State of California

The Resources Agency

Memorandum

To : Files, Independence Lake, Sierra County Date : August 26, 1992

From : Department of Fish and Game - Region 2

Subject : 1992 Summary of CTL Spawning Surveys

A total of seven surveys were conducted on Independence Creek during the 1992 season. Surveys were begun on April 23 and ended August 14. The May 27 and June 16 surveys were conducted by Ms. Ann Carlson and Ms. Wendy Thompson of the U.S. Forest Service, Tahoe National Forest. All other surveys were conducted by myself.

Water volumes in the creek were above average early in the season and declined rapidly to the low levels which have come to characterize the stream late in the season. Water flows were adequate to maintain surface flow in the creek during the survey period, and young-of-the-year CTL should have access to Independence Lake this fall.

It was noted that many more Lahontan (Tahoe) suckers (LSKE) appear to be using the stream this year than in the recent past. The reasons for this change are unknown.

Below is a summary of survey data information:

Date	Estimated Flow	Prevailing H_2O Tem (°F)	Observed Fish Species in Creek
4/23	14	42°	CTL, LRS
5/15	14	42°	CTL, LRS
5/27	NA	44°	CTL,LRS,LSKR,BK
6/5	9	47°	BK,LRS,LSKR
6/16	5	49°	BK,LRS,BK-fry
7/13	6	52°	LRS,BK-fry,LSKR
8/14	2	56°	LRS,CTL-fry

The mean size of the spawning CTL was smaller, with fish in the 14-16 inch size class comprising 56% of the run. Far fewer numbers of the 22-24 inch size class (8%) were seen as compared to surveys since 1988.

Changing environmental conditions in the Independence Creek watershed are the likely cause for the changes monitored in this survey. Every effort should be made to survey the system as early in the year as possible, preferably beginning in early April. Further information is necessary to determine whether the timing and composition of the CTL spawning run is shifting to survive changing environmental conditions.

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Date	Total Occupied Redds	Redds w/2 fish	Redds w/1 Fish	Total Unoccupied Redds	Other Live CTL	Total Carcasses	Live CTL Spawn
4/23	4	2	2	5	6	0	12
5/15	16	11	5	15	4	1	31
5/27	12	9	3	22	3	1	24
6/5	3	1	2	16	4	8	8
6/16	0	0	0	19	0	15	0
7/13	0	0	0	12	0	0	0
8/14	0	0	0	4	0	0	0

Visual Estimates of Total Length of CTL Spawners (inches)

Date	< 12	14-16	18-20	22-24	> 24	Total
4/23	2	8	0	2	0	12
5/15	2	18	8	3	0	31
5/27	1	9	13	1	0	24
6/5	0	7	1	0	0	8
6/16	0	0	0	0	0	0
7/13	0	0	0	0	0	0
8/14	0	0	0	0	0	0
Totals	5	42	22	6	0	75
% of Total	7%	56%	29%	8%	0%	100%

The 1992 surveys yielded several notable changes in the character of the CTL spawning run. Drought conditions allowed access to the stream far earlier than in past years. These earlier surveys suggest that the CTL run itself may be shifting to a late-spring phenomenon, with its peak around mid-May. Total observed CTL numbers for this year is more than triple what have been monitored over the past three years, with 57% of the fish occurring before May 15.

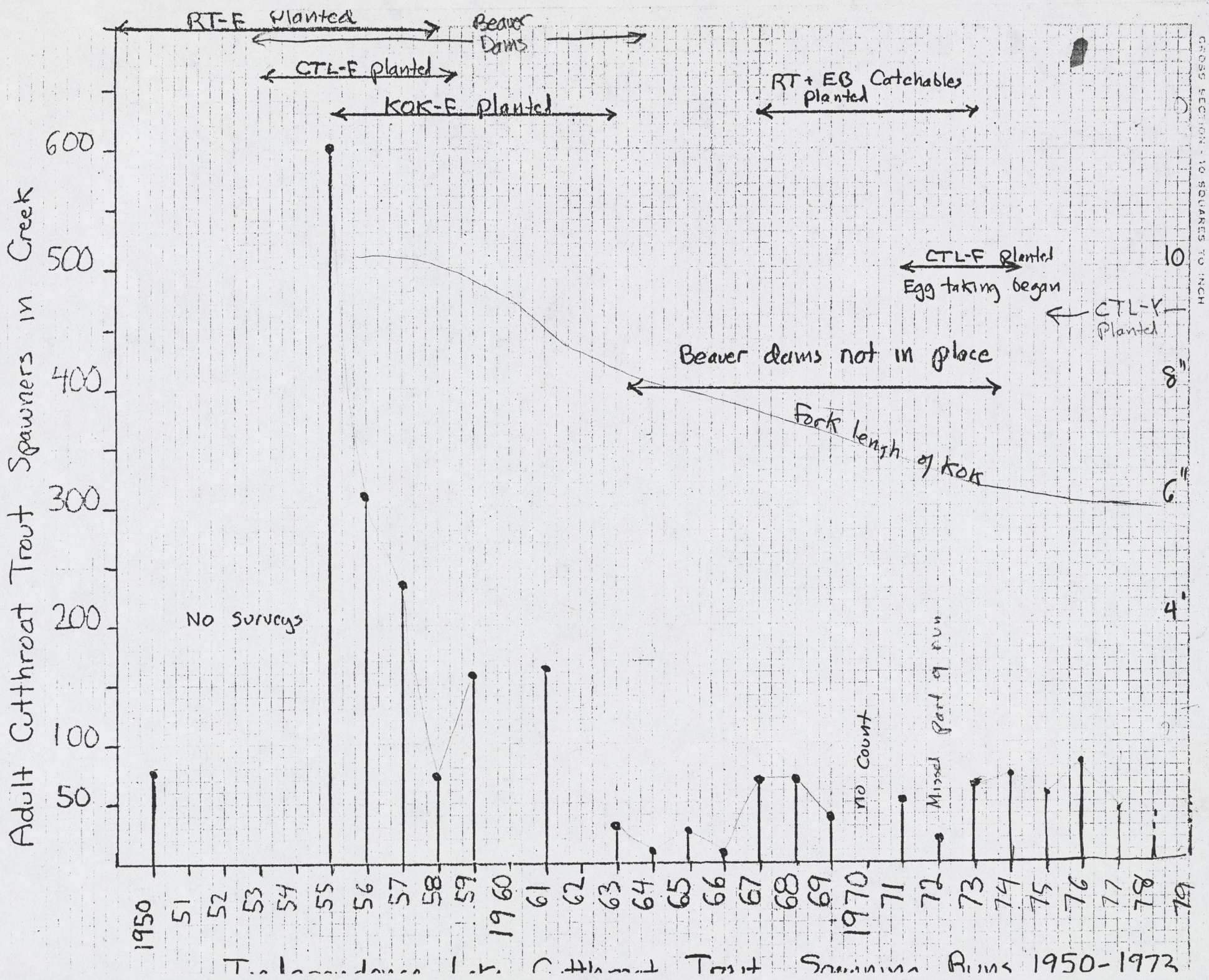
RT-F planted
Beaver Dams
CTL-F planted
KOK-F planted
RT + EB Catchables planted
CTL-F planted
Egg taking began
CTL-Y Planted
Beaver dams not in place
Fork length of KOK
Adult Cutthroat Trout Spawners in Creek
600
500
400
300
200
100
50
10
8"
6
4"
No Surveys
no count
Missed part of run
1950
51
52
53
54
55
56
57
58
59
1960
61
62
63
64
65
66
67
68
69
1970
71
72
73
74
75
76
77
78
79

Table 3. Indices of Lahontan Cutthroat Trout Abundance in Independence Lake, 1973-1991

Year	No. of CTL Planted	Gillnet Index	Sport Caught CTL (landings)	Mean No. of Spawners Observed Per Survey	Total No. of CTL Sightings
1973	21,500 F				84 weir 1/
1974	None	3			78 weir 1/
1975	75,000 Y		26		59 weir 1/
1976	6,200 Y		327		83 weir 1/
1977	17,500 Y	3	261		20 weir 1/
1978	None		150		40 weir 1/
1979	12,000 Y		-		
	1,400 C				316 planted
					18 wild 2/
1980	1,500 C	2	80		524 planted
					25 wild 2/
1981	1,900 C	6	26	24	72 2/
1982			97	27	164 2/
1983			36	8	50 2/
1984		2	81	18	74 2/
1985				8	40 2/
1986				17	69 2/
1987			12	23	92 2/
1988				6	46 2/
1989				21	160 2/
1990				4	22 2/
1991				11	61 2/

F = Fingerlings Y = Yearlings C = Catchable-sized trout

1/ All fish passing through the weir were trapped and counted

2/ This figure represents the sum of fish observed on each survey and should not be considered as a spawner count, particularly since a varying but unknown number of spawners are recounted during subsequent surveys. The data may serve as an index of relative abundance. (both live fish and carcasses were counted).

Population trend data will continue to be collected in the future to evaluate the effectiveness of habitat and fish population restoration activities.

