux).

ANALYSIS OF VARIANCE

| SOURCE | D.F. | M.S. | F-RATIO | PROB. |
|---|---|---|---|---|
| BIRD | 7 | 478.26 | 36.99 | 0.00 |
| PHASE | 3 | 28.87 | 2.23 | 0.11 |
| RESIDUAL | 21 | 12.92 | | |
| TOTAL | 31 | | | |

Table 28. Analysis of variance of activity of eight carbofuran treated birds (2 mg/kg) under continuous bright light (300 lux).

ANALYSIS OF VARIANCE

| SOURCE | D.F. | M.S. | F-RATIO | PROB. |
|---|---|---|---|---|
| BIRD | 7 | 236525.0 | 62.8 | 0.00 |
| WEEK | 8 | 5683.8 | 1.5 | 0.17 |
| RESIDUAL | 56 | 3765.7 | | |
| TOTAL | 71 | | | |

Table 29. Daily food consumption and mean (±S.D.) of eight carbofuran treated birds (2 mg/kg) under continuous bright light (300 lux).

MEAN DAILY FOOD INTAKE (GRAMS)

| PHASE | BIRD 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | MEAN (±S.D.) |
|---|---|---|---|---|---|---|---|---|---|
| PRETREATMENT | 13.7 | 16.4 | 15.0 | 15.5 | 18.2 | 17.9 | 19.8 | 14.6 | 16.4 (±2.1) |
| TREATMENT | 12.6 | 13.6 | 14.3 | 14.8 | 16.8 | 15.7 | 18.0 | 13.5 | 14.9 (±1.8) |
| POSTTREATMENT | 12.6 | 15.2 | 15.5 | 16.0 | 17.4 | 16.8 | 20.6 | 14.8 | 16.1 (±2.3) |

Table 30. Analysis of variance of food consumption of eight carbofuran treated birds (2 mg/kg) under continuous bright light (300 lux).

ANALYSIS OF VARIANCE

| SOURCE | D.F. | M.S. | F-RATIO | PROB. |
|---|---|---|---|---|
| BIRD | 7 | 22.05 | 33.65 | 0.00 |
| COLLECTIONS | 4 | 9.21 | 14.05 | 0.00 |
| RESIDUAL | 28 | 0.65 | | |
| TOTAL | 39 | | | |

Table 31. Body weight and mean (±S.D.) of eight carbofuran treated birds (2 mg/kg) under continuous bright light (300 lux) at the end of each phase.

BODY WEIGHT (GRAMS)

| PHASE | BIRD 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | MEAN (±S.D.) |
|---|---|---|---|---|---|---|---|---|---|
| ACCLIMATION | 188.4 | 191.9 | 213.9 | 199.8 | 198.0 | 193.0 | 202.4 | 177.9 | 195.2 (±10.6) |
| PRETREATMENT | 194.7 | 193.4 | 213.0 | 201.1 | 198.2 | 198.4 | 203.4 | 177.2 | 197.4 (±10.2) |
| TREATMENT | 200.0 | 201.6 | 214.7 | 200.2 | 197.6 | 195.3 | 194.3 | 180.0 | 198.0 (± 9.6) |
| POSTTREATMENT | 197.9 | 205.7 | 214.8 | 199.8 | 195.5 | 199.5 | 197.7 | 181.8 | 199.1 (± 9.3) |

Table 32. Analysis of variance of body weight of eight carbofuran treated birds (2 mg/kg) under continuous bright light (300 lux).

ANALYSIS OF VARIANCE

| SOURCE | D.F. | M.S. | F-RATIO | PROB. |
|---|---|---|---|---|
| BIRD | 7 | 376.03 | 20.83 | 0.00 |
| PHASE | 3 | 29.61 | 1.64 | 0.21 |
| RESIDUAL | 21 | 18.04 | | |
| TOTAL | 31 | | | |

Table 33. Analysis of variance of activity of eight carbofuran treated birds (10 mg/kg) under continuous bright light (300 lux).

ANALYSIS OF VARIANCE

| SOURCE | | | | |
|---|---|---|---|---|
| BIRD | 7 | 123118.5 | 19.4 | 0.00 |
| WEEK | 8 | 12852.8 | 2.0 | 0.06 |
| RESIDUAL | 51* | 6334.6 | | |
| TOTAL | 66* | | | |

* Missing data due to equipment failure. Should be 56 and 71 for residual and total, respectively.

