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BEHAVIORAL THERMOREGULATION IN RAINBOW TROUT, SALMO GAIRDNERI, AS A FUNCTION OF DIETARY REGIME.

Michael A.H. GREGORY

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ABSTRACT

Behavioral thermoregulation in Rainbow trout, Salmo gairdneri, as a function of dietary regime.

Michael A.H. Gregory

Experiments were conducted with the objective of studying the thermoregulatory behavior of juvenile Rainbow trout, Salmo gairdneri, as a consequence of dietary restriction. An electronic, aquatic shuttlebox with continuous water replacement was developed to facilitate these studies. This device is equipped to provide a temporal, thermal gradient ranging from 7° to 27°C.

Test fish, 10 to 12 cm in length, were divided into three groups and fed either 0 or 2% of their wet weight or to satiation, on a once daily basis. This regime was maintained for two weeks at a constant acclimation temperature of 13.0°C (±0.5°C) prior to testing and was continued during the four to five day experimental phase in the shuttleboxes.

The average temperatures selected for each of the test groups were 14.1°C (S.D. = 1.2°C), 16.8°C (S.D. = 0.8°C), and 18.6°C (S.D. = 1.0°C) for the 0%, 2% and ad libitum diets respectively. The thermal preference was found to vary with the time of the day on a diel basis, the greatest differences being observed in the unfed test group. All three test groups displayed similar thermal preferences at three different times of the day, 6:00-8:00 a.m., 12:00-2:00 p.m., and 9:00 p.m., with divergent temperature selection during the remainder of the

twenty-four hour cycle.

Evidence is presented to support the hypothesis that the differences in thermoregulatory behavior between the test groups are due to compensatory adjustments effected to optimize metabolic energetics with respect to nutritional status.

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INTRODUCTION

In 1947, the eminent Canadian fish physiologist F.E. Fry postulated that fish might select their ambient temperature conditions in accordance with the thermal optima of various metabolic processes. In the intervening years several field studies (eg. Brett 1971; McDonald, 1973) have noted that at least some salmonids do undergo daily thermoregulatory changes which can be correlated with metabolic efficiency (Fry, 1947). Laboratory work has shown that the optimum temperature for a fish is a function of its nutritional status. However no work has as yet convincingly demonstrated that fish will actually modify their thermoregulatory patterns in conjunction with the energetic provisions of a particular feeding regime.

This thesis reviews the efforts of the author to address the hypothesis that fish may seek alternative thermoregulatory behavior when faced with restricted rations. The work described herein can be divided into two phases. The first phase consisted of designing and testing an operational electronic aquatic shuttlebox incorporating a system of continual water replacement. The second phase was composed of a series of experiments investigating the thermoregulatory behavior of juvenile Rainbow trout, Salmo gairdneri, as a consequence of several variables including test period duration and dietary restriction.

Review

(1) Thermal Biology and Fish

The capacity of most fish species to maintain a body temperature which is significantly different from the ambient water temperature is severely limited by their anatomical design and physiological function (Fry, 1971; Crawshaw, 1976). The vast majority of fish species can maintain no more than a 1°C difference between their core temperature and the ambient temperature (Dean, 1976). Most of the heat generated by the metabolic processes of the fish is lost through the body wall and fins, with a small amount being lost at the gills (Crawshaw, 1976).

For most piscine physiological processes which have been studied, there is a temperature dependence such that functional efficiency is maximal at some temperature, declining above or below this temperature (Brett, 1971; Fry, 1971). Consequently, in the face of a temperature change, fish must respond in a fashion which allows them to maintain some degree of metabolic homeostasis. Such responses may be in the form of a capacity adaptation involving changes in certain operational rate-functions. These may be coupled with alterations in the upper and lower lethal thermal limits, i.e. resistance adaptations. Both such responses are commonly termed acclimatory processes (Fry, 1971).

The lethal thermal limits represent the incipient lethal levels and effectively function as the boundaries of what are known as the zones of thermal resistance (Prosser, 1973; Brett, 1952). These

regions encompass temperatures at which a fish can survive for an indefinite period of time, but which are not normally frequented by the organism (Prosser, 1973). Within these zones of resistance is the zone of tolerance containing the temperature range commonly associated with the species (Brett, 1952).

Death from relatively low temperature is usually a consequence of a variety of metabolic and physiological breakdowns including loss of ionic pump functioning, inadequate energy production, breakdown of coupled reactions, and decreased cardiac and respiratory activity resulting in hypoxia (Prosser, 1973). A similar variety of breakdown produces mortality at excessively high temperatures. These include reduced oxygen transport by hemoglobin, increased cell membrane permeability, and enzyme and DNA denaturation.

Enzymes have thermolabile tertiary and quaternary structures, thus increasing temperatures cause progressive denaturation of protein structure (Prosser, 1973). With increasing denaturation there is a decreasing reaction rate, since the concentration of the undenaturated enzymes decrease. However, the increasing temperature increases molecular motion thereby acting to counter the decreasing reaction rate. As a result of these two counter-balancing actions, each enzymatically catalyzed reaction has a specific thermal optimum at which the rate is maximal.

Enzymes which can acclimate, at least partially, are usually those associated with energy liberation, such as those necessary for glycolysis, fatty oxidation, and electron transport (Prosser, 1973).

Enzymes which do not show acclimation are usually involved in the degradation of metabolic products (Prosser, 1973). Different tissues and physiological functions also show varying degrees of acclimation. For example, the Rainbow trout shows partial acclimation, while its liver shows overcompensation and its gills show no compensation (Evans et al., 1962). Some crayfish species show partial acclimation in the heart and gills, but none in the hepatopancreas (Bowler, 1963).

Besides these metabolic changes as a result of acclimation, it is possible to observe a shift in the zone of thermal tolerance, including the preferred and incipient lethal temperatures (Reynolds, 1980). Acclimation to a higher temperature will consequently increase the length of time an organism can survive at elevated temperature and raises the maximum temperature it can survive for a given period of time. However, a temperature will ultimately be reached that will be lethal to the organism, regardless of acclimation temperature. This is known as the 'ultimate incipient lethal temperature' (Brett, 1952).

Extensive work by Cherry et al. has shown that these tolerance zone shifts vary in their degree of displacement according to the group to which the fish species belongs (Cherry et al., 1975 and 1977). With decreasing acclimation temperatures, the difference between the acclimation temperature and the preferred temperature increases for cyprinids and centrarchids, and decreases for salmonids (Cherry et al., 1975). The reverse trend occurs with rising acclimation temperatures (Cherry et al., 1977). The difference between the upper and lower avoidance temperatures also increased with decreasing acclimation temperatures. This would indicate a different

response to ambient temperatures, and provides a basis for classification.

Taking a cue from these distinctions, Hokanson has devised three classes; stenothermic, mesothermic, and eurythermic; each defined according to criteria for gonadal growth, spawning, the physiological optimum temperature, and the ultimate upper incipient lethal temperature (Hokanson, 1977). Each class heading is prefixed by 'temperate', indicating the ability of most temperature climate species to survive near-freezing temperatures.

Such a classification based on temperature requirements for fish is useful in understanding ecological relations. In effect, the scheme recognizes three temperature niches for fish in a temperate climate. Cherry points out that the eurythermic minnows have the option of choosing the habitat with least predation, without a temperature limitation during most of the year (Cherry et al., 1975). Hence, they could isolate themselves, at least partially, from stenothermal predators, such as trout, since these would be very strongly limited by temperature. However, they could not effectively isolate themselves from eurythermic predators such as bass and sunfish, as these species could generally inhabit the waters they wish with little regard to temperature (Cherry et al., 1975).

(2) Behavioral Thermoregulation of Fish

Acclimatory processes, by definition, involve metabolic adaptations as a consequence of sustained exposure to a particular temperature. Fish are not limited in mobility and thus are not

constrained to remain at any particular 'site'. The thermodiscriminatory abilities of fish are known to be acute (Greer and Gardner, 1970 and 1974) and readily demonstrable in the form of temperature preference or avoidance. When such responses are performed in an integrative, functional manner, they embody what is operationally termed behavioral thermoregulation (Reynolds, 1977; McCauley, 1977; McCauley et al., 1973).

The indices most frequently focused upon in studies of behavioral thermoregulation are the 'preferred temperature' and the so-called 'final preferendum'. The preferred temperature can be defined as the temperature selected as a function of a particular acclimation state (Fry, 1971; McCauley and Casselman, 1981). Consequently, the final preferendum provides some indication of the temperature that a species is likely to be found in, as well as the temperature that a species requires for optimal functioning of critical activities (Magnuson et al., 1979; Reynolds, 1979; Lemke, 1977; Hokanson et al., 1977; Crawshaw, 1975; Prosser, 1973; Fry, 1971; McCauley and Huggins, 1979). Both preferred temperature and final preferendum are routinely determined in the laboratory using a 'gravitational' approach. This method involves exposing the test fish to a temperature gradient for a period of time, usually several days, allowing a stable distribution to develop, representative of a final preferendum (Reynolds, and Casterlin, 1979; Fry, 1971).

Research Objectives

The author's work had two objectives at the outset. The first objective of this study was to examine the phenomenon of behavioral thermoregulation in response to varying dietary rations. Though a vast amount of work has been done on the effects of temperature on numerous physiological and metabolic processes, very little has been done on the influence of the bioenergetic component of temperature selection (Javaid and Anderson, 1967; Reynolds and Casterlin, 1979; Brett, 1979). Because temperature can act as a controlling factor as well as a directive factor, there is clearly the potential for some form of feedback mechanism allowing a fish to modify its acute temperature preference in accordance with some particular bioenergetic state of the moment. Even though it has been shown in various studies that metabolic rates in ectotherms shift with changes in daily food ration (eg. Brett, 1979), few workers have examined the role of the daily ration on behavioral thermoregulation. What little has been done is somewhat paradoxical and rather inconclusive.

Examining temperature selection in a horizontal gradient with the marine fish Girella nigricans, Duodoroff observed that two weeks of starvation did not significantly alter the temperature preference (Duodoroff, 1938). Atlantic salmon, Salmo salar, also displayed no noticeable change in thermoregulatory behavior as a consequence of starvation over a period of 22 days (Javaid and Anderson, 1967). On the other hand, the same researchers found that both Salmo gairdneri and Salvelinus fontinalis selectively decreased their temperature

preference over the course of a two to three week starvation period (Javaid and Anderson, 1967).

The second objective of this study was to develop an experimental apparatus and methodology which would allow for such a study to be performed with confidence.

There are several disadvantages to the traditional spatial temperature gradient, including maintenance of relatively large bodies of water (Neill et al., 1972), 'desensitization' of fish to ambient temperature (Reynolds, 1977), labor-intensive monitoring systems, and a tendency for spatial drift of the temperature gradient itself.

One alternative devised by Neill et al. (1972) consists of an aquarium divided into two chambers by a partition. The partition contains a passageway permitting the resident test fish to traverse the partition. In so doing, the fish activates an electronic monitoring system such as photoelectric cells, which in turn control heating or cooling elements. The apparatus is designed to cool or warm, depending on the direction of passage taken by the fish, both chambers at equal rates (Neill et al., 1972: McCauley, 1977). Throughout the heating and cooling bouts, a temperature differential usually of several degrees centigrade is maintained in order to provide the test fish with a proximate orientation factor (Neill et al., 1972; Reynolds, 1977). This type of device affords the researcher the opportunity to electronically record such parameters as rates of passage, activity, and temperature preference automatically and continuously for periods of several days. In effect, the test fish operates the device, selecting its ambient thermal environment

continuously, all the while being electronically monitored. This methodology has been used with great success by several workers including Magnuson et al. (1972), Reynolds (1976, 1977, 1978, 1979, 1980) with Casterlin (1976, 1978a, 1978b, 1979b), with McCauley, Casterlin and Crawshaw (1976), with Casterlin, Mathey, et al. (1978), with Casterlin and Millington (1978) and with Covert (1977), and Beitinger et al. (1975).

This apparatus, commonly known as an aquatic shuttlebox (Neill et al., 1972; McCauley, 1977; Reynolds, 1977; Gregory et al., 1984) has a number of advantages over the traditional spatial temperature gradients. It can provide continuous information relating temperature preference in terms of precision and stability as a function of the time of day. It also affords a continuous thermal challenge requiring the experimental fish to actively control its ambient temperature.

The shuttlebox designs used to date were considered inadequate for our purposes for several reasons. The rates of temperature change range from 3 to 5°C per hour in most designs (Neill et al., 1972; Beitinger et al., 1974). A more rapid rate of temperature change would provide a greater thermal challenge with a concomitantly faster rate of reinforcement of the shuttling response, resulting in potentially tighter control of thermoregulatory behavior. Additionally, most current shuttleboxes involve static systems with no water replacement. This feature was deemed undesirable given that dietary rationing studies might require relatively long periods of experimental testing time. Thus it was considered necessary to

provide a system of continual water replacement in the shuttlebox used in this study.

With these considerations in mind, a design was sought which would permit realization of the stipulated experimental objectives regarding behavioral thermoregulation.

MATERIALS AND METHODS

The focal point of this work is our electronic shuttlebox with its water flow-through system. The development of this apparatus involved extensive system testing coupled with frequent modification. Two of the models are described herein. They represent two stages in the evolution of the experimental system, and for the purposes of this report will be referred to as Models 1 and 2. The experimental design of the relevant studies on behavioral thermoregulation is provided in the accompanying section under methods.

Shuttlebox - Model 1

The experimental shuttlebox consists of two chambers, 23.0 cm in length, 20.0 cm in height, and 20.0 cm in width, connected by a passageway located in a partition separating the two chambers (Figure 1a and b). This passageway, positioned 9.0 cm from the bottom, is 4.0 cm in width and 5.0 cm in length. The depth of water in the passageway is a function of the depth of water in the entire shuttlebox. Placed in one of the walls of this passageway is a pair of photoelectric transistors (Darlington #114F1), 1.5 cm from the bottom of the passageway and separated by a space of 2.5 cm. These photocells receive light from a pair of fiber-optic light guides (Valtec #112-3) which terminate in the equivalent position in the opposing wall of the passageway. The light guides are connected to a light source equipped with a red filter, located adjacent to the shuttlebox. A schematic diagram of the photocell control unit is

shown in Figure 2.

A probe equipped with a temperature transducer (Analog Devices #AD590) is positioned near the passageway in the partition. This probe is in turn connected to a regular flatbed recorder (Canlab #R2970-3) which records the temperature continuously throughout the experiment. A schematic of the electronic circuitry for this unit is shown in Figure 3.

Each chamber of the shuttlebox (Figure 4) is fitted with twelve holes arranged in two horizontal rows of three holes per side panel. The holes are placed at 6.5 cm intervals, the rows being 4.5 cm and 9.0 cm from the bottom respectively. These holes are formed by 2.5 cm lengths of P.V.C. pipe (7 mm I.D.) which begin in the wall and project to the exterior. To these projections, Tygon tubing is connected. These tubes lead to a drain. Each piece of tubing is equipped with a hose clamp, permitting regulation of the water outflow. There is also one outlet positioned 15 cm from the bottom of each end panel to provide for the overflow control.

Four 160 cm P.V.C. tubes (7 mm I.D.) run horizontally along the interior of the side panels, at a distance of 6.5 cm from the bottom. Each such pipe is perforated with seven 3 cm holes spaced at 2 cm intervals, facing the center of the chamber.

Located above the shuttlebox are two reservoirs, headboxes, containing water of two different temperatures. These headboxes both lead to a third, smaller reservoir, positioned below the headboxes (Figure 5). Water flow from the headboxes is regulated by means of a pair of electric (solenoid) valves (Skinner valve #V52DB2017), one

Figure 1: Diagrams illustrating longitudinal (a) and cross-sectional (b) features of shuttlebox, where:

A - inlet pipe

B - water level (adjustable)

C - outlet ports

D - perforations

E - partition

F - floor of passageway

G - light source (equipped with red filter)

H - fiber optic light guides

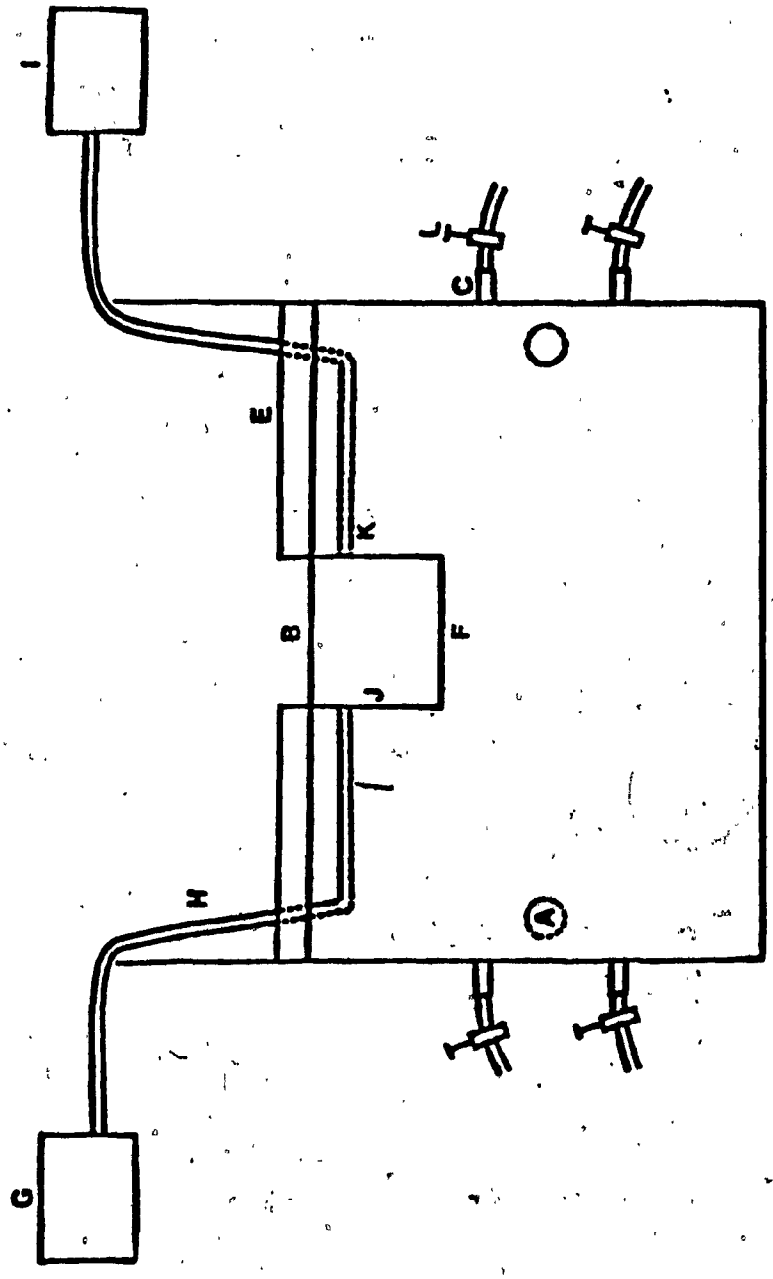
I - photocell - activated water temperature controls

J - wall of passageway

K - photocell

L - hose clamp.