5) McCloud Redband Trout Status. Fish population surveys involving electrofishing are periodically conducted on the more important redband streams in the McCloud River drainage. These are Trout and Swamp creeks, each with two miles of habitat, and Sheepheaven Creek, with about a half-mile of suitable habitat. The Sheepheaven Creek population has been adversely affected by drought and is limited to less than a hundred trout. Swamp Creek, a formerly barren stream, contains a good population of redband trout - over one thousand fish. Trout Creek which was chemically treated to remove nonnative trout also supports a good population of redband trout, though nonnative brown trout have become reestablished in the

Genetic Analysis of Kern River Rainbow Trout Populations With a Note on Two Cutthroat Populations

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Report to

California Department of Fish and Game
Threatened Trout Committee

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Introduction

Three forms of the rainbow trout are generally recognized to exist in the Kern River basin. These are: the Volcano Creek golden trout (a.k.a., South Fork golden trout) classified as Oncorhynchus mykiss aquabonita (formerly Salmo gairdneri aquabonita; S. aquabonita); the Little Kern River golden trout classified as O. mykiss whitei (formerly S. g. whitei; S. whitei); and the Kern River rainbow trout classified as O. mykiss gilberti (formerly S. g. gilberti). This alignment of the Kern River basin trout as subspecies has been confirmed by studies by Gold and Gall (1975), Smith (1981) and Berg (1987).

The extensive genetic analyses by our laboratory and summarized in Berg 1987) indicate that the Kern River rainbow trout is intermediate genetically to the Little Kern golden trout and coastal rainbow trout. The most plausible explanation for this observation is that the Kern River rainbow trout originated as an hybrid between ancestral inhabitants of the Little Kern River and later invading coastal type rainbow trout. Alternatively, the Kern River rainbow trout could have served as the progenitors of the Little Kern golden trout which have since followed a separate evolutionary pathway in isolation. The possibility exists today for fish from the Little Kern River system to migrate downstream into the Kern River, events which would be expected to maintain a degree of similarity between the two groups.

The purpose of the present study was to follow up on the extensive sampling carried out in the late 1970's and early 1980's. Eleven Kern River population were sampled in 1991. A few of these samples were obtained from populations included in the earlier studies. In addition, the earlier studies provided data for other populations from the Kern Basin and

for rainbow trout from coastal California populations. Thus, the early work provided comparative information. This report provides an overview of the genetic relationships of Kern River rainbow trout to other rainbow trout, compares the results of repeated sampling from several Kern River populations, and examines the 1991 samples in detail.

Material and Methods

Populations:

Data for a total of 31 population samples were utilized in the study. Groups of trout represented included Kern River rainbow trout, Volcano Creek golden trout, Little Kern River golden trout, and coastal rainbow trout.

Eleven populations were sampled in 1991 by the Department of Fish and Game and the fish delivered to the Animal Science laboratory at Davis. The samples provided (with sample size) were:

Nine-mile Creek (n=23)

Bone Creek above Highway 190 (n=11)

Bone Creek below Highway 190 (n=13)

Freeman Creek (n=23)

Junction Meadow (n=16)

Kern River at Kern Flat (n=27)

Kern River at Peppermint Creek (n=27)

Peppermint Creek (n=25)

Rattlesnake Creek at Bonita Flat (n=25)

Upper Funston Meadow mx=16)

Red Rock Creek (n=3)

Three of the eleven Kern River samples had been collected in the 1978-80 time period (referred to hereafter as the '79 samples). In addition, a sample was obtained from Peppermint Creek in 1985. Thus, the four samples (with sample size) available for direct comparisons of genetic characteristics were:

Nine-mile Creek (n=20)

Kern River at Kern Flat (n=18)

Rattlesnake Creek (n=24)

Peppermint Creek (n=16)

Seven samples from other areas of the Kern River were included in the '79 collections. These were used along with the 1991 collections to establish a broad overview of the rainbow trout populations of the Kern River. The samples (with sample size) were:

Soda Creek (n=24)

Lower Osa Creek (n=27)

Forks of the Kern (n=34)

Kern Lake (n=18)

Grasshopper Flats (n=29)

Hell Hole Creek (n=25)

Salmon Creek (n=24)

Data for representative samples of three other groups of rainbow trout were used for

comparative analyses of the genetic characteristics of Kern River fish and those of other rainbow trout lineages. The three groups and the nine samples selected as representative (with sample size) were:

Volcano Creek golden trout:

Golden Trout Creek (n=16)

Volcano Creek (n=19)

Mulkey Creek (n=31)

Little Kern golden trout:

Lower Wet Meadow Creek (n=24)

Middle Wet Meadow Creek (n=21)

Deadman Creek (n=14)

Coastal rainbow trout:

Devil Creek (n=20)

Big Creek (n=21)

Gualala Creek (n=57)

Methods of Analysis

Genetic variation at loci for selected enzyme systems was detected using standard starch-gel electrophoresis techniques (Berg and Gall 1988; Bartley and Gall 1990). Proteins were assayed from blood, eye, heart, liver, and muscle. A total of 84 loci were examined for the eleven 1991 Kern River samples. Nine were excluded from the analysis due to difficulties in reliably scoring these systems. Thirty-two loci were common to the data for

the '79 and 1991 collections as well as for the samples used for comparative purposes. Of the 32 loci, 10 represented five duplicate pairs that had been treated as single loci under the old methodology. Thus, there were effectively 27 loci available for comparative analyses, of which 21 were polymorphic (showed variation) in at least one population.

Genetic variability was assessed by calculating allele frequencies for each locus. Genetic identities (I) between all sample pairs were estimated using the method of Nei (1978). These estimates were then averaged arithmetically to obtain genetic identity estimates within and among samples for various groups of trout. Genetic diversity (G_{ST}) was estimated from total gene diversity (H_T) and within sample heterozygosity (H_S) following the methods outlined by Nei (1973) and Charkraborty and Leimar (1987).

Results

Genetic Variation:

Of the total of 75 loci included in analyses of the 1991 Kern River samples, 34 loci were monomorphic, and 41 were polymorphic. The nine loci excluded from the analyses were:

AAT-1,2, G3PDH-2, IDDH-1, IDDH-2, MDHp-1, MDHp-2, AND PGM-3,4.

The 34 monomorphic loci were:

AAT-4, mAAT-2, ACP-2, ADA-2, ADH, mAH-3, ALAT-2, CK-2, CK-5, FBALD-3, FBALD-4, GAPDH-1, GAPDH-2, GAPDH-5, GAPDH-6, αGLU, ßGALA-1, ßGALA-2, G3PDH-2, GR, HAGH, IDH-1, LDH-1, LDH-2, LDH-3, LDH-4, αMAN, mMDH-1, MDHp-3, MPI, PGK-1, PGM-1, TPI-2

and TPI-4.

The 41 polymorphic loci are listed in Table 2 along with their frequencies of occurrence in all the 1991 samples.

Thirty-two loci were common to both the '79 and 1991 data sets. Of the 27 effective loci that could be used for genetic analysis, 21 were polymorphic in at least one population. The polymorphic loci used in this analysis included:

ADH, CK-1, DPEP-1, DPEP-2, G3PDH-1, GPI-1, GPI-2, GPI-3, IDH-2, IDH-3,4, LDH-3, LDH-4, MDH-1,2, MDH-3,4, MDHp-3,4, PA-1,2, PHAP, PDGH, PGM-2, SOD-1, and TAPEP.

Loci monomorphic in all 31 populations were:

CK-2, G3PDH-2, IDH-1, LDH-1, LDH-2, and PGM-1.

Overview of Relationships:

The genetic identity among samples was summarized in a dendrogram based on an unweighted pair-wise averaging clustering analysis (Figure 1). The results clearly show the distinctness of the Volcano Creek and Little Kern golden trout (bottom of the figure). The coastal rainbow trout also establish a separate group, along with Bone Creek, indicating distinct genetic differentiation among the three groups used for comparison purposes.

For the most part, the Kern River Rainbow Trout (KRRT), from both the '79 and 1991 collections, formed a fairly tight group, with genetic identities of 0.99 or above. In addition, populations located within the main Kern River itself (or very close to it) formed a group with high genetic identity, regardless of their distance along the Kern River. These

include Kern Flat, Kern at Peppermint, Forks of the Kern, Kern Lake, Grasshopper Flats, along with Upper Funston Meadow, Soda Creek, and Lower Osa Creek.

A significant exception was the population in Bone Creek, which showed a closer genetic relationship to coastal rainbow trout than Kern River trout. Their genetic identities with coastal trout were surprisingly high, averaging around 0.990. These results may reflect a recent introduction into Bone Creek (planned or otherwise) or an evolutionary history distinct from typical Kern River rainbows.

Another exception to the consistent genetic similarity among Kern River trout was the sample from Freeman Creek which showed little identity with any other population in the study. Its ancestry is clearly distinct from KRRT. The allele frequencies observed suggests a strong hatchery influence, as discussed in a subsequent section.

Within the Kern River group, the samples from Peppermint Creek and Ninemile Creek appear to be considered outliers, as does Salmon Creek. While Red Rock Creek grouped with Peppermint Creek in the dendrogram, this is most likely erroneous, a consequence of the small sample size (n=3).

Average Genetic Identity:

The average genetic identities within and among the five population groups are presented in Table 1. The results are very similar to those observed from the dendrogram. The within group genetic identities (values on the diagonal of Table 1) are high for all ----- groups, although the average genetic identity among the Kern River samples collected in 1991 was the lowest of all five groups. Clearly, the samples obtained from the Kern River

area in 1991 represented a greater diversity of genetic types than did the '79 samples. Two samples, Bone Creek and Freeman Creek, account for most of this discrepancy (as discussed in a latter section).

The Kern River samples ('79 and 1991) show little homology with the Volcano and Little Kern groups, but show moderate homology with the Coastal Rainbow group. Of the three comparison groups, KRRT are most distinct from Volcano Creek populations. The results agree with Berg's (1987) hypothesis that KRRT arose as a hybrid between coastal and Little Kern forms. However, it also is possible that KRRT fish were the progenitors of the Little Kern River fish in the distant past.

