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Behavioral Thermoregulation and Growth in Juvenile Rainbow Trout (*Oncorhynchus mykiss*) Fed Different Rations

James A. Bruce

A Thesis

in

The Department

of

Biology

Presented in Partial Fulfillment of the Requirements
for the Degree of Master of Biology at
Concordia University
Montréal, Québec, Canada

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ABSTRACT

Behavioral Thermoregulation and Growth in Juvenile Rainbow Trout (*Oncorhynchus mykiss*) Fed Different Ration

James Anthony Bruce

This project examines the general notion that the temperature preferences of fish shift as a function of dietary intake. For several decades it has been assumed that such shifts adjust the energy budget relative to energy intake whereby either growth is maximized or the loss of weight minimized. This "bioenergetic hypothesis" was tested by measuring the relative growth rate of individual fish which had the opportunity of freely regulating their ambient temperature.

Underyearling rainbow trout, *Oncorhynchus mykiss*, were assigned to one of three dietary groups representing rations provided in excess, in a restricted amount or not at all. The study was run for 20 days during which each test fish regulated its ambient temperature through means of a shuttlebox device. Food quantities were divided and provided at dawn and dusk in a 12:12 L:D photoperiod regime. Ambient temperature and shuttling activity were monitored continuously by computer. Individuals were wet weighed at 0, 10, and 20 days.

There was no evidence that shifts in daily thermal preferenda occur as a function of ration level. There was no correlation between food intake and thermal preferendum of fish and no correlation between food intake and thermal preferendum except for fish which were able to feed to satiation. Diel rhythmicity in mean hourly preferred temperature occurred at a slight, but significant bioenergetic cost. The precision with which fish regulated their ambient temperature was not significantly affected by differences in food intake. Collectively, these results negate the long-standing bioenergetic hypothesis. The range of daily mean preferred temperatures however, varied inversely with ration size and the combined pattern suggests that fish may "thermally" disperse within a heterothermal environment when food intake is not adequate.

The unwillingness to abandon the central preferred temperature is deemed significant because it is that temperature at which bodily functions have been shown to be collectively optimized. The significance of thermal cycling in juvenile Salmonidae remains unanswered. Some of the alternative suggestions to a bioenergetic explanation are considered.

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My tenure as a Graduate student was funded in part by a scholarship from Fonds F.C.A.R. This research was supported in part by grants from Natural Sciences and Engineering Research Council of Canada (NSERC), Fonds F.C.A.R., and the Department of Fisheries and Oceans of Canada, awarded to Dr. P. D. Anderson.

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INTRODUCTION

When given the opportunity, fish actively seek out and occupy certain ambient water temperatures in preference to others. The frequency with which these temperatures are occupied tend to be monomodal in their distribution (Reynolds and Casterlin, 1979a; DeWitt and Friedman, 1979). The temperature most frequently occupied within this distribution, characterized by some measure of central tendency, has become a conventional parameter of thermoregulatory behavior known as the "preferred temperature" or "thermal preferendum" (Fry, 1947).

When given a choice, the temperatures initially selected by fish depend largely on their thermal history (Fry, 1947, 1964; Reynolds and Casterlin, 1979a). However, the initial preferences are transitory. They shift and "gravitate" towards a temperature that is independent of thermal history. Whereas the short-term temperature selections of fish are referred to as "Acute Thermal Preferenda" (AcTP), the temperature to which fish eventually gravitate has been termed by Fry (1947) as the "Final Thermal Preferendum" (FTP).

Several properties of the FTP have been empirically defined. Foremost, it is species-specific (Fry, 1947, 1964; Beitinger and Fitzpatrick, 1979; Reynolds and Casterlin, 1979a; Houston, 1982) and can be used in general to classify fish as eurytherms (warm water), mesotherms (cool water) and stenotherms (cold water) (Hokanson, 1977; Magnuson et al.,1979). Furthermore, studies with hybrid fish populations have demonstrated that the FTP is genetically determined and is subject to natural selection (Goddard and Tait, 1976; Peterson et al., 1979; Koppelman et al., 1988). There is also an apparent correlation between the FTP and the temperatures at which many physiological activities proceed at an optimum or maximum rate. Activities related to foraging and predatory avoidance such as swimming velocity, swimming acceleration, cardiac scope and metabolic scope have been shown to be optimized at temperatures equivalent to the FTP (Brett, 1971; Beitinger and Fitzpatrick, 1979; Huey and Stevenson, 1979; Houston, 1982; Bergman, 1987; Jobling, 1988). These findings have led to the general notion that the principal goal of behavioral thermoregulation in fish is to achieve a core temperature at which bodily functions collectively operate with maximum efficiency (Brett, 1971; Beitinger and Fitzpatrick, 1979; Beitinger and Magnuson, 1979; Magnuson et al., 1979; Houston, 1982; Rudstam and Magnuson, 1985; Bergman, 1987; Christie and Regier, 1988).

There are certain conditions however, when fish appear to establish an FTP at levels other than those which correspond to physiological optima. For example, bacterial infections can cause fish to choose temperatures greater than their normal FTP (Reynolds et al., 1976). This hyperthermal response is thought to be analogous to the febrile responses in homeothermic organisms, aiding the immune system in its defense against bacterial infection (Reynolds et al., 1976). Hypoxic conditions have also been shown to displace the temperatures normally occupied by fish. Bryan et al. (1984) have found that low oxygen availability may cause fish to occupy cooler waters than when oxygen supplies are adequate. This hypothermal response presumably lessens the metabolic demands of fish and offsets, to some degree, the stress of an inadequate oxygen supply.

Food consumption rate is thought to be another factor which modifies thermoregulatory behavior in fish. Numerous physiological studies have shown that optimum growth temperatures in fish vary directly as a function of ration size (e.g. Brett et al., 1969; Elliott, 1976; Wurtsbaugh and Davis, 1977; Brett, 1979; Allen and Wootton, 1982). When fish are fed _ad libitum_, optimal growth temperatures are at their highest value. However, as ration levels are reduced, optimal growth occurs at progressively cooler temperatures which reach their lowest value when fish are not fed.

Many studies have shown that the optimum temperatures for growth in fish fed to satiation are coincident with the FTP (Brett, 1971; Stuntz and Magnuson, 1976; Beitinger and Fitzpatrick, 1979; Kellogg and Gift, 1983; Mac, 1985; Jobling, 1988). However, fish rarely achieve satiation under natural conditions (Brett, 1971, 1979; Groves and Brett, 1979). It has been hypothesized that, on limited rations, fish shift their FTP to correspond to the optimum growth temperature for the reduced level of food intake (Brett, 1971; Crowder and Magnuson, 1980). The underlying assumption of this hypothesis is that growth takes precedence over other bodily functions. This may be a strategy exploited by certain juvenile anadromous fishes. In their underyearling phase, size is a critical determinant in achieving the important life cycle events of smoltification and sexual maturation (Hoar, 1976).

A number of fish species have been found to cycle through a range in temperatures on a daily basis, occupying high (peak) and low (trough) temperatures at different times of the day (Reynolds and Casterlin, 1978b, 1979b,c; Reynolds et al., 1978; Reynolds et al., 1978; Kavaliers and Ralph, 1980; Kavaliers, 1982). At first glance, thermal cycling seems to defy the concept of FTP. However, Richards et al. (1977) and Houston (1982) have noted that the mean temperature of these cycles (i.e. the mesor) share the same properties of the FTP as defined by Fry (1947).

In some fish, diel rhythms entail a significant shift in temperature. For example, juvenile sockeye salmon (Oncorhynchus nerka) have been observed to migrate through a thermocline on a daily basis. The range in temperatures over which they migrate span 10-12°C (Brett, 1971; McDonald, 1973). Brett (1971) hypothesized that cycling represents a thermoregulatory strategy which permits, through temporal segregation, both the rate of growth and other bodily activities to be optimized when fish which consume a limited ration. Noting that the time spent in the warmer waters correlated with feeding, he suggested that the high temperatures enhance foraging success and, through an accelerated digestion rate, maximize the total amount of food fish can consume. Conversely, the time spent in the cooler waters correlates with a non-feeding period, during which growth is optimized because of a lower metabolic rate.

Empirical studies by Hokanson et al. (1977), Spieler et al. (1977), Clark (1978), Biette and Geen (1980), Cox and Coutant (1981), Spigarelli et al. (1982), Diana (1984), Thomas et al. (1986) and Wurtsbaugh and Neverman (1988) have confirmed that the growth rate of fish subjected to cycling temperatures is greater than when held at a constant temperature equal to the cycle's mesor (i.e. the daily mean temperature about which ambient temperatures are cycled). This growth advantage, however, is accrued only if the mesor of the temperature cycle is below the optimum constant temperature for growth. In addition, the growth advantage is limited to conditions where rations are below satiation, but above maintenance levels. Hokanson et al. (1977), Biette and Geen (1980) and Cox and Coutant (1981) have found that growth in fish fed _ad libitum_ when subjected to cycling temperatures occurs at an equal or slower rate than when held at the optimum constant temperature (i.e. the FTP). Biette and Geen (1980) have also found that the rate of weight loss in unfed fish is accelerated when subject to cycling temperature conditions.

The empirical evidence gathered to date on the issue of ration size and thermal cycling would suggest the following scenario. Under ad libitum conditions, fish would gain a bioenergetic advantage by holding temperatures constant at a level equivalent to the optimum temperature for growth (i.e. the FTP). When food intake is below satiation, growth would be enhanced by cycling provided that it is accompanied by a downward shift in the mesor. This shift in cycle mesor would correspond, by definition, to a downward shift in FTP. As ration levels fall below maintenance, it would be most advantageous to refrain from cycling and to occupy the coolest possible temperatures. However, the above studies did not test whether fish, by volition, would exercise the above options in accordance to food intake. It is this bioenergetic choice that is hypothesized by Brett (1971).

Few studies have explored whether the size of ration alters the thermoregulatory behavior of fish in order to achieve maximum growth. In general, the results reported to date have been equivocal. Doudoroff (1938) carried out an independent study on the marine fish Girella nigricans. He noted that 2 weeks of starvation had no detectable effect on the temperatures occupied by this species in a laboratory spatial gradient. Using a similar experimental protocol, Javaid and Anderson (1967b) reported a slight downward shift in the preferred temperatures of brook trout (Salvelinus fontinalis) and rainbow trout (Salmo gairdneri) when starved for 15-22 days. However, they reported an upward rather than a downward shift in preferred temperature of Atlantic salmon

(Salmo salar) when subjected to the same starvation conditions.

In the two latter studies, thermoregulatory tests were brief in duration (2 hours) and were conducted daily over the course of the experiment during which the fish were held at one temperature. Thermoregulatory tests of short duration are known to yield thermal preferenda that are highly dependent on the acclimation temperature (Fry, 1947, 1964; Cherry et al. 1975, 1977; Reynolds and Casterlin, 1979a; Houston, 1982). This limits the usefulness of Doudoroff's (1938) and Javaid and Anderson's (1967b) experiments in testing the bioenergetic hypothesis. Furthermore, the interpretation of Doudoroff's (1938) and Javaid and Anderson's (1967b) results is complicated by the fact that several fish were tested concurrently in the temperature gradient apparatus. More recent studies have shown that territorial behavior may have a profound effect on the thermal distribution of fish when collectively occupying a spatial temperature gradient (Beitinger and Magnuson, 1975; Medvick et al., 1981). Furthermore, it has been shown that the behaviors related to social hierarchy, aggression and territory size vary as a function of ration size, particularly among salmonidae species. (Symons, 1968; Slaney and Northcote, 1974; Dill, 1983).

Stuntz and Magnuson (1976) and Mac (1985) have studied thermoregulatory behavior as a function of ration in individual fish, thereby avoiding the problems associated with groups.

In addition, their experimental design provided a 24 hour period for fish to gravitate to their respective FTPs prior to the onset of thermoregulatory testing. In both the latter studies, the tests were conducted using a temporal thermal gradient device - known as a "shuttlebox". In this device, fish regulate ambient temperatures through response-contingent behavior. Fish are able to control the direction and magnitude of temperature change by shuttling. Shuttling involves a movement back and forth through an aperture in a partition located midway in a holding box. Shuttling events cause signals to be sent that change the direction of temperature change in the box. (Neill et al., 1972; Beitinger and Magnuson, 1975; Reynolds 1977a; Gregory and Anderson, 1984; Gregory, 1986).

This device avoids the pitfalls of spatial thermal gradient systems. In the latter, thermoselective behaviors are often confounded by orientations toward accessory factors such as light intensity, water flow, and - in vertical systems - hydrostatic pressure (McCauley, 1977). An added advantage to the shuttlebox device is that core temperatures in shuttling fish are closely reflected by ambient temperature readings (Reynolds et al. 1976). Thus, records of ambient temperature changes in the shuttlebox provide a reasonable estimate of the changes in core temperature experienced by fish.

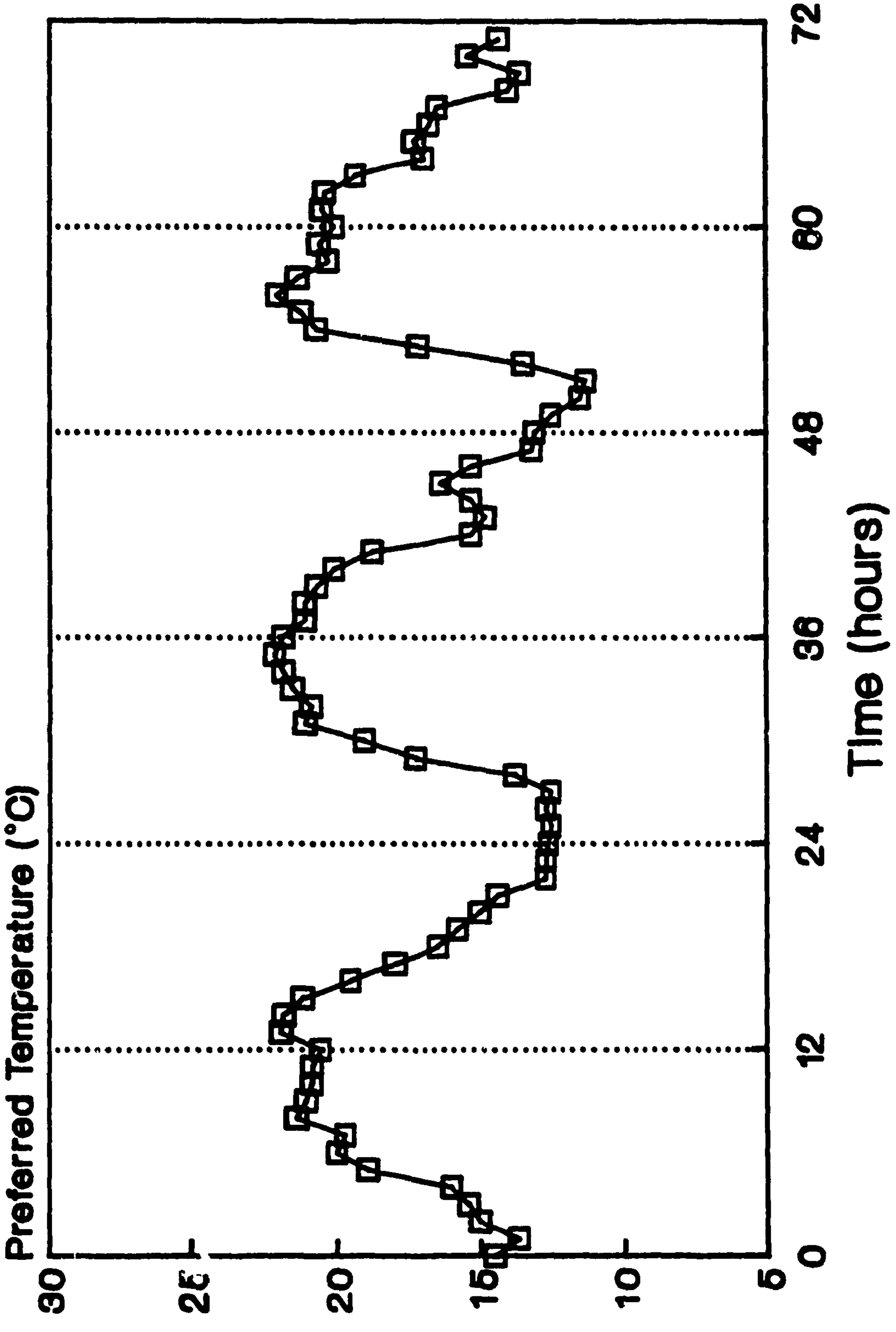
Thermoregulatory behavior in the shuttlebox studies of Stuntz and Magnuson (1976) and Mac (1985) were monitored continuously for 5 days and 1 day respectively following an initial 2 week growth period. Stuntz and Magnuson (1976) reported that bluegill sunfish (Lepomis macrochirus) preferred temperatures 1.4°C cooler when fed rations below maintenance levels (0 and 2% wet body weight/day) than when fed above maintenance (4 and 6% wet body weight/day). Mac (1985) demonstrated a positive correlation between FTP and food consumption over a range of ration provided to Salvelinus namaycush. However, the total shift in FTP was only 3.5°C for a ration reduction from 5.5% wet body weight/day to 0.3% wet body weight/day. The authors of both of these studies have noted that the observed shifts in FTP relative to ration size could have only provided a minimal growth advantage. Shifts to much cooler temperatures were predicted according to the bioenergetic hypothesis.

Neither Stuntz and Magnuson's (1976) nor Mac's (1985) study addressed the issue of whether thermal cycling is altered in relation to food consumption rate. Their respective investigations were limited to observations on the mean temperatures selected by fish on a daily basis.

In light of the importance of understanding the role of thermoregulatory behavior in the distribution of fish in their natural habitats (Ferguson, 1958; Brett, 1971; Reynolds, 1977; Magnuson et al., 1979; Beitinger and Magnuson, 1979; Crowder and Magnuson, 1980; Rudstam and Magnuson, 1985, Baltz et al., 1987; Christie and Regier, 1988), the bioenergetic hypothesis was examined once again in this study. However, unlike those of Stuntz and Magnuson (1976) and Mac (1985), a shuttlebox system was used which permitted thermoregulatory behavior, food consumption and growth to be measured concurrently and continuously over a 20 day period. Shuttlebox devices like those used by Stuntz and Magnuson (1976) and Mac (1985) employ a static water system. Without a regular replacement of water, wastes build up, water quality changes and consequently, the thermoregulatory behavior of fish becomes erratic after 4 or 5 days (Reynolds and Casterlin, 1979a, Richards et al., 1979). Gregory and Anderson (1984) and Gregory (1986) were able to circumvent this problem by incorporating a continuous water replacement system in their shuttlebox design. It was found that fish tested in the latter device thermoregulated effectively for periods up to 6 weeks (Gregory and Anderson, 1986). This feature allows the influence of thermoregulatory behavior on growth to be assessed directly.

The experimental animals of choice were juvenile rainbow trout (Oncorhynchus mykiss) because extensive information on their thermal biology and thermoregulatory behaviors is available. Furthermore, preliminary studies have shown that the temperature preferences of juvenile rainbow trout cycle on a daily basis (Figure 1). The range in temperatures over which they cycled resembled those reported in nature for other Oncorhynchus species (e.g. Brett, 1971; McDonald, 1973). This permitted the issue of whether fish, in conjunction with a downward shift in FTP, alter their cycling patterns in

Figure 1. The diel cycle in mean hourly preferred temperature of 11 juvenile rainbow trout over a 3 day period. The data are from a preliminary study done in February and March of 1986. All fish were fed a ration equivalent to 2% of their respective wet body weight 2 weeks prior to thermoregulatory testing. Thermoregulatory behavior was monitored in a shuttlebox system similar to that used in the present study. The photoperiod was 12:12 LD and incorporated a dawn and dusk event during which light intensity was gradually increased or decreased repectively over a 4 hour period.


Preferred Temperature (°C)
30
25
20
15
10
5
0
12
24
36
48
60
72
Time (hours)

relation to ration size to be addressed.

In addition to the FTP and cycling, a third parameter of behavioral thermoregulatory, thermoregulatory precision, was included in this study. Thermoregulatory precision is a measure of the degree to which fish tolerate fluctuations in their core temperature on a moment to moment basis (Neill et al. 1972; Beitinger et al., 1975). In a shuttlebox system, the upper and lower limits of this tolerance range correspond to the upper and lower temperatures which elicit a shuttling response. These thermal limits have been termed the "upper and lower turn-around temperatures" respectively (Neill et al. 1972; Beitinger et al. 1975). The greater the difference between these two limits, the less precisely fish are thought to be thermoregulating. Beitinger et al. (1975), Beitinger and Magnuson (1979) and Magnuson et al. (1979) have noted that the range of temperatures defined by the upper and lower turn-around temperatures typically span 70% of the temperatures occupied by a fish certered about its thermal preferendum. Since the mean plus or minus one standard deviation approximates this proportion of the thermal distribution, the upper and lower limits may be estimated by computing the standard deviation about the thermal preferendum (Magnuson et al. 1979; Gregory and Anderson, 1984). I have applied this concept by defining thermoregulatory precision as the standard deviation about the hourly mean preferred temperature. The less precisely fish thermoregulate, the greater the standard deviation

(Gregory and Anderson, 1984).

The effects of ration size on thermoregulatory precision in fish is currently unknown. However, among reptiles, it has been suggested that thermoregulatory precision may be lessened under conditions of low food availability (Huey and Slatkin, 1976). Based on the assumption that precise thermoregulation comes at an energetic and temporal cost to the animal, Huey and Slatkin (1976) hypothesized that a reduction in the level of precision under conditions of low food availability would allow reptiles to conserve energy and spend more time foraging.

Assuming that fish may respond thermally to reduced rations in a fashion similar to reptiles, thermoregulatory precision was examined in light of Huey and Slatkin's (1976) hypothesis. In a shuttlebox system and within a definable limit, thermoregulatory precision is a direct function of shuttling activity. Because shuttling activity represents an energetic cost to thermoregulating fish, its reduction in conjunction with a reduction in precision could result in energy savings. The implications of this hypothesis in relation to the bioenergetic hypothesis are also considered.

MATERIALS AND METHODS

2.1 Experimental Animals

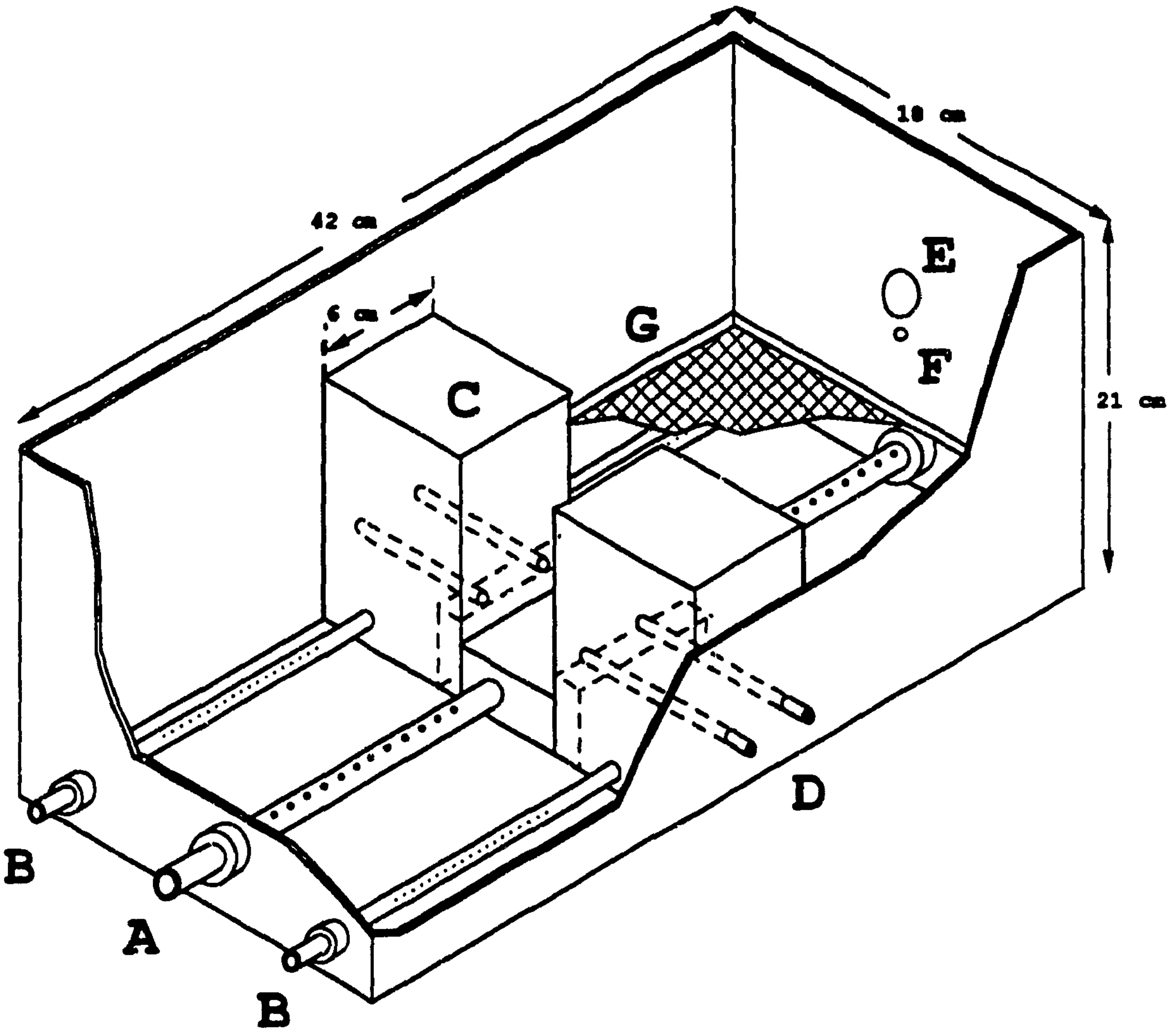
Underyearling rainbow trout (Oncorhynchus mykiss), measuring 8 to 10 cm Standard Length (SL), were purchased from a commercial hatchery, La Pisciculture Mt. Sutton, Sutton, Québec, in July, 1987. In the laboratory, they were held in a 200 L fiberglass tank for at least three weeks prior to experiments. During this holding period, water temperature was maintained at 12 ± 0.5 °C and a 12:12 light/dark (LD) photoperiod was provided using fluorescent lights. To minimize growth and size differences over the course of the project, they were fed to satiation on dry food pellets (Nutribec, P4) once every second or third day.

2.2 Apparatus

2.2.1 Thermoregulatory Shuttlebox

Thermoregulatory behavior was examined using a response-contingent paradigm. A fish regulated its temperature by shuttling through a photoelectrically monitored gate positioned midway in a holding chamber (Figure 2). The act of shuttling initiated a reversal in the direction of temperature change. This temperature reversal served to reinforce the likelihood of further appropriately-executed shuttles. A series of four shuttlebox systems were assembled, each operating independently of the other. This permitted four fish to be studied concurrently.

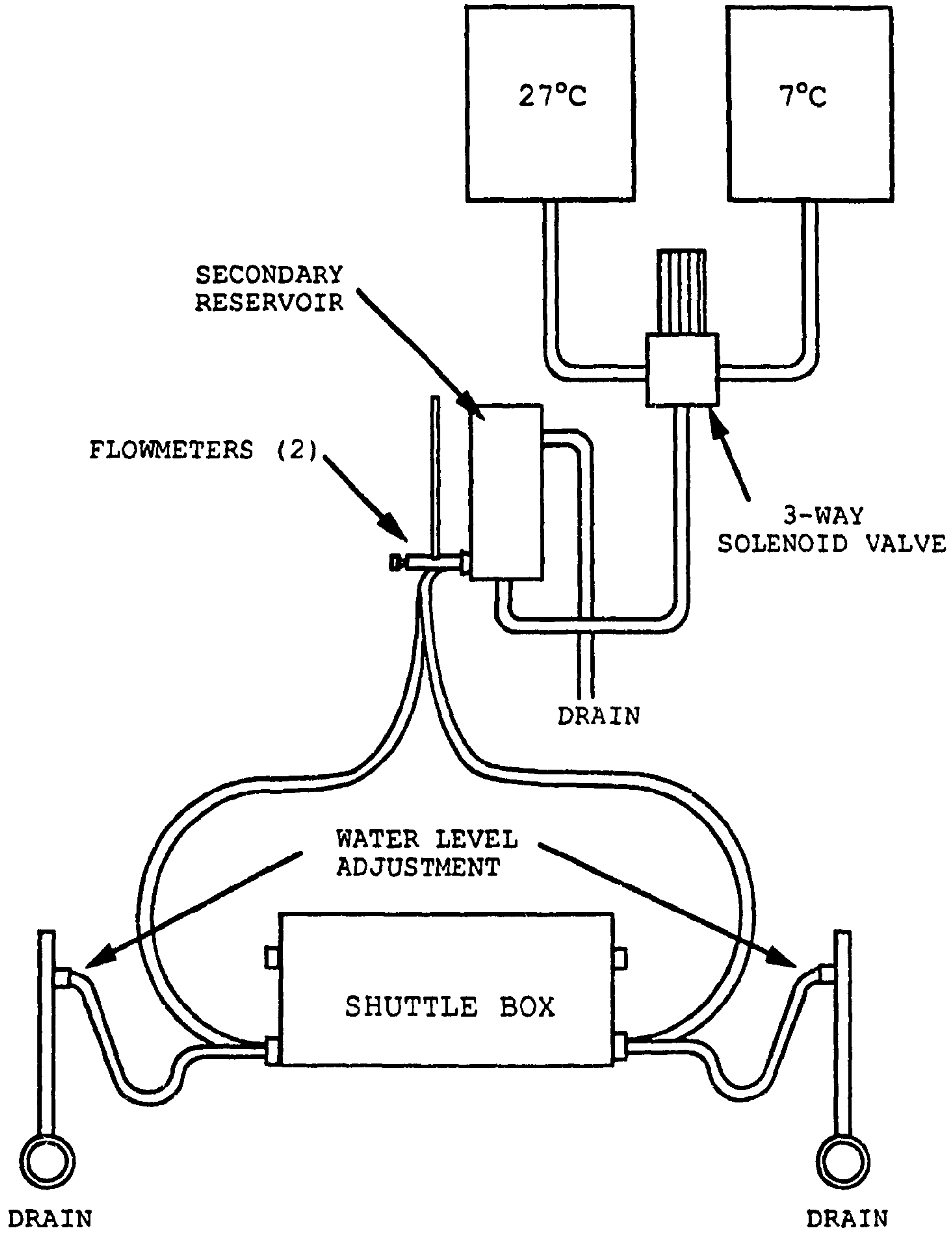
Figure 2. Schematic of the shuttlebox device. A, perforated pipe through which water entering each end then diffused into each chamber; B, perforated pipe through which water drained evenly from each chamber; C, 6 cm wide partition dividing the shuttlebox into two equal chambers; D, photoelectric cell system used to signal shutting events; E, overflow port; F, inlet port through which auxiliary reinforcement water was delivered, G, Nylon screen covering water inlet and outlet pipes.


42 cm
18 cm
6 cm
21 cm
C
G
E
F
D
B
A
B

The shuttlebox system was similar in design to that of Gregory and Anderson (1984) and Gregory (1986). Each consisted of a tank divided into two equal chambers by a partition (Figure 2). A 6 cm wide gate through the partition connected both chambers. Mounted on the inside wall of the gate were a pair of photoelectric cells. They were activated by light-beams emitted from a pair of corresponding optic fibers mounted on the opposite wall of the gate (Appendix I). The sequence of light-beam interruption caused by fish swimming through the gate signaled the completion and direction of each shuttle between chambers. These signals mediated the control of a three-way solenoid valve (Galteck, model no. 203-4314-4) which, in turn, controlled the flow of water from either of two head tanks located above the shuttlebox (Figure 3). One of these head tanks was a source of continuously replenished 7°C water and the other, 27°C water. A shuttle in one particular direction triggered the valve to direct the flow of cool water into the shuttlebox and in the opposite direction, it triggered the flow of warm water.

Water passing through the solenoid valve flowed into an intermediate head tank positioned midway between the source-water head tanks and the shuttlebox. This intermediate head tank served to stabilize water pressure. From this head tank, water passed through a pair of flowmeters and into opposite ends of the shuttlebox. The water flowrate into the respective chambers of the shuttlebox was set at 3.1 ± 0.1 L/h. This

Figure 3. Schematic of the water delivery system to the shuttlebox. Incoming water to each of the four shuttleboxes was supplied in sequence by two head tanks (50 cm long X 25 cm high X 15 cm wide), one continuously replenished with 7°C water and the other with 27°C. The flow of water from either head tank was controlled by a three-way solenoid valve, allowing only one source to flow through the system at one time. From the solenoid valve, water entered an intermediate head tank (5 cm long X 15 cm high X 10 cm wide) and subsequently flowed by parallel channels through a pair of flowmeters to both chambers of a shuttlebox. Water drained from the shuttlebox through a pair of tubes connected to the perforated outlet pipes at opposite sides of each chamber (Figure 1). The height of these tubes relative to the floor of the shuttlebox determined the water level within the device.


27°C
7°C
SECONDARY RESERVOIR
FLOWMETERS (2)
3-WAY SOLENOID VALVE
DRAIN
WATER LEVEL ADJUSTMENT
SHUTTLE BOX
DRAIN
DRAIN

exchange rate resulted in an average rate of change in temperature of 0.7°C/min.

Water entered the shuttlebox through a perforated pipe located centrally on the floor of each chamber. A pair of perforated pipes on either side of the inlet pipe served as conduits for outgoing water. This arrangement provided a uniform circulation of water throughout the shuttlebox and eliminated any measurable horizontal or vertical temperature gradients (Gregory and Anderson, 1984; Gregory, 1986). A nylon screen framed in PVC plastic was mounted flush with the floor of the gate and covered the water distribution pipes at the bottom of the shuttlebox. Fish occupied the uninterrupted space above the screen. The water level was maintained at 3.5 cm above the screen floor by adjusting the hydraulic height of the four drainage pipes located outside the shuttlebox (Figure 3). This height of water was just sufficient to submerge the fish completely and ensured that passage through the gate would interrupt the light beams.

Two auxiliary ports were located at either end of the shuttlebox and in direct line with the gate. The first, located 2 cm directly above the water inlet pipe, served as an auxiliary inlet. Bursts of warm or cool water released through these ports at the time of a shuttle aided the reinforcement process during the training period (Section 2.3.2). The respective temperatures of the water provided at opposite ends were 12°C and 22°C. The water sources for the latter were two holding tanks located above the shuttlebox complex. The flow was delivered in plastic tubing 5 mm in diameter. Water flow in these tubes was manually controlled. The second port was positioned 2.5 cm above the first port and provided a back-up outlet in case of an overflow.

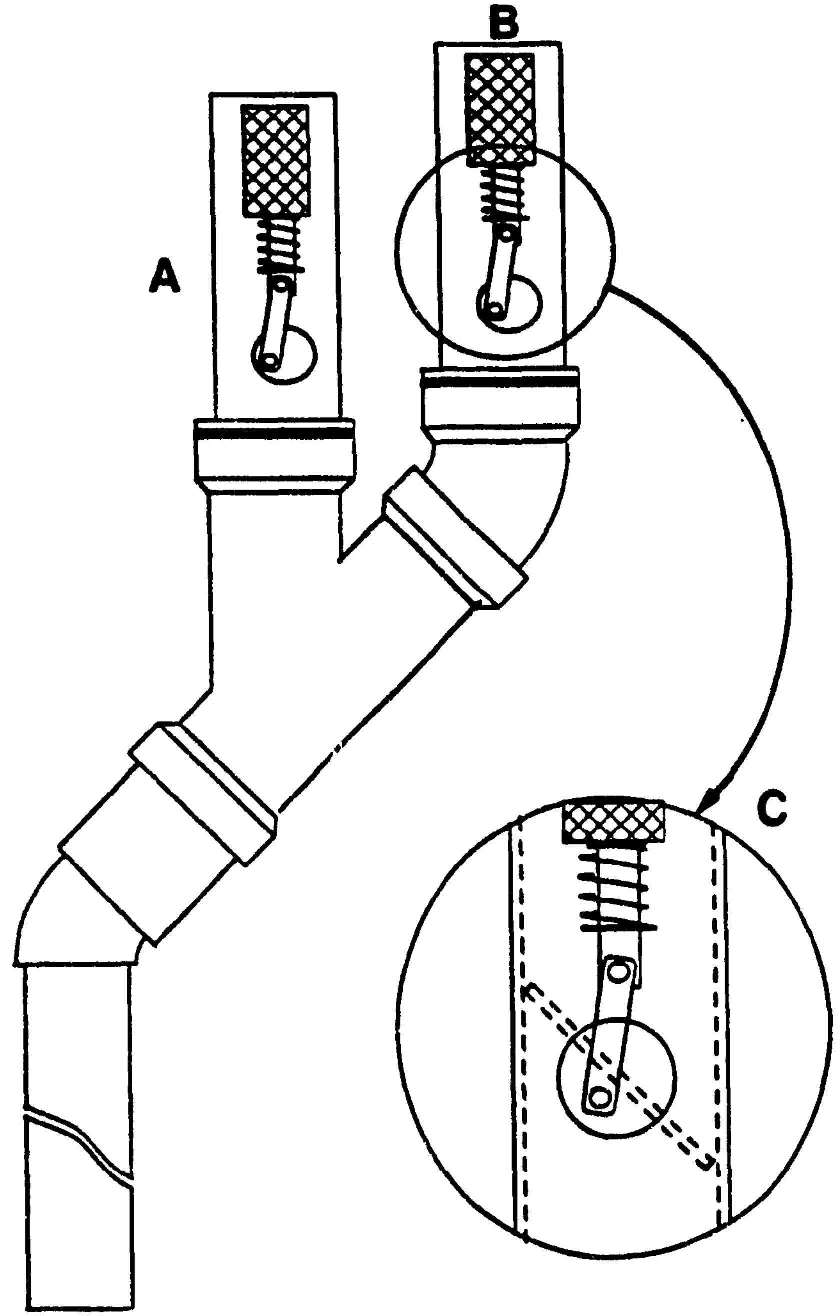
2.2.3 Automated Food Dispenser

A time controlled food dispensing device made of PVC plastic was suspended directly above the gate of each shuttlebox (Figure 4). The device consisted of two chambers, each fitted with a solenoid and valve. The outlets of these chambers converged above the center of the shuttlebox. The discharge of food from each chamber was controlled by a timer (Appendix I). One chamber held the morning ration which was delivered at 07:00 h and the other, the evening ration, delivered at 19:00 h. Similar devices were installed above the acclimation tanks.

2.3 Experimental Protocol

The study consisted of a series of 8 experiments carried out over the course of a one year period (August 1987 to July 1988). Four fish were tested concurrently during each experiment for a total of 32 fish. Each experiment consisted of 3 phases; an initial 20 day acclimation period, a 36 hour training and gravitation period and a 20 day thermoregulatory test period. Details of each phase are presented below.

Figure 4. Diagram of the automated food dispenser. Each chamber (A) of the food dispenser was charged with a specific amount of food which was released at preset times by activating a solenoid (B) controlled valve (C).


B
A
C

2.3.1 Acclimation

At the onset of each experiment, four test fish were randomly selected from the holding tanks, wet weighed in a tared water-filled container and isolated in separate 20 L flow-through acclimation tanks. The water temperature in these tanks was maintained at 17.0 ± 0.5°C. A 12:12 LD photoperiod was provided using a series of three 30 W incandescent light bulbs located directly above the acclimation tanks. To simulate dawn and dusk events between each light and dark phase of the photoperiod, light intensity was increased or decreased gradually over a 2 hour period using a mechanized variable transformer (Appendix I). Maximum light intensity at the water surface of each shuttlebox was 50 lx. All fish during the acclimation phase were fed dry, floating-type food pellets twice daily at the midpoint of each dawn (07:00 h) and dusk (19:00 h) event for a total daily ration of 1.0% of their respective wet body weight. Food was delivered by the automated food dispenser described in Section 2.2.3.

At the end of the acclimation period, each test fish was wet weighed as described above and immediately transferred to a shuttlebox. Food was not provided on the last day of the acclimation period to permit a more accurate measure of wet weight.

2.3.2 Training

Once transferred to their respective shuttlebox, each fish was left undisturbed for 1 hour to become familiar with their new surroundings. Water temperature in the shuttlebox during this initial hour was held constant at 17°C ± 0.5°C. Light intensity was at its maximum daytime level.

