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Radio-biotelemetry studies of circadian body temperature rhythms and activity levels [of the] zebra finch (Peophilia guttata)

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RADIO-BIOTELEMETRY STUDIES OF CIRCADIAN BODY TEMPERATURE RHYTHMS AND ACTIVITY LEVELS ZEBRA FINCH (POEPHILIA GUTTATA)

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INTRODUCTION

The measurement of body temperature is unique in that it is a dynamic measurement of the oxidative processes occurring in the body. Although most investigators agree on the importance of measuring body temperature, there seems to be little agreement on how this measurement should be made. King and Farner (1961), suggest that avian body temperature should be measured in the darkness in a post-absorptive state, and from the cloaca, proventriculus, or pectoral muscles. The author believes that avian temperature is best monitored with minimum physiological and behavioral disturbances and for periods of at least 24 hours so that the data obtained may represent a more complete pattern of normal body temperature and not an isolated event.

Activity levels, when considered in relation to body temperature, can be used as an index of possible increase or decrease in temperature caused by variation in muscular heat production. Variation in known activity levels may then be considered as an indication of physiological stress or behavioral abnormalities that arise owing to imposed experimental conditions.

The object of this study was to determine the normal thermoregulatory patterns of zebra finches (Poephilia guttata) in as near a normal physiological and behavioral state as possible. Surface body temperature and level of activity

were the two primary channels of information. Radio-biotelemetry allowed the reduction of external disturbance during long periods of continuous recording.

METHODS AND MATERIALS

Zebra finches are small Australian grass finches of the family Ploceidae. Zebra finches are described in nature by Immelmann (1965), as being ubiquitous Australian grass finches whose reproductive cycle is keyed to the rainy seasons of the Australian savannah. Zebra finches have been imported into the United States as an aviary bird, and have become a favorite of aviculturists as well as biologists.

There are several characteristics which make zebra finches a desirable test species. They are sexually dimophic and exhibit a generation time of 30 days when maintained in small flights (3' X 2' X 2') with adequate food and water, and near optimum temperature. Breeding will continue throughout the year with an average clutch size of four eggs, incubation period of 13 days, and fledging period of 13 days. Commercial finch seed with an occasional lettuce supplement meets the feeding requirements even during the reproductive periods. The fledglings complete juvenile molt and reach reproductive readiness within 20-40 days.

A flock of 40 zebra finches was raised by the author over a period of one and a half years. All finches when first obtained were weighed in a covered box to reduce struggling, banded, and paired. The pair was then placed indoors in a cage with the dimensions of 3' X 2' X 2' (the minimum flight for optimum breeding). Each cage contained a

nest box and nesting materials in the form of dried grass. A finch seed mixture of millet and Indian hemp comprised the primary food source. Supplements of lettuce, calcium in the form of egg shells, and cod liver oil mixed directly with the seed, were given at regular intervals. Under these conditions all but three of the twenty pair were successful in raising a clutch of fledglings.

During the year preceding the experiment a record of breeding attempts and successes was kept, so that data on each pair had been gathered prior to their being used experimentally. Behavioral observations were made and normal activities such as preening, gleening, and perch exchange were observed and compared with the findings of Morris (1954).

The most desirable method for obtaining continuous body temperature from the finches was determined to be radio-biotelemetry. Other techniques of temperature measurement were discarded due to the stress of restriction (Seyle 1950), the small size of the birds, and artifacts introduced by handling.

The skin of the dorsal feather tract was chosen as the area where body temperature would be measured. Steen and Enger (1957) suggested that surgical implantation in areas such as the pectoral muscles is most desirable for core temperature. However, the surgical procedures involved in placing a thermistor bead in the pectoral muscles of a bird

with an average body weight of 13 grams were considered too extreme for the limited benefits. Therefore, the dorsal feather tract was chosen because the surface temperature in feathered areas of small birds only varies slightly from core temperature (King and Farner 1961, Bartholomew and Dawson 1954a and 1954b, Irving and Krog 1955).

Various harnessing techniques were explored in an attempt to minimize disturbance during flying and perching. Cloth harnesses were judged inefficient because they added excess weight, became caught while flying, and came off easily. A simple technique of gluing the transmitters to the dorsal feather tract was adopted.

Test birds and controls were caught separately and placed under light Metaphane anesthetic. Metaphane (methoxyflurane) from Pitman-Moore, Inc. was chosen because it is an inhalent which when administered in small quanities will place the bird in a surgical plane of anesthesia for 10-15 minutes.

The finches were placed in an air tight container containing a piece of cotten soaked in Metaphane. The container was constructed of polyethylene and was $3\frac{1}{2}$ inches in diameter, thereby preventing flight. Zebra finches seem to exhibit three planes of anesthesia. Under light anesthesia the partial loss of palpebral, corneal, and cere reflexes without the loss of pedal and flight ability, so

that a bird escaping in this plane tended to fly at top speed directly into a wall or other obstacle. The second plane involved the loss of all reflexes, labored respiration, and shivering. In the third plane no respiration is evident and both body temperature and ratio of Metaphane to oxygen must be watched very carefully.

The birds were allowed to go into the second plane of anesthesia and then removed from the anesthetizing container. The wings were extended and taped down exposing the dorsal pterylae. Using fine forceps and a posterior pulling action the feathers were removed one at a time from the anterior .5 of an inch of the dorsal feather tract. Hemorrhage, when it did occur, was controlled by silver nitrate sticks.

When the plucking was completed, the finches were transferred to a recovery chamber. The chamber was constructed of plexi-glass pipe eight inches in diameter with the walls lined with foam rubber. A constant flow of oxygen was administered and the temperature of the chamber maintained at 38-40° C until all reflexes returned. The birds were then placed back in their cages and observed for 30 minutes to verify full recovery.

Twenty-four hours were allowed for the skin of the dorsal feather tract to recover and to lose the extreme sensitivity present immediately after plucking. At this time the birds were again placed under light Metaphane anesthetic

and the transmitter fastened to the back by Eastman 910 surgical adhesive (mono-2-cyanoacrylate monomer). An equal number of birds were subject to the same procedure except no transmitter was fastened to the back and a thin layer of Eastman 910 was applied to the exposed feather tract to check any effects of the anesthetic, handling, and/or glue. Test runs on the experimental animals were not begun until six hours after harnessing was completed to assure proper transmitter placement and adjustment.

The transmitter used (Spencer 1968) employs a Hartley blocking oscillator with a current drain of 0.2 milliampers from a 1.5 volt (RM 312) battery. This combination of Characteristics provides a temperature sensitive transmitter weighing 1.5 grams with a range of three meters and a life of up to 30 days. Figure 1., shows the transmitter assembled inside the coil before putting in beeswax. The finished transmitter was then activated and left running for a week in order to age the components and avoid response alterations due to aging during the test run.

The original transmitter design was used, however, several alterations were made in the construction to custom fit the transmitter to experimental needs (Figure 2). The coils were randomly wound on a 7/16 inch plastic coil forms using #38 wire were fixed with Q-Dope. Numerous frequencies may be obtained by placing ferrite chips next to the coil or

