

EVALUATION OF GROWTH INTERRUPTION AS A MEANS OF
MASS-MARKING HATCHERY TROUT

by
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A thesis submitted in partial fulfillment
of the requirements for the degree

of
Master of Science
in
Fish and Wildlife Management

MONTANA STATE UNIVERSITY
Bozeman, Montana

July, 1991

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of a thesis submitted by

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ACKNOWLEDGMENTS

Without the encouragement, support, and assistance of many people, this project would not have been possible. Dr. Robert White provided advice and critically reviewed this manuscript. Dr. Cal Kaya, Dr. Lynn Irby, William P. Dwyer, Roy Whaley, and Rich Comstock also reviewed the manuscript. Dr. Robert White and fellow students helped fin clip fish, collect scales, and measure fish. The staff of the Bozeman Fish Technology Center were invaluable in assisting with raising and providing food and space for the fish. Ron Skates, Creston Fisheries Assistance, USFWS provided an area to stock fish and assisted with recapture efforts.

Jeff Condiotty of BioSonics, Inc. was always willing and ready to provide technical assistance for use of the "OPRS".

I would particularly like to thank Roy Whaley of the Wyoming Game and Fish Department and William P. Dwyer of the Bozeman Fish Technology Center for providing aid in study design, facilities to work in, and moral support.

The majority of this project was funded by Wyoming Game and Fish Department.

TABLE OF CONTENTS

	Page
LIST OF TABLES.....	vii
LIST OF FIGURES.....	xi
ABSTRACT.....	xv
INTRODUCTION.....	1
METHODS.....	3
Experiment 1.....	3
Experimental Groups.....	3
Mark Retention.....	5
Experiment 2.....	6
Diet Treatments.....	6
Temperature Treatments.....	7
Processing and Analysis.....	8
Scale Preparation.....	8
Data Acquisition.....	9
Use of the OPRS.....	9
Radial Distance Measurements.....	11
Luminance Profiles.....	12
Data Analysis.....	12
RESULTS.....	15
Experiment 1.....	15
Post-treatment Comparisons.....	15
Radial Distance Analysis.....	15
Luminance Profile Analysis.....	17
Analysis with Selected Data Set.....	17
Hatchery and Cooper Lake Trout.....	17
Experiment 2: Diet Tests.....	18
Radial Distance Analysis.....	18
Variable Selection.....	18
Two Group Models.....	24
Three Group Models.....	26
Luminance Profile Analysis.....	31
Growth Comparisons.....	31
Experiment 2: Temperature Tests.....	34
Temperature Regimes.....	34
Radial Distance Analysis.....	38
Variable Selection.....	38
Two Group Models.....	42

TABLE OF CONTENTS (continued)

	Page
Three Group Models.....	44
Luminance Profile Analysis.....	44
Growth Comparisons.....	44
DISCUSSION.....	50
Radial Distance Work.....	50
Diet Groups.....	50
Temperature Groups.....	51
Mark Retention.....	53
Luminance Profile.....	54
Possible Problems With Experiment 1.....	55
Within Group Variability.....	55
Treatment Duration.....	56
Undetected "Marks".....	57
Scale Sampling Problems.....	58
Summary.....	59
REFERENCES CITED.....	61
APPENDICES.....	67
Appendix A--Variable Definitions.....	68
Appendix B--P-Values For Radial Distance ANOVA's.....	70
Appendix C--P-Values For Luminance Profile Transformation ANOVA's.....	77
Appendix D--Discriminant Analysis Data.....	83
Appendix E--Comparisons of Original, Hatchery- Reared, and Cooper Lake Cutthroat Trout Scale Patterns From Experiment 1.....	91

LIST OF TABLES

Table	Page
1. Percentage of each group used as "unknowns" to test model performance.....	14
2. Mean pre-treatment lengths of cutthroat trout used in Experiment 1, with t-test p-values comparing each group to control fish.....	15
3. Mean pre-treatment lengths of cutthroat trout used in 1988 feed experiments, with t-test p-values comparing each group to control fish.....	20
4. Discriminant analysis models, with associated Eigen values and classification arrays for 1988 feed experiments.....	24
5. Results of 1988 2-group feed model testing, using known cutthroat trout as "unknowns", with and without error classification correction factors. Numbers in parentheses represent 90 % confidence intervals for the estimate.....	25
6. Results of 1988 2-group feed model testing, switching "standards" with "unknowns", with and without error classification correction factors. Numbers in parentheses represent 90 % confidence intervals for the estimate.....	27
7. Discriminant analysis models, using 3 groups with associated Eigen values and classification arrays for 1988 feed experiments.....	28

LIST OF TABLES (continued)

Table	Page
8. Results of 1988 3-group feed model testing, using known cutthroat trout as "unknowns", with and without error classification correction factors. Numbers in parentheses represent 90 % confidence intervals about the estimate.....	29
9. Results of 1988 3-group feed model testing, switching "unknown" trout with "standards" for model building, with and without error classification correction factors. Numbers in parentheses represent 90 % confidence intervals about the estimate.....	30
10. Discriminant analysis models, with associated Eigen values and classification arrays for 1988 temperature experiments.....	42
11. Results of 1988 2-group temperature model testing, using known cutthroat trout as "unknowns", with and without error classification correction factors. Numbers in parentheses represent 90 % confidence intervals for the estimate.....	43
12. Results of 1988 2-group temperature model testing, switching "standards" with "unknowns", with and without error classification correction factors. Numbers in parentheses represent 90% confidence intervals for the estimate.....	45
13. Definitions of variables used in radial distance analysis. All measurements were made along longest anterior axis of the scale, drawn from focus to scale margin.....	69
14. Discriminant analysis, including variables used for discrimination, resultant Eigen values, and resultant classification arrays, for the 3 d versus control groups, Experiment 2.....	84

LIST OF TABLES (continued)

Table	Page
15. Discriminant analysis, including variables used for discrimination, resultant Eigen values, and resultant classification arrays, for the 5 d versus control groups, Experiment 2.....	85
16. Discriminant analysis, including variables used for discrimination, resultant Eigen values, and resultant classification arrays, for the 7 d versus control groups, Experiment 2.....	86
17. Discriminant analysis, including variables used for discrimination, resultant Eigen values, and resultant classification arrays, for the starved versus control groups, Experiment 2.....	87
18. Discriminant analysis, including variables used for discrimination, resultant Eigen values, and resultant classification arrays, for the cold water versus warm water temperature groups, Experiment 2. Scales with less than 7 circuli were left out of the data set.....	88
19. Discriminant analysis, including variables used for discrimination, resultant Eigen values, and resultant classification arrays, for the cold water versus fluctuating water temperature groups, Experiment 2. Scales with less than 7 circuli were left out of the data set.....	89
20. Discriminant analysis, including variables used for discrimination, resultant Eigen values, and resultant classification arrays, for the warm water versus fluctuating water temperature groups, Experiment 2. Scales with less than 7 circuli were left out of the data set.....	90

LIST OF TABLES (continued)

Table	Page
21. Comparisons of first 6 inter-circuli distances between original experimental cutthroat trout and hatchery-reared cutthroat trout. Sample size, mean, standard deviation, and p-values are shown.....	92
22. Comparisons of first 6 inter-circuli distances between original experimental cutthroat trout and cutthroat trout recovered from Cooper Lake approximately one year later. Sample size, mean, standard deviation, and p-values are shown.....	93

LIST OF FIGURES

Figure	Page
1. BioSonics' Optical Pattern Recognition System including a personal computer, second monitor, digitizing pad, frame grabber board, video camera, and microscope.....	10
2. Notched box and whisker plots for pre-treatment total lengths for each experimental group, Experiment 1. The central box covers the middle 50% of the data values, with "whiskers" extending out to the minimum and maximum values. The central line represents the median. A notch is added to each box corresponding to the width of a 95% confidence interval for the median and the width of the box is proportional to the square root of the number of observations in each data set (STSC, Inc. 1988).....	16
3. Notched box and whisker plots representing median, 95% confidence intervals, middle 50%, of and range for pre-treatment total lengths for each experimental group, Experiment 2 feed groups.....	19
4. Notched box and whisker plots representing median, 95% confidence intervals, middle 50%, and range of distance between 2nd and 3rd circuli, for each experimental group, Experiment 2 feed groups (variable 3).....	21
5. Notched box and whisker plots representing median, 95% confidence intervals, middle 50%, and range of distance between 3rd and 4th circuli, for each experimental group, Experiment 2 feed groups (variable 4).....	22

LIST OF FIGURES (continued)

Figure	Page
6. Notched box and whisker plots representing median, 95% confidence intervals, middle 50%, and range of distance between 4th and 5th circuli, for each experimental group, Experiment 2 feed groups (variable 5).....	23
7. Mean total length, with 95% confidence intervals, for each experimental group, Experiment 2 feed groups.....	32
8. Mean weight, with 95% confidence intervals, for each experimental group, Experiment 2 feed groups.....	33
9. Distribution of number of circuli per scale for each experimental group, Experiment 2 feed groups.....	35
10. Notched box and whisker plots representing median, 95% confidence intervals, middle 50%, of and range for pre-treatment total lengths for each experimental group, Experiment 2 temperature groups.....	36
11. Daily water temperature for cold water, fluctuating temperature, and warm water experimental groups, Experiment 2.....	37
12. Notched box and whisker plots representing median, 95% confidence intervals, middle 50%, and range of distance between 4th and 5th circuli, for each experimental group, Experiment 2 temperature groups (variable 5).....	39
13. Notched box and whisker plots representing median, 95% confidence intervals, middle 50%, and range of distance between 5th and 6th circuli, for each experimental group, Experiment 2 temperature groups (variable 6).....	40

LIST OF FIGURES (continued)

Figure	Page
14. Notched box and whisker plots representing median, 95% confidence intervals, middle 50%, and range of distance between 6th and 7th circuli, for each experimental group, Experiment 2 temperature groups (variable 7).....	41
15. Mean total length, with 95% confidence intervals, for each experimental group, Experiment 2 temperature groups.....	47
16. Mean weight, with 95% confidence intervals, for each experimental group, Experiment 2 temperature groups.....	48
17. Distribution of number of circuli per scale for each experimental group, Experiment 2 temperature groups.....	49
18. Resultant p-values from comparisons of each treatment group to the control group, using ANOVA, for all pertinent variables, Experiment 1 radial distance data.....	71
19. Resultant p-values from comparisons of selected trout in each treatment group, based on median growth rates, to selected trout from the control group, using ANOVA, for all pertinent variables, Experiment 1 radial distance data.....	72
20. Resultant p-values from comparisons of each treatment group to the control group, using ANOVA, for all pertinent variables, Experiment 1 radial distance data collected from trout after being reared in the hatchery an additional 8 months.....	73
21. Resultant p-values from comparisons of each treatment group to the control group, using ANOVA, for all pertinent variables, Experiment 1 radial distance data collected from trout after being reared Cooper Lake, Blackfeet Indian Reservation, an additional 12 months.....	74

LIST OF FIGURES (continued)

Figure		Page
22.	Resultant p-values from comparisons of each treatment group to the control group, using ANOVA, for all pertinent variables, Experiment 2 feed groups radial distance data.....	75
23.	Resultant p-values from comparisons of each treatment group to the control group, using ANOVA, for all pertinent variables, Experiment 2 temperature groups radial distance data.....	76
24.	Resultant p-values from comparisons of each treatment group to the control group, using ANOVA, for harmonics from transformed luminance profile data, Experiment 1.....	78
25.	Resultant p-values from comparisons of each treatment group to the control group, using ANOVA, for harmonics from transformed luminance profile data, Experiment 1 hatchery reared trout.....	79
26.	Resultant p-values from comparisons of each treatment group to the control group, using ANOVA, for harmonics from transformed luminance profile data, Experiment 1 Cooper Lake trout.....	80
27.	Resultant p-values from comparisons of each treatment group to the control group, using ANOVA, for harmonics from transformed luminance profile data, Experiment 2 feed groups.....	81
28.	Resultant p-values from comparisons of all treatment groups, using ANOVA, for harmonics from transformed luminance profile data, Experiment 2 temperature groups.....	82

ABSTRACT

Marking fish is a valuable technique which fisheries workers have used for centuries. Studies involving migration patterns, age and growth, stock identification, population abundance, stocking success, and mortality rates require later identification of fish involved. Despite importance of marking fish, there remains substantial need for improvement in types of marks used, methods of administering them and means of mark detection. Two groups of experiments were conducted to determine the feasibility of marking hatchery trout by inducing recognizable patterns on their scales using feed regime and water temperature manipulation. Difficulties with the first year's experiment precluded any separation between groups. Models used during the second year, based on scale pattern analysis, separated fish correctly, on the average, 95.6, 80.9, 91.2, and 96.4% of the time for experimental feed groups when compared with the control groups. Also, correct identification of group origin occurred, on the average, 97.6, 97.8, and 75.6% of the time for fluctuating water temperature experiments. My experiments have shown environmental conditions can be manipulated to induce "marks" on trout scales. Changes in feeding regime and, somewhat less precisely, water temperature effectively altered circuli spacing on treatment trout scales. These alterations were significant enough to produce a verifiable "mark" on trout scales. "Marks" were used to accurately predict fish group origin, constituting a simple, inexpensive, efficient, and harmless method of mass-marking trout in a hatchery setting. Several questions regarding this technique need further investigation. Size of fish to be marked, duration of marking period, duration of post-treatment period needed for detection of environmental changes, and scale sample selection are variables that need additional examination.

INTRODUCTION

Marking of fish is a valuable technique that has been used by fisheries managers for centuries. Studies involving migration patterns, age and growth, stock identification, population abundance, stocking success, and mortality rates require later identification of fish. Despite the importance of marking, substantial room for improvement remains in the types of marks used, methods of administering them, and means of mark detection.

Recent advancements in data acquisition techniques designed for rapid and systematic processing of optical patterns allow users to quickly and accurately process scale characteristic information. Numerous studies have used scale pattern information in attempts to separate stocks of fish in mixed-stock fisheries (Rowland 1969, Bilton 1971, Bilton and Messinger 1975, Cook 1982, Cook 1983, Fraidenburg et al. 1983, Cook and Guthrie 1987, Borgerson 1988, and Schwartzberg and Fryer 1989). These studies involved several species and met with varying degrees of success.

Fish raised in different environments often form distinguishable growth patterns on their scales (Cox-Rogers 1985, Whaley 1988, and Schwartzberg and Fryer 1989). These scale patterns could be manipulated in hatchery fish and possibly "read" as marks. Several studies suggest

manipulation of circuli spacing is possible. DeBont (1967) lists change in temperature, decrease in food availability, change in photoperiod, internal rhythms, and stress brought on by reproduction or migration as possible factors influencing circuli spacing.

Boyce (1985) reported exposure to extreme temperatures, shorter day length, and decreases in food availability tended to narrow circuli spacing in juvenile steelhead trout (*Salmo gairdneri*). Hogman (1968) reported an influence of photoperiod on scale patterns in coregonids. Other researchers found that feed manipulation measurably affected circuli spacing in rainbow trout (*Salmo gairdneri*) (Bhatia 1932, Gray and Setna 1930).

Work using trout from Speas Hatchery, Casper, Wyoming (Wyoming Game and Fish Department 1987) indicated feeding rate manipulation worked well to allow discrimination between groups of fish. Therefore, feeding rate and water temperature manipulation were examined in this research to determine if they could be used to mark hatchery fish.

Specific objectives of this study were to:

1. produce detectable marks on fish scales;
2. measure probability of detecting marks; and
3. test retention of marks over time.

METHODS

Experiment 1

During 1987 an experiment was conducted to determine if identifiable "marks" could be produced on trout scales by manipulating temperature and food supply. Ten thousand eyed eggs of Snake River cutthroat trout (*Oncorhynchus clarki*) were obtained from Jackson National Fish Hatchery and reared at Bozeman Fish Technology Center according to recommended hatchery procedures (Piper et al. 1982).

Experimental Groups

When fish reached an average size of 81 mm, they were divided into five experimental groups of 2,000 trout each. All fish were fin clipped and transferred to circular fiberglass tanks 1.8 m in diameter with water volume of 0.7 m³. Over 100 lengths were sampled in each group and tested for pre-treatment differences between mean group length. Feeding rates were calculated using the formula:

$$(\text{hatchery constant} \times \text{weight}) / \text{length} \times 0.01$$

(Piper et al. 1982). Feeding rates were recalculated each week, assuming typical growth rates for given water temperatures.

Experimental groups and identifying fin clips were:

1. control - left pectoral;
2. warm water - adipose;
3. cold water - left pelvic;
4. 1/3 feed - right pelvic; and
5. 2/3 feed - right pectoral.

Control group fish were held at 10°C and fed the full calculated ration. Warm and cold water groups were also fed the full ration but water temperatures were changed at the beginning of the experiment. Water temperature for the warm water group was raised from 10°C to approximately 12°C and held there for 18-d. Temperature was lowered to approximately 8°C for 18-d for the cold water group.

The two diet groups were held at a constant temperature of 10°C throughout the experiment. During the 18-d treatment, the 1/3-feed group received 1/3 of the calculated daily feed ration and the 2/3-feed group received 2/3 of the daily feed ration.

Following 18-d treatments, fish were allowed 1 month to resume normal growth. During this time all fish were reared in one outdoor raceway to assure identical post-treatment feeding and temperature regimes between groups.

Total length and scale samples were obtained from 400 fish in each treatment to ascertain if a "mark" had been formed. Scales were collected using a pocket knife to obtain scrape samples from the preferred area above the

lateral line and below the posterior insertion of the dorsal fin (Knudsen and Davis 1985). Coin envelopes were used to store scales. Fin-clip types were noted for each fish.

Mark Retention

To check for long-term retention of the "mark", two areas, (Cooper Lake and an outdoor raceway), were selected to rear the fish for about 1 year. After this additional growing period, fish were re-sampled to determine if the "mark" remained detectable.

Cooper Lake, on the Blackfeet Indian Reservation in northern Montana, is a productive closed basin lake which provides excellent rearing habitat for trout. One thousand cutthroat trout from each group were stocked into Cooper Lake in August, 1987 and sampled by gillnetting in August, 1988. Total length, weight, scale samples, and fin clip type were recorded for each fish recaptured.

To insure availability of fish in each treatment, 100 trout from each group were reared in an outdoor raceway until March, 1988. Total length, weight, scale samples, and fin clip type were collected at the termination of the rearing period.

Experiment 2

Based on first year results, treatments were modified and the experiment repeated in 1988. Cutthroat trout eggs were again obtained from Jackson National Fish Hatchery and raised at Bozeman Fish Technology Center. All fish were reared in 10°C water up to the beginning of the experiment. Fewer and younger fish were used. Diet and temperature changes were again used to attempt to produce "marks". Treatment variables were fluctuated rather than held constant. Treatments were of longer duration (40 d) and fish were given a longer post-treatment period to grow (Major and Craddock 1962, and Bilton 1974) before scale samples and other data were collected. No fish were kept in 1988 to check "mark" retention.

Diet Treatments

Two thousand trout, average total length 62 mm, were divided into five treatment groups. Fish were counted, 10 at a time, into each trough. Total lengths of 100 fish were measured to test for pre-treatment differences. Trout were acclimated to the new environment for 9-d before treatments were initiated. Troughs were rectangular with dimensions of 122 cm by 20 cm by 36 cm, with volume of 0.088 m³. Water temperature was kept at approximately 10°C throughout the experiment. Rations were calculated as

before.

Experimental groups were:

1. control;
2. 3 day cycle;
3. 5 day cycle;
4. 7 day cycle; and
5. no feed.

Control fish were fed the calculated standard ration each day throughout the experimental "marking" period. The 3-d treatment fish were fed the full calculated feed ration per day for 3 d, then not fed for 3 d. This was repeated throughout the experimental period. Similarly, two other treatment groups were on 5 d and 7 d cycles. The last group was not fed during the "marking" period.

After the "marking" period, all fish were given full feed rations for 58 d to allow additional growth before gathering data. Scale samples were taken and total length, weight, and treatment were collected from each fish as before.

Temperature Treatments

Three experimental temperature groups were tested in 1988. Four hundred fish were counted, 10 at a time, into each of three fiberglass tanks, 1.8 m in diameter with volume of 0.35 m^3 . Pre-treatment lengths were sampled on 100 fish in each group. All groups were fed a full ration

of feed throughout the experiment.

Experimental groups were:

1. cold water;
2. fluctuating water temperature; and
3. warm water.

Water temperature in the cold water group was reduced to approximately 8°C for 42 d. Temperature in the fluctuating group was raised to approximately 12°C for 1 week and dropped to approximately 8°C for the next week. This was repeated three times during the 6-week "marking" period. The warm water group was reared at approximately 12°C throughout the 6-week period. Feeding rates were adjusted weekly as described before.

At the end of the "marking" period, all temperatures were returned to 10°C. Fish were reared an additional 48 d before data were collected. Total length, weight, and treatment were recorded and scale samples taken.

Processing and Analysis

Scale Preparation

To improve readability, 100 scale samples from each treatment for both years were cleaned of mucous and other foreign substances. Scale samples were transferred to 2 dram vials filled with a mild digestive solution (Ihne 1970, Bowman 1978) and held for approximately 48 h. Vials

were agitated periodically to help break scale masses apart and aid in digestion of protein substances adhering to scale surfaces. The digestive solution was then replaced with distilled water until scales could be mounted between two glass slides held together with strapping tape (Major and Craddock 1962).

Data Acquisition

A BioSonics' Optical Pattern Recognition System (OPRS) was used to measure and record scale data. This package consists of a personal computer, second monitor, digitizing pad, frame grabber board, video camera, and microscope (Figure 1) packaged with software developed to efficiently process information from scale optical patterns (BioSonics Inc. 1985). A statistical package, along with statistical procedures specifically developed for scale analysis applications, is also included.

Use of the OPRS. Scale images magnified through a microscope are conveyed via the video camera to the frame grabber board where they are digitized. From here the digitized image is pipelined to computer RAM and re-displayed on the second monitor. This allows the operator virtually instantaneous images when moving from scale to scale.