Table 34. Daily food consumption and mean (±S.D.) of eight carbofuran treated birds (10 mg/kg) under continuous bright light (300 lux).

| | MEAN DAILY FOOD INTAKE (GRAMS) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| PHASE | | | | | | | | | |
| PRETREATMENT | 16.5 | 15.7 | 17.3 | 15.0 | 14.7 | 17.4 | 14.8 | 14.6 | 15.8 (±1.2) |
| TREATMENT | 7.2 | 12.7 | 10.6 | 12.0 | 9.2 | 5.6 | 11.5 | 9.2 | 9.8 (±2.4) |
| POSTTREATMENT | 17.0 | 15.8 | 19.3 | 16.0 | 16.1 | 19.4 | 20.6 | 16.4 | 17.6 (±1.9) |

Table 35. Analysis of variance of food consumption of eight carbofuran treated birds (10 mg/kg) under continuous bright light (300 lux).

ANALYSIS OF VARIANCE

| SOURCE | D.F. | M.S. | F-RATIO | PROB. |
|---|---|---|---|---|
| BIRD | 7 | 3.65 | 1.20 | 0.33 |
| COLLECTIONS | 4 | 72.96 | 24.06 | 0.00 |
| RESIDUAL | 28 | 3.03 | | |
| TOTAL | 39 | | | |

Table 36. Body weight and mean (±S.D.) of eight carbofuran treated birds (10 mg/kg) under continuous bright light (300 lux) at the end of each phase.

BODY WEIGHT (GRAMS)

| PHASE | BIRD 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | MEAN (±S.D.) |
|---|---|---|---|---|---|---|---|---|---|
| ACCLIMATION | 193.4 | 196.0 | 199.1 | 195.2 | 177.1 | 222.3 | 199.0 | 189.2 | 196.4 (±12.6) |
| PRETREATMENT | 192.6 | 193.4 | 200.2 | 199.4 | 178.6 | 223.6 | 200.1 | 190.6 | 197.3 (±12.8) |
| TREATMENT | 174.9 | 185.1 | 182.6 | 179.4 | 162.9 | 183.4 | 184.3 | 172.0 | 178.1 (± 7.7) |
| POSTTREATMENT | 193.1 | 186.8 | 186.0 | 189.9 | 182.0 | 218.1 | 195.2 | 190.7 | 192.7 (±11.1) |

Table 37. Analysis of variance of body weight of eight carbofuran treated birds (10 mg/kg) under continuous bright light (300 lux).

ANALYSIS OF VARIANCE

| SOURCE | D.F. | M.S. | F-RATIO | PROB. |
|---|---|---|---|---|
| BIRD | 7 | 422.88 | 14.64 | 0.00 |
| PHASE | 3 | 647.11 | 22.40 | 0.00 |
| RESIDUAL | 21 | 28.87 | | |
| TOTAL | 31 | | | |

Table 38. Analysis of variance of activity of four carbofuran treated birds (10 mg/kg) under light-dark photoperiod (14L:10D).

ANALYSIS OF VARIANCE

| SOURCE | D.F. | M.S. | F-RATIO | PROB. |
|---|---|---|---|---|
| BIRD | 3 | 28643.9 | 16.40 | 0.00 |
| WEEK | 6 | 3522.0 | 2.01 | 0.11 |
| RESIDUAL | 18 | 1746.3 | | |
| TOTAL | 27 | | | |

Table 39. Daily food consumption and mean (±S.D.) of four carbofuran treated birds (10 mg/kg) under a light-dark photoperiod (14L:10D).

MEAN DAILY FOOD INTAKE (GRAMS)

| PHASE | BIRD 1 | 2 | 3 | 4 | MEAN (±S.D.) |
|---|---|---|---|---|---|
| PRETREATMENT | 16.1 | 14.1 | 15.3 | 15.7 | 15.3 (±0.9) |
| TREATMENT | 10.9 | 11.1 | 10.6 | 12.5 | 11.3 (±0.8) |
| POSTTREATMENT | * | * | * | * | * |

* Missing data due to the death of all four birds.

Table 40. Analysis of variance of food consumption of four carbofuran treated birds (10 mg/kg) under light-dark photoperiod (14L:10D).

ANALYSIS OF VARIANCE

| SOURCE | D.F. | M.S. | F-RATIO | PROB. |
|---|---|---|---|---|
| BIRD | 3 | 0.93 | 1.26 | 0.43 |
| COLLECTIONS | 1 | 34.03 | 44.12 | 0.00 |
| RESIDUAL | 3 | 2.31 | | |
| TOTAL | 7 | | | |

Table 41. Body weight and mean (±S.D.) of four carbofuran treated birds (10 mg/kg) under a light-dark photoperiod (14L:10D) at the end of each phase.

| BODY WEIGHT (GRAMS) | | | | | |
|---|---|---|---|---|---|
| | BIRD | | | | |
| PHASE | 1 | 2 | 3 | 4 | MEAN (±S.D.) |
| ACCLIMATION | 196.1 | 165.0 | 180.5 | 170.6 | 178.0 (±13.6) |
| PRETREATMENT | 196.0 | 166.2 | 182.7 | 173.1 | 179.5 (±12.9) |
| TREATMENT | 185.0 | 168.9 | 162.9 | 166.9 | 170.9 (± 9.7) |
| POSTTREATMENT | * | * | * | * | * |

* Missing data due to the death of all four birds.