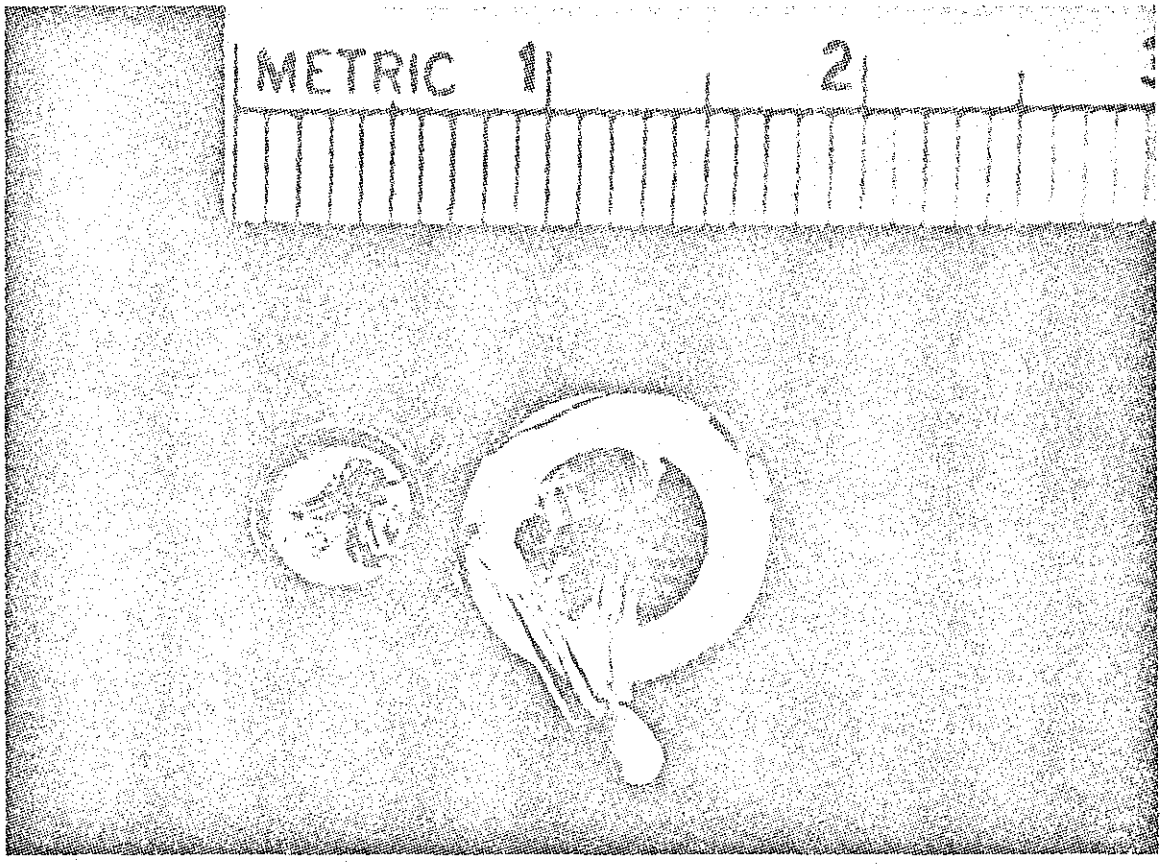
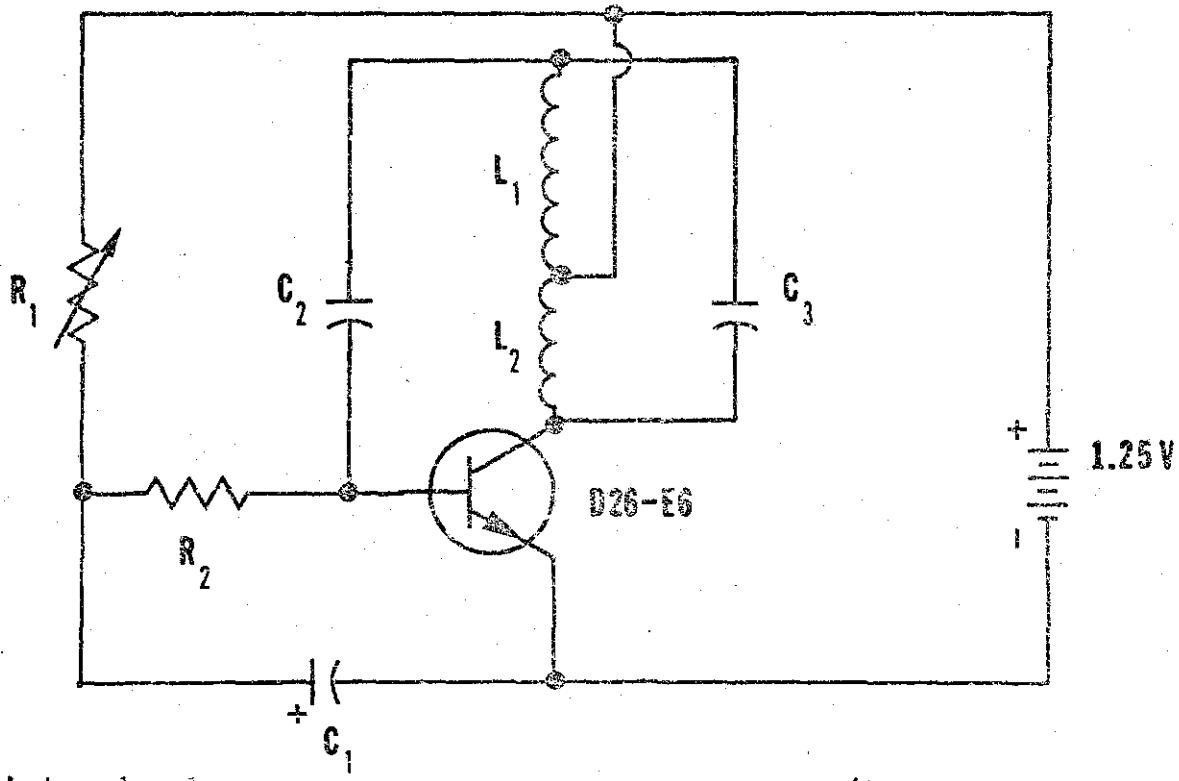


FIGURE 1

Assembled temperature transmitter (before potting in beeswax).

FIGURE 2

Thermally Stable temperature transmitter



R_1= 300K thermistor bead

R_2= 1K 1/8 watt

C_1= .001 MFD electrolytic

C_2= .001 MFD

C_3= .001 MFD

Male: L_1= 30 turns

L_2= 13 turns

Female: L_1= 25 turns

L_2= 8 turns

Transistor = D26-E6

Battery = 1.25 volts

reducing the numbers of winds. The latter technique was used to produce a male frequency of 90 KHz by winding L_1= 30 turns and L_2= 13 turns, and a female frequency of 120 KHz by winding L_1= 25 turns and L_2= 8 turns. The small diameter of the wire necessitates the use of Strip-Vare insulation remover (Walsco Electronics). After the entire process the finished product was checked for possible short circuits under a dissecting microscope.

The need for quality in micro-minature components can not be over stressed. In this application, all components had a tolerance of 5% or less. Components which do not meet these requirements may not provide the constant level of response required in research applications.

The NPN transistor used is a micro-tab D26-E6 made by General Electric and chosen for thermal stability, low base leakage current, and small size. R_2 of the RC pulsing circuit is a 1K 1/8 watt Allen-Bradley resistor with a low failure rate. The electrolytic C_1 is a .001 MFD tantalum sub-miniature from Components Inc. of Biddleford, Maine. In the original design C_1 was 1 MFD, making the pulse interval large and the pulsing frequency small. The temperature monitored was broken into frequency units from 1-100 pulses per second over a temperature range of 25° C to 40° C. The alteration of this capacitance to .001/MFD made the pulse interval very small (.0005 milliseconds) and the frequency of

pulsing very large. Over the same temperature range the frequency was now 2 X 10^3-20 X 10^3 pulses per second which greatly decreased error due to outside interference. Capacitances C_2 and C_3 were obtained as .001 MFD chips from Monolithic Dielectrics, Inc. A Yellow Springs Instrument Co. precision 300 K (at 25° C) thermistor bead was used as R_1. The power source was a RM 312 battery chosen for its small size, no fade characteristics, and superior operating life as compared to the much smaller RM 212. All components are glued to the inside of the transmitting coil and soldered with a 10 watt miniature soldering iron. The battery is attached by its circuit connections to the outside of the transmitting coil and the entire transmitter is potted in beeswax.

The transmitters were calibrated in a constantly stirred oil bath while the temperature was raised and lowered in 0.5° C increments from 30° C to 40° C. The frequency changes were recorded by tape recorder with the footage mark and corresponding temperature recorded. Each transmitter was monitored every 0.5° C up and down from 30° C to 40° C twice. The calibration recordings were played into a frequency counter and the frequencies recorded at every indicated footage mark. Therefore, every 0.5° C from 30° C to 40° C had four corresponding frequency values. A calibration graph for each transmitter was formed from these values by linear regressional analysis of individual 2° C segments.

Data collection from the harnessed birds was initially hindered by electromagnetic noise sources common in an unshielded building. A Faraday Cage (Bures, Petran, and Zachar 1967) was constructed to shield the receivers from unwanted electrical phenomena. A Faraday Cage acts as a large screen receiver which receives electromagnetic waves and shunts them to ground. The cage (Figure 3) was constructed of unanodized aluminium screening (6' X 4' X 4') entirely bolted together with the front section movable in tracks; the floor was particle board to avoid damage to the base screen. One corner of the cage was completely fused together by soldering and grounded. Only one corner must be grounded so that a ground loop is not formed. The result was a 90% reduction in all types of electromagnetic interference.

The cages for the test birds were all constructed of fiber glass screening with plexi-glass doors. Metal screening was not used, because it would have created a small Faraday Cage of the type described above which would shield transmissions from the receivers placed around the outside of the cage.

