OFFICE MEMO

TO: Lee & Rinne - raised temp **Date** = CTM

FROM: ~ 1°F / hr. 20° accl. : 29.5 all sp

SUBJECT: - fluctuate brooks actually higher 22-28 (96 hr. 50%)

brown RB 21-27

REMARKS:

- upper incipient lethal temp.
50% survive one week (raised 1-2° / wk.)
- all - Firehole & 2 hatch. 79°F = 27.2
- extrapolating CTM - < 2 hr. 85°
7 hr. - 82°

- Kaya 78. Prog. Fish cult.
Firehole R. / 2 hatchery strains
Thermal resistance

. Fisher, Browne, Cameron, Vyse. Genetics Firehole RB - T.A.F.S. 111(3): 312-316
- dumb - (alleles) - 3 loci monomorphic in hatch - polymorphic in Firehole
- no signif. allelic gene freq. diff = Ennis Hatch
- upper
- lower Firehole

Texas redband
-. Hunter et al Ibid - sterile coho -

★.

TX redband

Cat low Valley

1° F? / day - 50% perish

27.9°

55.8 / 56 / 50.2 = 82°

- 1 day 79 - 80 - 81 - 82

- resistance ? better

- but it not run together
 envir make select compare

OFFICE MEMO

TO: **Date**

FROM: Vanicl, R., S. Zoerlein, and L. Hesse. 1979.

SUBJECT: lethal and preferred temperatures of L. McConaughy rainbow trout versus domestic strain rainbow trout.

REMARKS: Nebr. Game Parks Comm, Tech. Ser. 7: 9-20.

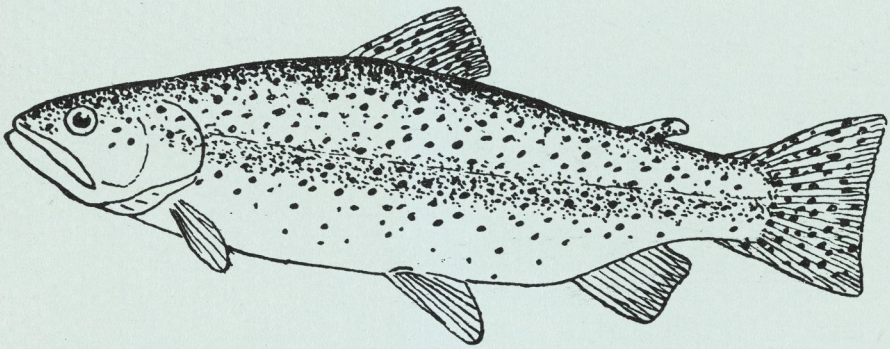
critical thermal maxima ultimate upper lethal temp.

aprx. 27° for McCon., slightly less than 26° for domestic

/ final prefer. 16.4-16.7 / 16.9-17.2

* monthly critical max 30.6 - 32.7 for McCon., increased fm. Apr. - Aug.

NEBRASKA RAINBOW TROUT



Nebraska Technical Series No. 7
NEBRASKA GAME AND PARKS COMMISSION
Eugene T. Mahoney, Director

Nebraska Rainbow Trout

Chapter 1

Lethal and Preferred Temperatures of Lake McConaughy Rainbow Trout Versus Domestic Strain Rainbow Trout

By
R. Vancil, G. Zuerlein and L. Hesse

Chapter 2

A Nitrifying Filter-Cooling Condenser System for Total Water Re-use in Cold-Water Fish Holding or Rearing Applications

By
L. Hesse, G. Zuerlein and R. Vancil

Chapter 3

Biochemical Genetic Analysis of Two Strains of Nebraska Rainbow Trout

By
J. Seeb and L. Wishard

1979
Nebraska Technical Series No. 7
Nebraska Game and Parks Commission
P.O. Box 30370
Lincoln, Nebraska 68503

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Project F-47-R Nebraska

PREFACE

The self-sustaining rainbow trout population living in Lake McConaughy and the North Platte River tributaries is unique to the Great Plains region. The population spends most of its adult life in the reservoir, but moves into tributary streams to spawn. Two spawning runs occur. The largest is from September through November followed by a reduced migration in March and April of the following spring. Rainbow trout fingerlings spend about a year in the stream before smolting and migrating to the reservoir.

Fishing for McConaughy rainbows in the tributaries and reservoir began in the late 1940's. Concern for the maintenance of this fishery prompted several investigations which were directed at development of a management plan. From these studies it was evident that the McConaughy rainbow possessed a selective advantage over hatchery origin fish stocked in the streams and reservoir.

Temperatures, normally too high for successful rainbow survival, were often encountered in the streams and the eutrophying Lake McConaughy. This study was designed to compare the temperature tolerance and preference of McConaughy rainbow trout with hatchery origin fish. Chapter 1 describes the results of this study. Chapter 2 describes the laboratory facility developed to hold fish for relatively long periods while conducting the experiments. Chapter 3 contains the results of studies designed to characterize the genetic differences between hatchery fish from Massachusetts and the self-sustaining McConaughy rainbow trout. The Pacific Fisheries Research Laboratory in Olympia, Washington, was contracted to perform an electrophoresis characterization of the frequency of biochemical genetic variants among the McConaughy and hatchery rainbow groups.

Contents

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance of all Nebraska Game and Parks personnel who were directly or indirectly involved with this study. Dirk Shoemaker provided assistance throughout the project on data collection as well as daily maintainence of fish stocks and equipment. Harold Sheldon and Mark Dicke contributed valuable services during the construction phase of the laboratory. The efforts of Brad Newcomb, Leigh Ann Devore (Retlesdorf) and Steve Satra were appreciated during data collection. Mel Taylor provided consultation during data processing. Dr. Harold Klaassen of Kansas State University offered some valuable suggestions on the dechlorination of tap water.

Chapter 1

Lethal and Preferred Temperatures of Lake McConaughy Rainbow Trout Versus Domestic Strain Rainbow Trout

By
R. Vancil, G. Zuerlein and L. Hesse

ABSTRACT

Investigations were conducted in 1978 to determine the temperature tolerance and preference of the McConaughy strain rainbow trout and make comparisons with a domestic strain. Lethal and preferred temperature tests were performed in the laboratory on both strains. Critical thermal maxima tests were conducted in the field on the McConaughy strain. The ultimate upper lethal temperature for the McConaughy strain was determined to be approximately 27°C while that for the domestic strain was slightly less than 26°C. The final preferenda for the McConaughy and domestic strains were 16.4 to 16.7°C and 16.9 to 17.2°C, respectively. A difference in mean ages of the two strains probably influenced temperature selection. The mean monthly critical thermal maxima for the McConaughy strain fish ranged from 30.6 to 32.7°C and increased throughout the season (April to August).

It appears that high temperature generally should not be a critical limiting factor for rainbow trout fingerlings in the North Platte Valley tributary streams.

INTRODUCTION

Trout from many sources across the country were stocked into the North Platte Valley streams between 1911 and 1945. The existence or extent of natural reproduction was unknown during this period. The closing of Kingsley Dam in 1945 created a new coldwater environment, and stream trout populations after 1945 were influenced by reservoir rainbow populations. Hatchery originated rainbow trout were stocked into the North Platte Valley streams between 1946 and 1967. However, during this period a population of self-sustaining migratory reservoir rainbows became established in the same streams where hatchery rainbows were being stocked. Investigations into the cohabitation of the wild and stocked rainbows led to the eventual termination of the stocking of hatchery trout. Information on finclipped recaptures of both strains returning to the spawning streams indicated poor survival of the domestic strain (Van Velson 1974). A fingerling stocking program, which utilized wild McConaughy brood stock, was initiated in 1968 in the North Platte tributary streams which did not support natural reproduction.

The spawning streams of the North Platte Valley are subjected to a variety of adverse conditions. During the agricultural season, flooding, irrigation return flows, and water releases from the numerous canals in the valley impact the streams. Turbidity becomes relatively high during this summer season. Water temperatures can exceed 24°C and diurnal fluctuations can be as much as 10°C (Van Velson 1974).

Lake McConaughy water quality poses potential limitations on rainbow trout. Threinen (1958) described 21.1°C (70°F) as the threshold above which distress occurred in rainbow trout. Suitable habitat for rainbow trout in reservoirs has been defined as water having a temperature of 21.1°C (70°F) or colder and a dissolved oxygen content of at least 3 mg/1 (Kirkland and Bowling 1966). Based on these criteria, Lake McConaughy trout habitat was found to be severely restricted by August in some years (Van Velson 1974, 1978).

Previous studies have shown that selective breeding can alter temperature tolerances (Gibson 1954, Donaldson and Olson 1957). It is conceivable that temperature tolerance could also be altered by natural selection. Previous studies have suggested that the McConaughy rainbow survived in the North Platte Valley when domestic fish could not. If the McConaughy rainbow trout has a higher temperature tolerance, improving its chance for survival, it would be valuable to define this tolerance.

The quantity of "trout water" in Lake McConaughy cannot be adquately determined until "trout water" for the McConaughy strain has been more precisely defined. In addition, Nebraska has isolated cold water streams in other sections of the state where trout presently exist in small numbers. The McConaughy strain might prove valuable to these marginal trout habitats. Once their temperature requirements are known, introductions of this strain can be assessed.

Establishing the temperature needs of this apparently tolerant strain will give other states the option of evaluating their stocking needs based on the knowledge that another strain of rainbow trout is available.