When a readable scale is found, the image is frozen and several enhancement and data extraction routines can be

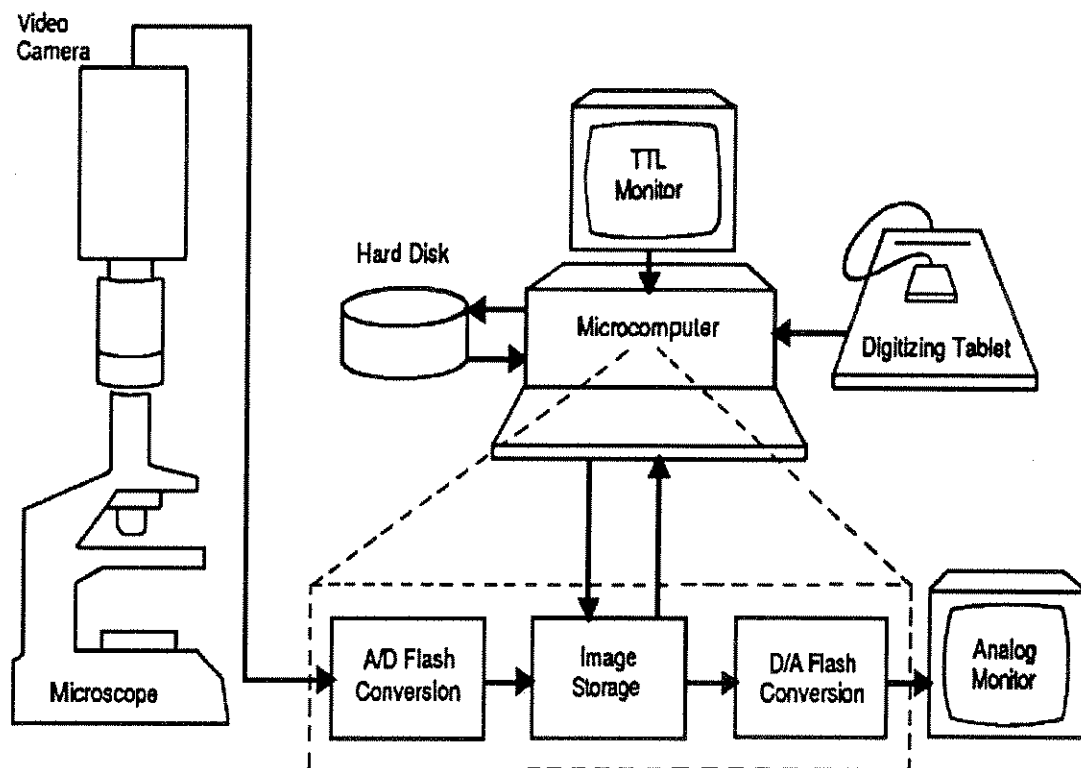


Figure 1. The Optical Pattern Recognition System including a personal computer, second monitor, digitizing pad, frame grabber board, video camera, and microscope (adapted from BioSonics, Inc. 1986).

run at the user's discretion. Image enhancement routines can be used to improve quality of the image, accentuate differences in scale features, and standardize light transmission through the scale. Data extraction routines convert selected scale features to variables stored in computer data files for later analysis.

Data extraction routines used for this project were radial distance measurements and luminance profiles. Radial distance measurements were attempted on 300 scale samples from each 1987 treatment and 200 scale samples from each 1988 treatment. Luminance profiles were attempted on 100 scale samples from each group both years. Occasionally scale samples were not valid either because no scales remained after the cleaning process or all scales present were unreadable.

Radial Distance Measurements. To obtain radial distance measurements a line was drawn from the center of the focus to the scale margin along the longest axis of the anterior portion of the scale. Software automatically marked each circulus along this axis. At times the program either missed a circulus or mistook extraneous material to be a circulus. This was corrected by adding or deleting marks. Data recorded to file included length, weight, condition factor, distances between each circulus, average spacing for each triplet of circuli (i.e., average spacing

between focus and 3rd circuli, 1st and 4th circuli, etc.), scale radius divided by fish length, scale radius divided by weight when available, scale radius, and number of circuli crossing the first 1/4 mm and 1/2 mm of the line drawn.

Luminance Profiles. Luminance profiles were more complicated. The software/hardware routine measured and recorded the amount of light transmitted through the scale. Hence, it was imperative that scales be free of mucous and extraneous material. Again, a line was drawn from focus to scale margin along the longest axis of the anterior portion of the scale. Light intensities along this line were automatically standardized and recorded to a data file as the luminance profile. Profiles were transformed using a Fourier series (BioSonics 1985) prior to analysis. Variables used for data analysis were the first 20 harmonics generated by the data transformation (Ross and Pickard 1988).

Data Analysis

Control group scales were compared to scales of all other groups. In some instances scales from non-control treatments were compared with scales from other non-control

treatments to determine if between treatment differentiation was possible.

OPRS software was modified to record inter-circuli spacing for the first 20 circuli, if they existed, as variables 1 through 20 (Appendix A, Table 13). Triplet distances were recorded as variables 21 through 38. Average inter-circuli spacing for the focus through the 5th circuli, the focus through the 7th circuli, and the 6th through the 10th circuli were recorded as variables 39, 40, and 41, respectively. Variables 42, 43, and 44 contained scale radius divided by total length, scale radius divided by weight, and scale radius. Variables 45 and 46 held the number of circuli in the first 1/4 mm and 1/2 mm of the axis line being measured.

All statistical analyses were done using STATGRAPHICS (STSC Inc. 1989). Analysis of variance was run for each variable generated. Low p-values from analysis of variance were used as indications of variables most useful in discriminating between groups.

If analysis of variance indicated between-group differences existed, linear discriminant models, using several combinations of variables, were built and tested. Generally, half of the measured scales were used as "standards" for building and testing models. One "best" model was chosen for each experiment based on a combination of highest Eigen values, success of classification arrays

at correctly identifying "unknowns", and similarities between groups. Correction factors were developed for each model (Cook and Lord 1978) by measuring the number of scales misclassified in the original training samples and adjusting final predictions accordingly.

Models were further tested with the remaining measured scales pertinent to the particular model. In each case, the model was used to predict group origin for varying percentages of scales from each group (Table 1). Percentages of correct predictions, with and without the correction factor, were recorded and averaged to get an overall rating for each treatment.

Growth measurements, (total length, weight and condition factor), were also compared between treatments. Analysis of variance and t-tests were used to compare distributions and means, respectively.

Table 1. Percentage of each group used as "unknowns" to test model performance.

Model	Group A	Group B	Group C
2 groups	50	50	-
	0	100	-
	100	0	-
	67	33	-
	33	67	-
3 groups	33	33	33
	100	0	0
	0	100	0
	0	0	100
	50	25	25
	25	50	25
	25	25	50

RESULTS

Experiment 1

Pre-treatment lengths of cutthroat trout ranged from 79.3 mm to 81.9 mm (Table 2). Control fish were slightly smaller than cold water fish, 1/3 feed fish, and 2/3 feed fish ($p < 0.05$). No significant difference existed between median length of control and warm water fish (Figure 2).

Table 2. Mean pre-treatment lengths of cutthroat trout used in Experiment 1, with t-test p-values comparing each group to control fish.

Group	N	Mean length	P-value	Std. dev.
Control	132	79.3 mm	-	7.68
Warm water	119	80.8 mm	0.1679	8.71
Cold water	172	81.1 mm	0.0470	7.65
1/3 feed	131	81.9 mm	0.0056	7.43
2/3 feed	188	81.3 mm	0.0158	6.95

Post-treatment Comparisons

Radial Distance Analysis. Radial distance analysis yielded poor results (Appendix B, Figure 18). Comparisons of circuli spacing between control and each test group (ANOVA) revealed no consistent differences which could be interpreted as obvious "marks". Discriminant analysis, using combinations of variables most different between groups, was applied to search for more subtle group

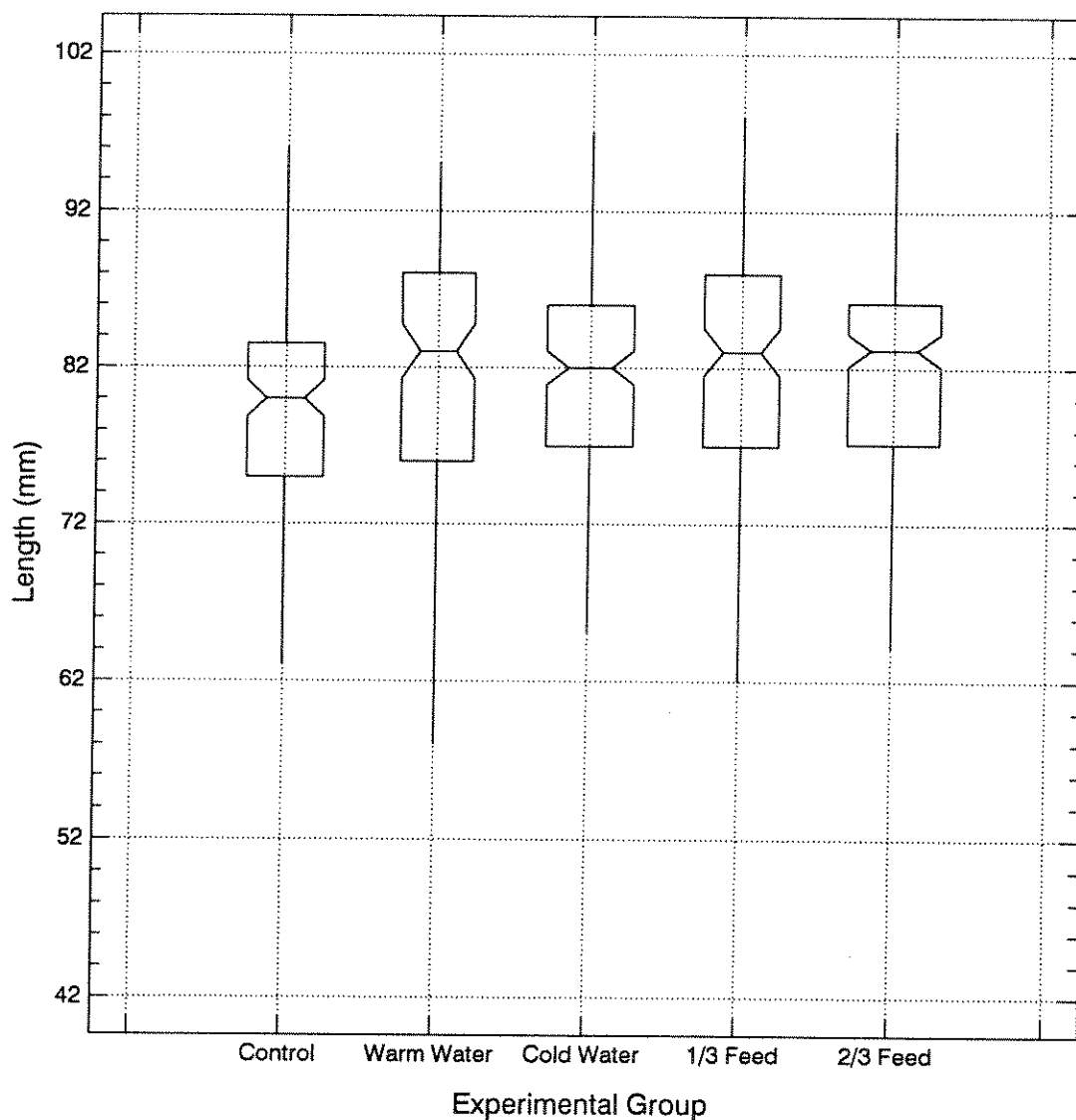


Figure 2. Notched box and whisker plots for pre-treatment total lengths for each experimental group, Experiment 1. The central box covers the middle 50% of the data values, with "whiskers" extending out to the minimum and maximum values. The central line represents the median. A notch is added to each box corresponding to the width of a 95% confidence interval for the median and the width of the box is proportional to the square root of the number of observations in each data set (STSC, Inc. 1988).

differences. This also met with poor success. Accurate discrimination between any groups was not possible.

Luminance Profile Analysis. Using the same group comparisons as with radial distance data (Appendix C, Figure 24), analysis again identified insufficient differences between groups and no discriminant analysis statistics were done. Thus, it was not possible to identify anything which could be termed a "mark".

Analysis with Selected Data Set. Because the wide variation in growth rates shown by the experimental fish could have obscured results, I attempted to test the impact of a narrower growth range. Fastest and slowest growing fish, as judged by number of circuli present on scales, were eliminated from the data set. Analysis was done as before using radial distance measurements. Again, no consistent differences between groups were found which could be identified as "marks" (Appendix B, Figure 19).

Hatchery and Cooper Lake Trout

The possibility existed that "marks" had been formed at scale margins and went undetected in early (48-d post-treatment) analyses. Thus, further examination was

done on scales from trout allowed a longer post-treatment growing period. Radial distance data (Appendix B, Figures 20 and 21) and luminance profile harmonics (Appendix C, Figures 25 and 26) from hatchery-reared and Cooper Lake trout scales again showed no meaningful differences between groups. Discriminant analysis was unsuccessful at identifying marks.

It was not possible to check mark retention since no "marks" had been identified. However, it was possible to test the assumption that scale circuli spacing does not change once formed. Comparison of original scale samples with scale samples from fish in the same groups raised in the hatchery and Cooper Lake showed no significant changes had occurred over time (Appendix E, Tables 21 and 22).

Experiment 2: Diet Tests

Mean pre-treatment lengths of test fish ranged from 61.9 mm to 63.0 mm (Figure 3). No significant differences between groups were found (Table 3).

Radial Distance Analysis

Variable Selection. Comparisons of each variable between groups revealed great enough differences to allow possible group discrimination (Appendix B, Figure 22).

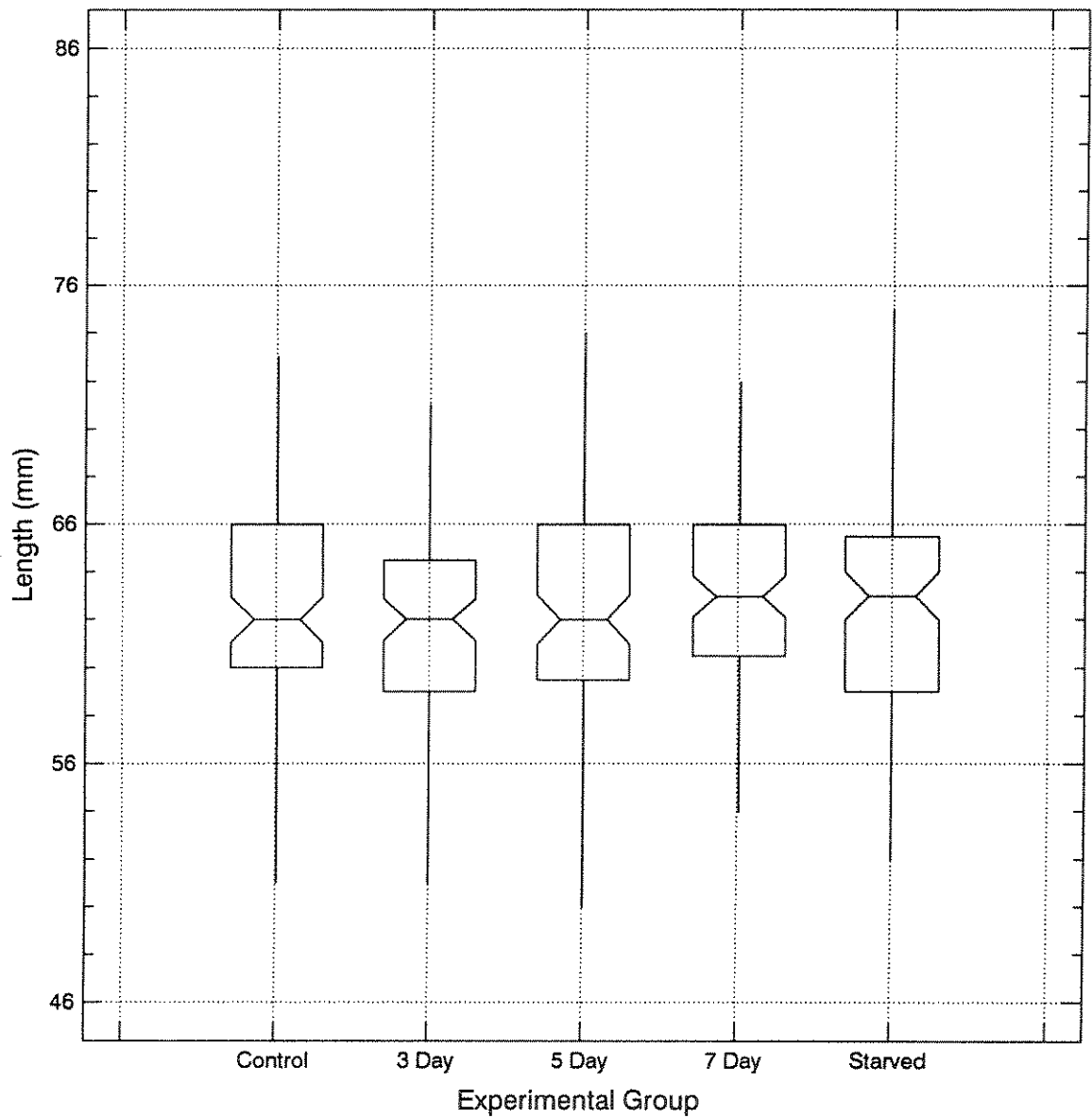


Figure 3. Notched box and whisker plots representing median, 95% confidence intervals, middle 50%, of and range for pre-treatment total lengths for each experimental group, Experiment 2 feed groups.

Table 3. Mean pre-treatment total lengths of cutthroat trout used in 1988 feed experiments, with t-test p-values comparing each group to control fish.

Group	N	Mean length	P-value	Std. dev.
Control	100	62.4 mm	-	4.59
3 day	100	61.9 mm	0.4463	4.68
5 day	100	62.4 mm	0.9778	5.51
7 day	100	63.0 mm	0.3634	4.41
Starved	100	62.7 mm	0.6163	4.71

Variables further tested for inclusion in the model were: intercirculi spacing just prior to the 2nd, 3rd, 4th, 5th, 6th, and 7th circuli; average spacing between the 2nd and 5th, 3rd and 6th, 4th and 7th, and 5th and 8th circuli; and the number of circuli counted along the first 1/4 mm of the scale axis (variables 2, 3, 4, 5, 6, 7, 23, 24, 25, 26, and 45; Appendix A, Table 13). Several combinations of these variables were examined. Eigen values, success of classification arrays, and similarities between data sets were used as an indication of model fitness (Appendix D, Tables 14, 15, 16, and 17). Variables 3, 4, and 5 (Figures 4, 5, and 6) generally yielded best results and were used for all models (Table 4). Roughly one half of the trout were used as "standards" to build these models.

Remaining trout were used as "unknowns" for model testing. Models were asked to predict group origin for scales entered as "unknowns". All 2-group models produced excellent results. Two 3-group models gave good results

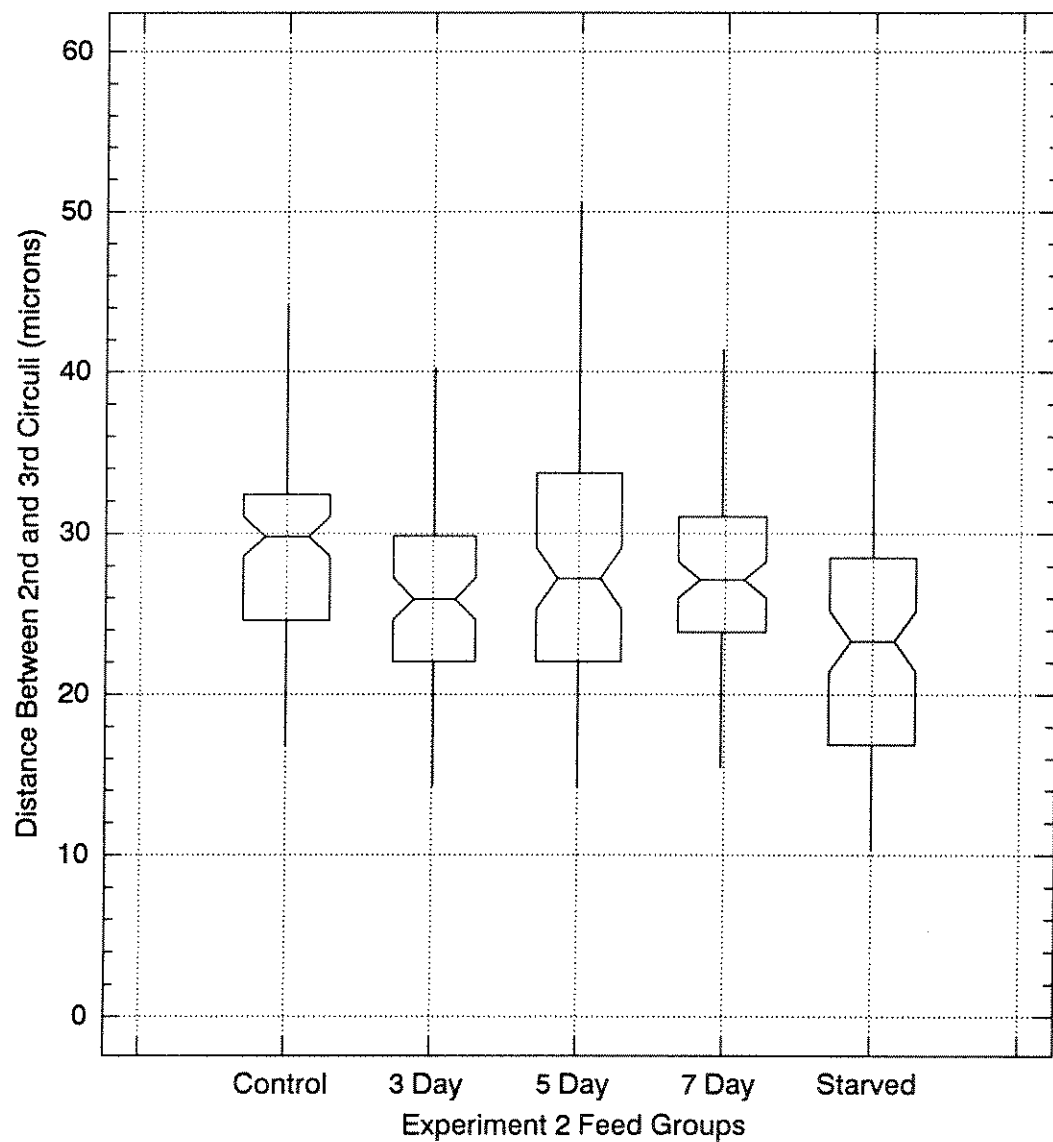


Figure 4. Notched box and whisker plots representing median, 95% confidence intervals, middle 50%, and range of distance between 2nd and 3rd circuli, for each experimental group, Experiment 2 feed groups (variable 3).