There appears to have been little change in the genetic identities among the groups over the last 12 years. Although the average genetic identity of KRRT with Little Kern trout appears to have dropped slightly while the average identity with Coastal Rainbows increased slightly, these differences are consistent with the difference observed in the within group genetic identities for the two Kern River sampling periods. Thus, it is unlikely that this apparent change is due to hatchery influences; it is more likely that the differences simply reflect the fact that different populations were sampled in the different studies.

The 1991 KRRT samples:

Based on analysis of data for the 41 polymorphic loci found in the 1991 samples, an estimate of 20.1 % was obtained for the Coefficient of Gene Diversity, G_{st}. This coefficient can be interpreted as an estimate of the percentage of genetic variation among all fish that can be attributed to average genetic differences between groups of fish and is a reflection

of reproductive subdivision of populations. This figure is unusually high for fish located within a single basin. For example, Berg and Gall (1988) found a value of 13.2 % for coastal rainbow while Bartley and Gall (1990) obtained an estimate of 6.1 % for California chinook salmon. Other studies have reported values in the range of 5 % to 12 % for many species. The high G_{st} value may reflect very different ancestries of populations in the 1991 sampling, an interpretation which is consistent with the dendrogram analysis. It also indicates that there is very low migration among at least subsets of the populations sampled.

A dendrogram representing the relationships among the populations studied in 1991 is presented in Figure 2. The genetic identity analysis utilized all 41 polymorphic and 34 monomorphic loci available. The average pair-wise genetic identity for the group of samples was 0.9796 (Table 1). In general, the dendrogram indicates relationships similar to those obtained in the comparative study using a reduced number of loci (Figure 1). One exception is Red Rock Creek which clustered with Peppermint Creek in the comparative study, but occurs as an outlier to most of the Kern samples in the 1991 analysis. As mentioned earlier, the Red Rock Creek sample size was too small to provide useful information regarding the most accurate placement of this population relative to the others.

The most interesting and obvious result of the 1991 analysis was that the samples collected from the main Kern River were much more similar to one another than to any of the tributaries. In fact, the three samples spanning the 36 miles of the main river, Kern at Peppermint Creek, Kern Flat, and Upper Funston Meadow, were almost identical and the sample from Junction Meadow was very similar to these three samples. Finally, the Kern River tributary, Rattlesnake Creek clustered with the Kern River group at a genetic identity

of .9956. All the remaining samples appear as outliers to what should be considered typical Kern River rainbow trout having genetic identities of less than .990 with the samples from the main Kern River. However, the samples from Ninemile Creek and its tributary Redrock Creek, and Peppermint Creek show a strong association with the group of samples taken from the main Kern River. The only fish that do not appear to be typical Kern River rainbow trout are those from the Upper and Lower Bone Creek samples. The sample from Freeman Creek presents a special problem; see the section on 1991 Samples Not Previously Examined for discussion.

Comparison of '79 and 1991 Samples

Rattlesnake Creek:

Although the '79 sample was collected from the upper portion of the creek, the 1991 sample, collected at Bonita Flat, was remarkably similar genetically to the sample of 13 years earlier. The only major exception was observed at the PGDH locus. A variant had not been detected at this locus in any of '79 Kern River Rainbow Trout (KRRT) collections, including the Rattlesnake Creek sample obtained in 1978. However, the 1991 collection contained of frequency of 0.220 for the PGDH(120) allele. This variant had been an important genetic marker for the Little Kern Golden Trout as they contain an average frequency of 0.460 for this allele. Our first thought was possible contamination from the Little Kern River by overzealous fishermen. However, if these fish had been introgressed with Little Kern golden trout over the last 13 years, we also would expect a corresponding decrease in the PA-1,2(105) allele frequency and an increase in the SOD(60) allele

frequency. In fact, the opposite occurred at these two loci. Thus, the observed frequency of the PGDH(120) allele must be assumed as a natural occurrence.

Another discrepancy was observed at the PHAP locus. The PHAP(90) allele occurred at a frequency of 0.125 in the 1978 sample but was absent from the 1991 sample. However, fish in neighboring tributaries, such as Osa Creek and Soda Creek, as well as those sampled at Forks of Kern show either a low frequency or the absence of the PHAP(90) allele. Thus, these differences can simply be explained as sampling error due to our relatively small sample sizes.

Of the loci screened in 1991 but not screened in 1978, the TPI-3(97) allele was observed at a frequency of 0.140 in Rattlesnake Creek; this allele also was seen at a frequency of 0.019 in the Kern Flat sample. Diagnostic loci for the KRRT, namely IDH-3,4, PA-1,2, TAPEP, MDH-3,4, and SOD, remained consistent with expectations and similar to the '79 collections.

Kern Flat

The Kern Flat sample of 1991 maintained its genetic similarity with the '79 sample at nearly every comparable locus. Only minor allele frequency differences were seen at a few loci, including MDH-3,4, PHAP, GPI-2, and IDH-2. Alleles at loci diagnostic for KRRT, such as IDH-3,4(74), SOD(60), and PA-1,2(100), actually showed slight increases in frequency indicating that there has been no introgression with fish stocked at this location over the past 13 years.

Of the new protein systems screened in 1991, the Kern Flat sample contained an

ACP-1(-350) variant, at a frequency of 0.093, that was not observed in any other Kern sample collected in 1991.

Because the Kern Flat sample had changed very little over the 13 year period (about 5 generations) and possesses Kern River rainbow trout genetic characteristics, it appears to be a solid representative of the typical species of the basin.

Ninemile Creek

The '79 sample from Ninemile Creek was collected at the uppermost part of the drainage, whereas the 1991 sample was taken much lower on the creek. Therefore, comparisons between the two collections may be misleading. The sample from Redrock Creek, a tributary of Ninemile Creek, consisted of only 3 fish; therefore conclusions from such a small sample could be erroneous and so will not be attempted. The 1991 sample from Ninemile Creek showed indications of some introgression with planted rainbows at several diagnostic loci. The upper Ninemile '79 sample appeared to be representative of the KRRT so further sampling of the area will be necessary to delineate the apparent coastal type rainbow trout contamination observed in the 1991 sample.

Alleles SOD(60) and IDH-3,4(74), diagnostic for KRRT, were at low frequency in the Ninemile 1991 sample (0.109 vs a KRRT average of 0.345 and 0.305 vs a KRRT average of 0.744, respectively). However, some variants commonly seen at low frequencies in KRRT fish were present in Ninemile 1991, including mAH-1(20) at 0.022, CK-3(105) at 0.065, DPEP-1(111) at 0.022, GPI-2(140) at 0.043, IDH-2(105) at 0.174, PDPEP(86) at 0.109, and TAPEP(124) at 0.152. Unusual characteristics for KRRT fish included a very high

frequency of ALAT-1(125) at 0.478, an allele observed at Junction Meadow at frequency of .0219, Kern at Peppermint at 0.048, Kern Flat at 0.037, and Upper Funston Meadow at a frequency of 0.019. A unique variant to the Kern basin was the CK-1(70) allele at a frequency of 0.065 in Ninemile Creek. A GPI-1(50) variant at a frequency of 0.217 also was observed in the Redrock Creek tributary at a frequency of 0.167, but nowhere else in the Kern River. The PGDH(120) variant allele was observed in Ninemile Creek at a frequency of 0.022 and in Rattlesnake Creek at a frequency of 0.220. The origin of these unusual variant alleles is not known.

Peppermint Creek: 1986 and 1991.

Peppermint Creek was sampled in 1986 and again in 1991, and although the collections were only about 2 generations apart, significant differences in the genetic structure of the population were observed. Considering diagnostic loci, the frequency of the IDH-3,4(74) allele was low in 1986 (0.453) and remained low (0.490) in 1991, compared to the average frequency of 0.744 typical of KRRT fish. The SOD(60) allele frequency dropped 20% from 0.655 to 0.458 over the 5 year period but was still higher than the KRRT average of 0.345. The frequency of PA-1,2(105) dropped from 0.422 in 1986 to 0.280 in 1991, well below the 0.501 common for KRRT. A common hatchery rainbow trout allele, IDH-3,4(45), appeared at an alarming high frequency of 0.140 and 0.160 in 1986 and 1991 samples, respectively, suggesting some past introgression.

Peppermint Creek appears somewhat genetically distant from other KRRT samples based on Nei's Genetic Identity (Figures 1 and 2) not so much because of its disparity at

diagnostic loci, but rather because of unusual variants at several other loci. Among these unique variants to the Kern basin and their frequencies in the 1991 samples, were: PGM-2(115) at 0.420, AH-1(110) at 0.220, GPI-1(183) at 0.080, DPEP-2(80) at 0.040, and MDH-1,2(42) at 0.030. In a genetic distance analysis, the allele frequencies at these loci would tend to make Peppermint Creek seem very different from other Kern River samples, even though allele frequencies at KRRT diagnostic loci were not that dissimilar. Thus, whether the Peppermint Creek fish represent typical KRRT remains an open question. Care must be taken not to discard a stock like this as introgressed fish when such anomalies in allele frequencies can be caused by long-term isolation from the parent stock. Information on planting activities and the history of other management activities is needed before final conclusions can be drawn. An assessment also must be made of what represents an unacceptable level of apparent introgression.

Other 1991 Samples not Previously Examined

Bone Creek:

Two samples were collected from Bone Creek in 1991. One was collected at a point above Highway 190 and is referred to as Upper Bone Ck., while the other was collected below Highway 190 and is referred to as Lower Bone Ck. Neither Bone Creek sample is representative KRRT in any way. The samples exhibited virtually no IDH-3,4(74) alleles, and a frequency of less than 0.050 of the SOD(60) allele, both diagnostic KRRT alleles. Alleles G3PDH(140), TAPEP(150), MDH-3,4(85), mAH-1(20), DPEP-1(111), and GPI(140),

all common variants in KRRT populations, were virtually absent in the Bone Creek samples.

