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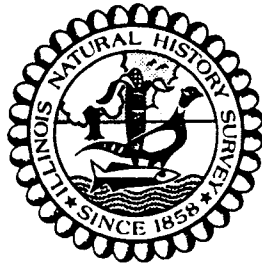
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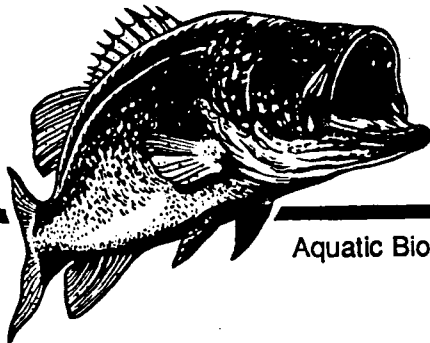
ILLINOIS NATURAL HISTORY SURVEY

**A GENETIC ANALYSIS OF THE
RAINBOW TROUT POPULATION
IN THE GRAND CANYON PORTION
OF THE COLORADO RIVER**



**Aquatic Biology Section
Technical Report**

Julie E. Claussen and David P. Philipp



**A Report to
Arizona Game and Fish Department**

October 1986

Aquatic Biology Technical Report 1986(5)

Illinois Natural History Survey
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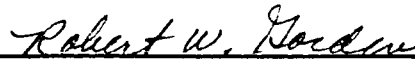
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October 1986

STUDY OBJECTIVES:

The major goal of this study was to use protein electrophoretic techniques to analyze the genetic variation existing within the adult rainbow trout population in the Colorado River from Glen Canyon Dam to Lake Mead and among the young-of-the-year rainbow trout produced in the tributaries along this stretch of river. Our specific objectives asked the following questions:

1. Are the adult rainbow trout from different regions of the Colorado River in the Grand Canyon genetically distinct?
2. Are the rainbow trout that spawn in each of the different tributaries genetically distinct from one another or from the general main channel population?
3. Is the supplemental stocking program for rainbow trout at Lee's Ferry having a significant impact upon the genetic structure of the main channel population?
4. What is the most likely source for natural recruitment of rainbow trout in this stretch of river?

METHODS AND MATERIALS:

Rainbow trout and their young-of-the-year were collected from the Colorado River in April and May 1986 from below the Glen Canyon Dam to Whitmore Canyon. Adults were taken from the main channel by electrofishing from a motorized raft. Young-of-the-year were collected in tributaries or backwaters by seining or by using a bank-electrofishing device. Adults were collected from the following main channel sites: river mile -4 (Watering Hole Canyon), river mile 35 (Vasey's Paradise), river mile 50 (Nankoweap), river mile 180 (Shinamo), river mile 132 (Dubendorff Rapid), river mile 136 (Deer Creek Falls), river mile 138 (Pancho's Kitchen), and river mile 188 (Whitmore Canyon). Young-of-the-year were collected from the following backwaters and tributaries: river mile -20 (Glen Canyon Backwater), river mile 52 (Nankoweap Creek), river mile 84 (Clear Creek), river mile 88 (Bright Angel Creek), river mile 89 (Pipe Creek), river mile 98 (Crystal Creek), river mile 134 (Tapeats Creek), and river mile 136 (Deer Creek).

Fish were weighed and measured, wrapped in foil, immediately frozen on dry ice, and shipped frozen to laboratory facilities at the Illinois Natural History Survey in Champaign, Illinois. Required tissue samples (white skeletal muscle, eye, and liver) were excised from each individual. Extracts were prepared and subjected to vertical starch gel electrophoresis and histochemical

staining as described in Philipp et al. (1979) to determine, for each individual, the genotype at each locus. The enzyme loci, tissue, and electrophoretic conditions under which the rainbow trout isozymes were analyzed are as follows:

