A SEROLOGICAL STUDY OF CUTTHROAT TROUT (SALMO CLARKI) FROM TRIBUTARIES AND THE OUTLET OF YELLOWSTONE LAKE

by

JAMES E. LIEBELT

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Approved:

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Head, Major Department

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Chairman, Examining Committee

_____
Dean, Graduate Division

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VITA

James E. Liebelt was born on December 8, 1938 in Kalispell, Montana, the son of Mr. and Mrs. B. A. Liebelt. He completed high school in May, 1956 and graduated from Concordia College with a Bachelor of Arts degree in Biology June, 1960. In April, 1963 he was employed as a chemist by the Anaconda Company in Great Falls, Montana. He married Jeanne Van Tighem of Great Falls, Montana in April, 1965 and they have a son, Michael. He entered the school of graduate studies at Montana State University in September, 1965 and received a Traineeship in September, 1966.

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ABSTRACT

Immunoelectrophoresis of blood serum pools collected during June and July, 1966 from spawning runs of Yellowstone cutthroat trout in 8 streams of Yellowstone Lake was done to determine if racial differences existed among these different spawning runs. Antigenic differences consisted of one precipitin line with the exception of 2 instances where 2 lines occurred. Five discrete populations are indicated. The fish from Cub Creek, Clear Creek, Columbine Creek and Yellowstone River outlet appear to be homogeneous and make up Population I. Fish from Pelican Creek, Yellowstone River inlet, Chipmunk Creek, and Grouse Creek comprise Populations II, III, IV, and V respectively. Factors such as geographic location and time of spawning in the streams may have influenced the mixing or segregation of spawning runs.

INTRODUCTION

A serological study was done on cutthroat trout taken during the spawning season from 7 tributaries and the outlet of Yellowstone Lake, Yellowstone National Park. The objective was to determine if populations could be distinguished from the different spawning areas on the basis of antigenic differences. Studies on trout of Yellowstone Lake show that fish of a given spawning run usually return to their home stream (Ball, 1955; McCleave, 1967). Cope (1957), in a study based on morphological characteristics, suggested that races of cutthroat trout inhabit Yellowstone Lake and each home to a particular stream during the spawning season.

The use of serological analyses to differentiate between various populations of fish has gained wide acceptance in recent years. Serological studies made on several species of trout and salmon have shown the presence of distinct populations. Calaprice and Cushing (1964) found that erythrocyte antigens of rainbow, brown, golden, and cutthroat trout were very distinct and therefore useful in identifying "... species, strains, and individuals." Blood group systems have been demonstrated for brown and rainbow trout by Sanders and Wright (1962) and for rainbow trout and sockeye salmon by Ridgway (1962). Serological differentiation in populations of sockeye salmon have been reported by Ridgway, Cushing, and Durall (1958). Antigenic differences between American and Asian stocks of sockeye salmon were found by Ridgway, Klontz, and Matsumoto (1962).

Serological relationships have been reported for a number of marine fish including Sardinops caerulea (Sprague and Vrooman, 1962), Sebastes

marinus (Sindermann, 1961), Clupea harengus (Sindermann and Mairs, 1959), Alosa pseudoharengus, Alosa aestivalis, Alosa sapidissima, Clupea harengus, and Brevoortia tyrannus (Sindermann, 1962), Squalus acanthias (Sindermann and Mairs, 1961), Raja ocellata (Sindermann and Honey, 1964).

METHODS

Between June 29 and July 13, 1966 blood was taken from 30 or more cutthroat trout (usually $\frac{1}{2}$ females and $\frac{1}{2}$ males) at the following locations: Yellowstone River outlet (1.6 km below Fishing Bridge); Pelican Creek (0.2 km above mouth); Cub Creek (0.8 km above mouth); Clear Creek (0.2 km above mouth); Columbine Creek (0.4 km above mouth); Yellowstone River inlet (8 km above mouth); Chipmunk Creek (0.4 km above mouth); Grouse Creek (0.2 km above mouth)(Fig. 1). The minimum straight line distance between areas sampled is about 1.6 km and the maximum about 29 km. The morphometry of Yellowstone Lake and its tributaries was described by Benson (1961).

