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NATURAL VARIATION IN SPOTTING, HYOID TEETH COUNTS, AND COLORATION OF YELLOWSTONE CUTTHROAT TROUT

SPECIAL SCIENTIFIC REPORT—FISHERIES No. 460

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE

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UNITED STATES DEPARTMENT OF THE INTERIOR, STEWART L. UDALL, SECRETARY
Fish and Wildlife Service, Clarence F. Pautzke, Commissioner
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ABSTRACT

Differences in hyoid teeth and spotting counts among samples from four related populations of Yellowstone cutthroat trout, *Salmo clarki lewisi* Girard, reached species and subspecies levels as defined by some workers. Body coloration varied significantly among fish in six spawning runs of Yellowstone Lake, Wyo. The use of coloration, spotting, and hyoid teeth counts in cutthroat trout for taxonomic purposes needs reevaluation.

Early descriptions of the cutthroat trout, *Salmo clarki*, stressed its brilliant body coloration and spotting, and the presence of basibranchial teeth. Field collections of this species are still being divided into subspecies and separated from rainbow trout (*Salmo gairdneri*) on the basis of these characters (e.g. Needham and Gard, 1959, and Quadri, 1959), even though wide variations in coloration, spotting, and number of hyoid teeth can be found within a single population. The present study was initiated in 1959 to determine the amount of variation in these characters within four related populations of Yellowstone cutthroat trout, *Salmo clarki lewisi* Girard. The results illustrate the extreme plasticity of the Yellowstone cutthroat in regard to the above characters and the hazards involved in separating field specimens into taxonomic divisions on the basis of one or two characters.

Populations studied

Collections of fish for meristic counts were obtained in 1960 from traps at the mouths of Arnica, Pelican, Grouse, and Chipmunk Creeks as fish entered these streams from Yellowstone Lake to spawn (fig. 1) and from Bear and Sedge Creeks, which are tributaries of Turbid Lake, and in 1961 from the Creston National Fish Hatchery at Kalispell, Mont. Turbid Lake is a large thermal spring and is an effective barrier to fish movement. Data on coloration were obtained in 1959 at traps on Arnica, Pelican, Clear, Cub, Chipmunk, and Grouse Creeks as the fish ran upstream from Yellowstone Lake to spawn.

Cutthroat in Yellowstone Lake are a composite group representing fish which hatch out in a number of different tributaries. Adults move into these tributaries to spawn and then return to the lake. The young migrate downstream to the lake usually during the first 2 years of life. The Creston hatchery has maintained a brood stock of cutthroat from eggs obtained at Yellowstone Lake in 1948. Fish of age group III in 1961 represented the fourth generation from wild stock. The fastest growing fish of each generation were retained for brood stock, but no other selection occurred and no mixing with other cutthroat strains was allowed.[1/]

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1/ Personal correspondence from Blendon H. Cook, Supt., Creston NFH, Kalispell, Mont.