Enzyme council number	Enzyme name	Locus	Tissue analyzed	Electrophoretic system ¹
1.1.1.1	Alcohol dehydrogenase	Adh-A	Liver	EBT
1.1.1.8	Glycerol-3-phosphate dehydrogenase	Gpdh-A ^a Gpdh-A ^b Gpdh-B	Muscle Muscle Liver	TC TC TC
1.1.1.14	Sorbitol dehydrogenase	Sdh-A	Liver	TC
1.1.1.27	Lactate dehydrogenase	Ldh-A ^a Ldh-A ^b Ldh-B ^a Ldh-B ^b Ldh-C	Muscle Muscle Eye Eye Eye	TC TC TC TC TC
1.1.1.37	Malate dehydrogenase	Mdh-A Mdh-B ^a Mdh-B ^b Mdh-M	Muscle Muscle Muscle Muscle	TC TC TC TC
1.1.1.40	Malic enzyme	Me-A ^a Me-A ^b	Muscle Muscle	EBT EBT
1.1.1.42	Isocitrate dehydrogenase	Idh-A ^a Idh-A ^b Idh-B ^a Idh-B ^b	Muscle Muscle Eye Eye	TC TC TC TC
1.1.1.43	6-Phosphogluconate dehydrogenase	6-Pgdh-A	Liver	TC
1.2.1.12	Glyceraldehyde-3-phosphate dehydrogenase	Gapdh-A Gapdh-C ^a Gapdh-C ^b	Muscle Eye Eye	TC TC TC
1.2.3.2	Xanthine dehydrogenase	Xdh-A	Liver	EBT
1.15.1.1	Superoxide dismutase	Sod-A	Liver	EBT

Enzyme council number	Enzyme name	Locus	Tissue analyzed	Electrophoretic system ¹
2.6.1.1	Aspartate aminotransferase	Aat-A	Muscle	TC
		Aat-B	Liver	TC
		Aat-M ^a	Muscle	TC
		Aat-M ^b	Muscle	TC
2.7.1.11	Fructose-1,6-diphosphatase	Fdp-A	Muscle	TC
		Fdp-B	Liver	TC
2.7.1.40	Pyruvate kinase	Pk-A	Muscle	TC
		Pk-B	Muscle	TC
2.7.2.3.	Phosphoglycerate kinase	Pgk-A	Muscle	TC
		Pgk-B	Muscle	TC
2.7.3.2	Creatine kinase	Ck-A ^a	Muscle	EBT
		Ck-A ^b	Muscle	EBT
		Ck-B	Eye	EBT
		Ck-C	Eye	EBT
2.7.4.3	Adenylate kinase	Ak-A	Muscle	EBT
2.7.5.1	Phosphoglucomutase	Pgm-A ^a	Muscle	EBT
		Pgm-A ^b	Muscle	EBT
3.5.4.4	Adenosine deaminase	Ada-A	Muscle	EBT
		Ada-B	Muscle	EBT
4.1.2.13	Aldolase	Ald-A	Muscle	TC
		Ald-C	Eye	TC
5.3.1.8	Mannose-6-phosphate isomerase	Mpi-A	Muscle	TC
5.3.1.9	Glucosephosphate isomerase	Gpi-A	Muscle	TC
		Gpi-B ^a	Muscle	TC
		Gpi-B ^b	Muscle	TC
-----	Esterase	Est-A	Eye	EBT
		Est-A	Liver	EBT
		Est-B	Liver	EBT
		Est-C	Liver	EBT

Enzyme council number	Enzyme name	Locus	Tissue analyzed	Electrophoretic system ¹
-----	Calcium binding protein	Cbp-A	Muscle	TC
-----	General protein	Prot-A	Muscle	EBT
		Prot-A	Eye	EBT
		Prot-B	Eye	EBT
		Prot-C	Eye	EBT

¹EBT: gel = 15.1% starch (w/v), 0.042 M Tris, 0.023 M borate, 0.00089 M EDTA, pH 8.6; electrode chambers = (top) 0.18 M Tris, 0.100 M borate, 0.0036 M EDTA, pH 8.6 and (bottom) 1.239 M Tris, 0.07 M borate, 0.0026 M EDTA, pH 8.6; electrophoresis = 17 h at 225 V, 4 C.

TC: gel = 18.6% starch (w/v), 0.0175 M Tris, 0.0058 M citrate, pH 7.0; electrode chambers = 0.075 M Tris, 0.025 M citrate, pH 7.0; electrophoresis = 18 h at 225 V, 4 C.

RESULTS AND DISCUSSION:

Allele frequencies at each of 60 loci were determined for each sample of adult rainbow trout (populations 1-8) and for each sample of young-of-the-year rainbow trout (populations 9-16) and are shown in Table 1. Of these 60 loci, 10 were found to be polymorphic. This level of polymorphism is similar to that reported for rainbow trout in other studies (Utter et al. 1973; Reinitz 1977; Busack et al. 1979; Allendorf and Phelps 1981).