1a



1b

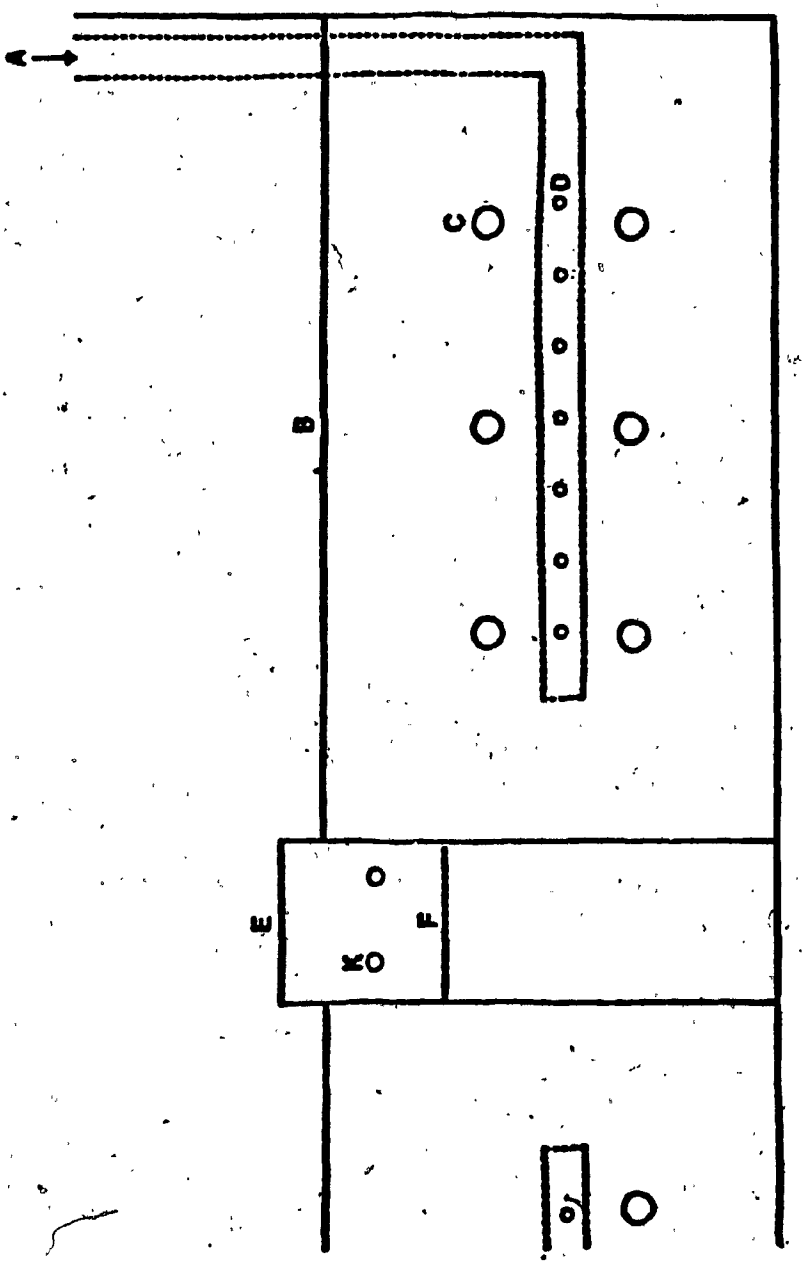


Figure 2: Schematic diagram of photocell activated water temperature control system, where:

A - fiber optic light guides (red-filtered)

B - photocells (GE-L14F1)

C - IC 7414

D - IC 7474

E - 4.7 K ohm

F - MPS-A12

G - solid state relay (CRYDOM S3714)

H - 22 ohm, 1W

I - 0.15 uf, 400 V

J - solenoid valve coil (Skinner valves #V52 DB2017).

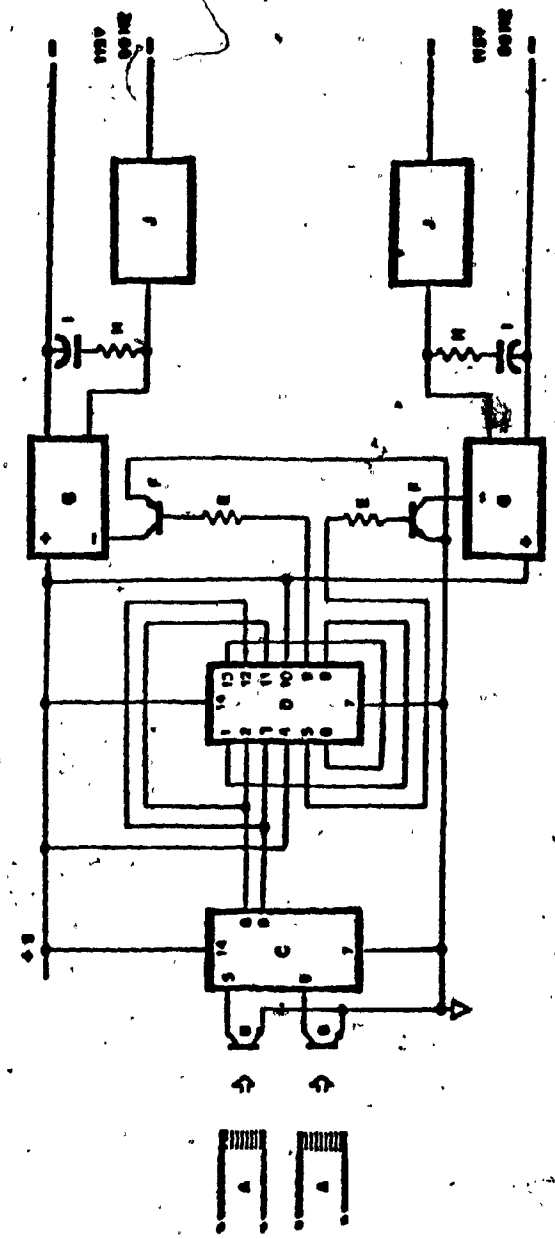
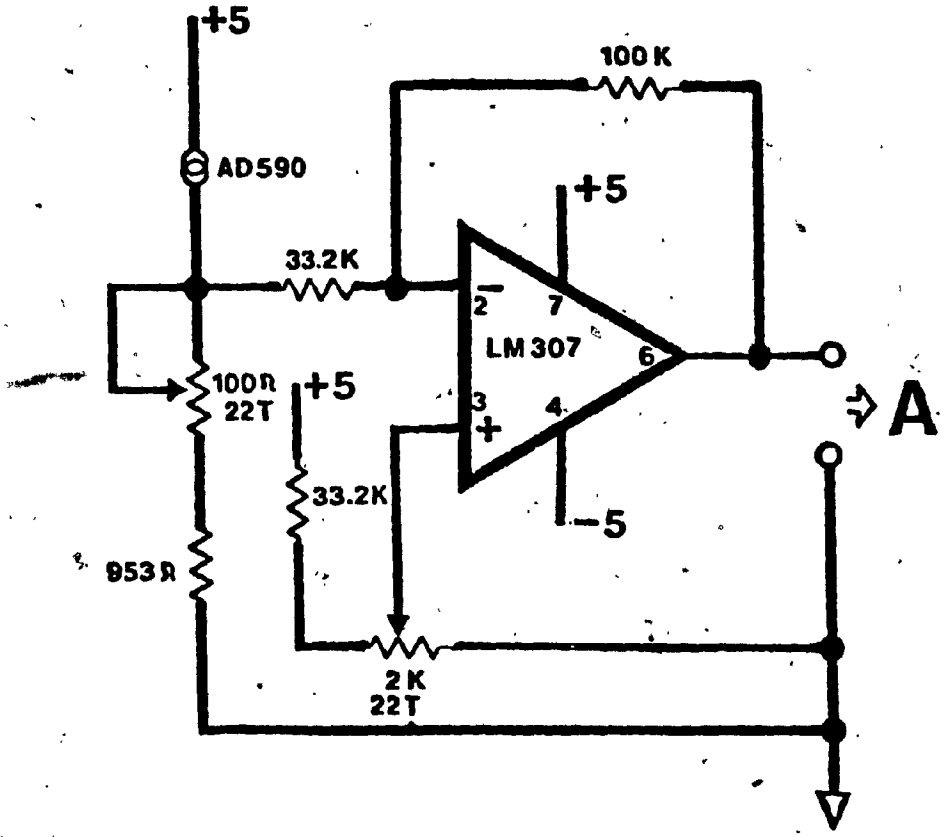


Figure 3: Schematic electronic temperature probe and amplifer where: terminals at (A) connect to recorder.


+5
100 K
AD590
+5
33.2K
2
7
LM 307
6
A
100Ω
22T
+5
3
4
33.2K
-5
953Ω
2 K
22T

valve per reservoir.

The smaller, third reservoir has four outlets, each of which is connected to one of the four perforated pipes positioned in the shuttlebox. Water flow into each pipe is regulated by means of a glass flowmeter (Manostat #36-514-310), one flowmeter per pipe. Water flow out of the box is accomplished via the twelve holes in each of the chambers. The water depth in the shuttlebox is normally set at 12 cm, through equalization of the rates of water inflow and outflow.

The third reservoir can act as a mixing chamber for the two water temperatures, by varying its volume via the standpipe. This adjustment provides some degree of regulation of the rate of temperature change in the shuttlebox. Additional control over the rate of change can be effected through the flowmeters. Both features must be adjusted in accordance with the experimental objectives.

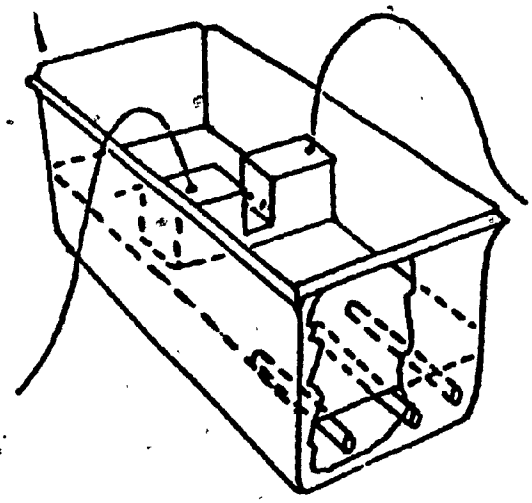
In practice, a fish swimming through the passageway will trigger the photocells. This event will open one solenoid valve and close the other. Swimming in the opposite direction will reverse the solenoid activity. In this manner, the fish may control its ambient water temperature. The photocell control unit is designed with the requirement that the fish swim completely through the passageway in order to effect a valve change.

Figure 4: Cut-away view of shuttleboxes.

a) Model 1

b) Model 2

(a)



(b)

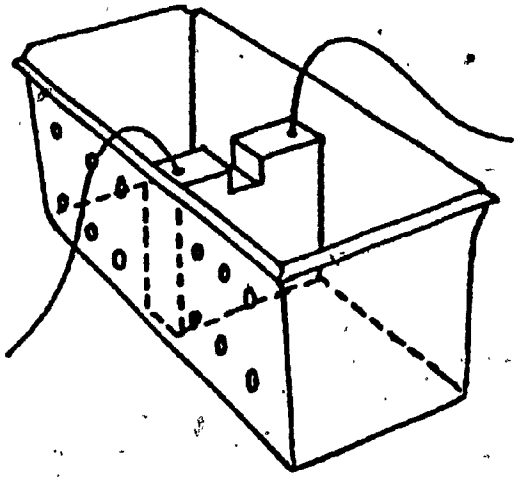


Figure 5: Flow diagram showing the water delivery system which permits a fish, through operant behavior within the shuttlebox, to titrate the thermal level of water, where:

A - headbox

C - headbox output

D - solenoid valve

E - mixing chamber

F - mixing chamber output

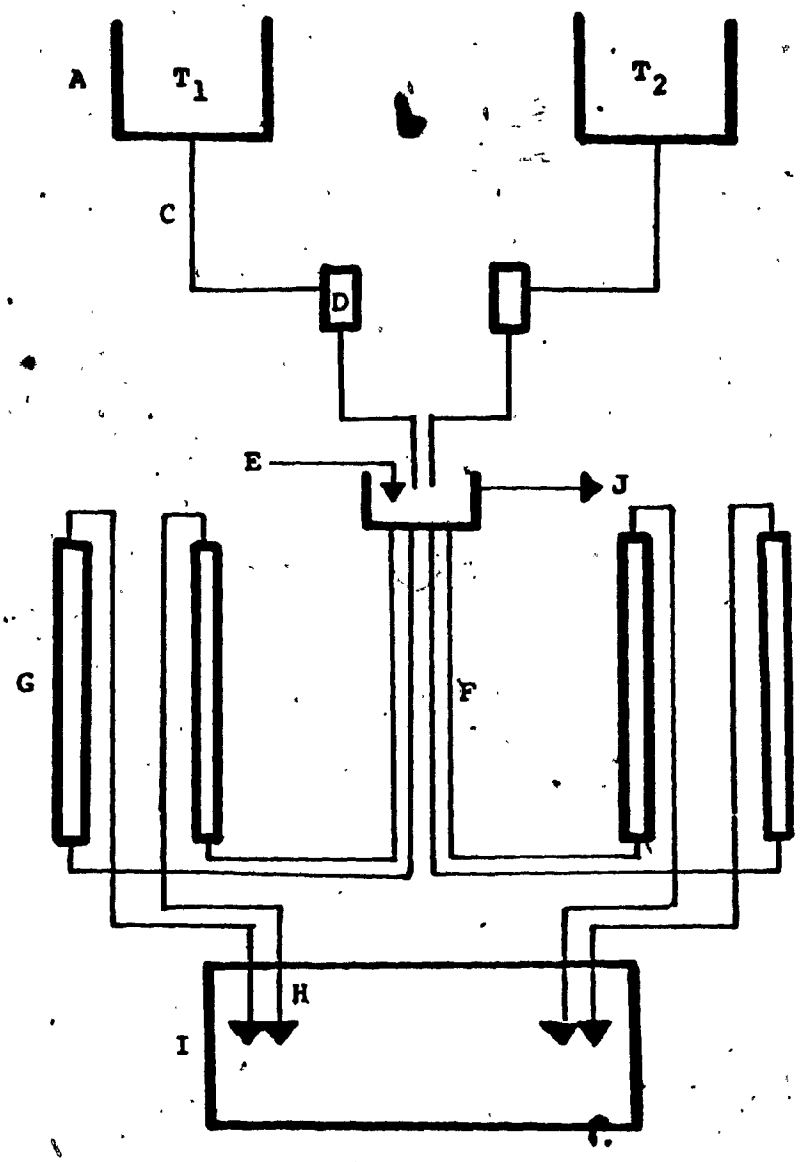
G - adjustable flowmeters

H - shuttlebox input pipes

I - shuttlebox

J - mixing chamber overflow outlet

T_1 and T_2 - cold and warm water sources respectively


A
T_1
T_2
C
D
E
J
G
F
H
I

Shuttlebox - Model 2

This apparatus consists of four identical and independent testing units. Each unit has several components; a shuttlebox, a single universal (3-way) valve, one mixing chamber, and the water conduit system equipped with manually regulated flowmeters. Additionally, each unit is monitored by a temperature sensitive chip located within a probe immersed in the shuttlebox. This probe transmits temperature information to a continuous recorder.

The shuttlebox itself consists of a watertight tank constituted entirely of polyvinylchloride (PVC) plastic. This unit is 42 cm in length, 21 cm in width and 18 cm in height. A partition is positioned medially within the tank, 17.2 cm from each end (refer to Figure 4b).

Projecting from each of the two end panels is a series of 3 PVC tubes, 16.5 cm in length (see Figure 6). Two tubes of 0.7 diameter I.D. (1.4 OD) are positioned 2.2 cm from the bottom and 2.0 cm from either side. The third tube is located along the longitudinal axis of the shuttlebox. This central tube is larger than the two peripheral tubes with a 1.0 cm I.D. (1.8 cm OD). All three tubes open through their respective mountings to the outside. The inner ends of all three tubes are sealed. Located along both sides of each tube is a series of perforations. These holes are sized and spaced in such a way that the rate of water flow through them is relatively consistent throughout the length of the tube (Figure 7). Also located in each of the two end panels are two other holes 0.8 cm in diameter and 1.5 cm in diameter, positioned 5.3 cm and 7.5 cm from the bottom respectively. Both are centrally located with respect to the sides.