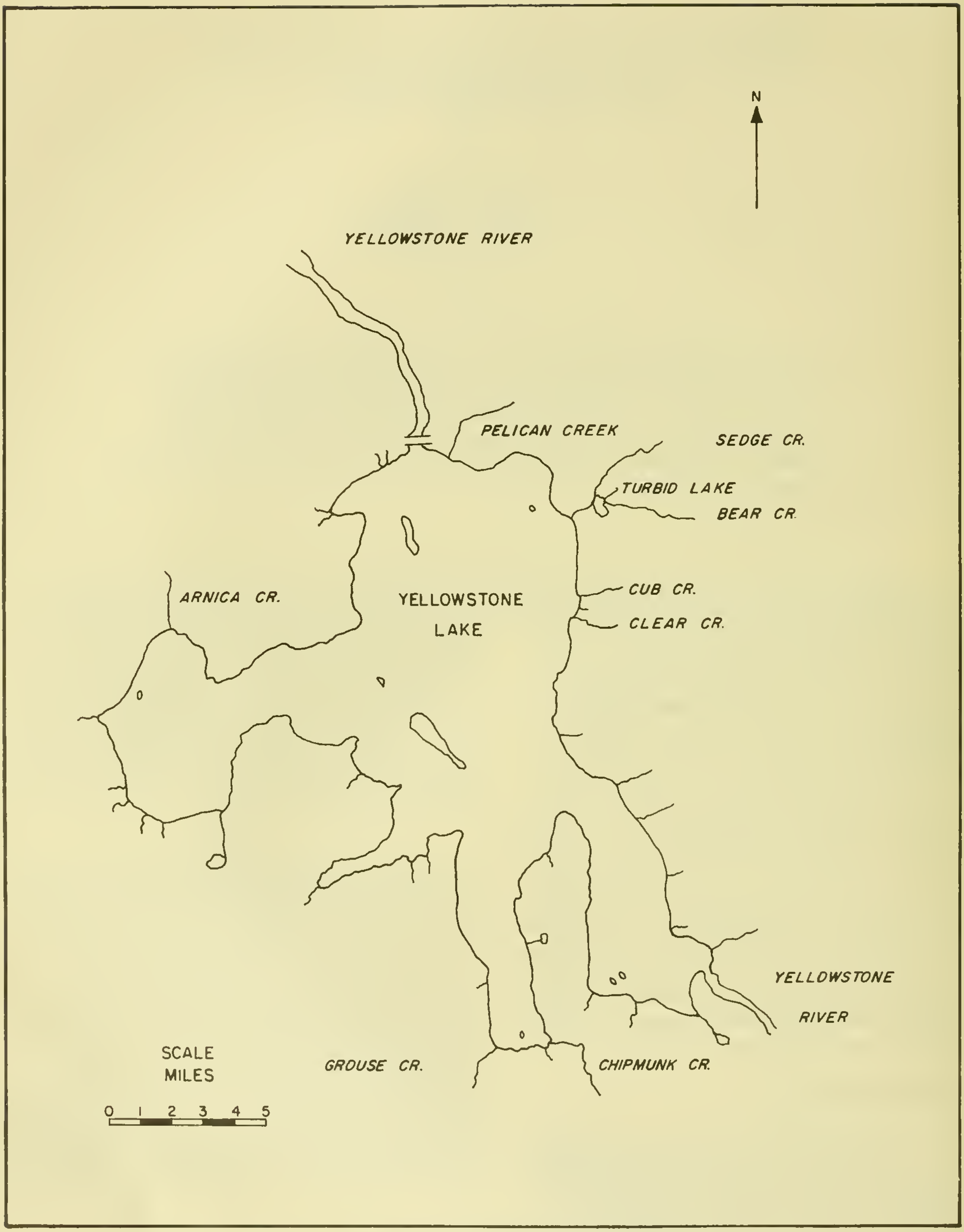

Figure 1:--Map of Yellowstone Lake including Sedge and Bear Creeks and Turbid Lake.

Bear and Sedge Creeks drain limited areas to the east of Yellowstone Lake and empty into Turbid Lake which lies about 2 miles from Yellowstone Lake. Summer stream flow in both streams is less than 5 cubic feet per second. The original populations of trout in both streams were probably once connected with the Yellowstone Lake population. Thermal activity of long duration[2/] in and around Turbid Lake has long since isolated these two populations.

Bear creek was stocked in 1939 and 1940 with 50,000 cutthroat trout from Yellowstone Lake[2/] but no other contamination of the original strain has occurred. This stream had an endemic population of cutthroat before 1939, as Kendall (1915) reported a native population there in 1914.