The mean number of alleles per locus ranged from 1.1 to 1.2 and the percentage of polymorphic loci ranged from 8.3 to 15.0 among all populations (Table 2). Also presented in Table 2 are the expected and observed heterozygosity levels for each population. Analysis of the observed values for genotype frequencies at each locus revealed no obvious deviation from expected values based upon the Hardy-Weinberg equilibrium equation. This concurrence with H-W equilibrium indicates that these populations exhibited mendelian segregation and confirmed that our locus and allele designations were correct.

The summary of the contingency table analysis at all loci (Table 3) indicates that the 16 adult and young-of-the-year samples do not represent sampling of a single panmictic population ($P <$

0.00001) but rather that genetic differentiation among these samples exists. However, the summary of F-statistics (Table 4) indicates that much of the genetic variation is within the samples rather than between them.

To assess the genetic relatedness of the 16 rainbow trout samples, the allele frequencies at each locus for each population were used to construct matrices of genetic distance and similarity using the modified Rogers coefficients (Wright 1978) as shown in Table 5. We then performed a cluster analysis using the unweighted pair group method and the modified Rogers distance values. The resulting dendrogram for the eight adult populations (Figure 1) indicates that the populations furthest from Glen Canyon Dam were most closely related. The two samples nearest to the dam, Watering Hole Canyon and Vasey's Paradise samples, were the most genetically distinct. This relationship of increased divergence to dam proximity most likely reflects the introduction programs for stocking hatchery-reared rainbow trout at Lee's Ferry.

Another dendrogram was similarly generated for the eight young-of-the-year samples (Figure 2). This dendrogram illustrates that the Deer Creek and Clear Creek young-of-the-year samples were the most genetically distinct.

A third dendrogram, combining all 16 samples (adults and young-of-the-year) (Figure 3) reveals several interesting relationships. First, the Tapeats Creek and Nankoweap Creek young-of-the-year samples clustered very closely and within the cluster containing the bulk of the adult samples. No other sample of young-of-the-year rainbow trout clustered so closely to the adult samples, which may indicate that these two tributaries contribute very heavily to the annual natural recruitment of rainbow trout in the main channel.

Second, the Watering Hole Canyon sample of adult rainbow trout was vastly different from the rest of the samples studied. Interestingly, the young-of-the-year sample taken near the dam (Glen Canyon sample) was more similar to the adult samples taken downriver than to the adults from Watering Hole Canyon, which further indicates that the genetic distinctness of this adult sample is due heavily to the introduction of hatchery-reared fish at Lee's Ferry. The closer relationship of the Glen Canyon young-of-the-year sample to the downstream adult populations also indicates that the hatchery-reared rainbow trout introduced at Lee's Ferry may not contribute heavily to the natural recruitment in the area.

Third, excluding the Watering Hole Canyon sample of adult rainbow trout, the six most disparate samples were all young-of-the-year samples (Glen Canyon, Bright Angel Creek, Pipe Creek, Crystal Creek, Deer Creek, and Clear Creek). In fact, Deer Creek and Clear Creek were the most genetically distinct samples analyzed. This indicates that the adult rainbow trout using these streams for spawning may be genetically distinct subpopulations from within the main channel population. That is, only a relatively few adults may return year after year to these small tributaries to spawn. Since the young-of-the-year produced in these tributaries differ genetically from the main channel adult rainbow trout, it appears that they may currently have only a minor impact upon annual recruitment.

SUMMARY:

1. Comparing samples of adult rainbow trout in the main channel, genetic relatedness increases as one proceeds downstream from the Glen Canyon Dam, reflecting a greater impact of introduced hatchery-reared rainbow trout to the population in areas closest to Lee's Ferry.

2. Comparing the young-of-the-year samples to the samples of adult rainbow trout revealed that two young-of-the-year samples (Nankoweap Creek and Tapeats Creek) were genetically similar to the adult samples (except Watering Hole Canyon), whereas the remaining six were quite distinct. This distinction indicates one of two possibilities: (a) most natural recruitment derives from adult rainbow trout that spawn in the main channel; or (b) if recruitment is derived mainly from tributary spawning, the runs up Nankoweap Creek and Tapeats Creek are the major contributors.