MATERIALS AND METHODS

Both domestic and McConaughy rainbow trout were obtained from Rock Creek Hatchery, Parks, Nebraska. The domestic strain fish were received as eyed eggs at Rock Creek Hatchery in early March, 1978 from Plymouth Rock, Massachusetts. The McConaughy strain eggs were collected from adults migrating up the North Platte River from Lake McConaughy between December, 1977 to February, 1978. Eggs of both strains were hatched in 11°C well water and the fry were later transferred to rearing ponds at 14°C. The domestic strain fish were fed a commercial trout feed while the McConaughy strain fish were fed the same with ground beef liver added as a supplement.

One thousand fish of each strain were transported to the laboratory on May 9, 1978. Domestic fish numbered approximately 1100/kg, while McConaughy fish numbered about 1000/kg. Fish of both strains averaged 43 mm TL and were fed four times daily an amount of food totaling 4% of body weight per day throughout the experiment. All fish were held for 10 days in a stock tank prior to testing. Domestic water circulated through this holding tank at an exchange rate of once every 52 minutes. Incoming water was charcoal filtered to remove chlorine and the filters were backflushed monthly. Holding tank temperature varied from 11°C in April to 16°C in August.

At the time lethal testing began the domestic fish were 3 months of age and averaged 53.8 mm TL with a range of 40

to 75 mm. The McConaughy strain fish were 3.5 to 4 months of age with a mean TL of 59.2 mm and a range of 40 to 104 mm. At the time of the preferred temperature testing, the domestic rainbows were 4 months of age with a mean TL of 64.9 mm and a range of 52 to 79 mm. The McConaughy strain rainbow trout were 4.5 to 5 months of age and ranged from 55 to 88 mm TL with an average of 67.4 mm.

Air temperature in the test facility was held at 18-20°C. A 12-hour lighting cycle was provided with timer controlled fluorescent lights.

Prescribed acclimation temperatures of 10°, 15°, 20°, and 23°C were established for each strain in eight 189 l glass aquaria. Each aquarium had an undergravel filter operated by air pressure, which removed settled solids and provided circulation, and aeration. Diatomaceous earth filters were used daily to remove the fine particulate matter and free floating stages of any aquatic parasites. Parasitic and bacterial disease was effectively negated during the entire study. The temperature was monitored with a tele-thermometer equipped with a remote probe. All fish were acclimated for at least one week prior to any test.

A nitrifying filter-cooling condenser system was designed for this investigation. Details of this system are presented in Chapter 2.

Lethal Temperature Testing

Lethal temperature tests were performed in a series of 23 aquaria. Each was equipped with a combination heater/thermostate, airstones, a carbon corner filter, and an undergravel filter. Water, air stones, and carbon were replaced between tests to maintain good water quality. Test temperatures ranged from 22-30°C (by 2° intervals) for fish acclimated at 10°C; 24-30°C for fish acclimated at 15°C; 26-30°C for fish acclimated at 20°C; and 26-32°C for fish acclimated at 23°C. Attempts were made to control test temperatures to within ± 0.5°C of the stated temperatures. Some fluctuations did occur, but tests were postponed if the amount of deviation from the desired temperature approached 1.0°C. Three complete replicates of each test were performed. Six fish were tested at a time in each aquarium and were discarded after each experiment.

The fish were placed in their respective test tanks, and the time in minutes elapsed until 50% mortality (median lethal tolerance or TL50) occurred was recorded for each test (Brett 1952, 1956; Fry et al. 1946). Tests were terminated after one week. Mean TL50 values of each strain were compared by Student's t test and ultimate upper lethal temperatures for the two strains were determined graphically.

Preferred Temperature

The preferred temperature tests were performed in a vertical gradient tank similar to that reported by Brett (1952). Inside dimensions of the tank were 111 cm high, 80 cm wide, and 17 cm deep. The gradient was formed using a closed cycle cooling coil at the bottom and a closed cycle heating coil at the top. The front of the tank was divided into 12 cells by wires stretched horizontally across the plexiglass. A temperature probe was inserted into the water column in each cell. Water temperatures in all cells were monitored on a 12 channel tele-thermometer.

The procedures for determining preferred temperatures and final preferenda were similar to that reported by Brett (1952) and McCauley and Tait (1970). Ten fish of a single strain from a single acclimation temperature were introduced into the preference tank at a level of temperature equal to their acclimation temperature. After all fish behaved in a normal manner, the lights were turned off and the fish were left undisturbed for 30 to 45 minutes. Following this adjustment period, a 35mm camera and electronic flash were used to obtain a photograph of the fish at a rate of once every 10 minutes for 2 hours. After each photograph, the temperatures in all cells were recorded. Darkness was maintained throughout each 2 hour sequence to reduce territorial behavior. The electronic flash did not appear to alter behavior. Analysis of variance was used to determine the effects of strain on temperature preference. Acclimation temperature was included in the analysis as a covariate.

Critical Thermal Maxima

Critical thermal maxima (CTM) were determined for McConaughy strain rainbow trout one day each month in 1978 from April to August. Fingerlings and smolts for the sample were seined from Nine Mile Creek, a North Platte Valley stream which supports natural reproduction.

Critical thermal maxima (CTM) for the McConaughy strain rainbow trout was determined in a manner similar to that described by Holland et al. (1974) and Huntsman and Sparks (1924). Five or six fingerlings were tested every two hours on each test day and a limited number of smolts were also tested. Testing began at dawn and concluded by late afternoon when ambient stream temperature reached its peak. Fish were captured with a 6 mm mesh seine immediately prior to each test period. A container of ambient water holding one fish was heated until the fish's equilibrium was lost. CTM was reached if the fish recovered in ambient water. The test was rerun on another specimen if this criterion was not met. The water was heated at a rate of 2.6°C

per minute (± 0.73 SD). Fingerlings ranged from 27 mm to 163 mm TL and averaged 86.7 mm TL. The mean TL of smolts was 216.6 mm with a range of 154 mm to 255 mm.

All data analyses were performed on an IBM 370 computer.

RESULTS

Acclimation temperatures fluctuated from the prescribed values; actual mean acclimation temperature, standard deviation, and coefficient of variation were calculated for each acclimation tank (Table 1).

Table 1. - Mean acclimation temperature, standard deviation, and coefficient of variation for each prescribed acclimation temperature.

Strain	Acclimation Temperature (°C) Prescribed	Mean Observed ± SD		Coefficient of Variation (%)	Number of Observations
McConaughy	10	9.4	±0.2	2.4	7
	15	16.1	±0.9	5.4	7
	20	19.7	±1.0	5.0	7
	23	22.7	±2.0	8.6	6
Domestic	10	10.1	±0.7	6.6	28
	15	16.2	±1.2	7.2	29
	20	20.7	±1.5	7.4	27
	23	23.1	±1.0	4.1	21

Lethal Temperature Tolerances

The difference in mean TL50 between strains proved to be insignificant ($P>0.05$) for all tests (Table 2). McConaughy fish acclimated at 20°C and 23° survived week long test temperatures of 26°C; the domestic fish did not survive at this temperature. At higher acclimation temperatures McConaughy rainbows have a higher upper lethal temperature than the domestic variety.

Brett (1952) demonstrated a highly significant linear relationship between the common logarithm of TL50 and test temperature (temperature causing death). This was true in the present study. Regression analyses of the log transformed TL50 with test temperatures for each strain at each acclimation temperature showed a highly significant correlation ($P<0.001$). This relationship of TL50 to test temperatures for each strain is presented in Figure 1. The solid line represents the range of test temperatures that resulted in 50% mortality. The dotted line represents the temperature at which mortality no longer occurred (upper lethal temperature).

Upper lethal temperatures were assumed to fall mid-way between the lowest temperature at which a mean TL50 was determined and the adjacent test temperature at which all three replications survived the 1-week test. An adjustment was made for the McConaughy fish acclimated at 20°C, since

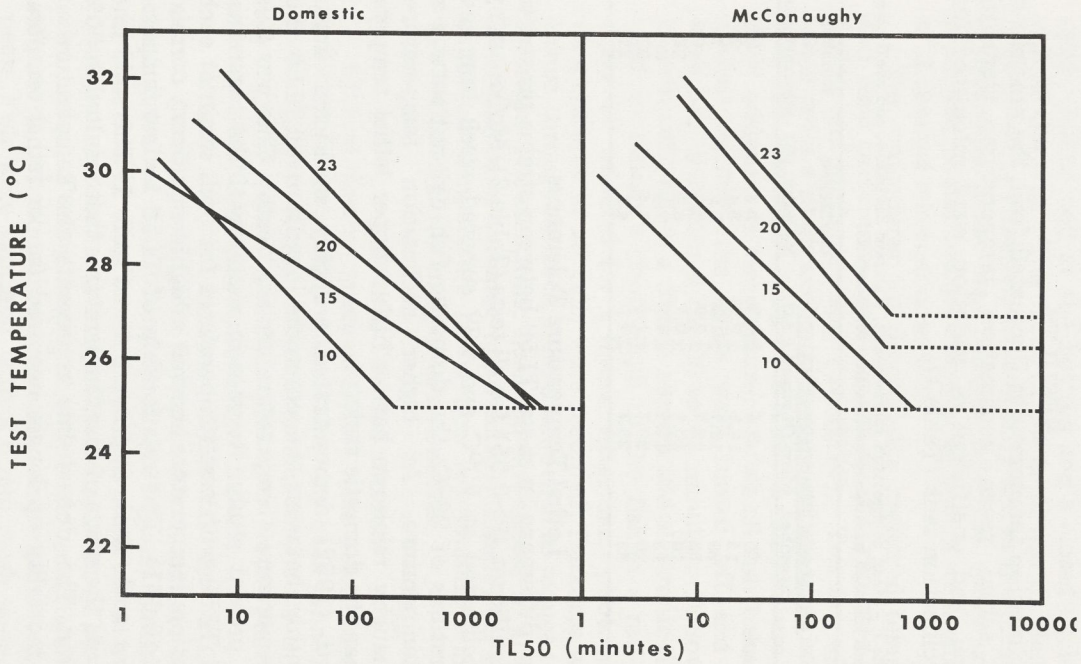


Figure 1. Relationship of median lethal tolerance to high test temperatures for each strain of rainbow trout. The solid oblique lines represent this relationship within the range of test temperatures resulting in 50% mortalities. Each line represents an individual acclimation temperature. The dotted horizontal lines approximate the temperatures at which mortality no longer occurs from high temperatures (upper lethal temperatures).