After this initial hour, a training session was initiated to help promote the response-contingent shuttling behavior required of fish to control ambient temperatures. Training began by cycling temperatures within each shuttlebox using a manual override system to operate the solenoid valves. The cycle started with an increase in temperature and ranged 2°C above and below the acclimation temperature. The amplitude of the cycle was increased each hour by 2°C until the absolute limits of temperatures available to the shuttlebox were reached (i.e. 7°C and 27°C). When, during the course of this cycling program, a fish was observed to traverse the gate completely, it was immediately rewarded with a 3-5 s spurt of water from the auxiliary inflow system. Depending on the chamber entered, the spurts were either 22°C or 12°C. In addition to this auxiliary reinforcement, a reversal in the direction of temperature change within the shuttlebox was also initiated (primary reinforcement). The auxiliary reinforcement accommodated the slight latency period (5-10 sec) which occurred between the onset of the primary reinforcement and each shuttling event. Training was continued until each fish was able to maintain its respective ambient temperatures within a 6°C range. Depending on the individual, training required 4 to 6 hours.

At the end of the training period, manual control of the solenoid valve system was abandoned along with the auxiliary reinforcement schedule. Once fish assumed control of the system, they were left undisturbed until 0:00h of following day. This period permitted each fish to "gravitate" to its respective FTP and to develop "proficiency" in regulating its ambient temperature. No food was given during this initial phase in the shuttlebox.

2.3.3 Thermoregulatory Test Period

2.3.3.1 Behavioral Thermoregulation

The next 20 days in the shuttlebox constituted the thermoregulatory test period. Ambient water temperature within each shuttlebox was under the full control of the resident fish. All fish were left undisturbed except for a brief, random period each day to siphon the shuttleboxes clean and to recharge the food dispensers with a known amount of food. The experiment was also briefly interrupted on day ten to wet weigh the fish. The photoperiod and the feeding schedules for fish receiving a ration were identical to those used during the acclimation period.

2.3.3.2 Feeding

Each test fish was assigned one of three rations; *ad lipitum*, restricted (1.0 to 1.5% wet body weight per day) and no food. Because four fish were tested simultaneously, one fish was designated as a replicate for one of the rations. Ration regimes were assigned during each experiment according to the schedule outlined in Table 14 of Appendix V.

The food pellets were of the dry floating type and were comprised of 40% crude protein, 9% crude fat and 2% crude fiber. They were selected for uniformity in size and averaged 88 mg/pellet ± 7(SD). Because the pellets remained intact for periods up to 36 h when in water, an estimate of the actual rate of food consumption for each fish was obtained by subtracting the number of uneaten food pellets collected each day by siphon from that which was provided.

Prior to weighing on day 10, the scheduled dusk and dawn feedings of days 9 and 10 respectively were denied to permit a more accurate weight measurement. Feeding was resumed at dusk on day 10 with ration levels increased to compensate for the weight gained during the initial 10 days of the test period. A similar protocol of food denial was carried out on days 19 and 20 so that again, accurate wet weights could be determined. As before, all fish were weighed in a tared water-filled container under red light.

3.3.3 Data Acquisition

Ambient water temperature and the number of shuttling events were recorded continuously throughout the test period using a microcomputer (Microcom). Each shuttlebox was equipped with a single temperature probe (Analog Devices # AD590) positioned near the passageway (Appendix I). Signals from the probe were monitored by the computer via an analog/digital converter (CYDBEC Inc.). Temperature measurements were taken at 60 sec intervals. At the end of each hour, the data set for each fish was averaged and a standard deviation computed. Also recorded at hourly intervals were the total number of shuttling events.

Data recording always started at 0:00h the day after training (Section 2.3.2) and was continued uninterrupted for the entire 20 day test period. Because all data were collected by computer, it was possible to conduct the thermoregulatory tests in a room isolated from the remainder of the lab.

2.4 Data Analysis

All statistical analyses were carried out using BMDP and SPSS-x procedures.

2.4.1 Temperature Preference

Following each 20 day thermoregulatory test, daily sets of hourly mean preferred temperatures recorded for each of the respective test fish were averaged. This provided a daily mean preferred temperature for each of the 20 days of the test. Effects of ration regime and shuttlebox on daily mean preferred temperatures (DMPT) were evaluated using a Mixed Between-Within-Subjects Analysis of Variance test (Tabachnick and Fidell, 1983). Because only 29 fish were included in the analysis (Section 3.2), ration regime and shuttlebox factors were examined in separate two-way ANOVA tests. To prevent an inflated experiment-wise error rate, the Alpha level for each test was set at 0.025 (Sokal and Rohlf, 1981).

Because of a brief power failure, only 17 continuous days of data were collected during the test period of the second experiment (Section 3.2). As a result, the within-subject factor (time) was restricted to 17 levels (i.e. days), allowing the data of the second experiment to be included in the general analysis. As is apparent in Figures 4, 5 and 6, the daily mean preferred temperatures during the latter 3 days of the experiments run to completion did not differ from the previous 17 days. Thus, the time restriction was deemed not to have a significant influence on the outcome of the analysis.

The ANOVA test requires that all levels of the within-subjects factor be independent of each other (i.e. unautocorrelated). This assumption, tested using Bartlett's Test of Sphericity (Tabachnick and Fidell, 1983), was found to be violated in both analysis. To compensate for this violation, the Greenhouse-Giesser Epsilon Correction Factor (computed by the BMD P2V program) was used to "adjust" the degrees of freedom in the MS_{error} term of the within-subjects factor (Dixon, 1981).

The variance in DMPTs of each ration group was used as a measure of the thermal preference range (TPR) which is defined as FTP ± 1 SD.

2.4.2 Diel Rhythmicity

The time series of hourly mean preferred temperature for each fish were subjected to Univariate Spectral Analysis (BMD PT1 program of Dixon 1981) to test for patterns of rhythmicity. Each time series was Fourier transformed to represent the squared amplitudes of a set of discrete Fourier frequencies (i.e. the inverse of each observation number in the time series). For each Fourier frequency, a group of neighboring frequencies was taken and their corresponding squared amplitudes averaged. This average value, referred to as a spectral density estimate, reflects the contribution of the central frequency of the group to the temporal variance in the time series. When these spectral density estimates are plotted against the corresponding frequency, a spectrogram is formed. The frequency with the highest density reflects the overall cycling tendency of the time series. A more comprehensive discussion of this technique can be found in Dixon (1981) and Bloomfield (1976).

The number of neighboring frequencies included in each group - the bandwidth - determines the resolution and statistical stability of the spectrogram (Dixon, 1981). For this particular analysis, the bandwidth, as determined by the BMD PT1 program, was 0.072 cycles/hour (i.e. 7 frequencies on either side of the central frequency). Each of the respective spectrograms was examined for prominent peaks at frequencies 0.0417 cycles/hour (1 cycle/day) and 0.0833 cycles/hour (2 cycles/day), wich would indicate a periodicity in temperature cycling of 24 and 12 hours respectively.

Besides periodicity, other aspects of rhythmicity were examined using a "rhythmicity" index. I developed this index to determine a cycle's phase, as indicated by the occurrence of peak temperatures relative to light or dark hours. This index also provided a measure of each cycle's range (amplitude x 2). The index is based on the relative difference between the average temperatures occupied during the 5 hours centered about 12:00 h and that centered about 0:00 h. Values of large magnitude were considered as an indication of a broad cycle in temperature preference while those approaching zero suggested an arhythmic pattern. In addition, the phase of a cycle was considered nocturnal if the index was negative (i.e. the fish selected the warmest temperatures during the dark phase) and diurnal if the index was positive (i.e. the fish selected the warmest temperature during the light phase).

Phase differences between individual test fish were examined in the following manner. The rhythmicity index for each day of the test period was pooled for each fish and averaged. If the cumulative value was positive, the fish was classified as diurnal and if the value was negative, then the fish was classified as nocturnal. The influence of the shuttlebox and ration level on the phase distribution of individuals were examined independently using a Log-Likelihood Ratio test of independence (Sokal and Rohlf, 1981).

The range of each daily cycle was examined independently of the phase by taking the absolute value of the index. Although it is actually a measure of the cycle range, this quantity is subsequently referred to as the "amplitude" of a diel cycle.

The effects of ration level and shuttlebox device on cycle amplitude were evaluated over time using a Mixed Between-Within Subjects Analysis of Variance test. A weak but statistically significant correlation was observed between the amplitude and precision indexes (Appendix III). Because correlations between dependent variables can lead to a greater probability of committing an Type I error, this analysis would normally warrant a multivariate approach (Tabachnick and Fidell, 1983). However, because the proportion of the variance that was actually shared by both indexes was minimal ($r^2 = 0.068$) this approach was abandoned in favor of the simpler univariate approach. Because of the constraints outlined in Section 2.4.1, ration level and shuttlebox effects were analyzed in separate ANOVA tests. Accordingly, the Alpha level was set to 0.025 in order to prevent an inflated experimentwise error rate. In addition, the within-subjects factor (time) was restricted to 17 days (Section 3.2). Because the distribution of cycle amplitudes was found to be highly positively skewed, the data were transformed using the log(x + 1) transform to satisfy the assumption of normality. As before, the MS_{error} term related to the within-subjects factor was adjusted using the Greenhouse-Giesser Epsilon Correction Factor (BMD P2V program of Dixon 1981).

2.4.3 Thermoregulatory Precision Index

The precision with which fish regulate their ambient water temperature is reflected by the hourly variance in occupied temperatures (Gregory and Anderson, 1984). On this premise, the standard deviation about each computed hourly mean preferred temperature was used as an index of thermoregulatory precision (TPI). Hourly indices were averaged on a daily basis for statistical analysis.

The effects of ration level and shuttlebox on thermoregulatory precision were evaluated using the same analysis of variance protocol outlined in Section 2.4.1. Each of these factors were tested in an independent two-way Mixed Between-Within-Subjects ANOVA, using days 1 through 17 as the within-subjects factor. The experiment-wise rate was set at 0.025 and the MS_{error} term related to the within-subjects factor was adjusted using the Greenhouse-Giesser Epsilon Correction Factor (BMD P2V program of Dixon 1981).

2.4.4 Shuttling Activity

The hourly recordings of shuttling frequency for each fish were pooled to yield an hourly rate of shuttling activity for each 24 hour period. Shuttling activity was only examined in relation to thermoregulatory precision since shuttling was found to be of little consequence to the daily mean preferred temperatures selected by fish ($r^2 = 0.024$) and the cycling pattern as measured by the rhythmicity index ($r^2 = 0.026$). The equation relating shuttling activity to thermoregulatory precision was based on that of Beitinger et al. (1975) and is as follows;

$$2 \times TPI = \frac{R}{S} \times 60 \text{ min} \qquad (1)$$

where,

TPI = thermoregulatory precision index

R = rate of temperature change (°C/min)

S = Shuttling rate (shuttles/hour)

2.4.5 Food Consumption and Growth

Average relative growth rate of each fish over the 20 day test period was calculated using the following equation (Warren, 1971);

$$\text{Growth (\%/day)} = \frac{(W_f - W_i)}{\frac{1}{2}(W_f + W_i) \cdot t} \times 100 \qquad (2)$$

where,

W_i = Initial wet weight

W_f = Final wet weight

t = time in days

A similar equation, also provided by Warren (1971), was used to calculate the average relative food consumption rate for each fish;

$$\text{Consumption rate (\%/day)} = \frac{R}{\frac{1}{2}(W_f + W_i) \cdot t} \times 100 \qquad (3)$$

where,

R = sum of all food consumed

The gross efficiency with which food energy is converted to biomass was calculated as the ratio of growth rate to the rate of food consumption (Warren, 1971).

A Standard Multiple Regression test (BMD P2R program of Dixon 1981) was used to assess the effects of grand mean preferred temperature, grand mean precision index, grand mean amplitude, and average relative food consumption rate on average relative growth rate. The respective contributions of each independent variable to the predictability of growth were evaluated on the basis of individual Semipartial Correlation Coefficients (sr_i^2). As the latter were not provided by the BMD P1R program, they were computed using the following equation (Tabachnick and Fidell, 1983);

$$sr_i^2 = \frac{T_i^2}{df_{res}} (1 - R^2) \qquad (4)$$

where,

T_i = Student t statistic

df_{res} = residual degrees of freedom

R^2 = coefficient of multiple determination

RESULTS

3.1 Acclimation Period

All rations (2 - 4 pellets/day) provided during the acclimation period were consumed by the test fish. The average food consumption rate during this period was 1.04%/day ± 0.23(SD) and was found not to differ significantly among the eight trials ($F_{7,21} = 1.52$, $P = 0.213$). The average growth rate for the test population was 0.92%/day ± 0.44(SD). Final wet weight at the end of acclimation averaged 20.70g ± 5.55(SD). The size of post acclimated fish was found not to differ significantly among the eight trials ($F_{7,21} = 0.84$, $P = 0.565$).

All test fish at the end of the acclimation period appeared to be in good condition. They were brightly colored and had prominent parr marks, indicating that all were pre-smolt (Hoar, 1976).

3.2 Thermoregulatory Test Period

Of the 32 fish tested, 3 were not included in the analysis. Of the latter, one had died of unknown causes after 12 days in the shuttlebox and a second was excluded because of a malfunction in its shuttlebox. A third test fish failed to learn the shuttling operant as it had consistently occupied temperatures at either of the extremes available in the shuttlebox (7°C and 27°C) and rarely shuttled.

A brief power failure during the second trial terminated data acquisition on day 18 of the test period. As a result, only 17 days of data were continuously recorded. Because shuttlebox operation was only momentarily affected, the trial was continued until day 20 so as to obtain comparable growth measurements.

Mixed Between-Within-Subjects Analysis of Variance revealed no significant differences in daily mean preferred temperature, thermoregulatory precision index and log(x + 1) transformed rhythmicity index (amplitude) among the four thermoregulatory shuttleboxes (Appendix IV). A log likelihood ratio test of independence showed that the incidence of nocturnal and diurnal rhythms was also independent of shuttlebox. It was therefore concluded that the thermoregulatory behavior of fish in this experiment was independent of the shuttlebox in which they were tested.

3.2.1 Temperature Preference

Considerable differences in daily mean preferred temperature (DMPT) were observed between individuals within each ration test population (Figures 5, 6 and 7). In addition, DMPTs of individual test fish frequently varied over the 20 day test period. Analysis of variance (Table 1) however, showed that the daily average preferred temperatures for the three test lots were remarkably constant through time. Furthermore, as revealed by the lack of a significant interaction, there were no differences in DMPTs between each ration group over time.

Figure 5. Daily mean preferred temperatures of 10 juvenile rainbow trout fed to satiation over the 20 day thermoregulatory test period. (———), indicates the upper and lower limits of the thermal preference range (FTP ± 1 SD) and (- - -) outlines the range encompassing 95% of the data distribution (FTP ± 2 SD).

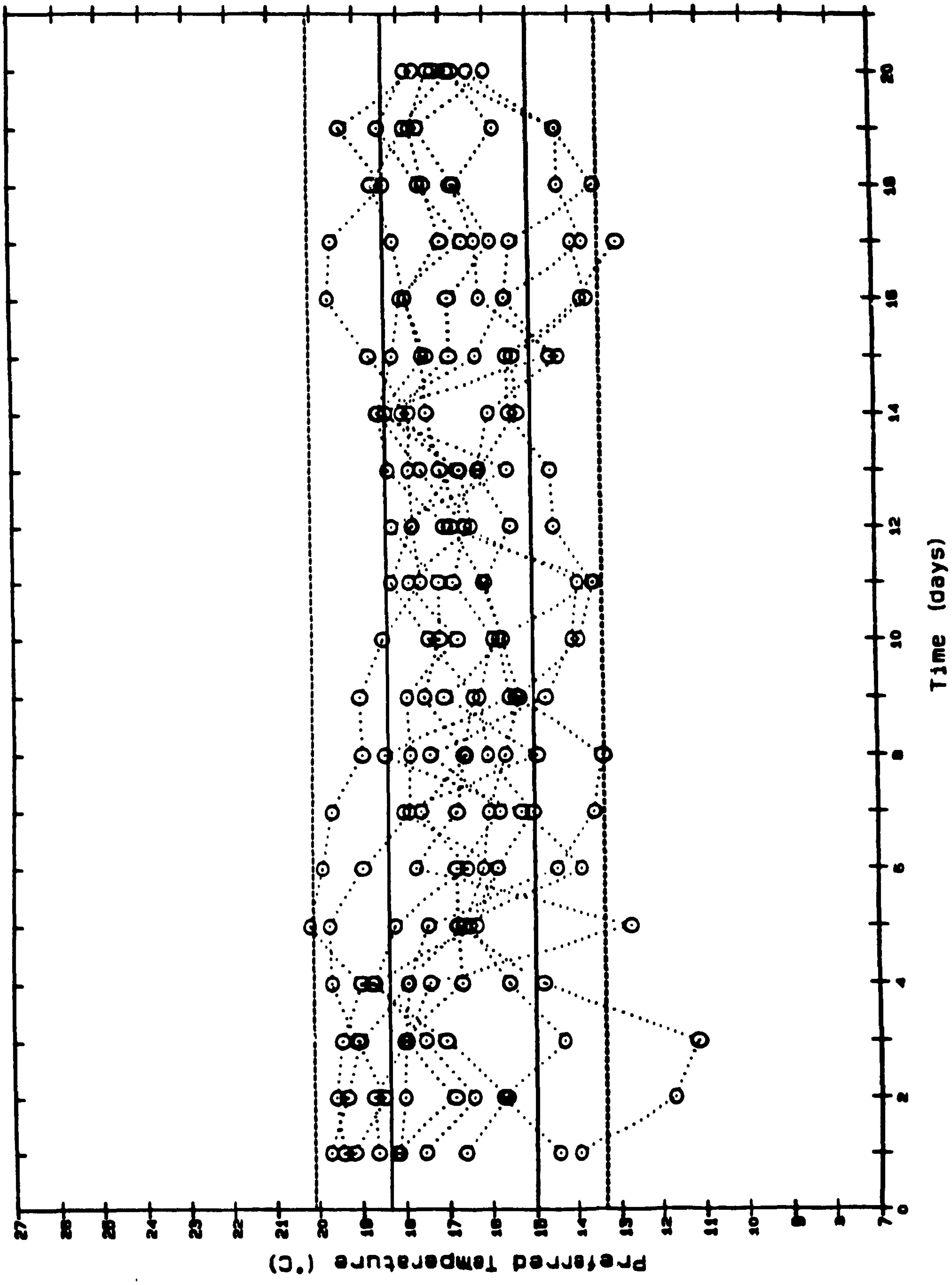

Preferred Temperature (°C)
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
Time (days)
0
2
4
6
8
10
12
14
16
18
20

Figure 6. Daily mean preferred temperatures of 9 juvenile rainbow trout fed a restricted ration (1.30 %/day) over the 20 day thermoregulatory test period. (———) indicates the upper and lower limits of the thermal preference range (FTP ± 1 SD) and (- - -) outlines the range encompassing 95% of the data distribution (FTP ± 2 SD).

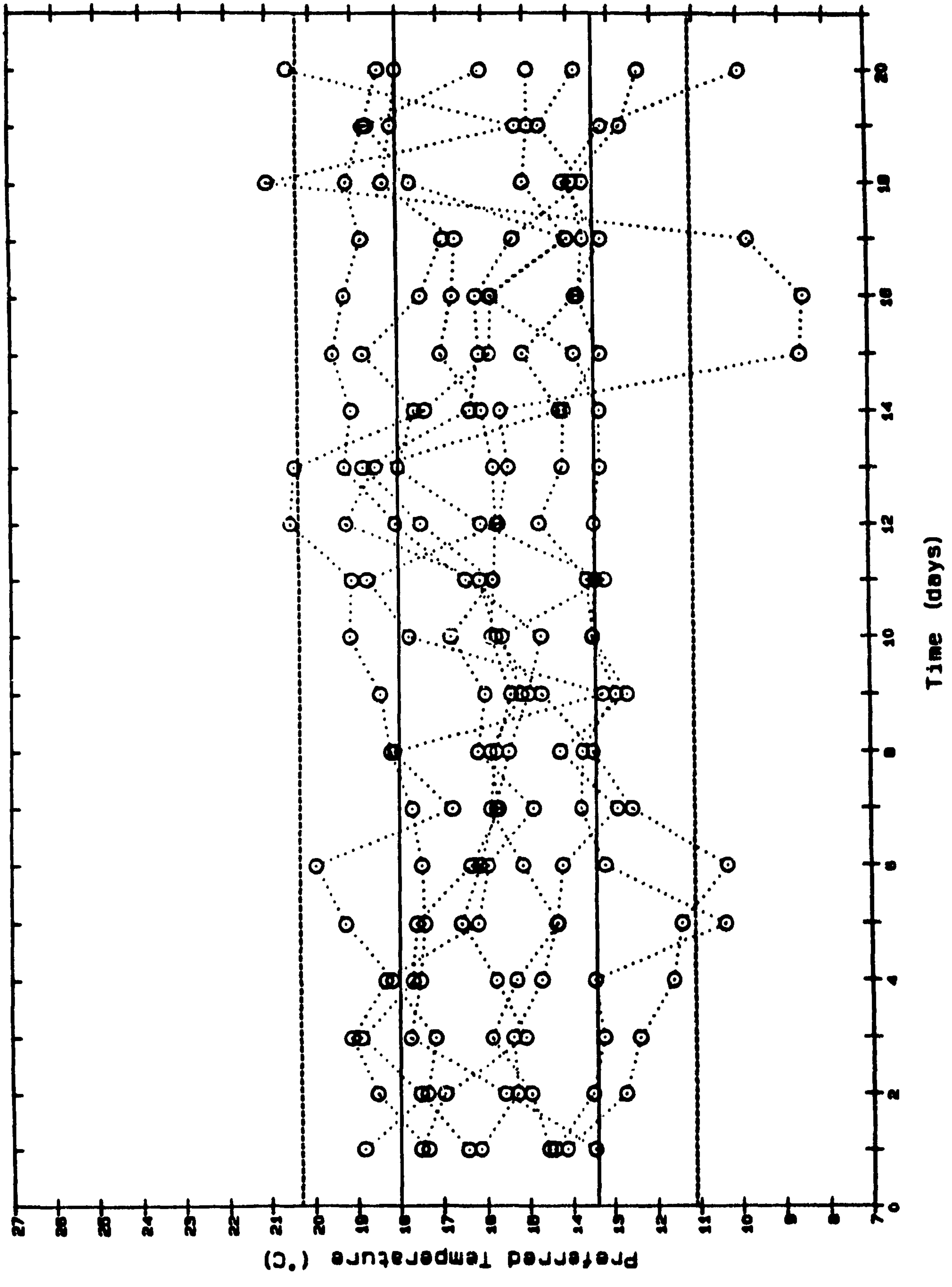
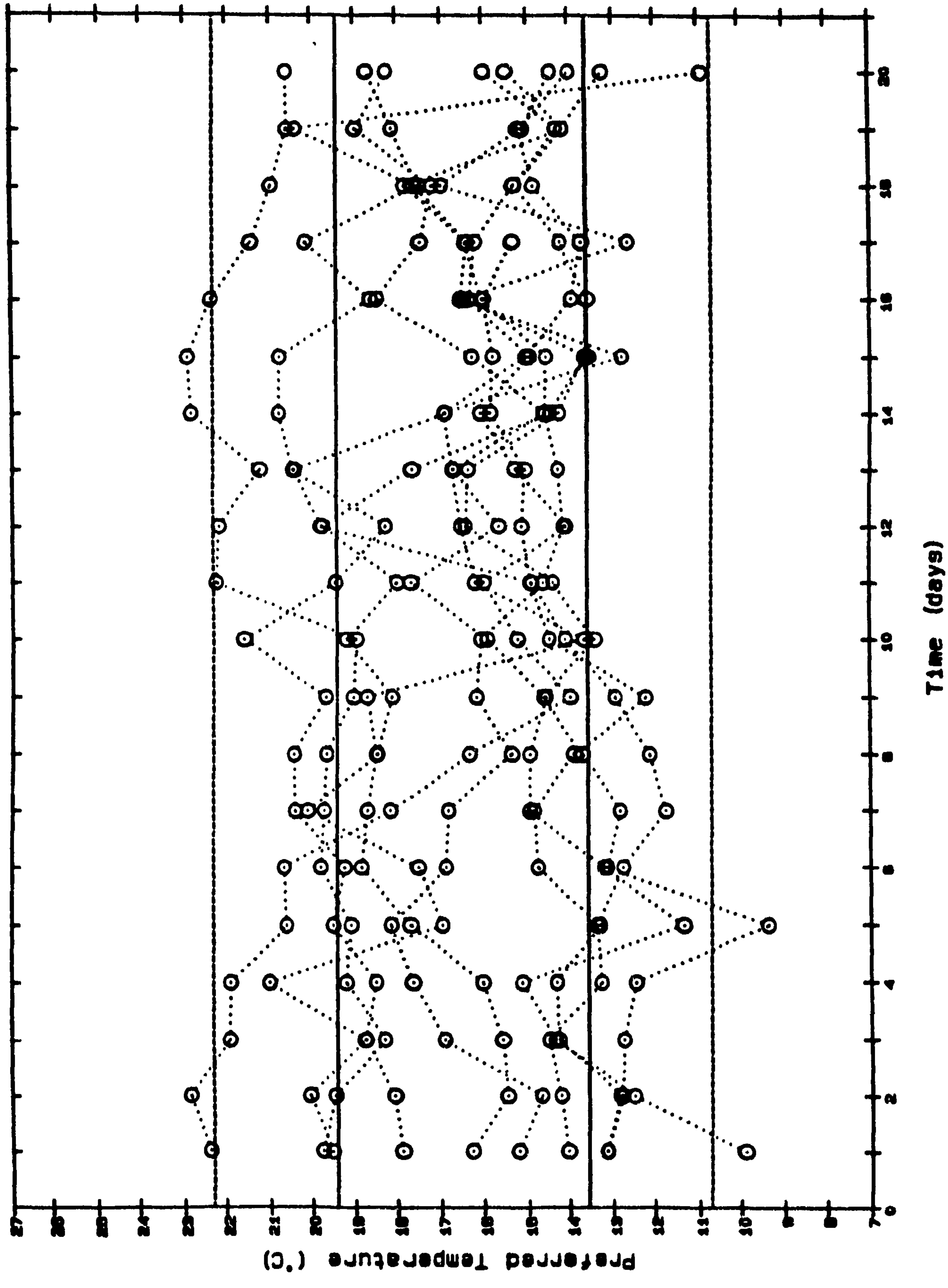

Preferred Temperature (°C)
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
Time (days)
0
2
4
6
8
10
12
14
16
18
20

Figure 7. Daily mean preferred temperatures of 10 juvenile rainbow trout deprived of food over the 20 day thermoregulatory test period. (———), indicates the upper and lower limits of the thermal preference range (FTP ± 1 SD) and (- - -), outlines the range encompassing 95% of the data distribution (FTP ± 2 SD).


Preferred Temperature (°C)
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
Time (days)
0
2
4
6
8
10
12
14
16
18
20

The average DMPT for the 10 fish fed to satiation was 16.5°C. The 9 fish fed the restricted regime selected an average DMPT of 15.7°C and the 10 unfed fish selected a DMPT averaging 16.7°C. Analysis of variance (Table 1) showed that these differences in group means were not statistically significant. Consequently, all DMPT data were pooled. The combined data were normally distributed (Figure 8) with a grand mean of 16.4°C.

3.2.2 Thermal Preference Range

Although group means did not differ significantly, there was a difference in the variance of DMPT selected by each ration group (Figures 5, 6 and 7). Among the fish fed *ad libitum*, this variance defined a thermal preference range of 3.4°C , the upper and lower limits of which were 18.4°C and 15.0°C respectively (group mean ± 1 SD). When computed for the restricted ration group, the upper limit of the thermal preference range was 18.0°C, a value similar to the *ad libitum* group. The lower limit however, was shifted downwards to 13.4°C, resulting in an increased breadth of 4.6°C. Among the unfed fish, the thermal preference range was increased further to 5.8°C. This increase was the result of a displacement in both the upper and lower limits, the former shifting upwards to 19.4°C and the latter downwards to 13.6°C. Bartlett's test of homoscedasticity (Zar, 1974) on the pooled DMPTs revealed that these inter-group differences in variance were highly significant ($B_C = 50.38$, $P < 0.001$).

Figure 8. Frequency distribution of the daily mean preferred temperatures of 29 juvenile rainbow trout pooled over the 20 day thermoregulatory test period (n = 568).

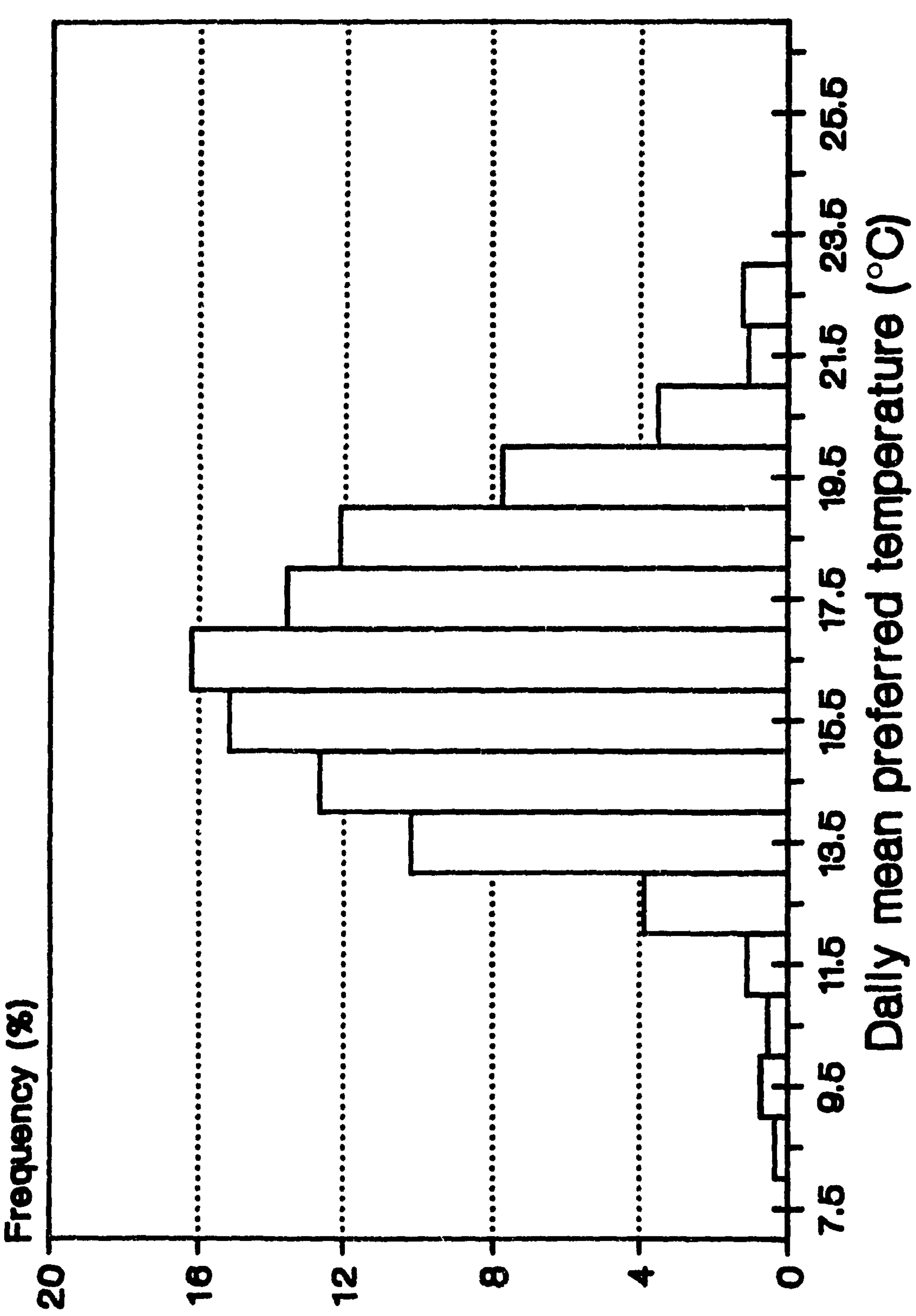

Frequency (%)
20
16
12
8
4
0
7.5
9.5
11.5
13.5
15.5
17.5
19.5
21.5
23.5
25.5
Daily mean preferred temperature (°C)

Table 1. A summary of the Mixed Between-Within-Subjects Analysis of Variance used to examine the effects of ration regime (*ad libitum*, restricted and unfed) on the daily mean preferred temperatures of 29 juvenile rainbow trout over the initial 17 days of the thermoregulatory test period.

Source of variation	df	MS	F	P
Ration	2	50.279	0.99	0.384
Error (1)	26	50.602		
Time (days)	16	4.348	1.40	0.240*
Time X Ration	32	1.810	0.58	0.788*
Error (2)	416	3.106		

* df adjusted using Greenhouse-Geisser $\epsilon = 0.2466$ to compensate for lack of sphericity ($P < 0.0001$)

To determine whether this increase in TPR is a function of the precision with which fish regulated their ambient temperatures in the shuttlebox, a scatterplot was constructed relating thermoregulatory precision and DMPTs (Figure 9). If TPR were a function of precision, one would expect the variance in DMPTs to increase with increases in TPI (i.e. as fish thermoregulate less precisely). However, as indicated by the constant variance in DMPT over the full range of TPIs, no such trend was apparent. This would suggest that the increases in TPR were not due to a loss in thermoregulatory precision.

3.2.3 Diel Rhythmicity

Spectral analysis of individual time series of hourly mean preferred temperature revealed a diel periodicity (1 cycle/day) in temperature preference in 24 of the 29 fish (Appendix V). Of the 24 fish, some cycled quite consistently (e.g. Figure 10), while others cycled periodically or sporadically over the course of the experiment (e.g. Figure 11). In 11 of the 24 fish, additional 12 hour cycles were observed. However, these latter cycles were often transient and always much weaker than the 24 hour cycle. The remaining 5 test fish were judged to be primarily arhythmic, as only very weak peaks or an absence of peaks were observed in their spectrograms (e.g. Figure 12). In most cases, the spectrograms revealed few other prominent cycles in thermal preference, suggesting that all temporal variations other than those occurring with 24, and 12 hour periods were random or non-cyclical in nature

Figure 9. Scatterplot of daily mean preferred temperatures versus daily thermoregulatory precision indexes. The greater the index value, the less precisely fish are thermoregulating.

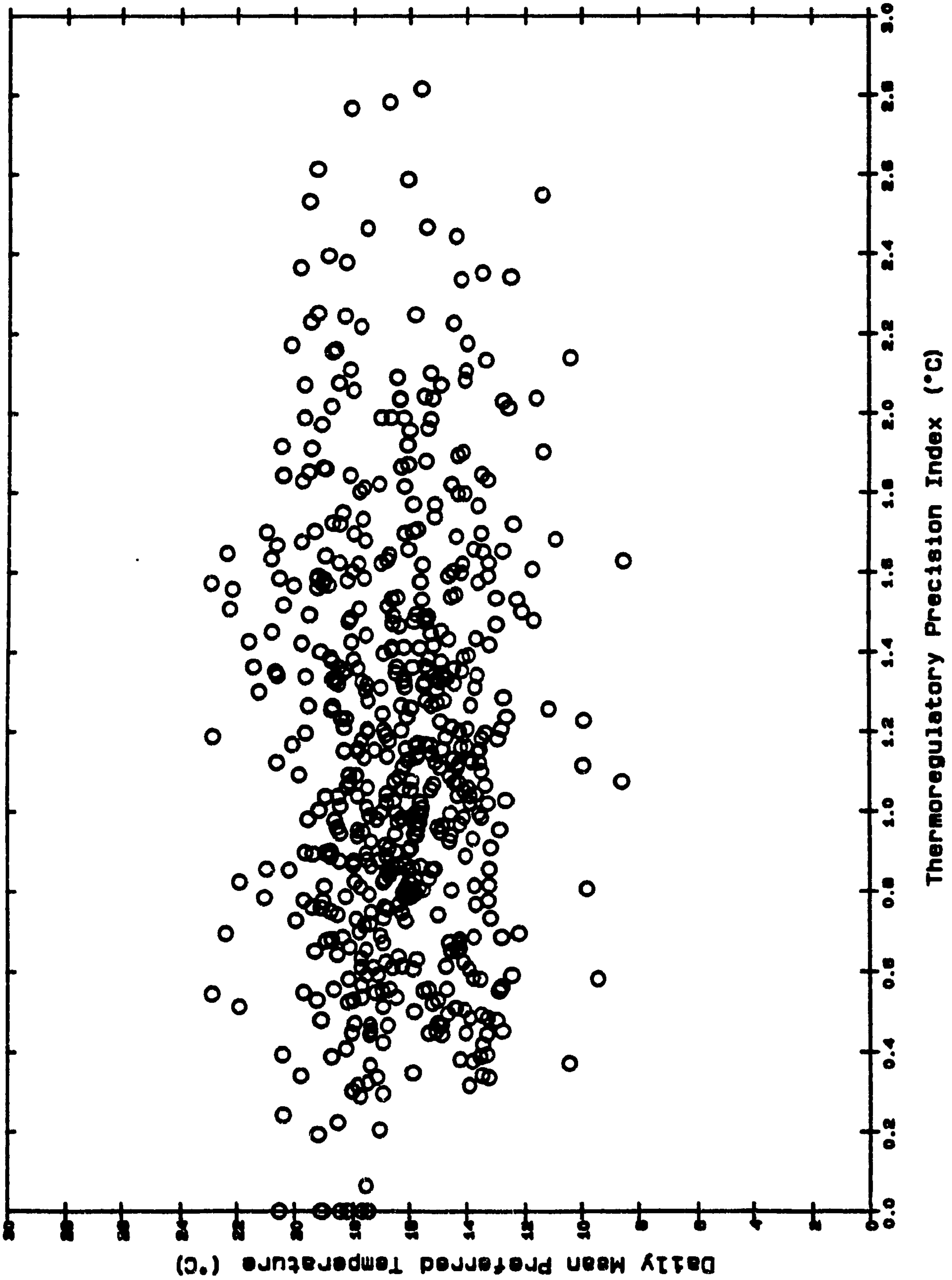
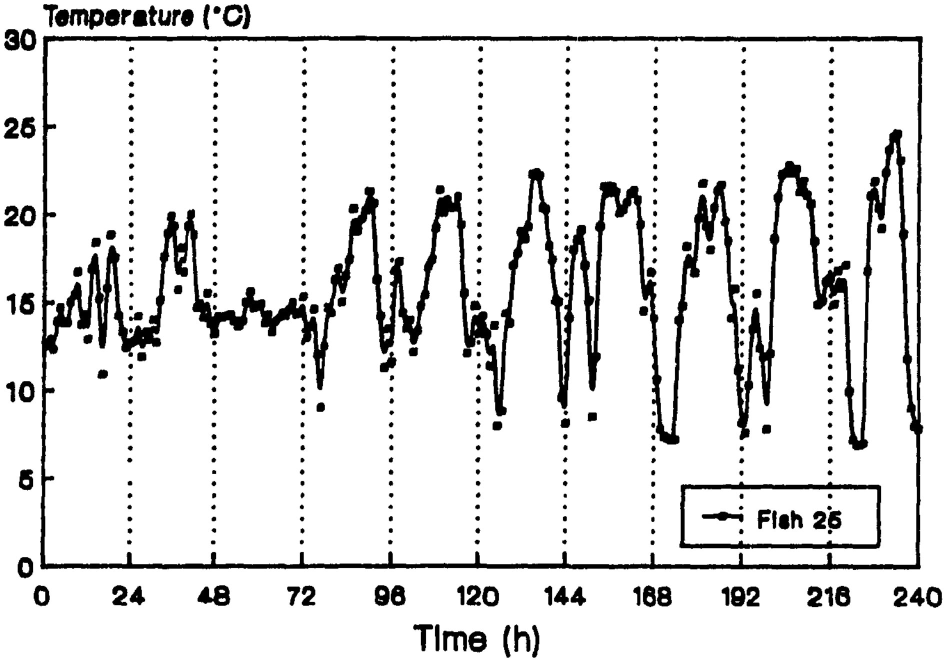

Daily Mean Preferred Temperature (°C)
Thermoregulatory Precision Index (°C)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0

Figure 10. Hourly mean preferred temperature of Fish #25 demonstrating a consistent diurnal pattern in temperature selection over a 20 day period. A, is the time series for the first 10 days and B, for the latter 10 days.