Cutthroat are abundant in Sedge Creek; this population is apparently uncontaminated, as a thorough search of all existing records revealed that no stocking of exotic strains of trout has occurred.[2/] Apparently the population has been isolated from Yellowstone Lake for many decades even though it is separated from the lake by only a few miles. Thus, the present study compares cutthroat from Yellowstone Lake with cutthroat stocks that have been separated from Yellowstone Lake for 13 years (Creston hatchery, four generations), for 21 years (Bear Creek), and for a long but unknown period of time (Sedge Creek).

Methods

Spawning females from Yellowstone Lake tributaries were taken from traps maintained at the mouth of these streams. Fish of both sexes were obtained from Sedge and Bear Creeks by applying a small amount of emulsified rotenone to the streams a short distance above their entrance into Turbid Lake. The number of specimens collected was controlled as the chemical immediately entered the highly toxic lake. Specimens from the Creston hatchery were selected at random from mature females and from yearling fish of both sexes.

The fish were preserved by immersing alive in a 10-percent solution of formalin after making a small slit in the abdomen to allow penetration of the preserving fluid. Measurements of total lengths were made from preserved specimens. Sex was determined by visual inspection of the gonads, using a microscope when necessary. As the differences between sexes in numbers of hyoid teeth and spots were not statistically significant, the sexes were combined in the Sedge Creek and Bear Creek samples for statistical comparisons with the other populations.

All hyoid teeth, regardless of size, were counted. Fused teeth with separate points were counted as two separate teeth. Special caution is needed in examining hyoid teeth (Miller, 1950; DeWitt, 1954) as they are frequently imbedded in the mucus covering the tongue and can be broken off easily. Accurate counts are difficult without damaging the specimen. In this study, the floor of the mouth was removed; sections were labeled and dried slightly with radiant heat to fully expose the teeth. Counts were made with a binocular microscope using a blue light. The teeth stood out in good relief, and the surface of the tongue could be examined thoroughly for evidence of missing teeth.

The spotting count was similar to the counts made by Quadri (1959) in defining differences between *Salmo c. lewisi* and *Salmo c. clarki*. All spots below the lateral line on the left side of the body were counted with the exception of spots smaller than a single scale (speckling). Parr marks were not counted, and fused spots were counted as one. Spots falling on the lateral line were included if more than half of the area of the spot was below the line. Spots on the head, tail, and lower fins were not enumerated. Ages of all fish were determined by scale examination with the exception of the known-age hatchery fish.

Data on coloration were collected in 1959 from live spawners of both sexes entering traps on 6 tributaries of Yellowstone Lake: Arnica, Pelican, Clear, Cub, Grouse, and Chipmunk Creeks. The predominant colors on the body above and below the lateral line were recorded for 10 percent of the fish on each run. The following major color types were observed in

2/ Personal correspondence from Oscar T. Dick, acting Chief Park Ranger, Yellowstone National Park, Wyo., dated February 14, 1961.

the Yellowstone Lake population: silver-white, silver-yellow, olive-white, olive-brown, yellow-yellow, and red-pink. A fish classified as silver-yellow would be predominantly silver on the back, blending into yellow on the lower sides and abdomen. All graduations of the above color were found, so some subjectivity was necessary in determining the proper category for certain fish.

Comparison of meristic counts

The samples contained the following age groups and ranges in total length (inches):

Area	Age group	Range	Mean
Yellowstone Lake	IV, V	14.7 - 15.2	14.9
Creston hatchery	I	4.3 - 5.8	5.0
	III	10.6 - 13.6	12.2
	IV	12.9 - 16.5	14.7
Bear Creek	III	5.8 - 7.9	6.7
	IV	7.4 - 9.7	8.1
Sedge Creek	II	3.8 - 5.2	4.3
	III	4.7 - 7.1	5.7
	IV	7.2 - 8.5	7.8
	V	-- --	8.7

Mean lengths by age group were not directly comparable among samples because all Yellowstone Lake fish were 15 inches total length ±0.1 inch, and the Creston hatchery fish represented the largest fish of their age groups. Bear Creek and Sedge Creek samples were selected completely at random.