Table 2. -Mean TL50 (median lethal tolerance) in minutes with standard deviations. Each mean determined from 3 replications.

Prescribed Acclimation Temp. (°C)	Test Temp. (°C)	Mean TL50 ± SD McConaughy		Domestic	
10	26	85.3 ±	6.4	88.0 ±	9.5
	28	6.7 ±	4.6	18.3 ±	6.4
	30	1.7 ±	0.6	4.0 ±	5.2
15	26	290.7 ±	109.3	2049.0 ±	2984.7
	28	48.7 ±	5.7	37.0 ±	18.5
	30	6.3 ±	4.0	1.7 ±	0.6
20	26	6929.0 ±	5457.7[a]	2160.0 ±	2096.1
	28	108.0 ±	17.1	134.0 ±	59.4
	30	25.7 ±	3.5	23.7 ±	18.8
23	26		——b	3101.3 ±	2258.5
	28	225.7 ±	93.1	203.0 ±	8.7
	30	38.3 ±	7.4	41.3 ±	16.6
	32	8.7 ±	5.5	11.7 ±	7.4

[a]Two replications went beyond the time limit of the test. (> 10080 min.).
[b]All replications exceeded the length of the test for the McConaughy strain.

only one TL50 was reached out of three replicate tests. The ultimate upper lethal temperature for the domestic strain appears to be less than, but approaching, 26°C (>24°C but <26°C). The ultimate upper lethal temperature for the McConaughy strain is definitely above 26°C and appears to approach 27°C (>26°C but <28°C).

Following each test, the total lengths (mm) of the dead fish and the fish remaining alive were recorded. The difference between the means was less than 1 mm. Total length did not appear to affect lethal temperature tolerance within the size range tested.

Preferred Temperature

Mean preferred temperatures of domestic fish were consistently higher than those chosen by the McConaughy strain over the entire range of acclimation temperatures (Figure 2). The 45° diagonal line, indicating all points where the acclimation temperature and the preferred temperature were equal, was used to determine the final preferenda. Analysis of covariance, treating acclimation temperature as a covariate and strain as the main effect, revealed that strain was a highly significant factor ($P<0.001$) (Table 3). A com-

Table 3. -Analysis of covariance for preferred temperature with mean acclimation temperature as a covariate and strain as a factor.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	Significance of F
Covariates	11.239	1	11.239	5.546	0.018
Acclim. Temp.	11.239	1	11.239	5.546	0.018
Main Effects	113.826	1	113.826	56.167	0.001
Strain	113.826	1	113.826	56.167	0.001
Explained	125.064	2	62.532	30.857	0.001
Residual	1856.331	916	2.027		
Total	1981.396	918	2.158		

plete summary of the mean preferred temperatures for each strain from each acclimation temperature is depicted in Table 4 along with standard deviations, skewness, and modal intervals. Final preferenda were also determined algebraically by solving the regression equation for each strain for the point where acclimation temperature and preferred temperature were equal. Depending on the method used, the final preferendum for the McConaughy strain was found to be from 16.4-16.7°C, while the domestic strain preferred 16.9-17.2°C.

It was assumed that time was not a factor in the temperature selected by the trout (i.e. preferred temperature did not change with time within the 2 hour test periods). This hypothesis was evaluated by simple linear regression of the individual preferred temperature tests. The regressions of

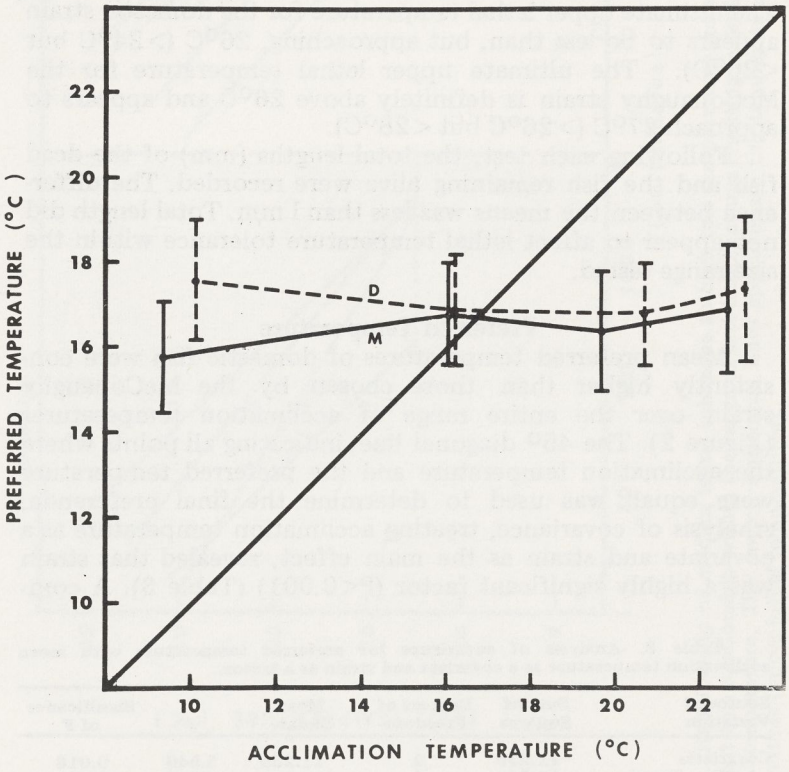


Figure 2. Mean preferred temperatures for each strain of rainbow trout at each acclimation temperature. Vertical lines indicate a range of ± one standard deviation from each mean. The broken line indicates the domestic strain while the solid line designates the McConaughy strain.

Table 4. — Summary of observed preferred temperatures (PT) for each strain at each acclimation temperature (AT).

Strain	AT (°C)	PT (°C) ± SD	Modal Interval	Skew-ness	N	Final Preferenda Graphic	Final Preferenda Algebraic
McConaughy	9.4	15.8 ± 1.3	15.0 - 15.9	1.47	130	16.7	16.4
	16.1	16.8 ± 1.2	16.0 - 16.9	1.45	123		
	19.7	16.4 ± 1.4	16.0 - 16.9	4.53	116		
	22.7	16.9 ± 1.5	16.0 - 16.9	0.71	118		
Domestic	10.1	17.6 ± 1.5	17.0 - 17.9	-0.23	130	16.9	17.2
	16.2	16.9 ± 1.3	17.0 - 17.9	0.02	119		
	20.7	16.8 ± 1.2	16.0 - 16.9	4.01	116		
	23.1	17.4 ± 1.7	16.0 - 16.9	1.48	67		

preferred temperature with time generally did not show significant correlation ($P>0.05$).

Critical Thermal Maxima

Critical thermal maxima of fingerling McConaughy strain rainbow trout showed a pronounced seasonal increase. At the beginning of the season the mean CTM's were 30.6-30.7°C, but increased to 32.7°C by the end of the season. A summary of mean CTM, ambient temperature, and total length is shown in Table 5. The CTM values were correlated with month. Ambient temperature and total length were also correlated with CTM over the season but not within any month.

In May, 1978 an equal number of smolts and fingerlings were tested. Ambient temperature remained constant due to overcast weather. The mean CTM's for the two age groups were compared by Student's t test. Since the mean CTM was expected, and observed, to be higher for the younger fish, a one-tailed t-test was selected. Mean CTM's for the fingerlings and smolts were 30.6°C and 29.7°C, respectively. This difference proved to be significant ($P<0.01$).

Table 5. —Means and standard deviations for variables in CTM analyses of age group 0 rainbow trout.

Month	Variable	Mean ± SD	N
April	CTM (min)	30.7 ± 0.95	23
	Amb. Temp. (°C)	13.3 ± 2.93	23
	Total Length (mm)	55.9 ± 9.30	23
May	CTM (min)	30.6 ± 0.39	12
	Amb. Temp. (°C)	11.8 ± 0.00[a]	12
	Total Length (mm)	68.1 ± 19.82	12
June	CTM (min)	31.5 ± 0.48	29
	Amb. Temp. (°C)	15.5 ± 2.74	29
	Total Length (mm)	80.0 ± 16.67	29
July	CTM (min)	32.7 ± 0.67	31
	Amb. Temp. (°C)	17.2 ± 2.58	31
	Total Length (mm)	96.5 ± 27.69	31
August	CTM (min)	32.7 ± 0.54	27
	Amb. Temp. (°C)	15.7 ± 1.13	27
	Total Length (mm)	117.0 ± 28.01	27
Months Combined	CTM (min)	31.8 ± 1.10	122
	Amb. Temp. (°C)	15.2 ± 2.84	122
	Total Length (mm)	86.7 ± 30.49	122

[a]No change occurred in ambient temperature during the day

DISCUSSION

Lethal Temperature Tests

The ultimate upper lethal temperatures of the two strains tested compare closely with previous studies on rainbow trout. Bidgood and Berst (1969) found the ultimate upper lethal temperature to be 25-26°C while Cherry et al. (1977) reported 25°C. These results are similar to the 26°C upper lethel temperature determined for the domestic fish in this study. The McConaughy trout strain's upper lethel temperature was slightly higher (27°C). Bidgood and Berst (1969) used fish 6-7 months of age which ranged in length from 37 mm to 92 mm. Cherry et al. (1977) used fish 50 mm to 100 mm in length and less than one year old.