The upper outlet can serve as an overflow outlet.

Sitting in a horizontal plane 3 cm from the bottom is a false floor consisting of a plastic mesh screen (0.3 cm hole size). This screen floor serves to divide the chamber vertically, the upper portion reserved for the experimental fish and the lower portion for the water conduits. This division eliminates the possibility of the fish seeking shelter amongst the conduits.

Partition

The partition, also fabricated from PVC, is 20.5 cm across, 3.2 cm in thickness, and 12.5 cm in height. A screen composed of plastic mesh (hole size 0.3 cm) is attached to the lower halves (the bottom 4.5 cm) of each side of the partition. This screen effectively limits fish passage from one side of the partition to the other but does not interfere with water flow. The presence of the partition reduces the tank to two chambers, 20.3 cm in width and 17.2 cm in length.

The partition contains an indentation in the center of the upper half, as shown in figure 8. This indentation is 4 cm in width and 3.2 in length (i.e. the thickness of the partition). The bottom of the indentation is located 3 cm from the bottom of the tank. When the tank is filled with the operational volume of water, this indentation serves as a passageway from one chamber to the other. As such, it is the only means by which the fish can move across the partition.

Placed in one of the walls of this passageway is a pair of photoelectric transistors (Darlington #114F1) 1.3 cm from the bottom of the passageway and separated by a space of 2.5 cm. The photocells

Figure 6: Transverse sectional view of Model 2 shuttlebox, where:

A - water inlet tube

B - water outlet tube

C - auxiliary outlet

D - water level control outlet

E - partition

F - passageway wall

G - water level

H - false floor (screen)

I - passageway floor

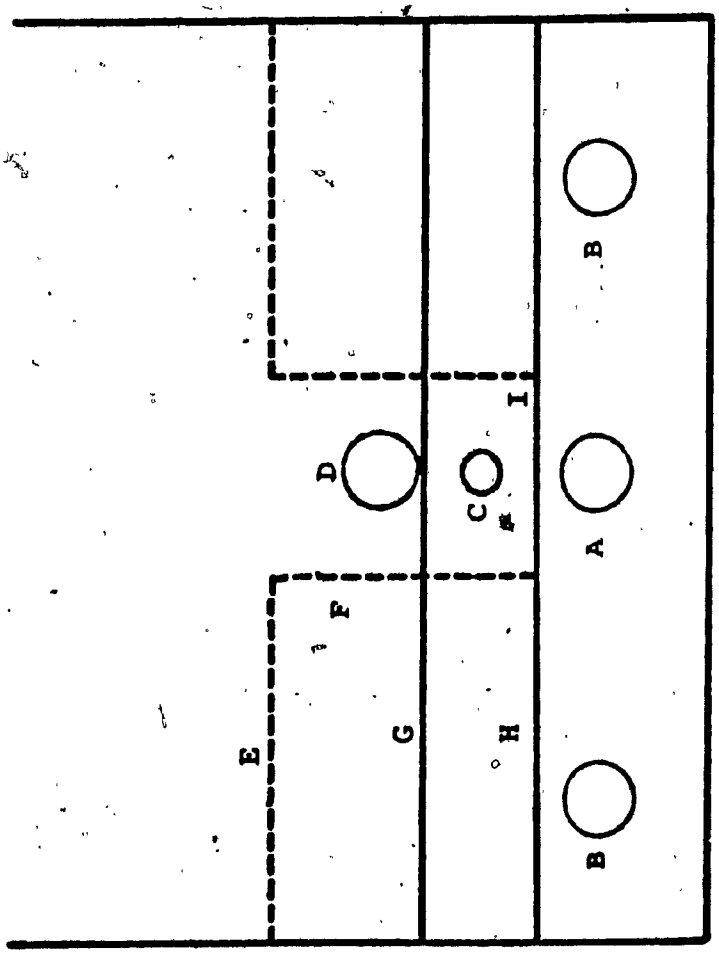

B
I
D
C
A
F
E
G
H
B

Figure 7: Longitudinal section view of Model 2 shuttlebox, where:

A - water inlet tube

B - water outlet tube

C - auxiliary outlet

D - water level control outlet

E - partition

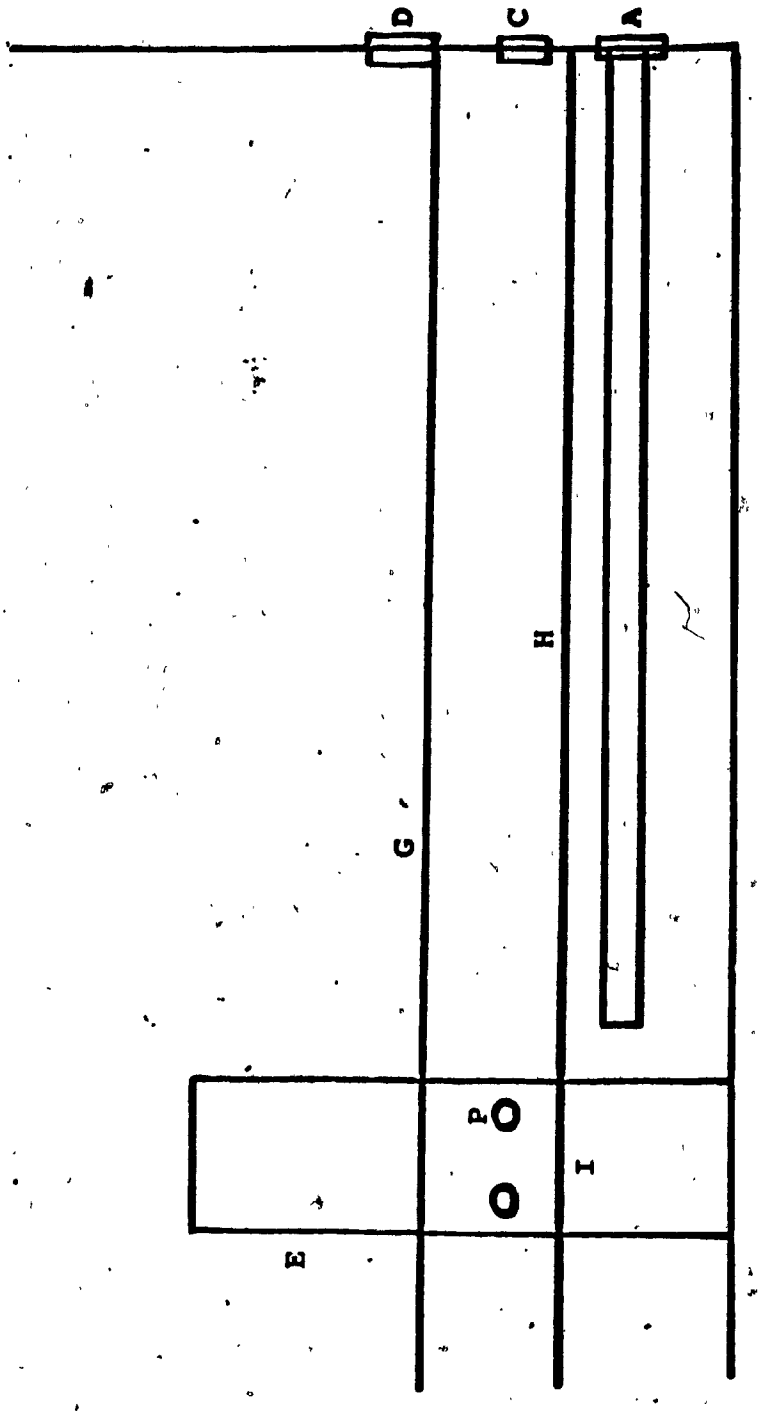
F - passageway wall

G - water level

H - false floor

I - passageway floor

P - photo transistors


D
C
A
H
G
P
I
E

receive light from the terminal ends of a pair of fiber-optic light guides positioned directly opposite the photocells in the facing wall of the passageway. The light guides are connected to a light source equipped with a red filter.

Water-Delivery System

Two water reservoirs are positioned above the apparatus, each provided with a continuous source of water maintained at specific temperatures. For studies of behavioral thermoregulation the two reservoirs would normally be kept at different temperatures. Both reservoirs lead to a universal (3-way) solenoid valve which in turn leads to a third reservoir positioned below the valve. This third reservoir acts as both a mixing chamber for the water it receives as well as a headbox, maintaining adequate water pressure to drive the system.

The volume of the mixing chamber is a function of the height of its standpipe. The flowrate of water into this chamber is a function of the height differential, i.e. the functional 'head' between the upper reservoirs and the lower mixing chamber.

The mixing chamber has two outlets besides the previously mentioned standpipe. Both outlets lead to the shuttlebox via similar yet independent routes composed of glass tubing each terminating on one of the two external mountings of the central-tubes in the box end panels. Thus water enters the shuttlebox by means of the central tubes. The flow rate of water into the shuttlebox is regulated by a pair of Manostat flowmeters (Manostat #36-514-310), one per tube.

Figure 8 Partition unit used in the Model 2 shuttlebox, where:

A - phototransistors

B - lens mountings

C - fiber optic light guides

D - electrical wiring

E - partition floor

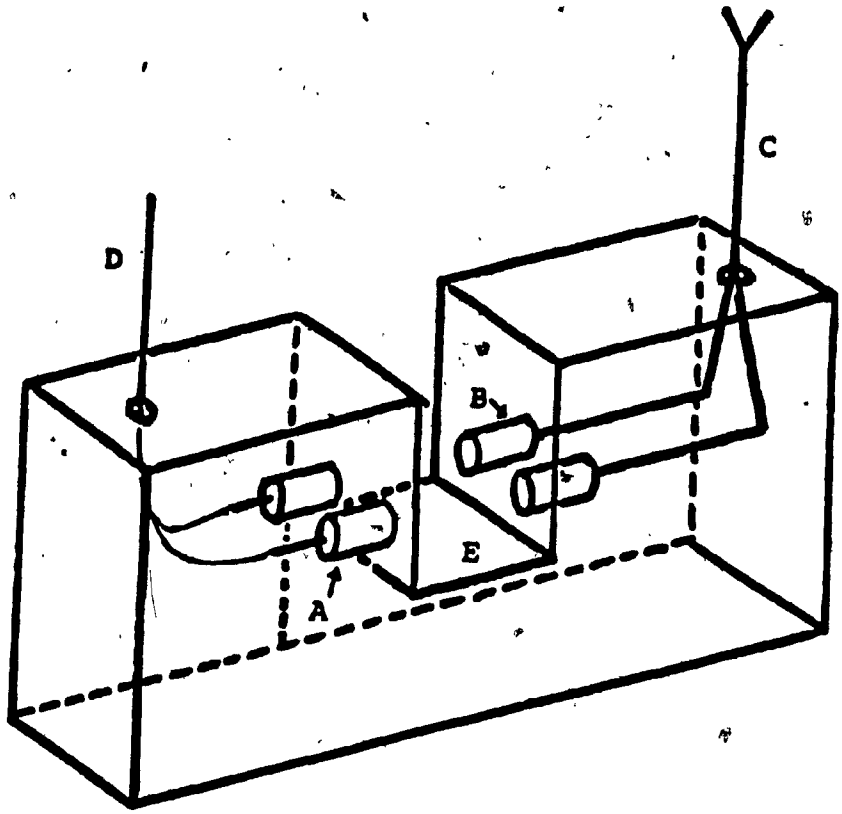

C
D
B
E
A

Figure 9: Schematic diagram of phototransistor control circuit, where:

A - light source (light guides)

B - phototransistors GE-L14F1

C - IC 7414

D - IC 7474

E - 4.7 K ohms

F - MPSA12

G - event marker

H - solid state relay

I - 3-way solenoid valve

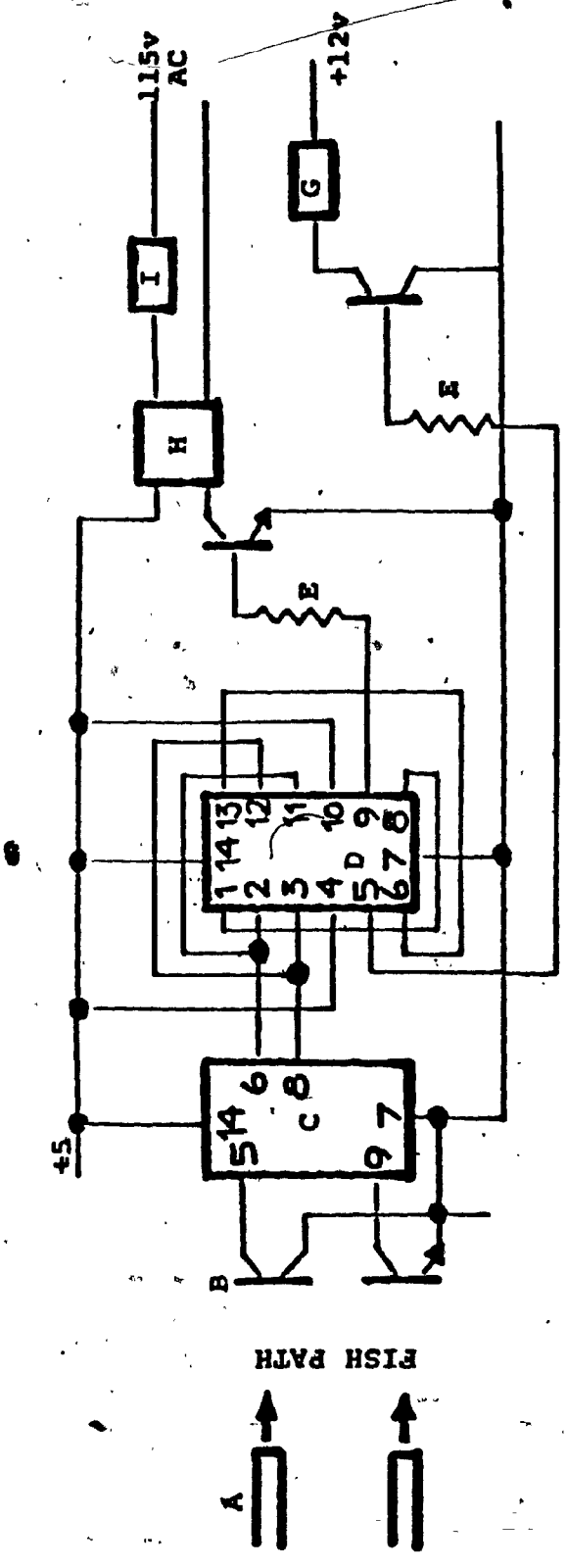

115v AC
+12v
G
I
H
F
E
D
C
B
A
+5
FISH PATH

Figure 10: Selection circuit for operational mode of Model 2 shuttlebox, where:

A - on

B - off

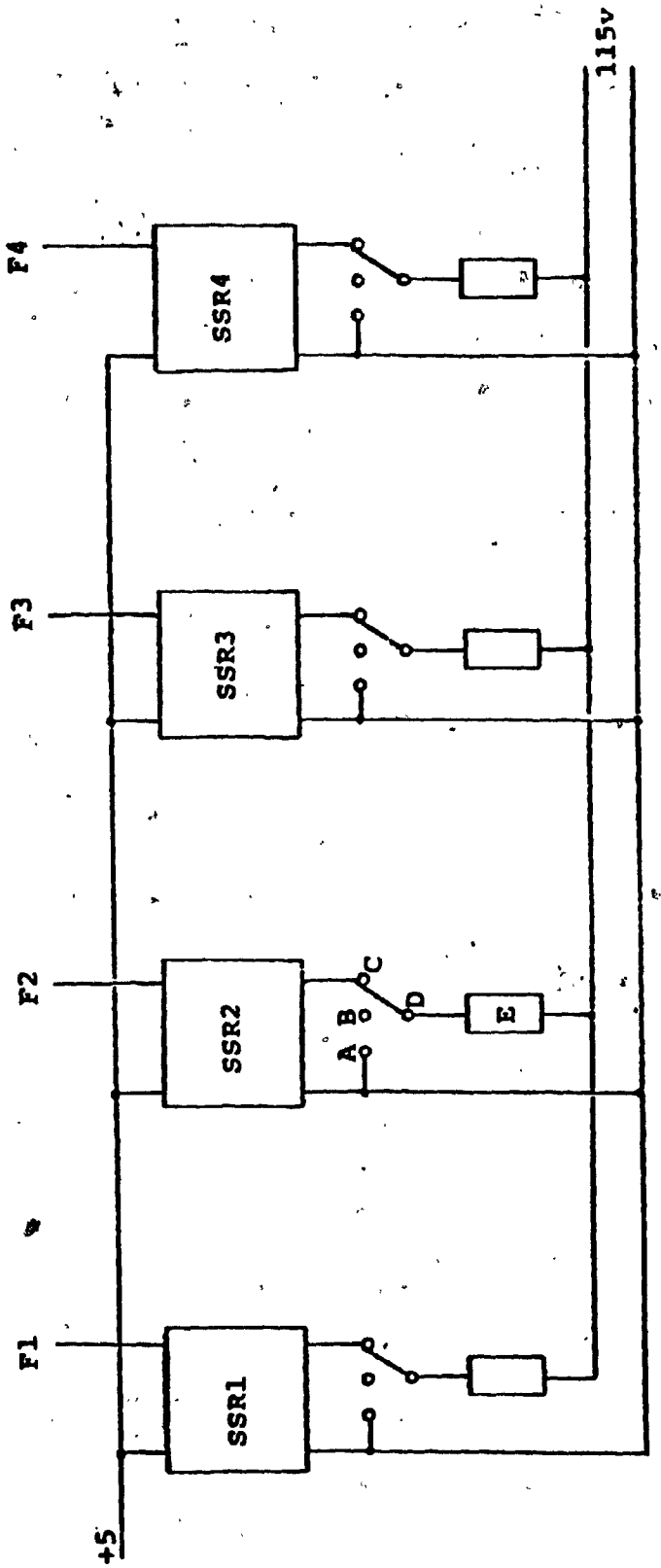
C - automatic operational mode

D - switch

E - solenoid valve

F - interface with control unit

SSR - solid state relay


115v
F4
SSR4
F3
SSR3
F2
SSR2
A
B
C
D
E
F1
SSR1
+5

Water-flow out of the shuttlebox is accomplished by the four peripheral tubes (two per chamber). These peripheral tubes act as conduits transporting the water taken in through the perforations to an exterior drain. Water flow through these conduits is regulated by means of adjustable pressure hose clamps mounted on the plastic tubing which is attached to the exterior projections of each of the four peripheral tubes. In practice, the water inflow should equal the water outflow, thereby maintaining a constant depth within the shuttlebox. The desired depth is a function of the size of the fish, i.e. the depth must permit the fish to pass easily through the passageway, and always within the range of the photocells.