Counts of spots below the lateral line and hyoid teeth in the above samples have been summarized by the method described by Hubbs and Hubbs (1953). This method combines the mean (x), range, standard deviation (s), and standard error ($s_{y.x.}$) of a sample into a single graph (fig. 2). The heavy line indicates the range in number in the particular sample for the character considered. The vertical line indicates the estimated mean number. The solid bar on each side of the mean represents twice the standard error ($s_{y.x.}$). The clear bars extending from the mean outward on each side represent one standard deviation (s) each. Adequate sample size is indicated whenever one white end bar approaches twice the length of the solid end bar. Sample size appeared to be adequate in all cases.

The greatest range in number of spots (15-149) was found in the combined sample from Yellowstone Lake; the collection of mature fish from Creston hatchery had the greatest range in number of hyoid teeth (18-68). The smallest ranges in numbers of spots (9-36) and teeth (2-12) were found in the Sedge Creek samples. Bear Creek fish were intermediate both in numbers of spots (24-68) and of hyoid teeth (2-20). The range in counts in samples from some areas failed to overlap, which indicates the large difference among samples in these two characters.

Sedge Creek fish had the lowest mean number of hyoid teeth (5.3) and spots (22.4), followed by Bear Creek (9.2, 43.0) and Yellowstone Lake (13.5, 84.3); Creston hatchery fish had the largest mean number of teeth and spots (39.8, 96.9). Four fish in the samples had no hyoid teeth, including a mature female of age group IV from Arnica Creek measuring 14.8 inches in total length and 3 immature fish of age group II from Sedge Creek measuring 3.1 to 3.2 inches in total length. Dymond (1928) reported that some cutthroat do not have hyoid teeth, but few, if any, workers have published data to substantiate his statement.

Data from the Sedge Creek and Creston hatchery samples suggest that the number of spots and hyoid teeth may increase with age (fig. 2). Sedge Creek samples showed increased mean numbers of teeth and spots with increase in age, having 4.0 teeth and 17.3 spots at age II, 5.2 teeth and 21.3 spots at age III, and 6.3 teeth and 24.5 spots at age IV. Creston hatchery fish had a similar pattern, with 14.2 teeth and 55.2 spots at age I, 33.7 teeth and 95.6 spots at age III, and 42.0 teeth and 98.2 spots at IV. This pattern of increased number of teeth and spots with age was not apparent in fish of age groups IV and V in Yellowstone Lake or in age groups III and IV in Bear Creek. Bear Creek samples had more spots on the average at age III than at age IV.

Only mature fish were used for comparison of teeth and spotting counts among populations