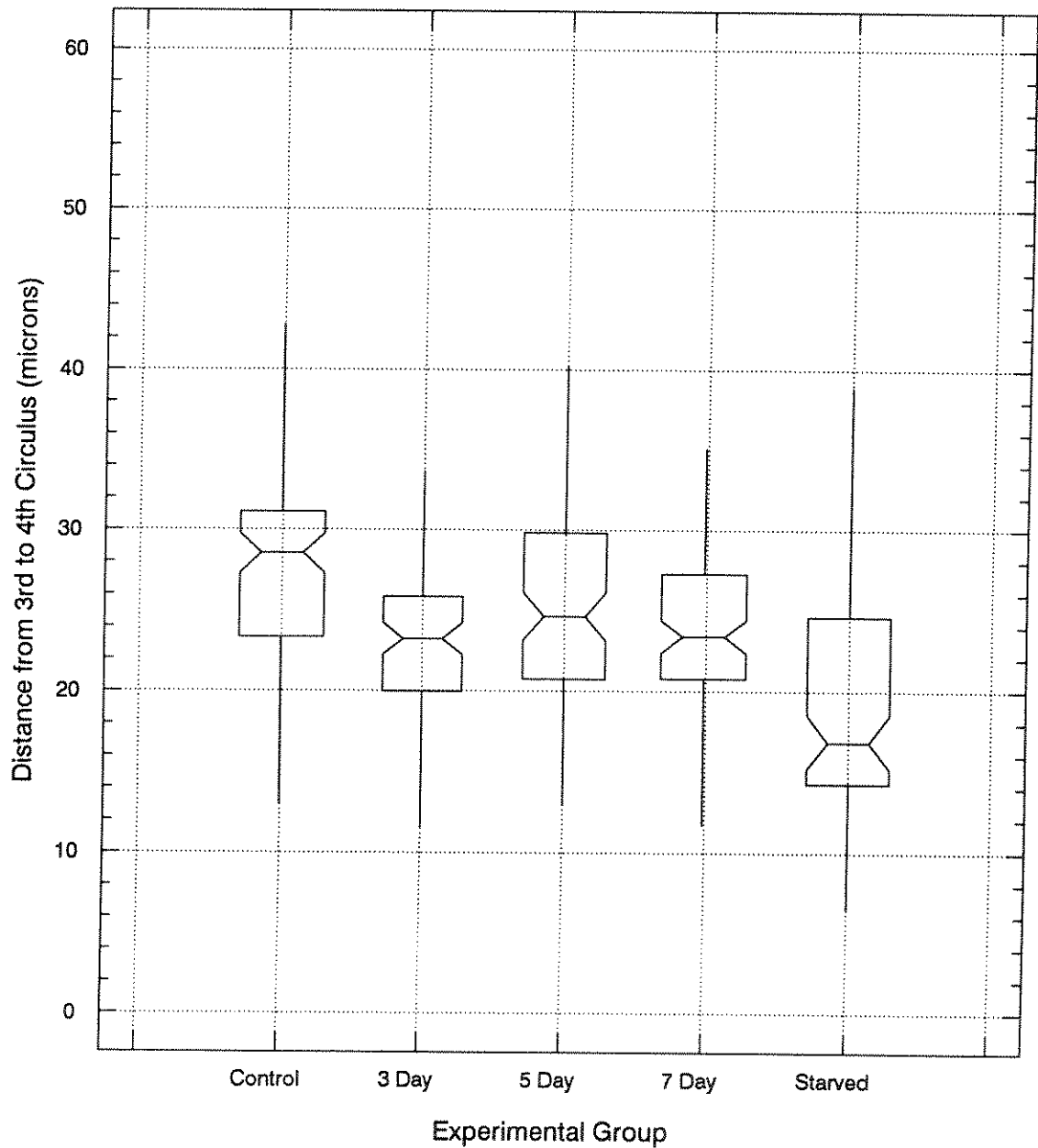


Figure 5. Notched box and whisker plots representing median, 95% confidence intervals, middle 50%, and range of distance between 3rd and 4th circuli, for each experimental group, Experiment 2 feed groups (variable 4).

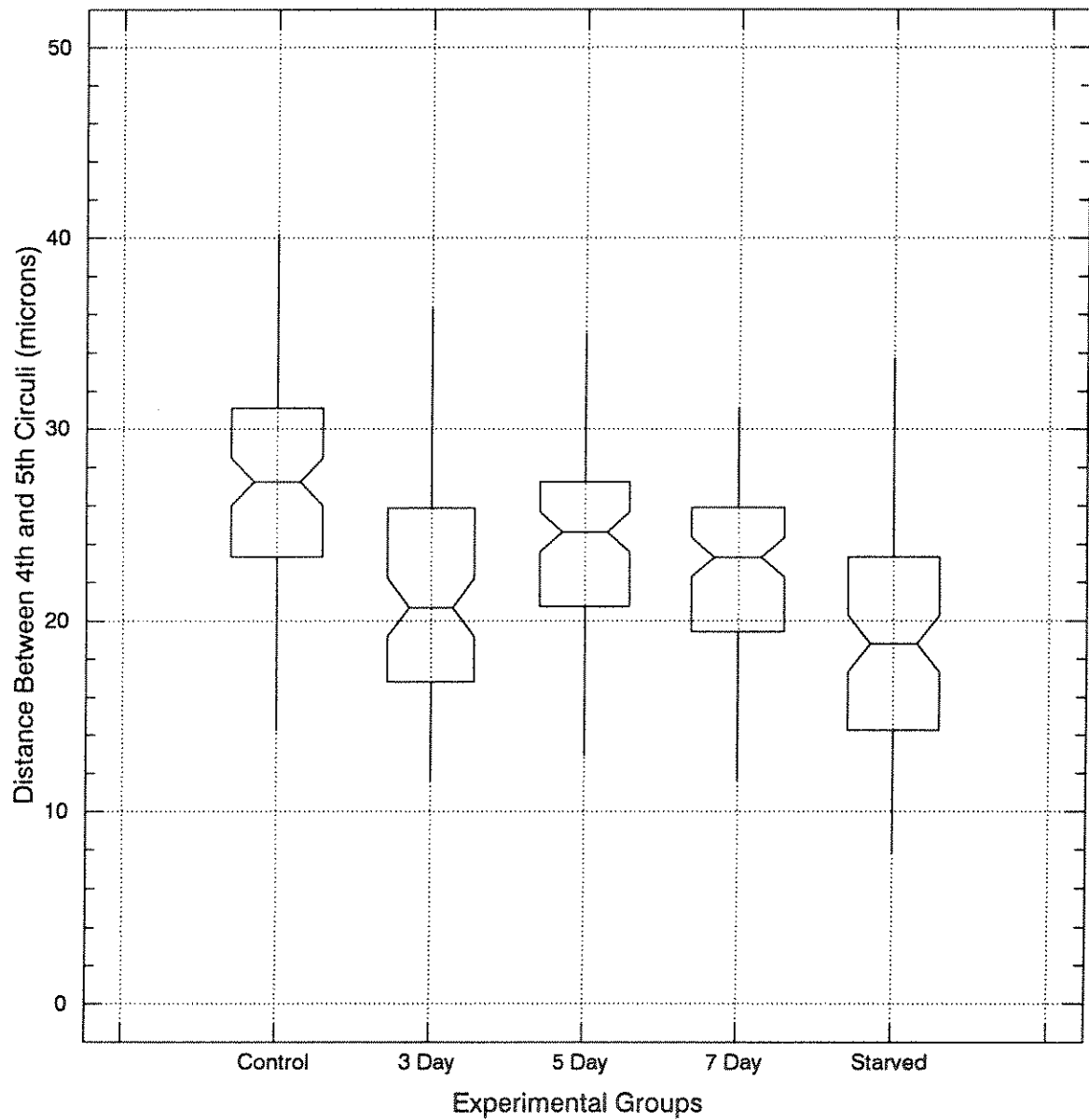


Figure 6. Notched box and whisker plots representing median, 95% confidence intervals, middle 50%, and range of distance between 4th and 5th circuli, for each experimental group, Experiment 2 feed groups (variable 5).

Table 4. Discriminant analysis models, with associated Eigen values and classification arrays for 1988 feed experiments.

Model	Eigen values	Classification array (percent)	
		Actual	Predicted
3-d versus control	0.3212		<u>3-d</u> <u>control</u>
		3-d	78.4 21.6
		control	25.5 74.5
5-d versus control	0.0784		<u>5-d</u> <u>control</u>
		5-d	64.2 35.8
		control	33.7 66.3
7-d versus control	0.2078		<u>7-d</u> <u>control</u>
		7-d	72.0 28.0
		control	31.6 68.4
Starved versus control	0.7291		<u>starved</u> <u>control</u>
		starved	78.7 21.3
		control	17.4 82.7

when constructed, but did poorly when further tested.

Two Group Models. Starved fish and fish on 3-d cycles were most easily distinguished from control fish (Table 5). Before error classification arrays were applied, these models correctly classified an average of 88.2 and 88.4% of the "unknowns", respectively. Correct classification rates increased to 96.2 and 98.4% for starved and 3-d cycle models, respectively, when correction factors (Cook and Lord 1978) were applied (Table 4). These increases approached significance ($p=0.1271$ and $p=0.0511$, respectively).

The 5-d versus control and 7-d versus control models also behaved satisfactorily. Correct classification

Table 5. Results of 1988 2-group feed model testing, using known cutthroat trout as "unknowns", with and without error classification correction factors. Numbers in parentheses represent 90 percent confidence intervals for the estimate.

	Natural			With error classification arrays		
Actual number	Predicted number in group		Percent correct	Predicted number in group		Percent correct
3-d versus control (con) model:						
<u>3-d</u> <u>con</u>	<u>3-d</u> <u>con</u>			<u>3-d</u>	<u>con</u>	
82 81	86 77	98		81 (54-107)	82 (56-109)	99
82 0	66 16	82		82 (66- 82)	0 (0- 16)	100
0 81	20 61	75		0 (0- 17)	81 (64- 81)	100
82 42	75 49	94		80 (58-102)	44 (22- 66)	98
47 81	56 72	93		41 (18- 64)	87 (64-110)	95
5-d versus control (con) model:						
<u>5-d</u> <u>con</u>	<u>5-d</u> <u>con</u>			<u>5-d</u>	<u>con</u>	
99 81	97 83	99		146 (68-180)	34 (0-112)	74
99 0	65 34	65		99 (46- 99)	0 (0- 53)	100
0 81	32 49	60		11 (0- 54)	70 (27- 81)	86
99 42	81 60	87		138 (67-141)	3 (0- 74)	72
50 81	65 66	89		80 (22-131)	51 (0-109)	77
7-d versus control (con) model:						
<u>7-d</u> <u>con</u>	<u>7-d</u> <u>con</u>			<u>7-d</u>	<u>con</u>	
88 81	80 89	95		64 (24-103)	105 (66-145)	86
88 0	55 33	63		70 (44- 88)	18 (0- 44)	79
0 81	25 56	69		0 (0- 26)	81 (55- 81)	100
88 42	67 63	84		64 (32- 96)	66 (34- 98)	82
47 81	53 75	95		38 (0- 61)	101 (67-128)	84
Starved (stv) versus control (con) model:						
<u>stv</u> <u>con</u>	<u>stv</u> <u>con</u>			<u>stv</u>	<u>con</u>	
66 81	64 83	99		64 (44- 84)	83 (63-103)	99
66 0	49 17	74		62 (50- 66)	4 (0- 16)	94
0 81	15 66	82		2 (0- 14)	79 (67- 81)	98
66 42	55 53	90		60 (44- 77)	48 (31- 64)	94
32 81	36 77	96		27 (11- 44)	86 (69-102)	96

occurred for an average of 80.0 and 81.2% of the "unknowns" using these models. Although correction factors improved this slightly to 81.8 and 86.2%, these increases were not

significant ($p=0.8476$ and $p=0.5253$).

Trout used as "unknowns" were switched with those used as "standards" and the discriminant analysis was repeated with similar results (Table 6). Correct classification rates for the starved, 3-d, 5-d, and 7-d models were 89.0, 85.2, 79.6. and 84.0%, respectively. After correction factors were applied, respective classification rates increased to 96.6, 92.8, 80.0, and 96.2% correct. Although correction factors improved prediction averages, these improvements were not statistically significant in the 3-d and 5-d models ($p=0.9666$ and $p=0.2248$). Only slightly significant ($p= 0.0706$ and $p=0.0902$) improvement was seen in the 7-d and starved models.

Three Group Models. Several discriminant models were built using three treatment groups to determine if separation between treatments, as well as between treatment and control, would be possible. Controls and all possible combinations of two more treatments were utilized in model building. Results indicated only two of these models, control versus 3-d versus starved and control versus 7-d versus starved, warranted further testing (Table 7). These models were again based on intercirculi spacing prior to the 3rd, 4th, and 5th circuli (variables 4, 5, and 6).

Best separation occurred using the control versus 7-d versus starved model. Correct classification occurred 75.4% of the time (Table 8). This improved to 86.1% correct when

Table 6. Results of 1988 2-group feed model testing, switching "standards" with "unknowns", with and without error classification correction factors. Numbers in parentheses represent 90 percent confidence intervals for the estimate.

Actual number	Natural		With error classification arrays	
	Predicted number in group	Percent correct	Predicted number in group	Percent correct
3-d versus control (con) model:				
<u>3-d</u> <u>con</u>	<u>3-d</u> <u>con</u>		<u>3-d</u> <u>con</u>	
98 88	80 106	96	75 (48-102) 111 (84-138)	93
88 0	60 28	88	76 (59- 88) 12 (0- 29)	86
0 98	20 78	80	0 (0- 18) 98 (80- 98)	100
88 48	69 67	86	74 (52- 96) 62 (40- 84)	90
43 98	49 92	96	36 (13- 58) 105 (83-128)	95
5-d versus control (con) model:				
<u>5-d</u> <u>con</u>	<u>5-d</u> <u>con</u>		<u>5-d</u> <u>con</u>	
95 98	89 104	97	45 (0-123) 148 (70-193)	74
95 0	55 40	58	71 (28- 95) 24 (0- 67)	75
0 98	34 64	65	0 (0- 51) 98 (47- 98)	100
95 48	72 71	84	60 (2-118) 83 (25-140)	76
58 98	68 88	94	19 (0- 89) 137 (67-156)	75
7-d versus control (con) model:				
<u>7-d</u> <u>con</u>	<u>7-d</u> <u>con</u>		<u>7-d</u> <u>con</u>	
100 98	104 94	98	114 (57-172) 84 (26-141)	93
100 0	74 26	74	100 (62-100) 0 (0- 38)	100
0 98	30 68	69	0 (0- 39) 98 (59- 98)	100
100 48	87 61	91	117 (69-148) 31 (0- 79)	89
50 98	68 80	88	52 (4- 99) 96 (49-144)	99
Starved (stv) versus control (con) model:				
<u>stv</u> <u>con</u>	<u>stv</u> <u>con</u>		<u>stv</u> <u>con</u>	
94 98	97 95	98	103 (75-130) 89 (62-116)	95
94 0	76 18	81	94 (76- 94) 0 (0- 18)	100
0 98	21 77	79	3 (0- 20) 95 (78- 98)	97
94 48	87 55	95	103 (80-125) 39 (17- 62)	94
48 98	59 87	92	52 (31- 75) 94 (73-117)	97

Table 7. Discriminant analysis models, using 3 groups with associated Eigen values and classification arrays for 1988 feed experiments.

Model	Eigen values	Classification array (percent)			
		Actual	Predicted		
3-d versus starved versus control	0.5483		<u>3-d</u>	<u>starved</u>	<u>control</u>
		3-d	42.1	36.4	21.6
		starved	16.0	70.2	13.8
		control	15.3	9.2	75.5
7-d versus starved versus control	0.5357		<u>7-d</u>	<u>starved</u>	<u>control</u>
		7-d	74.5	11.7	13.8
		starved	22.0	51.0	27.0
		control	8.2	22.5	69.4

correction factors were included (Table 8). The increase in accuracy was not statistically significant ($p=0.2139$).

The second three-diet group model, control versus 3-d versus starved, performed very similarly. Pre-correction factor results were 72.9% accurate while post-correction factor results were 83.1% accurate (Table 8). Improvement was not statistically significant ($p=0.2992$).

Reversing "unknowns" with "standards" and rerunning the analysis yielded somewhat similar results (Table 9). Best between-group separation again occurred using the control versus 7-d versus starved model. Classification of the "unknowns" was 77.6% correct, improving, nonsignificantly ($p=0.4455$), to 84.4% with error correction factors.

Control versus 3-d versus starved model correctly classified 86.8% of the "unknowns". Error classification

Table 8. Results of 1988 3-group feed model testing, using known cutthroat trout as "unknowns", with and without error classification correction factors. Numbers in parentheses represent 90 percent confidence intervals about the estimate.

Natural				With error classification arrays					
Actual number		Number in predicted group		Percent correct		Number in predicted group		Percent correct	
3-d versus starved (stv) versus control (con) model:									
3-d	stv	con	3-d	stv	con	3-d	stv	con	stv
82	66	81	54	87	88	50 (0-132)	91 (26-150)	88 (50-132)	84
82	0	0	26	41	15	49 (0- 88)	33 (0- 78)	0 (0- 21)	60
0	66	0	13	40	13	4 (0- 42)	56 (24- 66)	6 (0- 22)	84
0	0	81	15	6	60	0 (0- 46)	0 (0- 27)	81 (56- 81)	100
82	32	42	38	62	56	40 (0-103)	62 (13-111)	54 (23- 86)	73
36	66	42	37	63	55	36 (0- 99)	65 (17-114)	53 (22- 84)	92
36	32	81	37	44	79	30 (0- 96)	35 (0- 81)	95 (59-130)	89
7-d versus starved (stv) versus control (con) model:									
7-d	stv	con	7-d	stv	con	7-d	stv	con	stv
88	66	81	66	69	100	77 (0-159)	55 (15- 96)	103 (43-163)	91
88	0	0	35	19	34	68 (17- 88)	0 (0- 25)	20 (0- 55)	77
0	66	0	14	42	10	17 (0- 49)	49 (28- 66)	0 (0- 20)	74
0	0	81	17	8	56	0 (0- 47)	0 (0- 18)	81 (45- 81)	100
88	32	42	52	40	70	75 (7-144)	20 (0- 53)	66 (17-115)	85
47	66	42	42	53	60	50 (0-109)	48 (17- 80)	57 (14-100)	88
47	32	81	44	134	82	38 (0-106)	22 (0- 51)	100 (50-151)	88

Table 9. Results of 1988 3-group feed model testing, switching "unknown" trout with "standards" for model building, with and without error classification correction factors. Numbers in parentheses represent 90 percent confidence intervals about the estimate.

Natural				With error classification arrays			
Actual number		Number in predicted group		Percent correct	Number in predicted group		Percent correct
3-d versus starved (stv) control (con) model:							
3-d	stv	con	3-d	stv	con	stv	con
88	94	83	87	75	103	0	0
88	0	0	38	26	24	0	0
0	94	0	42	36	16	0	0
0	0	98	10	14	74	41	57
88	48	50	62	54	70	0	0
43	94	50	65	57	65	0	0
43	48	98	45	49	95	0	67
7-d versus starved (stv) control (con) model:							
7-d	stv	con	7-d	stv	con	stv	con
50	48	48	36	59	51	77	69
100	0	0	44	32	24	0	0
0	94	0	12	72	10	94	0
0	0	98	17	14	67	0	98
100	48	48	59	73	64	83	58
50	94	48	40	95	57	123	69
50	48	98	45	67	84	73	71
7-d versus starved (stv) control (con) model:							
7-d	stv	con	7-d	stv	con	stv	con
0	0	0	0	0	0	0	0
100	0	0	100	0	100	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
100	48	48	55	0	55	83	58
50	94	48	0	0	0	123	69
50	48	98	0	0	0	73	71

arrays decreased this, nonsignificantly ($p=0.3722$), to 59.8 % correct.

Luminance Profile Analysis

Transformed luminance profile data were not useful for group separation (Appendix C, Figure 27). Discriminant analysis results were poor and no further testing was done.

Growth Comparisons

Significant differences ($p<0.0001$) between mean lengths were found (Figure 7). Control fish averaged the longest (116.4 mm) and starved fish averaged the shortest (93.2 mm) at the conclusion of the experiment. Mean fish length of the 5-d test group, 109.5 mm, was slightly larger than the 3-d or 7-d test fish. There were no significant differences ($p=0.8095$) between mean lengths of the 3-d test group (107.2 mm) and the 7-d test group (107.4 mm).

Differences in mean weights were similar to lengths. Significant differences ($p<0.0001$) occurred between mean weights of all groups (Figure 8). Again, control fish were largest, averaging 16.9 g, and starved fish were smallest, averaging 9.0 g. Five day treatment fish were slightly larger (14.2 g) than either 3-d fish (13.0 g) or 7-d fish (13.1 g). No differences were found between 3-d and 7-d

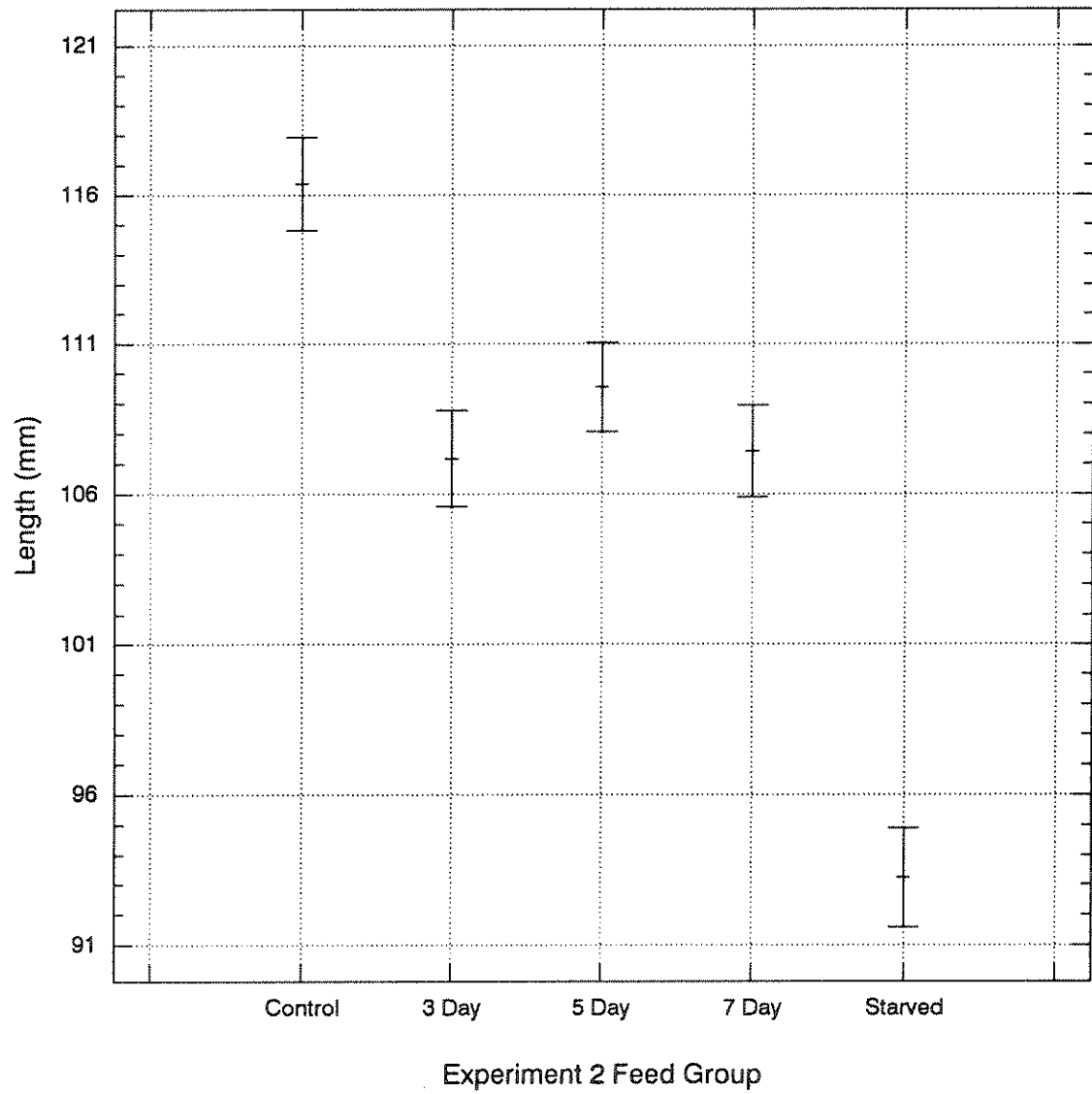


Figure 7. Mean total length, with 95% confidence intervals, for each experimental group, Experiment 2 feed groups.