RECOMMENDATIONS:

1. These data represent a single year's sampling. To confirm the validity of the genetic relatedness among these samples (particularly the distinctness of the young-of-the-year samples from individual creeks), the entire analysis should be repeated. Data concerning the degree of temporal (year-to-year) variation in the genetic structure of the young-of-the-year produced in single tributaries would be invaluable in assessing the actual contributions of these tributaries.

2. A more complete assessment of main channel spawning effort is needed, including sampling young-of-the-year to determine genetic structure of main channel breeding populations.

3. An assessment of the relative performances of hatchery-reared fish versus naturally spawned fish and the impact of these hatchery-reared fish on the natural annual recruitment would provide much of the data needed to evaluate the current supplemental introduction program.

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TABLE 2. GENETIC VARIABILITY AT 60 LOCI IN ALL POPULATIONS (STANDARD ERROR IN PARENTHESES)

POPULATION	MEAN SAMPLE MEAN NO. OF ALLELES PER LOCUS		PERCENTAGE OF LOCI POLYMORPHIC*	MEAN HETEROZYGOSITY	
	SIZE PER LOCUS	PER LOCUS		DIRECT-COUNT	HOMYBG EXPECTED**
1. WATERING HOLE	15.0 (0.0)	1.2 (.1)	15.0	.043 (.015)	.048 (.017)
2. VASEY'S PARADISE	17.0 (0.0)	1.2 (.1)	11.7	.032 (.013)	.035 (.014)
3. NANKOHEAP	23.0 (0.0)	1.2 (.1)	11.7	.040 (.017)	.038 (.016)
4. SHINAMO	23.0 (0.0)	1.2 (.1)	13.3	.048 (.020)	.042 (.016)
5. DUBENDORFF RAPID	23.0 (0.0)	1.2 (.1)	11.7	.042 (.018)	.038 (.016)
6. DEER CREEK FALLS	20.0 (0.0)	1.2 (.1)	15.0	.044 (.018)	.039 (.015)
7. PANCHO'S KITCHEN	20.0 (0.0)	1.2 (.1)	15.0	.042 (.016)	.042 (.015)
8. WHITMORE CANYON	20.0 (0.0)	1.2 (.1)	15.0	.041 (.015)	.039 (.015)
9. GLEN CANYON	10.0 (0.0)	1.2 (.1)	13.3	.037 (.017)	.034 (.015)
10. NANKOHEAP CRK.	20.0 (0.0)	1.2 (.1)	13.3	.034 (.014)	.037 (.014)
11. CLEAR CRK.	20.0 (0.0)	1.2 (.1)	13.3	.042 (.016)	.044 (.016)
12. BRIGHT ANGEL CRK	19.0 (0.0)	1.2 (.1)	13.3	.032 (.013)	.034 (.014)
13. PIPE CRK.	25.0 (0.0)	1.2 (.1)	13.3	.041 (.015)	.038 (.014)
14. CRYSTAL CRK.	6.0 (0.0)	1.1 (.0)	10.0	.036 (.015)	.034 (.014)
15. TAPEATS CRK.	20.0 (0.0)	1.2 (.1)	13.3	.038 (.016)	.038 (.015)
16. DEER CRK.	6.0 (0.0)	1.1 (.0)	8.3	.033 (.016)	.030 (.014)

TABLE 3. SUMMARY OF CONTINGENCY TABLE ANALYSIS AT ALL LOCI

LOCUS	NO. OF ALLELES	D.F.	CHI-SQUARE	P	G	P
GP-AB	2	15	8.252	.91328	8.184	.91618
LD-BB	2	15	52.636	.00000	27.448	.02529
LDH-C	2	15	37.607	.00103	36.030	.00175
MD-BA	4	45	98.799	.00001	77.336	.00193
MD-BB	3	30	26.083	.67089	11.229	.99927
MDH-M	2	15	26.803	.03038	10.293	.80094
ID-AB	2	15	23.143	.08115	23.313	.07772
ID-BA	4	45	76.840	.00217	41.444	.62338
ID-BB	2	15	18.043	.26043	19.863	.17724
SOD-A	2	15	13.403	.57122	13.278	.58085
(TOTALS)		225	381.609	.00000	268.419	.02505

TABLE 4. SUMMARY OF F-STATISTICS AT ALL LOCI

LOCUS	F(15)	F(17)	F(17)
GP-AB	.059	.074	.016
LD-BB	.320	.384	.095
LDH-C	.115	.033	.073
MD-BA	.101	.036	.059
MD-BB	.033	.060	.028
MDH-M	.058	.008	.048
ID-AB	.020	.027	.046
ID-BA	.111	.069	.037
ID-BB	.019	.049	.031
SOD-A	.104	.075	.026
MEAN	.060	.013	.044