Most of the fish sampled were in spawning condition, however, those from Chipmunk Creek and a few from Grouse Creek and the Outlet did not contain mature eggs or sperm. Cutthroat trout were dipped from traps or caught by angling in the streams. Following anesthetization with a solution of MS 222 (1 gm in 4 to 5 liters of water) total lengths were taken and blood was drawn directly from the heart of each fish using a syringe with an 18 gauge needle. One to 5 ml of blood were usually taken from each fish. After removal, the blood was discharged into numbered 75x12 mm Kahn precipitation tubes and kept on ice for 2 to 6 hours before it reached the field laboratory. The blood was processed immediately after reaching the field laboratory for serum removal. Blood clots were ringed in the tubes and centrifuged at 3500 rpm for 5 minutes. Serum was then removed with capillary pipettes, expelled into numbered screw cap vials and stored at about 4° C. The following day it was transported to the University

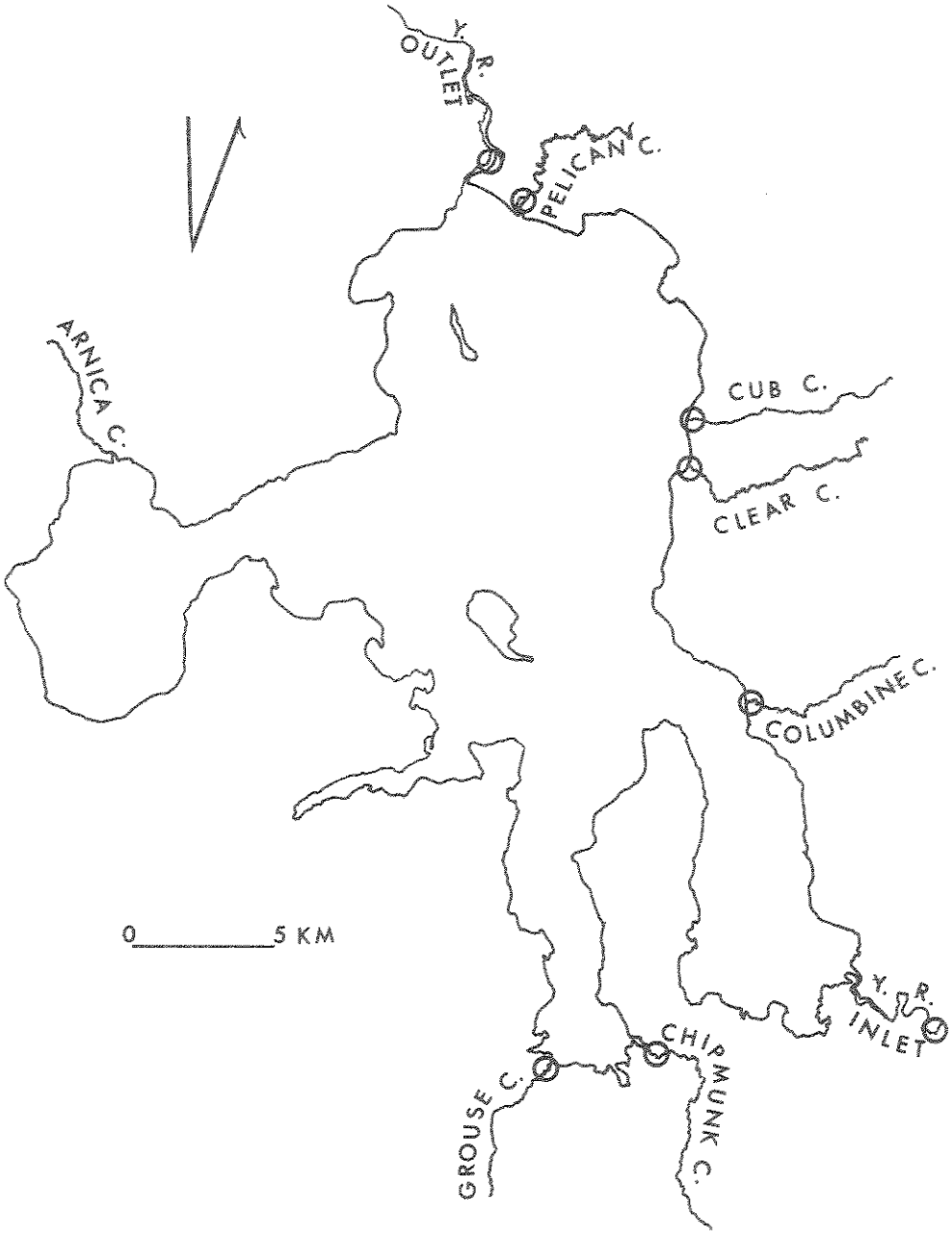

Figure 1. Map of Yellowstone Lake and major tributaries showing sampling locations (circles).

into glass vials and frozen. When 2 rabbits were used for a serum pool the antiserum was pooled.

The similarities and differences of the groups of pooled antigens were first tested using the Ouchterlony immunodiffusion method. Due to the complex nature of the reactions no clear distinctions could be made. In subsequent tests the immunoelectrophoretic method proved to be more successful. The procedure followed that of Campbell et al., 1964, with the exception of the voltage and running time which had to be determined by frequent trials. Separation of the components in cutthroat trout sera was accomplished using 7v/cm for a period of 90 minutes. It was found necessary to use absorbed antisera due to the relatively large number of components present and the close similarities of the patterns. Absorbtions were done by adding about 0.1 