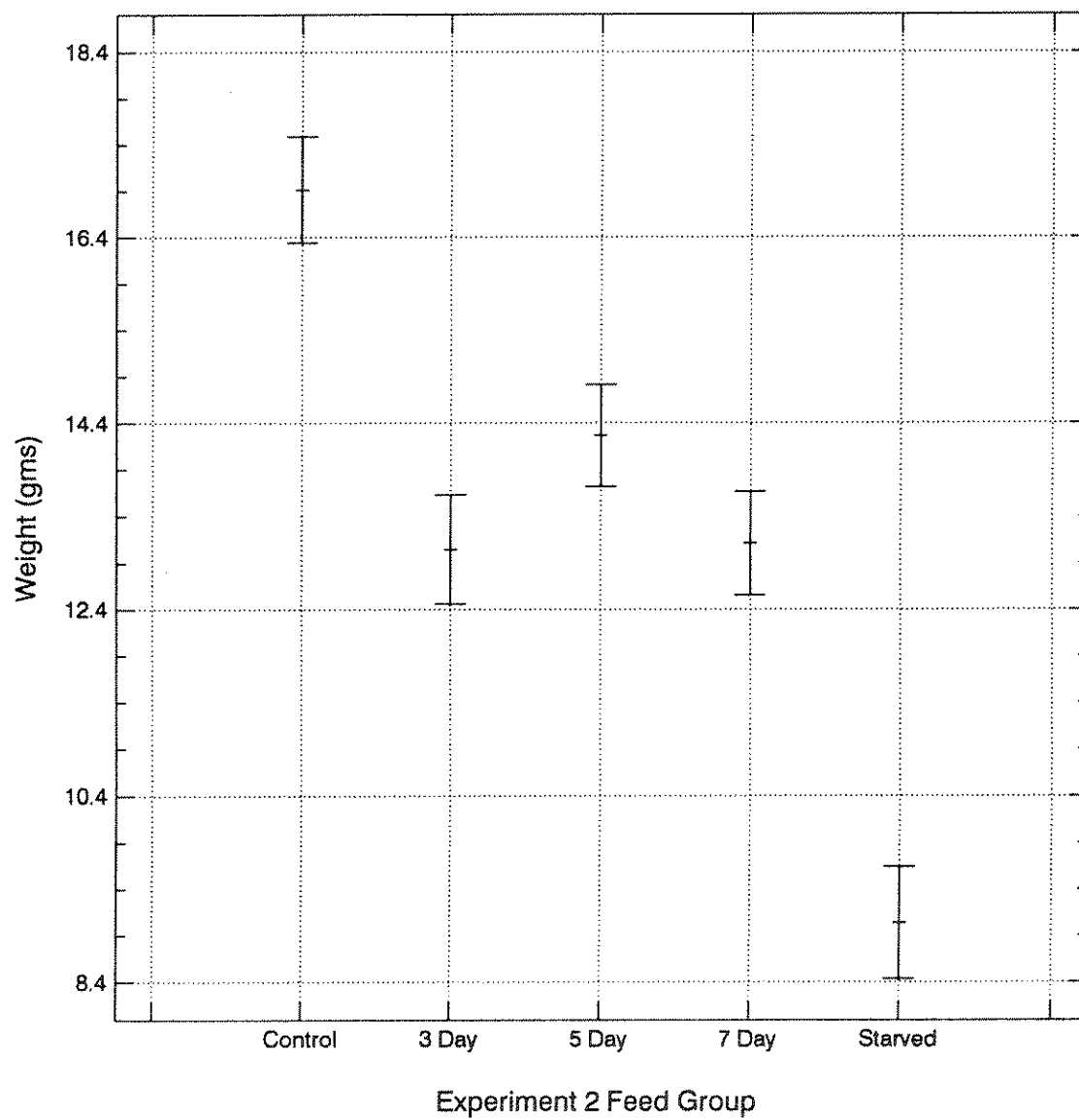


Figure 8. Mean weight, with 95% confidence intervals, for each experimental group, Experiment 2 feed groups.

mean weights ($p=0.8678$).

Number of circuli per scale was also significantly different ($p<0.0001$) between groups (Figure 9). A steady decrease in average number of circuli present occurred as the length of the feeding cycle increased. Control, 3-d, 5-d, 7-d, and starved fish had mean number circuli of 9.5, 9.3, 9.0, 8.7, and 7.3, respectively.

Experiment 2: Temperature Tests

Mean pre-treatment total length of experimental trout ranged from 62.2 mm to 64.5 mm. Lengths were significantly different between treatment groups ($p=0.0063$). Cold water fish began the experiment slightly smaller than either fluctuating or warm water fish (Figure 10).

Temperature Regimes

Water temperatures were held fairly close to intended values (Figure 11). Cold water treatment temperatures remained constant at approximately 7.8°C . Water temperature in the warm water treatment dropped to 7.8°C briefly on June 12, peaked on June 29 at 13.2°C , and averaged 11.1°C with minor fluctuations (Figure 11). Temperatures alternated from 7.4°C to 13.0°C on a weekly cycle in the fluctuating temperature treatment group (Figure 11).

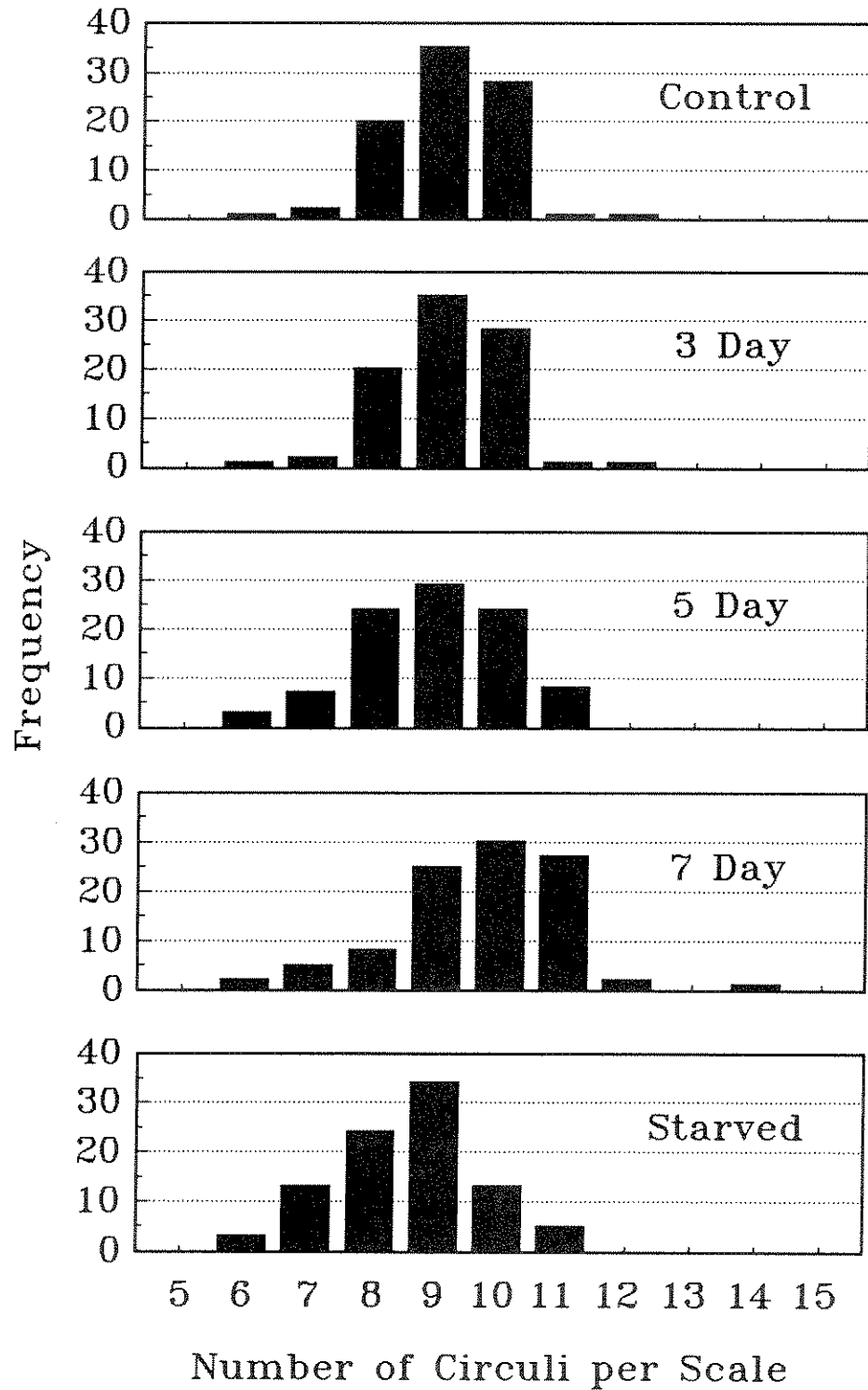


Figure 9. Distribution of number of circuli per scale for each experimental group, Experiment 2 feed groups.

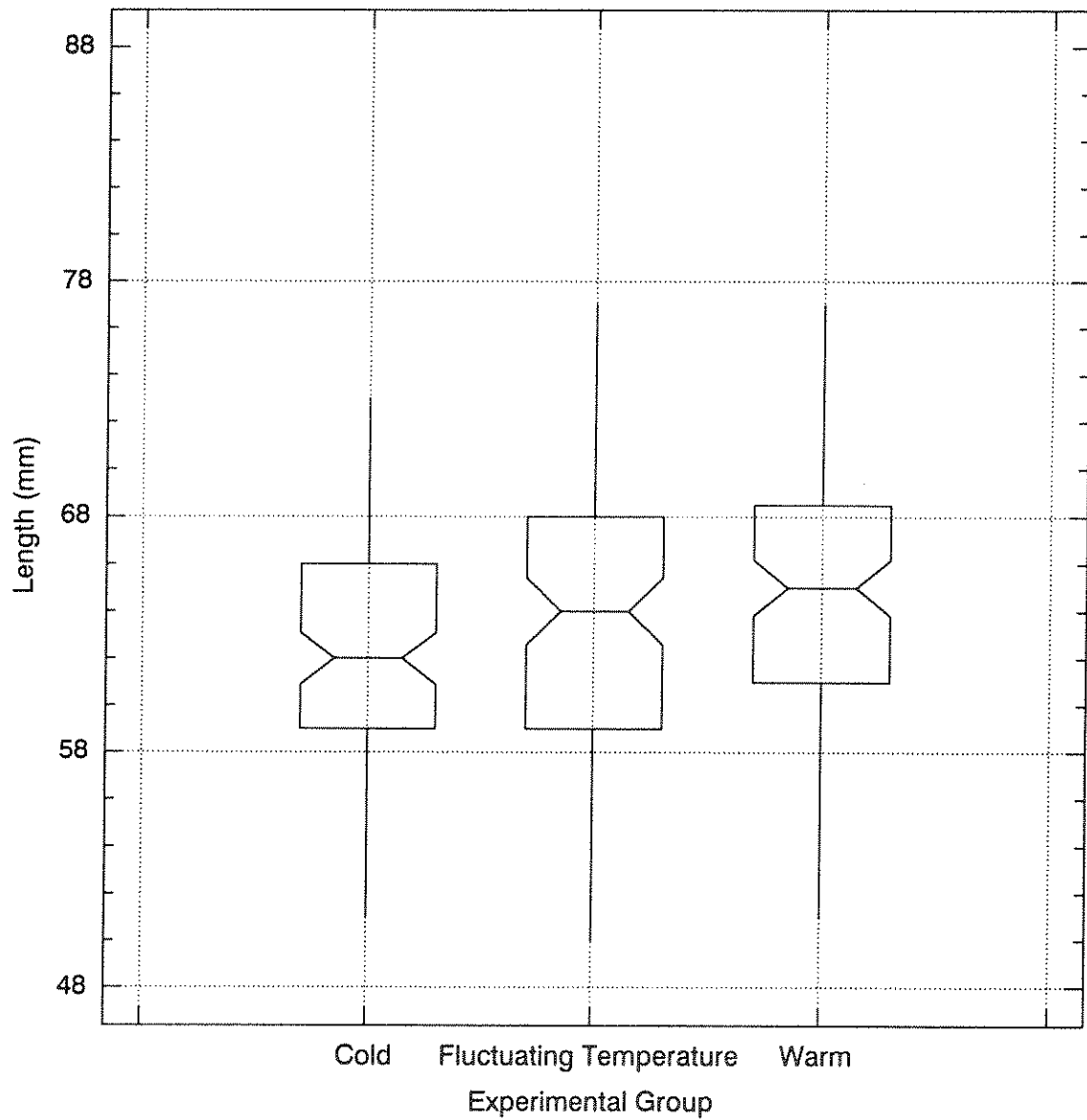


Figure 10. Notched box and whisker plots representing median, 95% confidence intervals, middle 50%, of and range for pre-treatment total lengths for each experimental group, Experiment 2 temperature groups.

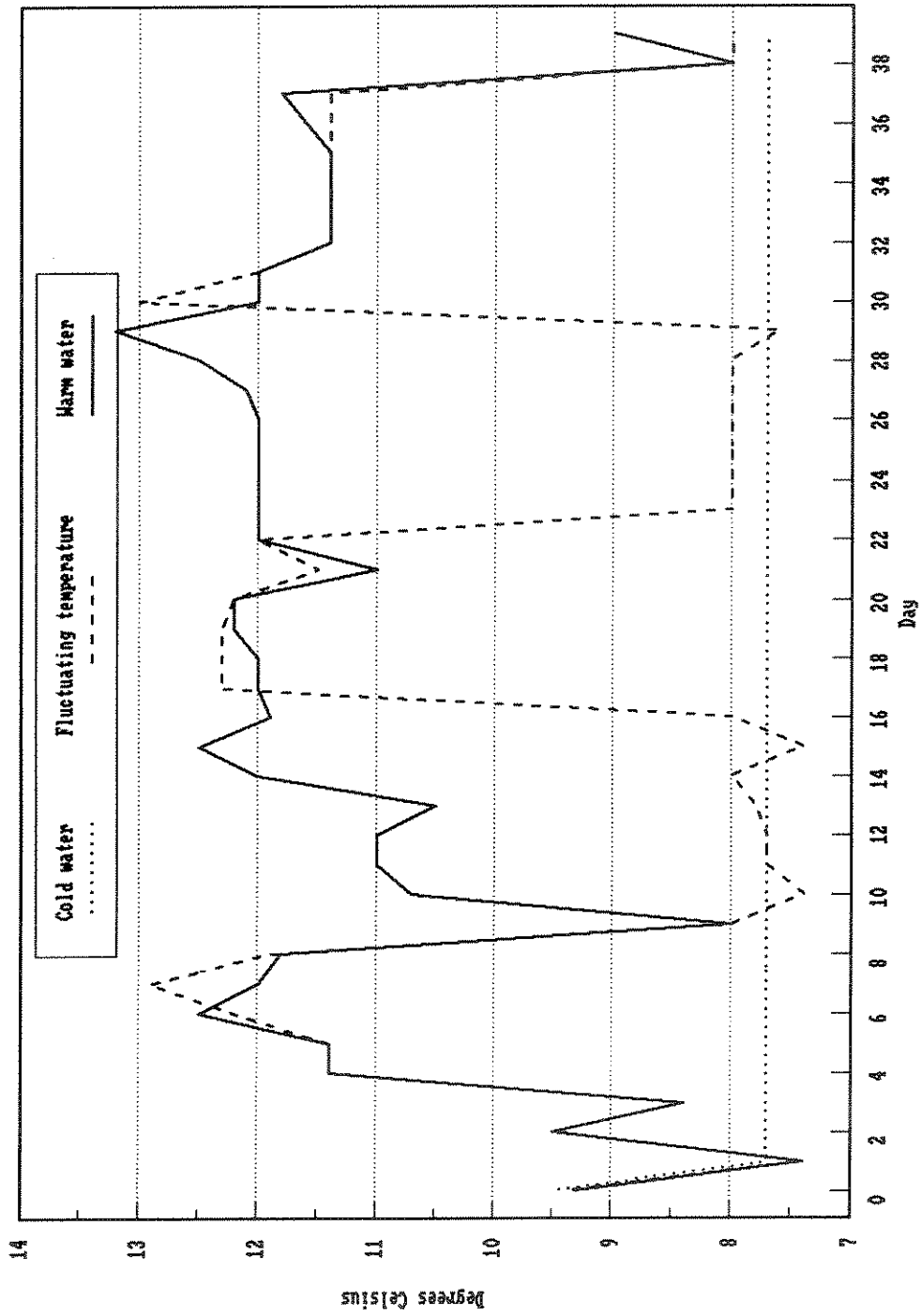


Figure 11. Daily water temperature for cold water, fluctuating temperature, and warm water experimental groups, Experiment 2.

Radial Distance Analysis

Variable Selection. Comparisons of each variable indicated consistent differences existed between groups and separation of groups might be possible (Appendix B, Figure 23). Intercirculi spacing, average spacing over triplets of circuli, scale radius divided by length, scale radius divided by weight, and the number of circuli counted in the first 1/4 mm along the scale axis (variables 5, 6, 7, 8, 9, 23, 24, 26, 27, 42, and 45; Appendix A, Table 13) demonstrated significant differences between groups. These variables were tested for inclusion in discriminant analysis models. As with previous data, several combinations of these variables were used. Again, Eigen values, success of classification arrays, and data similarities were employed as indicators of model fitness (Appendix D, Tables 18, 19, and 20).

Intercirculi spacing prior to the 5th, 6th, and 7th circuli (variables 5, 6, and 7; Figures 12, 13, and 14) yielded best results for Experiment 2 temperature trials and were used in final models (Table 10). Fish without values for these variables were excluded from remaining analysis. This amounted to 18 out of 555 samples, or 3.2%. Once more, about one-half of trout from each group were used as "standards" to build models and remaining trout were used as "unknowns" to further test models.

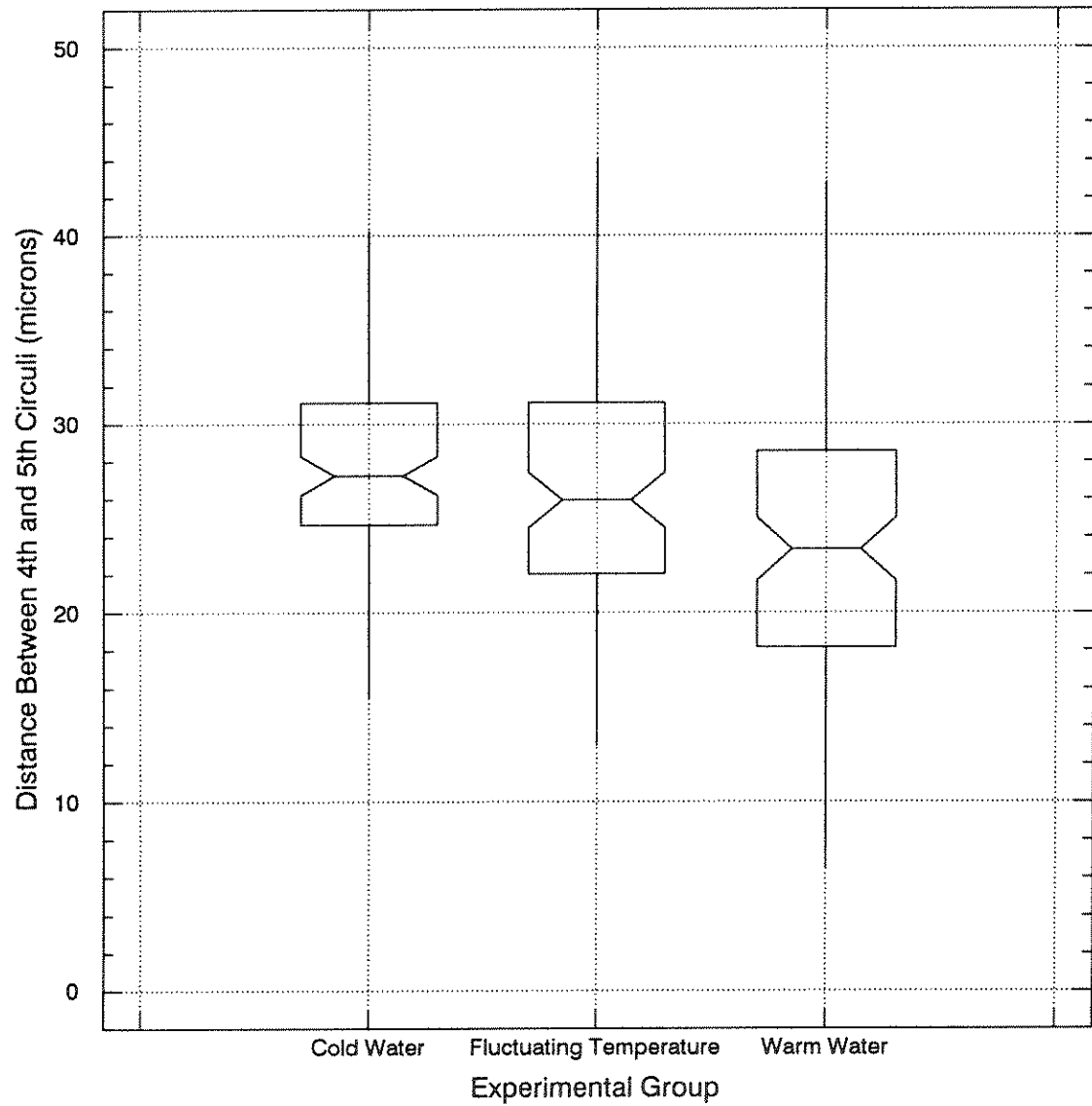


Figure 12. Notched box and whisker plots representing median, 95% confidence intervals, middle 50%, and range of distance between 4th and 5th circuli, for each experimental group, Experiment 2 temperature groups (variable 5).