The receivers were commercial Panasonic AM-FM transistor radios operated from an AC power supply. The receivers were chosen for their response to the transmitters (MacKay 1968). Line interference in the form of back-up voltages was controlled by Miller Capacity Line Filters 7816. The received signal was transmitted from the receivers using

(A) Method of photographing integrated temperature

(B) Method of frequency counting

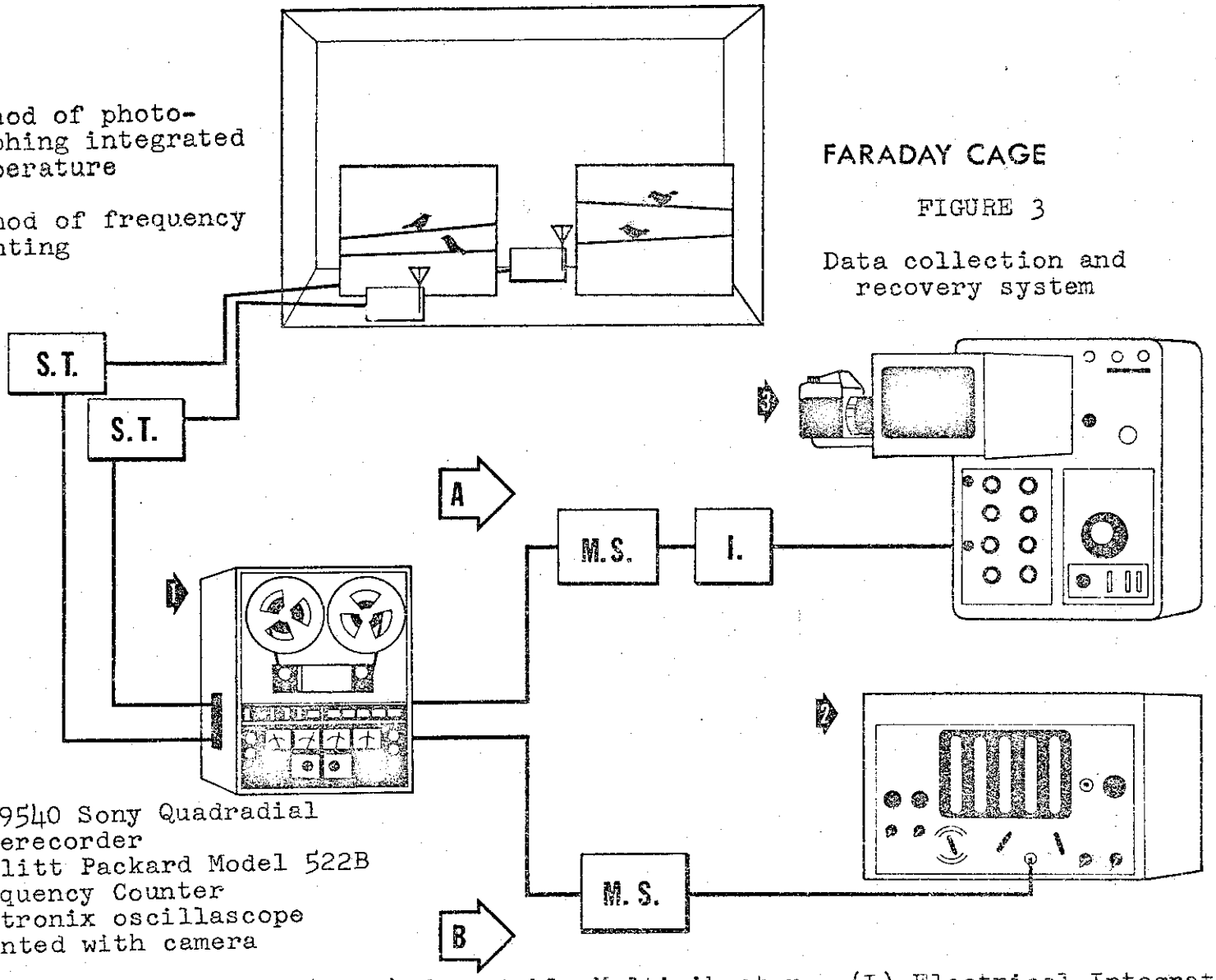


FIGURE 3

Data collection and recovery system

(1) TC-9540 Sony Quadradial Taperecorder
(2) Hewlitt Packard Model 522B Frequency Counter
(3) Tektronix oscillascope mounted with camera

(S.T.) Schmitt Trigger (M.S.) Monostable Multivibrator (I) Electrical Integrator

the ear phone plug as an output.

Although the Faraday Cage was very effective in shielding out interference, low level noise was detected in signals coming from the receivers. Therefore, the signals transmitted from the males and females that were picked up by the receiver were half-wave rectified and sent through Schmitt Triggers (Figure 3) (Malmstadt and Enke 1969) made from RCA CA 3001 micro-logic integrated circuits and preceded by a diode. Both Schmitt Triggers were set to fire above the level of noise present in the system, acting as high band-pass filters to eliminate low level noise.

The resulting signals were simultaneously recorded on a Sony Quadradial Tape recorder model TC-9540. The only interference at this point was sixty cycle interference and AC line back up which was eliminated by the application of a Miller 7815 line filter. The signals were recorded on Scotch Brand 290 half mill tape at 3 3/4 ips (low speed) so that sixteen hours of information could be recorded on the four channels of a single tape. The use of a tape recorder that directly records and stores an exact replica of the original electromagnetic event gave time during the test run to concentrate on data collection. The half mill tape proved highly useful for long periods of unattended recording; however, accidental stretching was not uncommon and ruined the tape for further use.

This model tape recorder proved to be ideal for recording bio-potentials. Tests showed a very low degree of "wow" and "flutter", thereby giving a near perfect reproduction of the recorded signals. The TC-9540 also has the feature of lacking an internal audio monitor, which is often a source of interference.

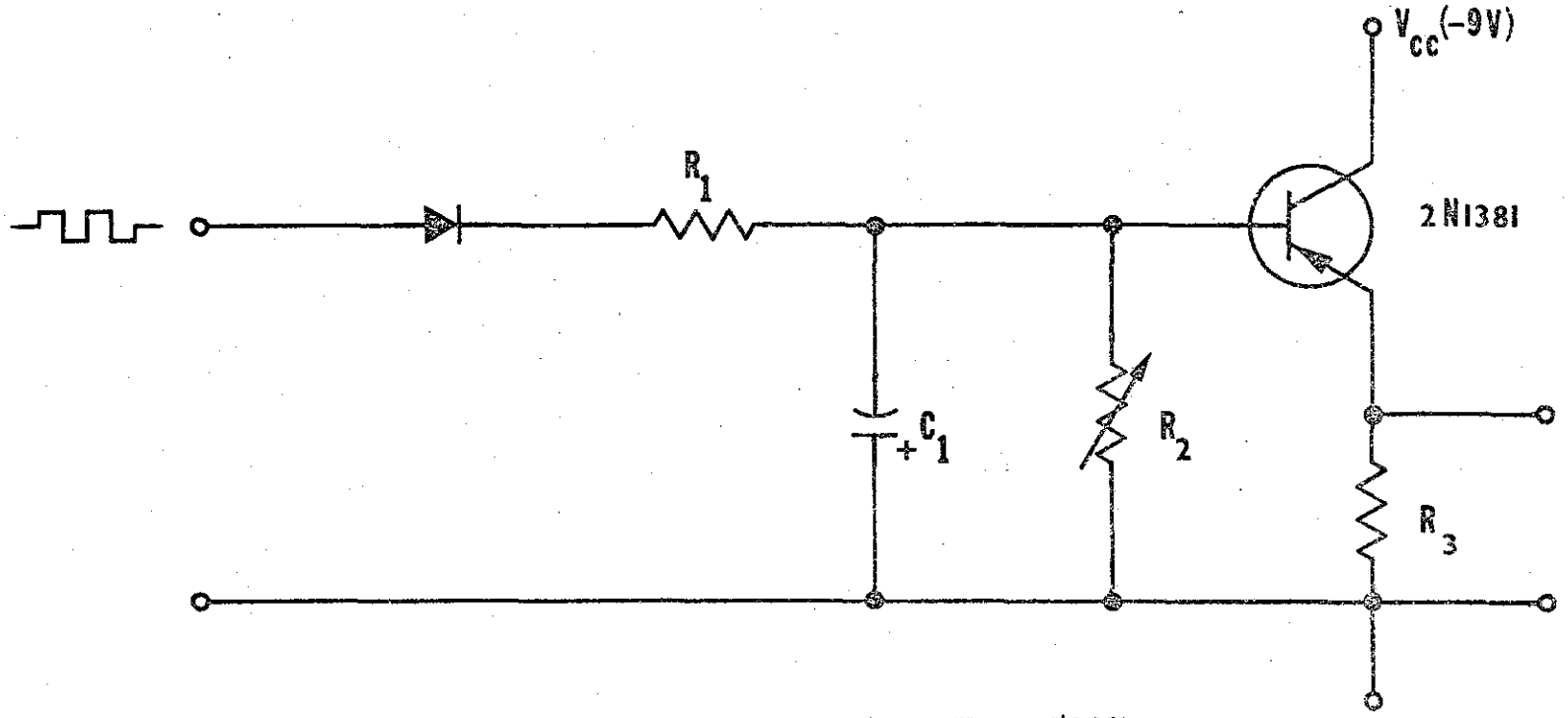
Data recovery time required one fourth the recording time because each tape was recorded at 3 3/4 ips (low speed) and played back at 15 ips (high speed). Three channels of information were taken from the recording, surface body temperature, activity levels, and subtle temperature change. The tape recorded information was shown by oscilloscope analysis to contain some aberrations in amplitude and duration of pulse.