cc of pooled cutthroat trout serum to 1.0 cc of antiserum and leaving at room temperature for one-half hour. These were then left at 4° C for periods ranging from 12 to 24 hours, centrifuged, and the absorbed antiserum drawn off. In some cases it was necessary to reabsorb the antiserum up to 4 times to ensure complete removal of all common antibodies which, if not removed, caused similar lines to appear on both antigen well sides. This was determined after the initial electrophoresis of pooled antigens and addition of absorbed antisera. The stained patterns were analyzed with the aid of a binocular microscope.

RESULTS

The results are based on antigenic differences using specifically absorbed antisera (cutthroat trout-antigen pool vs. rabbit anti-cutthroat trout serum). In all cases, the differences represent the least possible number of antigens present. Each antigen pool and its homologous antiserum was tested with the other 7 antigen pools. Differences consisted of a single line except for 2 instances where 2 lines occurred in close proximity to the homologous antigen well. These lines probably represent beta- or gamma-globulins (Figs. 2, 3). The minimum number of runs was 2, but certain tests were run as many as 8 times to help substantiate interpretations. Factors most likely responsible for the difficulties encountered include; poor rabbit immune response, repeated freezing and thawing of sera, excess of antigen or antibody. Others such as age or bacterial contamination of sera, improper absorbtion, masking and variability in intensity of lines, or changes in electrophoretic buffering solution may also have interfered in some of the runs. Table 1 shows the results of all tests.

Pelican Creek antiserum showed at least one antigenic difference with antigen pools of Cub Creek, Columbine Creek, Yellowstone River outlet, and Chipmunk Creek. No antigenic differences were indicated with antigen pools of Clear Creek, Yellowstone River inlet, or Grouse Creek.