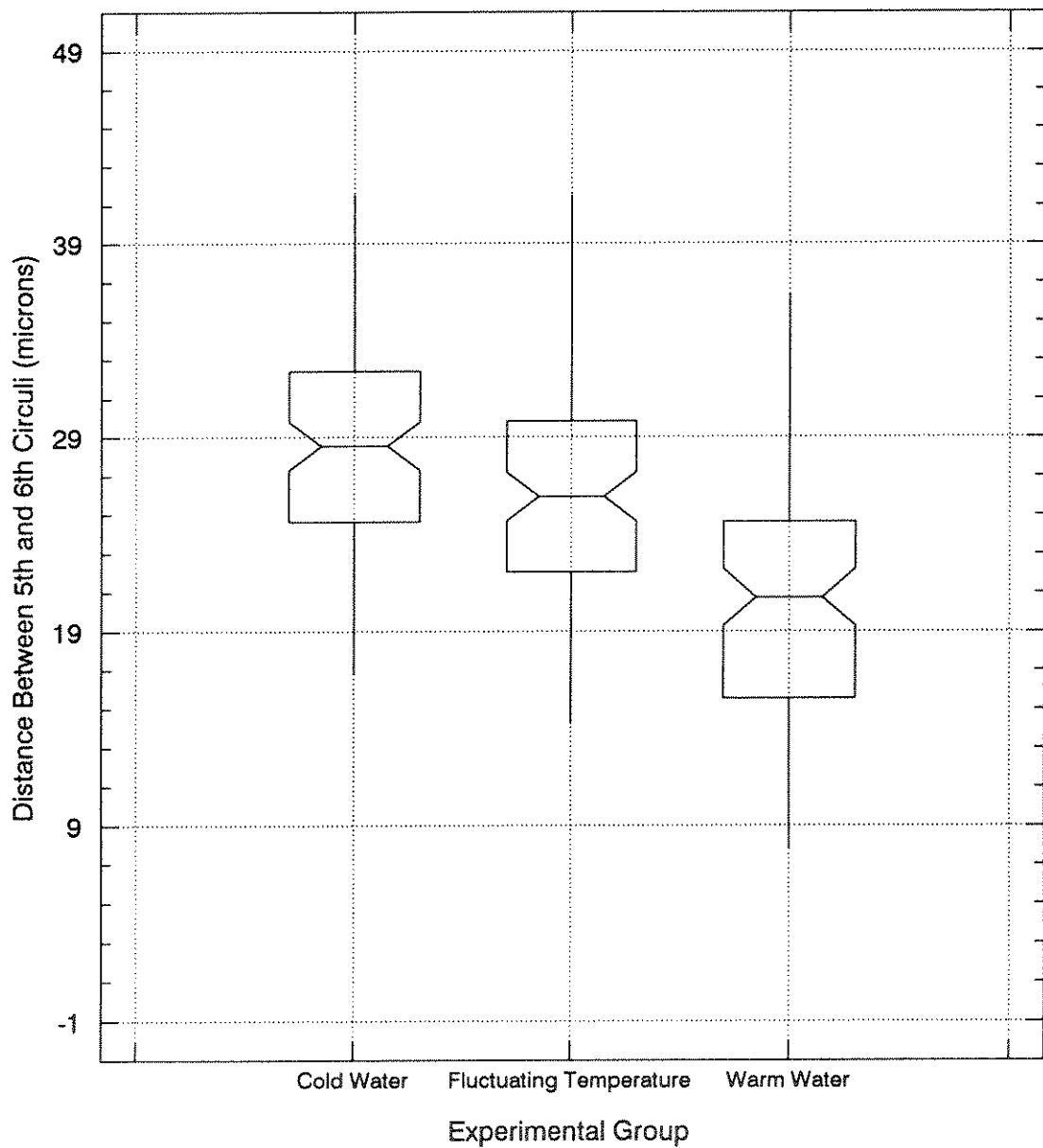


Figure 13. Notched box and whisker plots representing median, 95% confidence intervals, middle 50%, and range of distance between 5th and 6th circuli, for each experimental group, Experiment 2 temperature groups (variable 6).

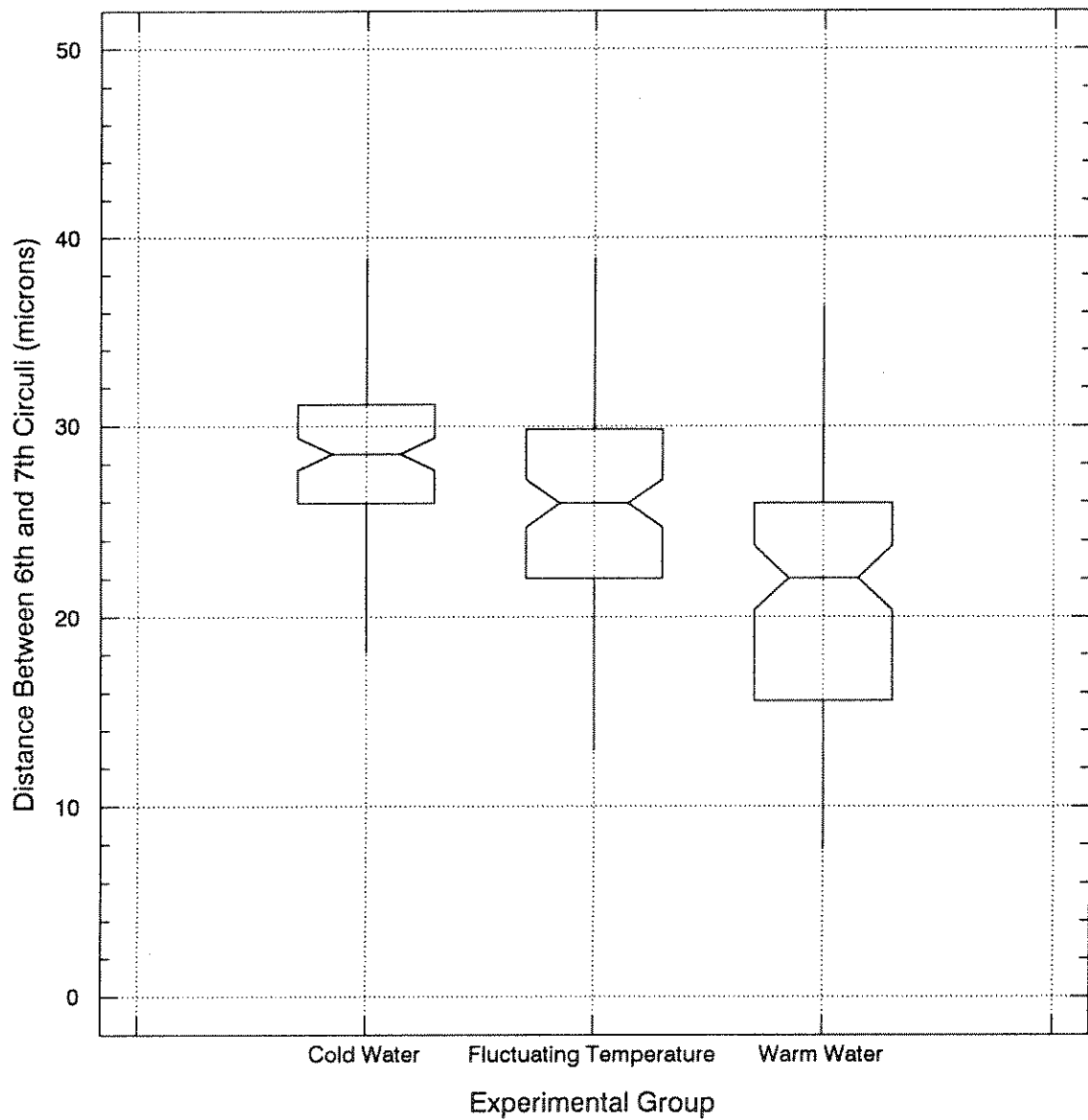


Figure 14. Notched box and whisker plots representing median, 95% confidence intervals, middle 50%, and range of distance between 6th and 7th circuli, for each experimental group, Experiment 2 temperature groups (variable 7).

Table 10. Discriminant analysis models, with associated Eigen values and classification arrays for 1988 temperature experiments.

Model	Eigen values	Classification Array (percent)		
		Actual	Predicted	
Cold water versus warm water	0.7899	Cold	<u>Cold</u> 85.3	<u>Warm</u> 14.7
		Warm	21.0	79.0
Cold water versus fluctuating (fluc) temperature water	0.0919	Cold	<u>Cold</u> 65.3	<u>Fluc</u> 34.7
		Fluc	37.2	62.8
Fluctuating (fluc) water temperature versus warm water	0.3437	Fluc	<u>Fluc</u> 69.2	<u>Warm</u> 30.7
		Warm	26.3	73.7

Two Group Models. Excellent separation was possible between cold water fish and fish from either other group (Table 11). The cold water versus warm water model correctly classified "unknowns" 89.2% of the time. This improved to 97.8% when error classification arrays were applied. Improvement was slightly significant ($p=0.0710$). The cold water versus fluctuating water temperature model correctly identified 80.4% of "unknowns", improving to 97.6% with error correction. This was a significant improvement ($p=0.0398$). Fluctuating water temperature versus warm water model correctly classified 83.6% of the "unknowns". This decreased, nonsignificantly ($p=0.3933$), to 75.6% when error classification arrays were used.

As with feed groups, trout used as "unknowns" were

Table 11. Results of 1988 2-group temperature model testing, using known cutthroat trout as "unknowns", with and without error classification correction factors. Numbers in parentheses represent 90 percent confidence intervals for the estimate.

	Natural		With error classification arrays		
Actual number	Predicted number in group	Percent correct	Predicted number in group	Percent correct	
Cold water (col) versus warm water (war) model:					
<u>col</u> <u>war</u>	<u>col</u> <u>war</u>		<u>col</u>	<u>war</u>	
86 77	95 68	94	93 (71-114)	70 (49- 92)	96
86 0	76 10	88	86 (74- 86)	0 (0- 12)	100
0 77	19 58	75	0 (0- 14)	77 (63- 77)	100
86 43	87 42	99	93 (75-111)	36 (18- 54)	95
45 77	57 65	90	46 (28- 64)	76 (58- 94)	99
Cold water (col) versus fluctuating water temperature flu) model:					
<u>col</u> <u>flu</u>	<u>col</u> <u>flu</u>		<u>col</u>	<u>flu</u>	
86 90	92 84	97	83 (10-156)	93 (20-166)	98
86 0	58 28	67	86 (36- 86)	0 (0- 48)	100
0 90	34 56	62	0 (0- 52)	90 (38- 90)	100
86 44	75 55	92	96 (35-130)	34 (0- 95)	92
45 90	67 68	84	46 (0-108)	87 (27-130)	98
Fluctuating water temperature (flu) versus warm water (war) model:					
<u>flu</u> <u>war</u>	<u>flu</u> <u>war</u>		<u>flu</u>	<u>war</u>	
90 77	103 64	92	141 (105-167)	26 (0- 62)	69
90 0	74 16	82	90 (68- 90)	0 (0- 22)	100
0 77	29 48	62	21 (1- 41)	56 (36- 76)	73
90 43	90 43	100	132 (99-133)	1 (0- 34)	68
44 77	66 55	82	82 (54-109)	39 (12- 67)	68

switched with trout used for "standards" and analysis was repeated (Table 12). Overall model performances were 79.6, 84.4, and 66.0% correct for cold water versus warm water, cold water versus fluctuating water temperature, and fluctuating water temperature versus warm water, respectively. With correction factors, these percentages increased to 90.2 and 94.6 for the first two models (Table 12). These changes were not significant ($p=0.2094$ and 0.2366 , respectively). In the fluctuating water temperature versus warm water comparison, applying correction factors tended to decrease model performance (50.4% correct, $p=0.4497$)

Three Group Models. Three group models were not attempted because of poor discrimination abilities between warm water and fluctuating water temperature groups.

Luminance Profile Analysis

Transformed luminance profile data indicated no variables were dissimilar enough to allow separation of groups (Appendix C, Figure 28). No further analysis was done on these data.

Growth Comparisons

Mean lengths were significantly different ($p=0.0034$) between groups (Figure 15). Fish raised in fluctuating water temperature (118.5 mm) were largest and fish from warm

Table 12. Results of 1988 2-group temperature model testing, switching "standards" with "unknowns", with and without error classification correction factors. Numbers in parentheses represent 90 percent confidence intervals for the estimate.

	Natural			With error classification arrays		
Actual number	Predicted number in group		Percent correct	Predicted number in group		Percent correct
cold water (col) versus warm water (war) model:						
<u>col</u> <u>war</u>	<u>col</u> <u>war</u>			<u>col</u>	<u>war</u>	
95 95	92 98	98	84 (62-107)	106 (83-128)	94	
95 0	75 20	79	86 (73- 95)	9 (0- 22)	91	
0 95	17 78	82	0 (0- 14)	95 (81- 95)	100	
95 49	84 60	92	86 (67-104)	58 (40- 77)	94	
50 95	56 89	96	42 (23- 62)	103 (83-122)	94	
Cold water (col) versus fluctuating water temperature (flu) model:						
<u>col</u> <u>flu</u>	<u>col</u> <u>flu</u>		<u>col</u>	<u>flu</u>		
95 94	88 101	96	76 (18-135)	113 (54-171)	90	
95 0	56 39	59	82 (43- 95)	13 (0- 52)	86	
0 94	32 62	66	0 (0- 40)	94 (54- 94)	100	
95 49	72 72	84	76 (28-124)	68 (20-116)	87	
50 94	60 84	93	32 (0- 83)	112 (61-144)	88	
Fluctuating water temperature (flu) versus warm water (war) model:						
<u>flu</u> <u>war</u>	<u>flu</u> <u>war</u>		<u>flu</u>	<u>war</u>		
94 95	34 155	68	0 (0-138)	189 (151-189)	50	
94 0	28 66	30	2 (0- 24)	92 (70- 94)	2	
0 95	6 89	94	0 (0- 18)	95 (76- 95)	100	
94 49	32 111	57	0 (0- 30)	143 (113-143)	34	
49 95	21 123	81	0 (0- 29)	144 (115-144)	66	

water (115.3 mm) were smallest. Mean length of fish in cold water was 116.0 mm.

Mean weights followed the same pattern (Figure 16). A significant difference ($p=0.0026$) existed between groups. Fish in fluctuating water temperature were largest (18.0 g) and fish in warm water were smallest (16.4 g). Cold water fish averaged 16.9 g.

Number of circuli present on scales was also significantly different ($p<0.0001$) among groups (Figure 17). Cold water fish had fewest, averaging 8.1 circuli per scale. Both fluctuating water temperature fish and warm water fish had 8.8 mean number circuli per scale.

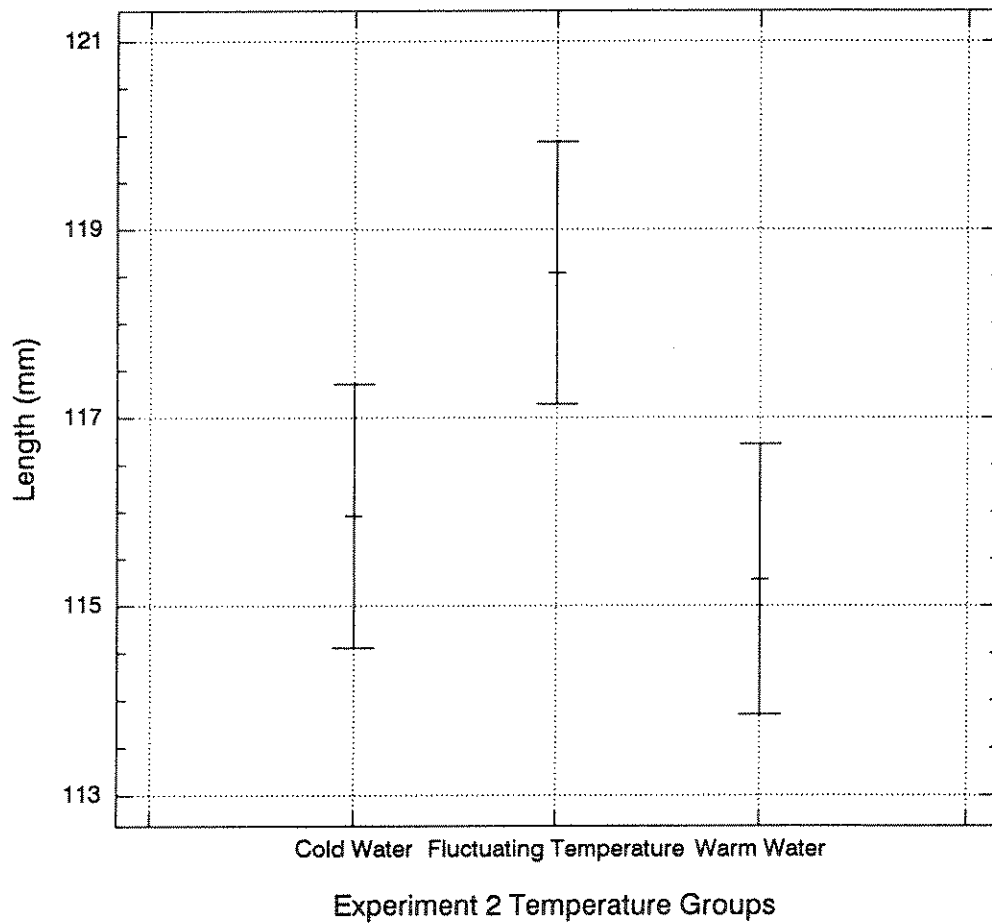


Figure 15. Mean total length, with 95% confidence intervals, for each experimental group, Experiment 2 temperature groups.

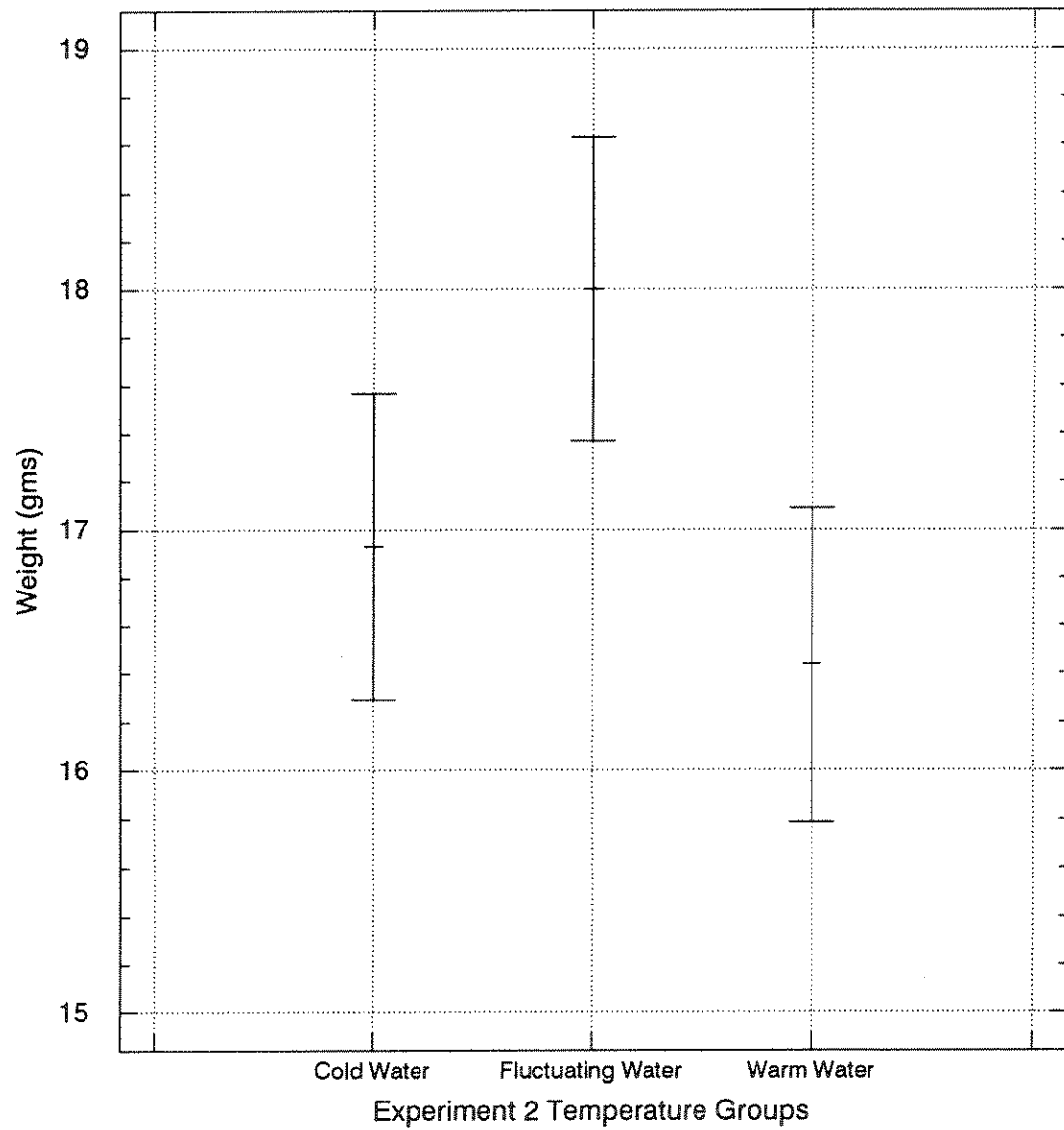


Figure 16. Mean weight, with 95% confidence intervals, for each experimental group, Experiment 2 temperature groups.