Preferred Temperature

A wide range of final preferenda have been reported by previous investigators (see Table 6). They suggested that much of this variation might be due to the type of gradient (horizontal vs. vertical) and the position of the gradient (in a vertical gradient tank). Evidence tending to negate these theories has been presented by McCauley and Pond (1971) and Brett (1952), respectively. Kwain and McCauley (1978) have shown age to be negatively correlated with preferred temperature. With each additional month of age for rainbow trout of 1 to 12 months, the preferred temperature was found to decrease by approximately 0.5°C. The information in Table 6 also tends to support this hypothesis.

The final preferenda in the present study of 16.4 to 16.7°C for the McConaughy strain and 16.9 to 17.2°C for the domestic strain fell well within the range of final preferenda shown for rainbow trout in Table 6. Fish younger than 4-5 months generally showed higher final preferenda while older fish generally showed lower final preferenda.

The previously mentioned relationship of age with final preferendum (Kwain and McCauley 1978) raises another consideration. Even though a highly significant difference in preferred temperatures was found between the two strains of rainbow trout, this difference may not be a result of a genetically determined temperature preference, but may, in fact, be related to the difference in age between the two strains.

Since the McConaughy strain fish were approximately 0.5-1 month older than the domestics, it is conceivable that the McConaughy fish would have a lower final preferendum based on Kwain and McCauley's (1978) finding that an increase in age of one month relates to a decrease in final preferendum of approximately 0.5°C.

Table 6. —Final preferenda of McConaughy and domestic strains of rainbow trout compared with those of previous studies.

Final Preferendum (°C)	Size	Age	Other Factors	Reference
20	38-76mm	3-4 mo		Javaid & Anderson 1967
19.2-19.8	50-100mm	<1 yr		Cherry el al 1977
18 - 20	----	1 mo	varying light intensity	Kwain & McCauley 1978
17 - 20	40-50mm	3 mo		McCauley & Pond 1971
18	-----	<1 yr		Cherry el al 1975
17.6-18.0	-----	4-5 mo	darkness	Kwain & McCauley 1978
16.9-17.2	52-79mm	4 mo	domestic strain	(present study)
16.4-16.7	55-88mm	4½-5 mo	McConaughy strain	(present study)
12-15	-----	12 mo	varying light intensity	Kwain & McCauley 1978
13.6	-----	>1 yr		Ferguson 1958
13.0	102-152mm	17 mo		Garside & Tait 1958
11-17	fingerlings	----		Mantelman 1958
11.3	(205g)	15 mo		McCauley et al 1977

Ferguson (1958) compared laboratory and field data on final preferenda for various fishes, and attributed the lack of agreement in final preferenda to age differences.

Reynolds (1977) also recognized that fishes in nature selected lower temperatures than did fishes in the laboratory, but he concluded that this difference could not always be attributed to size or age effects.

Horak and Tanner (1964) used vertical gill nets to determine distribution of fish in a reservoir. Rainbow trout ranging in size from 160-635 mm in total length were captured at temperatures from 5.0-23.9°C. The majority were determined to be at temperatures of 16.1-21.1°C with a modal interval of 18.9-21.1°C. (Due to the skewness of the distribution, the mean was slightly less).

Axon (1974) used vertical gill nets to determine that rainbow trout were found most frequently at 15.0-17.2°C. But these field studies involved older fish than were used in most of the laboratory investigations.

The temperature requirements of both strains were similar. Laboratory experimentation failed to demonstrate temperature as a factor providing a selective advantage to the McConaughy strain.

Critical Thermal Maxima

No critical thermal maxima data were obtained for the domestic trout, so comparison with the McConaughy strain is not possible. Additional data on the smolts would have been desirable since only 17 were tested. The inclusion of more smolts and additional sampling effort in March and early April would have permitted a more thorough analysis of the effects due to age and season.

Huntsman and Sparks (1924), Otto et al. (1976) and Holland et al. (1974) reported heating rates of 0.2°C per second, 0.3°C per second, and 1°C per second, respectively. A mean heating rate in the present study of 2.6°C per minute was considerably faster than reported in the earlier studies. Fry et al. (1946) reported that "on heating an animal slowly, the temperature attained before death takes place varies with the heating rate". Consequently, the CTM values reported herein could have been affected by the rate of heating.

SUMMARY AND CONCLUSIONS

The ultimate upper lethal temperature was determined to be approximately 27°C (>26° and <28°C) for the McConaughy strain and approaching 26°C (>24°C and < 26°C) for the domestic strain. Although the upper tolerances for each strain, when acclimated to high temperatures, were widely separated, statistical significance could not be demonstrated. The present findings do indicate that temperature is not a critical limiting factor to the immediate survival of McConaughy strain rainbow trout in the North Platte tributary streams based on previous stream temperature data (Van Velson 1978).

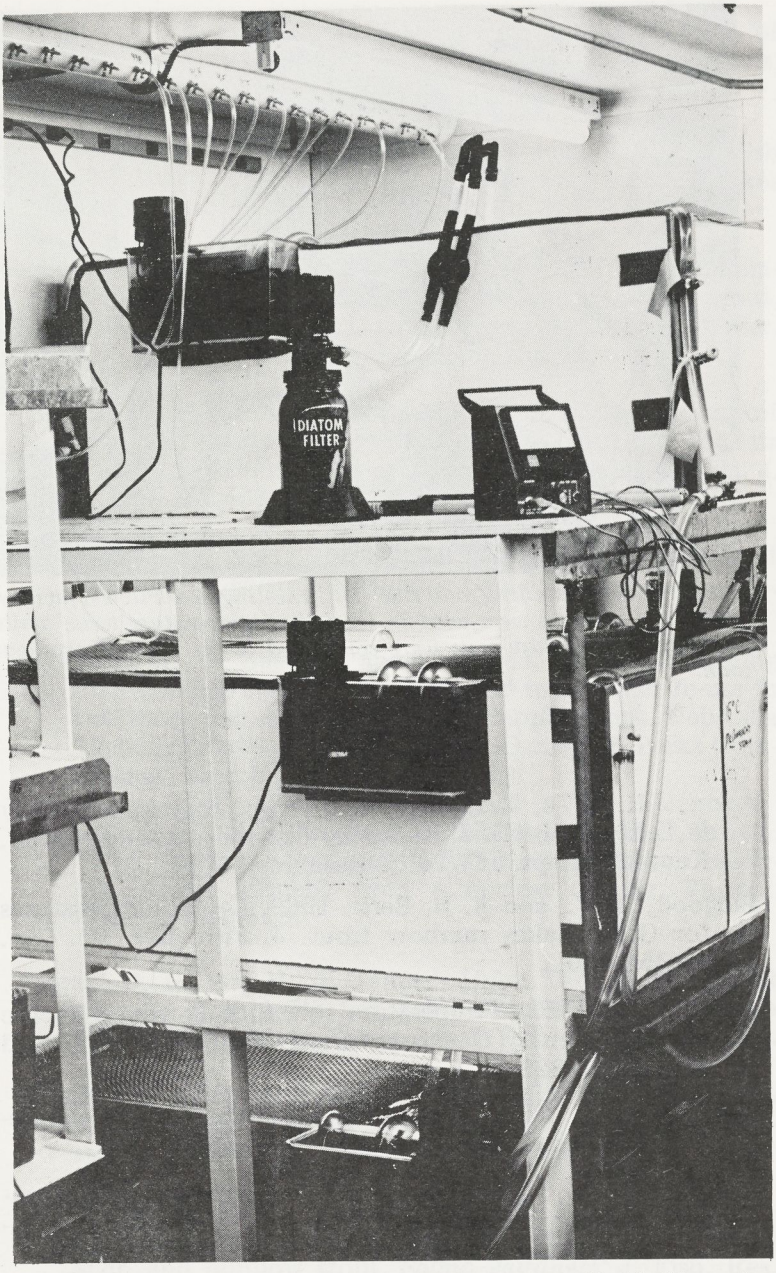
Final preferendums determined by two methods were found to range from 16.4 to 16.7°C for the McConaughy strain and from 16.9 to 17.2°C for the domestic strain. The overall preferred temperatures showed a highly significant difference between the two stains. One possible explanation for this difference was a difference in ages of the two strains of fish.

Mean monthly critical thermal maxima increased through the season. The range of these monthly means was 30.6 to 32.7°C. Smolts were found to have a significantly lower mean CTM than fingerlings.