Common hatchery rainbow trout alleles also were found in the Bone Creek fish. These included, with their frequencies: PGM-2(85) at 0.150, IDH-3,4(45) at 0.279, and MDH-3,4(75) at 0.125. The PA-1,2(105) allelic frequency averages 0.501 in KRRT, but occurred at a frequency of 0.712 in Bone Creek. Also, the Bone Creek sample possessed unique variant alleles not found in KRRT populations including (with their frequencies): CK-4(105) at 0.080, GPI-2(0) at 0.023, GPI-3(85) at 0.019, and TPI-1(-300) at 0.152. All of these characteristics taken together strongly indicate that the Bone Creek fish had an origin distinctly different from KRRT.

Freeman Creek:

Freeman Creek allele frequencies suggest that this stock may have introgressed considerably with stocked rainbow trout. The observed SOD(60) allelic frequency of 0.174 is about one-half the KRRT average frequency of 0.345. In addition, the SOD(140) allele, a common hatchery rainbow trout allele, was found at a frequency of 0.391, much higher than for any KRRT stock. Another common hatchery rainbow variant, PGM-2(85), was found in Freeman Creek at a frequency of 0.196. The TAPEP(150) allele found throughout the Kern River was absent from this population sample. The IDH-3,4(74) allele was found at a frequency of only 0.380 compared to the KRRT average frequency of 0.744. Unique to Freeman Creek fish were the GPI-2(46) allele found at very high frequency of 0.522 and the LDH-5(97) allele at a frequency of 0.217. These alleles probably were introduced from with hatchery rainbow trout.

Kern River at Peppermint Creek

This 1991 collection was taken from the Main Kern River at the confluence of Peppermint Creek. Based Nei's genetic identity analysis, these fish were most similar to those at Upper Funston Meadow, a sample cite over 35 miles upstream. Conversely, they possessed very few analogies with fish taken from Peppermint Creek, strongly indicating that few Peppermint Creek fish migrate and spawn in the main Kern River. Variant alleles commonly found in KRRT, and in the Kern River at Peppermint Creek sample (with their frequencies) are: ADA-1(93) at 0.214, AH-1(110) at 0.024, mAH-1(20) at 0.071, mAH-2(127) at 0.024, ALAT-1(125) at 0.048, G3PDH-1(140) at 0.071, IDH-2(105) at 0.048, mMDH-2(50) at 0.024, and TAPEP(124) at 0.119. A few alleles at diagnostic loci showed minor sampling differences from the norm for KRRT. The IDH-3,4(74) allele at a frequency of 0.560 was somewhat lower than the KRRT average of 0.744 while the SOD(60) allele at 0.429 was higher than the 0.345 average frequency for KRRT. The PA-1,2(105) allele at a frequency of 0.429 compared favorably with the average of 0.501 found for KRRT.

Upper Funston Meadow and Junction Meadow

These Funston and Junction Meadows samples were taken at the uppermost reaches of the Kern River. Genetically, these two samples look remarkably similar to the other Kern River samples obtained from other areas of the main Kern River (Figure 1). Upper Funston Meadow and Junction Meadow both exhibited a very high frequency of IDH-3,4(74)

(0.711 and 0.766, respectively) and a PA-1,2(105) frequency typical of KRRT (0.471 and 0.485, respectively). While the frequency of SOD(60) in Upper Funston Meadow was exactly equal to the average for KRRT (0.346), the frequency in Junction Meadow was low (0.063), one of the few unusual frequencies for these two samples. Other variant alleles commonly seen in KRRT were also observed in these samples, including AH-1(84), mAH-1(20), ALAT-1(125), DPEP-1(111), and G3PDH-1(140). Two alleles were unique to the Junction Meadow sample; these were PEPLT(110) at a frequency of 0.188 and TPI-3[103] at a frequency of 0.031. No evidence of hatchery rainbow trout influence was seen for either of these samples.

A Note on Two Cutthroat Populations

Four Mile Creek Paiute Cutthroats

The 23 fish from Four Mile Creek were homozygous for Paiute cutthroat alleles at all diagnostic loci, namely, at ADA-2, CK-2, DPEP-1, MDH-2, and MDHp-3,4. The fish were also homozygous at the PA-1,2, PGK-2, and SOD-1 loci for the common cutthroat alleles. Thus, there appears to be no evidence of rainbow trout introgression into this stock. In fact, the Four Mile Creek cutthroat population appears to be among the "purest" ever recorded.

The sampled fish were alarmingly lacking in heterozygosity. In fact, of the 84 loci examined only AAT-4 and AH-1 showed any polymorphism, and even for these loci, the alternate alleles were expressed in only one heterozygous fish. (While the data in Table 2 show IDH-3,4, MDH-1,2 and MDH-3,4 to be represented by 2 allelic forms in equal frequency, these are each duplicated loci fixed for alternate alleles.) Thus, 22 of the 23 fish sampled were homozygous at all loci analyzed. Assuming that the fish analyzed represent a random sample of the population, these data suggest that the population has undergone an extreme genetic bottleneck. This is often taken as evidence of vulnerability to extinction. However, if the population appears healthy and viable, its future is probably not in jeopardy. The population should be monitored carefully and further may merited further study.

Heenan Lake Cutthroat

The results of genetic analysis of the Heenan Lake Lahonton cutthroat were quite different from those obtained for the Four Mile Creek Paiute cutthroat. Unfortunately, the sample size was extremely small, and due to an error in processing the tissue, identification of the two sources of Heenan Lake cutthroat was lost. Of the 12 fish analyzed, only 2 were homozygous at all loci considered. However, overall levels of heterozygosity were still low, and typical of cutthroat trout, with most fish only demonstrating one to four heterozygous loci out of the 84 loci analyzed. Two fish accounted for more than 50% of the variability in the population.

Only one fish showed clear indications of introgression, expressing rainbow trout alleles at both DPEP-1 and MDHp-3,4. All other eleven fish were homozygous for the common cutthroat alleles at diagnostic loci. However, nine of these eleven fish expressed alleles which are inferential of rainbow trout introgression, though the loci cannot be considered diagnostic due to a lack of data at these new loci for the general cutthroat species. Seven fish expressed rainbow forms of alleles at IDDH-1 and IDDH-2; unfortunately, resolution of IDDH was not good for these samples so our confidence in the scoring was not high. Four fish expressed the IDH-3(126) allele, which is observed in rainbow trout but we had not previously seen in any cutthroat population. Two fish expressed TAPEP(100), a common rainbow trout allele which we had not previously observed in cutthroat.

Our overall impression was that one fish was clearly of hybrid ancestry, another fish was highly likely to have been of hybrid origin, and eight fish were suspect. The two fish

that were homozygous at all loci analyzed showed no evidence of rainbow trout introgression. Of those that did show evidence of introgression, the percentage of rainbow trout type alleles appeared to be relatively small.

One of the original goals of the Heenan Lake analysis was to analyze the variation in resident lake cutthroat versus hatchery broodstock. Due to the error in processing of the samples, we were unable to distinguish between the two stocks and were forced to analyze them jointly. Because of this problem, and the fact the data were largely inconclusive for a majority of the sample, we were unable to determine whether rainbow trout introgression differed for the two stocks.

Table 1. Average genetic identities within and among four groups of rainbow trout native to California, calculated from pair-wise genetic identity (I) estimates. Values on the diagonal are average identities for samples within groups. Values off the diagonal are averages for samples from different groups (among group identity).

	Kern 1991	Kern 1979	Volcano	Little Kern	Coastal Rainbows
Kern 1991	0.9796				
Kern 1979	0.9835	0.9911			
Volcano	0.9393	0.9383	0.9890		
Little Kern	0.9614	0.9706	0.9156	0.9917	
Coastal Rainbows	0.9784	0.9715	0.9468	0.9407	0.9960

TABLE 2. Allele frequencies for the 1991 samples of rainbow trout taken from the Kern River, and for Four Mile Creek cutthroat, and Heenan Lake cutthroat populations. See Table 3 for symbols for sample names.