TABLE 5. MATRIX OF GENETIC SIMILARITY AND/OR DISTANCE COEFFICIENTS

BELOW DIAGONAL: ROGERS (1972) GENETIC SIMILARITY
 ABOVE DIAGONAL: MODIFIED ROGERS DISTANCE (WRIGHT, 1978)

POPULATION	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 WATERING HOLE	*****	.037	.039	.037	.049	.037	.042	.045	.050	.047	.056	.051	.062	.070	.049	.067
2 VASEY'S PARADISE	.988	*****	.037	.036	.041	.028	.039	.036	.048	.035	.049	.047	.049	.058	.038	.063
3 NAKKORFAP	.986	.989	*****	.023	.042	.036	.033	.036	.057	.031	.044	.052	.056	.063	.046	.055
4 SHIMAMO	.988	.989	.992	*****	.025	.027	.023	.023	.044	.028	.046	.044	.043	.057	.032	.051
5 DUBENDORFF RAPID	.984	.989	.987	.992	*****	.033	.027	.023	.038	.037	.061	.041	.036	.061	.027	.047
6 DEER CREEK FALLS	.988	.991	.990	.992	.990	*****	.028	.022	.033	.028	.043	.038	.037	.045	.030	.056
7 PANGHO'S KITCHEN	.986	.989	.989	.992	.991	.992	*****	.016	.038	.025	.050	.032	.038	.049	.028	.035
8 WHITTHORE CANYON	.985	.991	.988	.993	.993	.984	.996	*****	.034	.018	.046	.031	.027	.041	.019	.039
9 GLEN CANYON	.985	.986	.982	.986	.988	.989	.987	.989	*****	.046	.066	.027	.041	.049	.032	.053
10 NAKKORFAP CRK.	.985	.990	.990	.992	.989	.993	.991	.994	.986	*****	.036	.037	.034	.037	.028	.044
11 CLEAR CRK.	.981	.985	.987	.988	.983	.986	.984	.986	.979	.990	*****	.059	.051	.047	.051	.073
12 BRIGHT ANGEL CRK	.984	.988	.984	.985	.988	.988	.988	.989	.993	.987	.981	*****	.035	.039	.027	.037
13 PIPE CRK.	.980	.988	.983	.986	.990	.988	.988	.992	.987	.988	.983	.990	*****	.035	.026	.051
14 CRYSTAL CRK.	.978	.984	.984	.984	.982	.987	.986	.987	.986	.990	.986	.987	.988	*****	.041	.058
15 TAYLATS CRK.	.984	.990	.986	.991	.992	.990	.991	.993	.991	.991	.987	.991	.990	.987	*****	.046
16 DEER CRK.	.979	.982	.984	.983	.987	.983	.988	.986	.986	.985	.977	.989	.985	.985	.984	*****

FIGURE 1. CLUSTER ANALYSIS USING UNWEIGHTED PAIR GROUP METHOD: ADULTS
 COEFFICIENT USED: MODIFIED ROGERS DISTANCE (WRIGHT, 1978)

GOODNESS OF FIT STATISTICS

FARRIS (1972) η^2 = .108
 PRAGER AND WILSON (1976) η^2 = 11.767
 PERCENT STANDARD DEVIATION (FITCH AND HARGOLASH, 1967) = 15.610
 COPHENETIC CORRELATION = .806