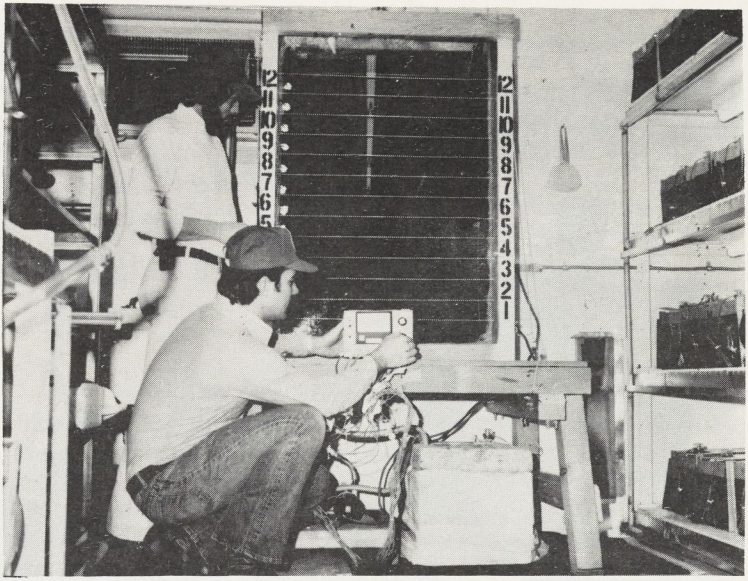
RECOMMENDATIONS

Future temperature investigations in the North Platte tributary streams should be aimed at delineating and quantifying the relationship of growth and overall production of rainbow trout to existing temperature regimes.

Future temperature investigations involving the McConaughy strain rainbow trout should place emphasis on the smolts captured from the North Platte River, it's tributaries and Lake McConaughy.



Attached to the 189 liter acclimation tanks were small side filters that contained activated carbon and diatomaceous earth filters. Cross siphon tubes connected each pair of tanks. The main plastic feed and return lines were used to cycle aquarium water to the biofilters outside the constant temperature room.



Authors Hesse, standing, and Zuerlein, kneeling, work in test facility. The small aquaria were used in the lethal temperature tests, while preferred temperature tests were conducted in the vertical gradient tank.

Literature Cited

Axon, J.R. 1974. Evaluation of the two story trout fishery in Lake Cumberland. Kentucky Fisheries Bulletin No. 60. Kentucky Dept. of Wildlife Resources. 69pp.

Bidgood, B. F., and A. H. Berst. 1969. Lethal temperatures for Great Lakes rainbow trout. J. Fish. Res. Bd. Can. 26:456-459.

Brett, J. R. 1952. Temperature tolerance in young Pacific salmon, Genus *Onchorhynchus*. J. Fish Res. Board Can. 9:265-323.

_____1956. Some principals in the thermal requirements of fishes. The Quart. Rev. Biol. 31:75-87.

Cherry, D.S., K. L. Dickinson, and J. Cairns, Jr. 1975. Temperature selected and avoided by fish at various acclimation temperatures. J. Fish. Res. Board Can. 32:485-491.

Cherry, D. S., K. L. Dickinson, J. Cairns, Jr., and J. R. Stauffer. 1977. Preferred, avoided, and lethal temperatures of fish during rising temperature conditions. J. Fish. Res. Board Can. 34:239-246.

Donaldson, L. R., and P. R. Olson. 1957. Development of rainbow trout brood stock by selective breeding. Trans. Amer. Fish. Soc. 87:93-101.

Ferguson, R. G. 1958. The preferred temperature of fish and their mid-summer distribution in temperate lakes and streams. J. Fish. Res. Board. Can. 15:607-624.

Fry, F. E. J., J. S. Hart, and K. F. Walker. 1946. Lethal temperature relations for a sample of young speckled trout, *Salvelinus fontinalis*. Univ. Toronto Studies, Biol. Ser. No. 54, Publ. Ont. Fish. Res. Lab., No. 64.

Garside, E. T., and J. S. Tait, 1958. Preferred temperature of rainbow trout (*Salmo gairdneri* Richardson) and its unusual relationship to acclimation temperature. Can. J. of Zool. 36:563-567.

Gibson, M. B. 1954. Upper lethal temperature relations of the guppy, *Lebistes reticulatus*. Can. J. Zool. 32:393-407.

Hesse, L. W., G. Zuerlein, and R. Vancil. 1979. A nitrifying filter-cooling condenser system for total water re-use in coldwater fish holding or rearing applications. Nebraska Technical Series No. 7, Nebraska Game and Parks Commission, Lincoln, Nebraska. p.21-28

Holland, W. E., M. H. Smith, J. W. Gibbons, and D. H. Brown. 1974. Thermal tolerances of fish from a reservoir receiving heated effluent from a nuclear reactor. Physiol. Zool. 47:110-118.

Horak, D. L., and H. A. Tanner. 1964. The use of vertical gill nets in studying fish depth distribution, Horsetooth Reservoir, Colorado. Trans. Amer. Fish. Soc. 93:137-145.

Huntsman, A. G., and M. I. Sparks. 1924. Limiting factors for marine animals. Relative resistance to high temperatures. J. Fish. Res. Board Can. 2:97-114.

Javaid, M. Y., and J. M. Anderson, 1967. Thermal acclimation and temperature selection in Atlantic salmon, *Salmo salar*, and rainbow trout, *Salmo gairdneri*. J. Fish. Res. Board Can. 24:1507-1513.

Kirkland, L., and M. Bowling. 1966. Preliminary observations on the establishment of a reservoir trout fishery.

Proc. 20th Ann. Conf. Southeast Game and Fish Com. p. 364-374.

Kwain, W., and R. W. McCauley. 1978. Effects of age and overhead illumination on temperatures preferred by underyearling rainbow trout, *Salmo gairdneri*, in a vertical temperature gradient. J. Fish. Res. Board Can. 35:1430-1433.

Mantelman, I. I. 1958. Distribution of the young of certain species of fish in temperature gradients. Fish. Res. Board Can. Translation Series, No. 257. 52pp.

McCauley, R. W., and J. R. Elliot, L. A. Read. 1977. Influence of acclimation temperature on preferred temperature in the rainbow trout *Salmo gairdneri*. Trans. Amer. Fish. Soc. 106-362-365.

McCauley, R. W., and W. L. Pond. 1971. Temperature selection of rainbow trout (*Salmo gairdneri)* fingerlings in vertical and horizontal gradients. J. Fish. Res. Board Can. 28:1801-1804.

McCauley, R. W., and J. S. Tait. 1970. Preferred temperature of yearling lake trout, *Salvelinus namaycush*. J. Fish Res. Board Can. 27:1729-1733.

Otto, R. G., M. A. Kitchell, and J. O. Rice. 1976. Lethal and preferred temperatures of the alewife *(Alosa pseudoharengus*) in Lake Michigan. Trans. Amer. Fish. Soc. 105: 92-106.

Reynolds, W. W. 1977. Temperature as a proximate factor in orientation behavior. J. Fish. Res. Board Can. 34: 734-739.

Threinen, C. W. 1958. Cause of mortality of a midsummer plant of rainbow trout in a southern Wisconsin Lake, with notes on acclimation and lethal temperatures. Prog. Fish Cult. 20:27-32.

Van Velson, R. C. 1974. Self-sustaining rainbow trout (*Salmo gairdneri*) population in McConaughy Reservoir, Nebraska. Trans. Amer. Fish. Soc. 103:59-64.

_____1978. The McConaughy rainbow. . . life history and a management plan for the North Platte Valley. Nebraska Technical Series No. 2, Nebraska Game and Parks Commission, Lincoln, Nebraska. 83 pp.

Chapter 2

A Nitrifying Filter-Cooling Condenser System for Total Water Re-use in Cold-Water Fish Holding or Rearing Applications

By
L. Hesse, G. Zuerlein and R. Vancil

Introduction

The investigation into temperature dynamics of a strain of rainbow trout *(Salmo gairdneri)* led to the creation of a new biological filter that combines nitrification with temperature control. The primary application is for laboratory research, but the need for water re-use systems in hatcheries may prompt the expansion of this system.

A quantity of quality water is essential when cold water fish species are being studied or reared. Since temperature control is difficult with a total flow through system, a closed system is often required.

Burrows and Combs (1968) experimented with oyster shell and rock substrate in a trickling filter, but found that the system had to be cleaned every other day. The U.S. Fish and Wildlife Service Salmon Cultural Laboratory near Longview, Wash., investigated the use of styrofoam balls as a nitrifying substrate. They were able to support a maximum fish loading of 0.08 kg per m^3 of media but void spaces were so small that clogging eventually disabled the system. We experimented with various combinations of filters using a large volume of charcoal, but found that the system quickly became overloaded with ammonia at low fish densities. Harris (1977) experimented with two biological filter media, polypropylene "Flexrings" and a rigid vinyl module called "Flocor". Under 90% re-use conditions Harris found that 0.98 m^3 of Flexring media would support 227 kg of 280 mm trout. Similar results were obtained with the Flocor units.

The present system was used to support fingerling rainbow trout at various acclimation temperatures throughout the course of a temperature experiment. At the conclusion of the study a monitoring and stocking program was undertaken to establish carrying capacity of this system.

The circulating pumps were capable of moving 12 liters of water per minute. The pump which carried water back to the aquaria was circuited through a float switch to avoid overflows in the system. A float in the submerged filter reservoir cycled the pump on and off to maintain a specified water level.

Temperature was controlled with thermostatically operated remote condensers. The 10° C tanks required a three-quarters HP compressor; the 15° C and 20° C tanks each had one-third HP compressors attached. Aquarium room temperatures varied slightly around 20° C and aquaria heaters were used, in tank, to work in conjunction with the cooling coil for more precise control.

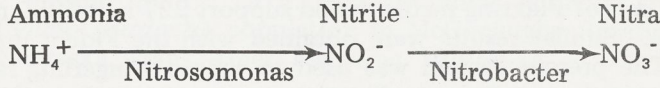
Fish were fed a commercial diet at 4% of their body weight per day.