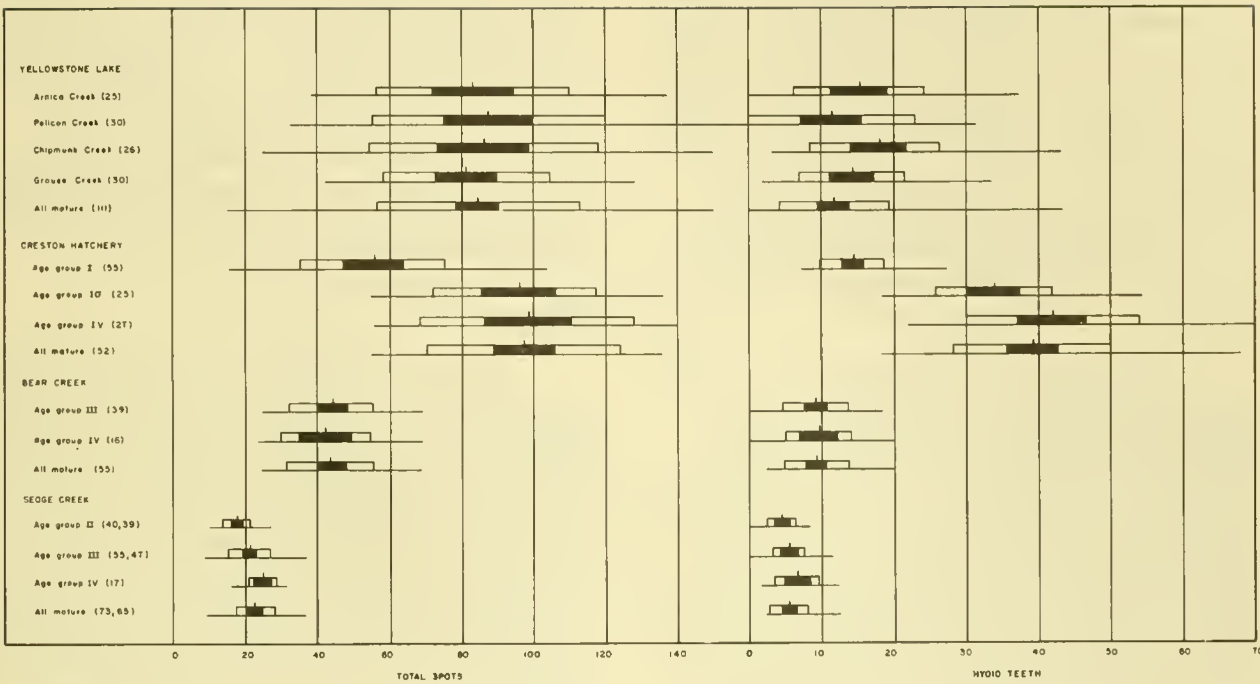

Figure 2:--Spotting and hyoid teeth counts from four populations of Yellowstone cutthroat trout. Number of fish in parenthesis. Range of variation is illustrated by the heavy horizontal line;the mean ($\bar{x}$) by the vertical line. The solid bar on each side of the mean represents 2 standard errors ($s_{y.x.}$). Length of the clear bar from the mean outward indicates 1 standard deviation (s) each.

in order to eliminate any variation associated with age. Samples from the four Yellowstone Lake tributaries were combined in the comparisons because differences among spawning runs in spotting and hyoid teeth were not statistically significant. The stocks in Yellowstone Lake tributaries were thoroughly mixed annually by hatching operations which continued until 1953. (Benson and Bulkley, 1962). Eggs were collected from fish in the spawning runs entering these and other tributaries of Yellowstone Lake, and eggs and fry were planted back into the streams. The eggs were not segregated by stream within the hatchery. This procedure caused considerable mixing of stocks among the various spawning runs in the lake. Cope (1957) found sufficient differences in size of fish and eggs, time of migration, etc., to describe them as distinct races, but significant differences in spotting and hyoid teeth have apparently not had time to develop.

Hubbs and Hubbs (1953) describe a simple method for determining statistically significant differences in samples when illustrated as in figure 2. This method of working directly from the graphs could not be used here. A careful scrutiny of data in figure 2 will reveal that the variances (S^2) for the samples of mature fish from different areas appear to be related to the size of the mean ($\bar{x}$) and hence cannot be pooled for testing differences. The fact that the variances are distinctly different indicates that the populations are quite different. The frequency distribution in certain samples (e.g., Pelican Creek hyoid teeth) is also skewed and deviates from a normal distribution. All meristic counts were converted to base-10 logarithms to fulfill the requirements of equal variance and normal distribution so that "t" tests could be made of differences between samples.

Although a statistically significant difference among populations is of interest, the amount of change (overlap) that has occurred since isolation is the factor of primary importance. The amount of overlap ($\underline{p}$) as defined by Royce (1957) is the probability of misclassifying an individual from one of two samples by use of the character in question. A $\underline{p}$ value of 0.5 indicates complete overlap of two samples, whereas a value of 0.0 indicates complete separation. Calculation of $\underline{p}$ is illustrated in appendix A.