		UBC	LBC	KPP	PEP	FRE	RAT	KFT	9MI	RRC	UFM	JMD	4MC	HLC
AAT1,2	110	.137	.077	.043	.050			.037	.044	.084	.019	.094		
	100	.863	.923	.957	.950	1.00	1.00	.963	.956	.916	.981	.906	1.00	.958
	80													.042
AAT3	110		.038											
	100	1.00	.923	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
AAT4	120												.977	.833
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.023	.167
mAAT1	-111	.045			.080				.022		.019			
	-100	.955	1.00	1.00	.920	1.00	1.00	1.00	.978	1.00	.981	1.00	1.00	.917
	-86													.083
mAAT3	[-110]			.048										
	[-100]	1.00	1.00	.952	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
ACP1	-350							.093						
	-100	1.00	1.00	1.00	1.00	1.00	1.00	.907	1.00	1.00	1.00	1.00		.042
	-35												1.00	.958
ACP2	200												1.00	1.00
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
ADA1	100	.727	.846	.786	1.00	.957	1.00	.852	.739	.667	.808	.875	1.00	.958
	93	.273	.154	.214		.043		.148	.261	.333	.192	.125		.042
ADA2	115												1.00	1.00
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
AH1	110			.024	.220									.083
	100	1.00	1.00	.976	.780	1.00	.980	.981	1.00	1.00	.962	.938	.978	.917
	84						.020	.019			.038	.063	.022	
mAH1	163													
	100	1.00	1.00	.929	1.00	1.00	.840	.926	.978	1.00	.904	.906	1.00	1.00
	20			.071			.160	.074	.022		.096	.094		
mAH2	127			.024										
	100	1.00	1.00	.976	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		.125
	60												1.00	.875

Table 2. Continued

		UBC	LBC	KPP	PEP	FRE	RAT	KFT	9MI	RRC	UFM	JMD	4MC	HLC
mAH3	250													.042
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.958
mAH4	119				.020						.019		1.00	.958
	100	1.00	1.00	1.00	.980	1.00	1.00	1.00	1.00	1.00	.981	1.00		.042
ALAT1	125			.048				.037	.478		.019	.219	1.00	1.00
	100	1.00	1.00	.952	1.00	1.00	1.00	.963	.522	1.00	.981	.781		
ALAT2	105													.083
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.917
CK1	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.935	1.00	1.00	1.00	1.00	1.00
	70								.065					
CK2	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
	85												1.00	1.00
CK3	[105]				.020			.037	.065	.500				
	[100]	1.00	1.00	1.00	.980	1.00	1.00	.963	.935	.500	1.00	1.00	1.00	1.00
CK4	[105]	.045	.115											
	[100]	.955	.885	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
DPEP1	111							.037	.022		.019	.031	1.00	.958
	100	1.00	1.00	1.00	1.00	1.00	1.00	.963	.978	1.00	.981	.969		.042
DPEP2	107										.019			
	100	1.00	1.00	1.00	.960	1.00	1.00	1.00	1.00	1.00	.981	1.00	1.00	1.00
	80				.040									
EST6,7	103			.038	.070			.028	.055	.084				
	100	.568	.846	.677	.470	.956	.850	.804	.782	.916	.721	.906	1.00	.979
	97	.432	.154	.275	.460	.044	.150	.158	.163		.279	.094		.021
	80							.010						
G3PDH1	140			.071				.074			.058	.063		
	100	1.00	1.00	.929	1.00	1.00	1.00	.926	1.00	1.00	.942	.938	1.00	1.00
G3PDH4	100	.955	.962	.857	.760	.891	1.00	.889	.826	.833	.750	1.00	1.00	.958
	81	.045	.038	.143	.240	.109		.111	.174	.167	.250			.042
GPI1	183				.080									
	100	1.00	1.00	1.00	.920	1.00	1.00	1.00	.783	.833	1.00	1.00	1.00	1.00
	50								.217	.167				

Table 2. Continued

		UBC	LBC	KPP	PEP	FRE	RAT	KFT	9MI	RRC	UFM	JMD	4MC	HLC
GPI2	140							.019	.043			.031		
	100	.955	1.00	1.00	1.00	.478	1.00	.981	.957	1.00	1.00	.969	1.00	.958
	46					.522								.042
	0	.045												
GPI3	115													.083
	100	1.00	.962	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.917
	85		.038											
IDDH1	950	.136			.040	.043	.100	.019	.109	.167		.031	1.00	.813
	100	.864	1.00	1.00	.960	.957	.900	.981	.891	.833	1.00	.969		.188
IDDH2	250												1.00	.708
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		.250
	40													.042
IDH2	[105]	.045	.231	.048			.020	.037	.174					
	[100]	.955	.769	.952	1.00	1.00	.980	.963	.826	1.00	1.00	1.00	1.00	1.00
IDH3,4	126	.068	.077	.012	.010			.010	.033					.084
	100	.682	.596	.368	.340	.566	.190	.260	.565	.583	.289	.203	.500	.416
	88												.500	.500
	74		.019	.560	.490	.380	.810	.693	.305	.333	.711	.766		
	45	.250	.308	.060	.160	.054		.037	.097	.084		.031		
LDH4	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.958
	72													.042
LDH5	100	1.00	1.00	1.00	1.00	.783	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	97					.217								
MDH1,2	130												.500	.500
	100	1.00	.981	1.00	.970	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.500	.500
	42		.019		.030									
MDH3,4	119					.076						.016		
	100	.704	.808	.904	.960	.869	1.00	.962	.869	.916	.914	.936	1.00	1.00
	95	.159	.057						.033		.019	.032		
	85		.019	.084	.040	.055		.019	.087	.084	.067	.016		
	75	.137	.116	.012				.019	.011					
mMDH2	100	1.00	.962	.976	.920	.935	1.00	.944	.956	1.00	1.00	1.00	1.00	1.00
	50		.038	.024		.065		.019	.022					
	-150				.080			.037	.022					

Table 2. Continued

		UBC	LBC	KPP	PEP	FRE	RAT	KFT	9MI	RRC	UFM	JMD	4MC	HLC
MDHp3,4	116												.500	.479
	105			.048				.056	.043				.500	.500
	100	1.00	1.00	.952	1.00	1.00	1.00	.944	.957	1.00	1.00	1.00		.021
PA1,2	105	.750	.673	.429	.280	.109	.437	.500	.337	.333	.471	.485	1.00	1.00
	100	.250	.327	.571	.720	.891	.563	.500	.663	.667	.529	.515		
PDPEP2	100	.864	1.00	1.00	1.00	1.00	1.00	1.00	.891	1.00	.904	.937	1.00	1.00
	86	.136							.109		.096	.063		
PEPLT	110											.188	1.00	1.00
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.813		
PGDH	120						.220		.022					
	100	1.00	1.00	1.00	1.00	1.00	.780	1.00	.978	1.00	1.00	1.00	1.00	1.00
PGK2	120								.022					
	100	.545	.577	.810	.980	.870	1.00	.852	.608	.333	.885	.750		
	90	.455	.423	.190	.020	.130		.148	.370	.667	.115	.250	1.00	1.00
PGM2	115				.420									
	100	.818	.885	1.00	.580	.804	1.00	1.00	1.00	.833	1.00	1.00	1.00	.958
	85	.182	.115			.196				.167				.042
PGM3,4	120		.019	.012		.054			.108	.333		.015		
	115	.363	.115	.393	.560	.511	.570	.444	.250	.250	.576	.594	1.00	.521
	110	.091	.212	.190	.180	.163	.050	.102	.283	.333	.039	.125		.167
	105	.546	.654	.393	.250	.272	.380	.426	.348	.084	.366	.203		.312
	100			.012	.010			.028	.011		.019	.063		
SOD	170							.019	.022	.333				
	140		.115	.071	.333	.391		.056	.109					
	100	.955	.808	.500	.208	.435	.480	.722	.761	.167	.654	.938	1.00	1.00
	60	.045	.077	.429	.458	.174	.520	.204	.109	.500	.346	.063		
TAPEP	124		.038	.119			.340	.148	.152	.167	.115	.188	1.00	.917
	100	1.00	.962	.881	1.00	1.00	.660	.852	.848	.833	.885	.813		.083
TPI1	-100	.773	.923	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	-300	.227	.077											
TPI3	[103]											.031		
	[100]	1.00	1.00	1.00	1.00	1.00	.860	.981	1.00	1.00	1.00	.969	1.00	1.00
	[97]						.140	.019						

[] measured from heterodimer band

Table 3. Listing of symbols used to identify samples (column headings) in Table 2.

Symbol	Sample Name as outlined in Text
UBC	Upper Bone Creek
LBC	Lower Bone Creek
KPP	Kern River at Peppermint Creek
PEP	Peppermint Creek
FRE	Freeman Creek
RAT	Rattlesnake Creek
KFT	Kern Flat
9MI	Ninemile Creek
RRC	Redrock Creek
UFM	Upper Funston Meadow
JMD	Junction Meadow
4MC	4 Mile Creek Paiute Cutthroat
HLC	Heenan Lake Cutthroat