Therefore, the recorded data was played through monostable multivibrators made from Fairchild micrologic circuit FGS 914 (Malmstadt, Enke, and Toren 1963). The monostable multivibrators produced a pulse of constant amplitude and duration which could be counted or integrated with a minimum of error.

The pattern of temperature change was displayed by the method marked (A) in Figure 3. Recordings of periods of temperature transition were played through a monostable multivibrator into a simple electrical integrator, Figure 4 (Runion 1970 personnal communication). The pulse information was electrically summed, amplified by the oscilloscope to the

FIGURE 4

Electrical integrator. (Runion, H.I., 1970, Personnal Correspondence)



R_1= 470 ohms

R_2= 100K to 500K

R_3= 820 ohms

C_1= 20 MFD electrolytic

Transistor = 2N1381 or equivalent

Battery = 9 volts

CRT screen, and the temperature change photographed. The integrated frequency of the temperature before the change occurred was set as the base line from which increase or decrease could be observed. Periods of rapid temperature change noted in general data recovery were investigated in this manner.

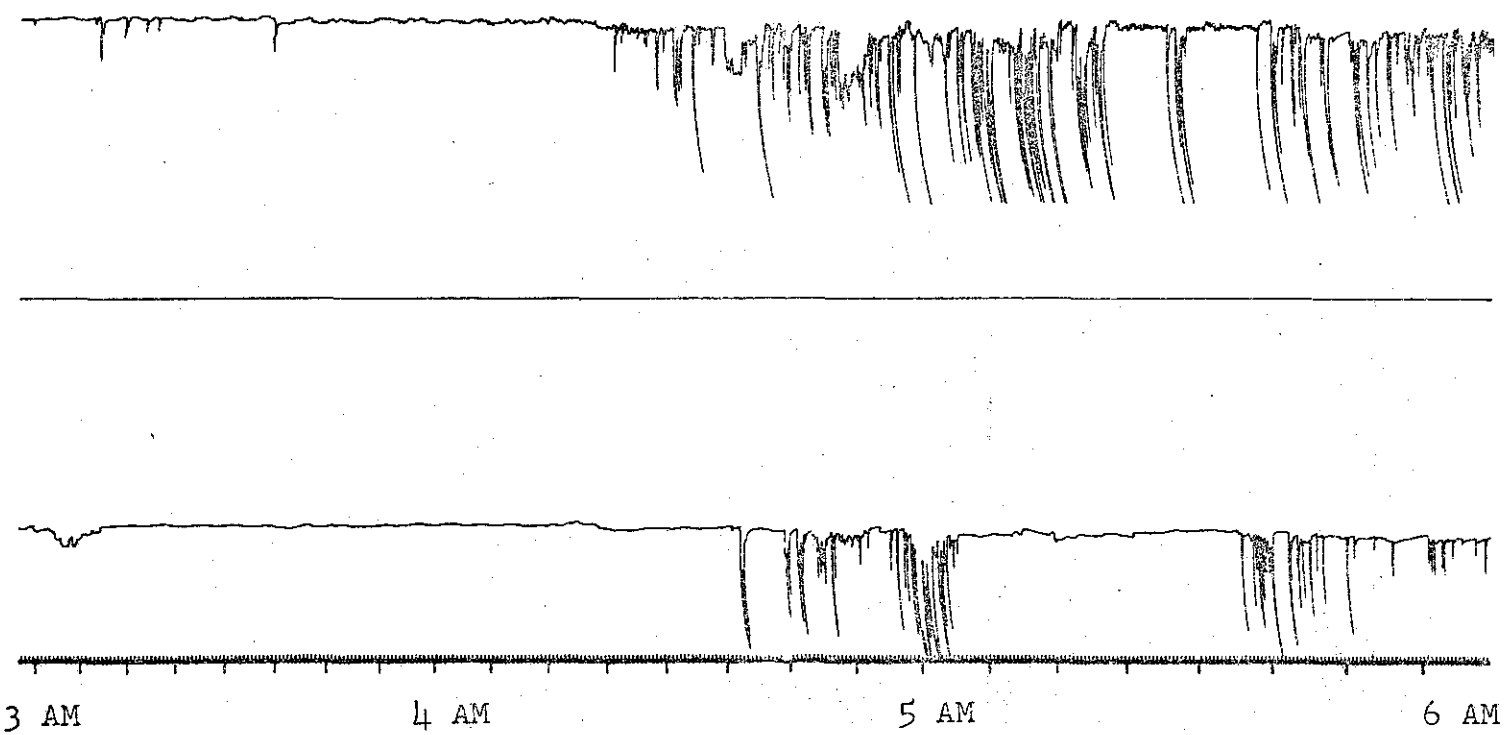
All recorded temperature information was played through the channel marked (B) in Figure 3. The information was transmitted through a monostable multivibrator into the digital frequency counter indicated by arrow (2) in Figure 3. The Hewlitt Packard model 522B Electronic Frequency Counter was set to display the number of pulses per second. Data was recorded as the number of pulses per second and sampled every eighty feet as indicated on the footage meter. Forty samples were taken over a three hour period so that a temperature reading was obtained every 4.5 minutes, for each test run. The data was read off the digital light display as crude frequency and converted to equivalent temperatures from the transmitter calibration graphs.

While two channels were being sampled for frequency information the other two channels were being played into the Grass Model 7B Oscillograph. The transmitting coil of the temperature transmitters when changed in orientation to the ferrite bar antenna of the receivers caused a brief null in the received signal. The nulls recorded were due to the inability of the receiver to adjust to quick changes in the

transmitting coil due to flight of the bird. The signal played into the Grass oscillograph was amplified and integrated on polygraph paper. The null transmissions caused high amplitude deflections of the pen, thereby graphing out an activity level for the recorded period, see Figure 5. The activity level recordings were analyzed by making a count of the number of columns containing one or more null deflections. Figure 5 shows a recording made from three to six o'clock in the morning. The first part of the graph has no null deflections and indicates a period of very low activity; null deflections begin as the birds leave the nest at sunrise.

FIGURE 5

Oscillographic recordings of male and female (zebra finch) activity levels between 3 AM and 6 AM during run 3



Upper trace: Male

Lower trace: Female

RESULTS

Fourteen birds were monitored during seven test runs (Table I). 604 hours of temperature and activity data were collected on both male and female finches. Room temperature was maintained at approximately 21° C and the natural photoperiod was recorded for each test run. Definite patterns of activity and thermoregulation were apparent throughout all test runs.