Another useful measurement is the percentage of one sample which might belong to another sample; this percentage has been assigned the Greek letter omega (Ω) by Royce. A condition of complete overlap of two populations is indicated by an Ω value of 100 percent. With equal sample size and equal variance, $\Omega = 200\ \underline{p}$. Our samples are not of equal size, but value of $\underline{p}$ and approximate values of Ω for populations compared in this study are presented in table 1. Some samples are obviously very divergent; for example, the percentage of the Sedge Creek sample that could theoretically belong to the Creston hatchery stock is 1.20 percent on the basis of spotting counts and 2.50 percent on the basis of hyoid teeth counts. In contrast, Creston and Yellowstone Lake samples were quite similar, with an overlap in spotting of 81.6 percent and in hyoid teeth counts of 35.0 percent.

Spotting

Spotting differences among samples were apparent not only in number of spots below the lateral line, which is indicative of the amount of spotting on the entire body, but also in distribution of spots. The relative number of spots on the anterior part of the body varied among individual fish. To illustrate this difference among populations, the number of spots anterior to a line drawn perpendicularly from the insertion of the ventral fin to the lateral line was compared with the total number of spots on the side of the body below the lateral line (table 2). In the Sedge Creek collection an average of less than 4 percent of the total spots below the lateral line were on the anterior part of the body. Spotting was more evenly distributed on fish from Bear Creek, Yellowstone Lake, and Creston. The Creston fish had the largest percentage of spots anterior (21.24), indicating a relatively even distribution of spots on the average fish in this population.

The regression of the number of spots anterior to the ventral fins to the total number of spots was determined to see if another measure of the amount of overlap between samples could be obtained. Assumptions necessary to determine statistical differences and to calculate the degree of overlap in spotting distribution were difficult to fulfill. Logarithmic transforma-

Table 1:--Probability of misclassification (p) and percent overlap of four populations based on number of spots below lateral line and number of hyoid teeth.

Character and area	Bear Creek		Creston Hatchery		Yellowstone Lake	
	P	Percent overlap	P	Percent overlap	P	Percent overlap
Spots:						
Sedge Creek.....	0.130	26.00	0.006	1.20	0.029	5.80
Bear Creek......	--	---	0.087	17.40	0.180	36.00
Creston Hatchery	--	---	---	---	0.408	81.60
Hyoid teeth:						
Sedge Creek.....	0.320	64.00	0.013	2.50	0.250	50.00
Bear Creek......	--	--	0.296	59.20	0.390	78.00
Creston Hatchery	--	--	--	--	0.175	35.00

Table 2:--Number of spots on the section of body below lateral line and anterior to insertion of ventral fins (area 1) and on left side of body below lateral line (total) of mature fish from 4 populations. Number of fish in parenthesis.

	Sedge Creek (73)		Bear Creek (55)		Creston Hatchery (52)		Yellowstone Lake (111)	
	Area 1	Total	Area 1	Total	Area 1	Total	Area 1	Total
Mean no.	0.795	22.410	7.945	43.000	21.557	96.920	16.000	84.279
Median	0.0	23.0	7.0	42.0	20.5	96.5	15.0	84.0
Range	0-5	9-36	0-23	19-68	0-56	55-135	0-44	24-149
Percent in area 1	3.54	---	18.47	---	21.24	---	17.85	---

tion of spotting counts did not provide variances which were statistically comparable, and distribution about the regression line for some samples such as Sedge Creek was highly skewed. This deviation from the normal distribution for Sedge Creek was due to the large number of fish with no spots on the anterior part of the body. Only two comparisons fulfilled the requirement of similar variances, which again illustrates the large difference among the samples. The Creston hatchery and Yellowstone Lake samples were comparable, but the overlap approached 100 percent with p=0.50, indicating no difference in location of spots on the body in the two populations. Creston and Bear Creek samples had an overlap of approximately 57.5 percent with p=0.288.