Cub Creek and Clear Creek antiserum showed at least one antigenic difference with antigen pools of Pelican Creek, Chipmunk Creek, and Grouse Creek. The antiserum of Columbine Creek and Yellowstone River outlet gave similar results with Pelican Creek and Grouse Creek antigen pools but at

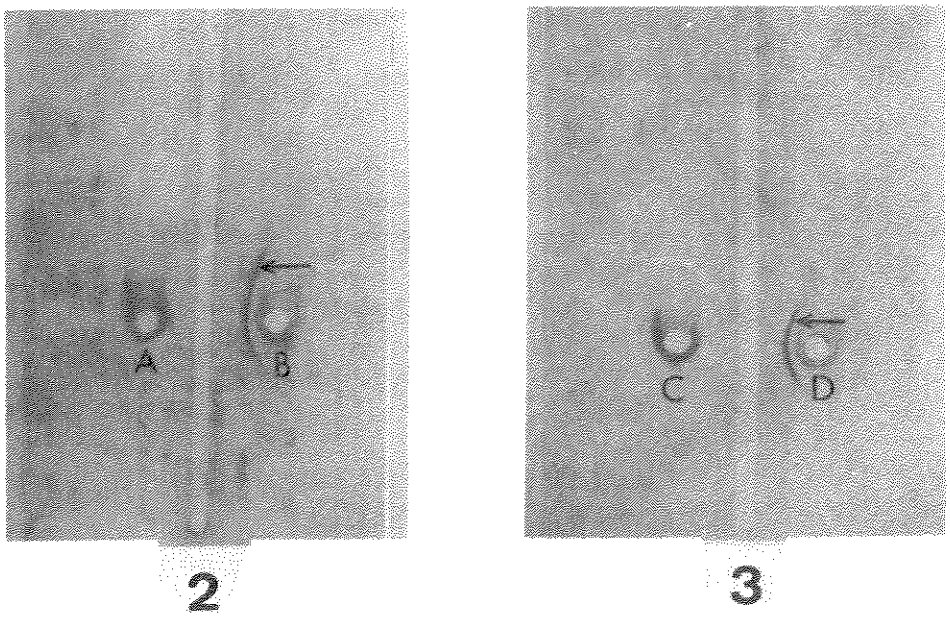

Figure 2. Immunoelectrophoretic pattern of Cub Creek antiserum absorbed with Chipmunk Creek antigen pool. A - Chipmunk Creek antigen pool well. B - Cub Creek antigen pool well. The line (arrow) shows at least one antigen difference between Cub Creek and Chipmunk Creek fish.

Figure 3. Immunoelectrophoretic pattern of Clear Creek antiserum absorbed with Chipmunk Creek antigen pool. C - Chipmunk Creek antigen pool well. D - Clear Creek antigen pool well. The line (arrow) shows at least one antigen difference between Clear Creek and Chipmunk Creek fish.

Table 1. Numbers of absorbtions, runs, and antigenic differences of antigen pools vs. specific absorbed antiserum in the populations of Yellowstone cutthroat trout.

Antigen Pools

Specific Absorbed Antisera	Population		Cub Creek	Clear Creek	Columbine Creek	Yellowstone River Outlet	Pelican Creek	Yellowstone River Inlet	Chipmunk Creek	Grouse Creek
Cub Creek	I	A*	Control-no difference	2	2	2	3	3	1	2
		R*		4	5	7	4	8	2	3
		D*		0	0	0	1	0	1	1
Clear Creek		A	1	Control-no difference	2	1	1	2	1	1
		R	5		6	3	2	5	2	2
		D	0		0	0	1	0	1	1
Columbine Creek		A	2	2	Control-no difference	2	4	4	1	2
		R	4	5		4	8	5	3	3
		D	0	0		0	1	0	2	1
Yellowstone River Outlet		A	3	3	3	Control-no difference	3	3	3	3
		R	6	5	5		4	6	2	2
		D	0	0	0		1	0	2	1
Pelican Creek	II	A	1	1	1	2	Control-no difference	2	2	2
		R	2	6	2	6		7	4	7
		D	1	0	1	1		0	1	0
Yellowstone River Inlet	III	A	1	1	4	1	1	Control-no difference	1	1
		R	4	2	6	4	3		2	3
		D	1	1	0	1	1		1	1
Chipmunk Creek	IV	A	1	1	1	1	1	1	Control-no difference	1
		R	5	2	3	3	5	3		3
		D	1	1	1	1	1	1		1
Grouse Creek	V	A	2	2	2	2	4	3	2	Control-no difference
		R	3	5	3	6	5	5	6	
		D	1	1	1	0	1	0	1	

* The numbers are listed in the order of A - number of absorptions, R - number of runs, D - number of antigenic differences.

least 2 antigenic differences were shown with the Chipmunk Creek antigen pool. These 4 antisera showed no antigenic difference with the remaining 4 antigen pools.