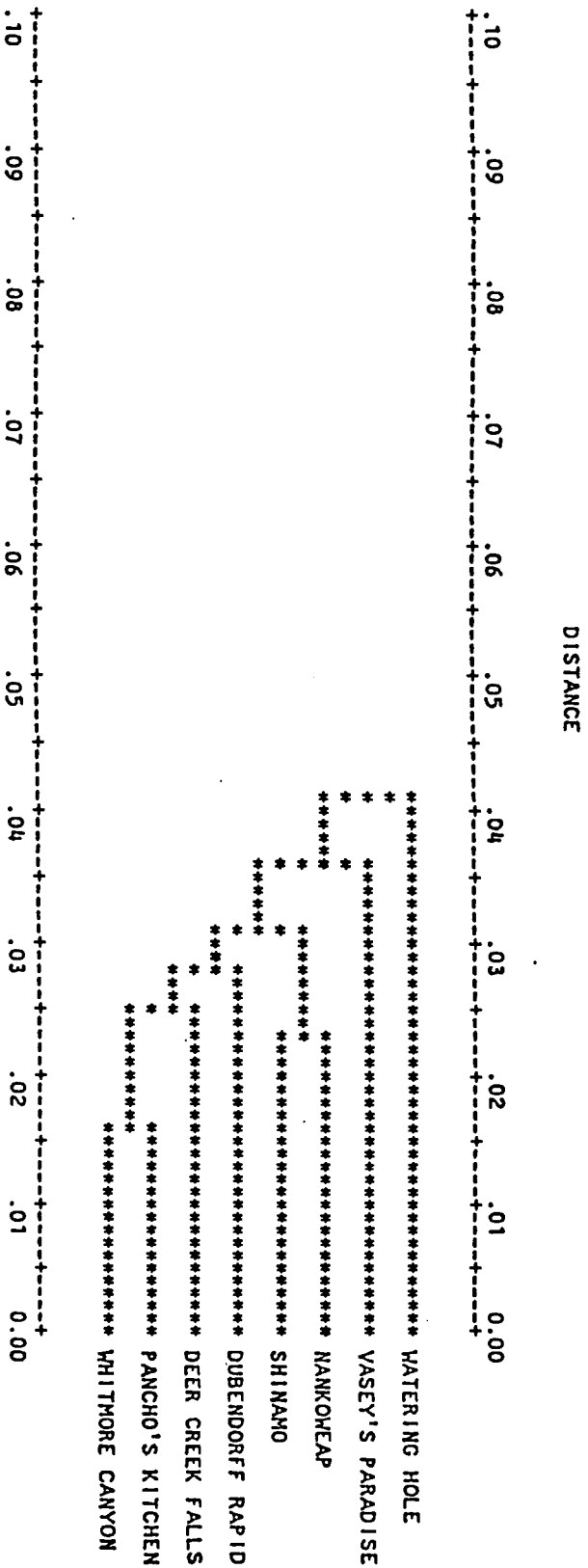


FIGURE 2. CLUSTER ANALYSIS USING UNWEIGHTED PAIR GROUP METHOD: YOY
 COEFFICIENT USED: MODIFIED ROGERS DISTANCE (WRIGHT, 1978)

GOODNESS OF FIT STATISTICS

FARRIS (1972) "F" = .158
 PRAGER AND WILSON (1976) "F" = 13.108
 PERCENT STANDARD DEVIATION (FITCH AND MARGOLASH, 1967) = 17.750
 COPHENETIC CORRELATION = .767