A


Temperature (°C)
30
25
20
15
10
5
0
0
24
48
72
96
120
144
168
192
216
240
Time (h)
Fish 25

B

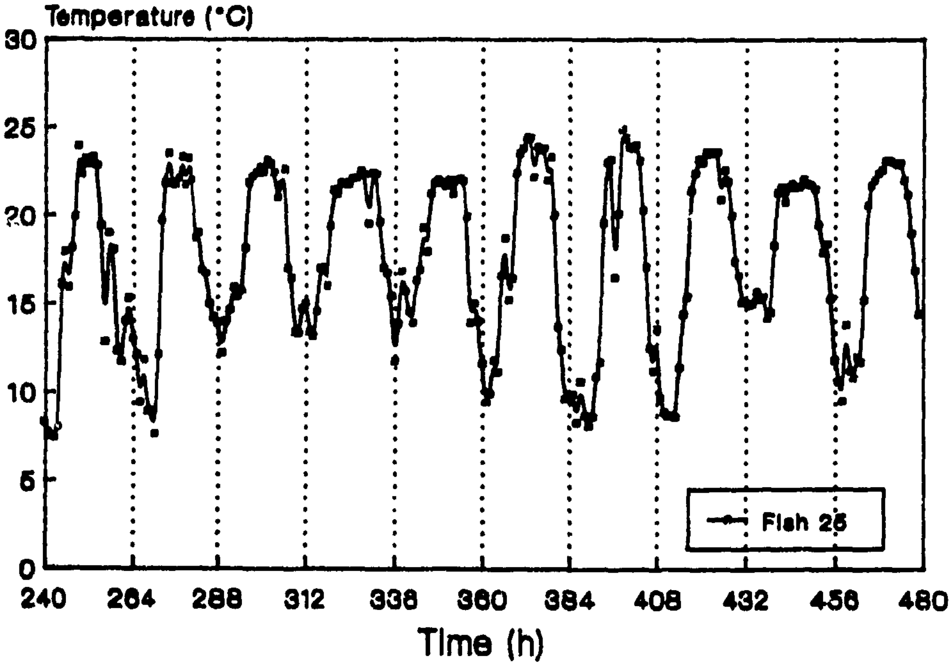

Temperature (°C)
30
25
20
15
10
5
0
240
264
288
312
336
360
384
408
432
456
480
Time (h)
Fish 25

Figure 11. Hourly mean preferred temperature of Fish #2 demonstrating a periodic nocturnal pattern in temperature selection over a 20 day period. A, is the time series for the first 10 days and B, for the latter 10 days.

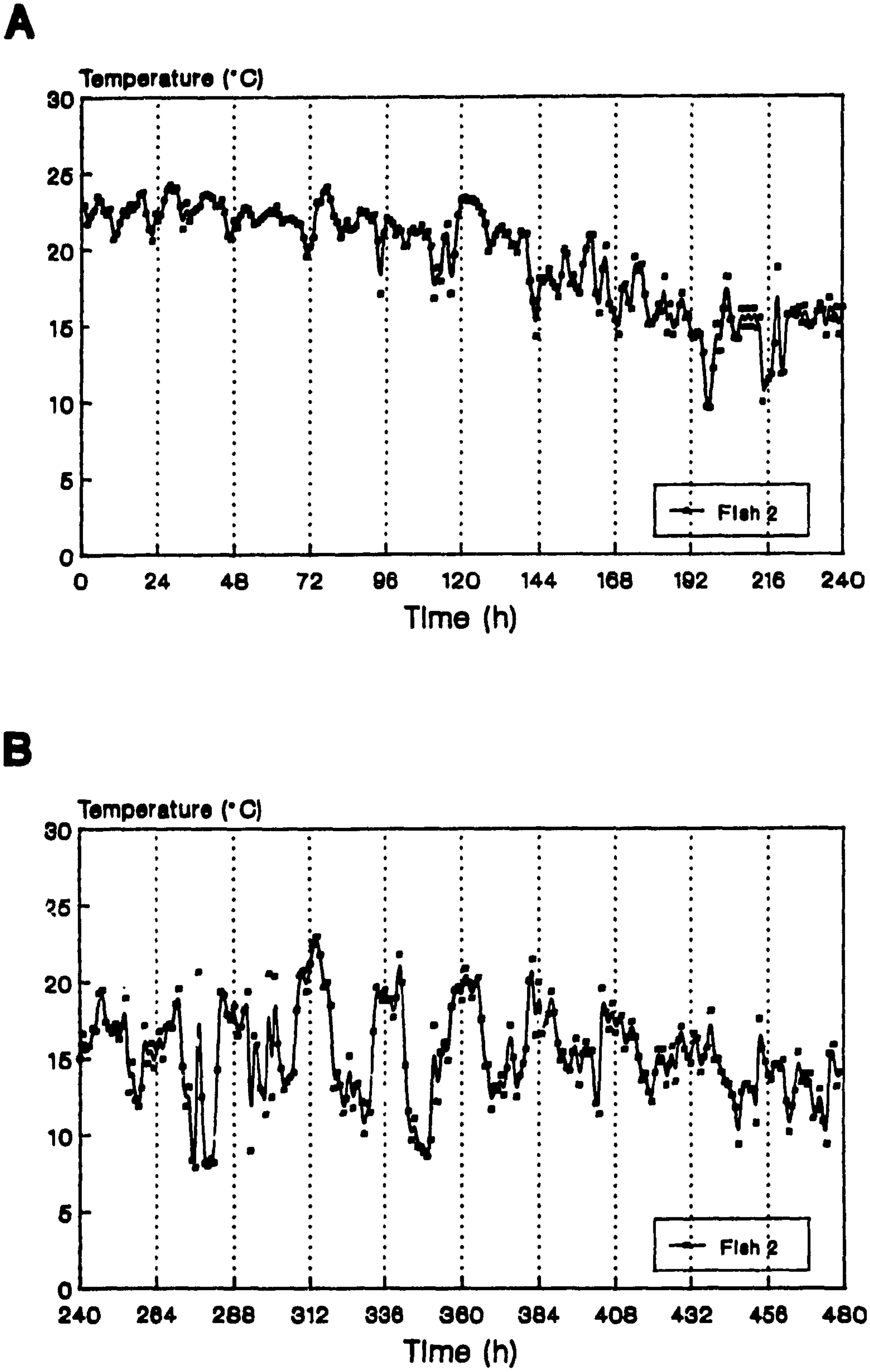
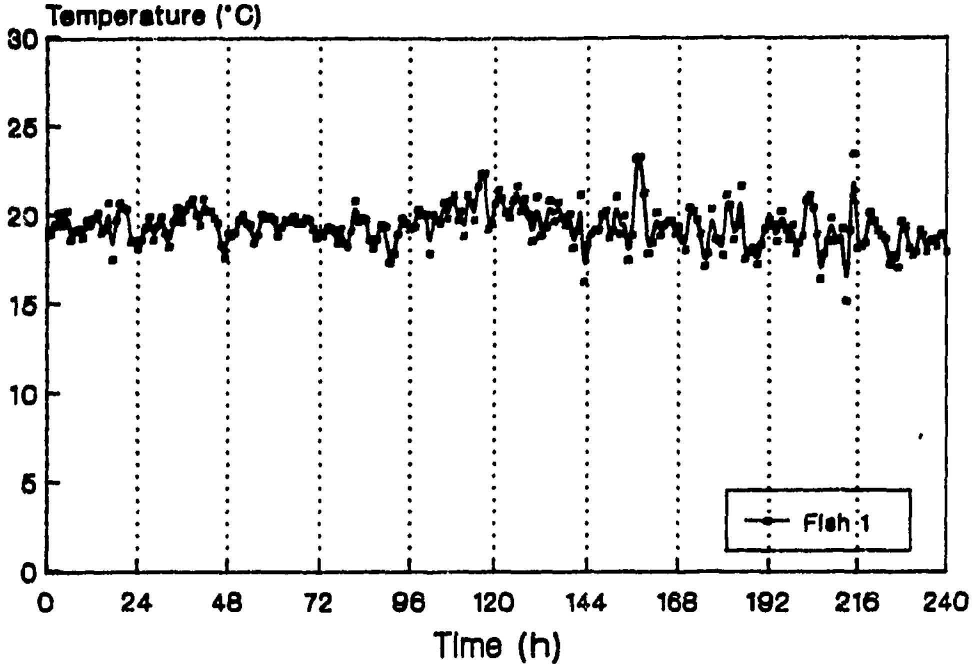
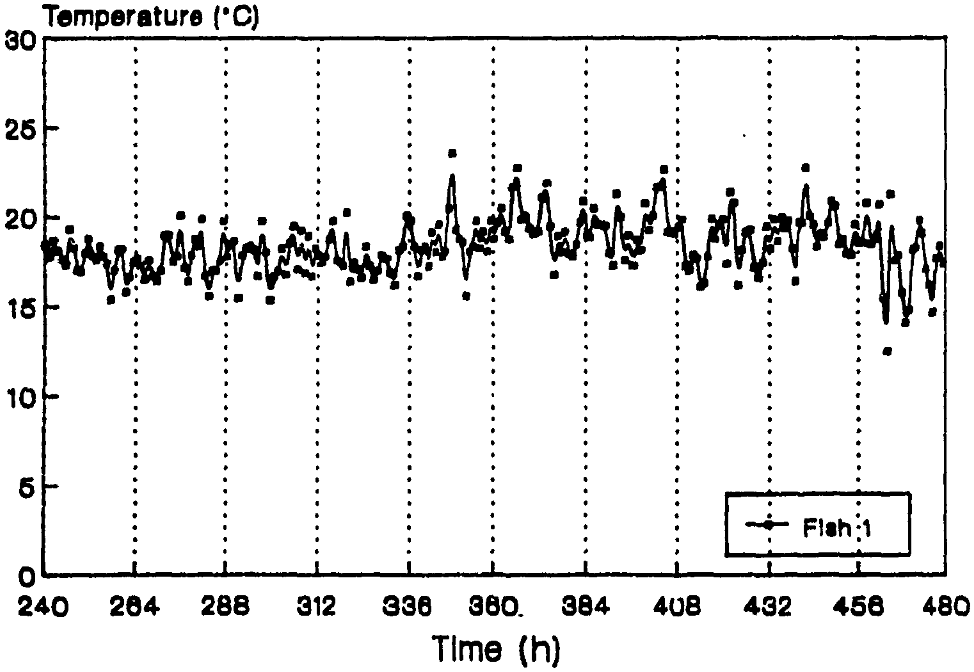

A
Temperature (°C)
30
25
20
15
10
5
0
0
24
48
72
96
120
144
168
192
216
240
Time (h)
Fish 2
B
Temperature (°C)
30
25
20
15
10
5
0
240
264
288
312
336
360
384
408
432
456
480
Time (h)
Fish 2

Figure 12. Hourly mean preferred temperature of Fish #1 demonstrating an arhythmic pattern in temperature selection over a 20 day period. A, is the time series for the first 10 days and B, for the latter 10 days.

A


Temperature (°C)
30
25
20
15
10
5
0
0
24
48
72
96
120
144
168
192
216
240
Time (h)
Fish 1

B


Temperature (°C)
30
25
20
15
10
5
0
240
264
288
312
336
360
384
408
432
456
480
Time (h)
Fish 1

(Bloomfield, 1976).

The amplitude and phase of each daily cycle expressed among the test population was highly variable both among individuals as well as temporally over the course of the test period. Because the shifts between peak and trough temperatures occurred crepuscularly in all cases (e.g. Figures 10 and 11), this variance in cycling was reasonably portrayed by the rhythmicity index. When all index values were pooled, the resulting frequency distribution was leptokurtic and symmetrical about a mean of 0.5°C (Figure 13).

Apparent in this frequency distribution was the equal prominence of both diurnal and nocturnal tendencies in thermal preference. Thirteen of the 24 fish which cycled their preferred temperatures were deemed nocturnal and the remaining fish diurnal. As revealed by a log-likelihood ratio test of independence, ration level (Table 2) had no apparent effect on the frequency of nocturnal or diurnal cycles.

A two-way analysis of variance (Table 3) also revealed that ration level had no significant effect on cycle amplitude. Neither was there a significant time effect, suggesting that temporal changes in amplitude were not consistent among individual test fish. In addition, no significant interaction effect was observed between ration level and time, indicating that inter-group differences in cycle amplitude were not manifested over time

Figure 13. Frequency histogram of the daily rhythmicity indexes of 29 juvenile rainbow trout pooled over the 20 day thermoregulatory test period (n = 568). Positive values indicate a diurnal peak in preferred temperature and negative values a nocturnal peak in preferred temperature. The magnitude of the index reflects the amplitude of the daily cycle.

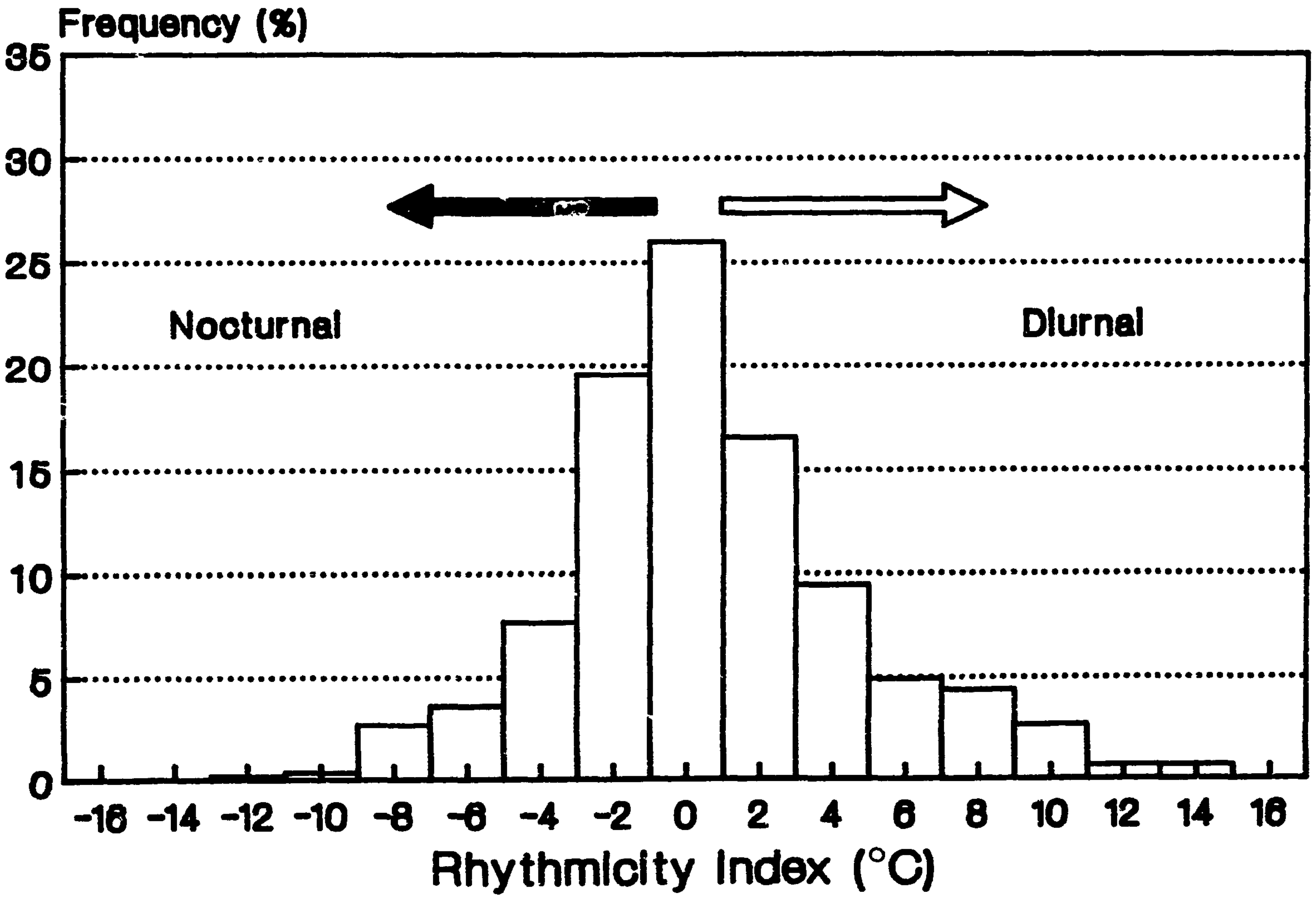

Frequency (%)
35
30
25
20
15
10
5
0
Nocturnal
Diurnal
-16
-14
-12
-10
-8
-6
-4
-2
0
2
4
6
8
10
12
14
16
Rhythmicity Index (°C)

Table 2. Incidence of nocturnal and diurnal rhythms in temperature preference as a function of ration regime. Uf, unfed; Res, restricted; Ad, *ad libitum*. Fish were judged to be cycling based on their respective spectrograms and cycle phase was determined from the computed 20 day mean rhythmicity index.

	Ration level			
Phase	Ad	Res	Uf	Totals
Diurnal	5	4	4	13
Nocturnal	3	4	4	11
Totals	8	8	8	24

G statistic = 0.169

Williams Correction Factor (q) = 1.084

$G_{adj} = G/q = 0.156$, $P > 0.90$

Table 3. A summary of the Mixed Between-Within-Subjects Analysis of Variance used to examine the effects of ration regime (*ad libitum*, restricted and unfed) on the daily cycle amplitude ($\log(x + 1)$ transformed rhythmicity index) of 29 juvenile rainbow trout over the initial 17 days of the thermoregulatory test period.

Source of variation	df	MS	F	P
Ration	2	1.117	2.14	0.138
Error (1)	26	0.522		
Time (days)	16	0.055	1.00	0.435*
Time X Shuttlebox	32	0.061	1.10	0.359*
Error (2)	416	0.055		

* df adjusted using Greenhouse-Geisser $\epsilon = 0.5300$ to compensate for lack of sphericity ($P = 0.0386$)

3.2.4 Thermoregulatory Precision

The thermoregulatory precision index (section 2.4.2) measured the daily average variance about the hourly mean temperatures selected by individual fish and is an indicator of how precisely ambient temperatures were regulated in the shuttlebox. Approximately 95% of these values were found to be below 2°C. When all data were pooled, the index averaged 1.1°C (Figure 14).

As revealed through analysis of variance (Table 4), thermoregulatory precision did not vary significantly over the 20 day test period. Furthermore, as indicated by a lack of a significant interaction effect, there were no temporal differences in precision among the three ration groups. However, there was an apparent tendency for the unfed fish to thermoregulate less precisely than those test fish which were fed either restricted or *ad libitum* rations (Figure 15). With the exception of day 14, this difference in precision was persistent throughout 20 day test period. When pooled within each ration group, the average TPI was identical between the *ad libitum* and restricted ration groups, both averaging 1.0°C. Among the unfed group, the TPI averaged 1.4°C. This difference, however was not statistically significant (Table 4).

3.2.5 Shuttling Activity

Daily shuttling rates varied considerably between individuals and on a day to day basis, ranging from 2 to 131 shuttles/hour. As illustrated in Figure 16, group mean shut-

Figure 14. Frequency distribution of the daily thermoregulatory precision indexes of 29 juvenile rainbow trout pooled over the 20 day thermoregulatory test period (n = 568).

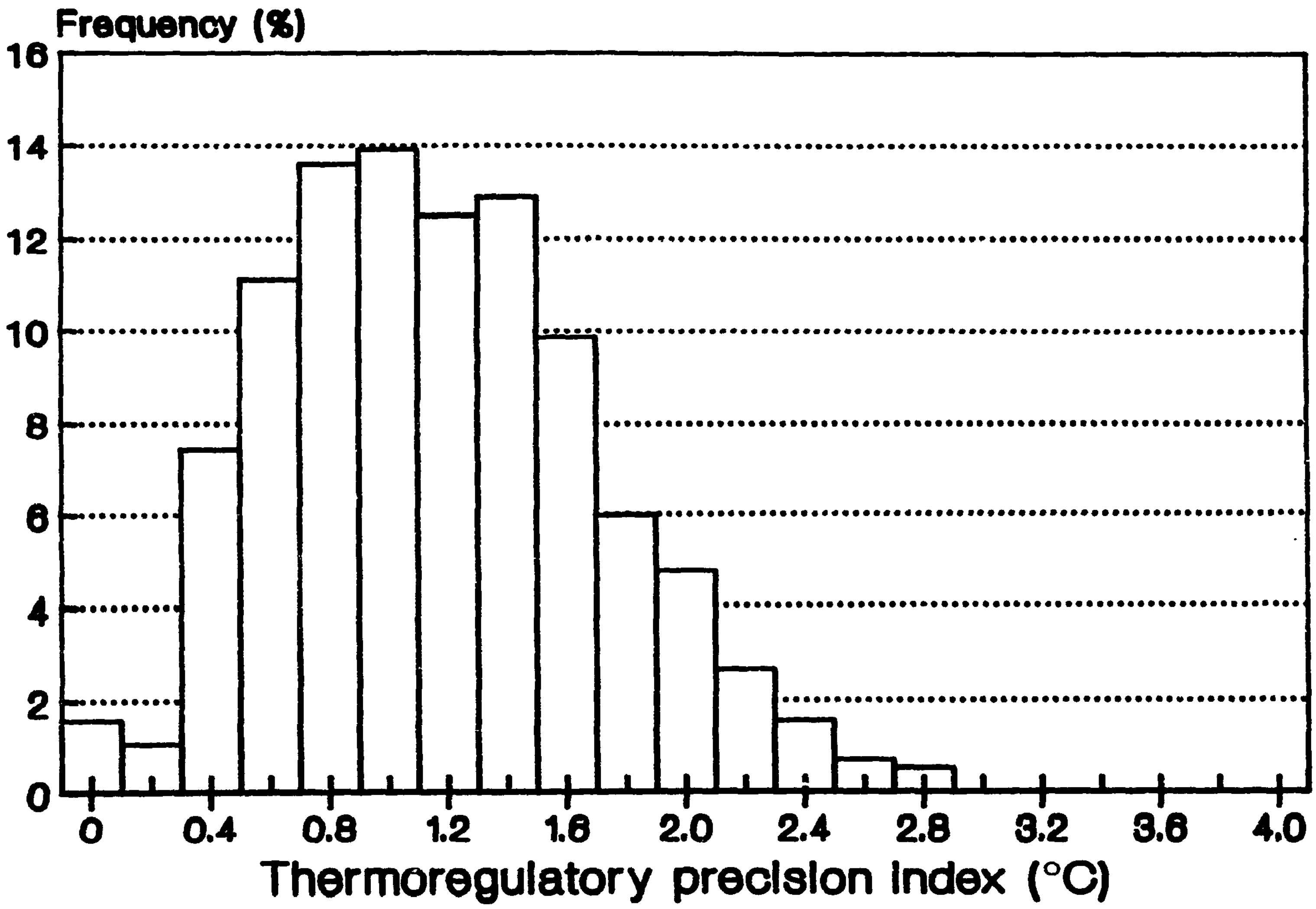

Frequency (%)
16
14
12
10
8
6
4
2
0
0
0.4
0.8
1.2
1.6
2.0
2.4
2.8
3.2
3.6
4.0
Thermoregulatory precision index (°C)

Table 4. A summary of the Mixed Between-Within-Subjects Analysis of Variance used to examine the effects of ration regime (ad libitum, restricted and unfed) on the daily thermoregulatory precision indexes of 29 juvenile rainbow trout over the initial 17 days of the thermoregulatory test period.

Source of variation	df	MS	F	P
Ration	2	6.539	2.56	0.097
Error (1)	26	2.558		
Time (days)	16	0.157	1.13	0.342*
Time X Ration	32	0.184	1.33	0.256*
Error (2)	416	0.139		

* df adjusted using Greenhouse-Geisser $\epsilon = 0.1833$ to compensate for lack of sphericity ($P < 0.0001$)

Figure 15. Mean thermoregulatory precision index for each ration group of juvenile rainbow trout (*ad libitum*, restricted and unfed) over the 20 day thermoregulatory test period.

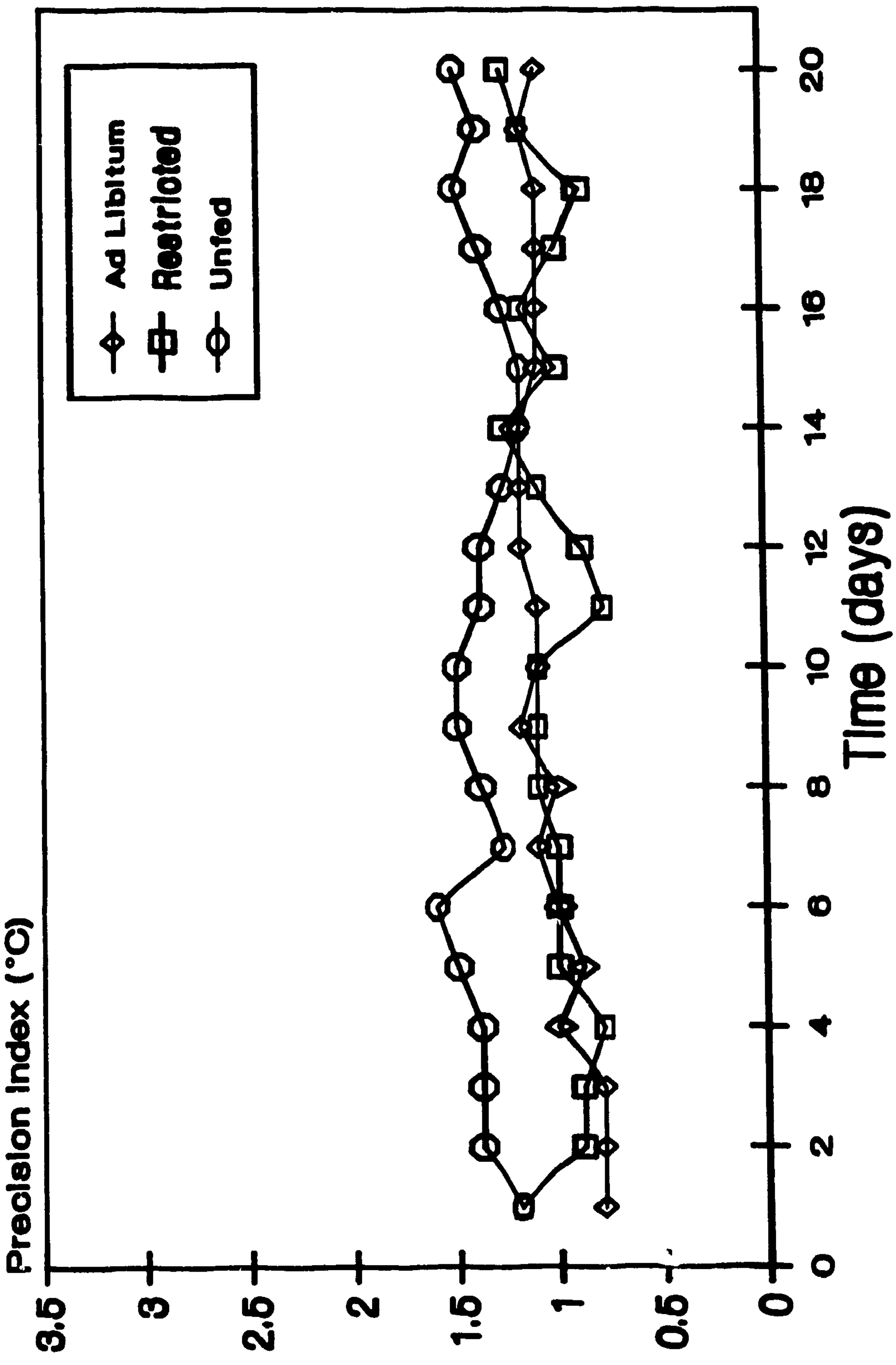
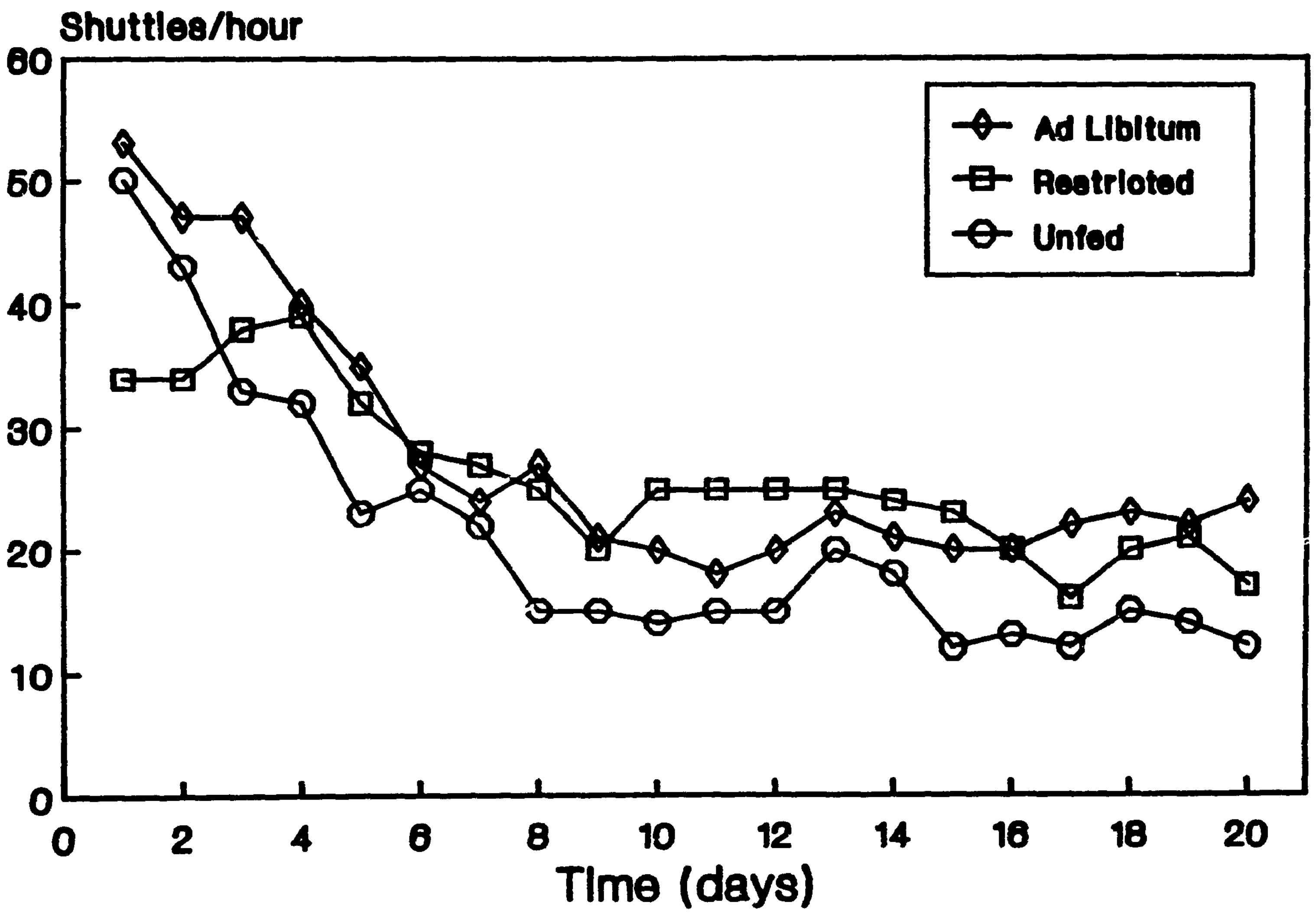

Precision Index (°C)
3.5
3
2.5
2
1.5
1
0.5
0
0
2
4
6
8
10
12
14
16
18
20
Time (days)
Ad Libitum
Restricted
Unfed

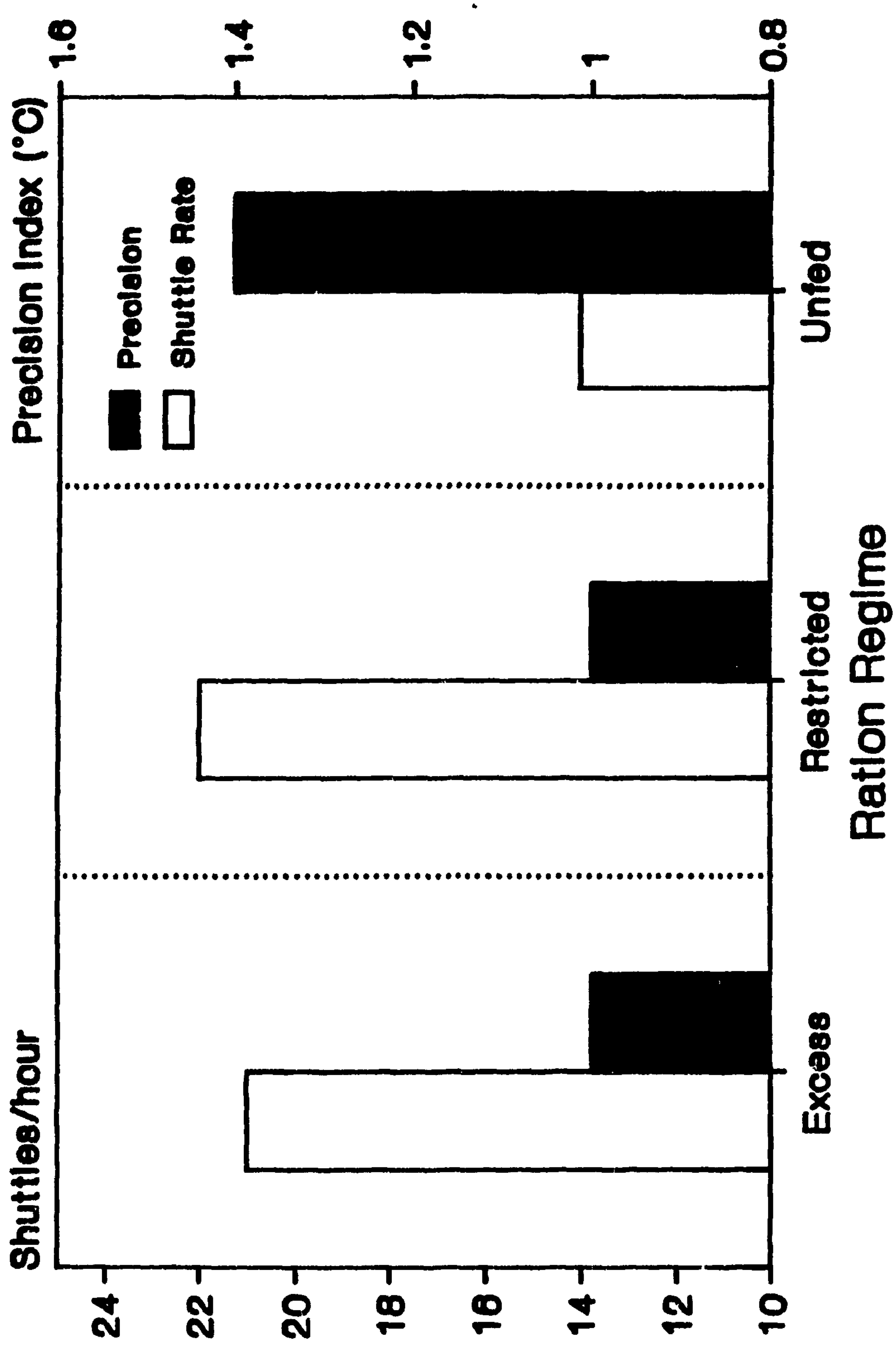
Figure 16. Mean daily shuttling rate for each ration group of juvenile rainbow trout (*ad libitum*, restricted and unfed) over the 20 day thermoregulatory test period.


Shuttles/hour
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
20
Time (days)
Ad Libitum
Restricted
Unfed

tling rates fell gradually during the initial 8-10 days of the test period. For the remaining 10-12 days, shuttling rates were remarkably constant. During this latter period, the average shuttling rate for unfed fish was consistently lower than those fed either restricted or ad libitum rations. In the former group, the 10 day average shuttling rate was 14 shuttles/hour. In the latter two groups, shuttling averaged 21 and 22 shuttles/hour respectively. However, One-Way Analysis of Variance of individual 10 day mean shuttling rates showed that these differences were not statistically significant ($F_{2,26} = 0.82$, $P = 0.42$).

Shuttling rate was highly correlated with thermoregulatory precision ($r_s = -0.489$, $P << 0.001$). This relationship was particularly evident during the latter 10 days of the test period. Based on equation 1, fish shuttling 21 or 22 shuttles/hour would have been able to maintain ambient water temperatures within a 2°C range. This corresponds well with the range of temperatures defined by the average precision index for both the ad libitum and restricted ration groups. In both cases, the precision index averaged 1.0°C, defining a 2.0°C range in temperatures (i.e. FTP ± 1 TPI). Among the unfed fish, shuttling 14 shuttles/hour would have allowed them, as predicted by equation 4, to maintain ambient temperatures within a 3°C range. This value is similar to the actual 2.8°C range empirically defined by ± 1 TPI (Figure 17).

Figure 17. 10-day mean shuttling rate and 20-day mean thermoregulatory precision index for fish fed *ad libitum*, a restricted ration, and deprived of food.


Shuttles/hour
24
22
20
18
16
14
12
10
Precision Index (°C)
1.6
1.4
1.2
1
0.8
Precision
Shuttle Rate
Excess
Restricted
Unfed
Ration Regime

3.2.6 Bioenergetics

3.2.6.1 Food Consumption

For those fed *ad libitum*, estimates of actual food consumption were obtained for all but one test fish. In the latter the unconsumed pellets were rarely left intact, making it impossible to determine the actual number consumed. As a result, this particular individual (Fish #1) was not included in the analysis. The average relative food consumption rate of fish fed restricted rations was 1.30 %/day. Fish fed *ad libitum* rations voluntarily limited their intake to a ration averaging 2.60 %/day.

As illustrated in Figure 18, 20 day mean preferred temperatures were, on the whole, independent of ration level ($r = 0.055$ $P >> 0.05$). However, a strong positive correlation was observed between both variables when the fish fed *ad libitum* were considered alone ($r = 0.70$, $P < 0.05$). Thus appetite (voluntary food consumption rate) in the *ad libitum* group was a function of the respective temperatures preferred by individual fish.

3.2.6.2 Growth

The average relative growth rate of fish fed *ad libitum* during the 20 day test period averaged 3.08 %/day. Fish fed a restricted diet grew at an average rate of 1.35 %/day. The unfed fish lost weight at an average rate of -0.69 %/day.

As illustrated in Figure 19, there was no apparent correlation between 20 day mean preferred temperature and growth

Figure 18. 20 day mean preferred temperature of individual juvenile rainbow trout (n = 28) relative to food consumption rate. Fish are grouped according to their assigned ration regime.