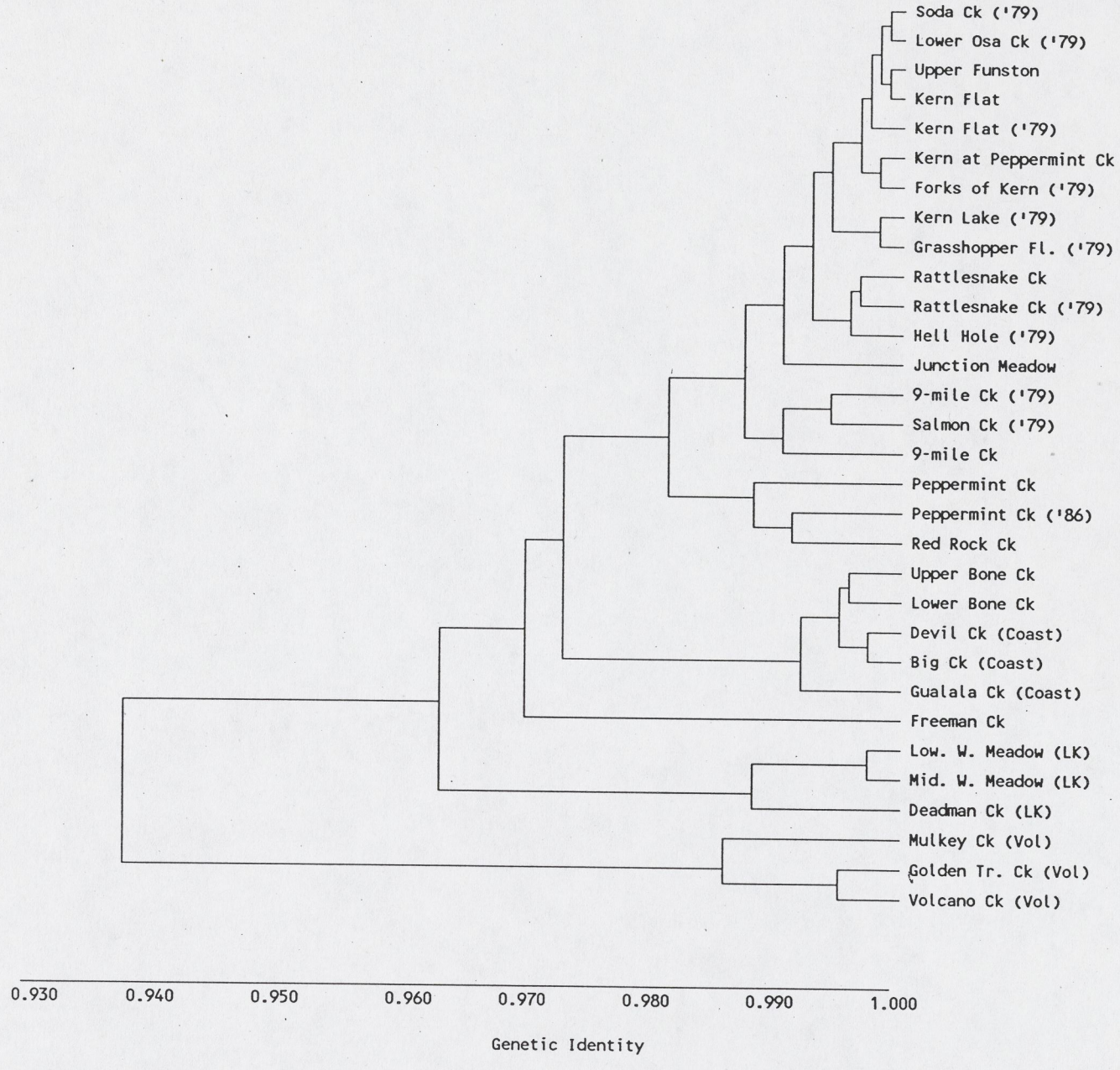


Figure 1. Dendrogram depicting the genetic relationships among rainbow trout sampled from the Kern River system in 1991, Kern River samples collected during earlier studies, and representative populations of Volcano Creek golden trout, Little Kern River golden trout, and coastal rainbow trout.

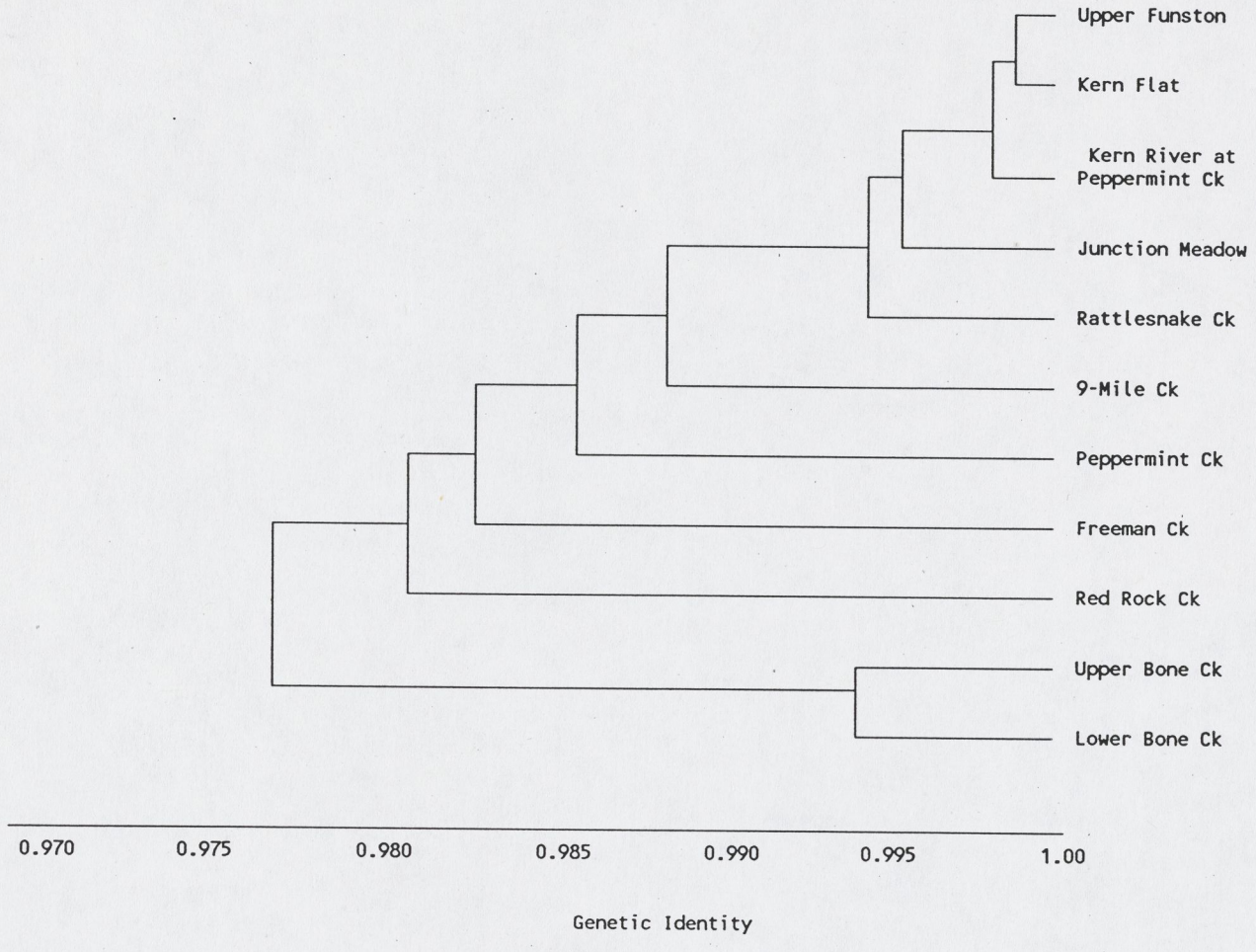
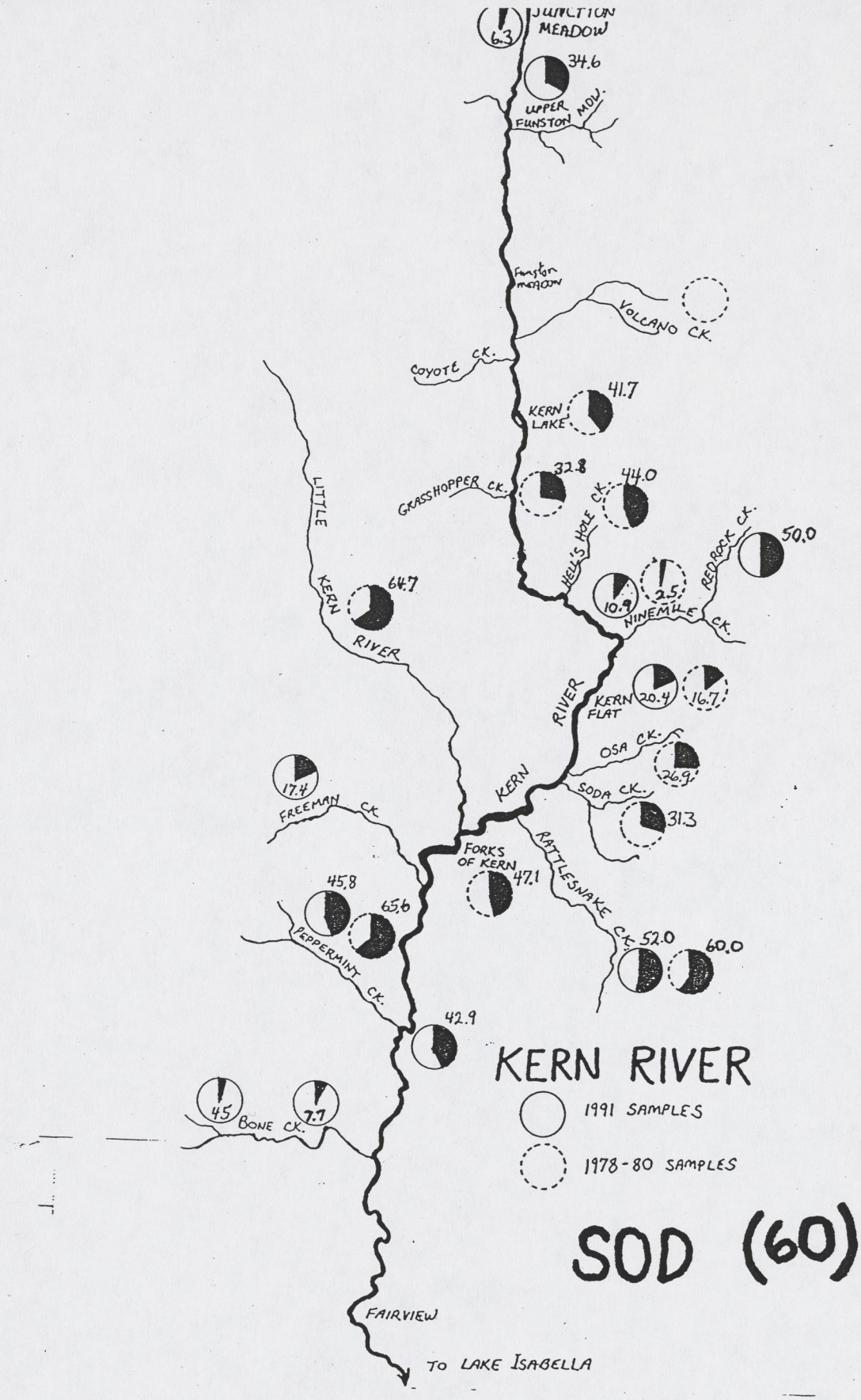
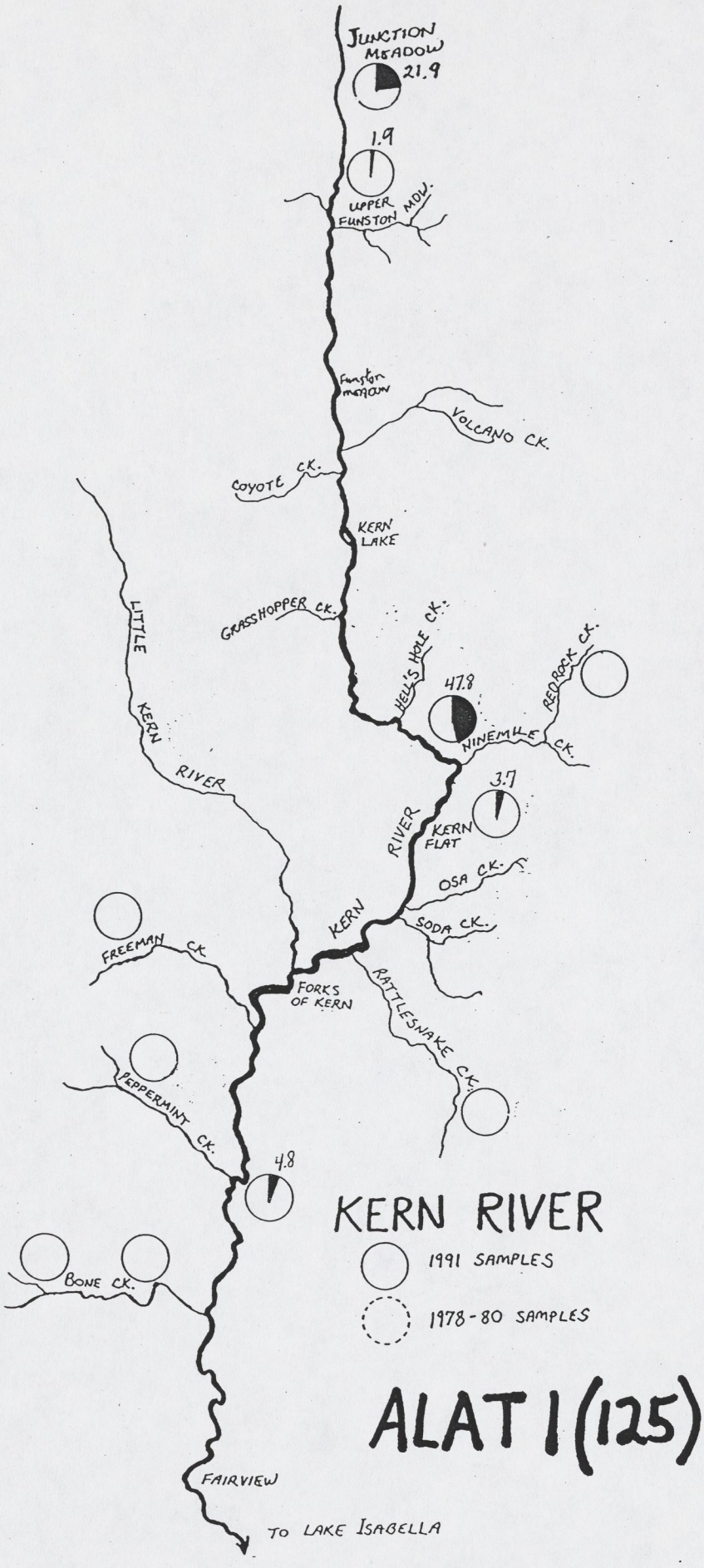