Table 42. Analysis of variance of body weight of four carbofuran treated birds (10 mg/kg) under light-dark photoperiod (14L:10D).

ANALYSIS OF VARIANCE

| SOURCE | D.F. | M.S. | F-RATIO | PROB. |
|---|---|---|---|---|
| BIRD | 3 | 388.2 | 13.24 | 0.00 |
| PHASE | 2 | 84.2 | 2.87 | 0.13 |
| RESIDUAL | 6 | 29.3 | | |
| TOTAL | 11 | | | |

Table 43. Analysis of variance of activity of four carbofuran treated birds (10 mg/kg) under light-dark photoperiod (14L:10D).

ANALYSIS OF VARIANCE

| SOURCE | D.F. | M.S. | F-RATIO | PROB. |
|---|---|---|---|---|
| BIRD | 3 | 231782.3 | 33.46 | 0.00 |
| WEEK | 8 | 31204.7 | 4.50 | 0.00 |
| RESIDUAL | 24 | 6926.7 | | |
| TOTAL | 35 | | | |

Table 44. Daily food consumption and mean (±S.D.) of four carbofuran treated birds (10 mg/kg) under a light-dark photoperiod (14L:10D).

MEAN DAILY FOOD INTAKE (GRAMS)

| PHASE | BIRD 1 | 2 | 3 | 4 | MEAN (±S.D.) |
|---|---|---|---|---|---|
| PRETREATMENT | 13.9 | 16.9 | 13.9 | 13.1 | 14.5 (±1.7) |
| TREATMENT | 7.9 | 8.5 | 9.8 | 10.2 | 9.1 (±1.1) |
| POSTTREATMENT | 15.3 | 19.0 | 14.3 | 13.6 | 15.6 (±2.4) |

Table 45. Analysis of variance of food consumption of four carbofuran treated birds (10 mg/kg) under light-dark photoperiod (14L:10D).

ANALYSIS OF VARIANCE

| SOURCE | D.F. | M.S. | F-RATIO | PROB. |
|---|---|---|---|---|
| BIRD | 3 | 4.28 | 1.54 | 0.29 |
| COLLECTIONS | 2 | 48.01 | 17.29 | 0.00 |
| RESIDUAL | 6 | 2.77 | | |
| TOTAL | 11 | | | |

Table 46. Body weight and mean (±S.D.) of four carbofuran treated birds (10 mg/kg) under a light-dark photoperiod (14L:10D) at the end of each phase.

| BODY WEIGHT (GRAMS) | | | | | |
|---|---|---|---|---|---|
| | BIRD | | | | |
| PHASE | 1 | 2 | 3 | 4 | MEAN (±S.D.) |
| ACCLIMATION | 180.6 | 175.9 | 155.4 | 159.9 | 168.0 (±12.2) |
| PRETREATMENT | 181.5 | 172.5 | 150.2 | 159.2 | 165.8 (±13.9) |
| TREATMENT | 175.5 | 172.7 | 147.5 | 162.9 | 164.7 (±12.6) |
| POSTTREATMENT | 181.7 | 176.7 | 151.0 | 160.3 | 167.4 (±14.3) |

Table 47. Analysis of variance of body weight of four carbofuran treated birds (10 mg/kg) under light-dark photoperiod (14L:10D).

ANALYSIS OF VARIANCE

| SOURCE | D.F. | M.S. | F-RATIO | PROB. |
|---|---|---|---|---|
| BIRD | 3 | 687.1 | 117.4 | 0.00 |
| PHASE | 3 | 9.0 | 1.5 | 0.26 |
| RESIDUAL | 9 | 5.8 | | |
| TOTAL | 15 | | | |

Table 48. Analysis of variance of activity of eight carbofuran treated birds (10 mg/kg) under continuous dim light (10 lux).

ANALYSIS OF VARIANCE

| SOURCE | D.F. | M.S. | F-RATIO | PROB. |
|---|---|---|---|---|
| BIRD | 7 | 352139.50 | 17.00 | 0.00 |
| WEEK | 8 | 45685.00 | 2.20 | 0.04 |
| RESIDUAL | 56 | 20707.55 | | |
| TOTAL | 71 | | | |

Table 49. Daily food consumption and mean (±S.D.) of eight carbofuran treated birds (10 mg/kg) under continuous dim light (10 lux).