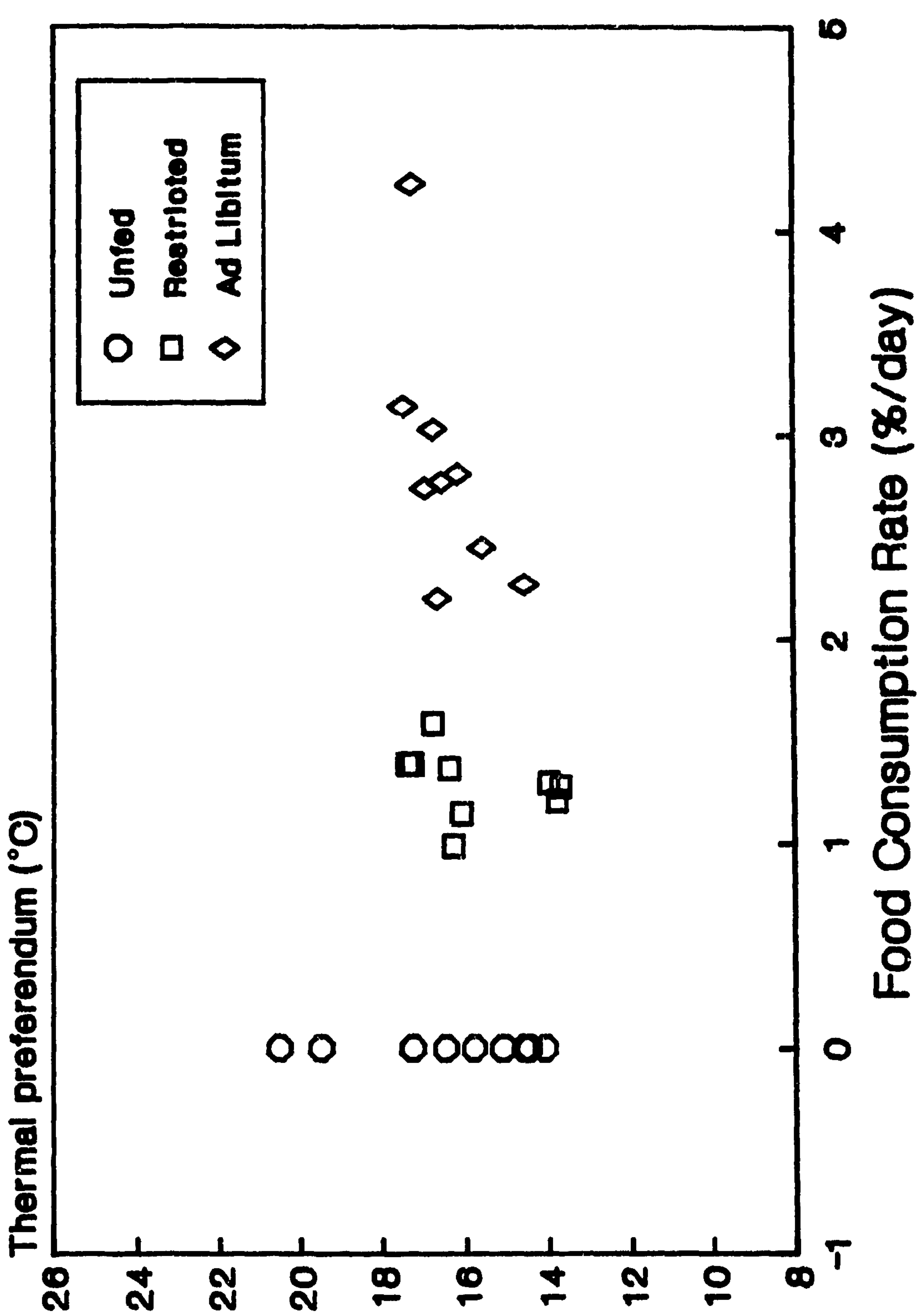
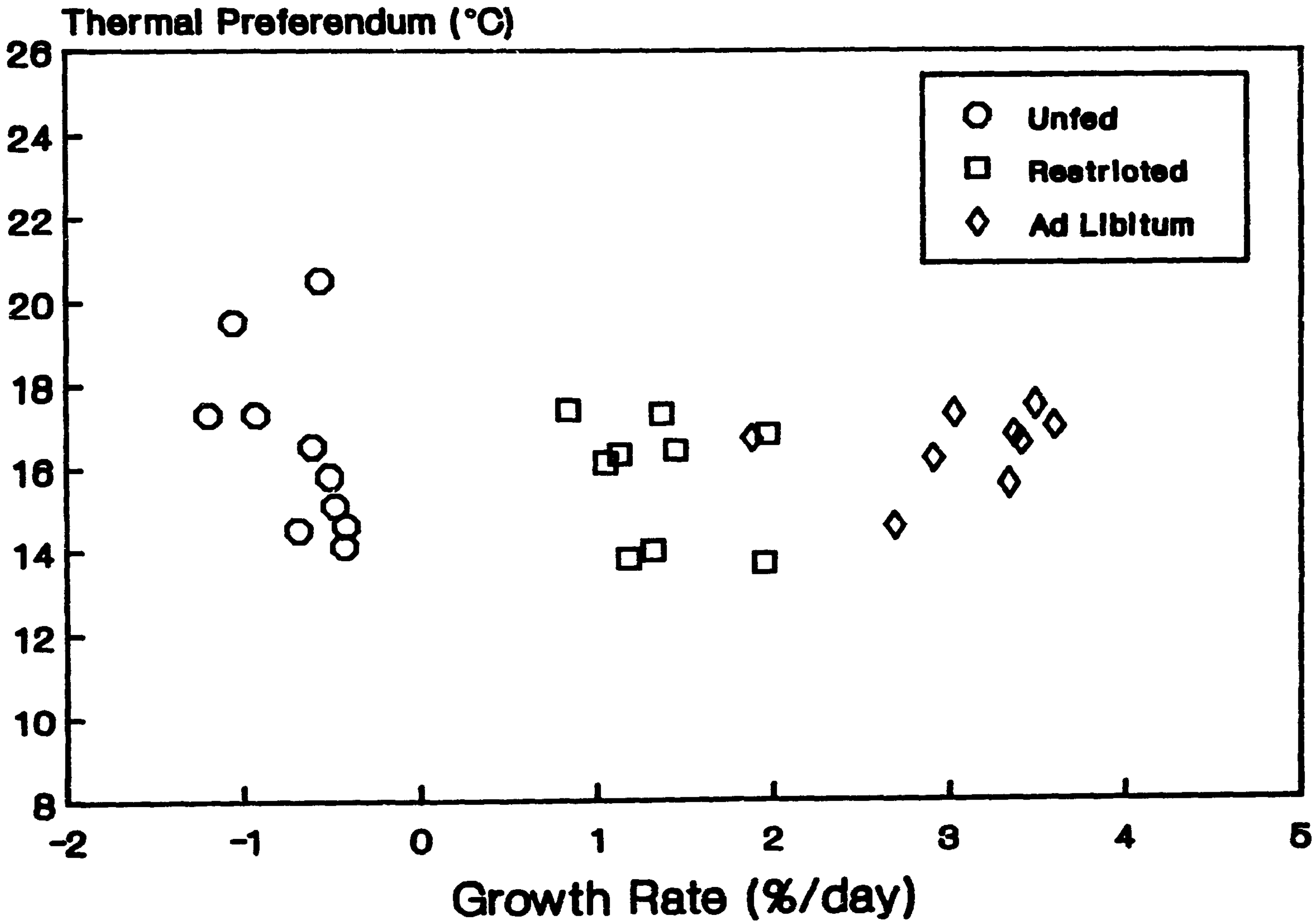

Thermal preferendum (°C)
26
24
22
20
18
16
14
12
10
8
-1
0
1
2
3
4
5
Food Consumption Rate (%/day)
○ Unfed
□ Restricted
◇ Ad Libitum

Figure 19. 20 day mean preferred temperature of individual juvenile rainbow trout (n = 28) relative to growth rate. Fish are grouped according to their assigned ration regime.


Thermal Preferendum (°C)
26
24
22
20
18
16
14
12
10
8
-2
-1
0
1
2
3
4
5
Growth Rate (%/day)
○ Unfed
□ Restricted
◇ Ad Libitum

($r = -0.068$, $P >> 0.05$). Similarly, when assessed within each dietary regime, correlations between preferred temperature and growth did not differ significantly from zero ($r = 0.272$, $P > 0.20$, $r = 0.090$, $P > 0.50$; and $r = -0.481$, $P > 0.10$ for fish fed *ad libitum*, restricted and unfed regimes respectively).

To evaluate the influence of preferred temperature (FTP), thermoregulatory precision (TPI), rhythmicity (RI') and actual food consumption rate on the growth of individual fish, a standard multiple linear regression test was conducted (Table 5). The results of the analysis are summarized in Table 6. Approximately 94 % of the variance in growth was explained by the regression model. A plot of residuals vs. predicted growth rates showed that the assumptions of normality, homoscedasticity and linearity were all satisfied (Figure 20).

The contribution of each independent variable to the variance in growth was determined by computing their respective squared Semipartial Correlation Coefficients (sr_i^2) (Section 2.4.5). Approximately 72% of the variance in growth was uniquely attributed to differences in the rate of food consumption. As indicated by the slope ($B = 1.23$), the greater the consumption rate, the greater the rate of growth. An additional 1.6% of the variance in growth was explained by the amplitude component of the rhythmicity index. However, as indicated by the negative slope ($B = -0.14$), diel cycles with a broad amplitude had a negative, rather than positive influence on growth. Fish grew less effectively at a given ration

Table 5. 20 day mean preferred temperature (FTP), 20 day mean rhythmicity index (Amplitude), 20 day mean thermoregulatory precision index (TPI), food consumption rate (Ration) and growth rate of 29 individual juvenile rainbow trout.

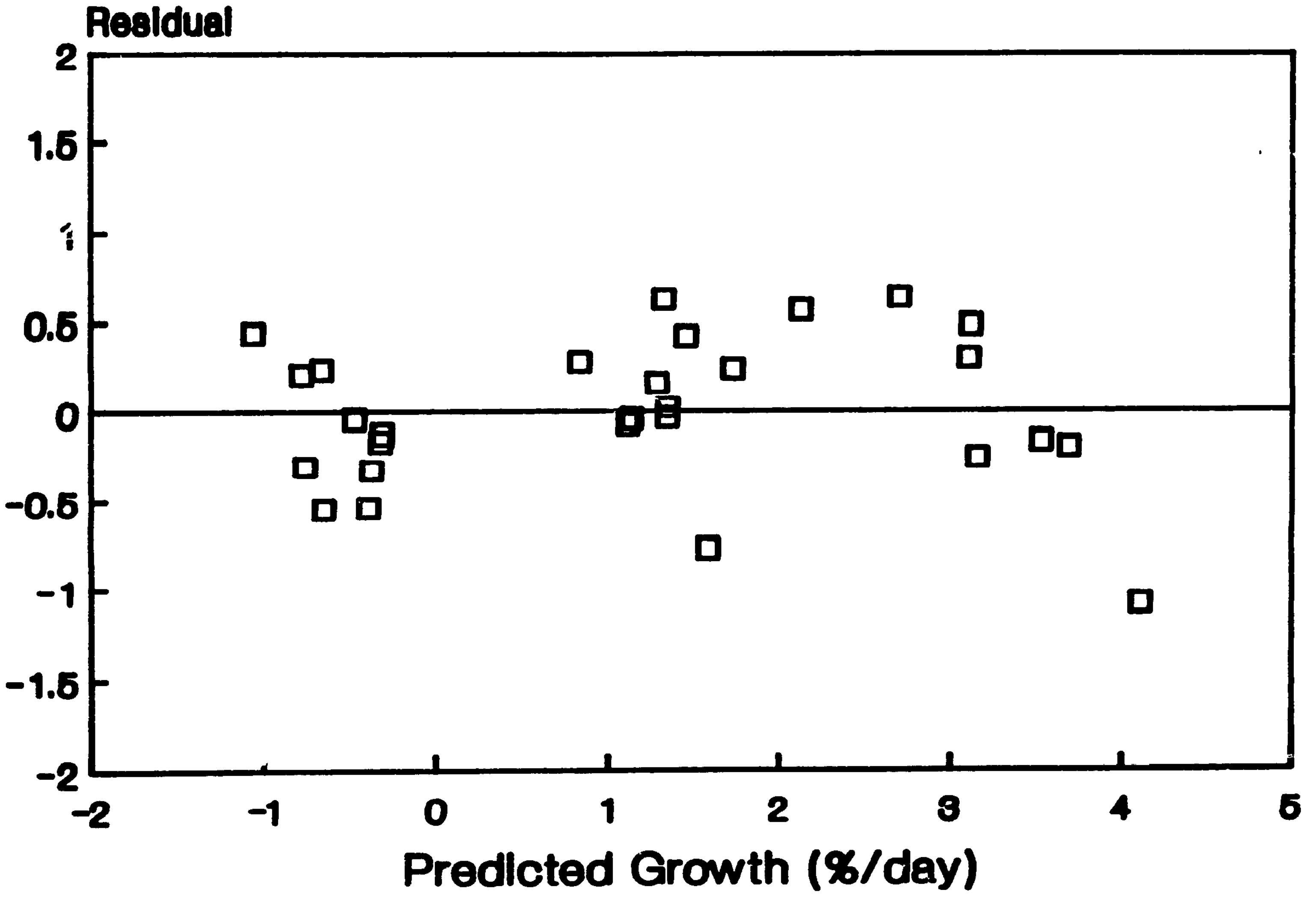
Fish no.	FTP (°C)	Amplitude (°C)	TPI (°C)	ration (%/day)	growth (%/day)
1	18.9	0.7	1.3	—	—
2	19.5	5.0	2.0	0.00	-1.06
3	17.3	2.7	1.6	0.00	-0.93
4	15.6	2.2	1.1	2.45	3.34
5	16.8	1.7	0.9	1.59	1.97
6	14.5	2.6	1.0	0.00	-0.69
7	14.7	4.4	1.4	2.27	2.69
8	13.7	1.8	0.4	1.28	1.95
9	17.3	4.3	1.3	0.00	-1.20
10	16.9	1.8	0.7	2.47	3.60
11	15.1	2.2	0.9	0.00	-0.48
12	16.4	2.7	0.9	1.37	1.45
13	16.6	2.1	1.0	2.77	3.41
14	16.2	2.2	0.7	2.81	2.91
15	15.8	3.2	0.8	0.00	-0.51
16	20.5	5.1	1.8	0.00	-0.57
17	17.3	2.5	1.4	1.39	1.37
18	17.3	7.1	1.3	4.23	3.03
19	17.4	1.0	0.5	1.39	0.84
20	14.6	4.4	1.4	0.00	-0.42
21	13.8	2.4	1.2	1.21	1.10
22	14.1	2.2	1.5	0.00	-0.43
23	16.3	2.6	1.2	0.99	1.13
24	16.1	2.1	1.4	1.15	1.05
25	16.7	8.0	1.4	2.20	1.88
26	16.8	1.5	0.6	3.03	3.37
27	16.5	6.8	1.5	0.00	-0.61
28	17.5	1.4	1.2	3.14	3.49
29	14.0	1.8	1.1	1.30	1.35

Table 6. Standard multiple regression of growth rate on 20 day mean preferred temperature (FTP), 20-day mean rhythmicity index (amplitude), 20-day mean thermoregulatory precision index (TPI) and food consumption rate (Ration) (n = 28).

Variable	B	S_i	sr^2 *	F	P
Intercept	1.18				
FTP	-0.07	0.06	0.004	1.59	0.221
Amplitude	-0.14	0.06	0.016	6.00	0.022
TPI	-0.03	0.28	0.000	0.01	0.909
Ration	1.23	0.08	0.717	263.41	0.000
	Unique variability =		0.737		
	Shared variability =		0.202		
			R^2 = 0.939	88.32	0.000

* df_{res} = 23

Figure 20. Relationship between the predicted growth rate based on the multiple linear regression equation, presented in Table 6, and corresponding residuals.


Residual
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2
-1
0
1
2
3
4
5
Predicted Growth (%/day)

level as the amplitude of the respective diel cycle increased. Both the respective 20 day mean preferred temperature (FTP) and the 20 day mean thermoregulatory precision index (TPI) did not have a significant effect on an individual's growth, each explaining less than 0.3% and 0.05% of it's variance respectively (Table 6).

Because the rate of food consumption of fish fed the restricted ration regime was similar to that provided during the acclimation period, a direct comparison of gross conversion efficiency was made. The average conversion efficiency coefficient for the limited ration group during the acclimation period was 0.97 ± 0.39 SD. During the experimental period the conversion coefficient was 1.04 ± 0.25 SD. A repeated measures T-Test showed that the conversion of food energy to growth did not differ significantly between the two phases of an experiment ($T = -0.478$, $P = 0.645$).

3.2.7 Seasonal Shifts

This study was carried out over the course of one year. Although ambient conditions in the laboratory were held constant during this period, the aging of fish was considered as a possible confounding variable. This issue was explored by grouping experiments and pooling data according to season, since Warner (1974) has indicated that underyearling rainbow trout are able to anticipate seasonal events through an internal "genetic clock". As indicated in Table 7, daily mean preferred temperatures, thermoregulatory precision indexes and shuttling activity, particularly during the latter 10 days of the test period, did not appear to shift on a seasonal basis. However, there was an apparent seasonal shift in the phase and amplitude of diel cycles. During the Fall and Spring, the rhythmicity index averaged 0.6 and 0.7°C respectively, indicating that fish tested during these two periods were primarily arhythmic. During the winter months, however, fish displayed a primarily nocturnal rhythm in temperature preference. The average amplitude of these cycles was 1.3°C. Conversely, primarily diurnal cycles in temperature preference were observed during the summer months, the amplitudes of which averaged 2.3°C.

Table 7. Mean and standard deviation of daily mean preferred temperatures (°C), daily rhythmicity index (°C), daily thermoregulatory precision index (°C), and daily shuttling rate (shuttles/hour) relative to the seasonal grouping of experiments.

	Thermal preference		Rhythmicity index		Precision Index		Shuttling Rate	
Season	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fall	16.7	1.6	0.6	2.8	1.2	0.6	32(18)	28(10)
Winter	16.0	1.6	-1.3	2.7	0.9	0.4	26(20)	20(12)
Spring	16.8	1.9	0.7	2.5	1.3	0.6	22(21)	16(13)
Summer	16.0	1.6	2.7	2.7	1.3	0.5	23(19)	18(12)

DISCUSSION

4.1 Final Thermal Preferendum

The grand mean of all DMPTs during the 20 day period was 16.4°C. This grand average reflects the mean temperature to which the test population, as a whole, gravitated over the 20 day thermoregulatory period and, by definition (Fry, 1947), represents the test population's final thermal preferendum (FTP). In general, previous determinations of Fry's (1947) "final thermal preferendum" have been limited to tests of 4 or 5 days (Reynolds and Casterlin, 1979a). This appears to be the first laboratory study to show that the FTP is a stable feature of thermoregulatory behavior over significant periods of time (3 weeks). This demonstration should dispel any doubts that the FTP is a persistent feature of thermoregulatory behavior in fish.

Because DMPTs did not differ significantly from the grand FTP calculated over the 20 day test period, comparisons can be made with those of the literature. Previously reported FTP values for rainbow trout range from 11.3°C to some unspecified value above 20°C (Table 8). Studies have shown that much of this variance in FTP may be attributed to differences in the age and/or size of the respective test populations (Kwain and McCauley, 1978; McCauley and Huggins, 1979). For the age/size range used in this experiment, FTP values fall between 18.1 to 20°C. This contrasts with the FTP determination in the present study of 16.4°C.

Table 8. Laboratory determined final thermal preferenda of rainbow trout (Oncorhynchus mykiss). Values in parenthesis are estimated. A temporal gradient refers to a shuttlebox device.

Author	Size	Acclimation (°C)	Duration (hours)	Thermal Gradient (°C)	FTP (°C)
Garside and Tait (1958)	1-2 years	10	2	Vertical	13
Javaid and Anderson (1967a)	5-6cm	20	2	Horizontal	(20)
McCauley and Pond (1971)	4-5cm	15-20	<24	Horizontal	18.4
"	4-5cm	15-20	<24	Vertical	18.4
Cherry et al. (1975)	5-12cm	18	2	Horizontal	18.1
McCauley and Huggins (1976)	≈200g	10	2	Horizontal	16.7
Cherry et al. (1977)	5-10cm	18-21	2	Horizontal	19.7
McCauley et al. (1977)	≈200g	5-25	7	Temporal	11.3
Kwain and McCauley (1978)	1-12 mo	10-20	3	Vertical	12-19
Gregory and Anderson (1984)	10-12cm	13	96	Temporal	18.1
Huggins et al. (1984)	20-25cm	10	2	Horizontal	13.4
"	"	10	2	Vertical	11.4
"	"	10	12	Temporal	13.8

The FTP of 16.4°C does appear to reflect closely the temperatures about which certain populations of juvenile rainbow trout have been found to congregate in field studies. Spigarelli and Thommes (1979) have reported that the average core temperatures of juvenile rainbow trout inhabiting an industrial thermal plume ranged from 14.5 to 19.1°C. This range was largely dependent on the available temperatures in the plume. However, during the Fall, when the photoperiod approximated that of the present experiment and when the plume's temperatures ranged from 12 to 21°C, the average core temperature of resident trout was 16.4°C, a value identical to the FTP determined in this project. In a more recent field study, Baltz et al. (1987) have reported a preference by a juvenile rainbow trout population for temperatures averaging 16.8°C when inhabiting a natural thermal plume.

In contrast, Horak and Tanner (1964) reported that juvenile rainbow trout in a thermally stratified lake occupied temperatures ranging between 19 and 21°C, values much higher than the respective preference noted in the studies of Spigarelli and Thommes (1979) and Baltz et al. (1987). However, it was also observed that a large population of adult rainbow trout coexisted with the juveniles in the study lake. Although adult rainbow trout generally prefer cooler temperatures than juveniles, their respective ranges of preferred temperatures overlap considerably (McCauley and Huggins, 1979). Because adult fish have been shown to exclude juveniles from commonly preferred thermal zones (Beitinger and Magnuson, 1975; Medvick et al., 1981), it is possible that the coexistence of adult trout limited the thermal distribution of juveniles to the upper layers of the thermocline. Alternatively, competition for other resources or an orientation towards other factors such as near-surface oxygen levels and light intensity may have contributed to the upward shift in occupied temperature (Fast, 1973; Magnuson et al., 1979).

Nevertheless, there is an overall agreement between the FTP of my study and the field occupied temperatures reported in the literature, despite all of the confounding variables which might have influenced the latter. This correlation between laboratory and field occupied temperatures in juvenile trout adds credence to the notion that temperature preference and thermoregulatory behavior play an important role in the distribution of temperate-zone fishes in heterothermal habitats (Ferguson, 1958; Beitinger and Magnuson, 1974; Brandt et al., 1980; Rudstam and Magnuson, 1985; Baltz et al., 1987)

The FTP of 16.4°C corresponds favorably with the thermal optima or maxima of a variety of functions in juvenile rainbow trout. Events such as active metabolic rate, swimming velocity, swimming acceleration and response latency times proceed most effectively at temperatures within or between 15°C and 20°C (Dickson and Kramer, 1971; Webb, 1978). The ability to learn would also appear to be most facilitated at temperatures in a similar range. In an experiment with juvenile rainbow trout acclimated to 6, 10 and 15°C, Munson et al. (1980) observed that the learning of a feeding task required less time at the highest temperature. Learned behavior in fish has been shown to provide a significant advantage in capturing prey (Ware, 1971; Godin, 1978; Werner et al., 1981). Thus, it is not unreasonable to assume that rainbow trout, in choosing ambient temperatures near or at 16.4°C, were establishing thermal conditions at which many of their bodily functions related to survival and foraging operate best.

Furthermore, the grand mean DMPT of 16.5°C selected by the group fed *ad libitum* corresponds remarkably with reported optimum growth temperatures for juvenile rainbow trout when fed to satiation. One study determined the optimum growth temperature on excess rations to be 17.2°C (Hokanson et al., 1977) and another reported a value of 16.5°C (Wurtsbaugh and Davis, 1977). In Wurtsbaugh and Davis's (1977) study, fish were held in tanks served by water from an outdoor creek. The temperature of this water was observed to fluctuate 1 to 3.5°C on a daily basis. This fluctuation in temperature is similar in amplitude to the average thermal cycle experienced by fish in the present experiment (Table 7). This similarity may explain the remarkable coincidence between this experiment's FTP and Wurtsbaugh and Davis's (1977) growth optimum temperature values.

A reduction in ration size, however, did not cause a corresponding downward shift in FTP as was predicted by the bioenergetic hypothesis (Brett, 1971; Crowder and Magnuson, 1980). FTPs of the groups fed the restricted and unfed regimes did not differ significantly from the 16.5°C FTP selected by the *ad libitum* group.

This result however, must be tempered in light of the fact that the variance in DMPTs differed significantly among the three ration groups. Heteroscedasticity among treatment groups can reduce considerably the power of analysis of variance tests to detect significant differences in group means. Although the group mean DMPT of fish fed *ad libitum* and those deprived of food were virtually identical, fish fed the restricted ration did show an apparent preference for slightly cooler temperatures. When the restricted and *ad libitum* ration groups were compared alone, the relative shift in FTP was in the direction predicted by the bioenergetic hypothesis. However, from respiratory quotient (Q_{10}) calculations, it was estimated that the observed 0.8°C difference in FTPs between *ad libitum* and restricted ration groups represented only a 5% reduction in standard metabolic rate (Dickson and Kramer, 1971). Thus, the decrease in metabolic rate corresponding to the shift in FTP does not appear to provide an adequate compensation for the 2 fold difference in ration size between the two groups. This contention receives support from the fact that the gross efficiency of food conversion in the restricted ration group was the same for both acclimation and test phases, despite the cooler temperatures occupied during the latter phase (17°C versus 15.7°C respectively). In addition, multiple regression analysis revealed that the temperatures selected by individual fish were of little consequence to growth. Thus, the apparent but slight difference in FTPs between the fish fed *ad libitum* and those fed a restricted ration appears to defy a bioenergetic explanation.

Although between groups, temperature preference was independent of food consumption, a significant correlation between these two variables was observed within the *ad libitum* group alone. This finding concurs with many previous studies in which maximum appetite was shown to increase positively with temperature. This correlation was observed to hold over a wide range in temperatures, with an optimum near the upper incipient lethal temperature (Brett, 1971; Elliott, 1976; Jobling, 1988). Thus, although ration size did not affect the FTP of juvenile trout, individual temperature preference did influence maximum appetite when food was available in excess. Fish preferring warmer temperatures consumed more food than those which preferred cooler temperatures. This relationship, however, does not necessarily suggest a bioenergetic advantage since, with increasing temperatures, there is also an increase in the energetic costs of maintenance. This increase in maintenance was evidently a factor in the present study because individual FTP and corresponding growth rates among the *ad libitum* group were not significantly correlated. Thus, in occupying temperatures warmer than the FTP, fish may consume more food, but they do not necessarily achieve a growth advantage.

The results of the present study relative to the issue of FTP and ration size appear to contradict those of Mac (1985). Mac's (1985) experimental design for preferred temperature studies in juvenile lake trout involved a series of 4 rations, one below maintenance, one at maintenance, and two above maintenance. There was not an unfed group, as in the present study. Although Mac (1985) reported a shift in FTP over a range in rations that was in accordance with the bioenergetic hypothesis, the total displacement in FTP was only 3.5°C. The lowest value attributed to this shift was determined from a bimodal frequency distribution in occupied temperatures. Fish in the lower of the two modes of this distribution were obviously occupying temperatures at the lowest limit of the cold water system. From my own preliminary studies with shuttleboxes, such a thermal residence pattern is characteristic of fish which do not shuttle adequately. There is therefore a likelihood that Mac's (1985) group of fish fed below maintenance levels did not adequately learn the shuttling task. Removing this group's data from Mac's determination limits the degree of shift in FTP to 2.0°C. Nevertheless, the apparent correlation between FTP and the level of daily intake remains.

The FTP trend observed by Mac (1985) may have been due to an effect of pre-test ration size on the rate of "gravitation" by fish in establishing their respective FTPs. The test period of Mac's (1985) study was only 2 days, of which the first day served as a gravitation period. In the pre-test growth phase, all ration groups were held at a constant low temperature of 10°C, several degrees below the eventually established FTPs. If the rate of gravitation had varied inversely with pre-test ration level, then it is possible that the 24 hour adjustment period was not adequate for all groups to reach their respective FTPs. Second-day measurements of thermoregulatory behavior would reflect acute thermal preferenda (AcTP) rather than FTPs and would explain the observed correlation. Unfortunately, the effects of ration size on the gravitation process are currently unknown, thus the contradiction between Mac's (1985) study and the present study stands to be resolved with further research.

4.2 Thermal Preference Range

Although ration size had no effect on the mean temperatures preferred by each ration group, it did have a significant effect on the respective range in preferred temperature (i.e. TPR). The TPR was found to be inversely related to ration size. As indicated in Figure 9, differences in thermoregulatory precision cannot account for the expansion in thermal preference range, because the variance in DMPTs of fish thermoregulating with a high degree of precision did not increase. Rather, precision levels decreased. Furthermore, differences in shuttling activity cannot account for the expansion in thermal preference range. At the onset of the experimental period, shuttling activity rates were very high, following which they gradually fell to an asymptotic level. Yet, despite this variance in shuttling, the range of thermal preference was consistent through time.

It has been noted that thermoregulation in fish in their natural habitat is possible only when spatial gradients in temperature exist (Neill, 1979). Therefore in heterothermal environments, spatial distribution is a reflection of thermoregulatory behavior, albeit that this distribution may be confounded by other factors (Magnuson et al. 1979; Houston, 1982). Furthermore, dispersal of fish under conditions of low food availability is a well documented phenomenon, particularly among Salmonidae species (Dill, 1983), including juvenile rainbow trout (Slaney and Northcote, 1974). It is therefore reasonable to assume that an expanded thermal range - as observed in the present study - would be reflected by spatial dispersion in nature. Dill (1983) has noted that dispersal not only decreases population density, thereby increasing an individual's home range, but also allows a population to expand into regions of the ecosystem not normally frequented. In a heterothermal environment, the latter would likely require a "willingness" on the part of individual fish to occupy temperatures further away from the FTP. The present experiment provides empirical evidence that these necessary thermoregulatory adjustments do occur. Thus, the progressive increase in TPR with ration size reductions would appear to be an adaptation which plays a role in dispersal within heterothermal habitats when food supply is limited or inadequate. Of course, the apparent ecological advantage of dispersal is an increased likelihood of encountering prey (Dill, 1983).

Although previously overlooked, there are other laboratory studies which support the pattern of TPR expansion with ration depletion observed in the present study. A reevaluation of the data reported by Stuntz and Magnuson (1976) has revealed that bluegill sunfish fed rations at or below 2% of their body weight occupied a dramatically greater temperature range (10°C) than those fed larger rations (4°C). This increase in thermal range alone can account for the reported 1.4°C difference in FTP between the two ration groups (Stuntz and Magnuson, 1976). A similar pattern can be found in the data published by Lillywhite et al. (1973) who studied an ectothermic amphibian. In this study, western toads (*Bufo boreas*), when fed to satiation, occupied temperatures spanning a range of 10°C. When left unfed for a period of 24 hours or more, the same individuals increased the range to 16°C.

Furthermore, there is a field study which describes an event where thermal range expansion occurred in response to low food availability. Spigarelli et al. (1983) found that the variance in occupied temperature of brown trout (*Salmo trutta*) in inhabiting a thermal plume was inversely related to the monthly impingement rates of alewife (*Also pseudoharengus*) and rainbow smelt (*Osmerus mordax*), their principal prey. These authors concluded that the expansion in thermal range was related to "increased foraging activities" in response to low overall prey density.

Thus, although limited, there is some evidence reported in the literature that supports the contention that spatial expansion under limited ration or starvation conditions is predicated by a broadening of the thermal range normally preferred by fish fed to satiation.

4.3 Diel Rhythmicity

4.3.1 Phase Shifts

A particularly puzzling aspect of the thermal cycling patterns recorded in the present study was the incidence of both nocturnal and diurnal rhythms. These differences in cycle phase were observed both within, as well as between, experiments. When experiments were grouped according to season, it was apparent that these phase differences followed, in part, a seasonal pattern. Fish tested during the Spring (Mar-May) and Fall (Aug-Nov) displayed a general tendency to be arhythmic. Fish tested during Winter (Jan-Feb) were primarily nocturnal and those during the Summer (June-July) were primarily diurnal.

The fish used in this study came from a single lot purchased as fingerlings and brought into the lab in early Summer where they were held at a constant temperature and under a constant lighting regime (Section 2.3.1). Cycling events which occur under such constant environmental conditions are said to be endogenous, being timed by an internal "clock" or "oscillator" (Brown and Graeber, 1982). Thus, the seasonal changes in thermal cycling observed in the present study may have been the consequence of an endogenous circannual rhythm.

When environmental conditions are held constant, endogenous rhythms are said to be "freerunning", cycling with a period that reflects the inherent periodicity of the internal clock. This inherent periodicity varies from individual to individual (Brown and Graeber, 1982). For example, Kavaliers (1982) has reported an endogenous circannual modulation in the amplitude of thermal cycling of white suckers (*Catastomus commersoni*). He noted that, under constant lighting conditions, the circannual period in amplitude size between individual fish varied from 290 to 325 days. Thus, assuming that the seasonal phase shifts observed in the present study were indeed the result of a freerunning endogenous circannual rhythm, the diel thermal cycles displayed by individual fish would not have been synchronized. This variance in periodicity would go far in explaining the thermal cycle differences that were observed both within and between experiments.

There is another factor which may have contributed to the high degree of variation in the thermal cycling phase. Eriksson (1978) has shown that low light intensities increase the probability of phase inversions in the diel rhythms of locomotor activity of brown bullheads (*Ictalurus nebulosus*). Muller (1978) observed similar phase inversions in the locomotory activity rhythms of turbots (Lota lota) when they were exposed to light intensities between 10 and 100 lx. The light intensity used in the present study did not exceed 50 lx, falling within the range reported by Muller (1978). Assuming that it affects thermoregulatory behavior as it does locomotor activity, the low light intensity used in the present study may have contributed also to the variance in the timing of diel thermal cycling.

Finally, it must be noted that, during these experiments, test fish were held in isolation for a period of 40 days. Kavaliers and Ross (1981) have shown that endogenous rhythms are more synchronized between individuals when together in a group than when alone. He suggested that cycling may be coordinated, at least in part, through social interactions. Thus, the isolation which occurred in the present study may have also contributed to the disharmony in thermal cycling.

The differences in cycle phase however, did not detract tests of the bioenergetic hypothesis. Cycle phase was found to be independent of ration regime. In addition, based on the normal frequency distribution of rhythmicity indexes (Figure 13), there was no compelling evidence which would indicate that diurnal cycling patterns were any different from nocturnal patterns. Furthermore, from the arguments of Hokanson et al. (1977), Clark (1978), Biette and Geen (1980), Cox and Coutant (1981), Spigarelli et al. (1982), Diana (1984), Thomas et al. (1986) and Wurtsbaugh and Neverman (1988), it is primarily the amplitude and mesor which determines the bioenergetic consequence of thermal cycling. Thus, cycle phase was not considered to be a confounding variable in this study.

4.3.2 Cycle Amplitude

Based on the bioenergetic hypothesis, it was predicted that only fish fed the restricted ration would cycle through a broad range in temperatures. Fish fed *ad libitum* and unfed fish were expected to cycle through a shallow range if at all. However, cycling occurred in all ration groups. Furthermore, analysis of variance showed that cycle amplitude (i.e. absolute value of the rhythmicity index) did not differ significantly between the three ration regimes. Therefore, the hypothesis that fish would modulate the amplitude of their diel rhythms as a function of ration size can be rejected.

These results, however, may have been confounded by the fact that cycle amplitudes also differed between seasons (see Section 4.3.1). Unfortunately, the small sample size per season prevented a within season test of the bioenergetic hypothesis. Nevertheless, there were no significant shifts in the mesors of cycles (note that mesors are equivalent to the daily mean preferred temperature, DMPT) as was predicted by the bioenergetic hypothesis. Furthermore, cycling, as measured by the amplitude, had a negative, rather than positive, effect on growth. Based on the latter evidence alone, the proposed bioenergetic explanation for thermal cycling can be rejected. It is concluded therefore that thermal cycling and associated diel migrations must serve some other function.

Some authors have suggested that diel rhythms in temperature preference in a heterothermal environment may lead to a temporal segregation of coexisting species, reducing the likelihood of inter/intra-specific competition and the risk of predation. This type of segregation has been demonstrated in the laboratory by Reynolds and Casterlin (1978b) for sympatric bass species (*Micopterus* sp.). They noted that the daily rhythms in temperature preference of largemouth (*M. salmoides*) and smallmouth (*M. dolomieui*) bass were complementary to one another; one displaying a nocturnal preference for peak temperatures and the other a diurnal preference. Assuming that both bass species prey on the same food types, Reynolds and Casterlin (1978b) hypothesized that this would minimize interspecific competition. In a field study, Northcote et al. (1964) reported a similar phenomenon between kokanee salmon (*Oncorhynchus nerka*) and the peamouth chub (*Mylocheilus caurinus*). The Kokanee were observed to occupy the warmer surface waters by day; the peamouth chub by night. Because both species preyed on the same organisms, they concluded that the opposing cycle patterns allowed both species to share a common food resource with minimal competition (Northcote et al., 1964).

Brandt et al. (1980) have suggested that opposing cycles in temperature preference may also minimize intra-specific interactions between different age classes of a species. From bottom trawling studies, they found that adult rainbow smelt (Osmerus mordax) generally occupied temperatures with a mode of 7-8 °C during the day and 11-16 °C at night. Larval smelt however, occupied a daily modal temperature of 13-14 °C and a nocturnal mode of 5-6 °C. Noting that adult smelt prey on their young, Brandt et al. (1980) have suggested that these opposing cycles may represent a mechanism through which cannibalism is minimized.

Clark and Levy (1988) have recently advanced a dynamic optimization model which attributes the diel vertical migration and thermal cycling of juvenile sockeye salmon (Oncorhynchus nerka) to a trade-off between feeding and predation risk. They assumed that predation risk is an inverse function of light intensity. Yet, light is also required for foraging. Thus, when migrating to the warmer surface waters at dusk, light intensities are sufficient for foraging but limiting to predation. Descending to the cooler dark waters at dawn reduces the risk of predation during daylight hours. During these crepuscular intervals, designated as "antipredation windows", the underyearling sockeye presumably balance the risk of predation against feeding.

Among these latter ecological correlates, inherent thermal cycling behaviors are generally thought to play a subsidiary role to daily migratory behaviors. Reynolds (1977b) and Levy (1987) have suggested that diel shifts in temperature may help guide fish during these daily migrations, whereby temperature serves as a proximate orientation factor. Alternatively, Hutchison and Maness (1979) and Spigarelli and Thommes (1979) have suggested that cycling tendencies may be a form of "anticipatory acclimation", whereby fish, through shifts in preferred temperature, adjust their metabolic machinery to accommodate anticipated thermal shifts in their environment. Either of these latter hypotheses can be used to explain the functional significance of thermal cycling.

4.4 Thermoregulatory Precision and Shuttling Activity

All test fish demonstrated a high degree of precision in regulating ambient water temperature. This is reflected by the relatively low standard deviations in the occupied temperatures recorded during each hourly interval of the test period. This variance, measured as the thermoregulatory precision index (TPI), was constant throughout the test period. Because the range in temperatures defined by the FTP ± 1 TPI approximates the range defined by upper and lower turn-around temperatures (Beitinger et al., 1975), this is a strong indication that all test fish thermoregulated effectively and consistently over time.

Average daily shuttling rates at the onset of the test period were high for all ration regimes exceeding the level which would have been necessary to hold ambient temperatures within a 2°C range (derived from equation 1). However, over the first half of the test period, average daily shuttling rates for each ration group dropped gradually and for the remaining 10 days, shuttling occurred at a relatively constant rate. Despite this gradual drop, each ration group's respective TPI remained relatively constant. Furthermore, during the latter 10 days of the test period, fish appeared to shuttle at a rate just sufficient to maintain temperatures within a 2-3°C range, similar in breadth to the range defined by the thermoregulatory precision index (equation 1). This finding would suggest that fish improved in the proficiency with which they performed the shuttling task as the thermoregulatory test period progressed, reaching an optimum after 10 days. Thus, over the course of the initial half of the experiment, fish were presumably gaining experience at regulating their ambient temperature, learning to minimize shuttling to that rate which is just sufficient to achieve a particular level of thermal consistency. If this interpretation is correct, then during the latter 10 days of the test period, shuttling occurred strictly in response to upper and lower turn-around temperatures. This is further evidence that all fish thermoregulated effectively in the shuttlebox device.

Assuming that shuttling activity represents an energetic cost to thermoregulating fish, it was hypothesized that a reduction in ration size would lessen the precision with which fish thermoregulate and that this would, in turn, impart some energy savings. Although fish fed the restricted and *ad libitum* ration regimes thermoregulated with an equal degree of precision, there was a tendency for the unfed fish to thermoregulate less precisely than the other two ration groups. In addition, this difference in precision was associated with an apparent drop in shuttling activity. However, the differences in thermoregulatory precision and in shuttling activity were not statistically significant. Furthermore, the observed differences in shuttling activity were small and do not appear to represent a significant energy savings. This contention is supported by the fact that the precision with which individual fish thermoregulated was of no consequence to growth. Thus, it may be concluded that shuttling activity and thermoregulatory precision were not affected by the rations.

SUMMARY AND CONCLUSIONS

1) This is the first known study to examine concurrently the relationship between ration, growth, and thermoregulatory behavior in fish.

2) The juvenile rainbow trout which were included in the analysis executed the response-contingent behavior of shuttling consistently and regulated their ambient temperatures with a high degree of precision over a 3 week period.

3) The grand mean FTP for all fish was 16.4°C. This value corresponds to the reported field occupied temperatures of rainbow trout inhabiting heterothermal environments.

4) The grand mean FTP of 16.4°C approximates the optimum and maximum temperatures at which many physiological activities related to foraging and survival operate best.

5) The grand mean FTP of 16.4°C also agrees with the reported values for the optimum growth in rainbow trout when fed to satiation.

6) Ration size did not affect the FTP.

7) The range in occupied temperature varied inversely with ration size. This increased range cannot be accounted for by differences in thermoregulatory precision, nor by differences in shuttling activity.

8) Diel rhythmicity in hourly mean preferred temperatures (HMPT) was not altered by ration size.

9) The was no evidence that diel rhythmicity in HMPT is advantageous to growth. Rather, rhythmicity occurs at a slight but significant cost.
10) Seasonal shifts in the phase of diel cycling suggest an endogenous clock mechanism.
11) Thermoregulatory precision and associated shuttling activity were not affected by ration size.

These results demonstrate that fish do not alter their thermoregulatory response patterns to maximize growth relative to ration size. However, these results do offer insight to an alternative strategy. The range in temperatures which fish occupy increases as food consumption rate decreases. However, the test population, under all ration conditions, held to it's centrally preferred temperature of 16.4°C. It is proposed that the expansion in thermal preference range is a strategy which may be advantageous for foraging in a heterothermal environment.

The significance of thermal cycling was not disclosed in this study. Further studies are required on the alternatives to the bioenergetic explanation for this event.

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APPENDIX I

Figure 21. Schematic diagram showing the electronic circuitry used to register the movement of fish between the two ends of a shuttlebox. These signals are used to mediate the control of a three-way solenoid valve and were recorded by microcomputer. Four of these circuits were made, one for each of the four shuttleboxes.