Activity levels were plotted as the number of null deflections against time. Activity levels for all finches tested are plotted in Figure 6. All recorded activity could be put on to a single graph because of the consistent repetition of this pattern. The finches are completely inactive during the night and the only recorded movements were movements in the nest box. Although ten or less deflections were considered to be an indication of the lowest possible activity level, most night time recordings showed no nulls at all. Transition periods occurred at sunrise and sunset. Morning activity was begun as the birds first left the nest (Figure 5), so that the level of activity rose very quickly during this period. Sunset was accompanied by slowly decreasing numbers of flights, until the male and female entered the nest box for the night. The recorded pattern of activity for this period was the reverse of Figure 5. Daytime activity levels fluctuated around a mean of fifty

TABLE 1

TOTAL RECORDED HOURS OF SURFACE BODY TEMPERATURE AND ACTIVITY LEVELS OF MALE AND FEMALE ZEBRA FINCHES

Test Run	Sex	Total Recorded Hours of Activity	Total Recorded Hours of Temperature	Natural Photoperiod	Temperature °C
1	Male	48	48	11 hours of light	21°C-22°C
	Female	48	48		
2	Male	48	48	11 hours of light	20°C-22°C
	Female	48	48		
3	Male	54	54	12 hours of light	20°C-22°C
	Female	54	54		
4	Male	24	24	12 hours of light	20°C-22°C
	Female	24	24		
5	Male	24	24	12 hours of light	20°C-22°C
	Female	24	24		
6	Male	56	56	12 hours of light	20°C-22°C
	Female	56	56		
7	Male	48	48	12 hours of light	19°C-23°C
	Female	48	48		
	Total	604	604		

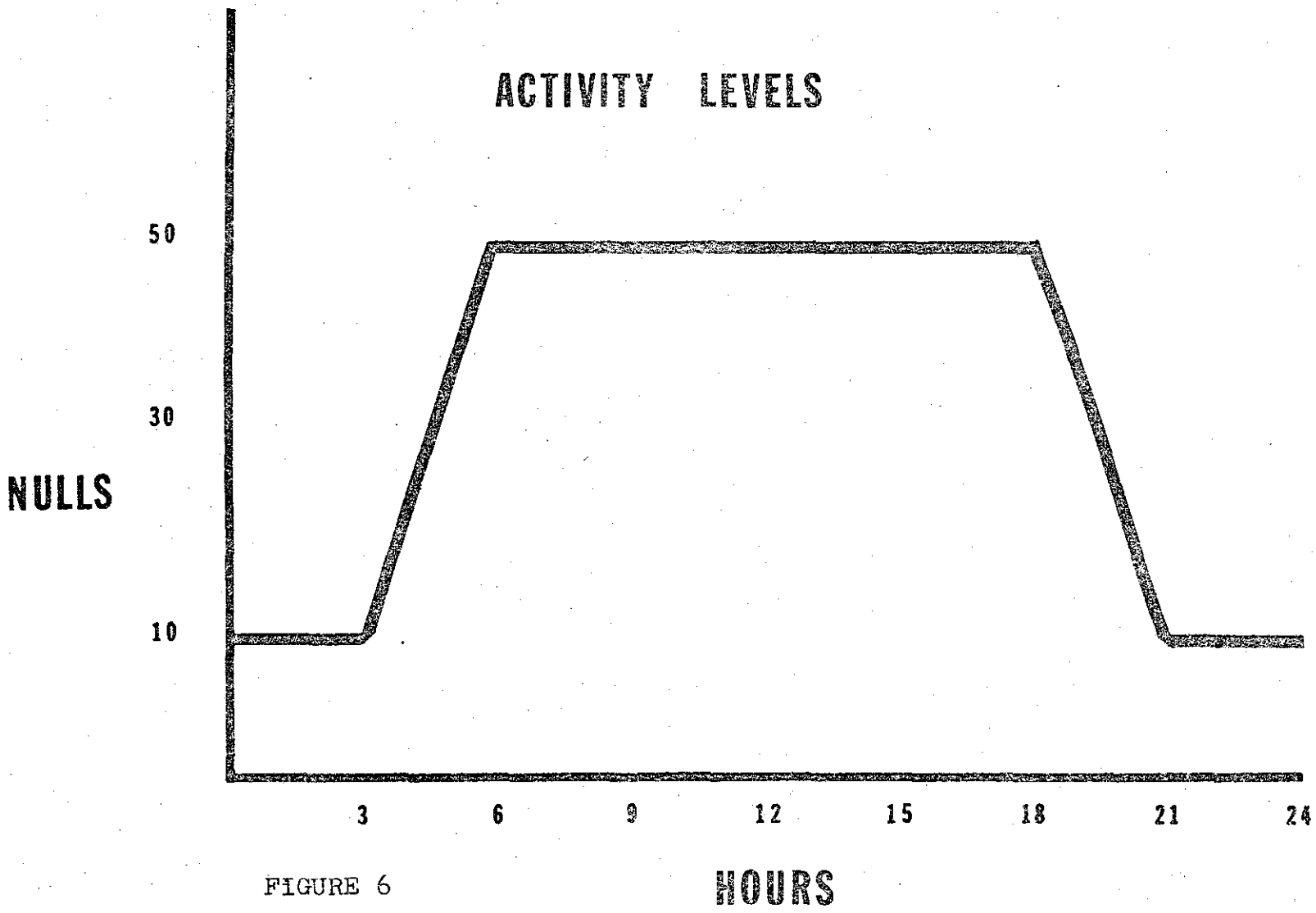


FIGURE 6

Circadian activity levels of zebra finches

nulls as shown in Graph I. No periods of complete inactivity during the daytime were recorded. The circadian activity levels indicated in this species are those of a typical diurnal bird, (Palmgren 1949) with activity levels beginning with first light and ending at dark.

Figure 7. is the surface body temperature of a male finch taken during 54 hours of continuous recording. The thermoregulatory phenomena has been graphed in three hour blocks to illustrate the general pattern of circadian temperature changes. The temperatures of the male during this run change around two periods of the day. The highest mean temperature occurs between 3 PM to 6 PM, after which the temperature steadily declines. The lowest mean temperature is tetween 12 AM to 3 AM. The differences between the high diurnal and low nocturnal temperatures were found to be statistically significant at the 0.05 level when tested by student's t-test.

The female surface temperature (Figure 8) taken simultaneously with the male exactly follows the pattern recorded for the male (Figure 7). Both male and female graphs represent the temperature transition times characteristic of the temperature cycle. In early morning (6 AM to 10 AM) there is a temperature rise from the low nocturnal temperatures to a period of high diurnal temperatures (10 AM to 6 PM). In the evening a temperature drop occurs between 6 PM and until 10 PM.