The Photocell - Mediated Valve Control

The photoelectric transistors in the passageway are connected to a central control unit which in turn operates the appropriate solenoid valve. The electronic circuitry of the control unit is functionally similar to that of the Model 1 except that there is the provision for an event marker (recording fish passages in either direction) and a single 3-way solenoid valve is used instead of two 2-way valves (see Figure 9). The four independent control units are connected as illustrated in Figure 10.

This system operates in the same manner as its analog in the Model 1, that is, a fish passage triggers a change in the solenoid valve, switching it from one of its water sources (reservoirs) to the other. The direction of the fish passage is coded according to the sequence of activation of the photo-transistors, which in turn

Figure 11: Schematic diagram of the temperature probe circuit, where:

A - MC1403 (2.5 v REF)

B - 0.01 uf

C - 8.87 K ohms

D - 26.7 K ohms

E - offset control - 10 K ohms, 22T

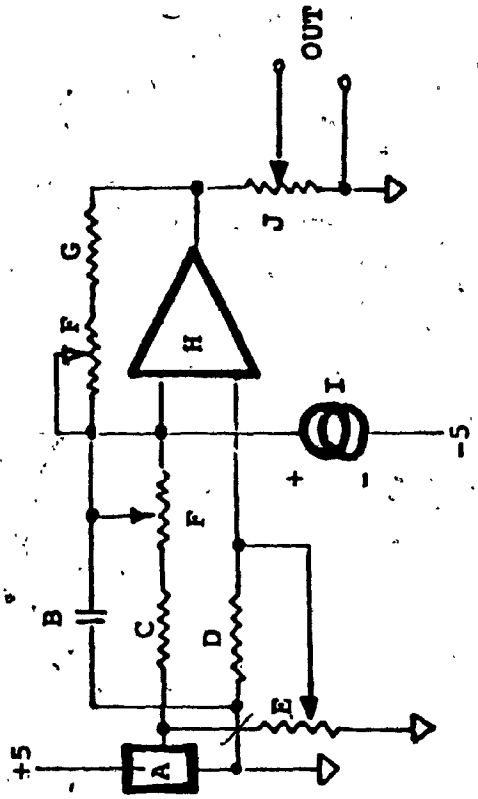
F - 500 K ohms, 22T

G - 9.76 K ohms

H - TL071

I - AD590 temperature sensor

J - 10 K ohms, 22T


OUT
J
G
F
H
I
-5
+
-
F
B
C
D
E
+5
A

dictates the reservoir to be triggered.

As can be seen from Figure 10, there is a provision for manual control of the valves. This allows the experimenter to operate the system independently of the test fish's activity.

Temperature Recording

A temperature sensitive micro-chip (AD 590) is located in a probe positioned at a depth of 3 cm near the passageway. Information is relayed by the circuit diagrammed in Figure 11 to a continuous recorder. The device used in conjunction with Model 2 was Narco Continuous Recorder #DMP 4-A (E & M Instrument Co.).

Experimental Fish

The fish used in this project were juvenile Rainbow trout, Salmo gairdneri, obtained from Pisciculture Mount Sutton (Sutton, Quebec). Fish were divided into groups of 50 and held in 200 L fiberglass tanks for 2 to 4 weeks prior to the implementation of experimental conditions. During this holding period, the water temperature was 13 ± 0.5°C and the photoperiod was 12 hours light, 12 hours dark. Both environmental parameters were regulated automatically. All fish were fed ad libitum with Martin Feed Mills #3 Trout food on a daily basis. The feeding times were randomized throughout the photophase.

METHODS

Experiment 1

The purpose of this experiment was to test the efficacy of the Model 1 shuttlebox through temperature preference testing. Rainbow trout, 10-12 cm in length (11.0-18.0 gm) were acclimated to 13.0 ± 0.5°C and a 12:12 photoperiod for a minimum of 3 weeks. During this time period, they were fed ad libitum once daily (Martin Feed Mills #3).

The apparatus (Model 1) was adjusted such that the rates of both warming and cooling were approximately 1°C/min. The two reservoirs were provided with 7° and 27°C water respectively.

A total of 8 fish were tested individually according to the following protocol: after a thirty minute period allowing the fish to familiarize itself with the interior, the shuttlebox was activated, permitting the 27°C water to flow in. All fish were tested for a minimum of three days, in accordance with the guidelines set forth by Reynolds and Casterlin (1979). The fish were not fed during the test period, as recommended by Richards (Richards et al., 1977).

Experiment 2

The purpose of Experiment 2 was to assess what alterations, if any, in the patterns of behavioral thermoregulation occurred as a result of dietary restriction under conditions of continuous exposure to the experimental contingencies for periods significantly longer than the conventional test periods. Juvenile Rainbow trout, 10-12 cm

(11.0-18.0 gms) were acclimated to the conditions described in Experiment 1 for a period of at least three weeks. Following this acclimation period, single fish were introduced into the shuttlebox (Model 1).

Individual experimental fish were introduced into the shuttlebox following the protocol outlined in Experiment 1. The apparatus was adjusted to provide rates of warming and cooling approximately 0.5°C/min. The two reservoirs were provided with 7°C and 27°C water respectively. A total of six fish were tested with the objective of maintaining normal thermoregulatory behavior for as long as possible without jeopardizing the health of the fish. Three of the fish were fed ad libitum on alternate days throughout the test period. The remaining three fish were not fed during the experimental period.

Experiment 3

The purpose of this series of tests was to examine in further detail the thermoregulatory trends observed in Experiment 2. Juvenile Rainbow trout 10-12 cm in length were acclimated to 13.5 ± 0.5°C and a 12:12 photoperiod for a period of at least three weeks prior to beginning experiments. Throughout this time they were fed once daily ad libitum with Marine Feed Mills #3 food.

For experimental purposes, the trout were then divided into three groups of twelve and held for two additional weeks (14 days) on different feeding regimes. The fish of one of the test groups were fed ad libitum on a daily basis, while a second group received only 2% of their wet weight in food. The third group was not fed during this

two-week period. Constant acclimation conditions (13.5°C and 12:12 LD) were maintained throughout the period.

Following this two-week schedule, each group was tested by placing individual fish in the shuttlebox (Model 2), following the protocol outlined in Experiment 1, and determining the temperature preferendum. Because of the design of the Model 2 system, four fish could be tested concurrently. Throughout the experimental period in question however, only three units were operated simultaneously, due to technical problems in one of the shuttleboxes. The fish were maintained continuously in the shuttleboxes for periods of four days (five in the case of Group #1). While in the shuttleboxes, the appropriate feeding regimes were continued without interruption. Feeding sites within the shuttleboxes were randomized in order to prevent bias.

RESULTS

Experiment 1

The mean preferred temperatures for each of six fish tested in Experiment 1 are shown in Table 1. Two of the test fish were unable to thermoregulate properly in the apparatus because of technical problems and were thus discounted. These mean temperatures were calculated on the basis of six measurements per hour for the entire twenty-four hours of the third day. The third day data only was used because it was assumed to be more representative of the actual preferred temperature of the fish (Reynolds & Casterlin, 1979). The final temperature preferendum, calculated as the mean of means, was 18.1°C with a standard deviation of 0.6°C.

Experiment 2

This preliminary study was conducted with the objective of examining the impact of dietary restriction upon thermoregulatory behavior. No working time limit was imposed, as this was considered to be an effort in preliminary data collecting. Because dietary restriction might not show any effect on thermoregulation over the short term, these experiments were run for as long as it was possible to maintain fish in apparent good health. Unfortunately, only one fish from Group #1 and two fish from Group #2 were able to effectively thermoregulate in the shuttleboxes for more than seven days. All three of these fish continued to thermoregulate for at least 24 days. Indeed, Fish #2 of Group 2 effectively shuttled in the apparatus for

TABLE 1

Third-day temperature preferendum for each of six Rainbow trout, 10-12 cm in length (pre-experimental acclimation temperature = 13 ± 1°C). Six measurements were made per hour for a period of 24 hours.

Fish #	Temperature Preferendum (°C)	S.D. (±)
1	18.2	0.9
2	17.4	1.2
3	17.8	0.6
4	18.0	1.1
5	19.1	0.5
6	17.9	0.3
Group Mean	18.1	0.6

42 days, at which point it was removed because of time restrictions. Due to an assortment of technical problems including fluctuations in reservoir water temperature, water flow-rates, recorder malfunctions and phototransistor failures, three fish were unable to exceed seven days experimental time in the shuttlebox. Though substitutions were attempted following aborted trials, none were adequately successful in generating additional data, as the technical problems persisted. Further substitution was deemed unnecessary in view of the amount of time already invested in such a preliminary study. Efforts to remedy the various technical problems led to the development of the Model 2 shuttlebox utilized in the next series of experiments (Experiment #3).

In order to obtain some idea of thermoregulatory trends over the extended experimental time of several weeks, temperature measurements at 12 hour intervals were obtained from the physiograph-recorder. As two of the fish (#1 Group 1; #1, Group 2) were run in the apparatus for 24 days, only the first 24 days of data for fish #2, Group 2 were used for ease of comparison. These temperature readings, shown in Table 2, were graphed in Figures 12a, b, and c. Figure 12a displays the thermoregulatory pattern of Fish 1, Group 1 over the entire 24 day period. Group 2 data demonstrated a trend of gradual cooling during the middle third of the experimental period. This trend became especially well-defined after approximately 10 days in the apparatus. Following some five to eight days of these decreasing temperatures, there was an indication of some stability in temperature selection, as evidenced by a consistency in selected temperatures, which remained

TABLE 2

Temperatures (°C) selected by juvenile Rainbow trout in Experiment 2. Observations recorded at 12 hr intervals.

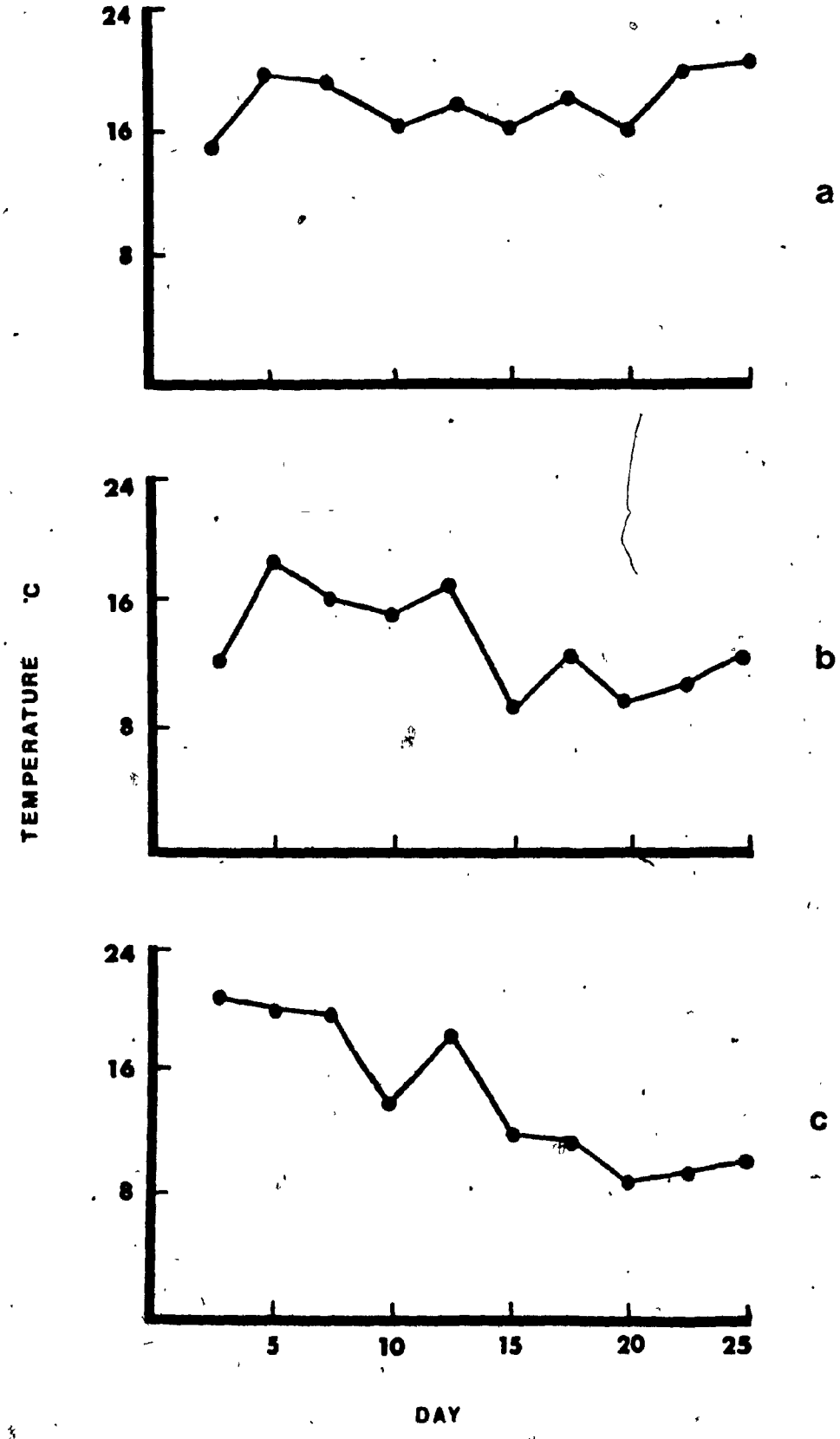
Observation No.	Group 1 Fish 1	Group 2 Fish 1	Group 2 Fish 2
1	19.2	13.2	17.0
2	15.3	10.5	18.5
3	18.7	10.4	20.5
4	18.2	9.5	19.2
5	15.0	11.0	21.4
6	14.4	9.5	20.4
7	17.6	18.0	18.2
8	19.6	18.4	17.6
9	19.2	18.6	19.5
10	19.8	17.5	19.2
11	17.0	16.5	20.8
12	16.8	17.7	19.5
13	13.4	17.2	18.5
14	15.7	14.6	17.2
15	18.9	15.6	18.8
16	18.4	18.8	17.1
17	19.0	18.4	15.8
18	20.7	14.6	17.2
19	17.1	15.2	14.6
20	16.7	14.4	20.5
21	18.6	14.8	20.0
22	16.5	10.4	20.0
23	17.0	12.8	20.0
24	18.6	13.8	19.0
25	17.4	17.0	18.0
26	18.0	14.4	17.0
27	18.2	10.6	16.2
28	19.1	9.0	17.5
29	17.8	9.2	13.8
30	16.3	9.3	13.1
31	17.1	9.2	12.8
32	16.5	10.2	14.5
33	16.0	11.2	12.0
34	15.6	10.6	10.8
35	17.7	14.2	12.8
36	17.4	16.4	13.2
37	18.0	9.6	12.3
38	16.9	9.8	11.0
39	15.8	10.0	9.8
40	15.9	10.0	9.0
41	18.0	10.0	9.6
42	17.2	12.0	10.3
43	19.2	10.6	9.4
44	18.3	10.0	9.7
45	20.7	10.0	9.2
46	21.8	10.0	9.4
47	20.5	10.0	9.4
48	20.2	11.5	9.4

Figure 12: Selected temperatures by juvenile Rainbow trout, recorded at 12 hr intervals.

a) Fish #1, Group 1

b) Fish #1, Group 2

c) Fish #2, Group 2


24
16
8
a
24
16
8
b
TEMPERATURE °C
24
16
8
c
5
10
15
20
25
DAY

close to the lowest temperatures available.

To ascertain the validity of these observations, values for the slopes of the regression lines were determined for each of the three fish. The equations for these regression lines, as well as their slopes are shown in Table 3.

The slope of the line described by Equation #1 was 0.27, indicating a relatively flat regression line. The slopes of the lines described by Equations #2 and #3 were -0.15 and -0.30. Both Group 2 lines showed inverse linear correlation, the line for Fish #2 data being especially pronounced with a correlation coefficient of -0.86.

Experiment 3

Daily mean temperature preferences were calculated for each fish of each group for days, two through five (day five in the case of Group 1). Readings from the first day were discounted, so as to allow 24 hours for fish accustomization to the experimental protocol.