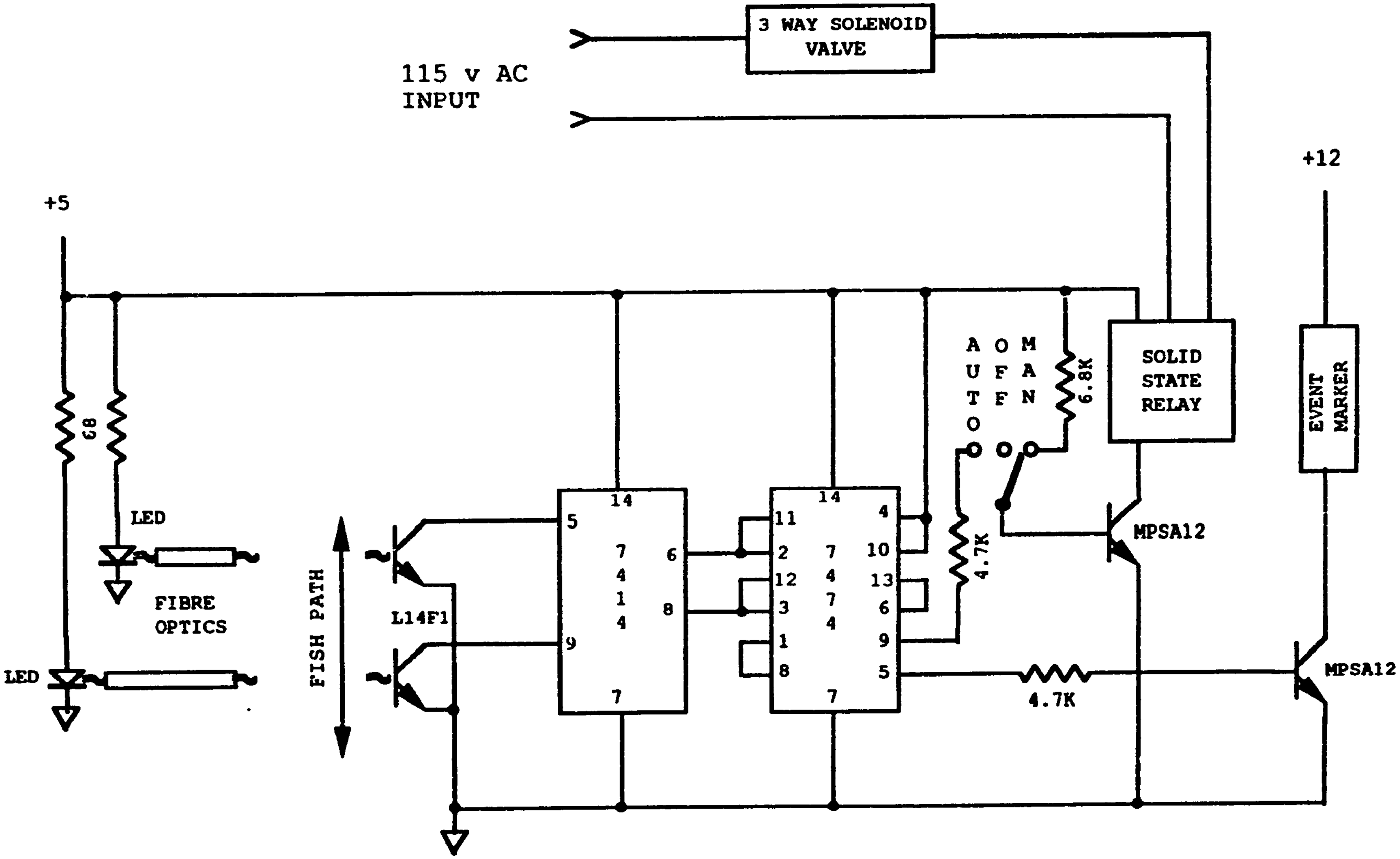

3 WAY SOLENOID VALVE
115 v AC INPUT
+12
+5
68
LED
FIBRE OPTICS
LED
FISH PATH
L14F1
5
14
7414
6
8
9
7
11
2
12
3
1
8
14
7474
4
10
13
6
9
5
7
AUTO
OFF
MAN
4.7K
6.8K
SOLID STATE RELAY
MPSA12
EVENT MARKER
MPSA12
4.7K

Figure 22. Schematic diagram of the temperature sensing device used to record ambient water temperature within a shuttlebox. The output of this device was monitored by a microcomputer. Four of these devices were made, one for each of the four shuttleboxes.

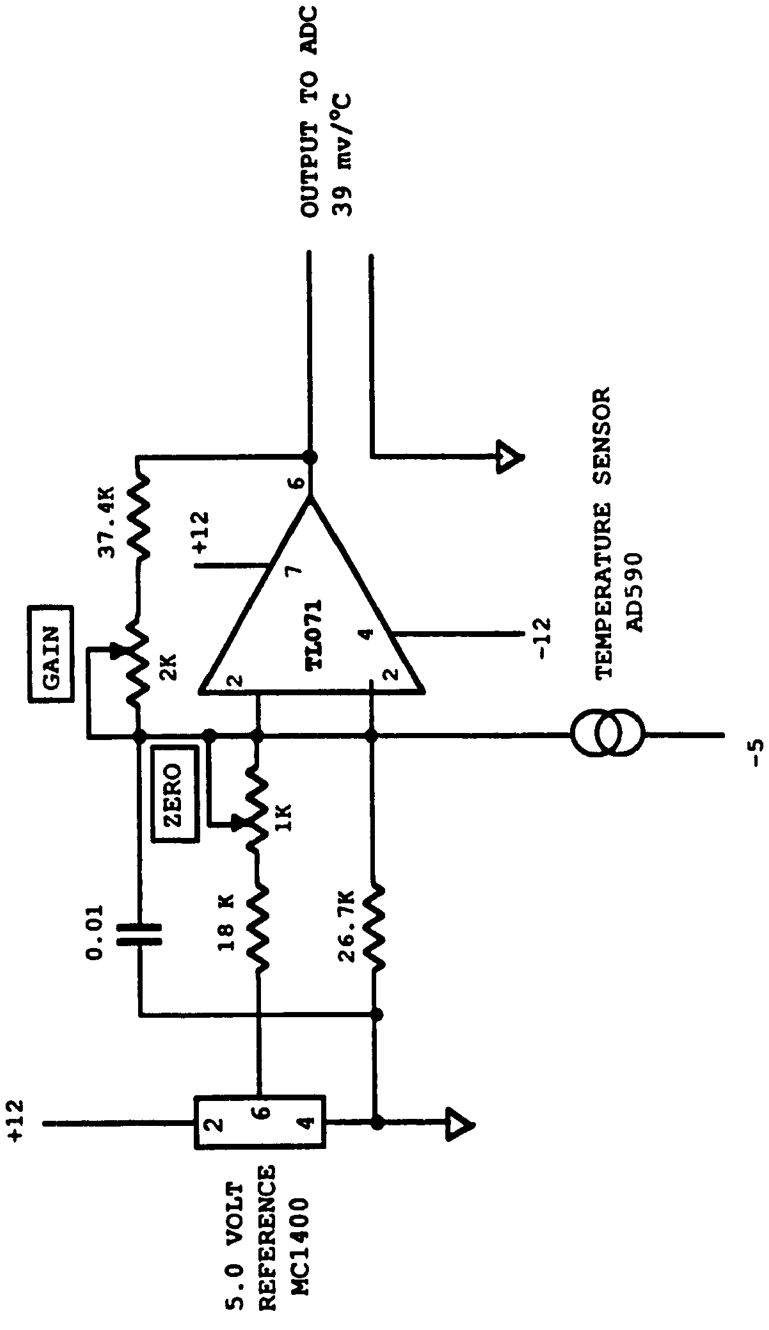

OUTPUT TO ADC
39 mv/°C
37.4K
+12
6
7
TL071
4
2
2
-12
GAIN
2K
ZERO
1K
0.01
18 K
26.7K
TEMPERATURE SENSOR
AD590
-5
+12
2
6
4
5.0 VOLT
REFERENCE
MC1400

Figure 23. Schematic diagram of the lighting system. A reversible motor was used to drive a variable transformer, gradually increasing or decreasing light intensity over a 2 hour period to simulate dawn and dusk conditions. The timing of these events were controlled by an electronic clock.

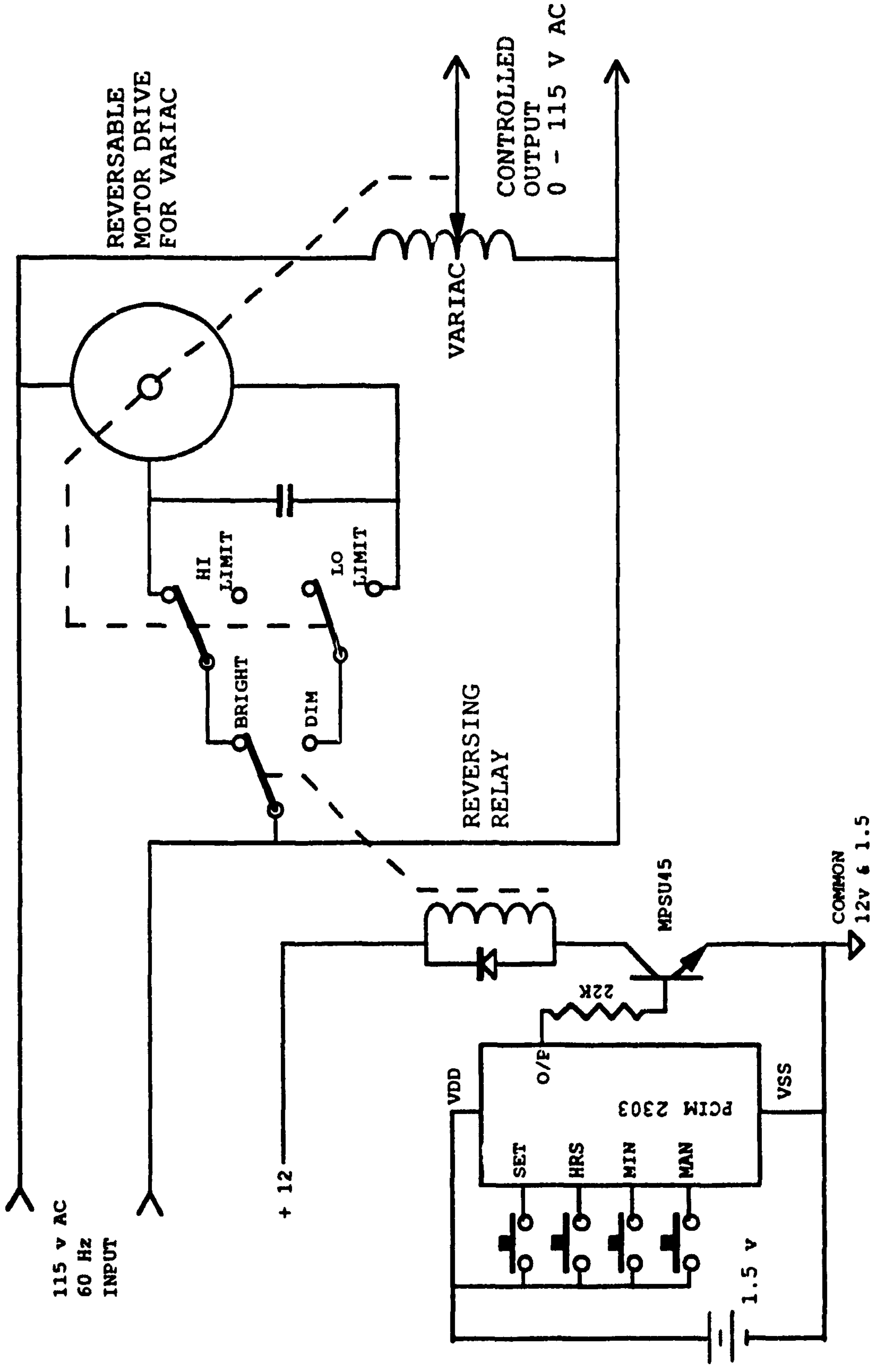
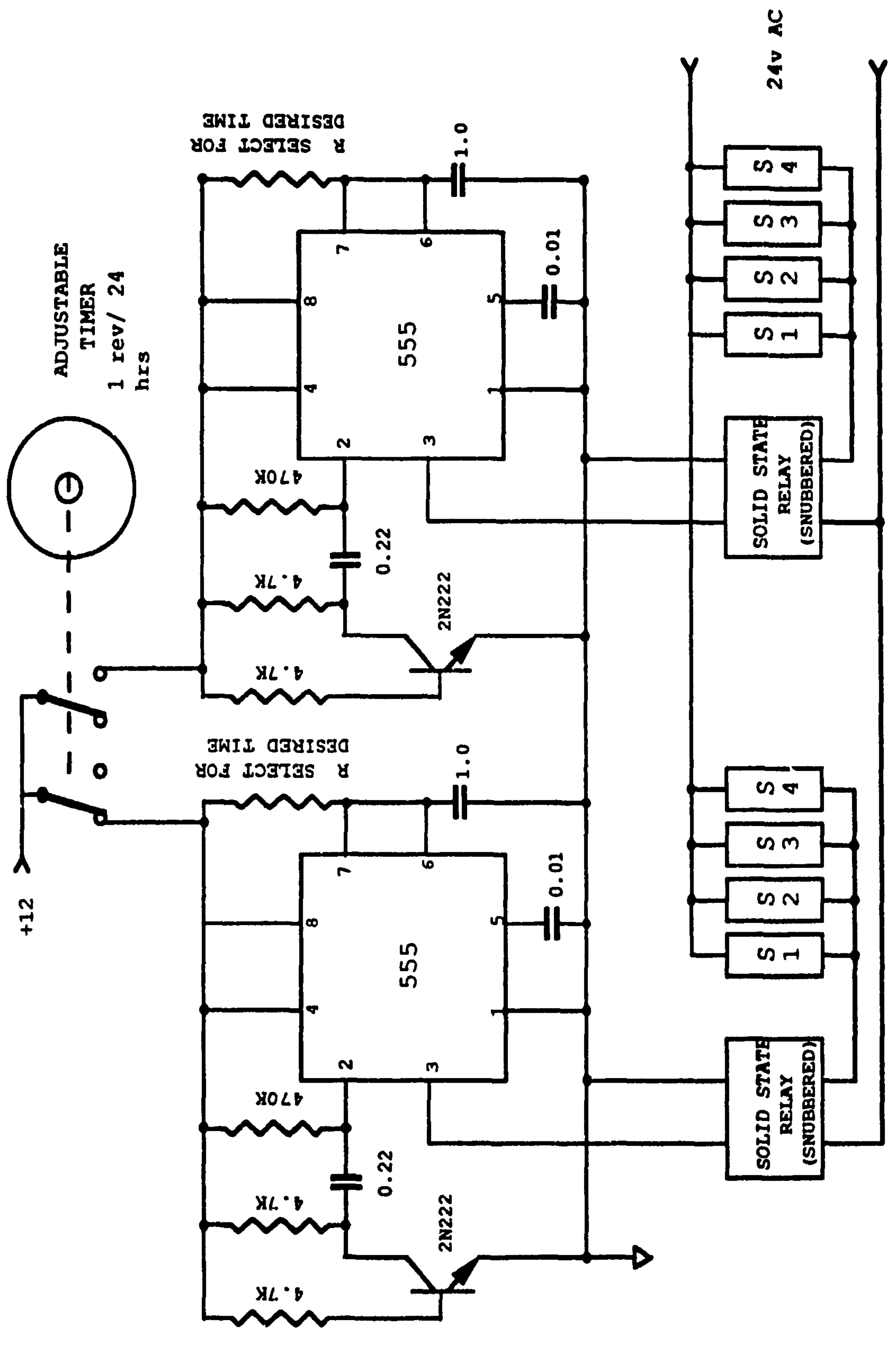

REVERSABLE
MOTOR DRIVE
FOR VARIAC
CONTROLLED
OUTPUT
0 - 115 V AC
VARIAC
HI
LIMIT
LO
LIMIT
BRIGHT
DIM
REVERSING
RELAY
MPSU45
COMMON
12v & 1.5
22K
O/P
VDD
VSS
PCIM 2303
SET
HRS
MIN
MAN
+ 12
1.5 v
115 v AC
60 Hz
INPUT

Figure 24. Schematic diagram of the electronic timer used to control the release of food from the automatic food dispensers.


ADJUSTABLE TIMER
1 rev/ 24 hrs
+12
R SELECT FOR DESIRED TIME
1.0
0.01
555
7
6
8
5
4
1
2
3
470K
4.7K
0.22
2N222
4.7K
R SELECT FOR DESIRED TIME
1.0
0.01
555
7
6
8
5
4
1
2
3
470K
4.7K
0.22
2N222
4.7K
24v AC
S 4
S 3
S 2
S 1
SOLID STATE RELAY (SNUBBERED)
S 4
S 3
S 2
S 1
SOLID STATE RELAY (SNUBBERED)

APPENDIX II

A computer program was devised to monitor shuttling activity and ambient water temperature within each of the four shuttleboxes. Ambient temperature measurements, taken on a per minute basis, were averaged at hourly intervals and stored on floppy disk. Similarly, the total number of shuttling events during each 60 min period was also stored on floppy disk.

Because of the limited capacity of the microcomputer, the program was divided into two parts. The first part (PROGRAM #1) prompted the experimenter for information about each experiment, such as it's duration and starting time, and stored on floppy disk. This information was subsequently retrieved and used in the second part of the program (PROGRAM #2) which performed all of the measurements and computations.

PROGRAM #1

Prompts for information about the trial

```
10    CLEAR: D$ = CHR$(4): DIM CODE$(4):
20    HOME: VTAB(1): PRINT TAB(9);"Type in the following;"
30    VTAB(5): INPUT "       Trial number......";EX$
40    IF LEN (EX$) = 1 THEN EX$ = "0" + EX$
50    FOR Z = 1 TO 4
60          VTAB(8): PRINT "    Ration code for shuttlebox #"
              Z;: INPUT "......";TR$(Z)
70          SH$(Z) = "0" + STR$(Z)
75          CODE$(Z) = "T" + EX$ + TR$(Z) + SH$(Z)
80          VTAB(14): PRINT TAB(3); "Code name identifying fish
              in shuttlebox #";Z;"....";CODE$(Z)
90    NEXT
```

```
100  HOME: VTAB(11): PRINT TAB(6); "How long is this
       trial to last (in days)....";: INPUT " ";D
110  HOME: VTAB(11): PRINT TAB(6); "Enter the starting day
       number (0, 10, 20, etc.)....";: INPUT " ";ST
120  HOME: VTAB(11): PRINT TAB(6); "How many days worth of
       data are to be stored"
130  PRINT TAB(8);"in each file (up to 4)....";: INPUT " ";FL
140  FL = FL * 24
150  HOME: VTAB(11): PRINT TAB(6); "At what time is the
      trial to start (in hours)....";: INPUT " ";U
160  U = U - 1: IF U = -1 THEN U = 23
```

Stores information on floppy disk (DOCUFILE)

```
170  PRINT D$;"OPEN DOCUFILE,D2"
180  PRINT D$;"WRITE DOCUFILE"
190  FOR Z = 1 TO 4
200        PRINT CODE$(Z)
210  NEXT
220  PRINT D: PRINT ST: PRINT FL: PRINT U
230  PRINT D$;"CLOSE DOCUFILE"
240  PRINT D$;"RUN WORK.PRG,D1"
```

PROGRAM #2

Goes to the start-up subroutine at the end of the program

```
10   CLEAR: GOSUB 640
```

Time allocation subroutine

```
20   GOSUB 240: GOSUB 30: GOTO 60
30   IF SEC = 50 THEN GOSUB 150: RETURN
40   IF MIN = 30 THEN PRINT: IF SEC < 2 THEN GOSUB 360:
     IF CL = FL THEN GOSUB 560
50   RETURN
```

Registers movement of fish through passageway

```
60   Y = FN DEVICE(9)
70   IF Y < 0 THEN Y = Y + 65536
80   FOR Z = 1 TO 4
90         IF Z = 1 THEN STAT(Z) = FN R1(FN DIGITAL(Y))
100        IF Z = 2 THEN STAT(Z) = FN R2(FN DIGITAL(Y))
110        IF Z = 3 THEN STAT(Z) = FN R3(FN DIGITAL(Y))
120        IF Z = 4 THEN STAT(Z) = FN R4(FN DIGITAL(Y))
130        IF STAT(Z) <> PREV(Z) THEN SHUT(Z) = SHUT(Z) + 1:
             PREV(Z) = STAT(Z)
140  NEXT Z: GOTO 20
```

Measures ambient water temperature

```
150  COUNT = COUNT + 1
155  Z = 1 TO 4: SAMPLE = 0
160        FOR I = 1 TO 30
170              Y = FN DEVICE(Z)
180              IF Y < 0 THEN Y = Y + 65536
190              SAMPLE = SAMPLE + FN ANALOG(Y)
195        NEXT I
200        AMB(Z,CT) = INT(SAMPLE/30)/10
210        IF AMB(Z,CT) > RH(Z) THEN RH(Z) = AMB(Z,CT)
220        IF AMB(Z,CT) < RL(Z) THEN RL(Z) = AMB(Z,CT)
230        SUM(Z) = SUM(Z) + AMB(Z,CT)
235  NEXT Z: RETURN
```

Determines current time

```
240 PRINT D$"IN#5"
250 PRINT D$"PR#5"
260 INPUT TIME$
270 PRINT D$"IN#0"
280 PRINT D$"PR#0"
320      HOUR = VAL(MID$(TIME$,12,2))
330       MIN = VAL(MID$(TIME$,15,2))
340       SEC = VAL(RIGHT$(TIME$,2)
350  HOME: VTAB(12): HTAB(10): PRINT TIME$
355  RETURN
```

Statistics and storage on floppy disk

Computes mean and variance

```
360  FOR Z = 1 TO 4
370       HMT(Z) = INT(SUM(Z)/COUNT * 10)/10
380       FOR L = 1 TO COUNT
390             SS(Z) = SS(Z) + (AMB(Z,L) - HMP(Z))^2
495       NEXT
400       VAR(Z) = INT(SS(Z)/(COUNT - 1) * 100)/100
```

Stores recorded data on floppy disk

```
410       PRINT D$;"APPEND";FILENAME$(Z);C$
420       PRINT D$;"WRITE";FILENAME$(Z)
430       PRINT HOUR; PRINT HMT(Z); PRINT VAR(Z); PRINT RH(Z);
            PRINT RL(Z); SHUT(Z)
440       PRINT D$;"CLOSE";FILENAME$(Z)
445  NEXT Z
```

Produces a printout

```
450  PRINT D$;"PR#1"
460  PRINT "      TIME : "TIME$
470  PRINT: PRINT "  CHANNEL      MEAN TEMP     VARIANCE";
      "       RANGE          SHUTTLES"
480  PRINT: FOR Z = 1 TO 4
490        PRINT; TAB(6);Z;TAB(17);HMT(Z);TAB(29);VAR(Z);
           TAB(42);RL(Z);TAB(10);"TO";TAB(16);RH(Z);TAB(29);
           SHUT(Z)
500  NEXT Z
510  PRINT: PRINT: PRINT;D$"PR#0": PRINT CHR(13)
```

Resets all variables to zero

```
520  FOR Z = 1 TO 4
530        SS(Z) = 0: VAR(Z) = 0: RH(Z) = 0: RL(Z) = 27:
           HMT(Z) = 0: SHUT(Z) = 0
540        FOR L = 1 TO COUNT: AMB(Z,L) = 0:NEXT
550  NEXT
555  CL = CL + 1: COUNT = 0: RETURN
```

Creates a new file for data storage every 1 to 3 days

```
560  IF DAY = D THEN END
570  DAY = DAY + (FL/24): CL = 0
575  IF DAY > 30 THEN C$ = ",D1"
580  FOR Z = 1 TO 4
590        FILENAME$(Z) = CODE$(Z) + STR$((ST + DAY))
600        HOME: VTAB(12)
605        PRINT; TAB(3);"INITIALIZING FILE.....";FILENAME$(Z)
610        PRINT D$;"OPEN";FILENAME$(Z);C$
620        PRINT D$;"CLOSE";FILENAME$(Z)
630  NEXT Z: RETURN
```

Start-up subroutine

Reads data from DOCUFILE

```
640  D$ = CHR(4): C$ = ",D2": DIM CODE$(4): DIM FILENAME$(4)
650  PRINT D$;"OPEN DOCUFILE,D2"
660  PRINT D$;"READ DOCUFILE"
670  FOR Z = 1 TO 4
680        INPUT CODE$(Z): NEXT
690  INPUT D,FL,ST,U
700  PRINT D$;"CLOSE DOCUFILE"
710  DAY = 0: GOSUB 560
```

Defines all variables

```
720  DIM SHUT(4): DIM STAT(4): DIM PREV(4): DIM SUM(4):
     DIM HMT(4): DIM VAR(4) DIM SS(4): DIM RH(4): DIM RL(4):
     DIM AMB(4,60)
740  FOR Z = 1 TO 4
745        RH(Z) = 0: RL(Z) = 27: PREV = 0: NEXT
```

Loads Digital/Analog Converter operating program

```
750  HOME: VTAB(12): PRINT TAB(10); "LOADING DAC SUBROUTINE"
760  PRINT D$;"BLOAD BAUD 4800.OBJO,D1"
770  CALL 768
```

Definition of program functions

```
780  DEF FN SCREEN(X) = SCRN (POS (0),2 * PEEK (37)) + 16 *
                       SCRN (POS (0),2 * PEEK (37) + 1) -
                       128
790  DEF FN R4(X) = INT ((X - INT (X/256) * 256)/128)
800  DEF FN R3(X) = INT ((X - INT (X/128) * 128)/64)
810  DEF FN R2(X) = INT ((X - INT (X/64) * 64)/32)
820  DEF FN R1(X) = INT ((X - INT (X/32) * 32)/16)
830  DEF FN DEVICE(X) = USR (X - 1)
840  DEF FN ANALOG(X) = INT (X/256)
850  DEF FN DIGITAL(X) = X - FN ANALOG(X) * 256
```

A continuous loop delaying the start of the program until designated time

```
860  GOSUB 240
870  IF HOUR = U THEN IF MIN = 31 THEN RETURN
880  GOTO 860
```

APPENDIX III

The approach used for data analysis was highly dependent on the degree of inter-correlation among the three dependent variables (DV) that were used to describe thermoregulatory behavior (daily mean preferred temperature, thermoregulatory precision index, and log(x + 1) transformed rhythmicity index). Highly correlated DVs lead to inflated F statistics when analyzed separately, thus increasing the chances of committing a Type I error (Tabachnick and Fidell, 1983). As shown in Table 9, both the precision and rhythmicity indices were independent of the daily mean preferred temperature. However, the index variables were significantly correlated with one another. To have maintained statistical purity, analysis of the two index variables would have warranted a multivariate approach (Tabachnick and Fidell, 1983). However, because the variance shared by the two index variables was minimal ($r^2 = 0.068$), this approach was abandoned in favor of the much simpler univariate approach.

Table 9. The interrelationships between the three dependent variables used to describe thermoregulatory behaviour in juvenile rainbow trout as determined through correlation analysis (n = 568). DMPT = daily mean preferred temperature, RI'= log (x + 1) transformed rhythmicity index, TPI = thermoregulatory precision index.

	DMPT	RI'	TPI
DMPT	1.000		
RI'	-0.014	1.000	
TPI	0.003	0.260*	1.000

* $r \neq 0$, $P < 0.001$

APPENDIX IV

A mixed Between-Within-Subject Analysis of Variance test was used to determine whether daily mean preferred temperature (Section 2.4.1), the thermoregulatory precision index (Section 2.4.2), and the log(x + 1) transformed rhythmicity index (Section 2.4.4) differed among the four shuttleboxes. Each variable was tested independently. The four shuttleboxes served as the between subject factor and time (in days) as the within-subject factor. The test was restricted to 17 levels (days) so as to include all individuals in the analysis (refer to Section 2.4.4).

A summary of the analysis is presented in Tables 10, 11, and 12. Daily mean preferred temperature, thermoregulatory precision, and cycle amplitude were found not differ significantly among the four shuttleboxes. Furthermore, the lack of a significant time-shuttlebox interaction suggested that temporal differences did not arise over the course of the experimental period. It was therefore concluded that the thermoregulatory behaviors expressed by the test population of rainbow trout were independent of the shuttlebox used.

A log-likelihood ratio test of independence showed that the incidence of nocturnal and diurnal rhythms were independent of the four shuttleboxes (Table 13).

Table 10. A summary of the Mixed Between-Within-Subjects Analysis of Variance used to examine the effects of shuttlebox device on the daily mean preferred temperatures of 29 juvenile rainbow trout over the initial 17 days of the thermoregulatory test period.

Source of variation	df	MS	F	P
Shuttlebox	3	31.518	0.60	0.623
Error (1)	25	52.867		
Time (days)	16	4.659	1.58	0.183*
Time X Shuttlebox	48	3.482	1.18	0.307*
Error (2)	400	2.957		

* df adjusted using Greenhouse-Geisser $\epsilon = 0.2611$ to compensate for lack of sphericity ($P < 0.0001$)

Table 11. A summary of the Mixed Between-Within-Subjects Analysis of Variance used to examine the effects of shuttlebox device on the daily cycle amplitude (log(x + 1) transformed rhythmicity index) of 29 juvenile rainbow trout over the initial 17 days of the thermoregulatory test period.

Source of variation	df	MS	F	P
Shuttlebox	3	1.029	0.34	0.799
Error (1)	25	3.060		
Time (days)	16	0.167	1.14	0.336*
Time X Shuttlebox	48	0.112	0.77	0.646*
Error (2)	400	0.146		

* df adjusted using Greenhouse-Geisser $\epsilon = 0.1856$ to compensate for lack of sphericity ($P < 0.0001$)

Table 12. A summary of the Mixed Between-Within-Subjects Analysis of Variance used to examine the effects of shuttlebox device on the daily thermoregulatory precision indexes of 29 juvenile rainbow trout over the initial 17 days of the thermoregulatory test period.

Source of variation	df	MS	F	P
Shuttlebox	3	0.415	0.71	0.554
Error (1)	25	0.583		
Time (days)	16	0.061	1.11	0.355*
Time X Shuttlebox	48	0.067	1.23	0.227*
Error (2)	400	0.054		

* df adjusted using Greenhouse-Geisser $\epsilon = 0.4782$ to compensate for lack of sphericity ($P = 0.0034$)

Table 13. Incidence of nocturnal and diurnal rhythms in temperature preference as a function of shuttlebox device. Fish were judged rhythmic based on their respective spectrograms and the cycle phase was determined from the computed 20 day mean Rhythmicity Index.

	Shuttlebox				
Phase	1	2	3	4	Totals
Diurnal	2	2	4	4	11
Nocturnal	2	3	3	4	13
Totals	4	5	7	8	24

G statistic = 0.165

Williams Correction Factor (q) = 1.151

$G_{adj} = G/q = 0.143, P > 0.90$

APPENDIX V

Spectral Analysis (BMD PT1 program of Dixon, 1981) was used to examine the time sequences of hourly mean preferred temperature of each test fish for patterns of rhythmicity. A brief discussion of this technique can be found in Section 2.4.4. The spectrograms for each the 29 juvenile rainbow trout tested in this experiment are presented below in Figures 25 to 53. The corresponding ration regime and trial date for each fish can be found in Table 14.

Table 14. Schedule of experiments.

Date	Fish	Shuttlebox	Ration
August/87[1]	1	1	Excess
	2	3	Unfed
	3	4	Unfed
November/87	4	1	Excess
	5	2	Restricted
	6	3	Unfed
	7	4	Excess
January/88	8	1	Restricted
	9	2	Unfed
	10	3	Excess
	11	4	Unfed
February/88	12	1	Restricted
	13	2	Excess
	14	3	Excess
	15	4	Unfed
March/88[2]	16	1	Unfed
	17	3	Restricted
	18	4	Excess
May/88[3]	19	2	Restricted
	20	3	Unfed
	21	4	Restricted
June/88	22	1	Unfed
	23	2	Restricted
	24	3	Restricted
	25	4	Excess
July/88	26	1	Excess
	27	2	Unfed
	28	3	Excess
	29	4	Restricted

[1] Fish excluded because of shuttlebox malfunction
[2] Fish died on day 12 of unknown causes
[3] Fish judged not to have learned shuttlebox operation