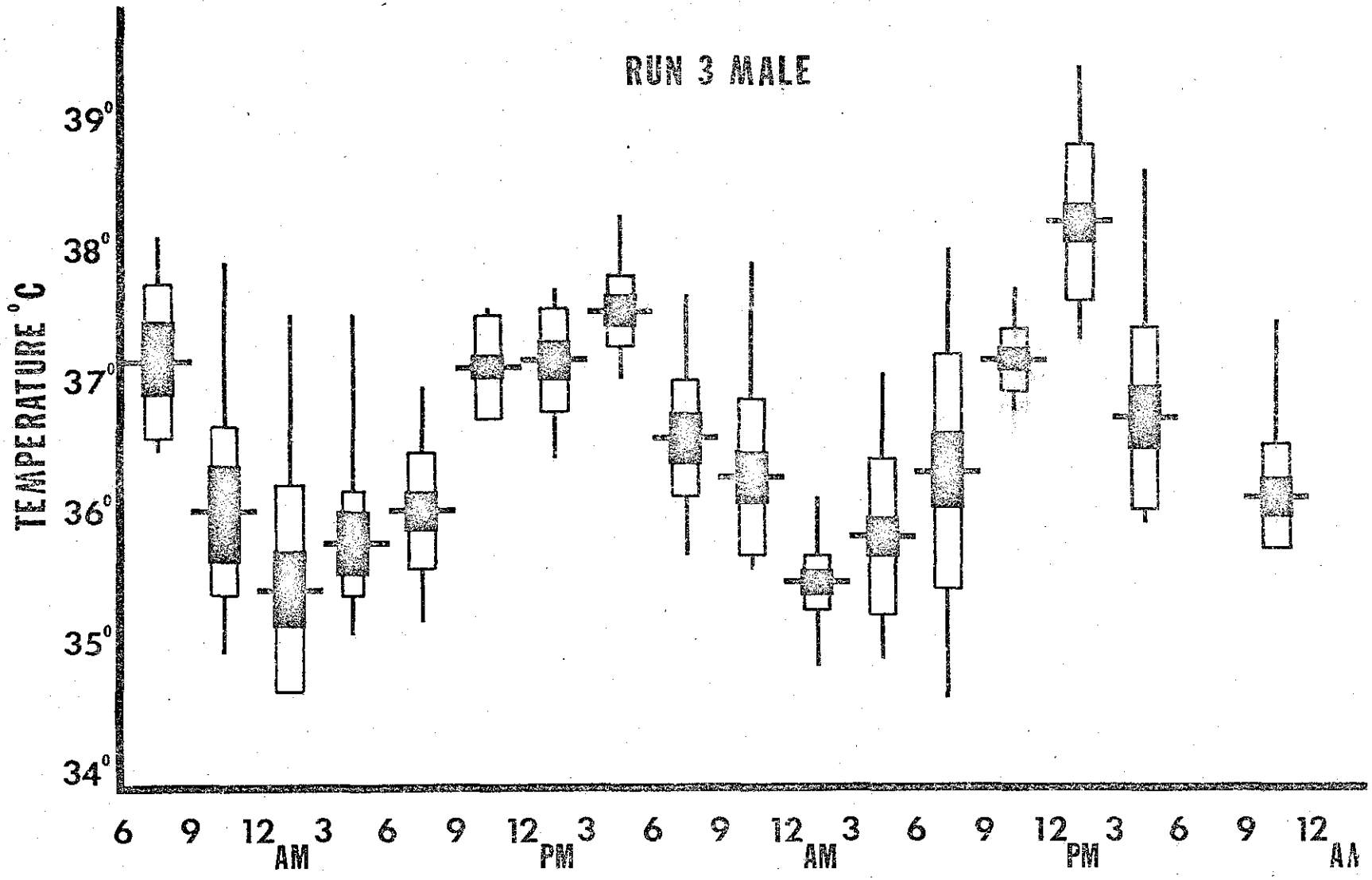


FIGURE 7

Surface body temperature of a male zebra finch during run 3. Solid black rectangle, standard error; open rectangle, standard deviation; horizontal bar, mean; and vertical bar, range

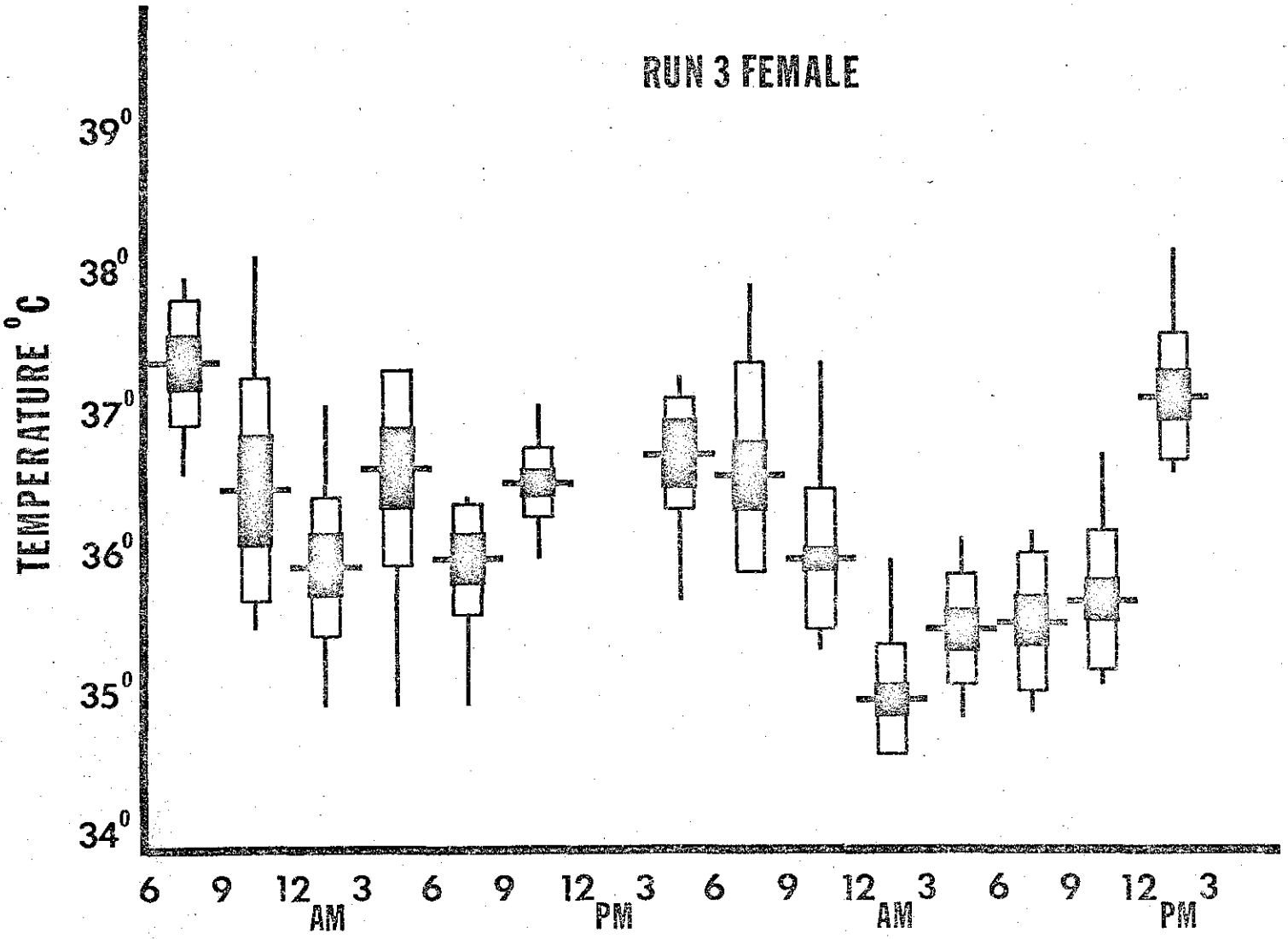


FIGURE 8

Surface body temperature of a female zebra finch during run 3. Solid black rectangle, standard error; open rectangle, standard deviation; horizontal bar, mean; and vertical bar, range

Figures 9 and 10 (Run 5) illustrate the transition period beginning in early evening. Recordings were made from male and female finches from 6 PM to 6 AM on two consecutive nights. The nocturnal temperature lows for both sexes are recorded between 12 AM and 3 AM just as in run three. The differences between the diurnal and nocturnal temperature means are 1.6° C and 1.6° C for the males during run five and 1.75° C and 1.7° C for the male in run three (Figure 7). The female (Figure 10) exhibits the same pattern as in Figures 7, 8 and 9 as well as high and low temperature means of 1.65° C and 1.6° C. The pattern of surface temperature change was the same through all the test runs with the differences between male and female thermoregulatory rhythms and levels determined not to be significantly different at 0.05 level when tested with student's t-test.

Run six (Figures 11 and 12) illustrates the characteristic pattern observed in all seven test runs. The data were recorded continuously for 56 hours and graphed in two hour blocks. The complete thermoregulatory cycle is completed twice in this run. Periods of high diurnal temperature previously described are isolated into the time block 4 PM to 6 PM. The nocturnal lows are recorded between 12 AM and 4 AM. The male and female body temperature patterns are approximately the same varying only slightly in magnitude.