These data, shown in Tables 4, 5, and 6 were then used to determine whole group daily mean preferred temperatures by finding the mean of all of the individual mean preferred temperatures. These results are shown in Table 7. Figure 13 portrays these daily means according to group.

In order to determine whether there were actual significant differences between each of the lines plotted in Figure 13, a post-hoc test, the Neuman-Keuls procedure, was performed. This analysis necessitated calculation of the mean temperatures for the entire experimental period for each group. These means of means are shown in

TABLE 3

Regression line equations for Experiment 2 juvenile Rainbow trout.

Group	Fish	Regression Line	Correlation Coefficient	Slope	S.E.
1	1	$y = 0.027(x) + 17.2$	0.188	0.027	0.023
2	1	$y = -0.154(x) + 15.9$	-0.548	-0.154	0.038
2	2	$y = -0.305(x) + 21.7$	-0.868	-0.305	0.028

TABLE 4

Daily mean temperatures (°C) for Group 1 fish (unfed).

Fish	Day 2 Mean	Day 2 S.D.	Day 2 Var.	Day 3 Mean	Day 3 S.D.	Day 3 Var.	Day 4 Mean	Day 4 S.D.	Day 4 Var.	Day 5 Mean	Day 5 S.D.	Day 5 Var.
1	14.8	5.3	27.7	14.8	5.2	27.1	14.0	6.2	37.9	14.0	5.4	29.1
2	17.0	4.1	17.1	15.5	4.8	23.4	14.2	4.4	19.2	13.1	4.6	21.1
3	15.0	5.4	29.2	15.0	5.5	29.8	14.0	5.1	26.1	13.5	4.3	18.2
4	13.6	4.8	23.3	14.2	4.5	20.4	12.4	4.8	23.4	12.9	4.3	18.3
5	16.0	4.6	21.0	14.4	4.7	21.8	11.0	3.0	9.3	12.2	4.4	18.9
6	14.5	4.2	18.0	14.9	4.6	21.4	14.7	5.1	25.8	13.9	5.4	28.6
7	14.3	5.5	29.8	13.8	4.8	23.3	13.4	4.1	17.1	12.2	3.4	11.9
8	13.7	5.0	25.1	14.0	5.0	24.8	14.9	4.5	20.4	13.9	4.6	20.7

TABLE 5

Daily mean temperatures (°C) for Group 2 fish (daily ration equal to 2 per cent of wet weight).

	Day 2			Day 3			Day 4		
Fish	Mean	S.D.	Var.	Mean	S.D.	Var.	Mean	S.D.	Var.
1	16.2	3.9	15.2	16.5	2.4	6.0	15.7	3.0	8.9
2	16.5	3.1	9.6	15.7	2.8	7.8	16.6	3.0	9.2
3	16.4	3.1	9.8	16.9	3.2	10.3	16.5	3.0	9.0
4	16.2	3.6	13.2	16.9	3.9	15.2	16.1	4.2	17.6
5	16.2	4.3	18.9	16.8	4.3	18.8	16.1	3.9	15.1
6	17.3	4.8	23.3	17.3	3.9	14.9	17.5	2.9	8.7
7	17.9	2.8	8.0	18.0	3.2	10.1	18.8	2.5	6.2

TABLE 6

Daily mean temperatures (°C) for Group 3 fish (fed ad libitum)

	Day 2			Day 3			Day 4		
Fish	Mean	S.D.	Var.	Mean	S.D.	Var.	Mean	S.D.	Var.
1	18.3	3.8	14.3	18.2	3.1	9.6	20.5	3.6	13.3
2	17.7	2.6	6.7	18.5	3.8	14.3	18.6	3.4	11.6
3	19.9	3.4	11.6	18.8	2.8	7.7	18.2	2.4	6.0
4	16.6	4.5	20.7	17.2	3.6	13.0	18.3	4.7	21.7
5	19.8	3.2	10.1	19.5	2.6	6.9	20.5	2.9	8.3
6	18.8	3.6	12.9	18.7	2.4	5.5	17.9	2.4	5.7
7	19.5	3.1	9.4	17.8	3.3	11.2	18.0	2.9	8.7
8	18.6	3.7	13.6	19.0	2.8	7.8	18.7	2.1	4.5

TABLE 7

Group mean of the daily preferred temperature

(i.e. mean of individual daily mean preferred temperatures).

Group #	Day	Mean	S.D.	Var.	S.E.
1	2	14.86	1.15	1.33	0.40
	3	14.57	0.57	0.32	0.20
	4	13.57	1.29	1.69	0.46
2	2	16.67	0.67	0.45	0.25
	3	16.87	0.70	0.49	0.26
	4	16.76	1.06	1.13	0.40
3	2	18.65	1.12	1.26	0.40
	3	18.46	0.72	0.52	0.25
	4	18.84	1.06	1.13	0.37

Figure 13: Group daily mean preferred temperatures (mean of mean) for Experiment 3. (● = Group 1; ■ = Group 2; ▲ = Group 3).

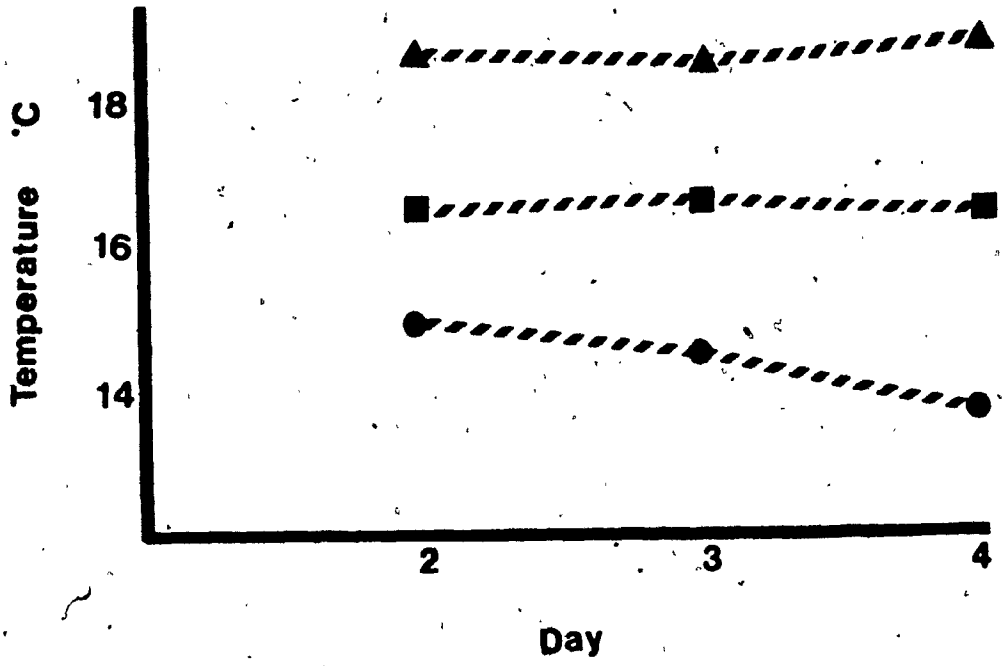

Temperature °C
18
16
14
2
3
4
Day

Table 8. As can be seen from Table 9, the differences between the means is greater in each case than the critical values determined via the Neuman-Keuls procedure. Consequently, it was concluded that the differences between the group means were significant.

In order to explore the causes of the differences between the groups, two factors were cross-examined using a two-way ANOVA test with repeated measures dependent on one factor. The potential source of variation between groups was assumed to be diet ration, while the potential source of variation within groups according to data was assumed to be the day in the experiment on which the measurements were taken. As can be seen in Table 10, the F ratio for ration as a factor was 68.17 with an F-value of 3.49 at 2 and 20 degrees of freedom. The experimental day as a parameter had an F-ratio of only 1.129 with an F-value of 3.23 at 2 and 40 degrees of freedom. The relatively large F-ratio for factor A given its F-value, and compared with the rather small F ratio for factor B indicates that the diet ration parameter was primarily responsible for the observed variation seen in Tables 4, 5, and 6.

The Omega test, which checks for strength of association, was then conducted in order to more accurately interpret the findings of the ANOVA test. This test demonstrated that the variance associated with ration (factor A) accounted for approximately 64.5% of of the observed variance, while a mere 0.1% could be attributed to factor B (see Table 11). The total variance associated with the experimental parameters in question was 67.6%.

TABLE 8

Mean temperatures (mean of means) for entire experimental period (grouped data).

	Mean (°C)	S.D.	Var.	n	Experimental period (days)
Group 1	14.1	1.2	1.4	8	5
Group 2	16.8	0.8	0.6	7	4
Group 3	18.6	1.0	1.0	8	4

TABLE 9

Post-hoc test on temperature means using the Neuman-Keuls Procedure.

Group #	1	2	3	
Group Mean of Means (°C)	14.1	16.8	18.6	r
14.1		2.7	4.5	3
16.8			1.8	2
18.6				

n_K = 7.3 (harmonic mean)

$$S_A = \sqrt{\frac{MS_A \times \text{subject within groups}}{mp}} = \sqrt{\frac{1.571}{7.3 \times 3}} = 0.26$$

r = 2	0.26	x	2.95	= 0.767	(0.05)
	0.26	x	4.02	= 1.04	(0.01)
r = 3	0.26	x	3.58	= 0.931	(0.05)
	0.26	x	4.64	= 1.206	(0.01)

TABLE 10

Two-way analysis of variance with repeated measures dependent upon one factor testing variation between groups due to ration and within groups due to day (Experiment 3)

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F-Ratio
Between Subjects		22		
Factor A*	214.1652	2	107.0826	68.1702
Subjects Within/Groups	31.4162	20	1.5708	
Within Subjects				
Factor B**	1.4008	2	0.7004	1.1298
A x B Interaction	6.2652	4	1.5663	2.5267
B x Subjects Within/Groups	24.7957	40	0.6198	

F-Ratio

Factor A*	(2,20) =	68.1702	F value (5%) =	3.49
Factor B**	(2,40) =	1.1298	F value (5%) =	3.23
A x B Inter.	(4,40) =	2.5267	F value =	2.61

* Factor A = Temperatures selected as a function of ration.
** Factor B = Temperatures selected as a function of test day.

TABLE 11

Test for strength of association for two-way ANOVA examining the variance in temperature selection associated with either ration or test day (estimate of Omega squares).

Variance associated with Factor A* =	64.5%
Variance associated with Factor B** =	0.1%
Total variance associated with both parameters =	67.6%

* Factor A = Temperatures selected as a function of ration.
** Factor B = Temperatures selected as a function of test day.

Tables 12 through 14 list the hourly mean temperature preferenda for the entire experimental period for each fish of each group. Each temperature shown represents the mean of the temperatures for a given hour on days two through four of the experimental period.

In order to better appreciate the hourly temperature preferenda as a function of the experimental design, the hourly means for all fish within each group were averaged to produce a group hourly mean temperature (tables 15, 16, and 17). These data, plotted in Figure 14, showed an apparently distinct thermoregulatory pattern for each of the three test groups, with a tendency toward convergence at certain specific times of the day.

To further elucidate the causes of these observed trends, parameters of ration and time of day were subjected to an ANOVA test (Table 18). When the results of this test were tested for strength of association (Table 19) the variance associated with the ration (factor A) was only 7.9%, whereas that associated with time of day was 67.5%. Furthermore, interactions between both parameters were responsible for 13.3% of the variance in the data. These data are especially compelling considering that the total variance associated with the experimental parameters was 88.7%, indicative of a reliable experimental protocol.

The consistency of the convergences in the temperature preferenda shown in Figure 14 in all experimental groups suggests the possibility of a rhythm of thermoregulatory behavior within the 24 hour period. Thus, all temperature recordings from Experiment 3 were examined for possible rhythms of whole-hour frequencies. The frequencies or

TABLE 12

Cumulative hourly mean temperature preferenda (°C)

for individual fish (Group 1)

Time of Day	Fish # 1	2	3	4	5	6	7
00:00h	8.1	13.5	10.5	10.5	13.3	12.0	10.3
01:00	8.0	12.7	8.4	9.4	8.8	8.9	8.0
02:00	8.8	9.9	8.1	9.8	8.3	8.7	8.7
03:00	9.1	8.9	8.4	10.8	8.6	8.9	9.1
04:00	12.5	8.9	8.8	10.8	10.1	9.3	9.1
05:00	19.7	13.2	11.9	15.2	13.7	13.3	18.2
06:00	21.7	18.0	18.7	15.4	17.5	19.5	12.5
07:00	20.3	20.1	20.6	18.8	18.5	22.0	20.7
08:00	20.3	20.4	23.4	16.6	19.0	21.3	21.4
09:00	16.6	20.9	18.4	14.0	16.0	17.5	14.0
10:00	9.6	19.5	14.8	12.8	14.7	11.0	11.0
11:00	9.3	16.9	10.6	12.6	12.4	9.7	9.4
12:00	18.1	18.8	16.2	15.0	18.3	14.6	16.9
13:00	21.7	19.8	20.2	20.5	21.8	21.0	20.8
14:00	17.8	18.7	19.0	16.4	20.3	15.0	13.4
15:00	14.5	14.1	13.6	13.6	13.7	11.4	9.6
16:00	11.2	12.1	16.9	14.3	9.3	13.6	15.7
17:00	12.7	11.5	10.0	10.4	11.2	14.6	20.1
18:00	18.0	15.4	8.9	9.7	11.8	10.7	13.1
19:00	19.0	18.4	13.4	8.8	14.6	11.2	9.8
20:00	21.5	18.6	18.5	15.7	17.4	14.5	18.3
21:00	20.9	18.5	20.5	17.3	20.8	17.2	18.8
22:00	14.3	12.1	19.6	20.8	20.4	17.2	16.8
23:00	9.6	9.8	8.5	13.5	12.6	9.6	10.0

TABLE 13

Cumulative hourly mean temperature preferenda (°C)

for individual fish (Group 2)

Time of Day	Fish # 1	2	3	4	5	6	7
00:00h	10.7	13.3	15.7	14.7	11.7	10.0	17.3
01:00	13.7	13.7	13.7	13.0	12.0	12.3	15.7
02:00	12.3	12.0	11.3	12.3	11.7	13.0	15.6
03:00	12.0	17.0	14.0	13.0	11.7	15.3	14.5
04:00	15.0	16.7	17.0	14.0	9.3	16.7	17.0
05:00	11.3	16.3	17.0	17.3	16.0	17.7	17.5
06:00	15.7	13.7	19.7	17.3	20.0	17.7	19.4
07:00	18.3	17.0	18.0	15.3	21.0	19.3	21.8
08:00	15.0	16.7	19.7	15.3	18.7	20.0	23.4
09:00	18.0	20.7	18.0	19.0	20.3	18.0	20.0
10:00	17.0	20.3	15.7	16.0	19.0	19.0	19.3
11:00	19.0	19.0	15.0	15.7	18.3	18.0	17.3
12:00	18.3	17.0	17.0	21.3	22.0	21.3	20.0
13:00	17.0	17.3	16.3	20.7	18.3	19.3	21.1
14:00	16.7	16.7	14.7	18.3	19.0	17.7	19.3
15:00	19.0	15.7	16.0	18.0	16.7	17.3	19.0
16:00	15.3	17.0	18.0	15.0	15.7	16.3	18.2
17:00	18.0	14.0	20.0	15.3	16.0	17.3	15.5
18:00	14.7	14.0	17.3	16.3	16.3	15.7	16.8
19:00	15.3	18.3	21.0	17.0	17.3	16.7	17.0
20:00	17.3	19.7	17.7	19.3	17.3	16.7	18.8
21:00	19.0	17.7	20.3	21.3	17.7	23.3	19.8
22:00	18.3	16.0	11.7	20.7	16.0	23.0	21.1
23:00	13.0	9.7	10.0	10.7	9.3	9.7	14.8

TABLE 14

Cumulative hourly mean temperature preferenda (°C)

for individual fish (Group 3)

Time of Day	Fish # 1	2	3	4	5	6	7	8
00:00h	17.7	14.0	15.0	14.3	19.0	18.0	16.3	17.7
01:00	16.3	14.0	15.7	18.3	22.0	18.7	14.0	16.7
02:00	12.3	14.3	16.7	10.0	23.0	17.0	14.3	15.0
03:00	12.0	14.0	16.0	8.7	23.7	18.3	18.0	17.0
04:00	14.0	14.7	15.0	13.3	18.7	16.0	14.7	18.7
05:00	17.0	16.3	17.7	16.0	20.0	18.7	16.3	20.0
06:00	20.0	17.0	17.7	19.0	19.3	18.3	18.3	20.0
07:00	20.7	18.3	19.3	19.0	20.0	17.3	19.3	19.7
08:00	21.7	20.0	22.0	18.0	18.7	20.0	20.0	17.3
09:00	21.0	22.0	22.0	22.3	19.3	18.0	21.7	19.7
10:00	22.0	22.7	22.3	18.0	21.0	17.7	19.3	20.7
11:00	21.0	21.3	20.7	17.7	20.0	16.3	21.0	19.7
12:00	19.7	22.7	20.7	13.3	20.0	17.7	20.3	21.0
13:00	19.3	20.3	17.7	14.0	17.7	19.7	19.3	21.0
14:00	19.3	20.0	19.0	18.7	20.3	22.0	21.0	18.7
15:00	21.3	16.7	18.0	17.7	20.0	23.0	21.7	20.3
16:00	21.0	17.0	16.3	19.7	20.7	19.7	18.7	14.0
17:00	20.7	19.0	19.7	20.7	19.3	20.3	18.0	18.0
18:00	21.0	18.3	19.7	20.7	19.3	18.0	17.3	18.3
19:00	20.0	18.0	17.0	19.3	17.3	18.3	21.3	18.7
20:00	20.0	18.7	19.3	20.0	19.3	16.7	17.3	19.7
21:00	19.3	20.0	21.0	19.7	19.3	17.3	16.7	16.7
22:00	18.3	19.7	21.7	18.7	19.7	17.0	19.3	17.3
23:00	18.3	19.3	17.7	20.0	20.7	16.7	18.7	18.0