Figure 25. Spectrogram of Fish 1

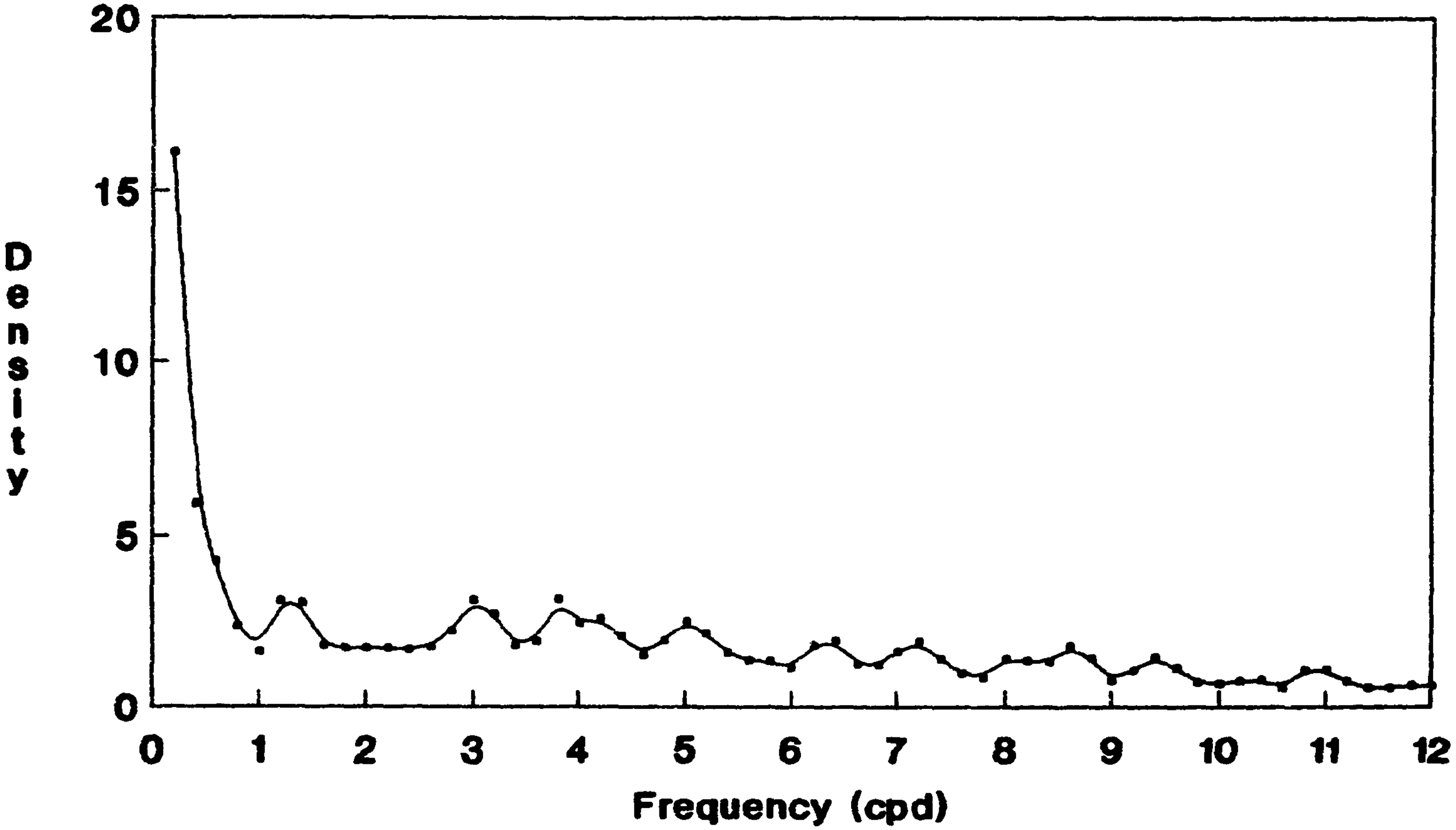


Figure 26. Spectrogram of Fish 2

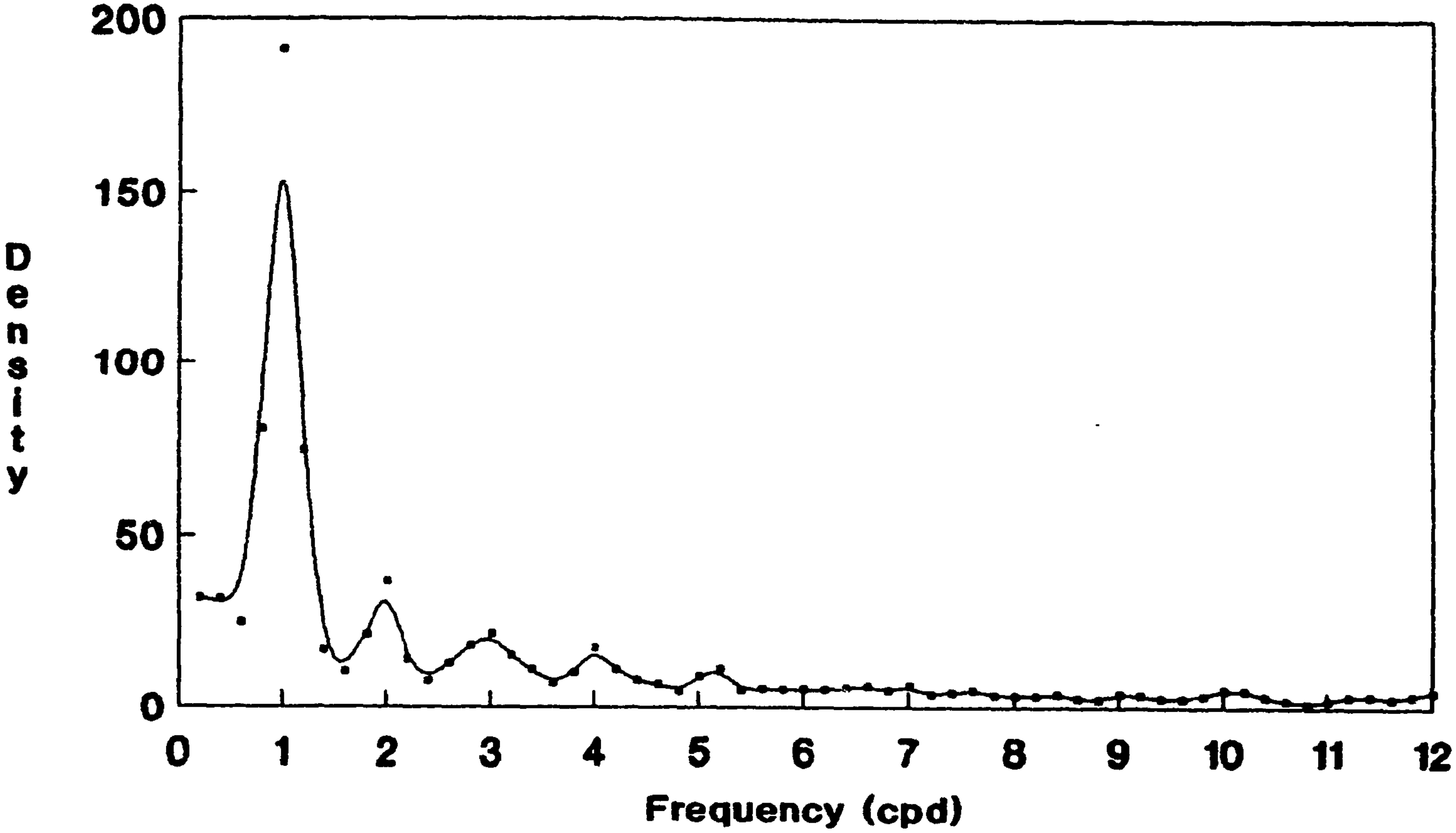


Figure 27. Spectrogram of Fish 3

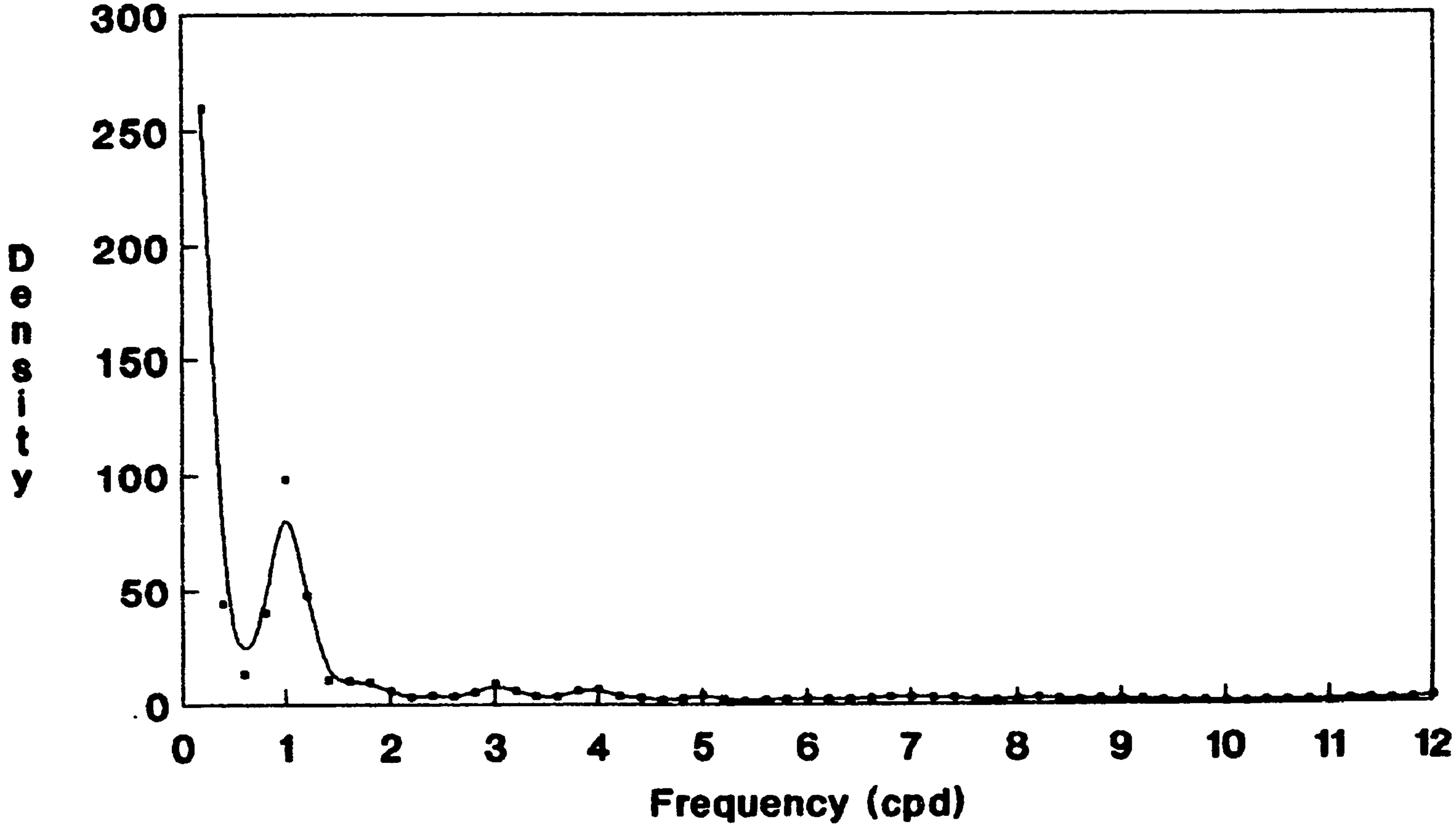


Figure 28. Spectrogram of Fish 4

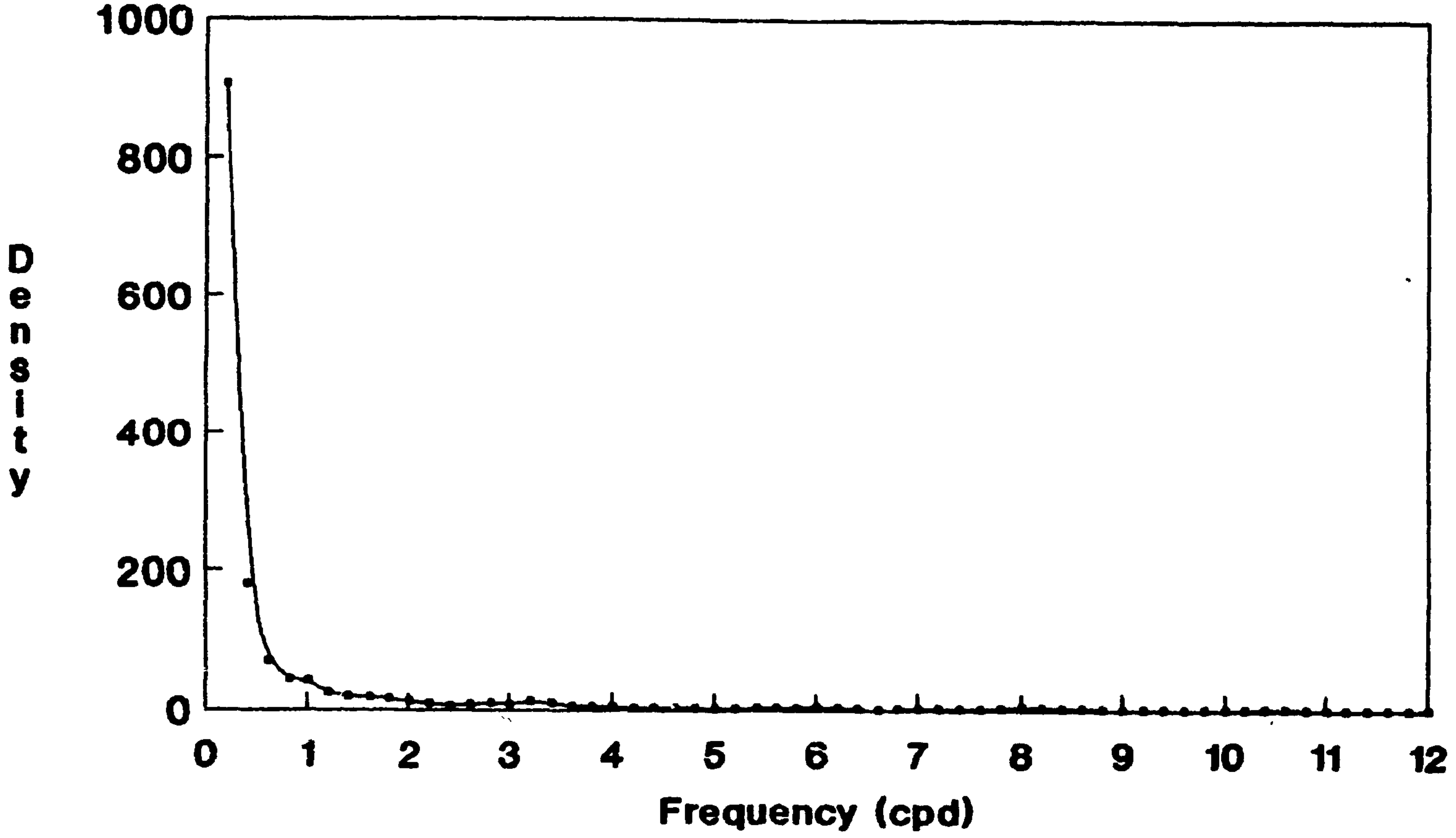


Figure 29. Spectrogram of Fish 5

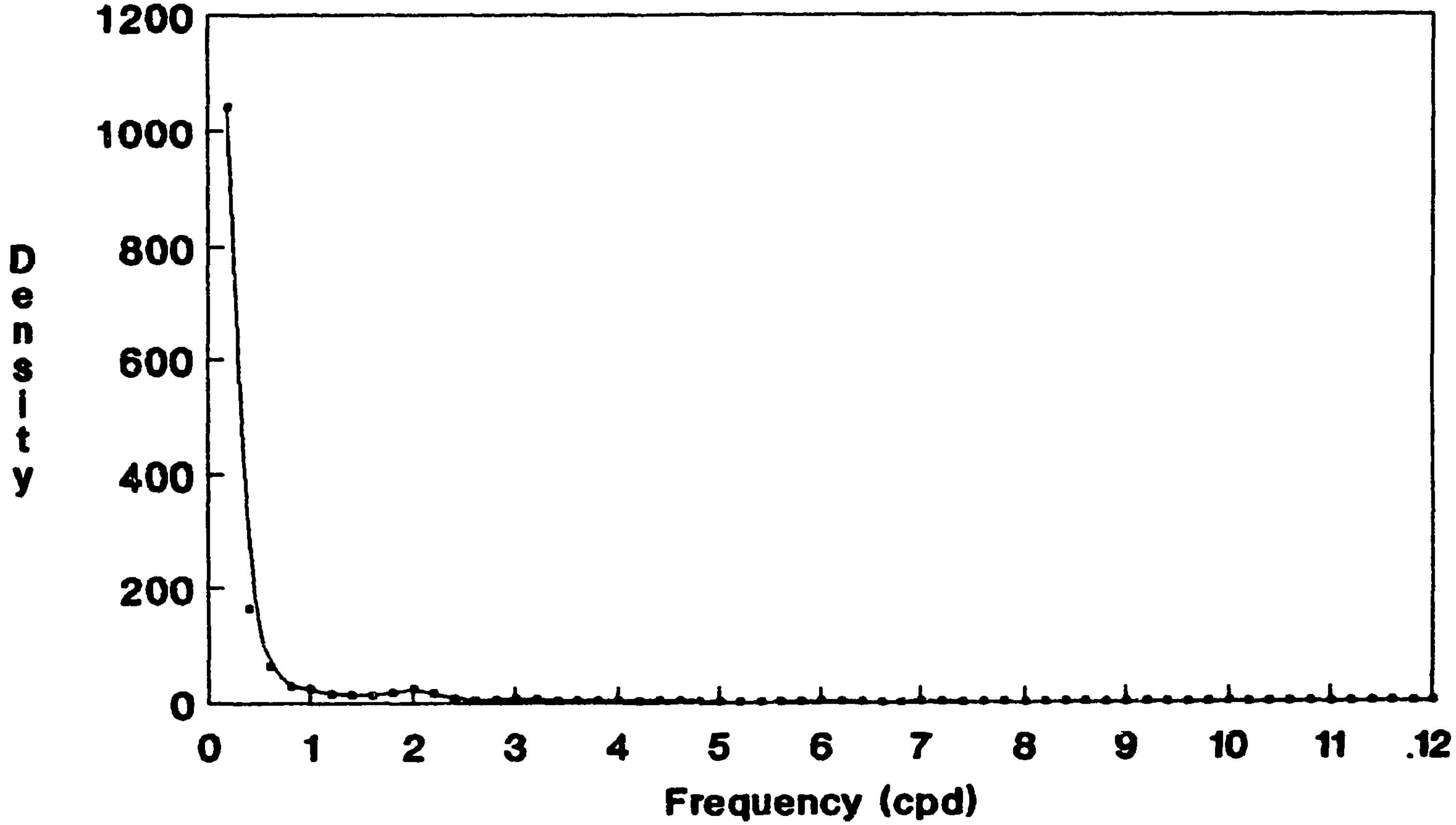


Figure 30. Spectrogram of Fish 6

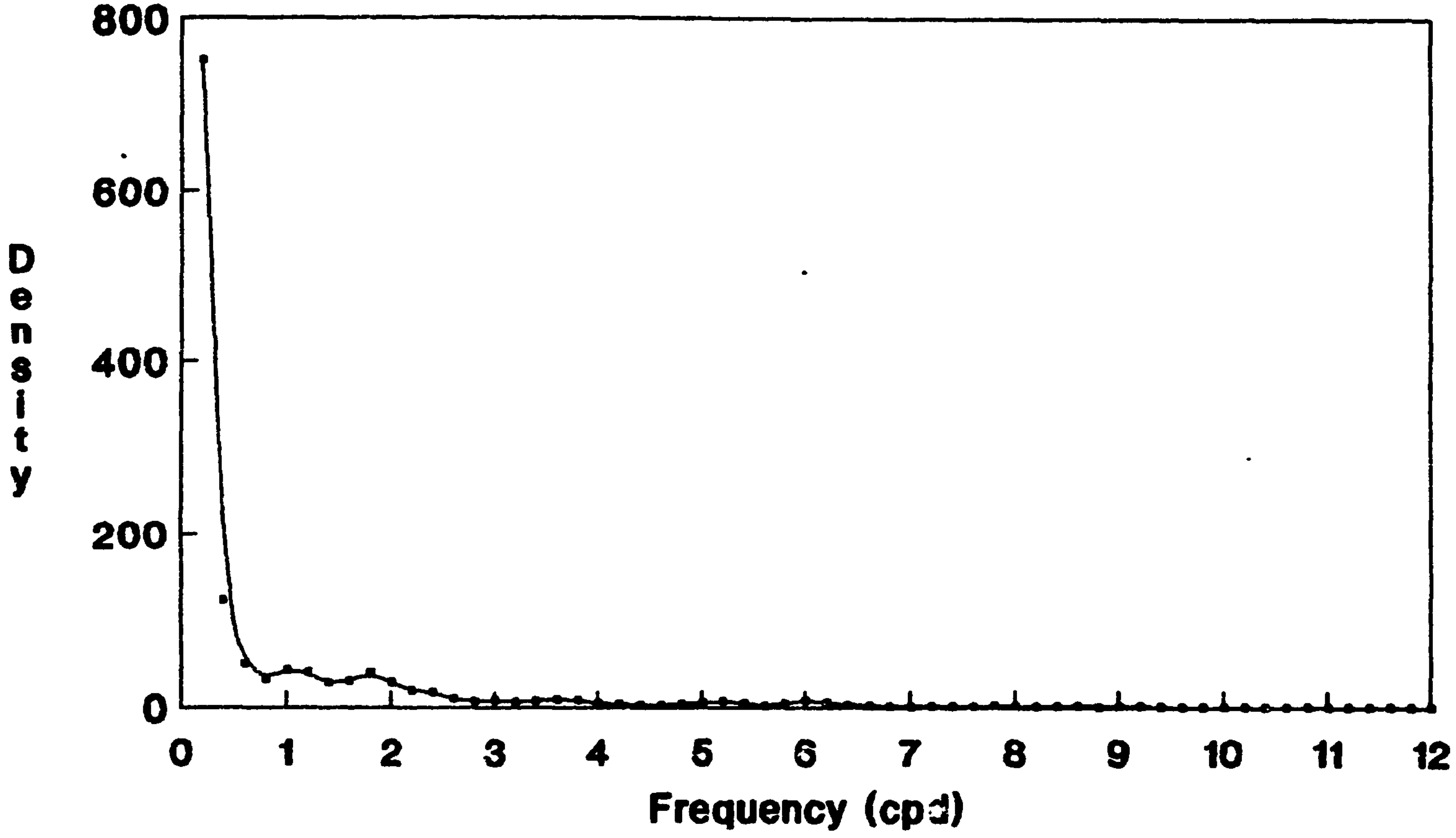


Figure 31. Spectrogram of Fish 7

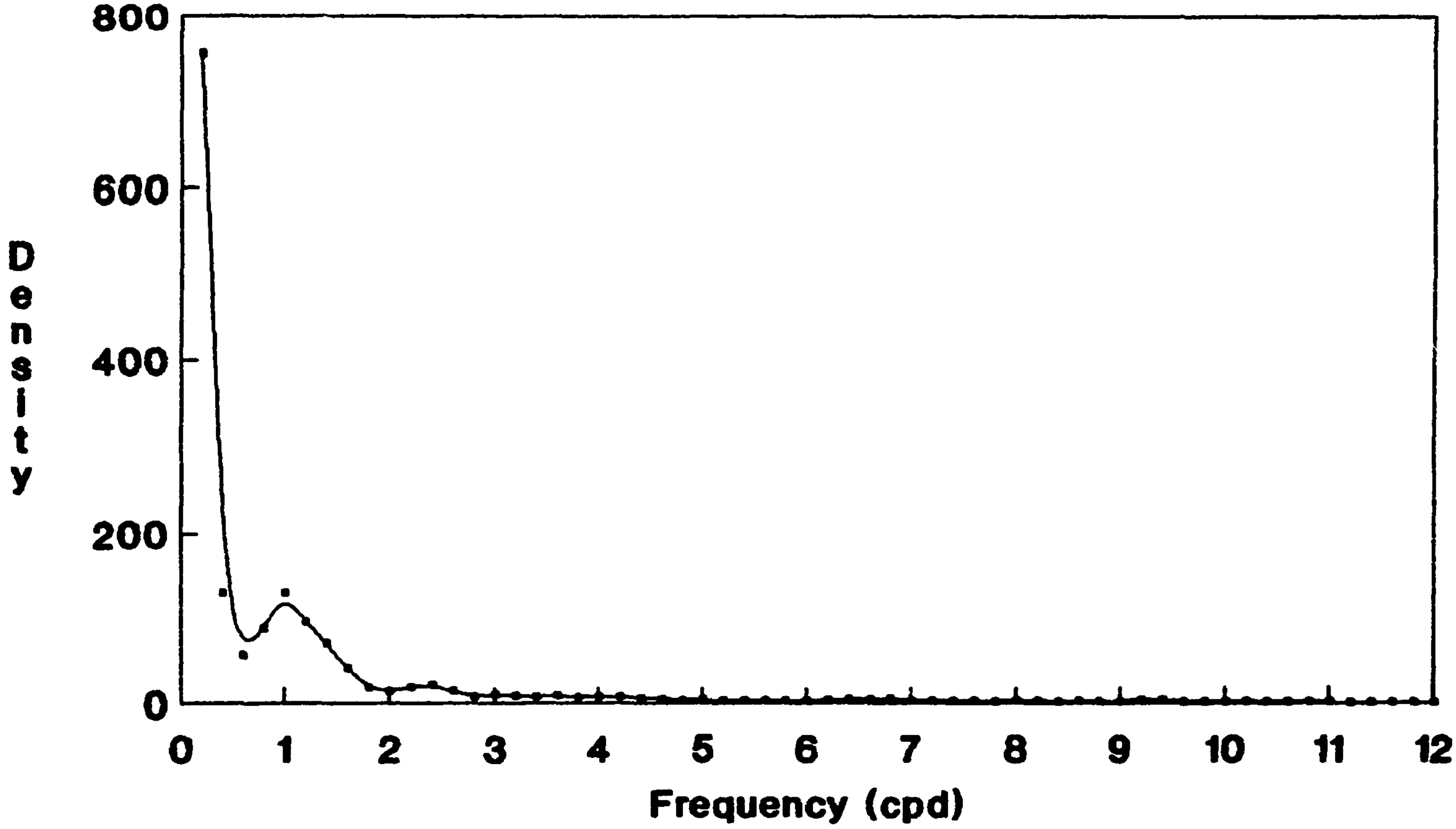


Figure 32. Spectrogram of Fish 8

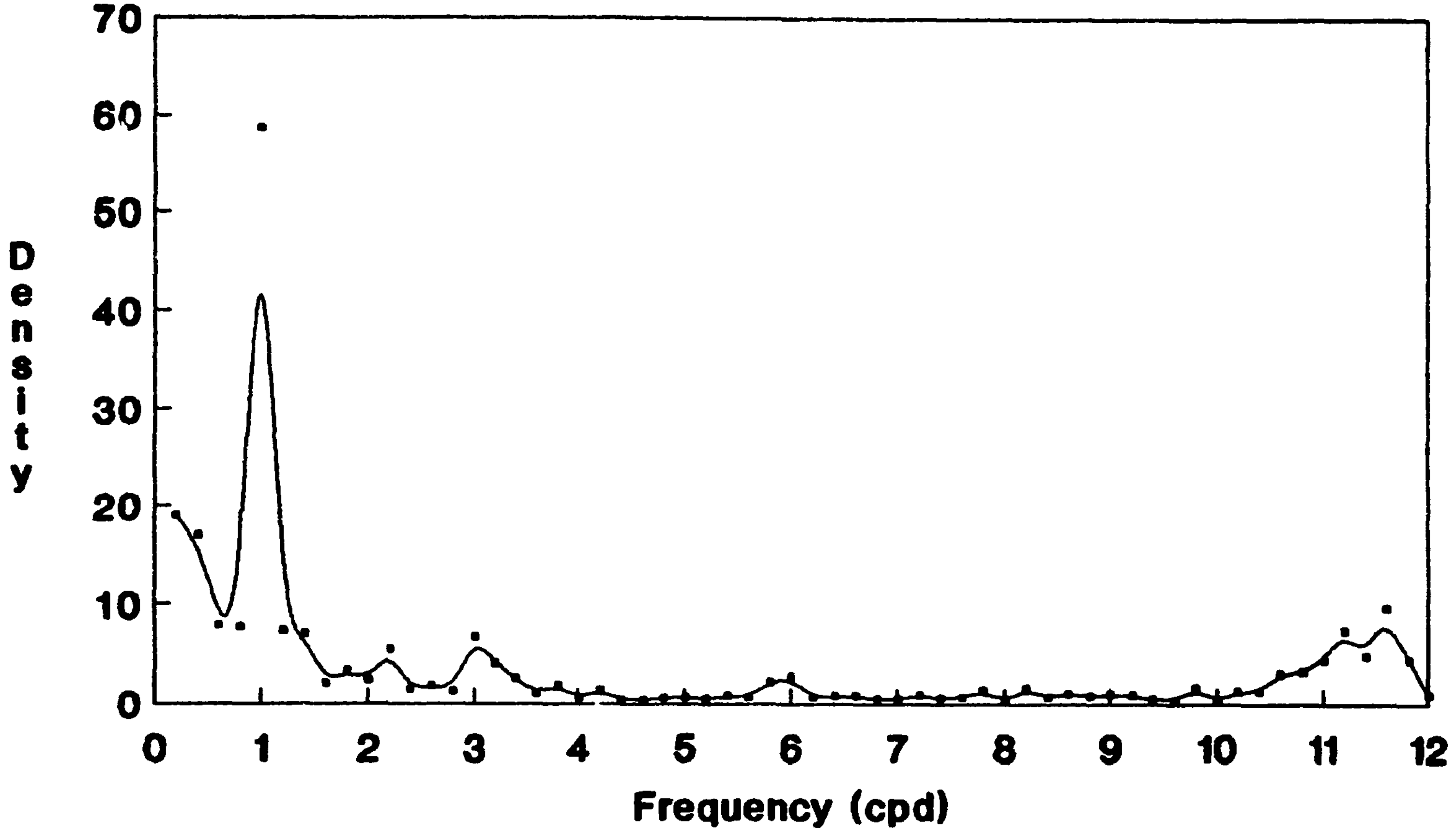


Figure 33. Spectrogram of Fish 9

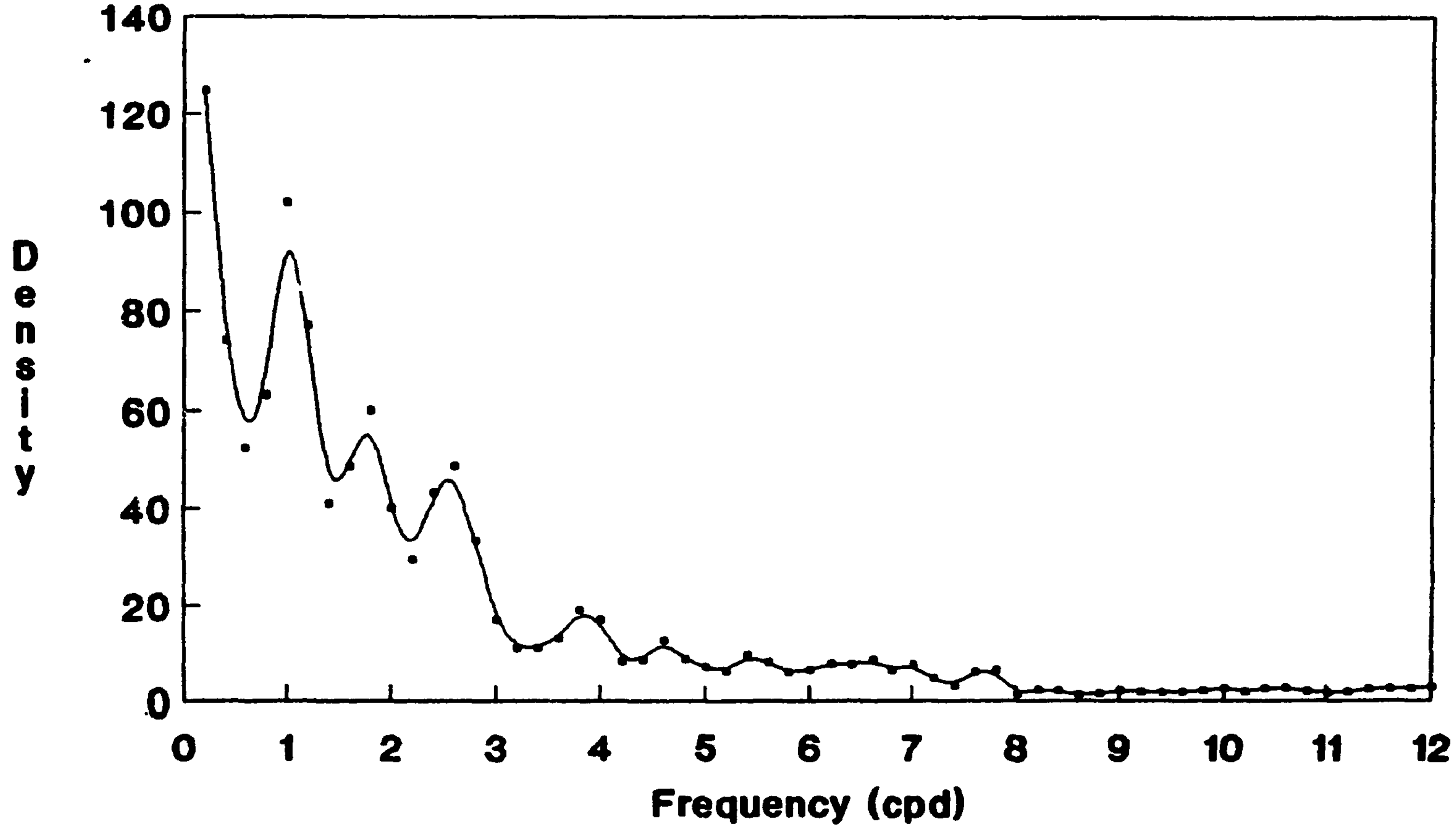


Figure 34. Spectrogram of Fish 10

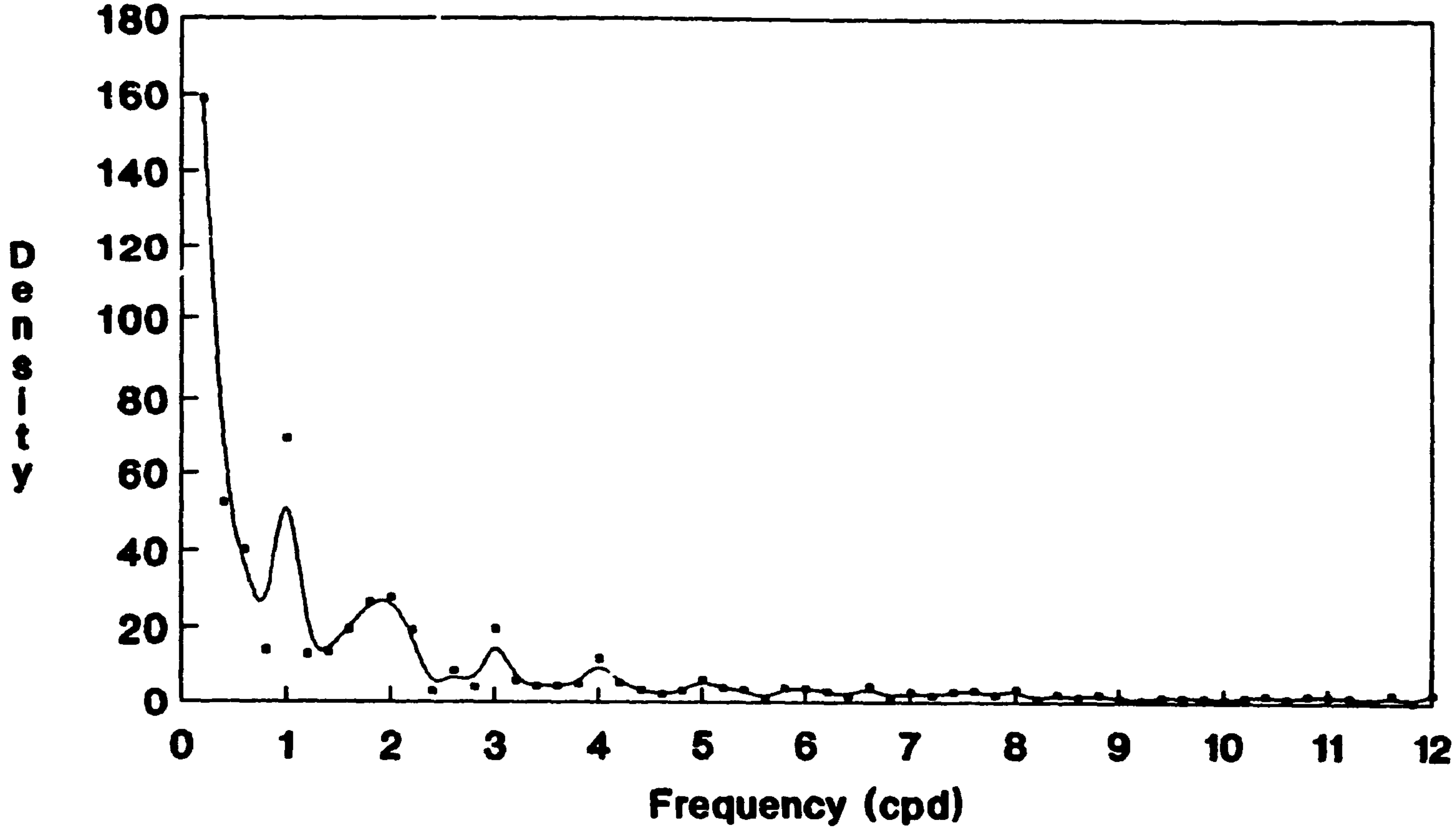


Figure 35. Spectrogram of Fish 11

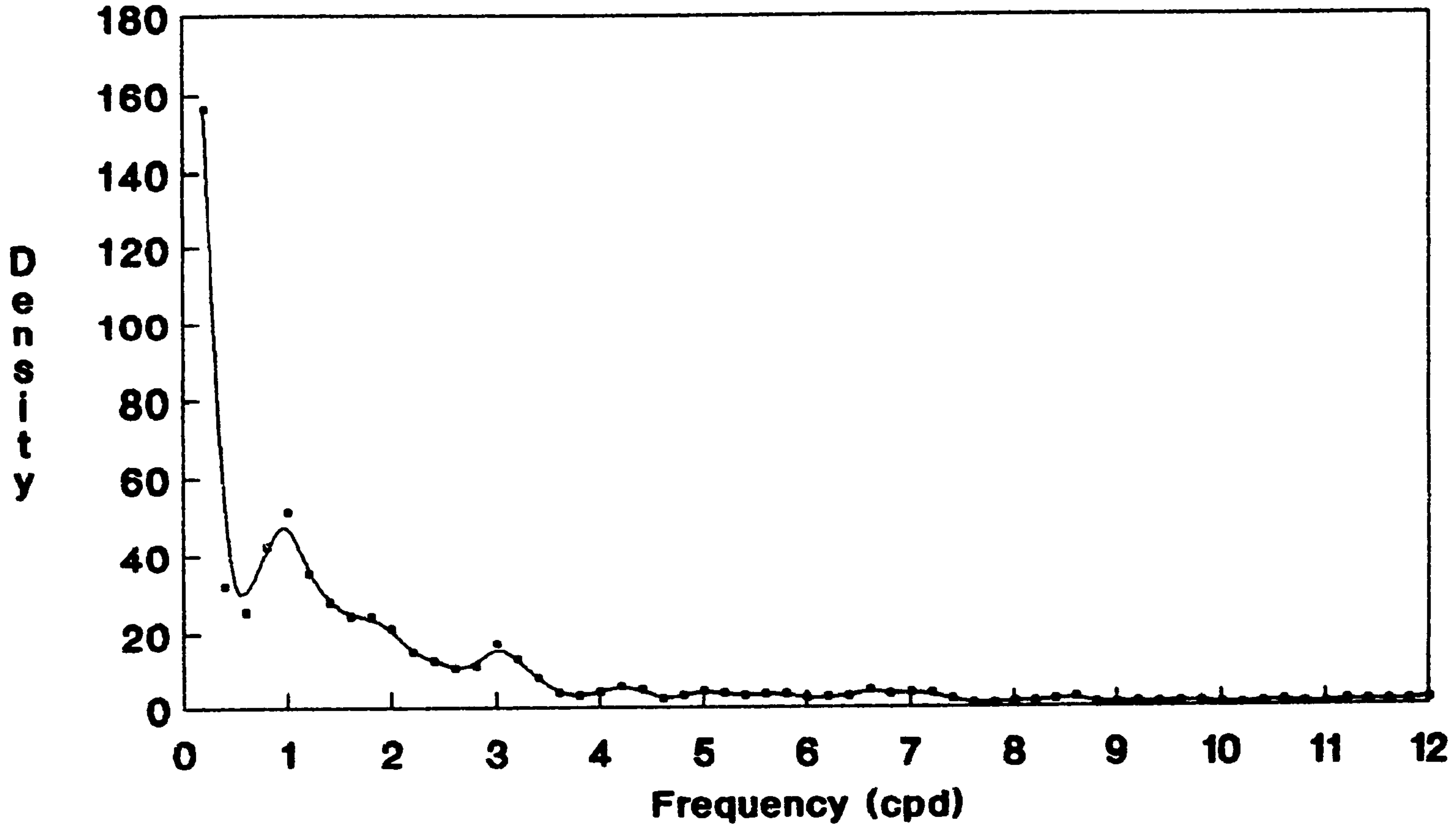


Figure 36. Spectrogram of Fish 12

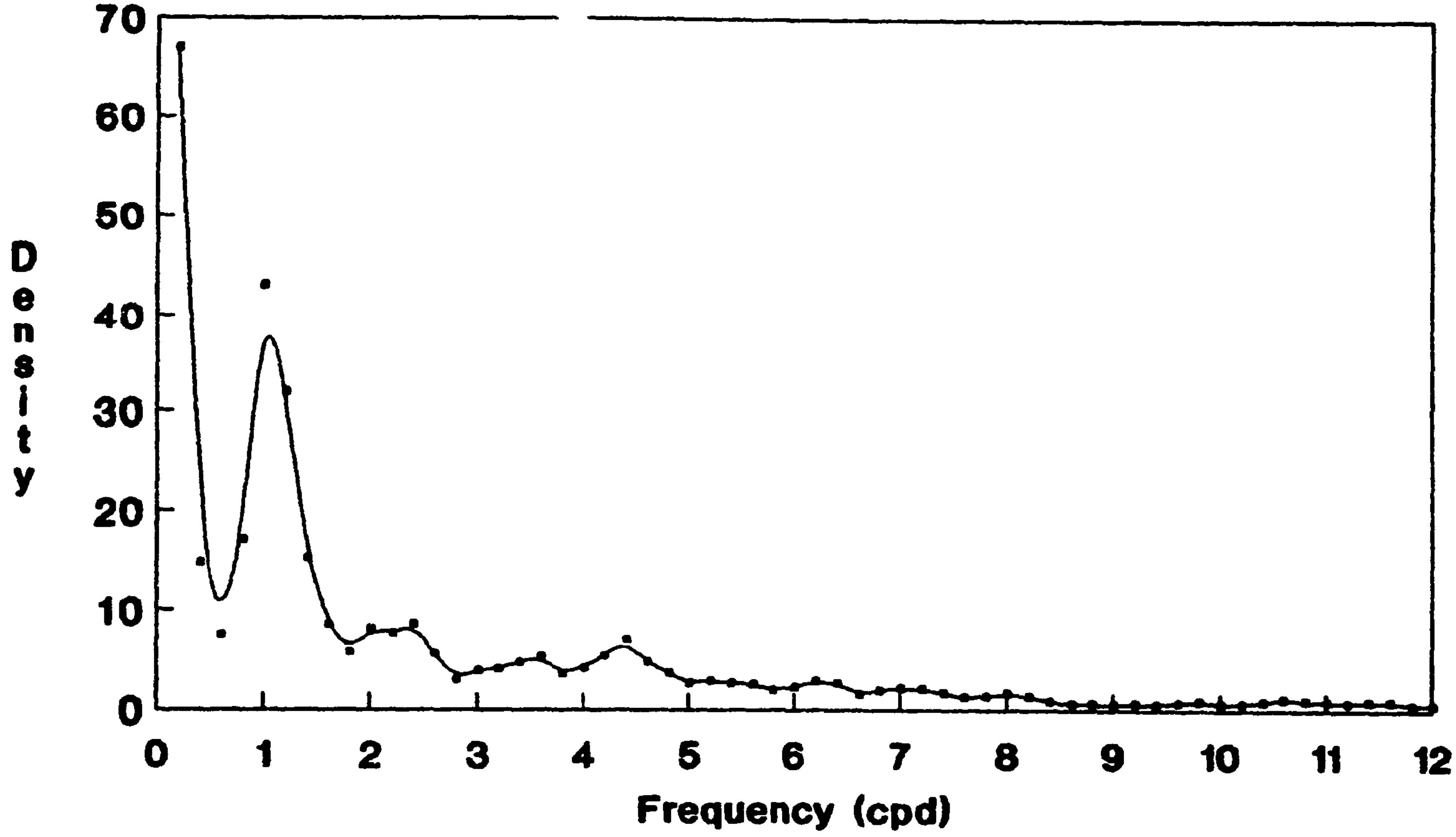


Figure 37. Spectrogram of Fish 13

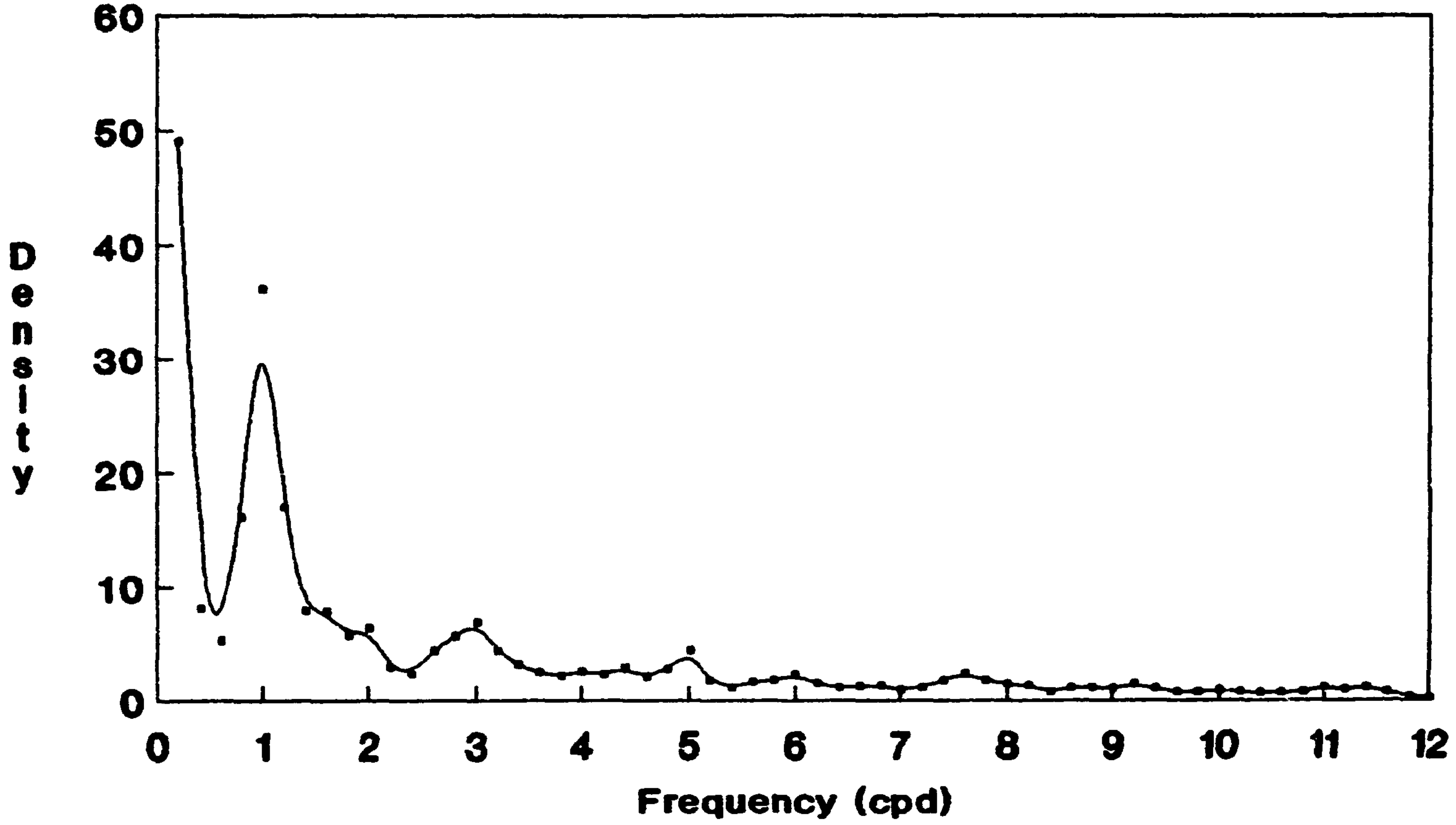


Figure 38. Spectrogram of Fish 14

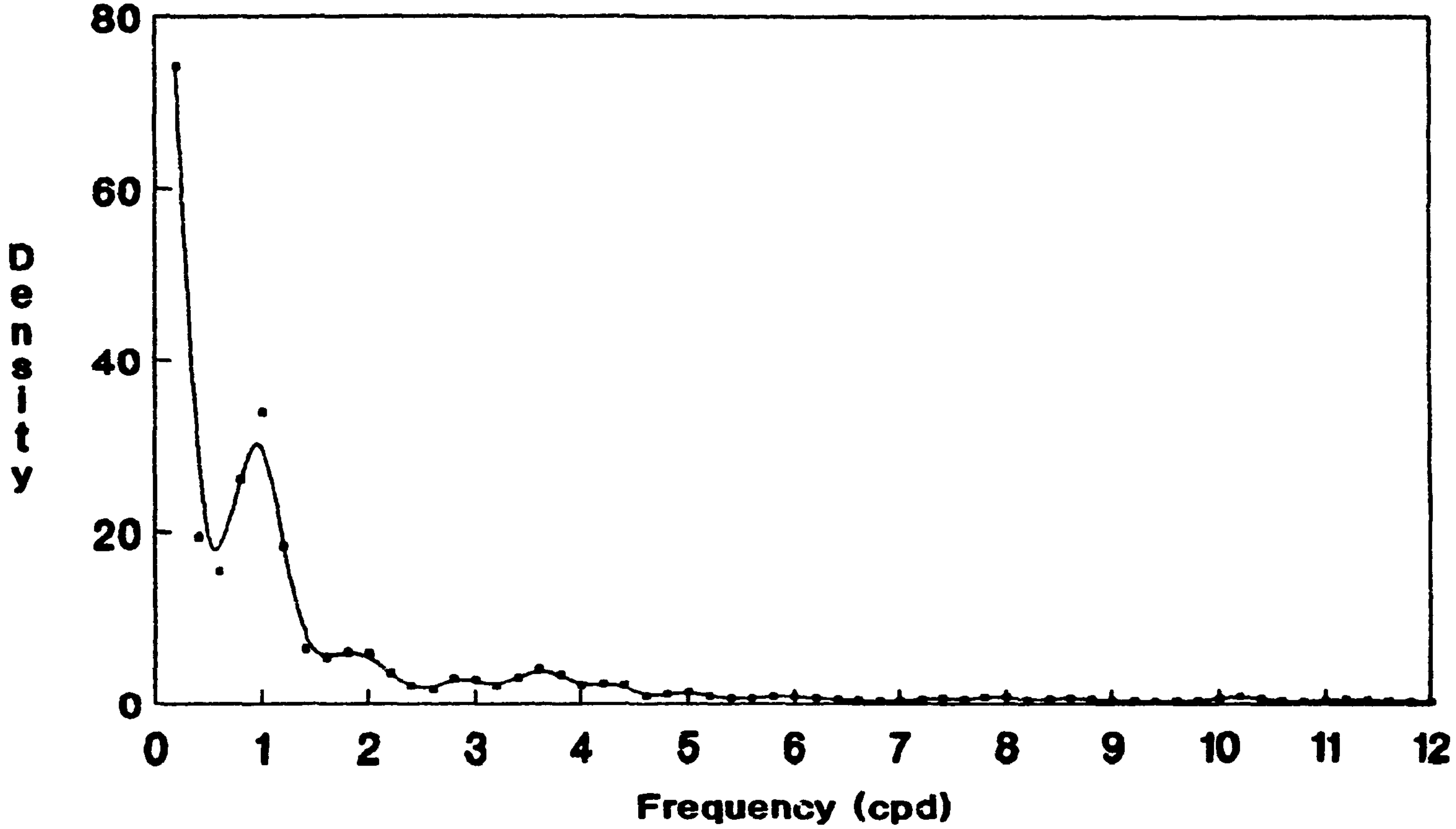


Figure 39. Spectrogram of Fish 15

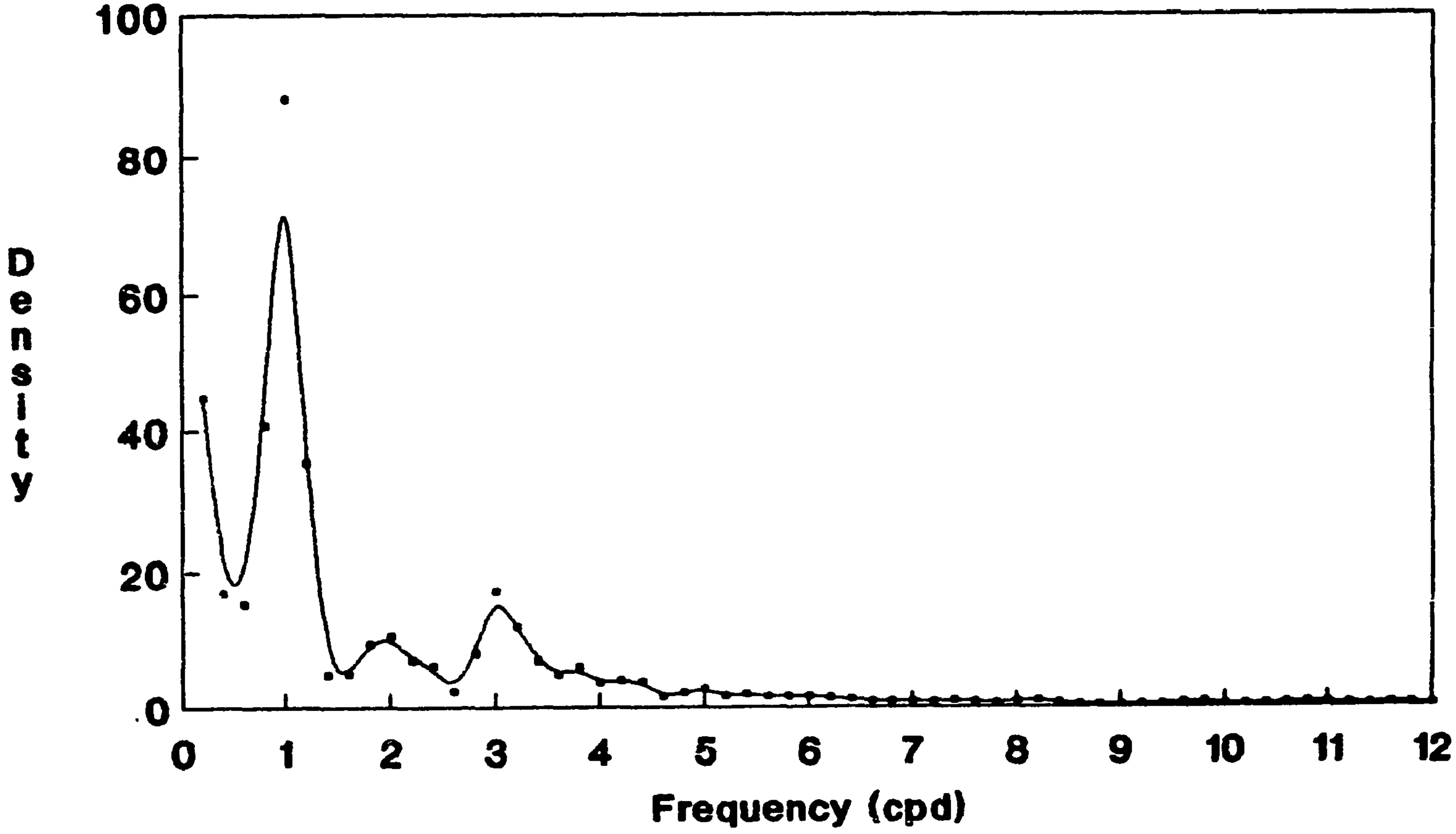


Figure 40. Spectrogram of Fish 16

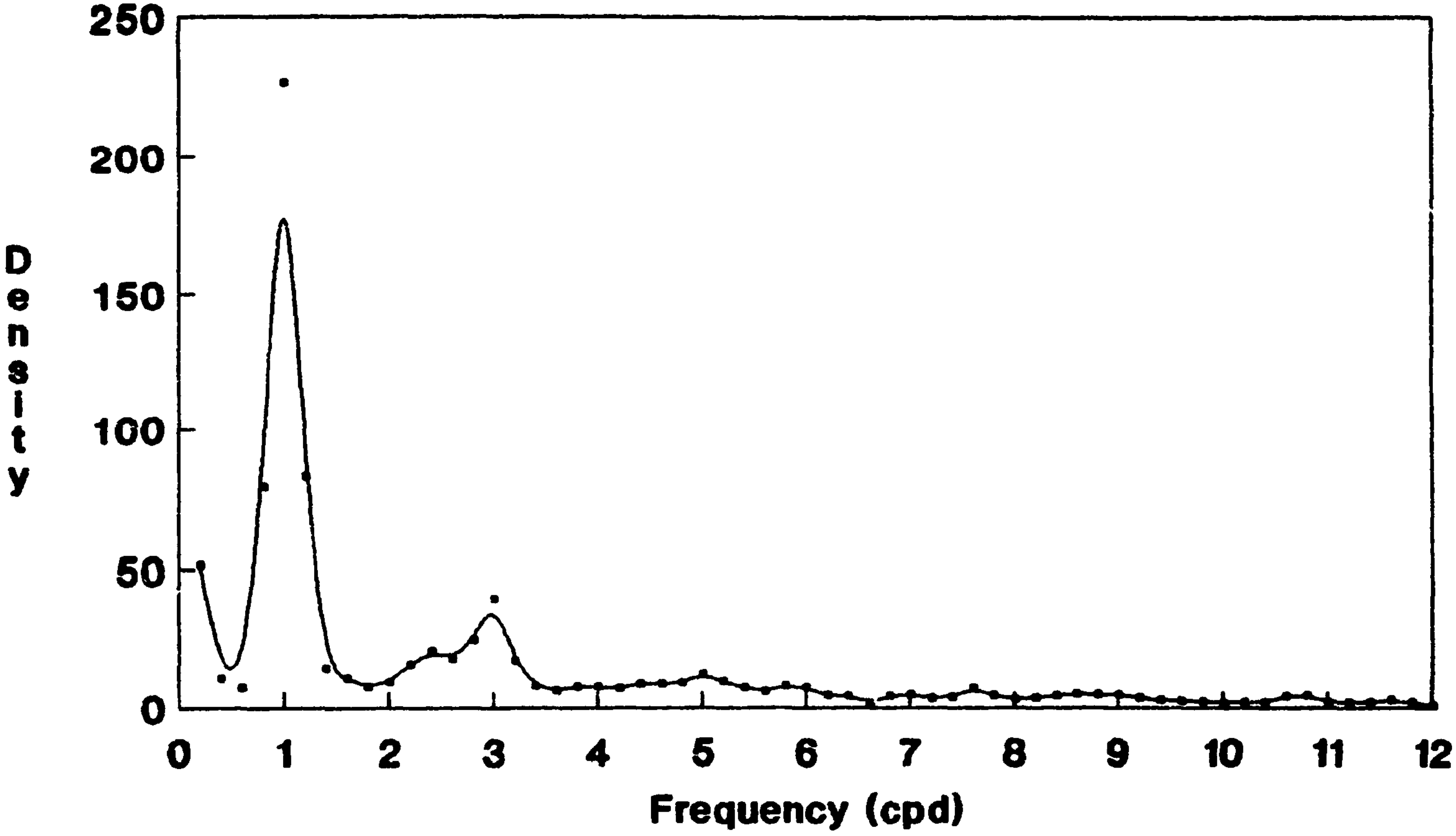
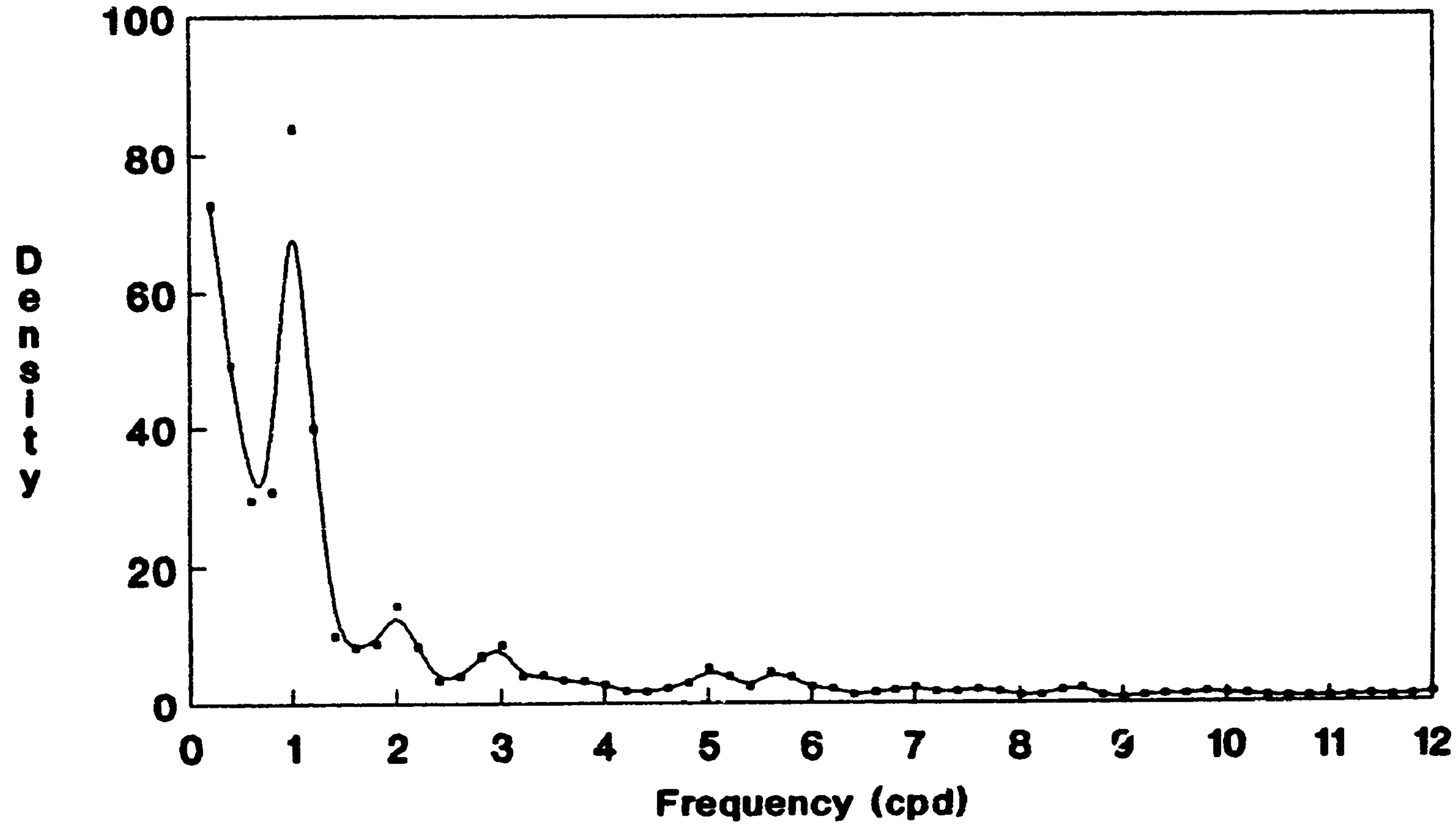