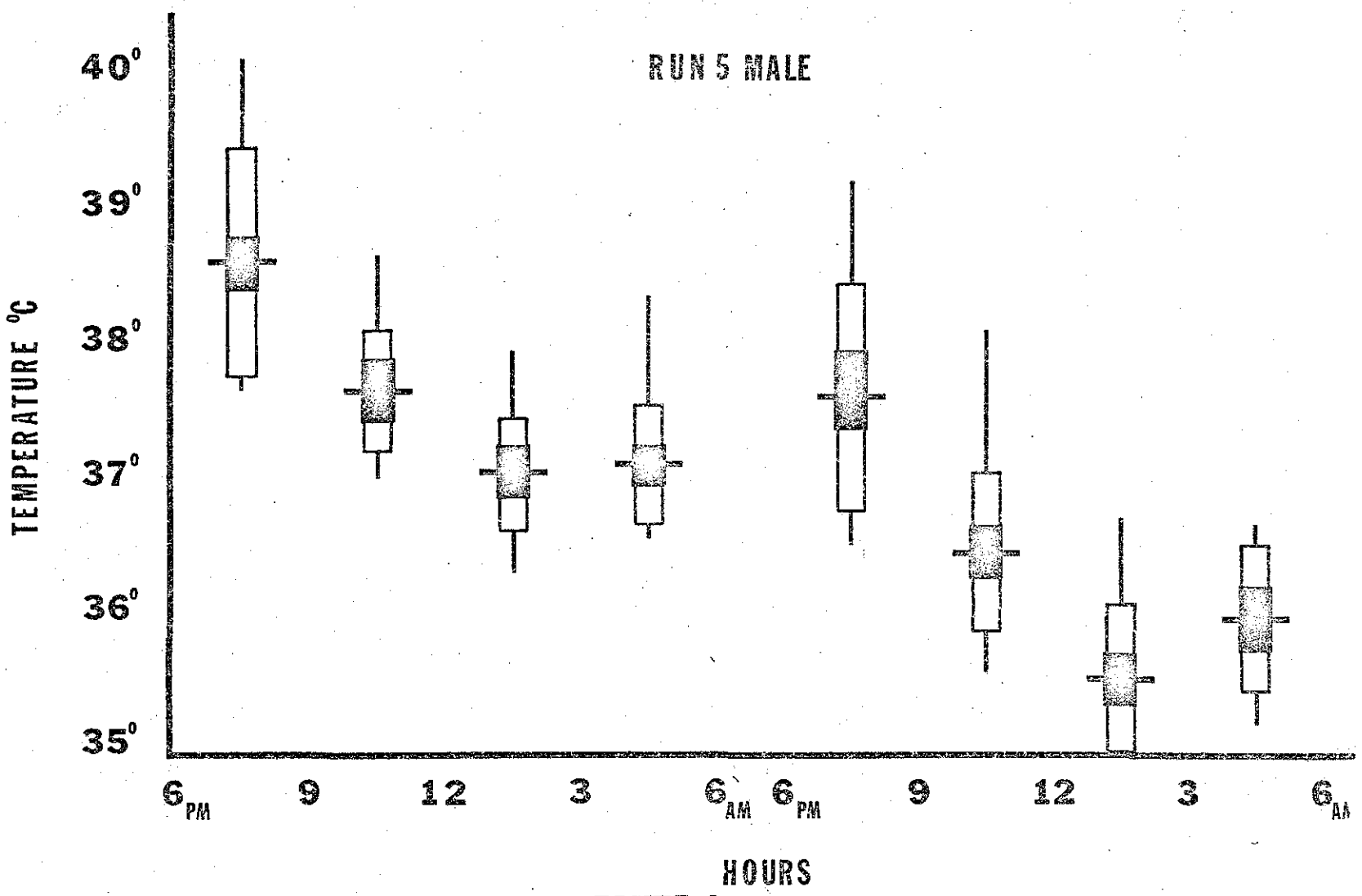


FIGURE 9

Surface body temperature of a male zebra finch during run 5. Solid black rectangle, standard error; open rectangle, standard deviation; horizontal bar, mean and vertical bar, range

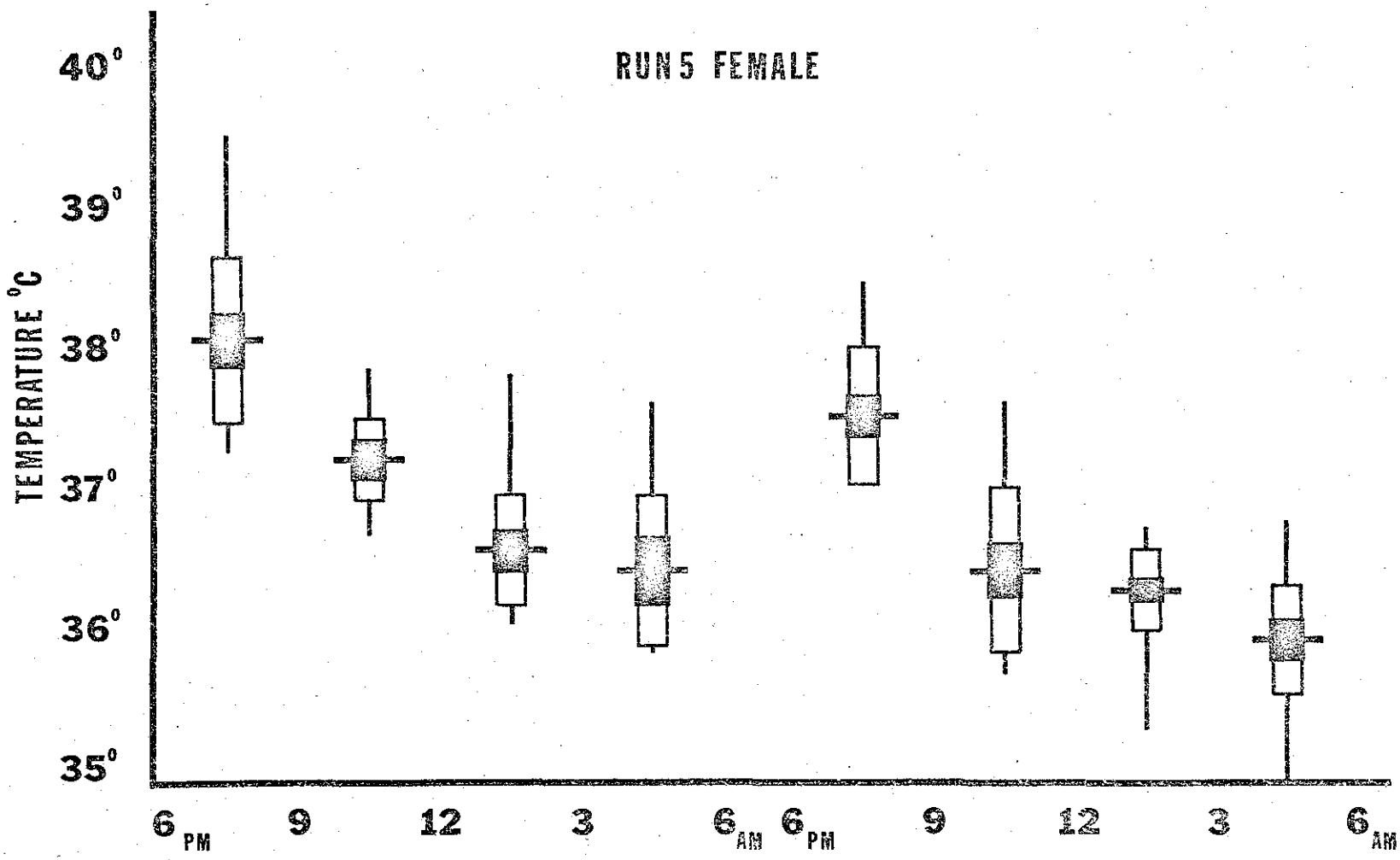


FIGURE 10

Surface body temperature of a female zebra finch during run 5. Solid black rectangle, standard error; open rectangle, standard deviation; horizontal bar, mean; and vertical bar, range