TABLE 15

Hourly temperature (°C) means derived from entire (5 day) experimental period (grouped data n = 8) for Group 1

Time of Day	Mean	Standard Deviation	Variance
00:00h	11.0	2.9	8.5
01:00	9.2	1.7	2.9
02:00	9.1	1.3	1.7
03:00	9.0	1.6	1.6
04:00	9.7	1.6	2.7
05:00	13.6	3.1	9.6
06:00	17.9	0.4	0.1
07:00	20.1	2.2	5.0
08:00	20.1	2.8	7.6
09:00	17.0	3.1	9.9
10:00	13.1	3.8	14.6
11:00	11.2	2.7	7.2
12:00	17.7	2.9	8.5
13:00	19.9	3.0	9.2
14:00	15.6	4.5	20.2
15:00	12.3	3.1	9.4
16:00	11.9	3.5	11.9
17:00	12.2	4.1	17.0
18:00	12.0	3.6	12.8
19:00	12.7	4.4	19.7
20:00	16.8	3.9	15.4
21:00	18.4	2.6	7.0
22:00	17.0	4.2	17.4
23:00	10.2	2.3	5.4

TABLE 16

Hourly temperature (°C) means derived from entire (4 day) experimental period (grouped data n = 7) for Group 2

Time of Day	Mean	Standard Deviation	Variance
00:00h	13.8	3.3	11.2
01:00	13.8	3.1	9.5
02:00	12.9	2.6	6.6
03:00	14.3	3.1	9.7
04:00	15.5	3.1	9.4
05:00	17.0	1.9	3.7
06:00	18.0	2.7	7.5
07:00	18.8	2.6	7.0
08:00	18.7	2.6	7.0
09:00	19.2	2.6	6.8
10:00	18.0	3.4	11.4
11:00	17.8	2.8	7.6
12:00	19.8	2.9	8.3
13:00	18.9	2.5	6.5
14:00	17.6	2.4	5.9
15:00	18.0	2.6	6.7
16:00	17.0	2.3	5.2
17:00	16.8	2.6	6.6
18:00	16.4	2.2	4.9
19:00	17.7	2.5	6.1
20:00	18.7	1.9	3.6
21:00	19.5	2.8	7.7
22:00	18.0	4.3	18.8
23:00	12.4	3.4	11.9

TABLE 17

Hourly temperature (°C) means derived from entire (4 day) experimental period (grouped data n = 8) for Group 3.

Time of Day	Mean	Standard Deviation	Variance
00:00h	16.6	3.2	10.0
01:00	17.0	3.9	15.0
02:00	15.4	4.7	21.6
03:00	15.8	5.2	27.5
04:00	15.3	2.8	7.7
05:00	17.4	2.0	4.1
06:00	18.5	2.2	4.7
07:00	19.1	2.9	8.5
08:00	20.0	2.2	4.8
09:00	20.9	2.2	4.9
10:00	20.4	2.7	7.1
11:00	19.9	2.8	7.7
12:00	19.4	3.4	11.7
13:00	18.3	3.6	13.2
14:00	20.3	2.5	6.3
15:00	19.7	3.1	9.8
16:00	19.0	3.1	9.6
17:00	19.7	2.3	5.5
18:00	19.4	3.3	10.8
19:00	18.8	2.2	5.0
20:00	18.8	1.9	3.8
21:00	19.0	2.3	5
22:00	19.2	2.6	6
23:00	18.7	2.6	6.8

TABLE 18

Two-way analysis of variance with repeated measures dependent upon one factor testing variation between groups due to ration and variation within groups due to time of day (Experiment 3)

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F-Ratio
Between Subjects		21		
Factor A*	1462.1287	2	731.0643	57.1144
Subjects Within/Groups	243.1997	19	12.7999	
Within Subjects		506		
Factor B**	2684.8945	23	116.7395	26.813
A x B Interaction	1024.9758	46	22.282	5.118
B x Subjects, Withn/Groups	1902.5405	437	4.3536	

F-Ratio

Factor A	(2,19)	= 57.1144	F value (5%): at D.F. 24/120	= 1.61
Factor B	(23,437)	= 26.813	F value (5%): at D.F. 40/120	= 1.50
A x B Inter.	(46,437)	= 5.118		

* Factor A = Diet ration.
** Factor B = Time of day.

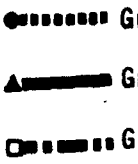
TABLE 19

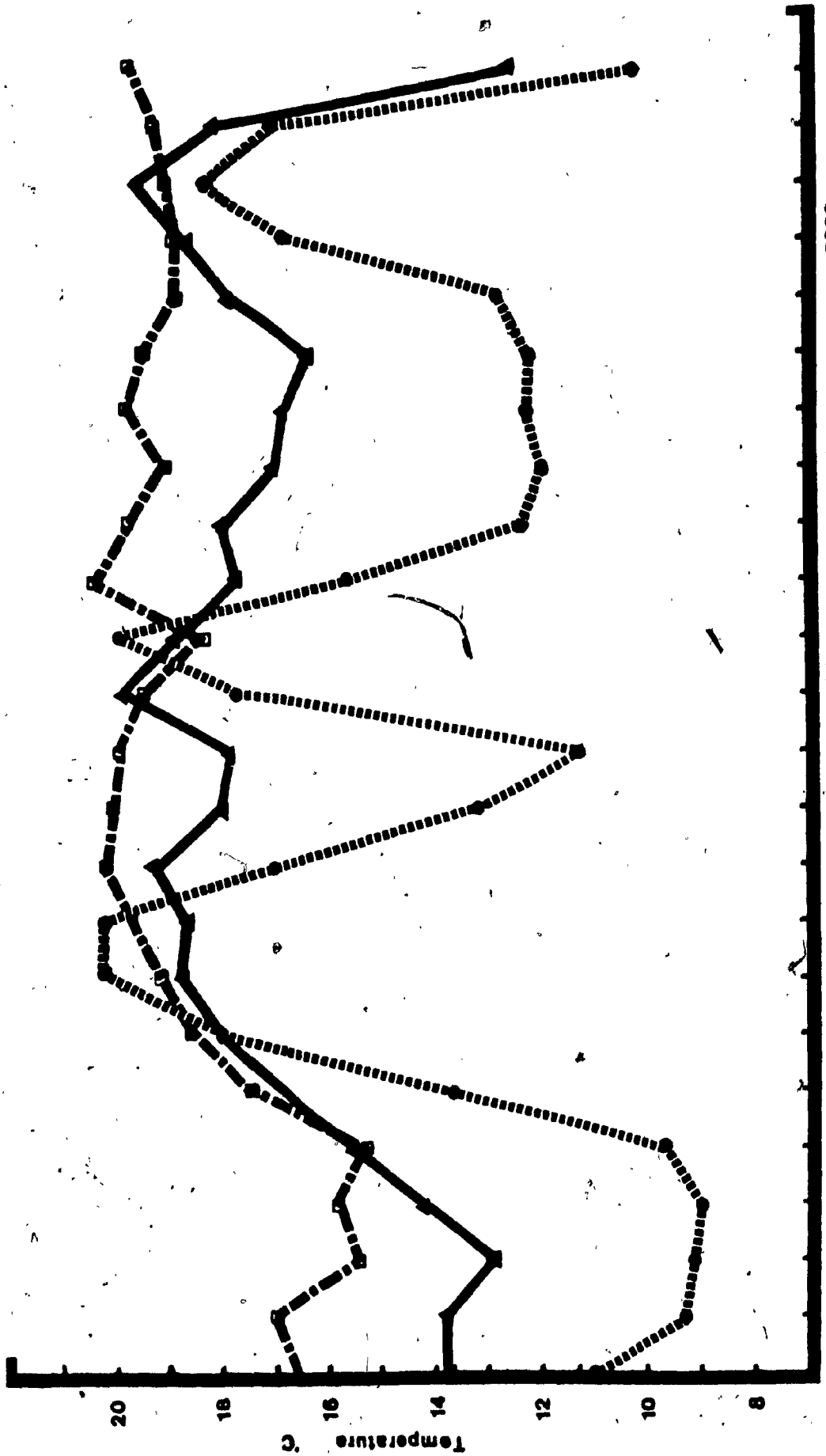
Test for strength of association for two-way ANOVA examining the variance in temperature selection associated with either ration or time of day (Table 18) (estimate of Omega squared)

Variance associated with Factor A*	=	7.9%
Variance associated with Factor B**	=	67.5%
Variance associated with A x B Interaction	=	13.3%
Total variance associated with treatments	=	88.7%

* Factor A = Diet ration.
** Factor B = Time of day.

Figure 14: Pattern of temperature selection averaged over the 24 hours of the day. Data is shown for all three test groups.




Temperature °C
20
18
16
14
12
10
8
0400
0800
1200
1600
2000
Time of day hr.

periods tested ranged from 4 to 29 hours inclusive, thus providing a range greater than circadian periodicity. Following the procedure outlined in Appendix 1, standard deviations were determined for each of the twenty-six periods tested. These standard deviations were then plotted (Figs. 15, 16 and 17) according to the method developed by Enright (1965) and re-worked by Williams and Naylor (1967). Such a periodogram serves to provide a means for comparison of the various frequencies being tested. Relatively large values for the standard deviations are indicators of rhythmicity with respect to the frequency in question.

From the data represented in Table 20 and Figures 15, 16, 17 the most consistently outstanding periodicity is manifested about the twenty-four hour cycle. Frequencies of eight, twelve, and sixteen hours also show some tendency toward periodism, though to a somewhat smaller degree than the twenty-four hour period. Moreover, the greatest degrees of all periodicities occur in the data for the unfed fish (Group 1), with the least degrees (i.e. most strongly single cycle) being evident in the fish fed ad libitum (Group 3).

TABLE 20

Standard deviations for periods of four to twenty-nine hours (Experiment 3)

Period (hrs) (frequency)	Standard Deviation Group 1	Group 2	Group 3
4	1.0	0.6	0.5
5	0.3	0.2	0.3
6	1.4	0.7	0.3
7	0.5	0.4	0.3
8	2.2	1.0	0.6
9	0.4	0.5	0.3
10	0.6	0.4	0.7
11	0.8	0.8	0.5
12	2.5	1.6	0.9
13	1.3	0.9	0.9
14	1.0	0.6	0.7
15	1.0	0.9	0.4
16	2.2	1.2	0.8
17	1.0	0.7	0.7
18	1.5	0.7	0.6
19	1.0	0.9	0.8
20	1.4	1.0	1.0
21	1.5	1.2	0.9
22	1.8	1.4	1.2
23	2.8	1.9	1.4
24	3.7	2.4	1.6
25	2.6	2.2	1.8
26	1.8	1.9	1.8
27	1.2	1.7	1.7
28	1.5	1.6	1.7
29	1.5	1.5	1.5

Figure 15: . Periodogram for Group 1.

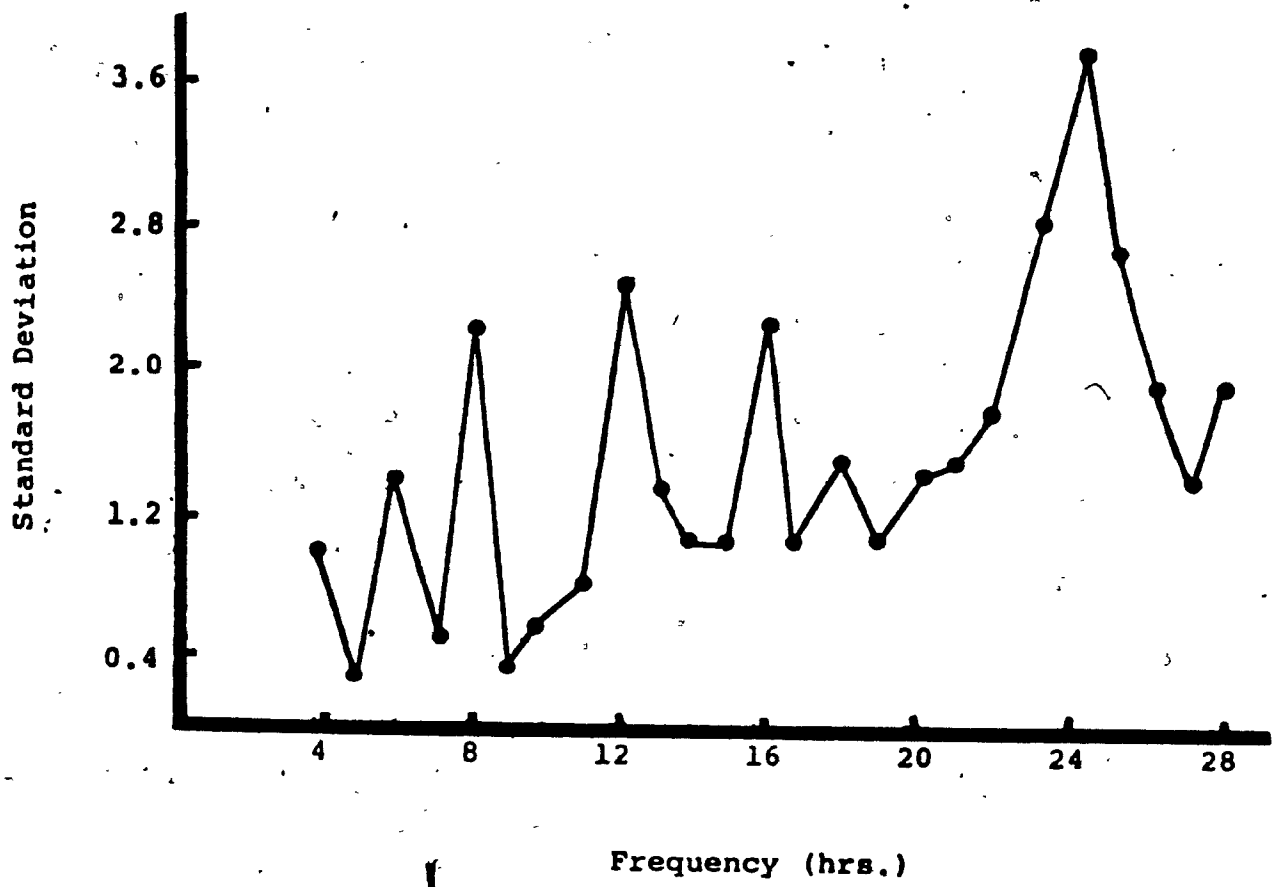

Standard Deviation
3.6
2.8
2.0
1.2
0.4
4
8
12
16
20
24
28
Frequency (hrs.)

Figure 16: Periodogram for Group 2.