Figure 41. Spectrogram of Fish 17



Figrure 42. Spectrogram of Fish 18

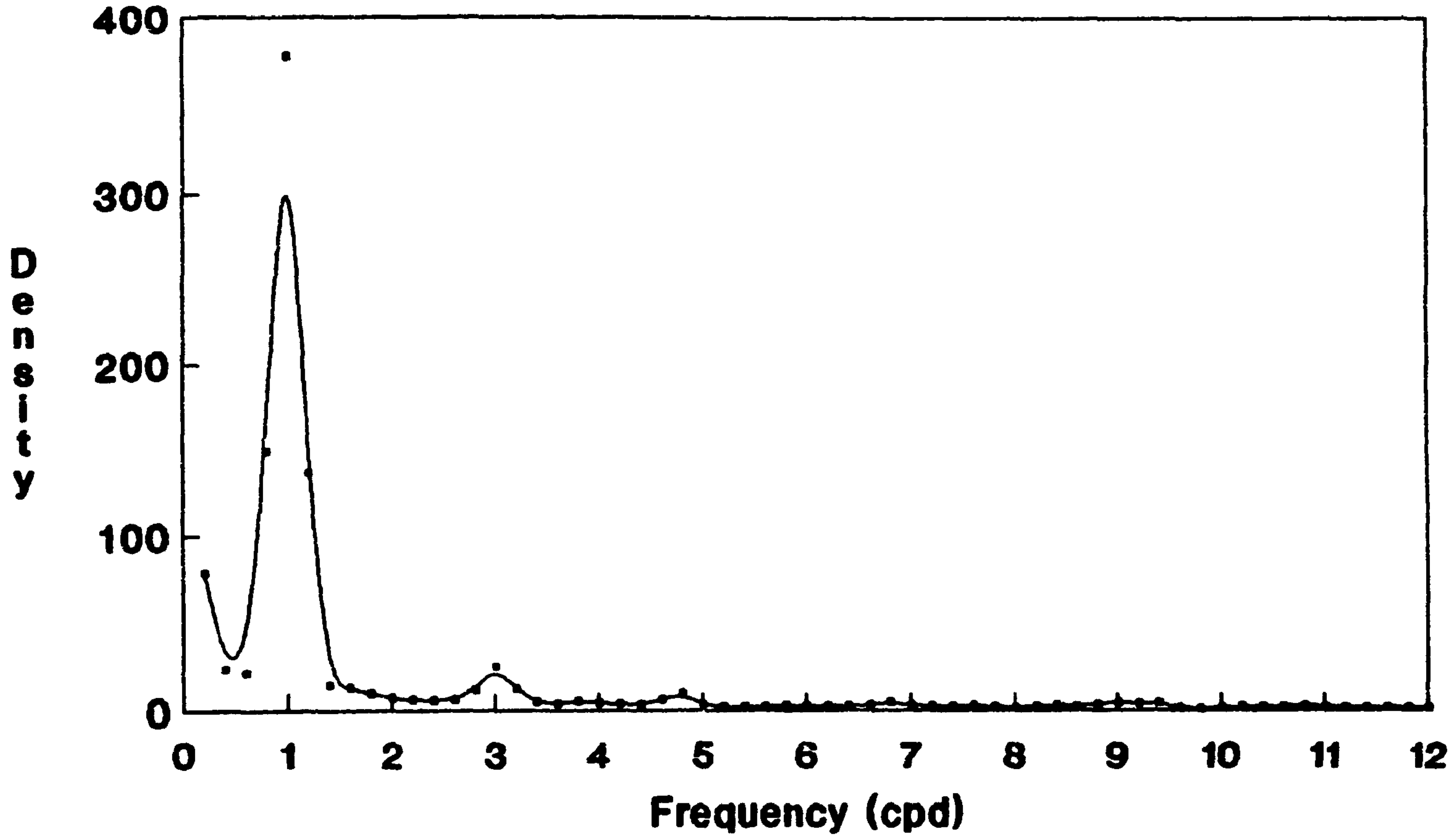


Figure 43. Spectrogram of Fish 19

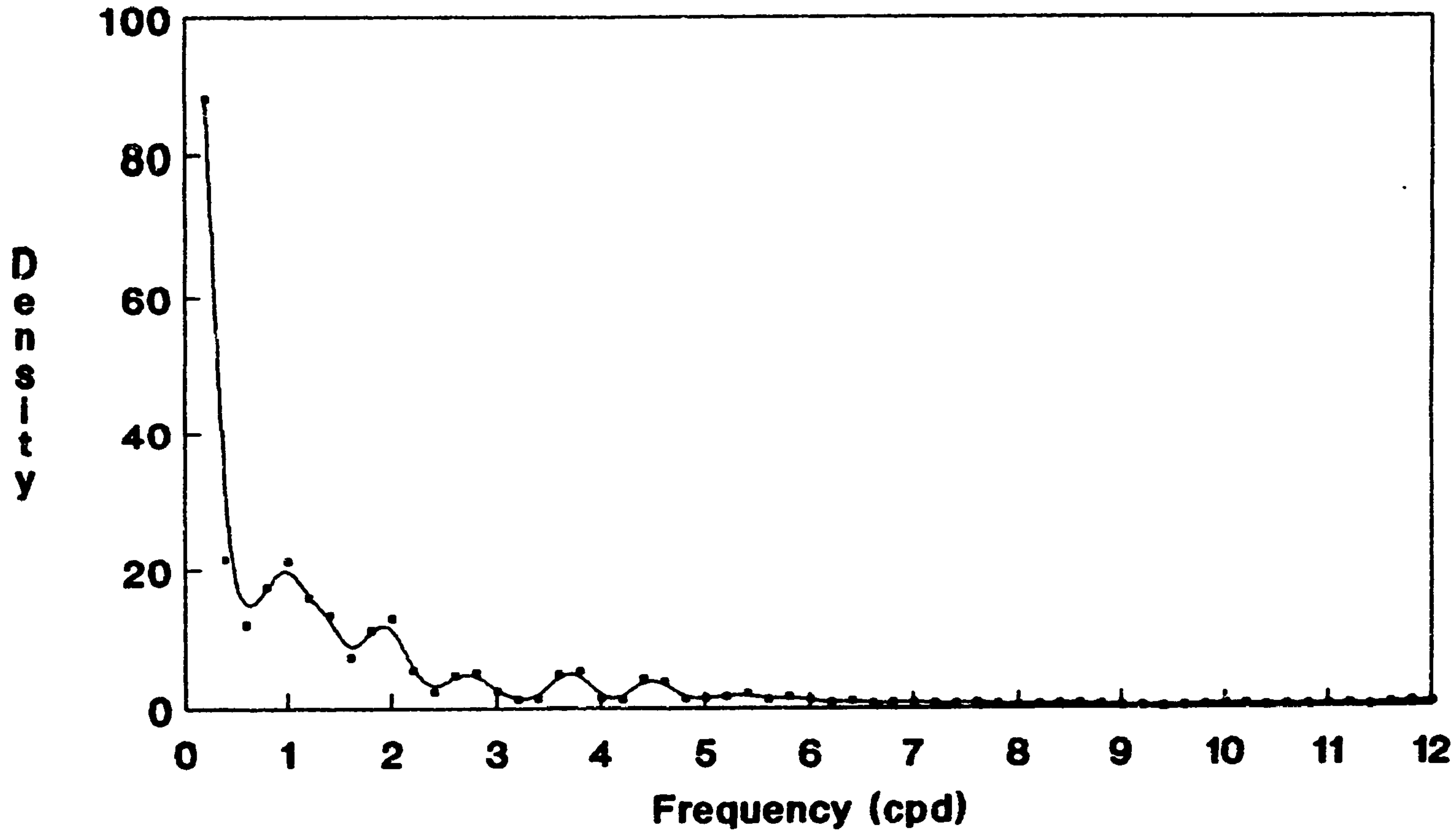


Figure 44. Spectrogram of Fish 20

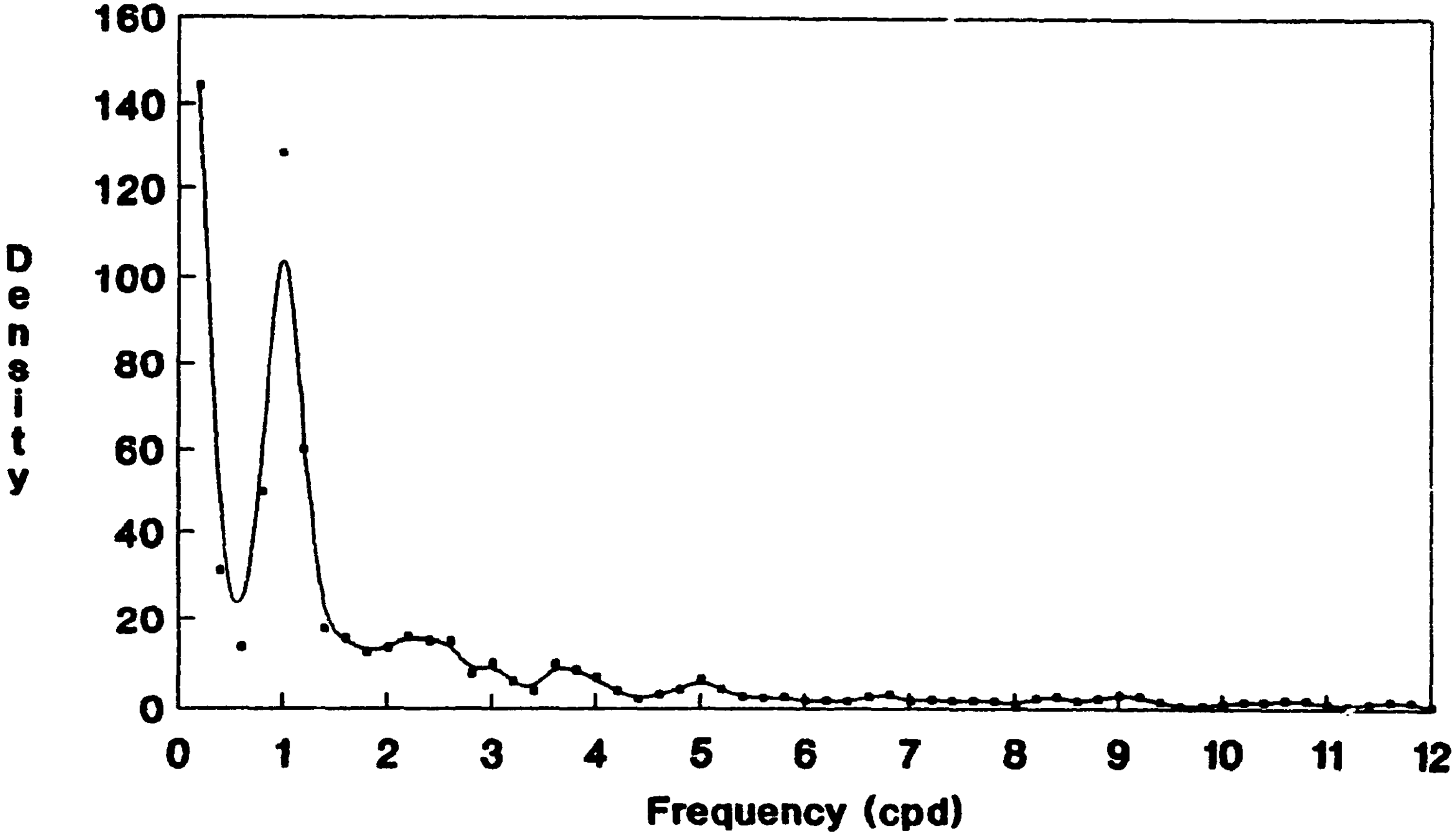


Figure 45. Spectrogram of Fish 21

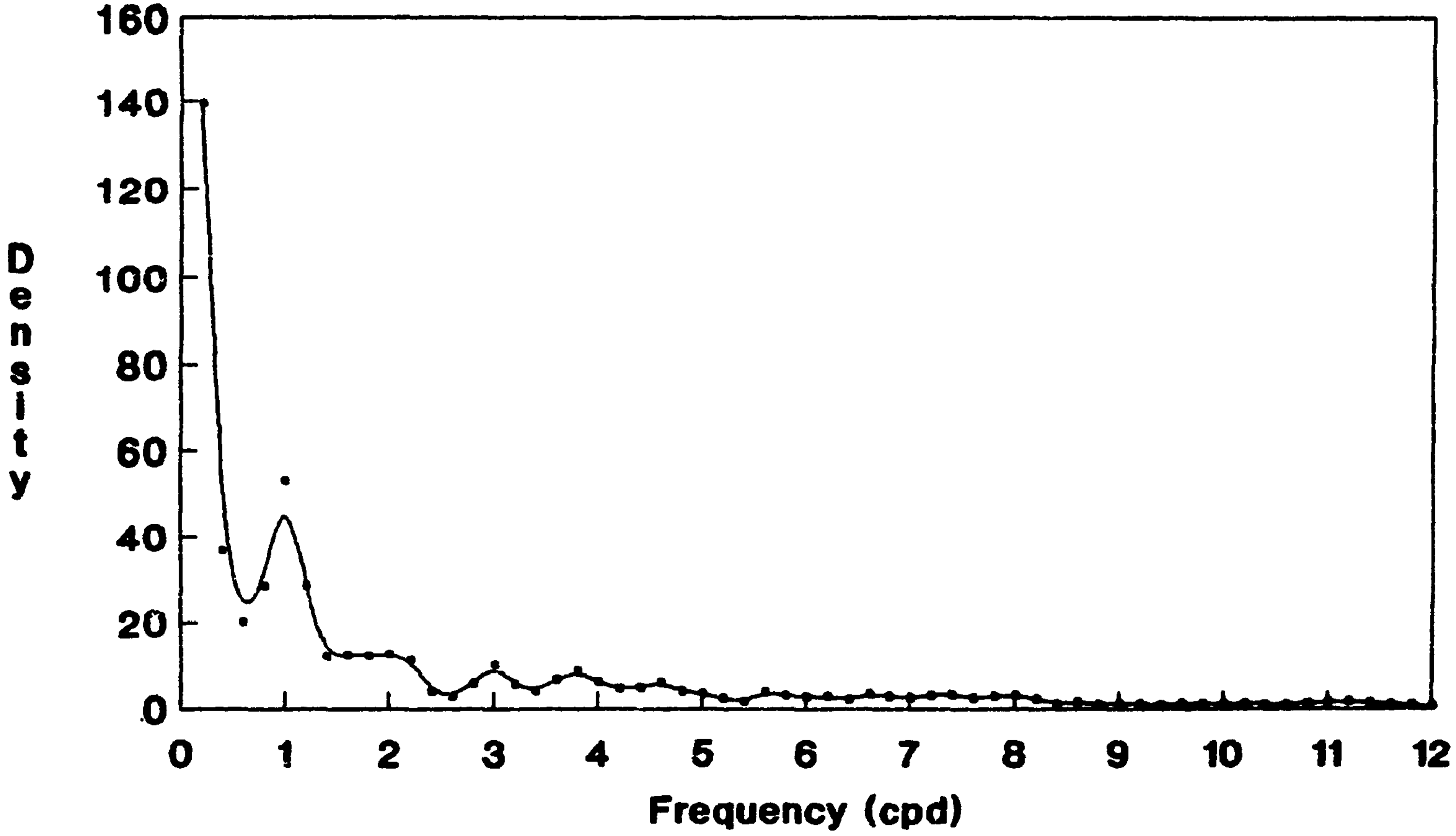


Figure 46. Spectrogram of Fish 22

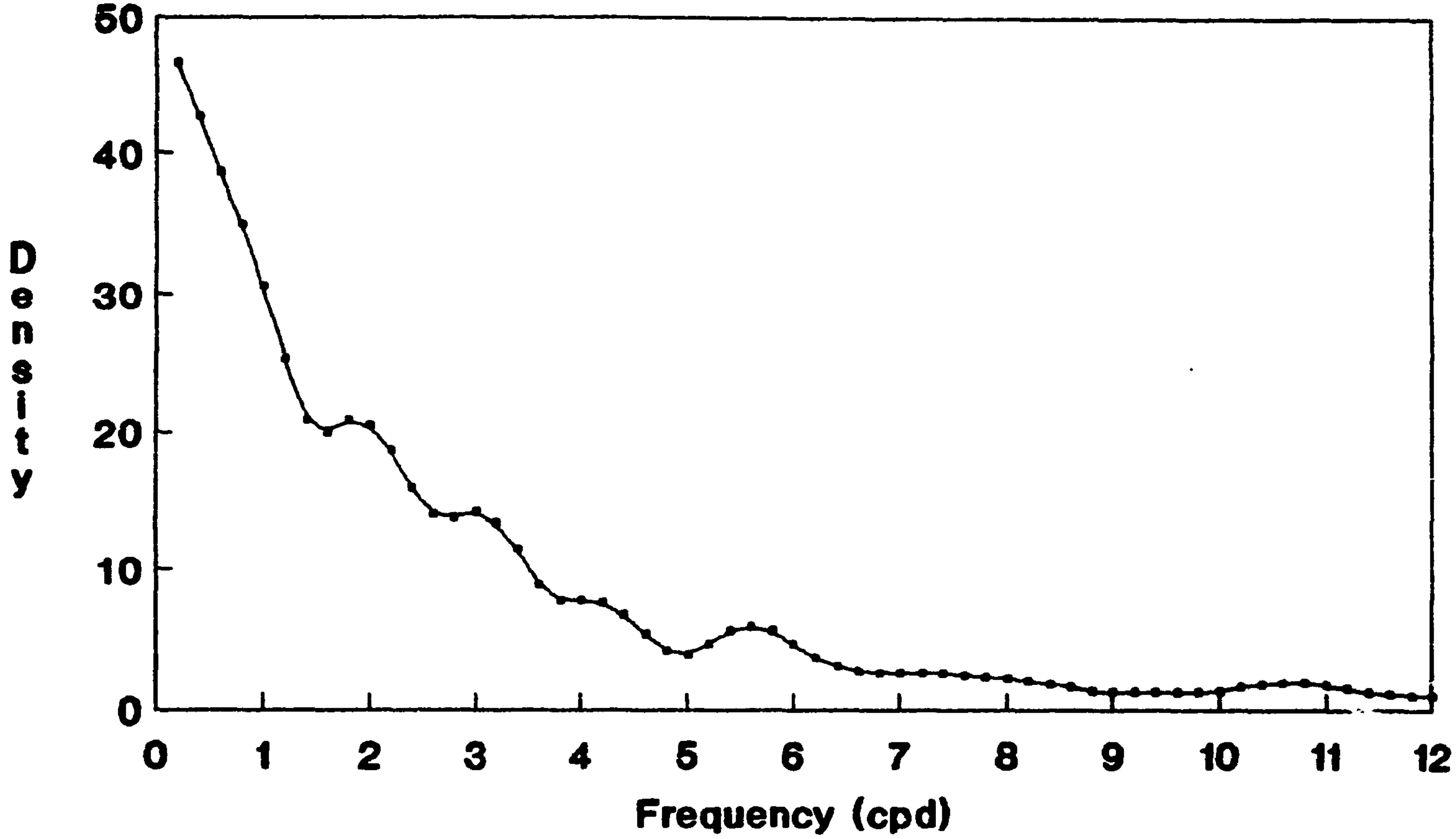


Figure 47. Spectrogram of Fish 23

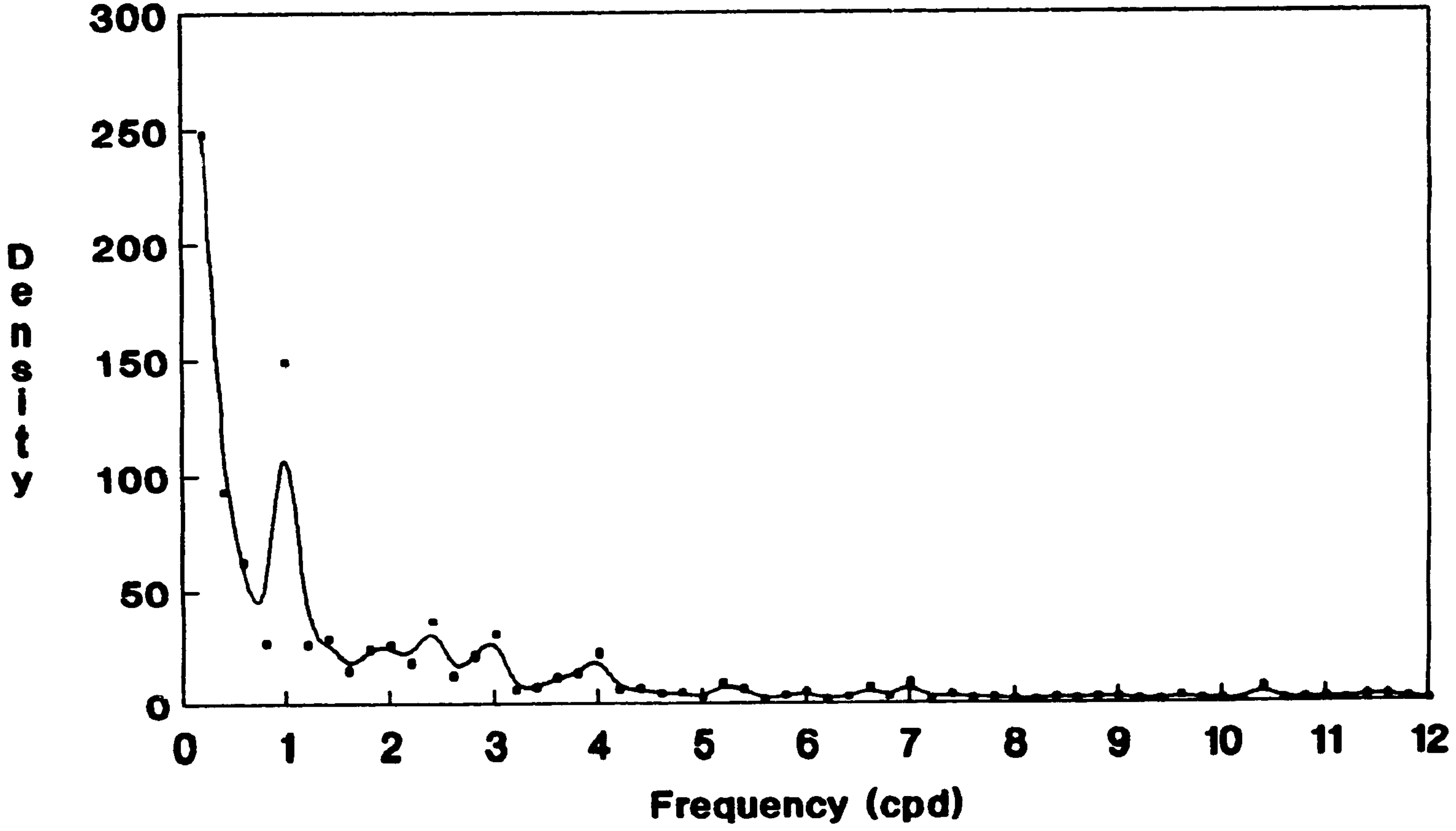


Figure 48. Spectrogram of Fish 24

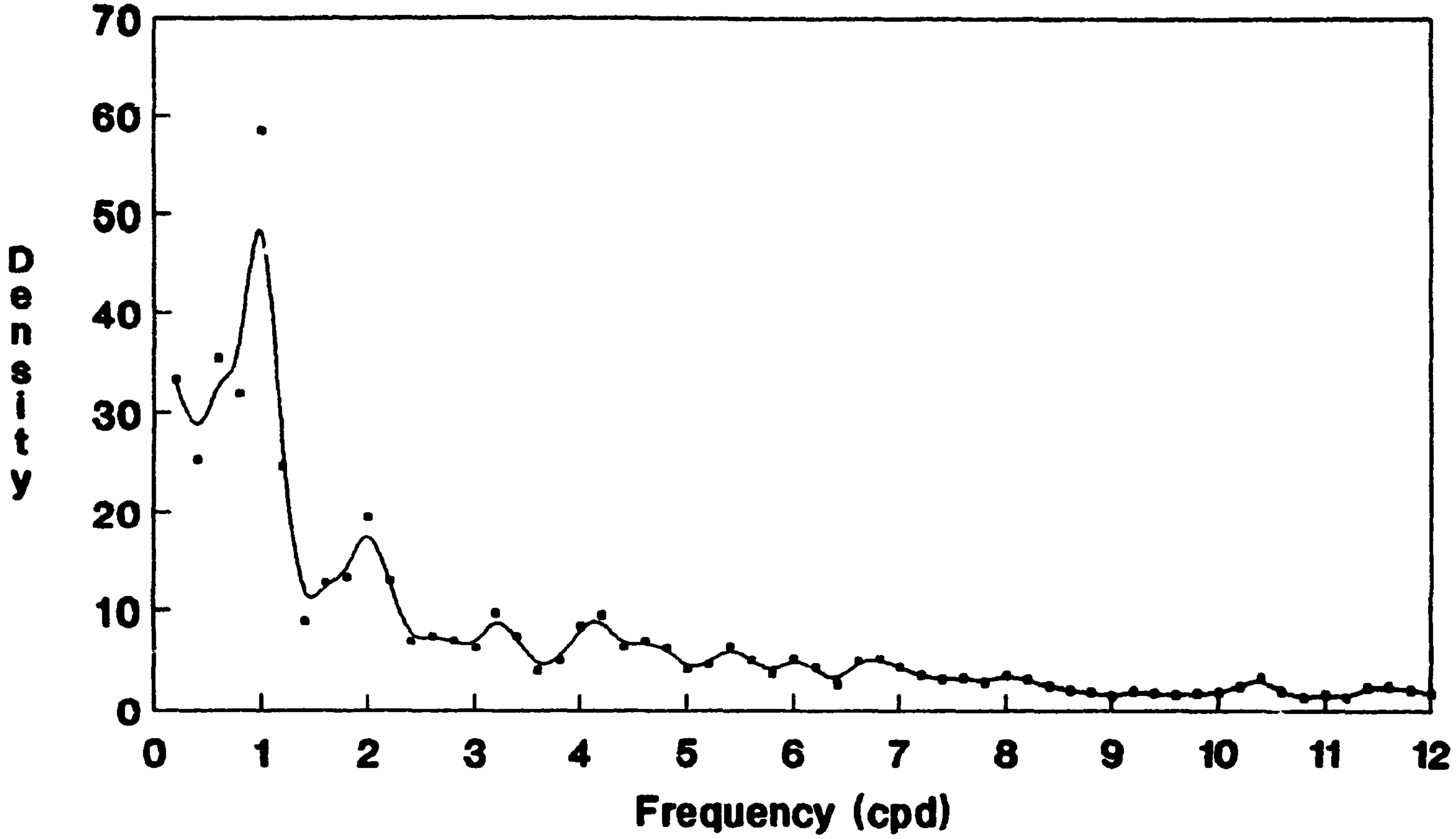


Figure 49. Spectrogram of Fish 25

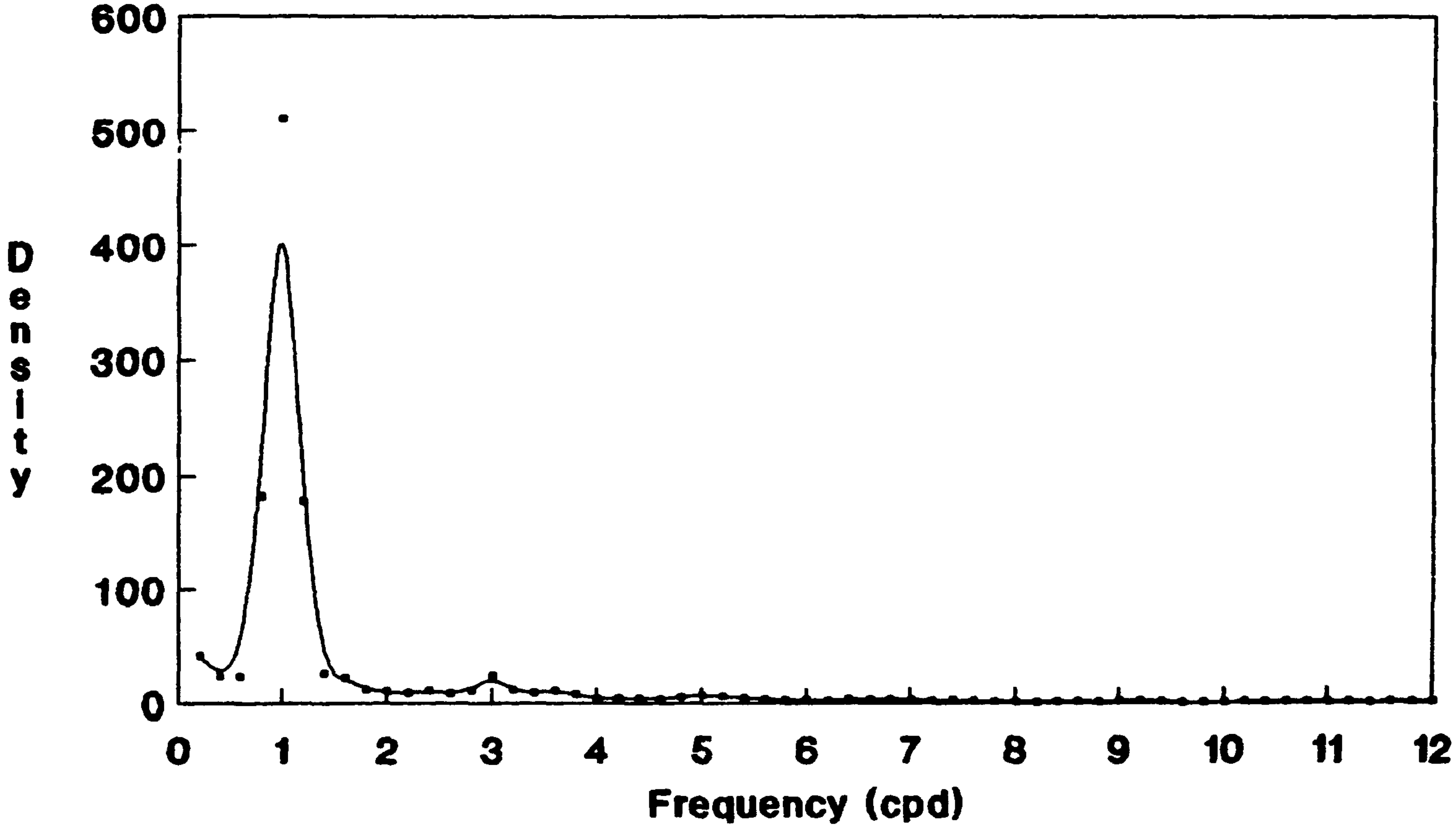


Figure 50. Spectrogram of Fish 26

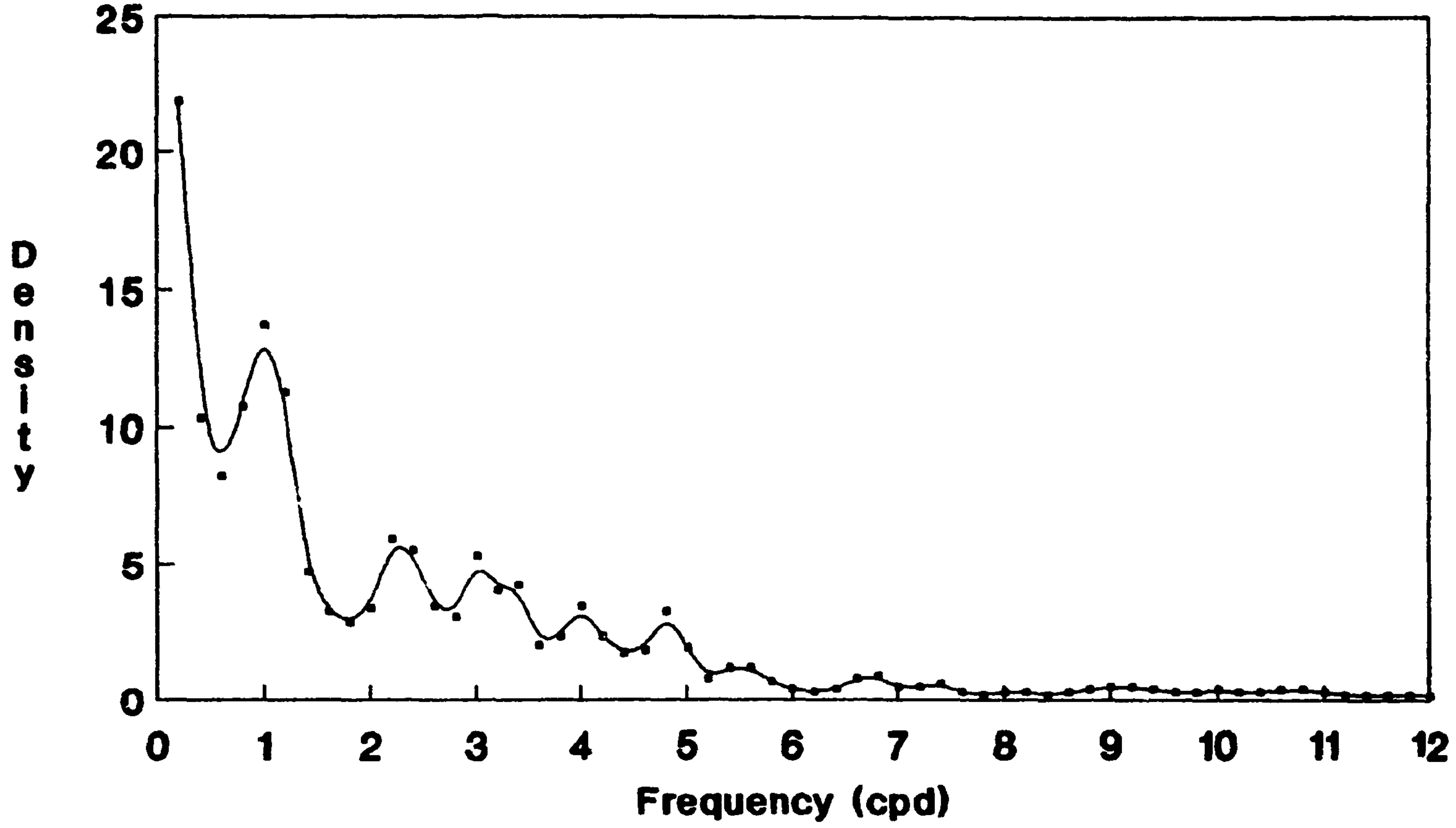


Figure 51. Spectrogram of Fish 27

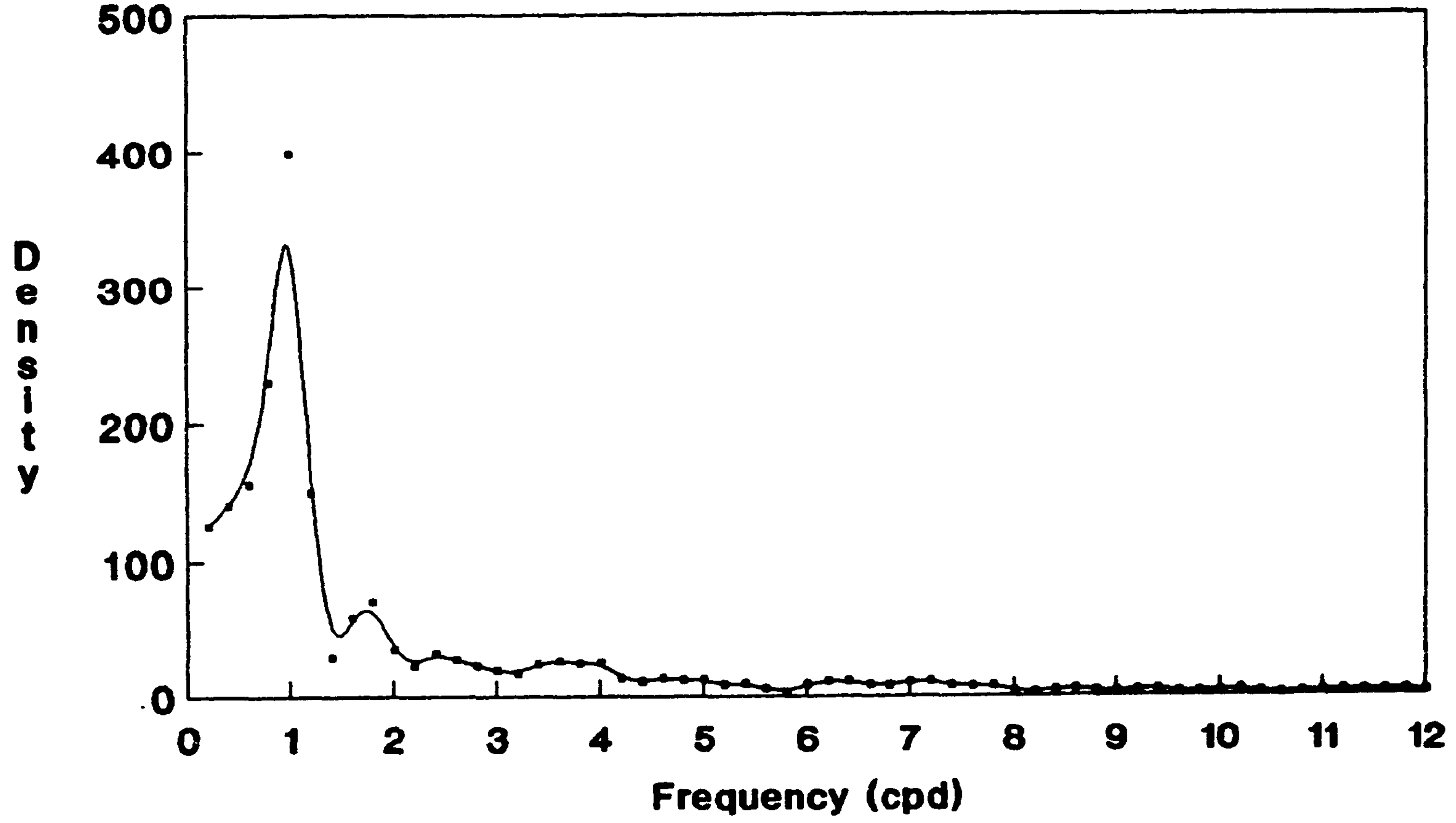
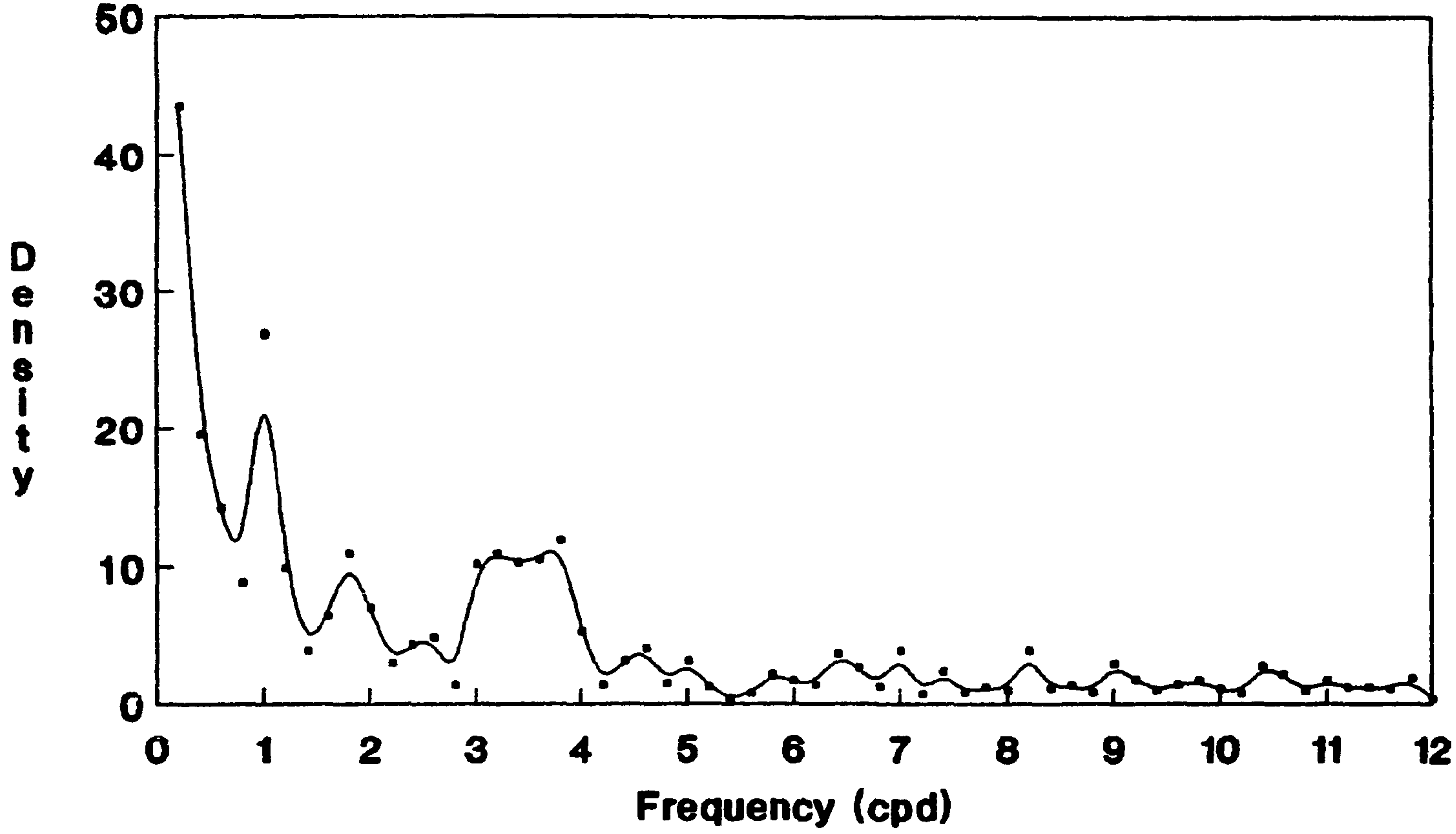


Figure 52. Spectrogram of Fish 28



APPENDIX VI

Table 15. Daily mean preferred temperature (°C) of juvenile rainbow trout over a 20 day thermoregulatory test period. Ration regime and trial date of each fish can be found in Table 14.

	Fish Number									
Day	1	2	3	4	5	6	7	8	9	10
1	19.4	19.5	22.4	18.2	16.5	13.2	13.9	14.5	17.9	18.3
2	19.6	20.0	22.8	18.0	17.5	12.8	11.7	13.5	18.1	16.9
3	19.4	18.8	21.9	18.0	18.9	14.3	11.2	13.2	18.7	17.9
4	19.0	18.5	21.9	17.9	17.7	14.3	14.8	13.4	21.0	17.4
5	20.2	19.5	20.6	17.4	17.6	13.3	16.8	10.4	17.0	12.8
6	19.9	19.2	20.7	14.5	16.4	14.8	13.9	13.2	17.6	17.7
7	19.6	20.4	18.2	13.5	15.7	14.9	15.3	13.8	20.1	16.8
8	19.0	20.4	16.3	13.3	15.9	13.9	18.5	13.7	18.5	16.7
9	19.0	19.7	14.0	15.4	15.2	12.2	15.5	12.9	18.1	16.4
10	18.5	21.6	15.2	13.9	15.6	14.5	15.8	13.5	19.2	17.4
11	17.5	19.4	16.0	13.5	13.2	14.9	13.5	13.4	18.0	18.3
12	17.7	18.3	14.1	17.0	16.1	15.1	14.5	13.4	19.7	17.8
13	17.8	20.5	15.3	16.2	18.0	15.1	14.6	13.3	17.7	16.7
14	17.8	20.8	16.1	15.3	17.6	15.8	15.5	13.3	14.5	18.0
15	18.7	20.8	14.9	15.4	18.8	15.8	15.5	13.9	13.6	16.9
16	19.6	18.6	16.3	13.6	17.4	16.0	13.8	15.9	16.4	16.9
17	19.5	20.1	16.2	14.0	16.9	15.3	12.9	14.0	12.6	15.9
18	18.4	17.5	15.3					15.0	17.0	16.7
19	19.3	18.1	14.1					14.9	15.1	17.5
20	17.6	18.7	13.2					14.9	14.4	16.0

Table 15 cont'd

Daily mean preferred temperature (°C) of juvenile rainbow trout over a 20 day thermoregulatory test period. Ration regime and trial date of each fish fish can be found in Table 14.

	Fish Number									
Day	11	12	13	14	15	16	17	18	19	20
1	13.1	17.3	19.7	18.7	15.2	19.7	17.5	19.2	13.5	14.0
2	12.8	18.6	19.3	18.8	14.7	19.5	16.9	18.5	15.6	14.2
3	12.7	19.1	19.1	18.1	16.9	18.3	15.1	19.1	17.8	14.5
4	12.5	18.4	17.9	16.7	17.7	19.2	15.8	19.6	17.5	13.3
5	9.4	16.2	16.7	16.8	18.1	19.1	16.6	19.7	17.4	13.4
6	13.2	15.9	16.2	16.8	16.9	19.8	16.2	18.9	17.5	12.8
7	12.8	14.9	16.0	15.8	16.8	19.7	15.8	17.9	17.7	11.7
8	13.7	16.2	15.6	17.3	15.4	19.7	15.5	17.8	18.2	12.1
9	14.6	16.0	15.3	14.8	16.2	19.0	15.0	17.9	18.5	13.0
10	15.9	16.8	15.7	14.1	16.1	19.0	14.7	16.8	19.1	14.1
11	17.7	15.8	16.1	13.9	14.6	22.2	16.5	17.8	19.1	14.9
12	15.6	17.5	15.4	16.6	14.1	22.2	18.1	16.4	20.5	19.8
13	16.7	18.8	16.3	17.5	14.3	21.2	19.2	16.7	20.4	20.4
14	14.6	16.3	16.0	18.6	14.6	22.8	19.0	18.4	17.4	16.9
15	16.2	16.1	14.4	14.6	14.6	22.9	19.4	16.3	15.9	15.0
16	18.5	16.2	16.2	15.5	16.0	22.3	19.2	15.5	15.8	13.9
17	17.5	15.3	16.3	13.7	16.4	21.4	18.8	15.4	14.1	13.7
18	17.2	13.7	16.8	14.3	17.8	20.9	19.1	13.4	17.7	14.8
19	18.9	14.6	15.8	14.4	14.1	20.6	18.7	14.4	18.7	15.2
20	18.2	13.8	16.9	17.1	15.4	20.6	13.4	16.4	16.0	14.0

Table 15 cont'd

Daily mean preferred temperature (°C) of juvenile rainbow trout over a 20 day thermoregulatory test period. Ration regime and trial date of each fish can be found in Table 14.

Day	Fish Number								
	21	22	23	24	25	26	27	28	29
1	14.2	9.9	18.7	14.6	14.5	16.7	16.3	17.5	16.2
2	12.7	12.5	17.4	15.0	15.7	15.6	15.5	16.5	15.3
3	12.4	14.3	17.2	15.9	14.4	17.1	15.6	17.5	15.4
4	11.6	15.2	18.2	15.3	15.6	18.8	16.0	18.7	14.7
5	11.4	11.3	19.2	14.3	16.6	16.4	17.7	18.3	14.4
6	10.4	13.2	19.9	15.1	15.8	15.8	18.9	16.6	14.2
7	12.6	15.0	16.8	15.9	18.0	15.0	18.7	17.6	12.9
8	13.5	15.0	18.1	15.7	14.9	16.1	18.5	16.6	14.3
9	14.7	14.6	13.2	15.4	17.1	16.2	18.7	17.5	12.7
10	15.7	13.5	17.8	15.9	15.7	15.9	13.7	17.1	13.5
11	16.1	14.4	18.7	15.8	16.2	16.8	16.2	17.1	13.6
12	19.2	16.4	15.7	15.7	16.9	16.6	16.5	18.3	14.7
13	18.5	16.3	15.4	15.8	18.3	17.1	16.7	15.5	14.2
14	14.2	14.2	15.6	16.1	18.5	17.4	16.9	18.6	14.1
15	15.1	13.6	8.6	16.9	18.2	17.5	12.8	17.3	13.2
16	13.9	13.6	8.5	16.7	18.0	17.8	16.5	17.9	13.8
17	13.2	14.2	9.8	16.6	16.6	17.0	16.4	18.2	13.7
18	14.1	15.3	21.0	18.3	17.5	17.4	17.6	18.6	13.9
19	13.2	14.3	15.2	18.1	18.5	17.7	20.4	17.9	12.7
20	9.9	15.9	20.5	18.0	17.8	17.3	10.9	16.8	12.3

Table 16. Rhythmicity index (°C) of juvenile rainbow trout over a 20 day thermoregulatory test period. Ration regime and trial date of each fish can be found in Table 14.

	Fish Number									
Day	1	2	3	4	5	6	7	8	9	10
1	0.7	0.8	-0.0	-0.5	3.4	-3.3	-7.7	-0.8	0.2	1.3
2	1.3	1.5	0.5	0.1	-2.0	-1.3	6.3	-2.3	1.3	-0.5
3	0.3	1.6	0.8	1.0	-0.7	0.5	3.7	-2.1	1.2	-0.5
4	-0.1	3.4	-0.5	1.1	-2.9	-0.6	0.9	-2.4	3.3	0.0
5	0.3	2.7	-1.6	-0.8	-3.8	-5.7	-2.9	-7.5	-6.3	-6.7
6	0.6	6.2	-0.0	-3.8	1.9	-2.6	1.4	-0.5	1.8	-1.7
7	1.0	6.2	1.5	-3.9	1.9	4.7	3.6	-1.3	4.9	-0.4
8	-0.1	7.2	-0.1	-4.9	0.6	-0.2	-1.6	-0.2	3.9	-1.4
9	-0.9	9.3	2.3	-2.9	2.9	-0.1	-0.1	-0.7	1.0	-1.5
10	-0.2	8.4	1.0	-4.7	-0.8	3.0	2.5	-1.1	1.9	-2.1
11	-0.3	1.4	1.0	2.0	1.9	-2.0	10.9	-1.9	-8.6	-1.7
12	0.3	1.2	-6.6	-1.8	0.0	-4.7	6.7	-0.6	-6.1	-1.1
13	-0.5	5.7	-6.0	1.3	0.7	-1.8	7.2	-0.9	-2.0	-1.4
14	-1.2	6.1	-8.2	1.3	1.5	-1.1	1.2	-0.5	-8.4	-1.1
15	1.3	7.3	-9.6	-1.4	1.6	-1.4	4.2	-2.3	-3.9	-3.7
16	-0.4	7.5	-5.4	4.7	1.3	-2.1	7.8	-2.2	-7.1	-1.1
17	-0.8	4.3	-2.8	1.5	-0.3	-8.3	6.3	-2.2	-4.2	-3.5
18	1.2	6.1	-2.2					-2.2	6.8	-2.0
19	0.8	4.7	-3.1					-1.9	-7.8	-1.1
20	-2.2	7.6	-0.5					-2.0	-5.0	-3.8

Table 16 cont'd

Rhythmicity index (°C) of juvenile rainbow trout over a 20 day thermoregulatory test period. Ration regime and trial date of each fish can be found in Table 14.

	Fish Number									
Day	11	12	13	14	15	16	17	18	19	20
1	-0.4	4.8	0.2	0.4	0.7	3.4	0.5	7.7	0.7	-1.2
2	-0.4	3.0	-0.5	0.0	-1.3	5.1	-2.3	7.0	-1.5	-3.2
3	-3.2	3.1	2.3	2.3	3.3	5.3	-1.0	7.2	-0.6	-1.0
4	0.1	-1.1	1.6	1.8	1.0	3.9	-2.9	6.7	-0.2	-5.0
5	-3.8	-2.4	-3.0	-1.6	1.6	8.8	-7.4	8.4	0.1	-0.5
6	0.2	-4.5	-1.6	-5.3	1.9	7.0	-7.9	9.7	0.4	-5.9
7	-3.2	-2.2	-0.5	-4.1	-0.3	8.0	-6.3	13.2	-0.4	-1.5
8	-2.3	-3.1	-3.6	-1.0	-3.7	8.1	-3.5	10.2	-0.8	-6.0
9	-1.7	-0.3	-0.2	-2.2	-2.9	7.2	-3.5	8.3	-0.9	-7.3
10	-0.8	-0.2	-1.6	-1.8	-4.0	5.4	-0.5	4.4	-0.5	-5.7
11	-1.3	-1.8	-2.3	-1.9	-7.4	6.3	-1.7	9.0	-1.2	-7.0
12	-2.6	-4.0	-2.7	1.5	-4.7	3.2	1.3	11.4	-0.0	-2.5
13	0.3	3.0	-2.2	-3.1	-7.8	5.7	0.3	6.4	1.3	1.2
14	-0.7	4.5	-3.7	-2.5	-6.7	3.0	-0.5	7.3	1.3	-1.1
15	-1.5	3.0	-0.7	-3.1	-6.5	1.8	2.0	4.0	-0.2	-3.9
16	-0.4	3.1	-4.8	-2.2	-4.7	5.4	-0.3	9.1	-0.4	-6.3
17	-0.1	4.0	-3.5	-2.3	-0.9	1.8	1.7	3.8	3.5	-6.7