Total ammonia was monitored with a specific ion analyzer equipped with an ammonia probe; pH and temperature were routinely monitored. The un-ionized ammonia fraction of total ammonia was calculated from published tables (Trussell 1972).

THE MEDIA

The trickling filter contained 0.2 m^3 of Ceramic Biosaddles. Ceramic media were chosen over plastic because the controlled porosity increased available surface area for bacterial attachment and the rough surface was advertised to provide quicker establishment of colonies. The smallest Biosaddles available were used as they were designed specifically for nitrification work and our application was not expected to produce a large biomass of solids. The media was randomly dumped and resulted in a 77% void space. The surface area per unit volume was 255.8 m^2/m^3. The only obvious disadvantage of the ceramic media over the plastic media was the weight difference. The ceramic media weighed 674 kg/m^3, 15 fold heavier than the smallest plastic media but only one-third as heavy as a similar volume of aggregate material.

The filter was seeded with a freeze-dried preparation of *Nitrosomonas* sp and *Nitrobacter* sp. The nitrification process was expected to proceed as follows (Mitchell 1974).



The filters were used to complete a temperature tolerance and preference investigation before this carrying capacity

study was initiated. The 45 days needed to complete the temperature study allowed bacterial colonies to become firmly established.

THE SYSTEM

Fish loading densities for three temperatures (10°, 15°, 20° C) were determined by stocking at random intervals while monitoring temperature, pH, and total ammonia. Un-ionized ammonia is toxic to fish and is also most difficult to measure directly. The fraction of total ammonia that is of the un-ionized form can be found when pH and temperature is known by using Table 1. As a design limit, the value of 0.0125 mg/1 un-ionized ammonia was used as a maximum allowable concentration. This was derived from work with rainbow trout chronically exposed for 9 to 12 months (Smith and Piper 1975).

THE FILTER AND COOLING RESERVOIR

Three separate systems were established, each a duplicate of Figure 1. The fish were held in two 189 l glass aquaria connected by cross tank siphons. Water to be filtered was drawn from the bottom of tank number 2 and pumped through a sprinkler head onto a trickle filter bed of the biofilter media; gravity carried the effluent into a second chamber that served as a secondary filter and cooling reservoir. The second reservoir was partially filled with a rock-oyster shell substrate in a submerged filter mode. Standing water above this bed was cooled by a remote condenser unit which pumped a refrigerant (Freon 12) through a copper coil in the water above the submerged bed. All copper lines in contact with aquarium water were cleaned with acid and sealed with epoxy paint. This chilled, filtered water was then pumped back into the top of aquarium number 1.

The containers used to hold the filter media were 176 liter, heavy gauge plastic, trash cans.

The cooling system effectively maintained the prescribed temperatures. The test temperature means (SD) were computed as follows for 59 observations at each temperature:

10.3C (1.1C)
15.6C (1.5C)
20.0C (0.9C)

All supply lines leading to and from the filters were insulated since these were outside the aquarium room where ambient temperature variation was often great.

1. 189 liter aquarium
2. 189 liter aquarium
3. Cross tank siphon
4. Circulating pump No. 1
5. Adjustable stop-cock (screw shutoff)
6. Sprinkler head
7. Trickle filter (176 liter)
8. Submerged filter and temperature regulating reservoir (176 liter)
9. Remote cooling condenser, Compressor
10. Strainer–dryer
11. Expansion Valve
12. Thermostat with capillary tube and bulb
13. Thermostat bulb
14. Copper cooling coil
15. Plastic float
16. Float switch
17. Circulating pump No. 2
18. Adjustable stop-cock (screw shutoff)
19. Filter intake
20. Filter outfall

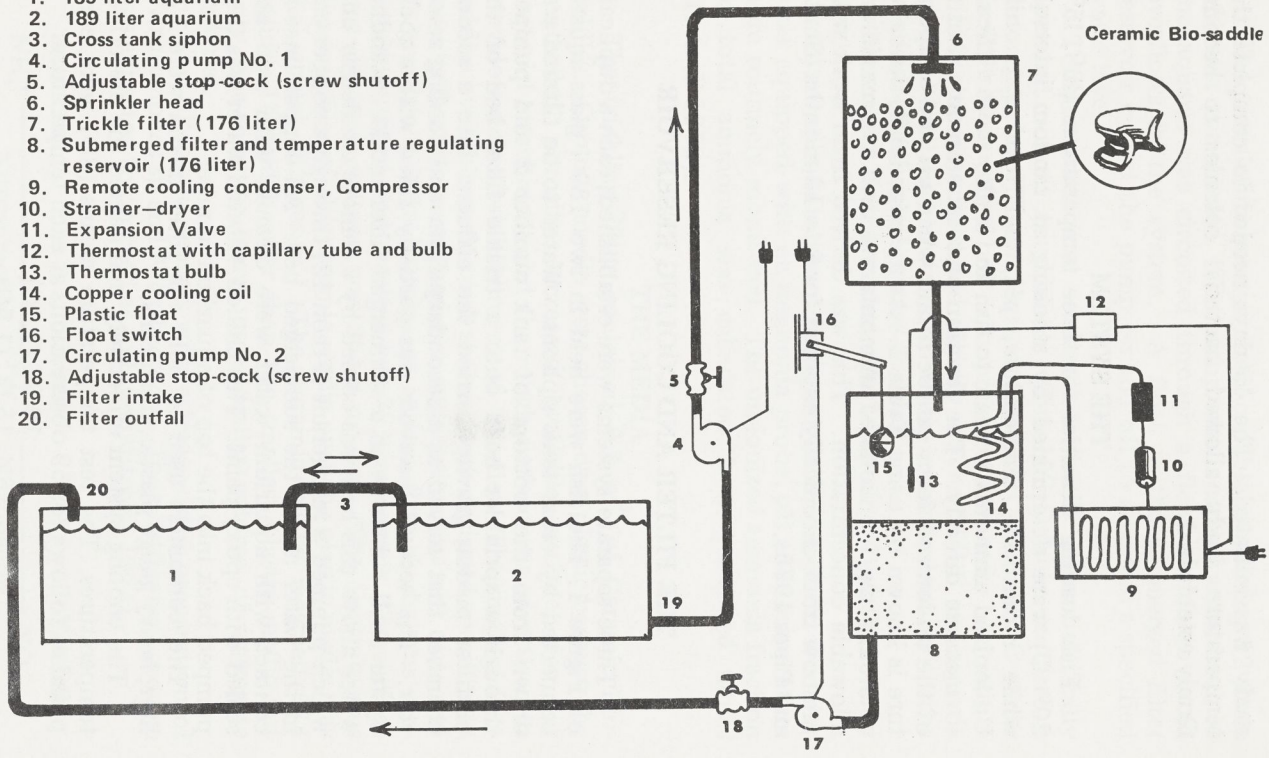


Figure 1. Packed biological reactor and temperature control reservoir in water re-use applications for holding or rearing freshwater fish.

Table 1. Percentage of un-ionized ammonia in aqueous ammonia solutions at different pH's and temperatures (Trussell 1972).