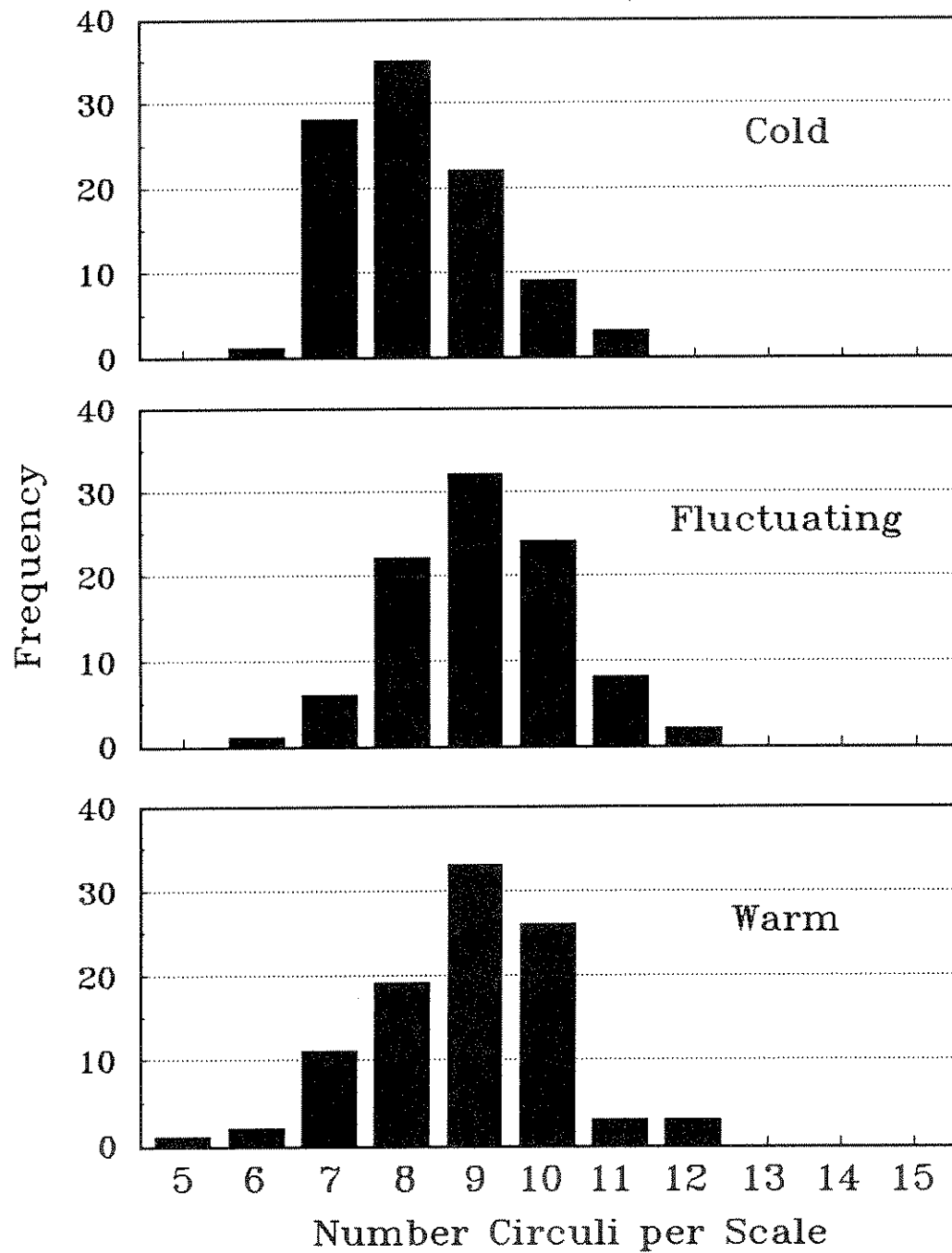


Figure 17. Distribution of number of circuli per scale for each experimental group, Experiment 2 temperature groups.

DISCUSSION

Radial Distance Work

Radial distance analysis provided good separation between both diet and temperature groups, showing trout can be "marked" by altering circuli spacing on scales. The use of error classification matrices consistently improved prediction accuracies but did not change interpretation. Although improvements were seldom statistically significant, probably due to small sample sizes, a real improvement using this technique likely does exist (Cook and Lord 1978, Cook 1982). Therefore, only the corrected outcomes of model accuracies will be discussed.

Diet Groups

Three of four diet models correctly classified over 90% of "unknown" fish in the 1988 tests. This is excellent discrimination. Acceptable levels of accuracy vary, depending on study objectives, but Myers et al. (1987) suggest accuracies of 75% and above are satisfactory.

Reducing food intake in cutthroat trout can successfully decrease circuli spacing on scales. All treatment groups had smaller mean inter-circular spacing previous to the 3rd, 4th, and 5th circuli than did control fish. Presumably, formation of these circuli (or a subset of these circuli) was affected by growth conditions during

the 6-week treatment period.

Although using feed reductions to "mark" fish also reduces growth, this should not be a serious problem. Marking periods need not be more than 3 or 4 weeks long, not enough to seriously reduce overall growth of fish. Major and Craddock (1962) starved fish for 0, 2, 4, 6, and 8 weeks. They found that condition factors recovered in all groups once feeding resumed.

Three-group models did not perform well in this study. However, this experiment was designed to compare each treatment with only the control group. The fact that three group models showed limited ability to correctly predict trout origin suggests it would be possible to have combinations of feed induced marks in the same study. Perhaps with various feeding regimes, models with several groups could provide accurate discrimination to allow for more than two experimental groups per study.

Temperature Groups

Excellent separation also occurred between all temperature treatments. An average of 94% of the cold water trout scales were identified correctly by the model used. Warm water and fluctuating water temperature fish also separated well, averaging approximately 88% correct.

Inter-circuli spacings used to separate water temperature groups were farther from the scale focus than

with feed groups. Fish for each treatment were obtained randomly from the same population. It is unlikely these fish had more circuli per scale than fish in feed groups prior to beginning treatments. Apparently, temperature affects circuli spacing indirectly through changes in metabolism (Gray and Setna 1930) and therefore, effects are recorded on scales after a greater time lag than with changes in feeding rates. Major and Craddock (1962) and Bilton and Robins (1971a, 1971b, 1971c) suggest a time lag occurred before results were recorded on scales when marking their fish using starvation periods. It appears this time lag is longer when using temperature as the experimental variable than when using feeding regimes.

In the 1988 experiments the warm water group of fish grew less than the cold water fish and the fluctuating water temperature fish grew the most. Unfortunately, trout in the cold water group began these trials slightly smaller than trout in the other two groups. I believe, however, this did not affect the outcome of the experiment. Mean inter-circuli spacings included in models were larger, not smaller, as would be expected for cold water fish than for either warm water or fluctuating water temperature fish. Also, inter-circuli spacing measurements used in final models were made on portions of scales formed after the experiment was begun and should not have been significantly affected by such small length differences (approximately 2

mm).

Mark Retention

I was unable to test mark retention since I was unsuccessful in marking trout in the first year experiment. However, these fish were sampled a year later and I was able to compare circuli spacing with original samples to verify that no changes occurred. This comparison showed no changes in spacing over time. Establishing that scale patterns do not change once formed is an important validation of this technique. In fact, Bilton (1974) found scale foci to be smaller in older sockeye salmon than in very young ones. He suggested some amount of time was needed between scale formation and final form of the scale.

It is possible scale resorption could also cause problems in mark recovery in some species. Although this will not be a problem in most cases, scale resorption is known to occur in salmonids and has been found somewhat frequently in Asian chum stocks (Bigler 1989).

To use this technique it is important that scales are representative of the populations to be identified. For example, Cook and Guthrie (1987) advised against using smolts to establish training samples for stock identification with sockeye salmon adults because differential mortality could significantly bias samples. This problem should also be weighed when considering

"marking" fish by altering scale growth patterns.

Luminance Profile

Luminance profile work did not produce expected results. Data recorded were intensities of light transmitted through the scale along a given axis. I believed this would be a more sensitive measure of differences between groups than radial distance measurements. If dissimilarities in scale formation had occurred but not resulted in a circulus being laid down, this technique presumably would detect these as changes in scale thickness.

However, discrimination between treatment groups was poor using this approach. Even if this method had produced adequate results, additional problems would limit its usefulness for trout scale marking. Time and tedium involved in preparing trout scale samples for processing were serious drawbacks. Scales must be free of mucous prior to being measured. The small size of scales involved in this study, often less than 1 mm, made cleaning and mounting scales extremely difficult. Although Whaley (1991) has developed a scale cleaning technique which appears to be more efficient, time required for scale preparation is still a disadvantage.

Another problem with luminance profile work concerns data analysis. Transformation of data into Fourier

descriptors results in biologically meaningless variables. Although it is not necessary for statistical analyses to be intuitive to be useful, generally the more straight forward analyses are, the more effective results will be in influencing relevant policies.

Possible Problems With Experiment 1

Reasons for lack of success in marking trout scales in Experiment 1 are only speculative but several possibilities exist: 1) too much within-group variability prior to exposure to experimental treatment; 2) inadequate duration of treatment; 3) formation of "marks" at the scale margin resulting in the mark being undetected; and 4) imprecise scale collection procedures.

Within Group Variability

Within group variability could be high if a wide range of fish sizes existed. Pre-treatment trout lengths ranged 45 mm to 98 mm for all groups. This range of 53 mm is approximately 65% of the average mean length for these groups. Smallest range for fish within one group was 39 mm, still over 48% of average length.

The number of circuli per scale was not counted prior to administering treatments; however, post-treatment circuli counts showed wide variation for fish within groups (4-14

circuli). All except the warm water group had a range of nine circuli. Circuli counts in the warm water group averaged 8 and ranged from 5 to 13 circuli. If "marks" had been formed near scale margins, they would be difficult to detect using this data set.

The impacts of within group variability were minimized in experiment 2 by using younger, and therefore smaller, fish. The smaller the fish, the less the variation in fish length and number of circuli per scale. The lower limit in fish size, however, must be large enough for scales to have formed on all fish. For cutthroat trout this is approximately 47 mm (Brown and Bailey 1952).

Another problem which contributed to within-group variation in experiment 1 feed groups was unequal distribution of fish feed. Feed rations were reduced to $1/3$ and $2/3$ of the calculated quantity under the assumption all fish would receive a smaller amount of feed. In actuality, aggressive fish received more of the food than non-aggressive fish. This probably contributed to masking any possible "marks". To avoid this problem all experiment 2 feed groups were given full rations when fed, but not fed every day.

Treatment Duration

Rate of circulus formation varies considerably between species, thus different treatment lengths may be required

for different species. Major and Craddock (1962) had excellent success using 2 and 4 week starvation periods to produce marks on scales of juvenile sockeye salmon. Boyce (1985) had success altering scale patterns on juvenile steelhead trout by restricting feed for 1 to 2 months and increasing it for 1 month. Similar results were obtained using juvenile sockeye, kokanee, coho, and chinook salmon (Bilton 1974). Gray and Setna (1930) and Bhatia (1932) showed feeding rates significantly affected patterns in yearling rainbow trout scales. However, both of these studies starved fish for over 4 months. It is possible that 3 week treatments were not long enough to produce detectable differences in scale growth of cutthroat trout at the size attempted (pretreatment mean length = 80.9 mm). Experiment 2 treatments lasted 6 weeks and were performed on slightly smaller fish (pretreatment mean length = 62.5 mm).

Undetected "Marks"

Apparently changes in scale pattern occur when fish resume feeding, not during the starvation period (Bilton and Robbins 1971a, 1971b, 1971c). Bilton and Robbins (1971b) starved sockeye for up to 30.5 weeks and found no record of the starvation period on scales of those fish. Major and Craddock (1962) suggested a time lag existed between cessation of experimental conditions and reflection of

environmental changes in scale patterns. Differences between groups may have occurred but were undetected because samples were collected too soon after treatment periods ended. However, after roughly one additional year of growth in the hatchery and Cooper Lake, no marks were observable. Other problems, such as handling stress and small sample sizes, could have obscured differences between groups.

Scale Sampling Problems

It is important to be fairly specific in scale collecting procedures. Scale characteristics vary widely between body regions on the same fish (Knudsen and Davis 1985). Scrape samples may not be a precise enough method for collecting scales because too large of a sampling area is involved. It is especially important to be aware of scale sampling area when scales for "standards" are being collected from young fish for use in later identifying adults. Differences in fish size could lead to scales being collected from an entirely different body area on adults, making accurate predictions impossible.

Clutter and Whitesel (1956) recommended taking a scale from the second row above the lateral line along the diagonal column extending downward from the posterior insertion of the dorsal fin. If that scale is regenerated, the corresponding scale from the opposite side of the fish could be collected. Being this exact would eliminate effect

of body region on scale characteristics. It could, however, prove too time consuming for management purposes. Scales taken from a restricted area in this general region would provide a good compromise without introducing too much variability (Knudsen and Davis 1985).

Taking scales from too small of an area may also result in an unusable sample. Regeneration cannot easily be checked on trout scales during collection. Since regenerated scales are useless, it is important to have large enough sample sizes to insure most samples will have some usable scales. In a coastal cutthroat trout (*Oncorhynchus clarki clarki*) age and growth study, Moring et al. (1981) recommended samples of at least 50 scales per fish to be 87% confident three non-regenerated scales would be present. Although the location from which scales are taken is more critical in a scale pattern analysis study than an ageing study, this does illustrate the need to be aware of possible regeneration problems.

Summary

This study has shown environmental changes, such as feed availability or water temperature, can be manipulated to induce "marks" on trout scales. Changes in feeding regime and water temperature effectively altered circuli spacing on treatment trout scales. These alterations were significant enough to allow a verifiable "mark" to be

produced on trout scales. Although temperature fluctuations generated adequate marks, feed interruptions produced more precise results. "Marks" were used to accurately predict fish group origin, constituting a simple, inexpensive, efficient, and harmless method of mass-marking trout in a hatchery setting. The actual marking of fish with this method can be done at no cost. Some costs would be incurred for scale handling and data analysis, but overall expenses would be minimal.

Several questions regarding this technique still exist and need further investigation. Size of fish to be marked, duration of marking period, adequate post-treatment period needed for environmental changes to be reflected in scale patterns, and scale sample selection are all variables that need additional examination.

One other problem which needs to be addressed is the effect of using juvenile fish to build standards, then trying to predict origin of these fish as adults. Differential mortality and/or problems with body location of scales sampled could seriously hamper the ability to recognize established "marks".

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APPENDICES

APPENDIX A

VARIABLE DEFINITIONS

Table 13. Definitions of variables used in radial distance analysis. All measurements were made along longest anterior axis of the scale, drawn from focus to scale margin.

Variable number	Definition
1	distance from focus to first circulus
2-20	non-cummulative measures of distance from previous circulus to current circulus (cc)
21	average spacing between focus and 3rd cc
22	" " " 1st and 4th cc
23	" " " 2nd and 5th cc
24	" " " 3rd and 6th cc
25	" " " 4th and 7th cc
26	" " " 5th and 8th cc
27	" " " 6th and 9th cc
28	" " " 7th and 10th cc
29	" " " 8th and 11th cc
30	" " " 9th and 12th cc
31	" " " 10th and 13th cc
32	" " " 11th and 14th cc
33	" " " 12th and 15th cc
34	" " " 13th and 16th cc
35	" " " 14th and 17th cc
36	" " " 15th and 18th cc
37	" " " 16th and 19th cc
38	" " " 17th and 20th cc
39	" " " focus and 5th cc
40	" " " focus and 7th cc
41	" " " 6th and 10th cc
42	scale radius* divided by length
43	scale radius* divided by weight
44	scale radius*
45	number circuli along first 1/4 mm of line
46	number circuli along first 1/2 mm of line

* Distance to last circulus was considered equal to scale radius. Actual scale radius was not measured.

APPENDIX B

P-VALUES FOR RADIAL DISTANCE ANOVA'S

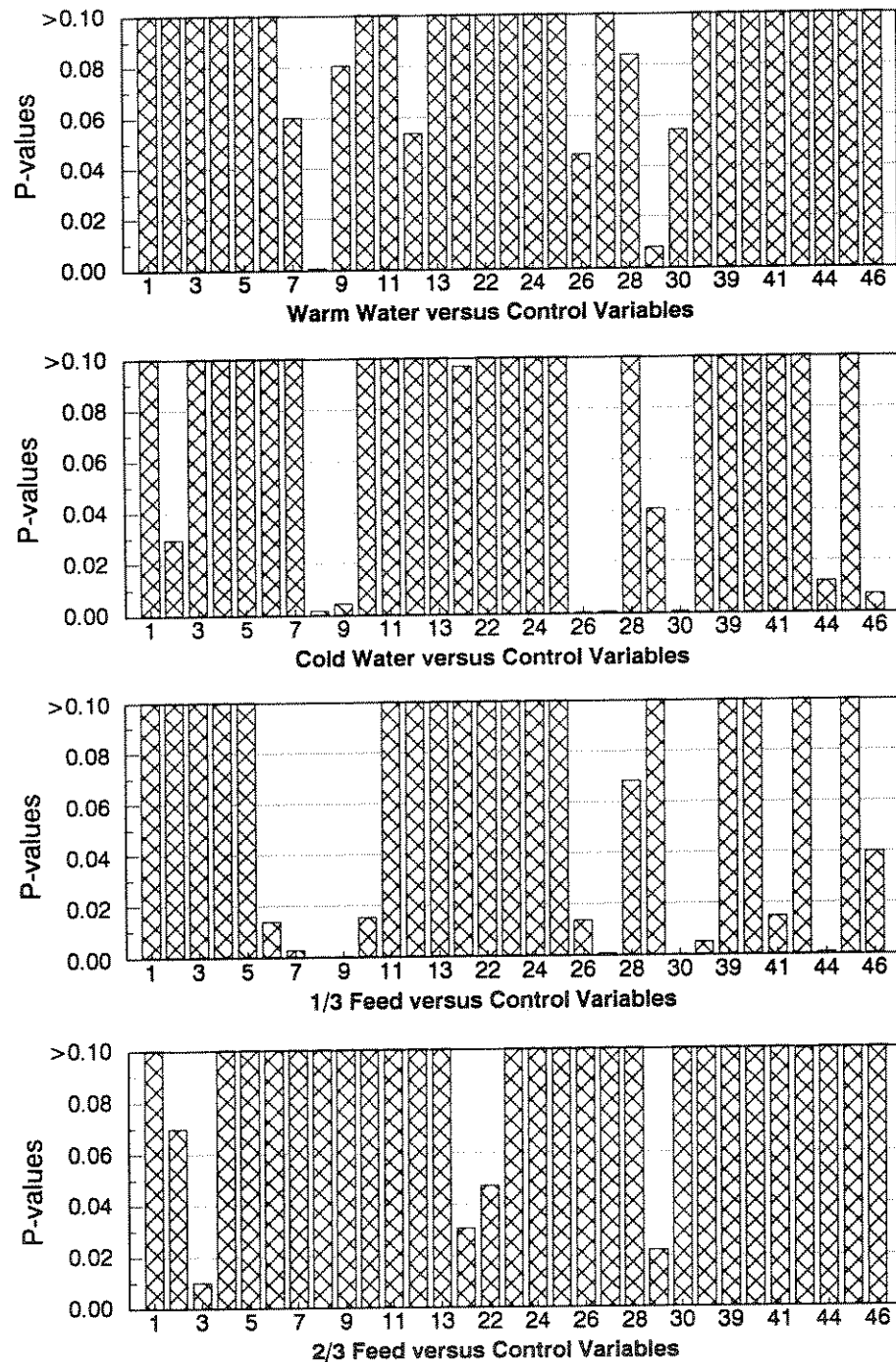


Figure 18. Resultant p-values from comparisons of each treatment group to the control group, using ANOVA, for all pertinent variables, Experiment 1 radial distance data.

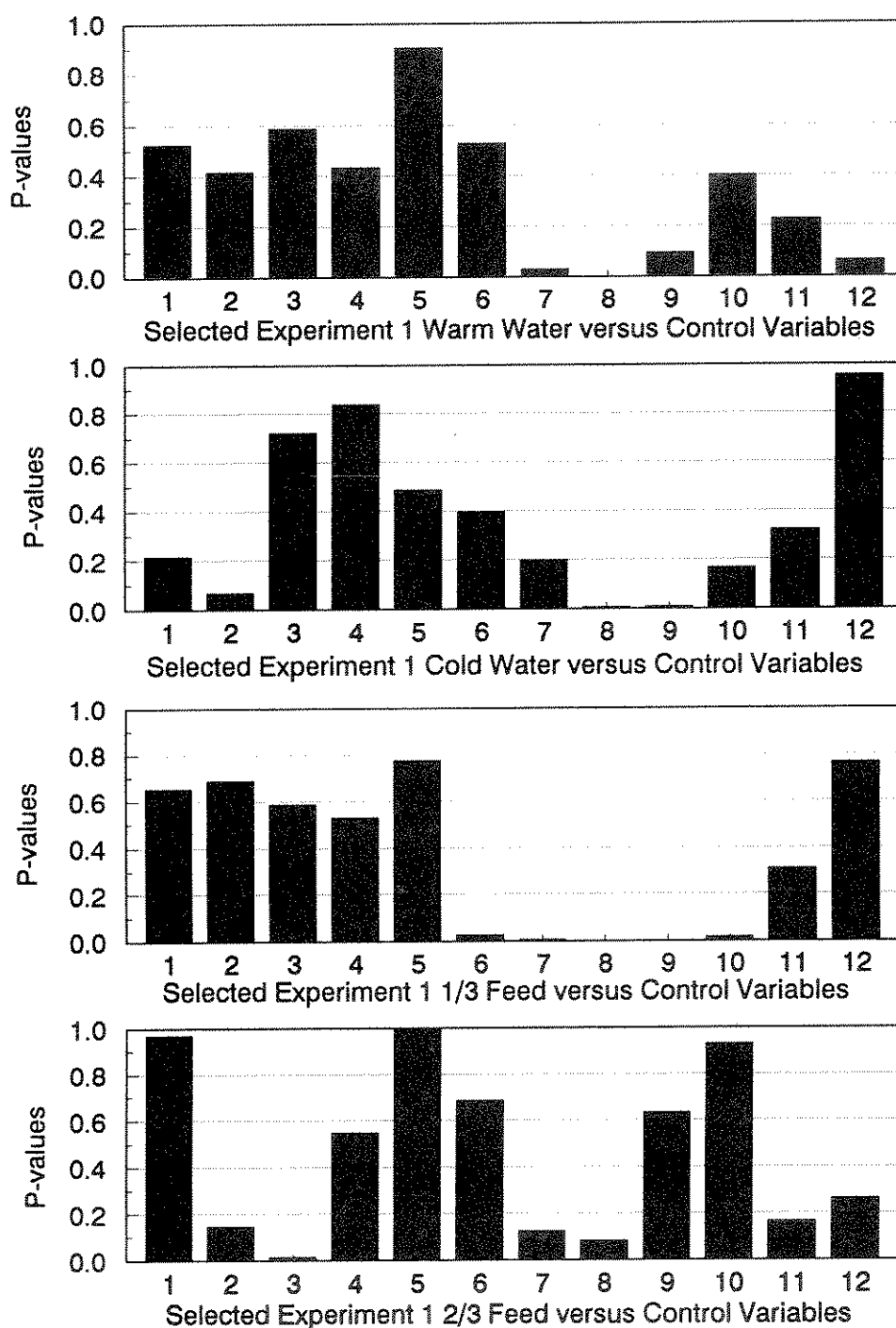


Figure 19. Resultant p-values from comparisons of selected trout in each treatment group, based on median growth rates, to selected trout from the control group, using ANOVA, for all pertinent variables, Experiment 1 radial distance data.