Coloration

DeWitt (1954) found all gradations of color among different populations of coastal cutthroat, *Salmo c. clarki*, and concluded that no single individual could represent this subspecies as far as coloration was concerned. Data collected in 1959 on coloration of live spawners entering Arnica, Pelican, Clear, Cub, Grouse, and Chipmunk Creeks illustrate the wide variation in coloration possible among Yellowstone cutthroat trout within the same population. Color patterns in the Yellowstone Lake fish varied by sex, size of fish, and spawning run (table 3). Prominent coloration was directly associated with size of fish. The silver-white phase was

Table 3:—Percent and mean total length (inches) of Yellowstone cutthroat belonging to different color phases.

Area	Number of fish	Color phase											
		Silver-white				Silver-yellow				Olive-white			
		Females		Males		Females		Males		Females		Males	
		Per-cent	Length	Per-cent	Length	Per-cent	Length	Per-cent	Length	Per-cent	Length	Per-cent	Length
Arnica Creek	179	3.9	13.6	22.9	13.9	--	--	--	--	6.1	14.5	17.9	14.7
Pelican Creek	1,625	6.3	14.9	46.6	14.6	--	--	--	--	6.0	15.1	3.7	14.9
Cub Creek	994	1.1	15.0	46.7	15.0	--	--	--	--	3.0	15.2	0.6	15.1
Clear Creek	304	0	--	46.0	15.2	--	--	--	--	2.6	15.3	--	--
Chipmunk Creek	622	1.0	13.6	13.0	14.8	0.8	14.9	13.7	15.0	0.6	14.9	3.5	15.1
Grouse Creek	767	0.2	14.1	12.0	14.7	0.8	15.1	17.2	15.0	0.5	15.0	2.0	14.8
		Yellow-yellow				Olive-brown				Red-pink			
Arnica Creek		13.4	14.6	15.1	14.7	3.9	14.4	2.8	15.0	6.1	14.4	7.8	14.6
Pelican Creek		11.8	15.6	12.6	15.1	0.5	16.1	0.2	15.2	3.1	15.6	4.2	15.0
Cub Creek		18.6	15.3	10.4	15.0	11.5	15.30	0.2	14.4	7.1	15.4	0.8	15.2
Clear Creek		18.4	15.9	8.2	15.4	21.7	15.4	0.3	14.6	2.6	15.6	0	--
Chipmunk Creek		13.5	15.4	21.9	15.2	2.7	15.8	1.0	15.3	14.0	15.6	9.3	15.1
Grouse Creek		15.8	15.5	17.1	15.0	1.7	15.5	0.7	15.1	19.3	15.4	12.8	15.1

found most frequently on young female fish. Few male spawners fell into this category, indicating that they were more highly colored than females. Females were predominantly silver when young and became yellowish, olive, or red with age. Males were mainly yellow or red. The olive-brown color phase was predominantly found on male fish.

Fish from streams in close proximity were more similar in frequency of occurrence of certain color phases than fish from streams far apart (table 4). Fish with silver-white coloration were most common in Pelican, Cub, and Clear Creek runs, indicating a large proportion of young spawners. The yellow phase was most abundant in Chipmunk and Grouse Creeks, followed by fish with red coloration. Arnica Creek had about equal amounts of silver-white, yellow, and red-pink fish. This wide range and variation in color among spawning populations of cutthroat trout in Yellowstone Lake makes it difficult to describe the coloration of a representative specimen from this subspecies. In fact, coloration of most cutthroat subspecies can be duplicated by individual fish from Yellowstone Lake, which suggests that the use of color as a major factor for separating cutthroat subspecies needs reevaluation.

Table 4:--Frequency of occurrence of different color phases of spawning cutthroat entering 6 Yellowstone Lake tributaries, 1959.