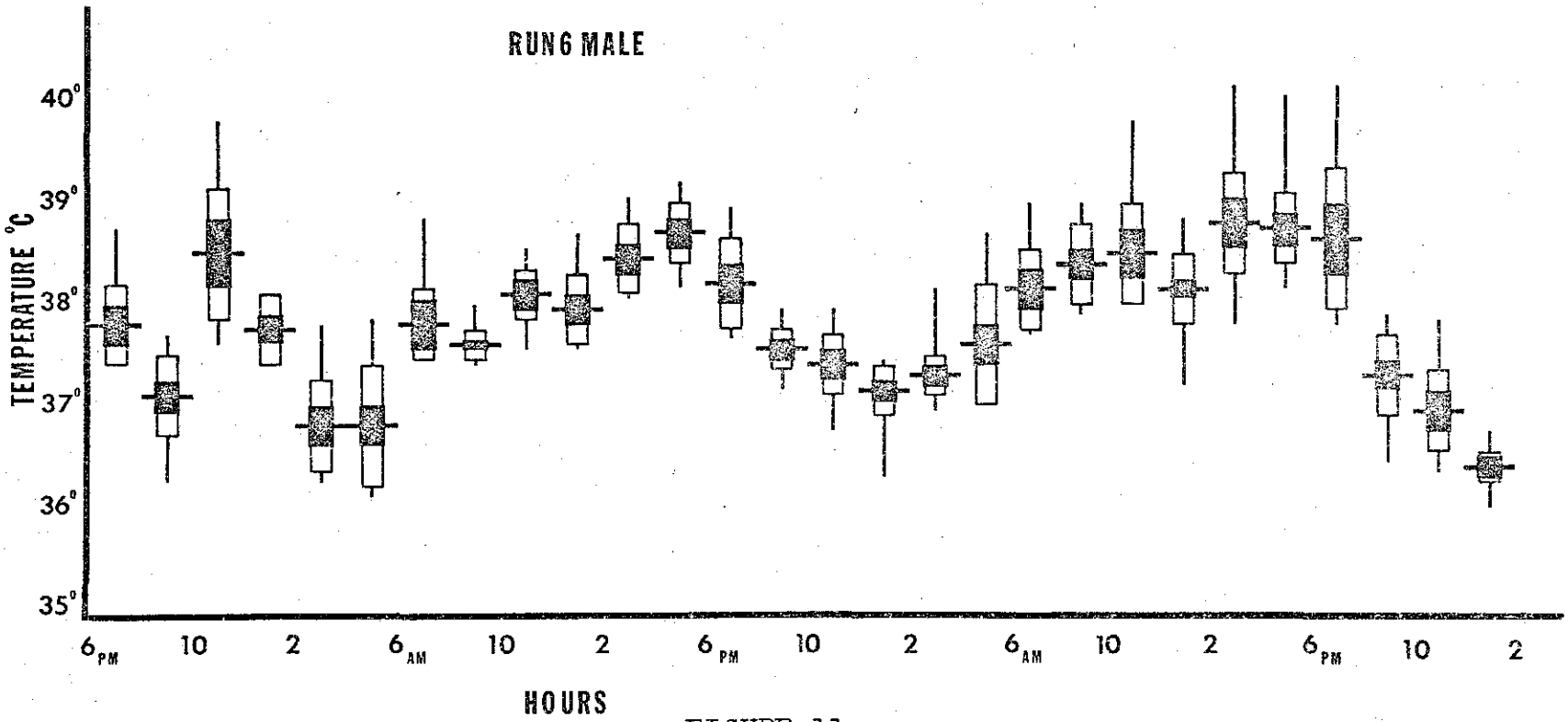


FIGURE 11

Surface body temperature of a male zebra finch during run 6. Solid black rectangle, standard error; open rectangle, standard deviation; horizontal bar, mean; and vertical bar, range

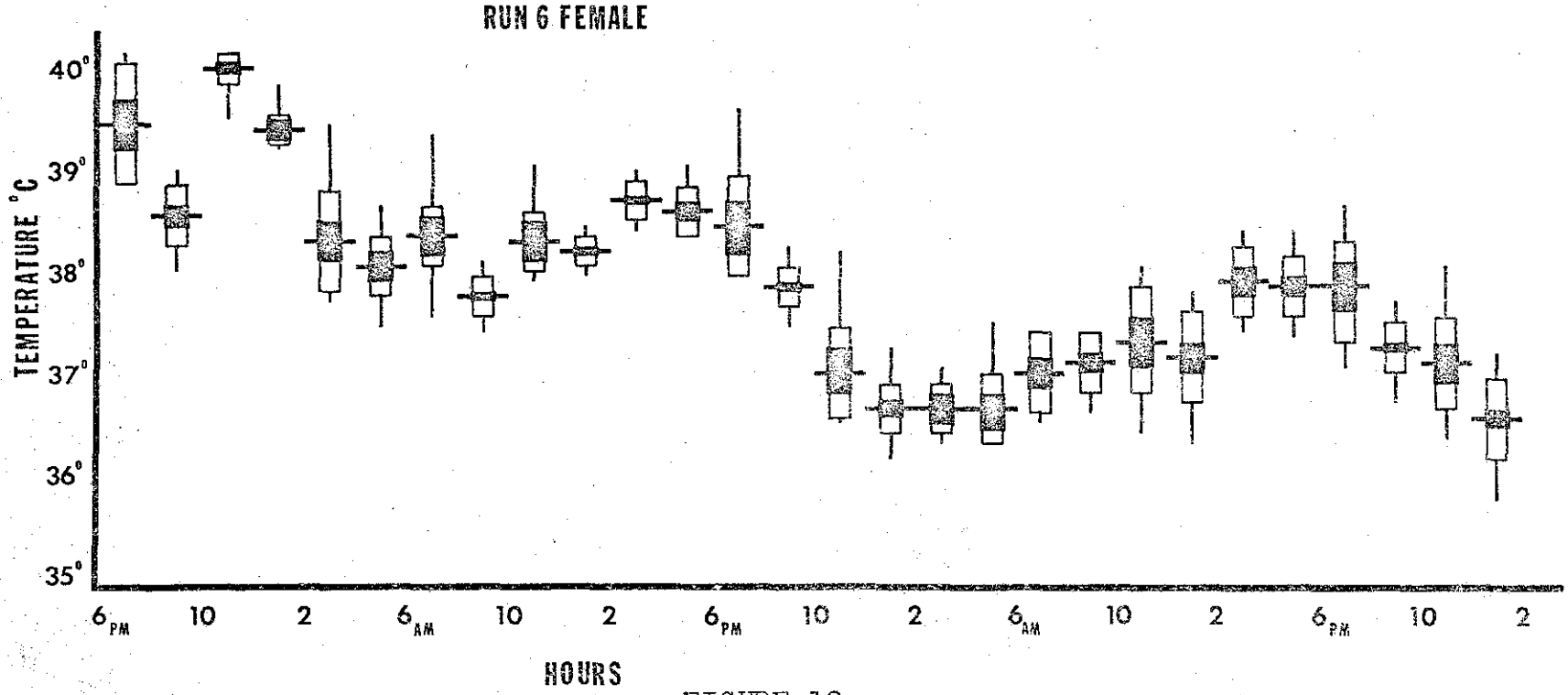


FIGURE 12

Surface body temperature of a female zebra finch during run 6. Solid black rectangle, standard error; open rectangle, standard deviation; horizontal bar, mean; and vertical bar, range

DISCUSSION

The characteristic pattern of surface body temperature in the zebra finch is a circadian cycle changing an average of 1.6° C between nocturnal lows and diurnal highs. The period of highest mean temperature occurs from 3 PM to 6 PM. The lowest temperature mean is from 12 AM to 3 AM during the nocturnal low level of activity. There appears to be no significant difference between male and female thermoregulatory cycles.

Circadian temperature cycles have been reported in the literature for several different species of birds (Bartholomew, and Cade 1957, Bartholomew and Dawson 1958, and Dawson 1958). Although there is nothing in the literature concerning thermoregulatory cycles in Old World Ploceid finches, Dawson (1954 and 1958) has described nocturnal low temperatures and diurnal high temperatures in several species of Fringillidae. The phenomena is not a new concept but one which is not fully explained.

Zebra finches, with average weights of 13 grams and high metabolic rates, must combat a high surface to volume ratio (Zeuthen 1953 and Kleiber 1947), in order to conserve energy. Zebra finches in the wild are subject to the Australian drought and the low nocturnal temperatures typical of a desert environment. These conditions would seem to point to the need for a mechanism for the conservation

of energy.

Inactivity during periods of darkness is a common method of energy conservation for most animals. A high level of activity is costly in that muscular heat production requires the use of metabolic stores. The high periods of temperature during 3 PM to 6 PM were not associated with higher levels of activity in this experiment. Therefore, the temperature increase was not due to muscular heat production but some other factor. Similar temperature phenomena occurs in the ruby-throated hummingbird, Archilochus colubris, (Pearson 1953) where the metabolic rate of the hummingbird reaches its maximum between 5 PM and 6 PM. Also Gambel quail (Lophortyn gambelii) exhibit a period of high temperature just before 6 PM (Woodard and Mather 1964). King and Farner (1961) state that the measurement of avian body temperature in the absorptive state will be higher than normal because of chemical thermogenesis. Zebra finches feed in the late afternoon at a time of little increase in activity but of a predictable increase in surface body temperature due to digestion. Although no explanation is provided for the increase in temperature before nocturnal quiescence in either Gambel quail or ruby-throated hummingbird it is suggested here that it is a similar phenomena to that occurring in zebra finches.

Torpidity in birds has been described for the white-throated swift, Anna hummingbird, and poor-will (Bartholomew,

Howell, and Cade 1957). These birds have adapted to periods of low ambient temperature and lack of food by dropping core body temperature, decreasing oxygen consumption, and slowing heart rate (Bartholomew, Hudson, and Howell 1962). Torpidity is used as a method of energy conservation during periods when the cost of maintaining normal body temperature would be too great. Nocturnal hypothermia and low levels of activity shown for the zebra finch in this experiment are mechanisms for energy conservation which are not unlike the more marked states of torpidity described above.

SUMMARY

The consistent circadian thermoregulatory cycle of zebra finches (Poephilia guttata) was measured by radiobiotelemetry while the birds were kept at optimum conditions. It was found that zebra finches exhibit maximum body temperatures between 3 PM and 6 PM, and minimum body temperatures between 12 AM and 3 AM with an average variation of 1.6° C. The circadian thermoregulatory cycles corresponded with high diurnal and low nocturnal activity levels.

The importance of obtaining information on complete body temperature cycles instead of on an isolated event is mandatory for a full understanding of normal thermoregulatory patterns and alterations in those patterns. Further investigations using the data presented above as a normal, and then varying some condition (food, water, or ambient temperature) would, in the author's opinion, show a previously unsuspected ability of zebra finches to conserve energy through nocturnal hypothermia and lowered levels of activity.

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