	(C) 5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
pH	(F) 41.0	42.8	44.6	46.4	48.2	50.0	51.8	63.6	55.4	57.2	59.0	60.8	62.6	64.4	66.2	68.0	69.8	71.6	73.4	75.2	77.0
6.5	.04	.04	.05	.05	.06	.06	.06	.07	.07	.08	.09	.09	.10	.10	.12	.13	.13	.14	.16	.17	.18
6.6	.05	.05	.06	.06	.07	.07	.08	.09	.09	.10	.10	.12	.13	.13	.15	.16	.17	.18	.20	.21	.22
6.7	.06	.07	.07	.08	.09	.09	.10	.11	.12	.13	.14	.15	.16	.17	.18	.20	.21	.23	.25	.26	.28
6.8	.08	.09	.09	.10	.11	.12	.13	.14	.15	.16	.17	.19	.20	.21	.23	.25	.27	.29	.31	.33	.35
6.9	.10	.11	.12	.13	.14	.15	.16	.17	.19	.20	.22	.23	.25	.27	.29	.32	.34	.36	.39	.42	.44
7.0	.12	.14	.15	.16	.17	.19	.20	.21	.24	.25	.27	.29	.31	.34	.37	.40	.42	.45	.49	.52	.55
7.1	.16	.17	.19	.20	.22	.23	.26	.27	.30	.32	.34	.37	.39	.42	.46	.50	.53	.57	.62	.66	.70
7.2	.20	.22	.23	.25	.27	.29	.32	.34	.37	.40	.43	.46	.50	.53	.58	.63	.67	.71	.77	.83	.88
7.3	.25	.27	.30	.32	.34	.37	.40	.43	.47	.51	.54	.58	.62	.67	.73	.79	.84	.90	.97	1.04	1.10
7.4	.31	.34	.37	.40	.43	.47	.51	.54	.59	.64	.68	.73	.78	.84	.91	.99	1.05	1.13	1.22	1.30	1.38
7.5	.39	.43	.47	.50	.54	.59	.64	.68	.74	.80	.85	.92	.98	1.06	1.15	1.24	1.32	1.42	1.53	1.63	1.73
7.6	.49	.54	.59	.63	.68	.74	.80	.85	.93	1.00	1.07	1.16	1.24	1.33	1.44	1.56	1.66	1.78	1.92	2.05	2.17
7.7	.62	.68	.74	.80	.86	.92	1.01	1.07	1.17	1.26	1.35	1.45	1.55	1.67	1.81	1.96	2.08	2.23	2.41	2.57	2.72
7.8	.78	.85	.93	1.00	1.08	1.16	1.27	1.35	1.46	1.58	1.69	1.82	1.95	2.09	2.26	2.45	2.61	2.79	3.01	3.21	3.39
7.9	.98	1.07	1.16	1.25	1.35	1.46	1.59	1.69	1.83	1.98	2.12	2.29	2.44	2.62	2.83	3.06	3.26	3.48	3.76	4.01	4.24
8.0	1.22	1.34	1.46	1.58	1.70	1.83	1.10	2.12	2.30	2.48	2.65	2.86	3.05	3.28	3.54	3.83	4.07	4.35	4.69	4.99	5.28
8.1	1.54	1.68	1.83	1.98	2.13	2.29	2.50	2.65	2.88	3.11	3.32	3.58	3.81	4.09	4.42	4.77	5.07	5.41	5.83	6.21	6.55
8.2	1.93	2.11	2.29	2.48	2.67	2.86	3.12	3.32	3.59	3.88	4.14	4.46	4.75	5.10	5.50	5.94	6.30	6.72	7.23	7.69	8.11
8.3	2.41	2.64	2.87	3.10	3.33	3.58	3.90	4.14	4.48	4.84	5.16	5.55	5.90	6.33	6.82	7.36	7.80	8.31	8.94	9.49	10.00
8.4	3.02	3.30	3.59	3.87	4.16	4.46	4.87	5.15	5.58	6.01	6.41	6.89	7.32	7.84	8.44	9.09	9.62	10.24	10.99	22.66	12.27
8.5	3.77	4.12	4.47	4.82	5.18	5.55	6.05	6.40	6.92	7.45	7.98	8.52	9.04	9.68	10.40	11.18	11.82	12.56	13.45	14.25	14.97
8.6	4.70	5.13	5.57	5.99	6.44	6.89	7.50	7.93	8.56	9.21	9.79	10.49	11.12	11.88	12.74	13.68	14.44	15.31	16.37	17.30	18.14
8.7	5.85	6.38	6.91	7.43	7.97	8.53	9.26	9.78	10.54	11.32	12.02	12.86	13.61	14.51	15.53	16.63	17.53	18.54	19.77	20.84	21.82
8.8	7.25	7.90	8.54	9.18	9.84	10.50	11.38	12.01	12.92	13.84	14.68	15.67	16.55	17.61	18.30	20.07	21.22	22.27	23.68	24.90	26.00
8.9	8.96	9.74	10.52	11.28	12.07	12.87	13.92	14.66	15.74	16.82	17.80	18.96	19.98	21.20	22.57	24.02	25.19	26.51	28.09	29.44	30.66
9.0	11.02	11.96	12.89	13.80	14.74	15.68	16.91	17.78	19.04	20.30	21.42	22.75	23.91	25.30	26.85	28.47	29.78	31.26	32.96	34.44	35.76

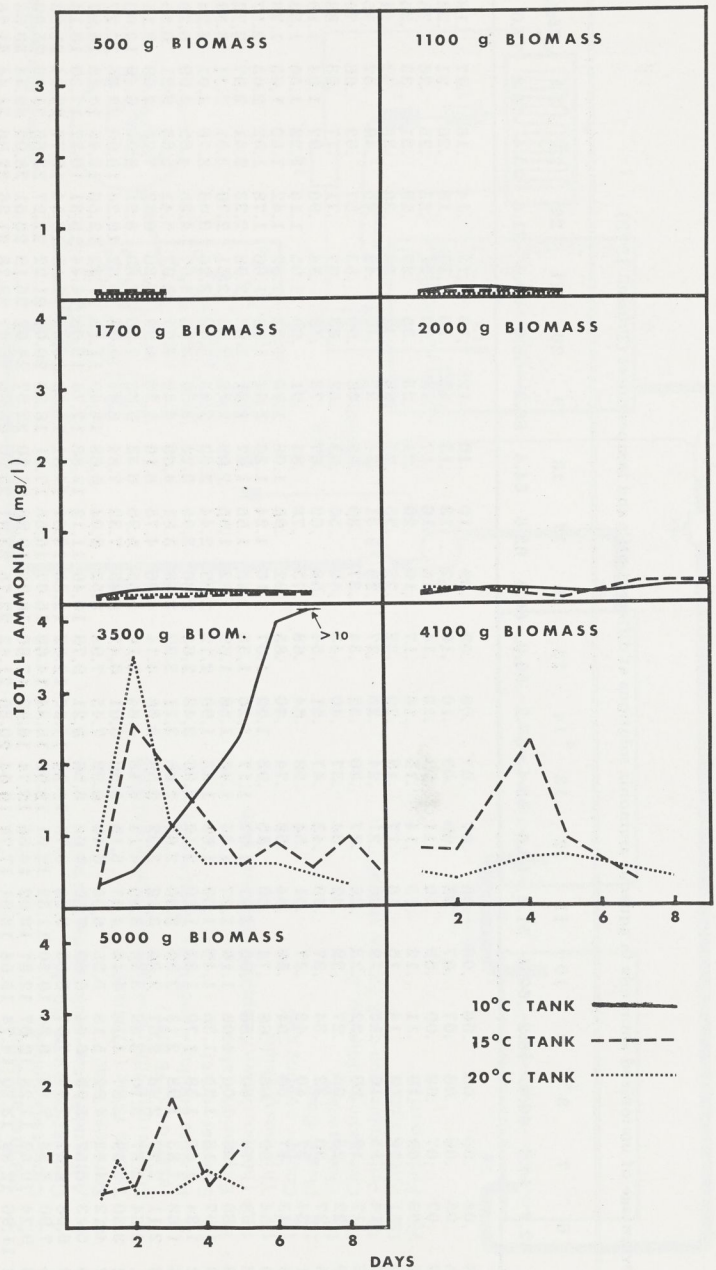


Figure 2. The relationship between increased biomass of rainbow trout and total ammonia for three different temperatures.

CARRYING CAPACITY

Total ammonia was unaffected by increased biomass until the standing crop reached 3.5 kg (Figure 2). Total ammonia should have been related to bacterial populations; as biomass increased, total ammonia was expected to increase, level off, and then decline. Temperature proved to be an important variable. The 10°C tanks experienced a drastic rise in total ammonia to more than 10.0 mg/1. Stressed fish appeared and many were removed in order to save the tank. At the time the study was terminated, biomass in the 10°C tanks had not been brought back up to 3.5 kg. It is apparent that water temperature could be an important limiting factor in the operation of this biofilter; 15°C tanks experienced a greater initial rise in total ammonia than 20°C tanks.

Fish fed more vigorously and completely in the 15° C water than in either the 20° C or 10° C tanks, although 20° water was apparently more favorable to bacterial colonization.

A highly significant correlation ($P<0.001$) was found to exist between total ammonia and biomass loading (Figure 3). The maximum stocked biomass resulted in slightly overcrowded conditions and yet it was felt that this loading was well below the system carrying capacity for ammonia levels.

The toxic, un-ionized form of ammonia made up a small portion of the total depending on temperature and pH. The percent of total ammonia made up by the un-ionized form can be obtained from Table 1. As previously discussed, 0.0125 mg/1 un-ionized ammonia has been determined to be

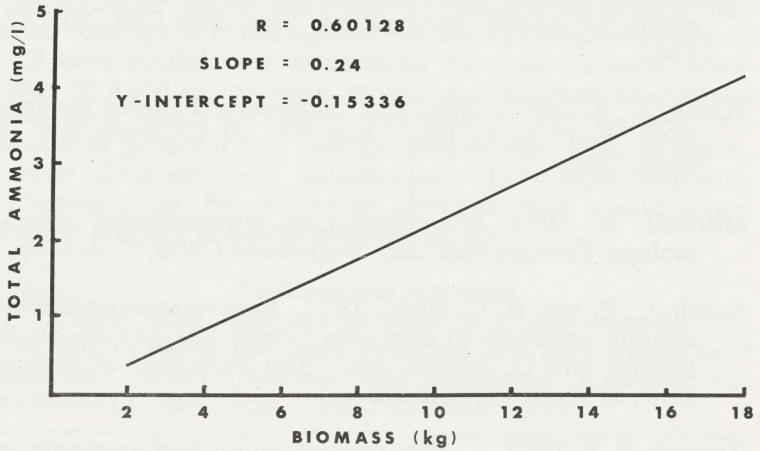


Figure 3. Simple linear regression of total ammonia with biomass of rainbow trout.

the maximum allowable concentration for chronic exposure to rainbow trout. Carrying capacity for a system of this type can be estimated by choosing a temperature and pH near the values expected to occur within the system, and determining the percent of un-ionized ammonia. The corresponding total ammonia reading where the above determined percentage equals 0.0125 mg/l un-ionized ammonia can be used with Figure 3 to establish an approximate carrying capacity. For example, a total ammonia reading of 4.7 mg/l (15° C and 7.0 pH) would be required to exceed the design limit of un-ionized ammonia. An approximate carrying capacity (20 kg) can be determined by satisfying the regression equation and solving for biomass:

$$\text{Biomass (kg)} = \frac{\text{Total ammonia} + .15336}{0.24}$$

It is evident from Table 1 that if either pH or temperature, or both, could be lowered, a resulting increase in carrying capacity could be expected.

Literature Cited

Burrows, R. E., and B. D. Combs. 1968. Controlled environments for salmon propagation. Prog. Fish Cult. 30:123-136.