MEAN DAILY FOOD INTAKE (GRAMS)

| PHASE | BIRD 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | MEAN (±S.D.) |
|---|---|---|---|---|---|---|---|---|---|
| PRETREATMENT | 11.5 | 14.1 | 12.9 | 13.8 | 13.6 | 16.3 | 14.5 | 13.8 | 13.8 (±1.4) |
| TREATMENT | 10.4 | 4.2 | 12.4 | 12.9 | 5.2 | 8.3 | 8.6 | 6.8 | 8.6 (±3.2) |
| POSTTREATMENT | 18.9 | 21.2 | 16.2 | 16.5 | 17.0 | 15.7 | 17.4 | 11.3 | 16.8 (±2.8) |

Table 50. Analysis of variance of food consumption of eight carbofuran treated birds (10 mg/kg) under continuous dim light (10 lux).

ANALYSIS OF VARIANCE

| SOURCE | D.F. | M.S. | F-RATIO | PROB. |
|---|---|---|---|---|
| BIRD | 7 | 4.36 | 0.56 | 0.77 |
| COLLECTIONS | 4 | 137.03 | 17.61 | 0.00 |
| RESIDUAL | 14 | 7.77 | | |
| TOTAL | 23 | | | |

Table 51. Body weight and mean (±S.D.) of eight carbofuran treated birds (10 mg/kg) under continuous dim light (10 lux) at the end of each phase.

BODY WEIGHT (GRAMS)

| PHASE | BIRD 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | MEAN (±S.D.) |
|---|---|---|---|---|---|---|---|---|---|
| ACCLIMATION | 175.0 | 190.2 | 180.7 | 182.2 | 182.1 | 176.8 | 151.6 | 162.9 | 175.2 (±12.3) |
| PRETREATMENT | 173.7 | 183.8 | 184.2 | 186.6 | 188.5 | 186.7 | 156.3 | 168.1 | 178.5 (±11.4) |
| TREATMENT | 157.7 | 124.6 | 176.5 | 172.6 | 183.5 | 176.8 | 154.1 | 164.7 | 163.8 (±18.8) |
| POSTTREATMENT | 195.0 | 193.8 | 190.1 | 191.2 | 190.1 | 190.5 | 163.5 | 163.6 | 184.7 (±13.2) |

Table 52. Analysis of variance of body weight of eight carbofuran treated birds (10 mg/kg) under continuous dim light (10 lux).

ANALYSIS OF VARIANCE

| SOURCE | D.F. | M.S. | F-RATIO | PROB |
|---|---|---|---|---|
| BIRD | 7 | 435.16 | 3.49 | 0.01 |
| PHASE | 3 | 615.22 | 4.93 | 0.00 |
| RESIDUAL | 21 | 124.59 | | |
| TOTAL | 31 | | | |

# THE EFFECTS OF CARBAMATES ON BOBWHITE (*COLINUS VIRGINIANUS*) ACTIVITY

by

RICHARD WAYNE FELTHOUSEN

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A series of experiments were undertaken to determine the effects of carbaryl and carbofuran on various aspects of adult male bobwhite (Colinus virginianus) activity. Variables tested included locomotor activity, free-running activity rhythms and diurnal activity patterns. Food consumption and body weight data were also collected. Four experiments were conducted under continuous bright light (300 lux), one experiment under continuous dim light (10 lux) and two experiments under a 14L:10D rectangular light-dark regimen. Carbaryl treated birds were kept under chamber conditions of 25°C and 65 percent relative humidity while carbofuran treated birds were maintained under chamber conditions of 20°C and 65 percent relative humidity. Birds were treated at daily dosages equivalent to 20 and 100 mg/kg carbaryl in diet and 2 and 10 mg/kg carbofuran in diet.

All birds were individually housed in activity cages constructed of plywood. An Esterline Angus 8-channel Minigraph continuous event recorder measured activity and monitored the bird's position in the cage.

Preliminary experiments, that allowed the birds to establish a self-selected free-running rhythm with regards to light intensities, were unsuccessful. Therefore, all free-running experiments were conducted under constant light conditions.

Quail receiving daily dosages equivalent to 20 and 100 mg/kg carbaryl in diet showed no significant changes in activity, food consumption or body weight. Quail ingesting 10 mg/kg of carbofuran in diet

showed a significant decrease in activity and food consumption under all photoperiods, however, body weight was only significantly decreased under continuous illumination experiments.

Under rectangular light-dark cycles, bobwhites exhibited a conspicuous bimodal activity pattern with major morning and evening peaks. Ingestion of carbofuran at 10 mg/kg in diet reduced peak amplitude but did not affect the timing.

Bobwhite exhibit a free-running activity rhythm when kept under constant conditions, the establishment and persistence of which appears to depend upon light intensity. At 300 lux most of the birds had interspersed activity throughout their periods. The effects of treatment on free-running periodicity were difficult to access because of spontaneous changes occurring in periodicity during pretreatment.