Yellowstone River inlet antiserum indicated at least one antigenic difference with all antigen pools except Columbine Creek. Chipmunk Creek antiserum indicated at least one antigenic difference with all antigen pools, whereas Grouse Creek antiserum indicated at least one antigenic difference with all antigen pools except Yellowstone River inlet and Yellowstone River outlet. All reciprocal tests showed at least one antigenic difference with antigen pools of Pelican Creek and one or 2 differences with Chipmunk Creek antigen pool.

DISCUSSION

Data show the presence of different serum antigens among cutthroat trout sampled which may indicate at least 5 populations.

Plausible explanations for the presence of these populations might be related to such factors as stream location and time of spawning. There are no apparent geographical barriers in Yellowstone Lake which would prevent cutthroat trout of the various spawning runs from mixing with each other. However, distance between tributary streams of a given lake may be a factor in the isolation or in mixing of spawning runs. The time of spawning may be more important than distance in isolating different populations.

The almost identical reactions of Cub Creek, Clear Creek, Columbine Creek, and Yellowstone River outlet antiserum (Population I) with all unrelated antigen pools indicate a homogenous population. The two antigenic differences shown by antiserum of Columbine Creek and Yellowstone River outlet with Chipmunk Creek antigen pool are not considered to be significant enough to further differentiate between fish from these creeks and fish from Cub Creek and Clear Creek. The streams from which the fish of Population I were sampled are located on the north and east shores of Yellowstone Lake. The absence of antigenic differences between Cub Creek and Clear Creek antigen pools may be the result of their mouths being only 1.6 km apart and having nearly coincident spawning runs. A tagging study by McCleave (1967), showed that 2.6% (29 of 1137) Clear Creek fish strayed into Cub Creek and 6.2% (119 of 1908) Cub Creek fish strayed into Clear Creek. Tagging studies of fish from several other spawning runs in

Yellowstone Lake indicated small percentages of the trout strayed during the spawning season (Ball, 1955; Cope, 1957; Ball and Cope, 1961).

The Pelican Creek cutthroat trout population (Population II) is discrete. When antiserum of Pelican Creek was absorbed with antigen pools of Clear Creek, Yellowstone River inlet, and Grouse Creek, no antigen(s) specific for Pelican Creek fish was left as shown by the negative tests. However, the reciprocal tests indicated at least one antigen specific for fish from each of these three streams. The difference shown by Population II may be accounted for by the earlier spawning run in the stream and not related to the location of Pelican Creek which is on the NE shore of Yellowstone Lake.

The spawning run from Yellowstone River inlet (Population III) in the South East Arm is a distinct population as indicated by the antigenic difference. The absence of antigenic difference between Yellowstone River inlet antiserum and Columbine Creek antigen pool and also in the reciprocal test may indicate that fish from the spawning runs of these streams have mixed. This could be since the mouth of Columbine Creek is only about 9 km from that of Yellowstone River inlet.

A discrete population is indicated for Chipmunk Creek (Population IV) and for Grouse Creek (Population V) which are located in the South Arm as shown by antigenic difference found with Population IV antiserum against Population V antigen pool and also in reciprocal tests. This situation occurs in spite of the fact their mouths are only 3 km apart and records

show the time of spawning to be almost the same in both streams (Ball and Cope, 1961). Chipmunk and Grouse Creeks are located the greatest distance from other streams which might explain their antigenic difference from other populations. The cutthroat trout from Chipmunk Creek constitute the most diverse population found on the basis of antigenic differences.

The isolation afforded by the arms may have been a significant factor in the establishment of these three populations.

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