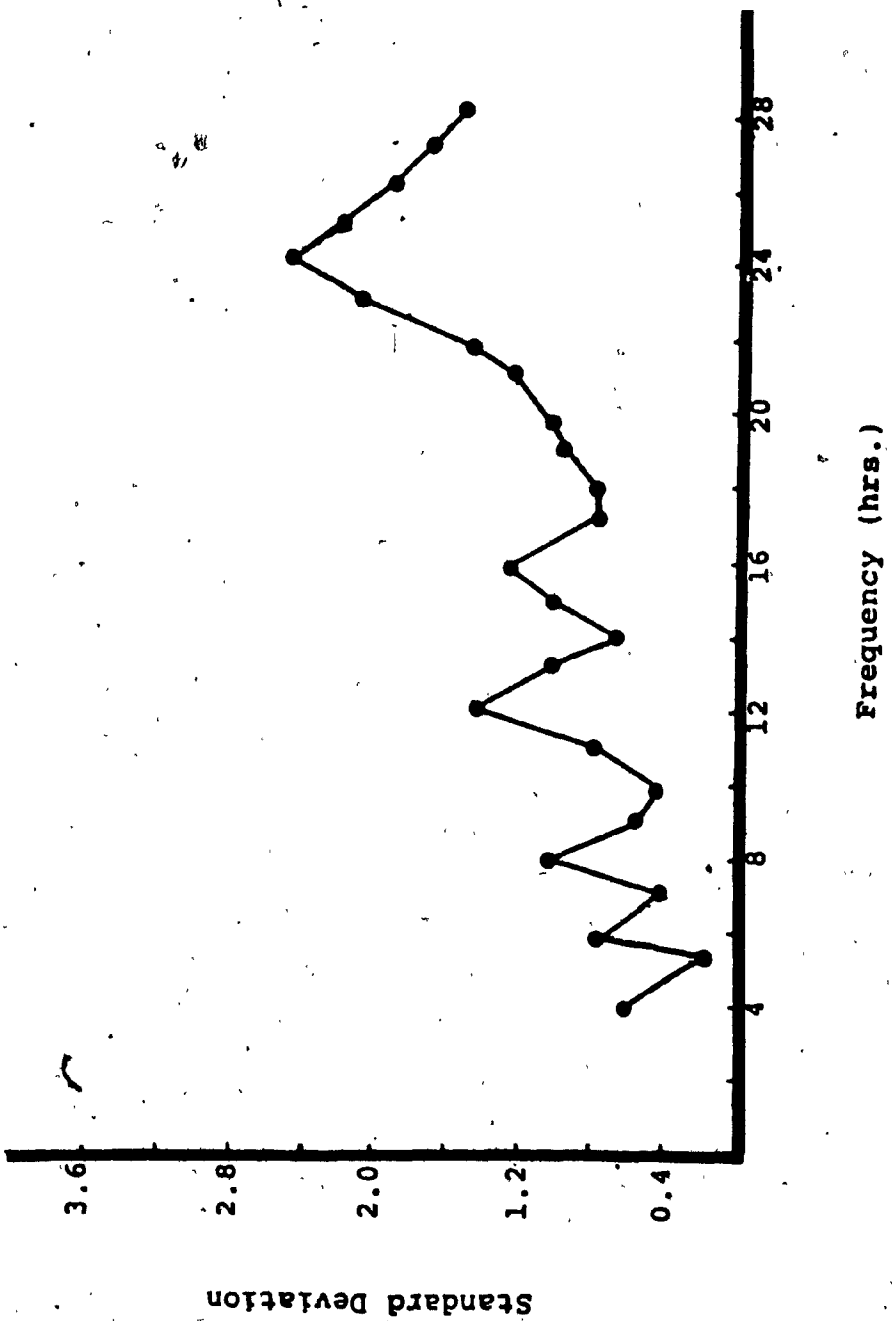
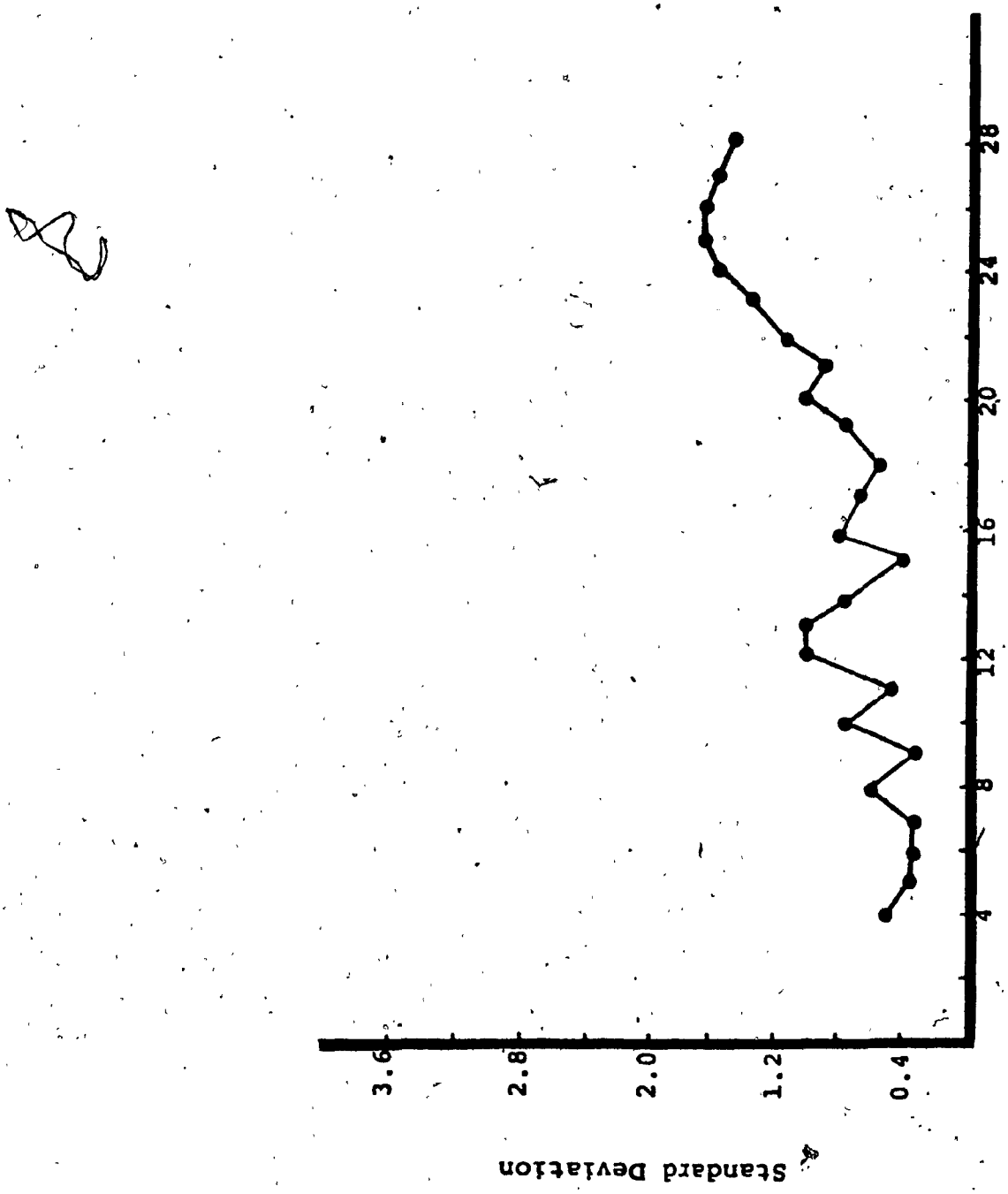

Standard Deviation
3.6
2.8
2.0
1.2
0.4
4
8
12
16
20
24
28
Frequency (hrs.)

Figure 17: Periodogram for Group 3.


Standard Deviation
3.6
2.8
2.0
1.2
0.4
4
8
12
16
20
24
28
Frequency (hrs.)

DISCUSSION

The work described in this thesis has been divided into two phases. The first phase involved the design and implementation of an effective functional shuttlebox methodology incorporating a system of constant water replacement. The second phase consisted of a series of experiments designed to investigate the themoregulatory behavior of Salmo gairdneri as a consequence of several variables including duration of test period and dietary restriction. This discussion will concern itself with the second phase.

(1) Thermal Preferenda for Experimental Fish

The trials comprising the first experiment were primarily geared towards trying to obtain valid thermal preferenda values for the test group of fish using our newly developed shuttlebox methodology.

The mean of means calculated from the data in Experiment 1 was 18.1 ± 0.6°C. This value agrees well with those obtained in the experimental analogs in the later tests. The fish fed ad libitum in Experiment 2 and 3 had mean preferred temperatures of 17.7 ± 1.7°C (Group 1, Fish #1) and 18.6 ± 1.0°C (Group 3) respectively. Similar values have been reported for this species in the literature.

Rainbow trout, Salmo gairdneri have been found to select temperatures ranging from 18°C to 22°C as fingerlings and 13°C to 18°C as adults when tested in the laboratory for final preferenda (Javaid and Anderson, 1967; McCauley and Pond, 1971; Garside and Tait, 1958; Cherry et al., 1975; McCauley and Huggins, 1976). While all salmonid

species occupy temperatures ranging from 4°C to 23°C in the field (Brett, 1971, Kaya et al., 1977; Cherry et al., 1975; McCauley and Pond, 1971), temperature preferences determined in the laboratory comprise a much smaller range. Numerous other salmonid species have also been investigated in light of their commercial importance. Brook trout, Salvelinus fontinalis, select temperatures ranging from 8°C to 18°C as fingerlings and 14.8°C to 15.7°C as adults, depending upon the season (Ferguson, 1958; Graham, 1949; Sullivan and Fisher, 1953; Javaid and Anderson, 1967; Cherry et al., 1975). Lake trout Salvelinus namaycush, prefer 11.7°C as juveniles and 11.8°C as adults (McCauley and Tait, 1970). Atlantic salmon, Salmo salar prefer 14°C to 18°C as juveniles (Fisher and Elson, 1950; Javaid and Anderson, 1967). Chinook, coho and sockeye salmon Oncorhynchus tshawytscha, O. kisutch and O. nerka were found to prefer 11.7°C, 11.4°C, and 14.5°C respectively when measured in the laboratory (Brett, 1952).

These results indicate both the similarity in temperature preferences amongst salmonid species, as well as the diversity in preferred temperatures within a species due to such factors as seasonal and ontogenic constraints. A relevant study conducted by Hokanson (1977) examined the parameter of growth with the objective of estimating the optimum temperature for growth. The experiments consisted of juvenile Rainbow trout maintained on ad libitum diets under one of six constant temperature regimes. The optimum temperature range for growth was found to be 17.2-18.6°C. These findings conveniently bracket the estimated temperature

preferences in all three of the experiments presented in this thesis. The similarity of our data to published values lends credence to both Shuttlebox 1 and 2 methodologies.

(2) Behavioral Thermoregulation as a Function of Food Ration

2a. Experiment 2 - Estimation of Onset of Altered Thermoregulatory Behavior in Response to Food Deprivation

Thermoregulatory studies utilizing shuttlebox methodologies as reported in the literature have rarely tested specimens for periods longer than four days. This is in part due to the fact that the experimental objective is usually little more than the estimation of the preferred temperature of the test species. As has been previously mentioned, fish will normally select for their thermal preferenda within 48 hours (Reynolds and Casterlin, 1979). Temperature data are thus typically sought for one or two days following this 48-hour period, as this period is assumed to be stable. Consequently, experiments run for periods of time which are significantly longer than three or four days are deemed unnecessary by most workers. Furthermore, because of the lack of water replacement, most experimental systems would be hard-pressed to maintain constant conditions for longer periods of time.

Fortunately, our experimental design incorporated continuous water replacement. As such, we were not limited to the duration of the conventional test periods. This feature proved to be of great value in Experiment 2, where fish were tested for periods of no less

than three weeks, and up to six in the case of one fish. Such unlimited experimental time permitted the study of behavioral thermoregulation as a consequence of food deprivation from the very onset of the deprivation phase.

The fish which did have their rations restricted showed no major thermoregulatory pattern changes during the first seven to nine days. Thereafter, however, there was a steady decrease in selected temperatures, such that the mean temperature dropped in a constant fashion for approximately five to eight days. Following this cooling period, temperatures remained relatively constant within a 1°C range of the coolest temperatures available. As mentioned in the Introduction, Javaid and Anderson (1967) also conducted temperature preference studies with Rainbow trout on starvation regimes. They found that the preferred temperature of the fingerlings, as tested in a spatial temperature gradient, decreased from approximately 22°C at the outset of the experiment to 18°C at the end of the first day. For the remaining 14 days of their experiment, the selected temperature remained within the same range.

The extent to which the work by Javaid and Anderson can be compared to our Experiment 2 is limited unfortunately for several reasons. To begin with, fingerlings of the size used by Javaid have been reported elsewhere as having temperature preferenda anywhere between 15 and 21°C, depending on such factors as age and illumination intensity (McCauley and Pond, 1971, Kwain and McCauley, 1978). The temperature preferenda observed by Javaid and Anderson easily fall within this range. Their results are further confounded by several

other factors. Groups of five or six individuals were tested simultaneously to obtain data points. The data were treated as that derived from independently tested individuals. A more accurate representation of the data might have involved estimation of the mean preferred temperature of the group as a group with no reference to individual preference. Experiments conducted by Beitinger and Magnuson (1975) have shown that intraspecific social influences such as social hierarchy phenomena can have a marked effect on individual thermoregulatory behavior. In one series of tests, juvenile Bluegill sunfish (Lepomis macrochirus) were displaced from the area of a horizontal temperature gradient containing their preferred temperature by a larger adult Bluegill (Beitinger and Magnuson, 1975). The mechanism of displacement consisted of various types of agonistic behavior on the part of adult Bluegills. Similar hierarchical structures reinforced by agonistic behavior have been reported for the species used in the author's work, i.e. Salmo gairdneri, as well as for the closely related species Salmo trutta (Jenkins, 1969). Hence, it would seem questionable to attribute the observed group temperature selection to individual temperature preferences in the manner put forth by Javaid and Anderson (1967).

Yet, another problem is that the thermal preference tests were not run in a continuous fashion but rather unfolded over the course of several hours during the day. These brief tests were conducted each day, with the test population spending the balance of its time in the 20°C acclimation-holding tanks. Given that no control values are provided by Javaid and Anderson, it is difficult to know whether their

fish had sufficient time to selectively lower their body temperature. Certainly, there is a limit to the rate at which an ectothermic organism can adjust its metabolism to a significantly different temperature. Had their experiments been permitted to run for a longer interval each day, it is more than possible that the mean selected temperatures would have been lower.

The temperature selection of Javaid and Anderson's test fish may have been additionally biased by the 20°C acclimation temperature maintained for one month prior to testing and throughout the experiment. Such an elevated temperature may have actually placed the test fish on a negative energy budget, depending on the amount of ration they were receiving. If so, then the data obtained by Javaid and Anderson would not accurately reflect the experimental objectives. Again, the lack of controls in their work is unfortunate.

Our work indicates that for a larger-sized trout (circa 10-12 cm), food deprivation perceptibly influences the thermoregulatory pattern only after a period of at least one week. From this point onwards, there is a constant gravitation towards cooler temperatures typified by a steady decrease in temperature selection. As the final temperature preferendum of the unfed fish in Experiment 2 was approximately equal to the lowest temperatures available, i.e. circa 9.0°C, it is possible that the fish would have elected to maintain even cooler temperatures had they been available.

It is interesting to note that the one successful fish fed ad libitum (Fish 1, Group 1) maintained a relatively constant thermoregulatory pattern with a mean approximately 4.8°C higher than

its acclimation temperature for the entire experimental period. This trend was substantiated by the findings in Experiment 3.

2b. Estimation of Diurnal Thermoregulatory Rhythm as a Consequence of Dietary Restriction

Given that a period of up to ten days at the acclimation temperature of 13.5°C was indicated for this size of Rainbow trout to alter its thermoregulatory pattern as a consequence of food deprivation, a pre-test rationing scheme of two weeks in duration was instituted in the next series of experiments (Experiment 3). This two-week dietary regime was then followed immediately by a four day (five in the case of Group 1) period in the shuttleboxes.

Within each of the groups tested in Experiment 3, no significant difference was observed between the daily mean preferred temperatures of any of the experimental days. Following from the trends observed in Group 2 of Experiment 2, it would seem that the test periods did indeed fall after a ration schedule of sufficient length of time to permit thermoregulatory adjustments given the opportunity. This confirms the experimental design rationale for pre-test rationing. Hence, testing time occurred during a phase in which the test fish was apparently prepared to seek metabolic options.

The Neuman-Keuls test demonstrated that the differences between the group mean temperature preferenda were significant (Table 9). Furthermore, these group means were of decreasing values concomitant with the restriction in ration. Thus the group fed ad libitum (Group

3) had the highest temperature preferendum, 18.6°C, while the group which was unfed for 25 days (Group 1) selected the lowest temperature preferendum, 14.1°C

The ANOVA and Omega squared analyses indicated that the experimental variable primarily responsible for these mean differences was in fact the feeding ration. Thus it would appear that restricting rations resulted in certain physiological responses on the part of the test fish such that the organism actively sought an alternate thermal environment when provided with the opportunity.

Fry has hypothesized that, all factors permitting, a fish will select a temperature which permits optimum growth for its nutrient status (Fry, 1971). Unfortunately, relatively little work has been done to elaborate this idea. Experiments conducted by Brett on young sockeye salmon, Oncorhyncus nerka, have shown that of 25 physiological processes measured (including standard and active metabolic rate, scope for activity, growth, and cardiac output) all but two have temperature optima of 15°C (Brett, 1971). However, he points out that this growth optimum will only occur if food rations are unlimited. With decreasing food rations, Brett states that growth optima would occur at progressively lower temperatures (Brett, 1971). His work shows that sockeye salmon fed ad libitum have a growth optimum at their final temperature preferendum, approximately 15°C, but that on daily rations of 1.5, 3.0, 4.5 and 6.0% of their body weight the growth optima occur at 5, 8, 10, and 12°C respectively (Brett et al., 1969).

As previously mentioned, Hokanson et al. (1977) has found that

juvenile Rainbow trout (245 mg average wet weight) show optimal growth rates and food conversion efficiencies at temperatures between 17.2 and 18.8°C when held under conditions of constant temperature. However, at temperatures above or below this, both growth rate and conversion efficiency fell off dramatically, despite provision of food ad libitum (Hokanson et al., 1977).

As the fish in Group 3, fed ad libitum, had a preferred temperature of 18.6°C ± 1.0°C, it is tempting to conclude that they were in fact selecting their optimum temperature so as to obtain maximum growth. Similarly, Groups 1 and 2 may have selected concomitantly lower temperature with the objective of growth optimization.

Many organismic processes operate about a circadian rhythm. In an effort to determine whether such rhythmic effects were present in our data, mean selected temperatures were calculated for each fish of each group tested in Expermient 3 (Tables 12, 13 and 14).

These means were derived for each hour of the twenty-four hour day based on the hourly mean preferred temperatures of Days 2 through 4 (Days 2 through 5 for Group 1). When these data were further averaged to provide hourly mean preferred temperatures for each group, three apparently distinct patterns of thermoregulation emerged (Tables 15, 16, 17). These patterns are shown more clearly in Figure 14.

There are several striking aspects of the curves plotted in Figure 14. A definite distinction can be made between each of the groups. In particular, Group 1 displays a thermoregulatory pattern throughout the twenty-four hour day which has a relatively large

proportion of the day spend in the lowest available temperatures. Group 1 also shows rather rapid changes in selected temperature, undergoing shifts of as much as 10°C in as little as three hours.

As can be seen in Figure 14, these rapid changes in temperature selection occurred in conjunction with relatively brief excursions into warmer temperatures at certain specific times of the day. These same episodes appear to coincide temporally with similar trends in the thermoregulatory behavior of Group 2 and 3. In all three group plots, there is an apparent tendency toward convergence of selected temperatures at certain times of the day. There are marked convergencies occurring at 6:00 AM, 1:00 PM, and 9:00 PM. Conversely, there are very pronounced differences in temperature selection between the hours of 9:00 AM and 12:00 noon, 2:00 and 8:00 PM, and 11:00 PM and 5:00 AM.