Harris, L. E. 1977. Nitrifying bacterial substrates for hatchery water reuse. Spec. Rep. No. 40, Colorado Div. of Wildl. 29pp.

Mitchell, R. 1974. Introduction to environmental microbiology. Prentice-Hall Inc. Englewood Cliffs, N. J. 355p.

Smith, C. E. and R. G. Piper. 1975. Lesions associated with chronic exposure to ammonia. Pages 297-514. In W. E. Ribelin and G. Migaki, eds. The Pathology of Fishes. Univ. of Wisc. Press. Madison.

Trussell, R. P. 1972. The percent un-ionized ammonia in aqueous ammonia solutions at different pH levels and temperatures. J. Fish. Res. Board Canada 29:1505-1507.

Chapter 3

Biochemical Genetic Analysis of Two Strains of Nebraska Rainbow Trout

By
J. Seeb and L. Wishard[1]

Introduction

This report presents the results of a biochemical genetic analysis of two groups of Nebraska rainbow trout. One group was a domestic strain acquired from hatchery fish in Massachussetts; the other was a wild strain derived from various transplants during the period from 1911 to 1945 which now survives in the North Platte River drainage (McConaughy strain). This paper identifies biochemical genetic differences which distinguish the two strains and discusses the implications of such differences.

Electrophoresis was used to obtain the biochemical genetic data. These data allow a population or a species to be characterized by its frequency of biochemical genetic variants. Groups of individuals which share a common gene pool will have similar gene frequencies for these variants. However, if isolation between groups occurs, these frequencies may shift either by natural selection or such random processes as genetic drift, and the isolate may develop significantly different gene frequencies. These gene frequency differences can be used as an effective stock identification tool to identify non-interbreeding groups.

Methods and Materials

Muscle, liver, and eye samples from 100 domestic and 100 McConaughy fish were collected and kept frozen until analysis. Electrophoresis was conducted following the methods of Utter et al. (1974) and May (1975).

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[1]Pacific Fisheries Research, 6901 Libby Road Northeast, Olympia, Wash. 98506

During electrophoresis the various proteins were subjected to an electric current and subsequently migrated different distances through a starch gel according to their relative charge and structure. After electrophoresis, histochemical staining techniques described in Allendorf et al. (1977) were used to visualize the proteins.

For each electrophoretically detectable locus the mobility (distance travelled) of the most common protein type found in rainbow trout was used as a standard. The mobility of all other variants or alleles was calculated relative to this common allelic form which was designated with a mobility of 100. An allelic protein that migrated half as far as the common protein would be designated (50). In the case of multiple forms of the same enzyme, a hyphenated number is attached to the protein abbreviation. A list of the 30 loci scored for the two rainbow strains is given in Table 1.

Average genetic heterozygosity ($\overline{H}$) or the average proportion of the genome heterozygous per individual (Selander and Johnson, 1973) were estimated for the 2 strains by the formula:

$$\overline{H} = \left(L - \sum_{i=j}^{L} \sum_{j=1}^{A_i} P^2_{ij} \right) / L$$

where L is the number examined, A_i is the number of alleles at a particular locus, P_{ij} is the frequency of the jth allele at the ith locus. This is a good measure of the amount of total variation found within a population.

Results and Discussion

A broad screening of approximately 45 genetic loci was conducted on the two strains of rainbow trout. Of these 45 loci a total of 30 loci were consistently resolved in the two strains (Table 1). Seven of the 30 loci were polymorphic displaying at least 2 alleles at a particular locus (Table 2). These polymorphic loci provided the raw material necessary to genetically compare the two strains. Any significant differences in allele frequencies at the polymorphic loci were considered as strong evidence for genetic isolation and lack of interbreeding.

Chi-square contingency tests (2 x k where k is the number of alleles) were performed at each locus to test for significance. The results of the 7 possible chi square tests are

Table 1. A list of the enzymes and their abbreviations used in this study. Since different genes may code for the same product, each is given a numerical identifier which follows the abbreviation, i.e., LDH-2 is the second locus coding for lactate dehydrogenase.

Enzyme	Abbreviation
Alcohol dehydrogenase	ADH
Aspartate aminotransferase	AAT 1-3
Creatine kinase	CK
Beta-glucuronidase	BGD
Alpha-glycerophosphate dehydrogenase	AGP
Isocitrate dehydrogenase	IDH3,4
Lactate dehydrogenase	LDH 1-5
Leucyl alanine peptidase	LA
Leucylglycylglycine peptidase	LGG
Malate dehydrogenase	MDH 1-4
Malic enzyme	ME
Peptidase (glycyl leucine)	GL
6-phosphogluconate dehydrogenase	6PG
Phosphoglucomutase	PGM
Phosphaglucose isomerase	PGI 1-3
Phosphomannose isomerase	PMI
Sorbitol dehydrogenase	SDH
Tetrazolium oxidase	TO

given in Table 3. At least four loci[2] showed significant differences (IDH-3, MDH-3,4, AAT-1, and TO). Two loci (MDH-3,4 and AAT-1) were significant at less than the 0.001 level. The differences demonstrated at these four loci indicated that the two strains were indeed genetically distinct.

The McConaughy and domestic strains also differed in average heterozygosity ($\bar{H}$) which was the average amount of variation per individual. These values were calculated for both strains with the following results:

Strain	Average Heterozygosity ($\bar{H}$)
Domestic	0.057
McConaughy	0.037

[2] For purposes of discussion the variation in MDH-3, 4 will be assumed to have occurred at one locus.

Table 2. Allele frequencies for seven polymorphic loci for the McConaughy and domestic strains of Nebraska rainbow trout.

Population	N	AAT-1		AGP		PGM		TO		ME	
		100	120	100	140	100	90	100	152	100	80
McConaughy	99	1.00	.00	.97	.03	.91	.09	.87	.13	.99	.01
Domestic	100	.91	.09	.99	.01	.90	.10	.79	.21	1.00	.00

MDH-3-4				IDH-3			
100	67	118	95	100	38	67	171
.84	.15	.01	.00	.83	.14	.02	.01
.74	.25	.00	.01	.73	.21	.04	.02

Table 3. Results of the chi-square contingency tests.

Locus	df	X^2
AAT-1	1	18.66**
AGP	1	1.34
IDH-3	2	8.79*
MDG-3, 4	1	12.27**
ME	1	2.03
PGM	1	0.02
TO	1	4.45*

* Significant at the 0.05 level
** Significant at the 0.001 level

The domestic strain had variant alleles which occurred at generally higher frequencies than the McConaughy strain at nearly all loci. Reduced heterozygosity within a population of a particular species most likely indicates that the population has passed through periods of small population numbers in which variation was lost by random factors. A situation of small population numbers has probably occurred within the McConaughy strain judging from its relatively low heterozygosity value.

Gene frequency differences such as those exhibited by the domestic and McConaughy strains have proven very useful in mixed fishery analyses. Given the known baseline frequencies and the frequency of a mixture of strains, the proportion of each strain from the mixture can be estimated. This type of work is currently being developed and applied on several salmonid species using a modification of the maximum likelihood method developed by Milner (1977). A simulation model would be necessary, though, to determine the appropriate sample sizes and the associated confidence intervals surrounding the estimate, should a mixed fishery analysis be desired.

Conclusions

This report genetically characterized the domestic and McConaughy strains of rainbow trout using the biochemical genetics and the technique of electrophoresis. The domestic

and McConaughy strains have been shown to be genetically distinct. Not only are the allele frequencies different at 4 loci, but the domestic strain was overall more polymorphic than the McConaughy strain. The frequencies associated with the strains would remain constant through time, assuming no outside manipulation or drastic reduction in population numbers occurred. Thus the differences identified in this paper should provide useful management tools to identify, separate, and follow the progress of the McConaughy and domestic strains in Nebraska.

Literature Cited

Allendorf, F. W., N. Mitchell, N. Ryman, and G. Stahl. 1977. Isozyme loci in brown trout (*Salmo trutta L.*): detection and interpretation from population data. Hereditas 86: 179-190.

May, B. 1975. Eectrophoretic variation in the genus *Oncorhynchus:* The methodology, genetic basis, and practical application to fisheries research and management. M.S. Thesis, University of Washington, Seattle.

Milner, G. B. 1977. Biochemical genetic variation: Its use in mixed fishery analysis. In: Genetic Implications of Steelhead Management, Special Report 77-1, California Cooperative Fishery Research Unit, pp. 10-15 Ed. T. J. Hassler and R. R. VanKirk.

Selander, R. K. and W. E. Johnson. 1973. Genetic variation among vertebrates species. In Proceedings of the International Congress of Zoology, Monaco.

Utter, F. M., Hodgins, H. O., and F. W. Allendorf. 1974. Biochemical genetic studies of fishes: potentialities and limitations. In Biochemical and Biophysical Perspectives in Marine Biology, Vol. 1. Academic Press, London, New York. p. 213-237.

CTM - heated rate of 2.6°C/min
until equilib lost

Lethal Temp. Tol.
M&C. each 20, 28° survived week at 26°C
47°C
79°
domestics one survival.

- Upper lethal Tol. temp
ca. 27° >26 <28
- can survive no mol 26° for all week
- Domestic - ca 25°.
>24 <26°

Kramer & Dickson

Nebraska Game and Parks Commission
P.O. Box 30370
Lincoln, Nebraska 68503

McConaghy
Trout
lethal temp
electrophoresis
Tex. redband
Temp. notes

FIRST CLASS MAIL

Robert Behnke
Dept. of Fishery & Wildlife Biology
Colorado State University
Fort Collins, CO 80523