Stream	Color phase					
	Silver-white	Silver-yellow	Olive-white	Olive-brown	Yellow-yellow	Red-pink
Arnica Creek	2	--	3	5	1	4
Pelican Creek	1	--	3	5	2	4
Cub Creek	1	--	5	3	2	4
Clear Creek	1	--	4	3	2	4
Chipmunk Creek	4	3	5	6	1	2
Grouse Creek	4	3	5	6	1	2

Discussion and conclusions

As mentioned previously, coloration and spotting differences have often been used as major distinguishing features between subspecies of cutthroat trout, and the presence or absence of hyoid teeth has frequently been the deciding factor in calling a specimen a cutthroat, cutthroat-rainbow hybrid, or rainbow trout. Hence, natural variations in these characters within the same subspecies are of considerable importance.

On the basis of the amount of spotting below the lateral line, the four samples in this study ranged from an overlap of 1.20 ($p=0.006$) to 81.60 ($p=0.408$) percent. On hyoid teeth counts, the samples ranged from 2.50 percent overlap ($p=0.013$) to 78.00 percent ($p=0.390$). The significance of these differences is apparent when the usually accepted levels of p values for separating species and subspecies are noted. Ginsburg, as quoted by Royce (1957) suggested that a $p = 0.1$ would commonly be found between species, 0.3 between subspecies, 0.4 between races, and up to 0.5 between varieties. Mayr et al. (1953 suggest that $p = 0.1$ is the usual level of subspecific difference. Hubbs and Hubbs (1953) feel that a better level of subspecific difference is $p = 0.25$.

Considering spotting below the lateral line by Ginsburg s definition, Sedge Creek fish would be a separate species from the Yellowstone Lake and Creston hatchery stocks; Bear Creek fish would be a different species from the Creston hatchery stock. All except Yellowstone Lake and Creston hatchery fish would be classified as different subspecies. For hyoid teeth, Sedge Creek fish would be classified as a different subspecies from Yellowstone Lake and Creston; Bear Creek would be different from Creston; and Creston fish would be a different subspecies from Yellowstone Lake. Even on the level of subspecific difference ($p = 0.1$) selected by Mayr et al., Sedge Creek fish would be a distinct subspecies from Yellowstone Lake and Creston fish, and Bear Creek fish would approach subspecific differences from Yellowstone Lake and Creston populations. According to the levels of difference set by Ginsburg and by Hubbs and Hubbs, Creston hatchery fish were able to alter sufficiently in four generations to be classified as a different subspecies from their ancestral stock in Yellowstone Lake.

These comparisons indicate that the Yellowstone cutthroat trout is a very plastic subspecies so far as spotting, hyoid teeth, and body coloration are concerned and that wide natural variations in these characters can be expected. Data are insufficient to determine how much of the differences reported in this study are of actual genetic origin as environmental conditions were not constant among the four populations sampled. Some genetic change in spotting and hyoid teeth abundance is suggested as levels of difference varied directly with length of isolation in most comparisons. Controlled laboratory studies will be necessary to determine the cause of these differences, but their magnitude clearly indicates that the usually accepted levels for separating species and subspecies are not applicable to field collections of Salmo c. lewisi. The value of coloration, spotting, and hyoid teeth in classifying cutthroat subspecies obviously needs to be reassessed.

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Appendix--Calculation of Probability of Misclassification

Calculation of the probability (p) of assigning a fish to the wrong sample of two possible samples based on counted characters is accomplished in the following steps (Royce, 1957):

1. $$D = \frac{\bar{x}_1 - \bar{x}_2}{s_p}$$

   Where D is the distance between the means $\bar{x}_1$ and $\bar{x}_2$ in units of the standard deviation, and s_p is the square root of the pooled variances.

2. The probability P_t of having a "t" value as large as a calculated "t" where "t" = $\frac{D}{2}$ with $n_1 + n_2$ degrees of freedom is determined from a table of "t" values.

3. The probability of misclassification (p) = $\frac{P_t}{2}$ and ranges from 0.00 indicating no chance of misclassification to 0.50 indicating a 50:50 chance of misclassification and representing complete overlap of the two samples for the character in question.