The consistency of these convergencies and divergencies indicates the possibility of thermoregulatory behavior revolving around a circadian rhythm. The periodogram analysis elaborated by Williams and Naylor (1967) was used to investigate the possibility of such circadian rhythms. As shown in Figures 15, 16, and 17, this procedure provided concrete evidence for circadian rhythms in each group of test fish. All three graphs show a pronounced rhythmicity at the twenty-four hour period. There is also the suggestion of rhythmicity at the eight, twelve, and sixteen hour periods for Group 1. However, the deviations seen at these hours are much less than that of the twenty-four hour period. The same deviations are also seen in Groups 2 and 3, but to much lesser degrees than in the Group 1 periodogram.

Such periodogram analyses frequently have as an artifact such 'ghost' periods, existing because of procedural manipulation of data due to the fact that they are multiples of the 'real' period. Misinterpretation of their significance is unlikely however, as they are evidently smaller than the deviations shown about the real period(s) (Williams and Naylor, 1967; Enright, 1965).

The possibility of periodicity in the thermoregulatory behavior is reinforced by the results of several other statistical treatments. Tests for ANOVA (Table 18) and strength of association (Table 19) for the mean temperatures as a function of the time of day showed that the actual time of day was the principle determining factor in the temperature selection process. The major experimental variable, food ration, was only responsible for some 7.9% of the variance in temperature selection (Table 19) when considering thermoregulation during a twenty-four hour day.

Thermoregulatory behavior based on circadian rhythmicity has been observed by several workers in a number of fish species. Reynolds and Casterlin (1978) noted that the bowfin Amia calva has a distinctly diurnal pattern in its temperature selection. This species, when acclimated to 23°C under a LD 12:12 photoperiod, maintains a mean temperature of 31.3°C during the photophase and 29.6°C during the scotophase (Reynolds and Casterlin, 1978). The maximum temperature selected is 32°C and the minimum is 28.8°C, occurring during the photophase and the scotophase respectively.

The goldfish Carassius auratus, a crepuscularly active fish, shows a diurnal variation in its temperature selection when acclimated

to 21°C with a LD 12:12 photoperiod. However, unlike the bowfin, the peak in preferred temperatures, 29.7°C, occurs during the scotophase for goldfish (Reynolds and Casterlin, 1978). The range of temperatures selected varied between 26.0°C and 29°C, with a mean of 27.7°C.

The white sucker, Catostomus commersoni, a nocturnal species, also shows a diurnal pattern of thermoregulation. However, this fish prefers slightly higher temperatures during the photophase, i.e. a mean of 25°C as opposed to 24°C during scotophase (Reynolds and Casterlin, 1978). In none of these species was there any positive correlation between activity and thermoregulatory behavior.

Diurnal thermoregulatory rhythmicity has also been observed in other ectotherms. Regal (1967) found that three species of lizards, Klauberuia riversiana, Uma notata, and Phrynosoma cornutum, select lower temperatures during the scotophase. Such voluntary 'hypothermia' (Regal, 1967) was concomitant with torpidity, and hence resulted in a lowered metabolic state.

In comparison with the above-mentioned temperature selection differentials between the various phases of the photoperiod in the diurnally thermoregulating fish species, the results displayed in Experiment 3 show relatively larger temperature ranges. This is most evident in the unfed test fish (Group 1), which has an average diurnal fluctuation of more than 11°C. Such a temperature range in diurnal thermoregulatory behaviour has been observed in field studies of another salmonid species, the sockeye salmon (Brett, 1971).

Brett investigated the distribution of sockeye salmon

Oncorhynchus nerka in a lake with respect to time of day (Brett, 1971). Using round-the-clock tow-netting methods directed by echo-location, he was able to follow the daily habits of the sockeye. Typically, in late summer, young sockeye spend most of the photophase in the relatively cold (5°C) hypolimnion. At dusk they rapidly rise to the surface to feed, descending to the upper stratum of the thermocline by midnight (Brett, 1971). Here they remain until dawn, whereupon they again rise to the surface to feed. Immediately after feeding, they again descend, this time to the hypolimnion where they pass the day.

Thus the sockeye selects temperatures ranging from 5° to 17°C daily; a change of 12°C. Furthermore, approximately 60% of the day is spent in the hypolimnion, where temperatures range between 4° and 6°C.

The function of this rhythmic behavior has been investigated by several workers. Spieler et al. (1977) exposed goldfish, Carassium auratus acclimated to 15°C and either a winter LD 9.5:14.5 or spring LD 11.5:12.5 photoperiod, to a 9°C rise in temperature for a period of 4 or 8 hours at six different times of the day. Fish were then measured for weight changes and gonadal growth. Their finding was that there were significant differences in weight gain and gonadal growth depending upon the time of day that the ambient rise in temperatures occurred (Spieler et al., 1977). In fact, the interaction of temperature change and time in the photoperiod cycle produced both stimulatory and inhibitory effects, depending upon the phase relationship of the two variables (Spieler et al., 1977) Weight gain and gonadal growth are greatest during the final 4 hours of

darkness, though Spieler found the degree to which this occurred was dependent upon the lengths of the photophase and scotophase (Spieler, 1977).

Reynolds et al. (1978) studied the behavioral thermoregulatory pattern of goldfish, as a function of the time of day. This species typically exhibits a crepuscular locomotory rhythm and a unimodal diurnal thermoregulatory pattern. It is interesting to note that within the thermoregulatory pattern is a peak in preferred temperature during the latter part of the scotophase (Reynolds et al., 1978). The timing of this temperature peak seems to correspond exactly with the point in the photoperiod at which Spieler found goldfish to show the greatest amount of weight gain and gonadal growth in response to an increase in ambient temperature. Reynolds himself, referring to Spieler's findings, argues that a rhythmic growth response to cycled temperatures is of little adaptive value to the goldfish if there is not behavioral mechanism to 'take advantage of it', since temperature cycles in the aquatic environment would not be expected to have a pre-dawn temperature peak (Reynolds et al., 1978).

Some investigators have found that there exist diurnal fluctuations in the thermal tolerance of some species of fish. Spieler et al. (1977) tested groups of fathead minnows, Pimephales promelas, for their critical thermal maxima (CTM) during a 24 hour period, sampling every 4 hours. The fish, which had been acclimated to 14.5° ± 0.5°C and LD 14:10, were subjected to a temperature increase at the rate of 13°C/hr. The results showed that there was a highly significant correlation ($p < 0.001$) between susceptibility and

the point in the photoperiod at which the fish were tested (Spieler et al., 1977). For example, maximum susceptibility for this species was found to occur during late afternoon (18:00h) and evening (22:00h).

In a similar test of diurnal variation in temperature tolerance, Johnson exposed Gammbusia affinis affinis, acclimated to 18°C, LD 12:12, to a temperature increase of 0.3°C/min. Not only did he find diurnal fluctuations in susceptibility, but he also found tolerance differences between the sexes (Johnson, 1976). The results revealed that in general, females were more tolerant of the temperature increase, as they had higher CTM's than did the males.

Such fluctuations in thermal tolerance presumably reflect modulation of the sensitivity of certain metabolic processes. Consequently, it is possible to conclude that temperature selection throughout the 24 hour cycle is not equally specific at all points in time. That is, there may be certain times of the day when the degree of precision of the thermoregulatory process is less critical than at other times. The existence of such fluctuations in thermal tolerance in the thermoregulatory behavior of our test fish is a variable of potential significance, and one not isolated in our data.

Though all of the physiological reasons for such rhythmic variations in thermal tolerance are not known, there is speculation that an endocrine component may be involved in stress reactions, cortisol and prolactin, may play a role here (Spieler, 1976). He has shown the existence of both diurnal and seasonal changes in the level of serum prolactin and corticoids in the striped mullet, Mugil cephalus (Spieler et al., 1976). Similarly, McKeown and Peter have

found circadian rhythm-correlated prolactin (McKeown et al., 1976). In both cases, the level of serum prolactin increased with longer photophases.

Organic evidence providing further links between cause and effect with respect to the photperiod and temperature regulation has been provided by Kavalier et al. (1980). Pinealectomies were performed on white suckers (Catostomus commersoni), a species which, as has been previously mentioned, has a diurnal rhythm in temperature selection. The pinealectomized fish displayed no difference in temperature selection relative to a diurnal rhythm. The pineal gland is photosensitive, and is thought to function in the coupling of temporal organization of physiological behavior, and hormonal cycles with circadian rhythms through central nervous system modulations (Takashashi and Zatz, 1982). Hence, it would not be surprising if photoperiod modulation of the thermoregulatory behavior of at least some fish species was mediated through the pineal gland.

The thermoregulatory behavior observed by Brett in his previously mentioned work with the sockeye salmon is not unlike that displayed by the Rainbow trout in Expermient #3, especially by Group 1. Brett has suggested that the primary purpose of these diurnal habitat shifts is to better utilize energy resources (Brett, 1971). In his study, Brett calculated the 'gross conversion efficiency',i.e. the percentage of body mass produced form food, from the food ration and growth rate. The optimal temperature for maximum gross conversion efficiency, that is, the highest yield in body weight from food, occurs at 11.5°C for the sockeye salmon (Brett, 1971). It is

interesting to note here that the temperature Brett determined gross conversion efficiency to be greatest is about 3.5°C below the temperature at which the growth rate is maximal.

Brett concludes that since the physiological and growth optimum temperature apparently is 15°C, one could expect to find fish at this temperature, except when food ration is limited (Brett, 1971).

Since the metabolic rate would be reduced by at least one half in going from 17° to 5°C, according to the Van't Hoff Q10 relationship, Brett postulates that the diurnal thermal niche shifts may be a mechanism to optimize growth under restricted food ration conditions (Brett, 1971).

Support for this latter hypothesis does exist, in a limited form, in the work done by Javaid and Anderson (1967). In their study, fingerlings of three salmonid species; Atlantic salmon Salmo salar, Rainbow trout Salmo gairdneri, and Brook trout Salvelinus fontinalis, were starved for 22, 20 an 15 days respectively, after they had been acclimated to 20°C. Temperature selection tests in a horizontal gradient revealed that both Brook trout and Rainbow trout preferred lowered temperatures; 16° and 18°C respectively for the bulk of the starvation period. Atlantic salmon showed little or no change in selected temperature during the experimental starvation period, electing to remain in 20°C water; a temperature equivalent to its original acclimation temperature (Javaid and Anderson, 1967). Thus at least two of the three test species, including the species utilized in the author's work, preferred sub-acclimation temperatures when deprived of food.

Brett has found further support for his hypothesis in a study by MacDonald which examined growth rates of these same sockeye in the same lake. MacDonald calculated possible weight gain under conditions of the average temperature experienced by the fish and the possible maximum temperature, that is the surface temperature, under constant conditions and compared these with the observed weight gain (MacDonald, 1973). In general, the observed weights were from 105% to 350% less than the calculated possible weights (MacDonald, 1973). Since the calculated weight gains under average and maximum temperatures assumed an unlimited food ration, Brett concludes that the sockeye in question must have had a limited energy intake in order to account for their sub-maximal growth rate (Brett, 1971). Brett further argues that the food availability in the lake is not necessarily limited, but rather the fish simply don't eat as much as they theoretically could. Brett suggests that this reduced intake may be due to the decreased energy efficiency involved in catching the extra amount of food necessary to sustain maximum growth (Brett, 1971). Unfortunately, he provides no evidence to support either of these suggestions.

As a result of these investigations, Brett argues that these sockeye have 'evolved a pattern of thermoregulation peculiarly adapted to maximizing growth, through the selective pressure of bioenergetic efficiency' (Brett, 1971).

Buffenstein and Louw (1982) arrived at a similar conclusion as a result of their experimental work with varanid lizards. Varanus niloticus was observed to select the preferred temperature when given

the opportunity and provided with food in excess. In so doing, the lizards displayed a faster growth rate, greater food intake, and superior food conversion efficiency compared with animals restricted to lower temperatures (Buffenstein and Louw, 1982).

Hokanson et al. (1977) has conducted a series of experiments yielding results which serve as an interesting compliment to Brett's hypothesis. The crux of Hokanson's work consisted of comparing growth rates of juvenile Rainbow trout fed ad libitum held under conditions of either constant or diurnally fluctuating temperatures. A range of seven constant and six diurnally fluctuating cycles were used in his study. Hokanson concluded from these experiments that the growth of the Rainbow trout was accelerated by the cycled temperatures when the mean temperature of the cycle was below the optimum temperature for growth, as estimated under constant temperature conditions (Hokanson et al., 1977). Additionally, he noted that fluctuating temperatures inhibited growth when their means were above the constant temperature optimum. Hokanson attributes these results to differential rates of acclimation to increasing and decreasing temperatures.

Biette and Geen (1980) conducted a one-month study of growth as a function of the temperature and diet regime using underyearling sockeye salmon (Onchorhynchus nerka). Five different temperature regimes were imposed, including constant temperatures of 6.2, 11.3, 15.3, and 15.9°C, as well as a diel thermal cycle similar to that encountered by the species during their daily vertical migrations, oscillating between 4.5° and 17.5°C. Daily rations, provided twice daily according to the experimental group, consisted of one of eight

different rations ranging from 1.3 to 6.9% of the wet body weight.

In general, growth rates were superior under the cyclic temperature regime at all rations except the highest and lowest ration levels where growth was greater with the constant low and high temperatures respectively (Biette and Geen, 1980).

Spigarelli, Thommes, and Prepejchal (1982) exposed groups of adult Brown trout (Salmo trutta) to one of three different thermal regimes for a period of approximately eight weeks. The three regimes included a constant temperature of 13°C, the theoretical constant optimum temperature for growth in Brown trout, a diel temperature cycle regularly fluctuating between 9° and 18°C with a mean temperature of 12.5°C, and an arrhythmic fluctuating regime ranging from 4° to 11°C with a mean of 7.7°C, corresponding to the naturally occurring inshore water temperatures of a local lake, part of the Brown trout's natural habitat. All fish were fed to satiation twice daily.

Spigarelli found the growth rate to be greatest in those fish maintained in the diel temperature cycle (Spigarelli et al., 1982). The growth rates of the test groups subjected to either a constant 13°C regimes or the arrhythmic inshore temperature fluctuations were approximately the same.

The evidence generated by Hokanson (1977), Biette and Geen (1980), and Spigarelli, Thommes, and Prepejchal (1982) provides some very interesting information regarding growth rates as a function of ration and thermal regime. First of all, regularly fluctuating cycles with a diel rhythm produce the greater growth rates than constant

temperature regimes when food rations are sub-maximal. The sole exception to this was observed by Biette and Geen (1980) who found that there was a slightly greater growth rate at the constant temperature of 6.2°C than with the diel cycle, but only at the lowest ration level. Secondly, when food rations are available in surplus, growth is superior with a constant temperature regime compared with a diel cycle, only when the constant temperatures are relatively high. Finally, fluctuating temperature cycles are more stimulating to growth when compared with constant temperatures only when they occur with a diel rhythmicity (Spigarelli et al., 1982). This implies an innate diel rhythmicity in the metabolism which is positively expressed in the form of a diel rhythm in the thermoregulatory behavior. Thus it would seem that selection of temperature on a diurnally fluctuating basis does indeed have some advantage in terms of bioenergetic efficiency. Certainly, the data produced in Experiment 3 demonstrate that given the choice, Rainbow trout will select lower temperatures when subjected to dietary restriction. Furthermore, the rather pronounced tendency to select temperatures as a function of the time of day indicates that there is an attempt on the part of the fish faced with insufficient dietary resources to optimize its metabolic condition in conjunction with some diel cycle in an effort to achieve an optimal growth rate concomitant with the experimental conditions.

CONCLUSION

This thesis has presented evidence which lends itself to the hypothesis originally proposed by Fry (1947) and iterated by Brett (1971), that fish may select their ambient temperature with the goal of optimizing their metabolic energetics. Specifically, the author's work has demonstrated that salmonids will select lower temperatures during certain times of the day and maintain a lower overall daily mean temperature when faced with restricted rations. Furthermore, the altered thermoregulatory behavior appears to be organized in conjunction with a diel cycle. These patterns suggest that the thermoregulatory behavior is modified in such a way as to optimize the metabolic processes according to their own diel cycles.

Future work should pay heed to the necessity of elaborating the relationship between optimizing bioenergetics with growth as one of the experimental parameters.

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APPENDIX 1

Method for Computation of Periodogram

In the periodicity analyses employed for Experiment 3 data, sequences of temperature values (x_1, x_2, x_3, ..., x_n) were scanned for rhythms of whole-hour frequencies (f) from 4 to 29 hours inclusive. Each data scan gave the appropriate number (4 to 29) of mean hourly temperature values (X_1, X_2, X_3, ..., X_f) as follows:

	x_1	x_2	...	x_f
	x_{f+1}	x_{f+2}	...	x_{2f}
	x_{2f+1}	x_{2f+2}	...	x_{3f}
	x_{n-f+1}	x_{2-f+2}	...	x_n
means	X_1	X_2		X_f

These values were then utilized in the following equation to yield a value for the variance:

$$s^2 = \frac{1}{f-1} \sum X^2 - \frac{1}{f} \left(\sum X \right)^2$$

The standard deviation was computed from the variance. For a more detailed theoretical and procedural analysis of this form of periodicity estimation, the reader is advised to consult Williams and Naylor (1967) and, especially, Enright (1965).