18	5.2	3.2	-2.9	0.7	0.9	2.9	1.2	7.3	-0.9	-2.5
19	5.5	-0.1	-1.2	-2.6	-2.9	6.9	3.4	1.3	-2.1	-7.2
20	3.2	3.2	-3.7	-2.0	-0.8	2.7	2.6	0.2	-2.6	-11.3

Table 16 cont'd

Rhythmicity index (°C) of juvenile rainbow trout over a 20 day thermoregulatory test period. Ration regime and trial date of each fish can be found in Table 14.

	Fish Number								
Day	21	22	23	24	25	26	27	28	29
1	2.2	5.3	4.5	4.4	2.5	2.1	-1.0	1.4	-1.3
2	0.8	-6.5	1.8	3.1	5.0	0.7	7.6	2.6	-0.8
3	-2.9	-5.7	3.0	3.6	-0.3	2.6	10.3	-0.8	3.4
4	-5.6	4.1	4.7	4.9	4.9	0.9	7.0	-2.0	1.2
5	-0.5	4.7	3.9	3.6	4.9	-2.9	7.5	2.3	1.8
6	-1.4	2.3	-0.5	1.6	8.9	-1.8	5.4	1.8	2.9
7	-2.7	-0.1	-8.4	1.4	4.1	-3.3	10.3	2.7	-0.0
8	-5.5	-3.7	-3.1	-0.3	10.6	-2.9	5.6	1.5	0.3
9	-5.8	0.8	-3.2	-0.7	9.1	-1.9	0.8	1.1	-1.5
10	-2.5	0.9	-4.6	1.6	8.1	-1.1	10.6	0.3	1.3
11	-2.4	-3.7	2.1	1.7	13.0	-0.6	11.7	0.3	4.6
12	-0.4	0.7	3.8	-0.9	9.8	-0.6	9.2	-0.1	4.3
13	0.4	2.1	-1.5	0.6	8.9	-2.7	9.7	-2.0	2.7
14	-1.7	1.7	-0.6	-0.1	8.2	1.2	9.5	-1.9	2.0
15	-2.5	-0.5	-2.7	0.2	7.3	1.5	0.2	1.8	-0.1
16	-3.1	-0.2	0.8	0.7	13.5	1.7	13.6	0.0	0.6
17	-1.5	-0.1	0.4	-1.9	11.8	0.1	9.6	-1.6	-0.1
18	-1.0	-0.2	0.2	1.5	12.4	-0.1	2.8	1.7	2.4
19	-2.6	0.4	1.1	4.0	6.9	-0.4	3.2	1.2	2.8
20	-3.5	0.3	1.5	5.3	10.2	-1.6	-0.4	1.1	1.9

Table 17. Thermoregulatory precision index (°C) of juvenile rainbow trout over a 20 day thermoregulatory test period. Ration regime and trial date of each fish can be found in Table 14.

	Fish Number									
Day	1	2	3	4	5	6	7	8	9	10
1	0.9	1.9	0.7	0.6	0.9	0.9	1.1	0.5	1.1	0.4
2	1.0	1.6	0.5	0.3	0.7	1.0	1.5	0.6	1.4	0.4
3	0.8	2.0	0.5	0.5	0.7	1.0	1.3	0.5	1.3	0.5
4	0.8	2.1	0.8	0.7	0.6	1.1	1.3	0.5	0.8	0.4
5	0.9	2.5	1.1	0.8	0.7	1.2	1.2	0.4	0.7	0.6
6	1.1	2.6	1.3	1.1	0.9	1.2	1.0	0.3	1.7	0.6
7	1.3	1.8	1.5	1.2	0.8	1.3	1.1	0.4	1.2	0.9
8	1.6	1.8	2.0	1.1	1.0	1.0	1.0	0.6	1.7	0.6
9	1.6	2.1	2.2	1.4	0.9	0.7	2.0	0.5	1.8	0.5
10	1.4	1.4	2.0	1.3	1.0	1.1	1.5	0.4	1.6	0.7
11	1.4	1.9	2.0	1.0	1.4	1.4	1.1	0.4	1.4	0.8
12	1.3	2.4	2.1	1.3	1.2	1.1	1.6	0.3	1.4	0.8
13	1.6	1.9	2.1	1.3	0.9	1.0	1.5	0.4	1.7	0.9
14	1.5	1.6	1.9	1.3	1.1	0.9	1.9	0.4	1.3	0.9
15	1.4	1.5	2.1	1.3	0.8	0.9	1.5	0.3	1.0	0.7
16	1.2	2.2	2.0	1.6	1.0	0.9	1.7	0.3	1.0	0.5
17	1.3	2.2	2.0	1.2	0.8	0.4	1.5	0.4	1.2	0.6
18	1.7	2.5	2.0					0.5	1.6	0.8
19	1.7	2.8	1.9					0.5	1.3	0.9
20	1.6	2.2	1.8					0.4	1.5	0.9

Table 17 cont'd

Thermoregulatory precision index (°C) of juvenile rainbow trout over a 20 day thermoregulatory test period. Ration regime and trial date of each fish can be found in Table 14.

	Fish Number									
Day	11	12	13	14	15	16	17	18	19	20
1	0.9	0.9	0.5	0.6	0.5	1.7	0.9	1.0	1.1	2.1
2	0.7	0.6	0.7	0.4	0.5	2.2	1.0	1.0	0.8	2.3
3	0.5	0.5	0.8	0.4	0.5	2.2	1.3	0.8	0.3	2.2
4	0.6	0.7	0.8	0.5	0.5	2.3	1.0	0.9	0.1	2.1
5	0.6	1.1	0.8	0.6	0.7	2.0	1.5	0.8	0.0	2.3
6	0.7	1.1	1.1	0.9	0.7	2.4	1.3	0.9	0.0	1.7
7	0.6	1.0	1.1	0.6	0.8	1.8	1.2	1.0	0.0	1.6
8	0.7	0.8	1.0	0.5	0.8	2.0	1.5	1.2	0.0	1.5
9	0.7	0.9	1.2	0.6	0.9	1.9	1.3	1.1	0.0	1.5
10	0.9	1.0	1.0	0.5	0.9	1.9	1.6	1.6	0.0	1.8
11	1.2	0.8	1.2	0.6	0.9	1.5	1.3	1.4	0.0	1.2
12	1.0	1.2	1.2	0.9	1.1	1.6	1.5	1.4	0.0	0.3
13	1.2	0.9	1.2	0.6	0.7	1.3	1.6	1.6	0.4	0.2
14	1.0	0.8	1.3	0.7	0.9	1.2	1.6	1.3	0.7	0.3
15	0.7	0.8	1.0	0.7	0.8	1.6	1.5	1.9	0.8	0.7
16	0.9	0.8	1.3	0.6	0.8	1.6	1.6	1.3	1.0	1.1
17	1.2	0.9	1.3	0.8	1.0	1.4	1.4	1.5	0.9	1.3
18	1.2	0.8	1.0	1.1	0.7	1.7	1.4	1.6	1.0	1.3
19	1.0	1.1	1.1	1.1	1.0	1.3	1.3	2.4	1.3	1.1
20	1.1	1.1	1.2	0.3	1.3	1.7	1.2	2.1	1.7	1.2

Table 17 cont'd

Thermoregulatory precision index (°C) of juvenile rainbow trout over a 20 day thermoregulatory test period. Ration regime and trial date of each fish can be found in Table 14.

	Fish Number								
Day	21	22	23	24	25	26	27	28	29
1	1.6	1.2	1.6	1.8	1.4	0.9	1.0	1.0	1.8
2	2.0	2.3	0.5	1.1	1.0	0.9	1.4	0.9	0.9
3	1.7	1.9	0.5	1.7	0.7	0.9	1.5	1.3	0.5
4	2.0	1.7	0.5	1.4	1.6	0.9	1.9	1.7	0.5
5	2.5	1.9	0.5	1.8	1.5	0.6	2.2	1.4	0.5
6	2.1	1.6	0.7	1.8	1.5	0.5	2.4	1.4	0.7
7	2.0	1.2	0.8	1.4	1.6	0.5	1.3	1.3	1.2
8	1.7	0.9	1.1	1.7	1.5	0.8	1.6	1.4	1.2
9	1.4	1.2	1.6	2.0	1.8	0.6	2.2	1.3	1.0
10	1.2	1.8	1.8	1.8	1.6	0.8	1.4	1.0	1.2
11	0.7	1.7	0.7	0.8	1.7	0.8	1.4	1.0	1.1
12	0.2	1.5	1.4	1.1	1.4	0.6	1.1	1.2	1.3
13	0.2	1.1	2.5	2.2	1.2	0.6	1.5	1.3	1.4
14	0.4	1.2	2.8	2.6	1.3	0.5	1.2	1.0	1.4
15	0.5	1.3	1.1	2.0	1.6	0.3	1.2	0.9	1.0
16	0.5	1.8	1.6	2.8	1.7	0.3	1.0	1.0	0.9
17	0.8	1.6	0.8	2.0	1.5	0.2	1.5	1.1	1.0
18	0.6	1.0	0.9	1.2	1.1	0.4	1.8	1.0	1.4
19	0.8	0.7	1.4	2.1	0.9	0.6	1.5	0.9	1.3
20	1.1	1.1	1.6	2.1	1.2	0.6	1.7	1.1	1.5

Table 18. Daily mean shuttling rate (shuttles/hour) of juvenile rainbow trout over a 20 day thermoregulatory test period. Ration regime and trial date of each fish can be found in Table 14.

	Fish Number									
Day	1	2	3	4	5	6	7	8	9	10
1	99	88	112	83	73	18	78	76	53	42
2	95	59	131	90	70	14	18	54	30	44
3	97	35	96	70	87	14	25	51	34	39
4	79	21	94	40	69	12	55	44	25	32
5	88	17	74	34	53	7	50	37	17	15
6	79	15	81	13	38	8	27	42	14	11
7	50	28	48	7	42	9	35	49	17	8
8	43	20	21	5	33	7	43	32	12	15
9	35	11	15	7	29	10	22	27	16	15
10	44	17	15	8	32	7	19	48	14	12
11	30	24	27	9	14	6	18	43	14	12
12	35	19	27	8	26	6	12	40	17	14
13	30	16	27	6	36	8	20	40	17	8
14	33	16	25	6	29	6	12	47	11	11
15	34	12	16	4	41	7	17	46	6	11
16	32	6	24	4	24	7	7	50	6	5
17	22	10	26	4	26	7	18	38	5	12
18	15	6	27					47	8	7
19	14	6	26					46	6	13
20	18	6	23					45	6	13

Table 18 cont'd

Daily mean shuttling rate (shuttles/hour) of juvenile rainbow trout over a 20 day thermoregulatory period. Ration regime and trial date of each fish can be found in Table 14.

	Fish Number									
Day	11	12	13	14	15	16	17	18	19	20
1	28	23	64	36	126	26	35	38	10	32
2	33	33	70	51	114	23	34	33	13	24
3	21	28	62	37	78	15	18	46	45	20
4	18	28	58	36	89	11	26	23	66	15
5	10	18	45	21	63	10	15	25	58	16
6	15	17	32	5	57	8	14	15	56	17
7	13	16	26	15	50	11	17	14	48	12
8	14	14	22	47	28	7	14	13	45	14
9	17	14	18	7	36	6	17	8	26	12
10	17	14	19	4	32	7	16	8	34	14
11	15	14	22	5	27	5	18	11	34	14
12	12	15	20	9	21	9	25	14	25	32
13	8	18	21	33	32	9	28	13	21	56
14	9	17	16	26	34	8	22	14	15	49
15	8	21	12	8	21	5	21	8	11	27
16	14	18	23	20	24	4	19	4	7	17
17	11	15	42	6	24	6	22	4	5	14
18	11	16	34	4	25	7	19	5	9	18
19	9	17	29	13	25	8	19	10	8	19
20	9	14	31	48	20	8	21	17	6	11

Table 18 cont'd

Daily mean shuttling rate (shuttles/hour) of juvenile rainbow trout over a 20 day thermoregulatory test period. Ration regime and trial date of each fish can be found in Table 14.

	Fish Number								
Day	21	22	23	24	25	26	27	28	29
1	24	11	34	13	35	27	9	25	20
2	14	8	44	15	41	17	2	12	33
3	19	16	46	12	57	22	3	12	40
4	16	31	55	11	22	42	5	10	38
5	13	12	48	16	19	39	7	13	35
6	9	23	29	13	15	62	8	12	34
7	21	26	27	11	15	58	10	10	20
8	32	27	23	12	12	53	6	13	25
9	39	24	9	7	12	64	3	18	15
10	48	17	13	8	6	63	3	20	11
11	61	18	19	11	7	54	3	20	12
12	73	10	9	9	9	69	3	7	9
13	56	25	7	8	15	73	3	8	13
14	57	25	6	9	15	62	8	20	15
15	50	23	5	8	12	64	3	33	12
16	34	24	4	6	7	75	3	22	17
17	21	23	5	7	5	94	3	14	12
18	43	28	12	8	9	83	3	28	9
19	45	25	10	10	12	56	2	26	13
20	17	24	15	10	9	52	3	9	6