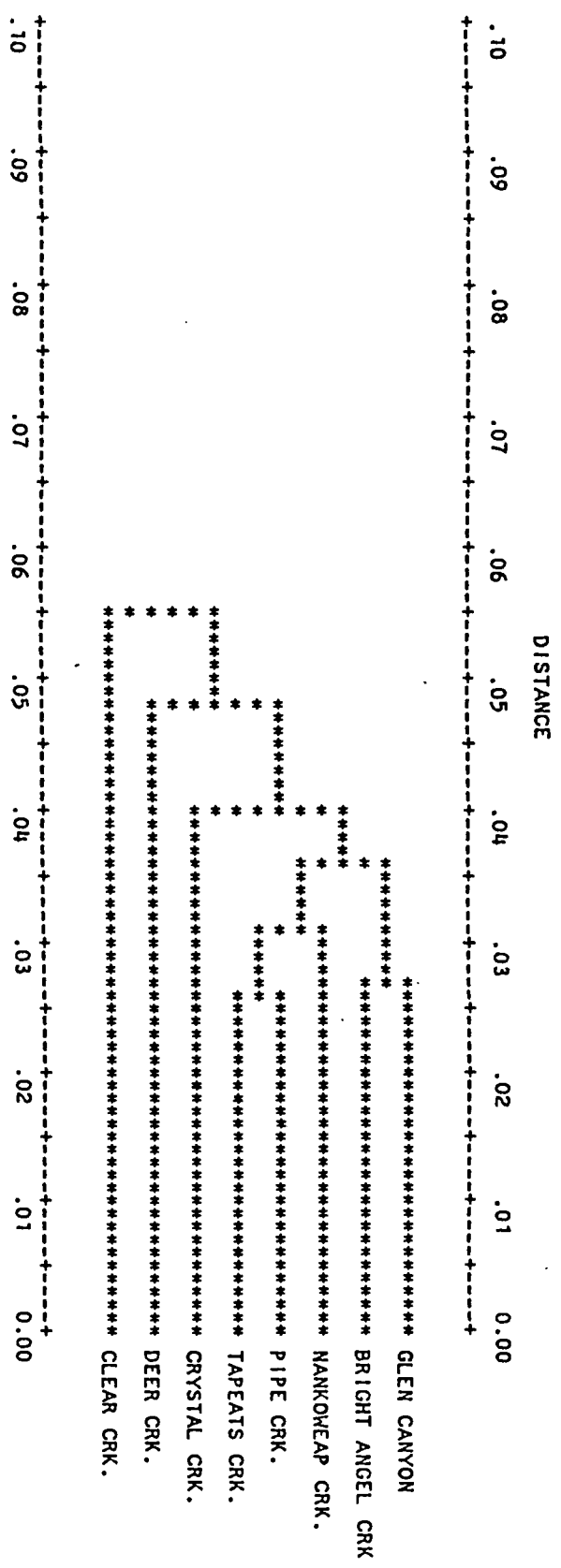


FIGURE 3. CLUSTER ANALYSIS USING UNWEIGHTED PAIR GROUP METHOD
 COEFFICIENT USED: MODIFIED ROGERS DISTANCE (WRIGHT, 1978)

GOODNESS OF FIT STATISTICS

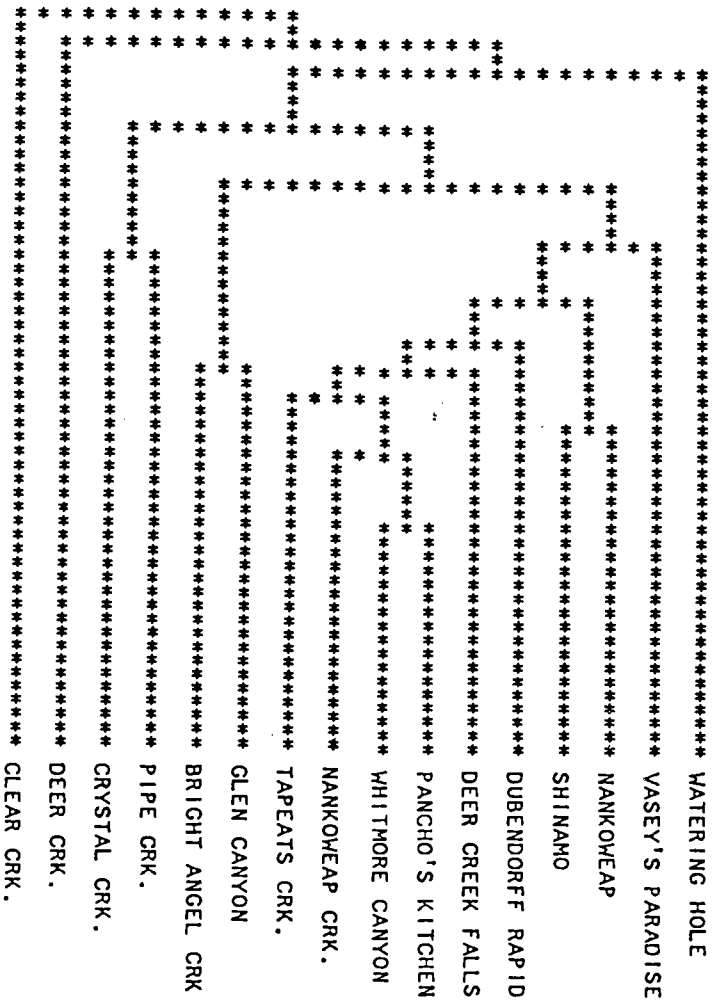
FARRIS (1972) "F" = .753

PRAGER AND WILSON (1976) "F" = 15.292

PERCENT STANDARD DEVIATION (FITCH AND MARGOLASH, 1967) = 20.374

COPHENETIC CORRELATION = .730

.10 .09 .08 .07 .06 .05 .04 .03 .02 .01 0.00
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 DISTANCE



.10 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 .09 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 .08 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 .07 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 .06 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
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