Figure 2. A dendrogram depicting the genetic relationship among samples collected from the Kern River system in 1991.

Appendix A

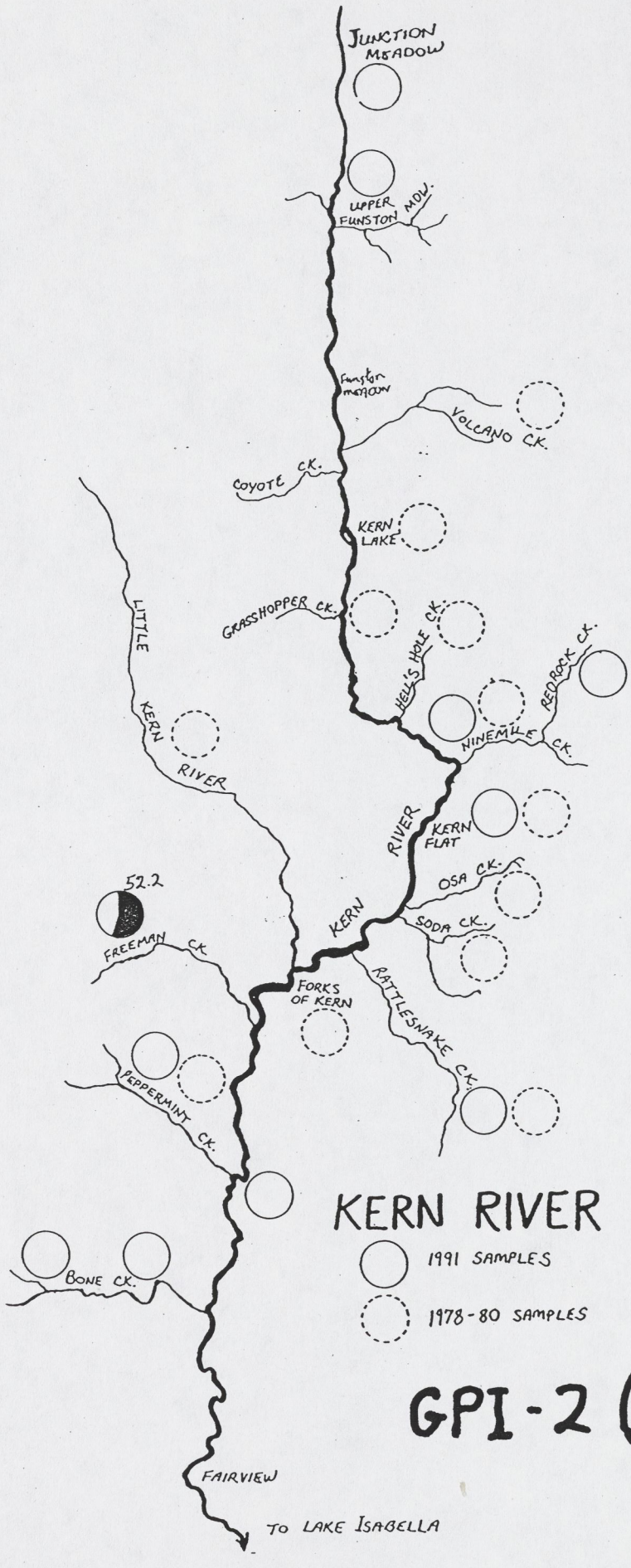
Maps of selected allele frequencies for Kern River populations sampled in 1978-80 and 1991.


JUNCTION MEADOW
6.3
34.6
UPPER FUNSTON MDW.
Funston meadow
VOLCANO CK.
COYOTE CK.
41.7
KERN LAKE
32.8
GRASSHOPPER CK.
44.0
HELL'S HOLE CK.
50.0
REDROCK CK.
10.9
2.5
NINEMILE CK.
LITTLE KERN RIVER
64.7
KERN RIVER
KERN FLAT
20.4
16.7
OSA CK.
26.9
SODA CK.
31.3
17.4
FREEMAN CK
KERN
FORKS OF KERN
47.1
RATTLESNAKE CK.
52.0
60.0
45.8
65.6
PEPPERMINT CK.
42.9
45
BONE CK.
7.7
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES
SOD (60)
FAIRVIEW
TO LAKE ISABELLA