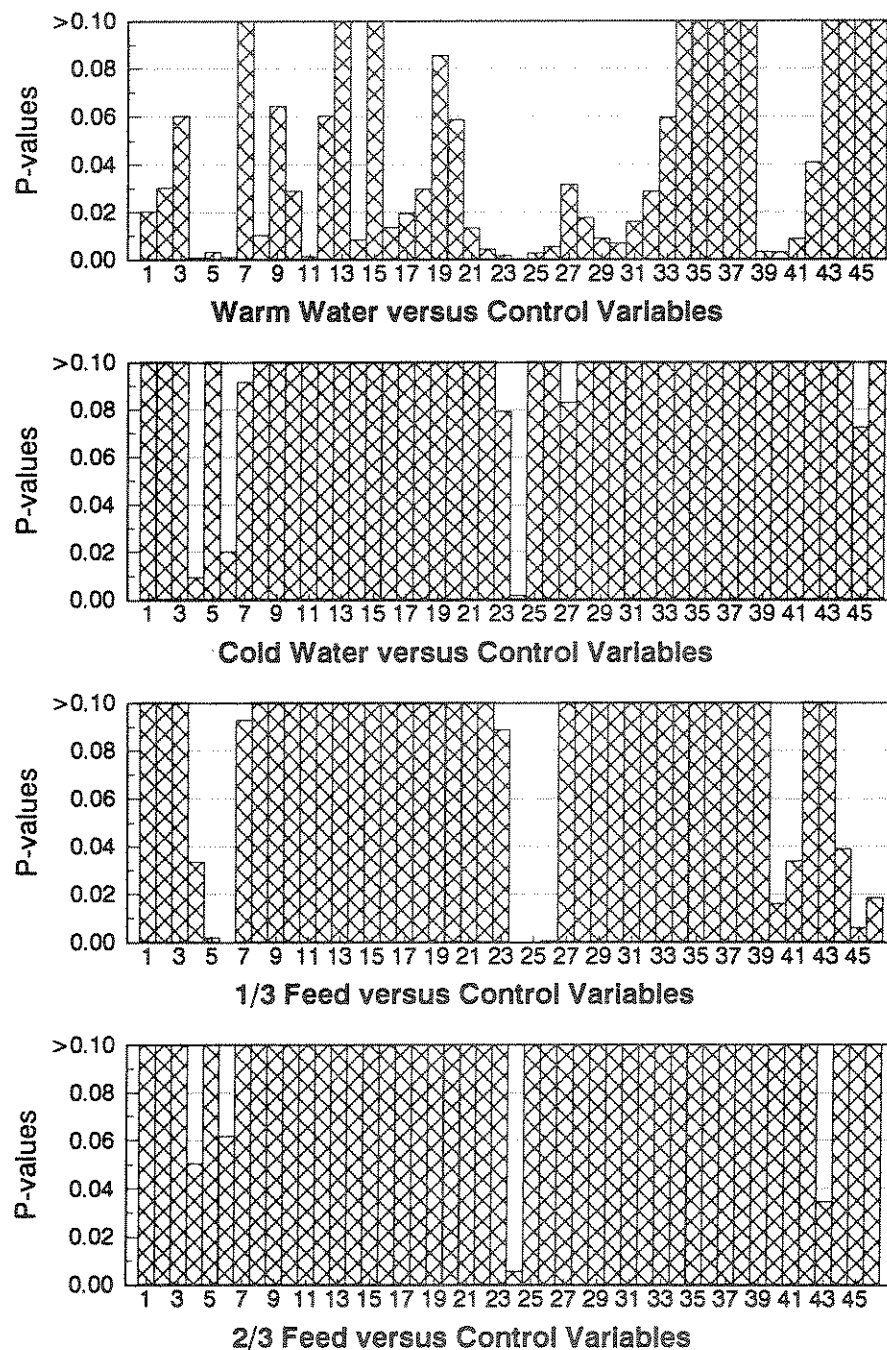


Figure 20. Resultant p-values from comparisons of each treatment group to the control group, using ANOVA, for all pertinent variables, Experiment 1 radial distance data collected from trout after being reared in the hatchery an additional 8 months.

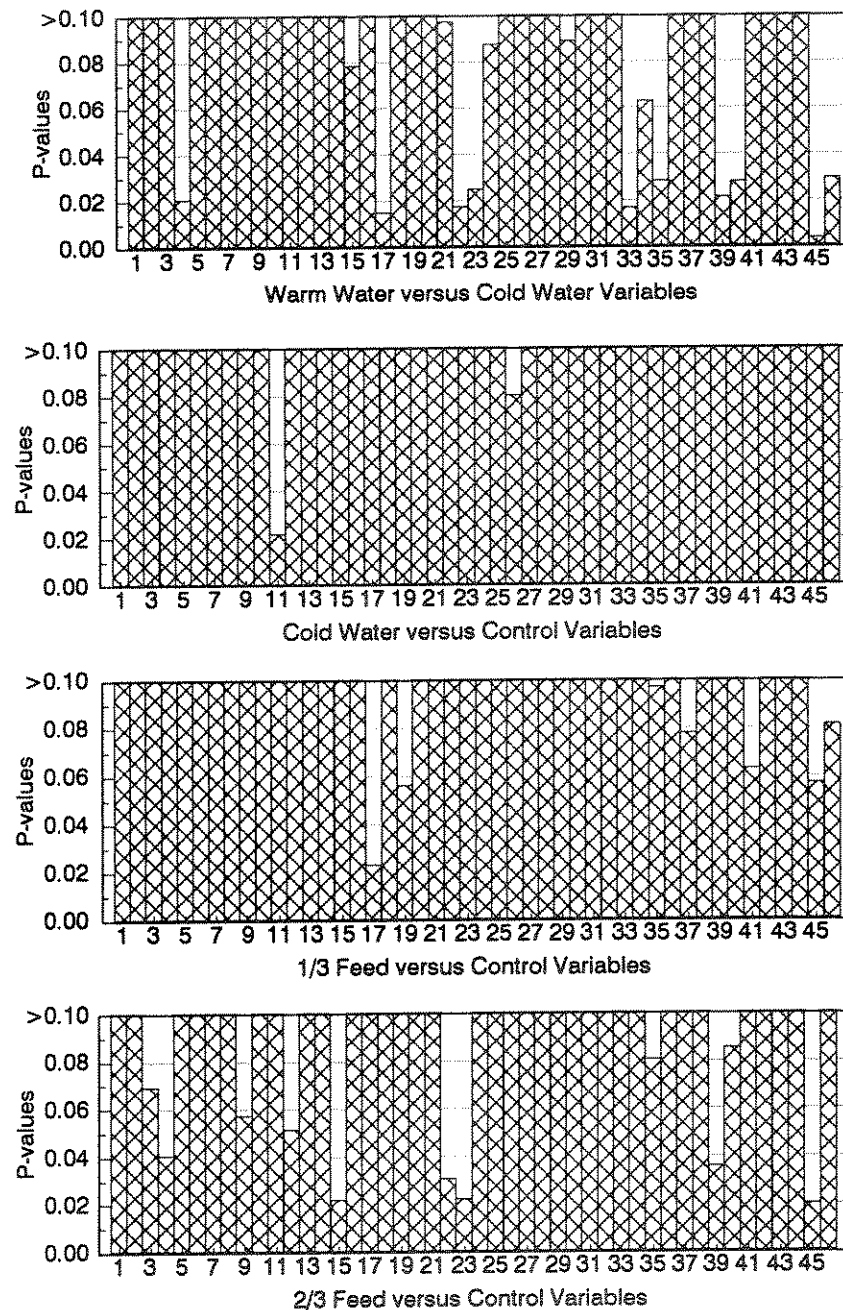


Figure 21. Resultant p-values from comparisons of each treatment group to the control group, using ANOVA, for all pertinent variables, Experiment 1 radial distance data collected from trout after being reared Cooper Lake, Blackfeet Indian Reservation, an additional 12 months.

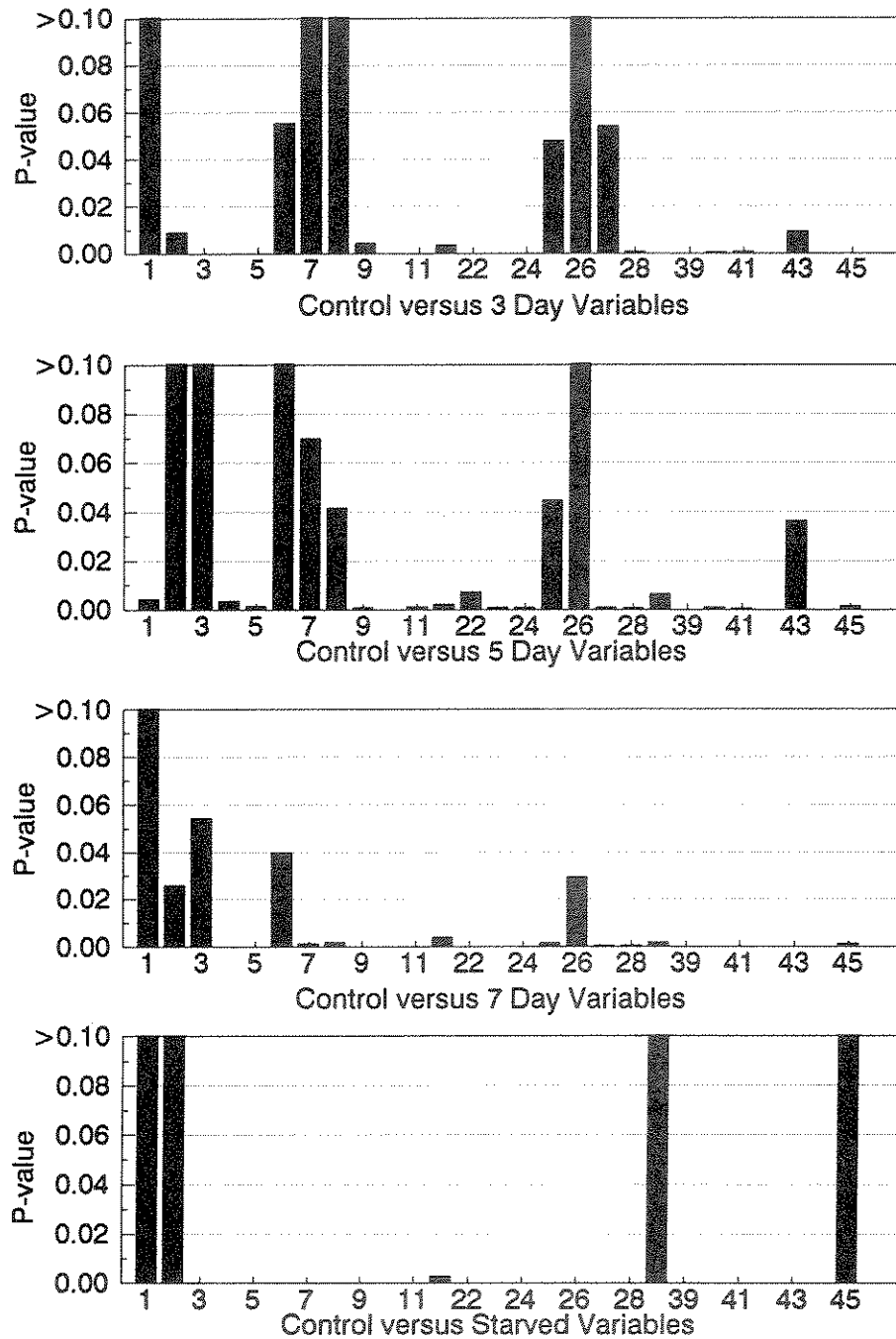


Figure 22. Resultant p-values from comparisons of each treatment group to the control group, using ANOVA, for all pertinent variables, Experiment 2 feed groups radial distance data.

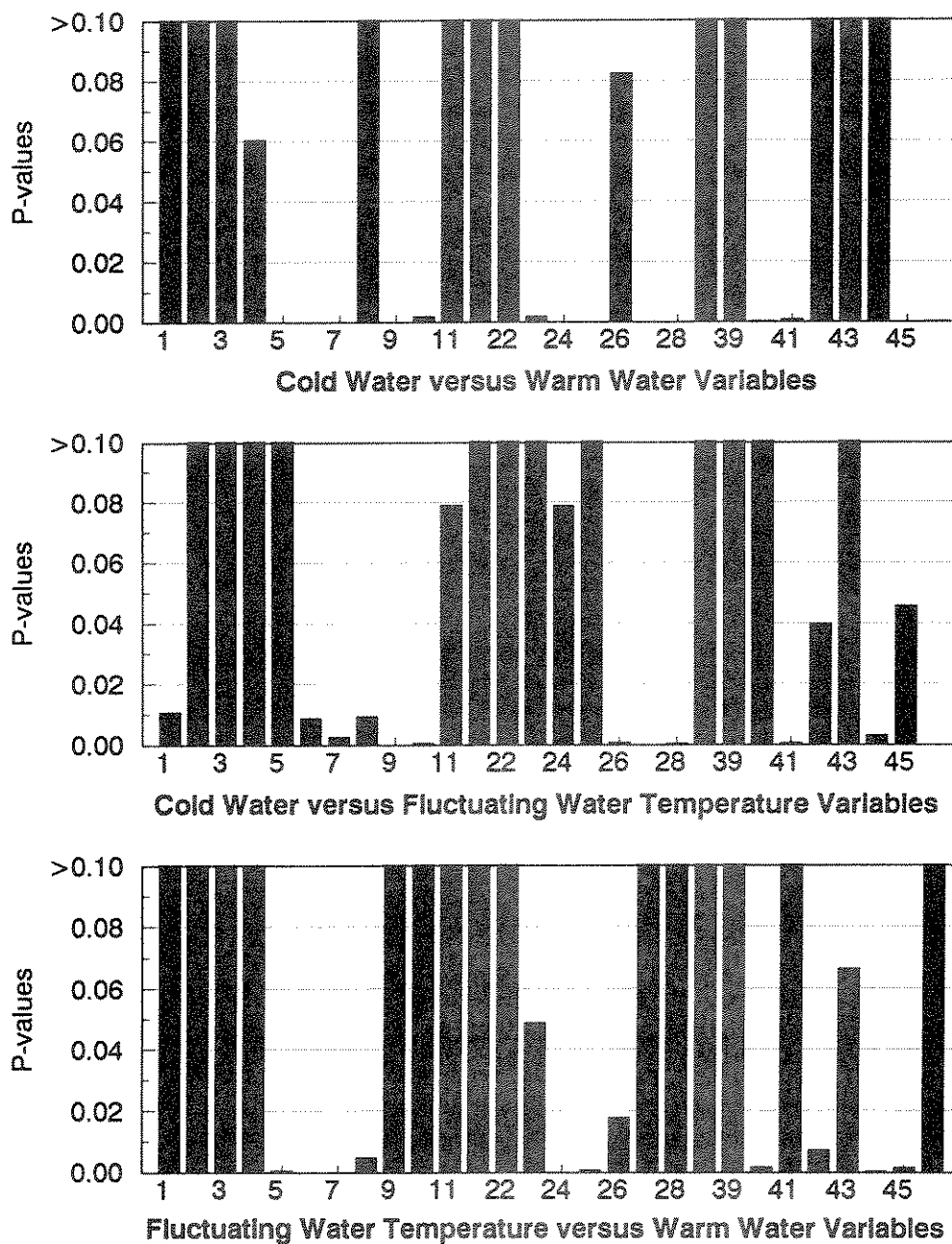


Figure 23. Resultant p-values from comparisons of each treatment group to the control group, using ANOVA, for all pertinent variables, Experiment 2 temperature groups radial distance data.

APPENDIX C

P-VALUES FOR LUMINANCE PROFILE TRANSFORMATION ANOVA'S

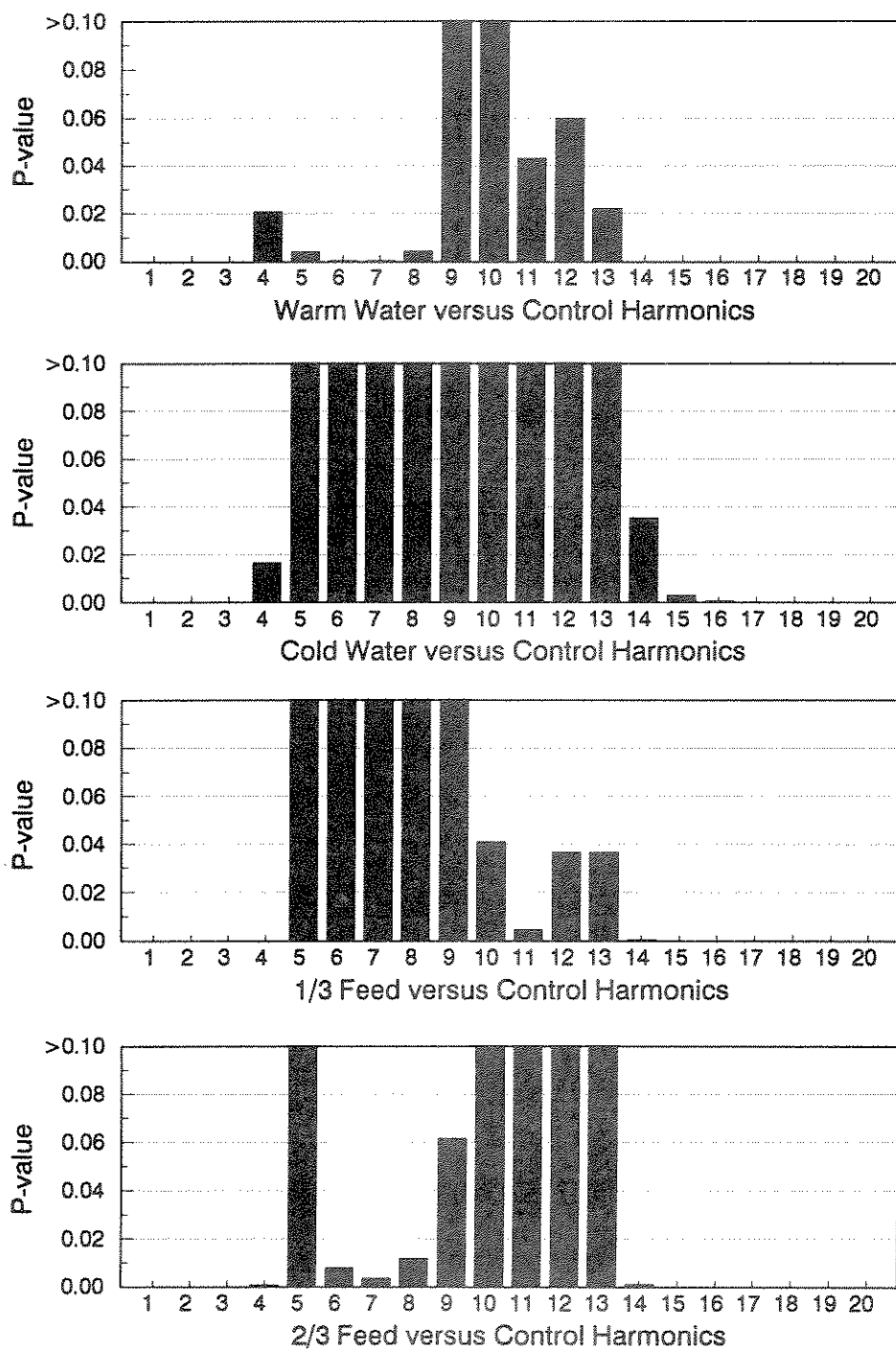


Figure 24. Resultant p-values from comparisons of each treatment group to the control group, using ANOVA, for harmonics from transformed luminance profile data, Experiment 1.

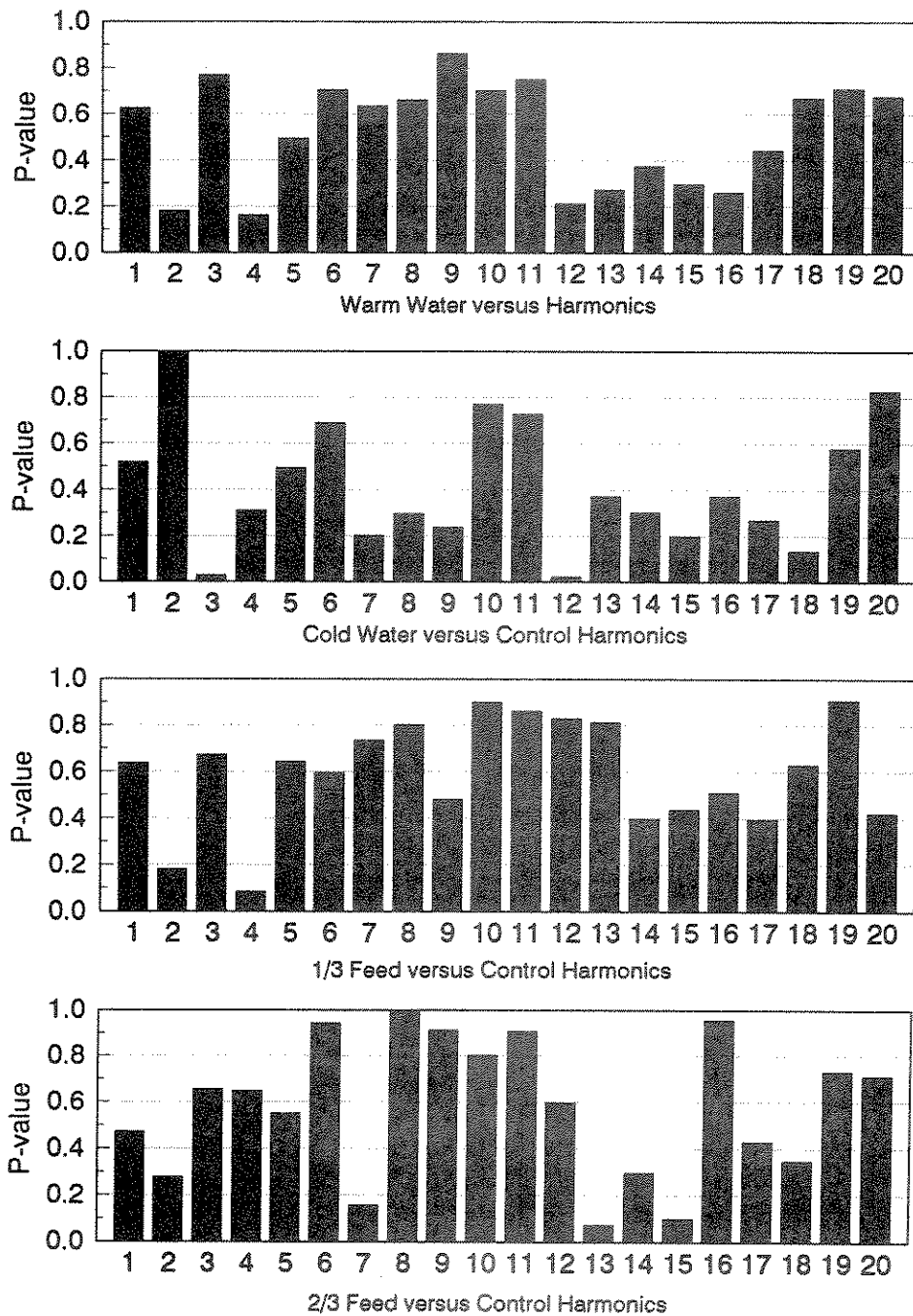


Figure 25. Resultant p-values from comparisons of each treatment group to the control group, using ANOVA, for harmonics from transformed luminance profile data, Experiment 1 hatchery reared trout.

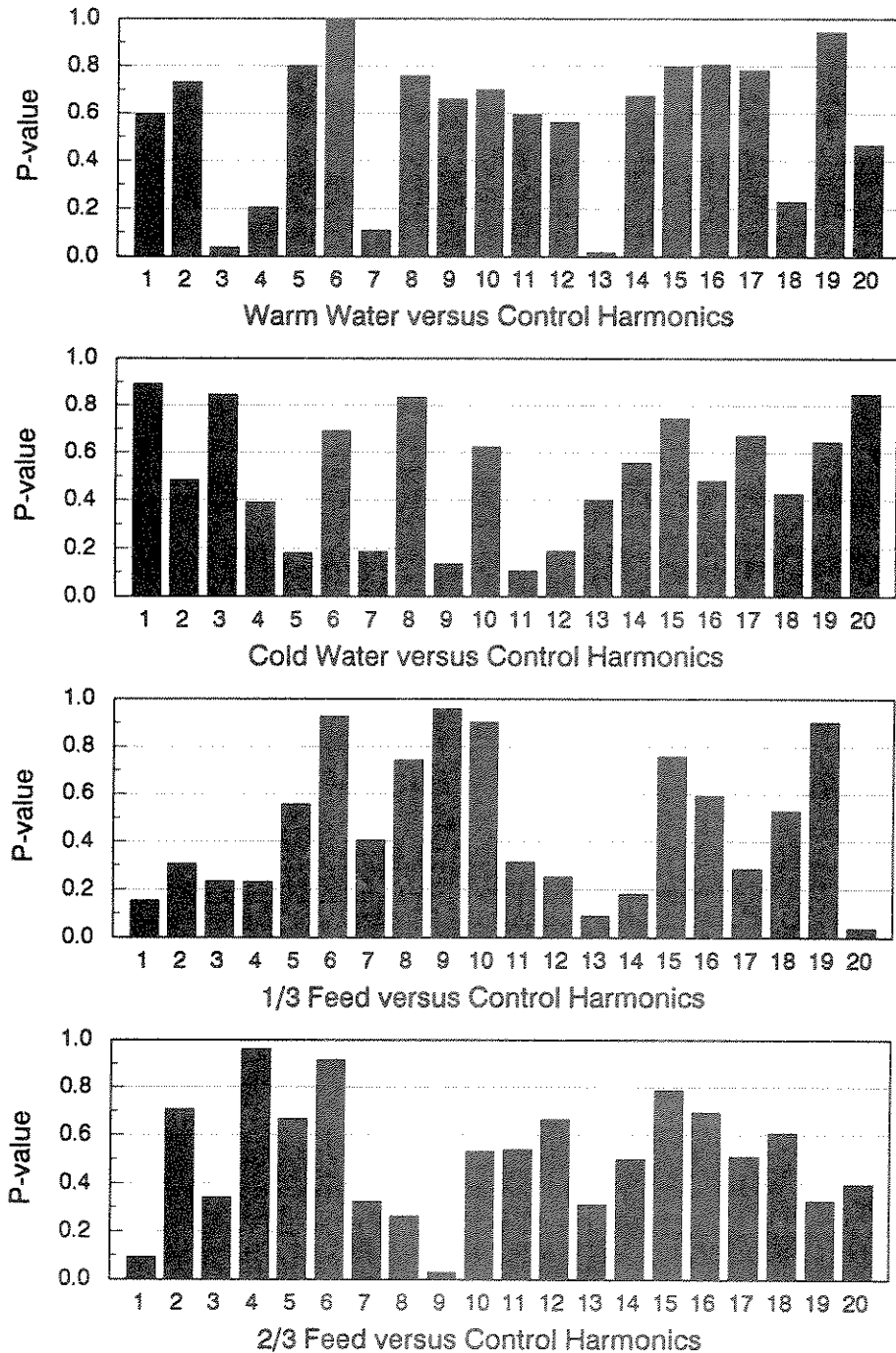


Figure 26. Resultant p-values from comparisons of each treatment group to the control group, using ANOVA, for harmonics from transformed luminance profile data, Experiment 1 Cooper Lake trout.

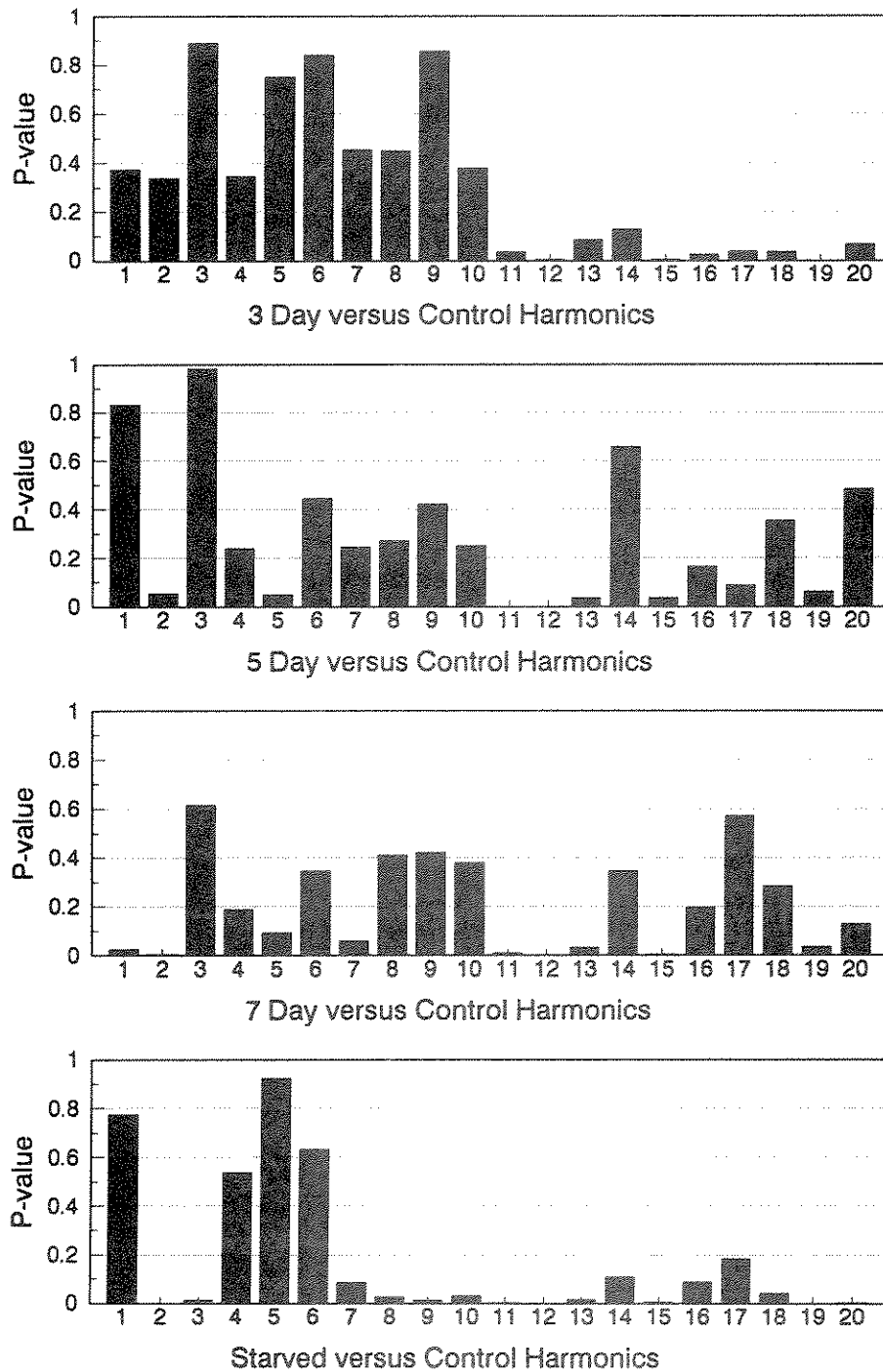


Figure 27. Resultant p-values from comparisons of each treatment group to the control group, using ANOVA, for harmonics from transformed luminance profile data, Experiment 2 feed groups.

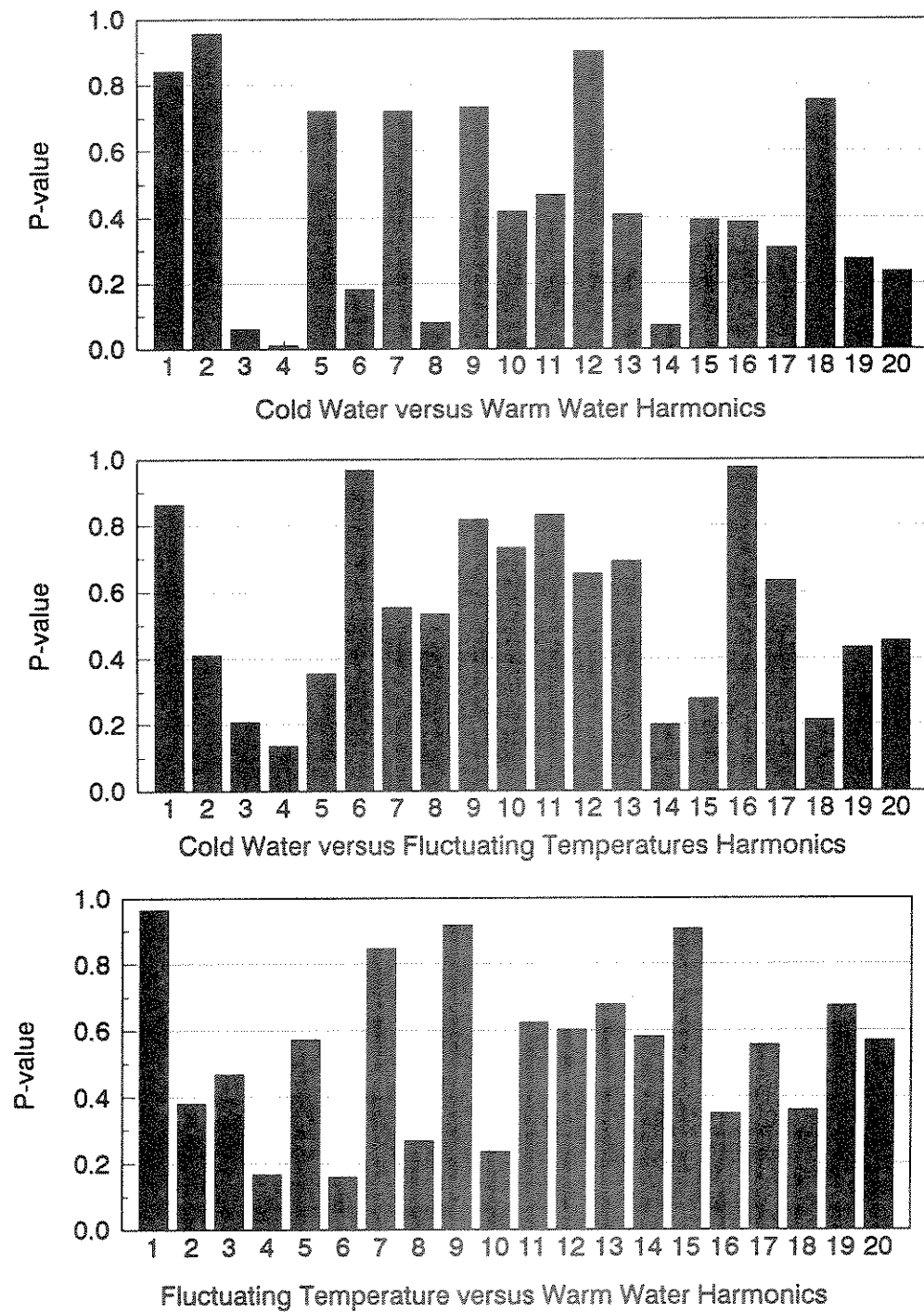


Figure 28. Resultant p-values from comparisons of all treatment groups, using ANOVA, for harmonics from transformed luminance profile data, Experiment 2 temperature groups.

APPENDIX D

DISCRIMINANT ANALYSIS DATA

Table 14. Discriminant analysis, including variables used for discrimination, resultant Eigen values, and resultant classification arrays, for the 3 d versus control groups, Experiment 2.

Variables	Eigen values	Classification array (percent)		
		Actual	Predicted	
2, 3, 4, 5, and 6	0.3260		<u>3 d</u>	<u>Control</u>
		3 d	77.3	22.7
		Control	26.5	73.5
2, 3, 4, and 5	0.3256	3 d	78.4	21.6
		Control	26.5	73.5
3, 4, 5, and 6	0.3219	3 d	78.4	21.6
		Control	25.5	74.5
3, 4, and 5	0.3212	3 d	78.4	21.6
		Control	25.5	74.5
3, 4, and 45	0.3236	3 d	76.1	23.9
		Control	25.5	74.5
22, 25, and 45	0.2097	3 d	70.5	29.5
		Control	30.6	69.4
22, 23, and 24	0.3162	3 d	78.4	21.6
		Control	26.5	73.5
7, 23, and 45	0.3194	3 d	72.7	27.3
		Control	26.5	73.5
5, 6, 7, and 45	0.2109	3 d	70.5	29.5
		Control	29.6	70.4

Table 15. Discriminant analysis, including variables used for discrimination, resultant Eigen values, and resultant classification arrays, for the 5 d versus control groups, Experiment 2.

Variables	Eigen values	Classification array (percent)		
		Actual	Predicted	
			<u>5 d</u>	<u>Control</u>
3, 4, 5, and 6	0.0797	5 d Control	64.1 33.7	35.8 66.3
4, 5, and 6	0.0784	5 d Control	62.1 34.7	37.9 65.3
3, 4, and 5	0.0797	5 d Control	64.2 33.7	35.8 66.3
3, 4, 5, and 45	0.0865	5 d Control	66.3 36.7	33.7 63.3
3, 4, 5, 6, and 45	0.0870	5 d Control	64.2 36.7	35.8 63.3
4, 5, 6, and 45	0.0869	5 d Control	66.3 36.7	33.7 63.7
4, 5, 6, 7, and 45	0.1026	5 d Control	64.2 41.8	35.8 58.2
5, 6, 7, and 45	0.0967	5 d Control	61.5 42.9	38.9 57.1
5, 6, and 7	0.0779	5 d Control	61.1 41.8	38.9 58.2
5, 6, 7, and 8	0.0864	5 d Control	62.1 43.9	37.9 56.1
23, 24, 25, and 26	0.898	5 d Control	62.1 33.7	37.9 66.3
24 and 7	0.0747	5 d Control	65.3 39.8	34.7 60.2
23, 26, and 45	0.0934	5 d Control	69.5 39.8	30.5 60.2

Table 16. Discriminant analysis, including variables used for discrimination, resultant Eigen values, and resultant classification arrays, for the 7 d versus control groups, Experiment 2.

Variables	Eigen values	Classification array (percent)		
		Actual	Predicted	
			<u>7 d</u>	<u>Control</u>
3, 4, 5, and 42	0.3301	7 d Control	77.3 26.5	22.7 73.5
2, 3, 4, 5, and 42	0.3366	7 d Control	75.0 28.6	25.0 71.4
2, 3, 4, and 5	0.2242	7 d Control	73.0 31.6	27.0 68.4
3, 4, and 5	0.2073	7 d Control	72.0 31.6	28.0 68.4
3, 4, 5, and 25	0.2371	7 d Control	71.0 30.6	29.0 69.4
3, 4, 5, and 45	0.2073	7 d Control	72.0 31.6	28.0 68.4
3, 4, 5, and 6	0.2108	7 d Control	70.0 29.6	30.0 70.4
4, 5, and 45	0.2015	7 d Control	69.0 29.6	31.0 70.4
2, 4, and 5	0.2214	7 d Control	71.0 29.6	29.0 70.4
2, 3, 4, 5, and 25	0.2475	7 d Control	70.0 29.6	30.0 70.4
2, 3, 4, 5, and 45	0.2254	7 d Control	73.0 31.6	27.0 38.4
2, 3, 4, and 24	0.2019	7 d Control	75.0 29.6	25.0 70.4
22, 23, 24, and 25	0.2188	7 d Control	72.0 28.6	28.0 71.4

Table 17. Discriminant analysis, including variables used for discrimination, resultant Eigen values, and resultant classification arrays, for the starved versus control groups, Experiment 2.

Variables	Eigen values	Classification array (percent)		
		Actual	Predicted	
			<u>Starved</u>	<u>Control</u>
3, 4, 5, and 42	0.8336	Starved Control	81.9 15.3	18.1 84.7
3, 4, and 5	0.7291	Starved Control	78.7 17.3	21.3 82.7
3, 4, 5, and 6	0.7601	Starved Control	79.8 16.3	20.2 83.7
3, 4, 5, 6, and 42	0.8457	Starved Control	80.9 14.3	19.1 85.7
3, 4, 5, 6, and 43	1.0500	Starved Control	81.9 12.2	18.1 87.8
3, 4, 5, and 45	0.9507	Starved Control	78.7 14.3	21.3 85.7
4, 5, 6, and 45	0.7929	Starved Control	77.7 15.3	22.3 84.7
4, 5, and 6	0.6352	Starved Control	76.6 19.4	23.4 80.6
2, 3, 4, and 24	0.6549	Starved Control	78.7 17.3	21.3 82.7
2, 3, and 4	0.5569	Starved Control	78.7 20.4	21.3 79.6
22, 23, 24, and 25	1.0920	Starved Control	85.1 13.3	14.9 86.7

Table 18. Discriminant analysis, including variables used for discrimination, resultant Eigen values, and resultant classification arrays, for the cold water versus warm water temperature groups, Experiment 2. Scales with less than seven circuli were left out of the data set.

Variables	Eigen values	Classification array (percent)		
		Actual	Predicted	
			<u>Cold</u>	<u>Warm</u>
4, 5, 6, and 7	0.7928	Cold	83.2	16.8
		Warm	18.9	81.1
5, 6, and 7	0.7899	Cold	85.3	14.7
		Warm	21.0	79.0
5, 6, 7, and 45	0.7901	Cold	85.3	14.7
		Warm	21.0	79.0
3, 4, 5, 6, and 7	0.7901	Cold	84.2	15.8
		Warm	18.9	81.1
5, 6, 7, and 8	0.8059	Cold	85.3	14.7
		Warm	20.0	80.0
3, 4, and 5	0.1530	Cold	67.4	32.6
		Warm	42.1	57.9

Table 20. Discriminant analysis, including variables used for discrimination, resultant Eigen values, and resultant classification arrays, for the warm water versus fluctuating water temperature groups, Experiment 2. Scales with less than seven circuli were left out of the data set.

Variables	Eigen values	Classification array (percent)		
		Actual	Predicted	
			<u>Fluctuating</u>	<u>Warm</u>
4, 5, 6, and 7	0.3812	Fluctuating Warm	71.3 22.1	28.7 77.9
5, 6, and 7	0.3437	Fluctuating Warm	69.2 26.3	30.8 73.7
4, 5, 6, 7, and 45	0.7901	Fluctuating Warm	73.4 24.2	26.6 75.8
3, 4, 5, and 6	0.2507	Fluctuating Warm	74.5 30.5	25.5 69.5
5, 6, 7, and 8	0.3702	Fluctuating Warm	73.4 27.4	26.6 72.6

APPENDIX E

COMPARISONS OF ORIGINAL, HATCHERY-REARED, AND COOPER LAKE
CUTTHROAT TROUT SCALE PATTERNS FROM EXPERIMENT 1

Table 21. Comparisons of first six inter-circuli distances between original experimental cutthroat trout and hatchery-reared cutthroat trout. Sample size, mean, standard deviation, and p-values testing for equal means are shown.

Group	Original trout			Hatchery-reared			P-value
	N	Mean	SD	N	Mean	SD	
Control							
200	66.92	11.09	55	65.74	9.89	0.4736	
	35.82	10.74		32.64	10.01	0.0494	
	32.67	9.79		29.43	8.56	0.0264*	
	27.44	7.46		29.20	6.88	0.1179	
	23.63	6.80		25.06	6.60	0.1642	
	23.06	6.72		24.85	4.71	0.0643	
Warm Water							
200	67.89	11.03	61	66.19	9.66	0.2781	
	34.56	10.62		31.99	8.32	0.0836	
	32.94	9.34		29.45	8.70	0.0099*	
	28.13	7.97		26.66	6.91	0.1960	
	23.78	6.15		23.70	4.59	0.9283	
	22.75	6.22		23.14	5.04	0.6543	
Cold Water							
200	65.57	11.50	94	64.62	8.84	0.4783	
	33.56	9.97		34.08	7.72	0.6501	
	32.84	9.33		29.86	7.84	0.0077*	
	27.60	7.60		26.16	6.68	0.1193	
	24.29	6.36		23.92	5.02	0.6197	
	23.58	6.40		22.92	4.90	0.3776	
2/3 Feed							
200	67.96	12.50	61	66.19	9.66	0.7272	
	33.90	10.42		31.99	8.32	0.1920	
	30.28	8.64		29.45	8.70	0.5138	
	28.16	8.98		26.66	6.91	0.2295	
	23.73	7.32		23.70	4.59	0.9748	
	22.63	6.28		23.14	5.03	0.5664	
1/3 Feed							
200	67.54	11.78	55	65.72	10.70	0.3014	
	35.69	9.94		31.94	9.03	0.0124*	
	31.56	9.28		31.39	8.22	0.9001	
	27.60	7.82		26.31	7.16	0.2699	
	23.32	6.78		21.18	5.99	0.0351*	
	21.41	6.54		20.33	4.26	0.2450	

*p-value < 0.05