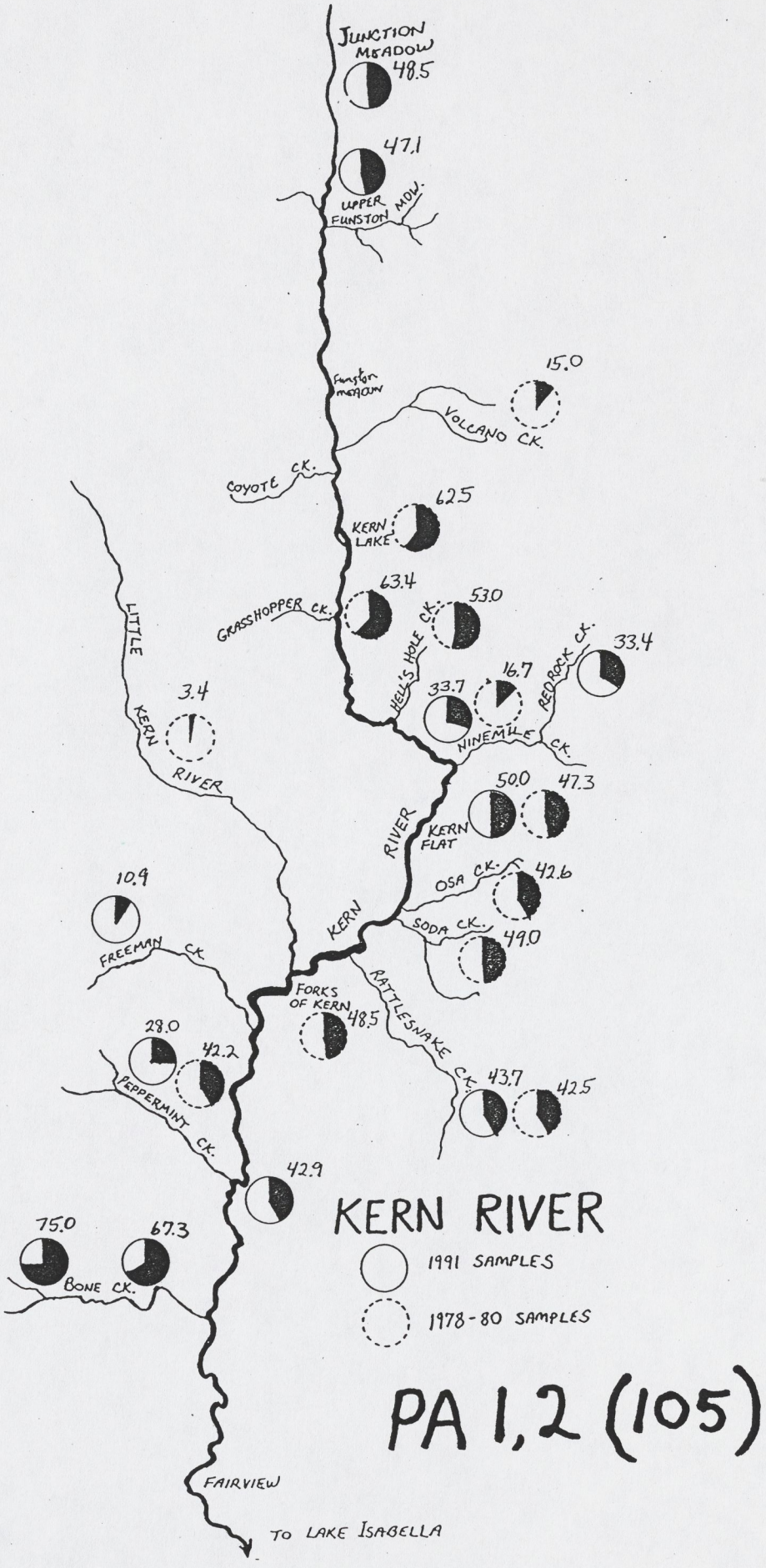

JUNCTION MEADOW
21.9
1.9
UPPER FUNSTON MDW.
Forster meadow
VOLCANO CK.
COYOTE CK.
KERN LAKE
GRASSHOPPER CK.
LITTLE KERN RIVER
HELL'S HOLE CK.
47.8
NINEMILE CK.
RED ROCK CK.
3.7
KERN FLAT
KERN RIVER
OSA CK.
SODA CK.
FREEMAN CK.
FORKS OF KERN
RATTLESNAKE CK.
PEPPERMINT CK.
4.8
BONE CK.
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES
ALAT 1 (125)
FAIRVIEW
TO LAKE ISABELLA

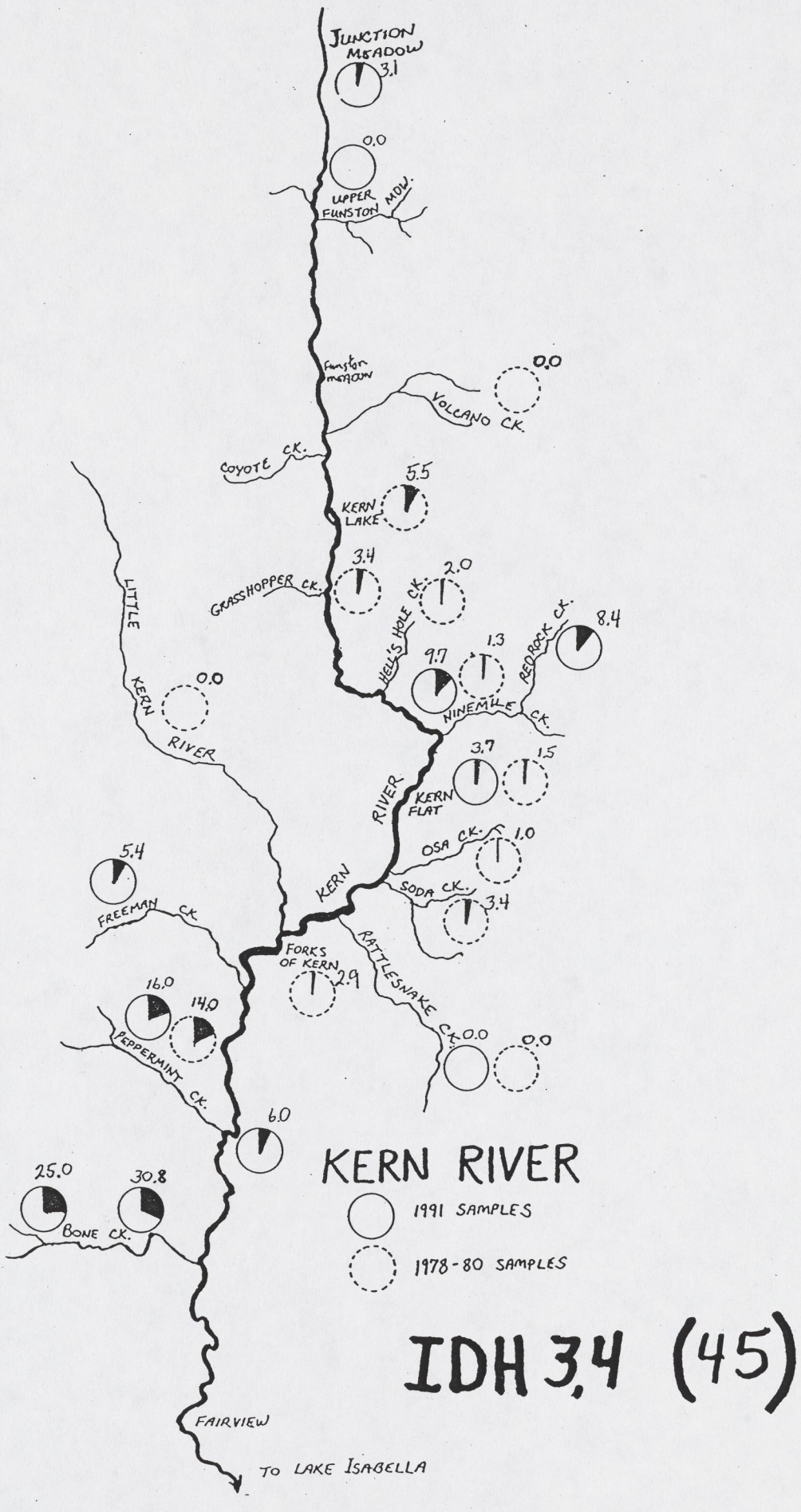

JUNCTION MEADOW
3.1
UPPER FUNSTON MDW.
Funston meadow
VOLCANO CK.
COYOTE CK.
5.6
KERN LAKE
1.7
GRASSHOPPER CK.
HELL'S HOLE CK.
REDROCK CK.
4.3
NINEMILE CK.
LITTLE KERN RIVER
KERN RIVER
1.9
KERN FLAT
OSA CK.
SODA CK.
KERN
FREEMAN CK
FORK'S OF KERN
1.5
RATTLESNAKE CK.
PEPPERMINT CK.
BONE CK.
FAIRVIEW
TO LAKE ISABELLA
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES

GPI-2 (140)


JUNCTION MEADOW
UPPER FUNSTON MDW.
Funston meadow
VOLCANO CK.
COYOTE CK.
KERN LAKE
GRASSHOPPER CK.
HELL'S HOLE CK.
REDROCK CK.
NINEMILE CK.
LITTLE KERN RIVER
KERN RIVER
KERN FLAT
OSA CK.
SODA CK.
52.2
FREEMAN CK
KERN
FORK'S OF KERN
RATTLESNAKE CK.
PEPPERMINT CK.
BONE CK.
FAIRVIEW
TO LAKE ISABELLA
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES

GPI-2 (46)


JUNCTION MEADOW
48.5
47.1
UPPER FUNSTON MDW.
Funston meadow
15.0
VOLCANO CK.
COYOTE CK.
62.5
KERN LAKE
63.4
GRASSHOPPER CK.
53.0
HELL'S HOLE CK.
33.4
REDROCK CK.
16.7
33.7
NINEMILE CK.
LITTLE KERN RIVER
3.4
KERN RIVER
50.0
47.3
KERN FLAT
OSA CK.
42.6
SODA CK.
49.0
10.9
FREEMAN CK.
KERN
FORKS OF KERN
48.5
RATTLESNAKE CK.
28.0
42.2
PEPPERMINT CK.
43.7
42.5
42.9
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES
75.0
67.3
BONE CK.
PA 1,2 (105)
FAIRVIEW
TO LAKE ISABELLA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COYOTE CK.
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KERN LAKE
3.4
GRASSHOPPER CK.
2.0
HELL'S HOLE CK.
8.4
REDROCK CK.
1.3
9.7
NINEMILE CK.
LITTLE KERN RIVER
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KERN RIVER
3.7
1.5
KERN FLAT
OSA CK.
1.0
SODA CK.
3.4
5.4
FREEMAN CK.
KERN
FORK'S OF KERN
2.9
RATTLESNAKE CK.
0.0
0.0
16.0
14.0
PEPPERMINT CK.
6.0
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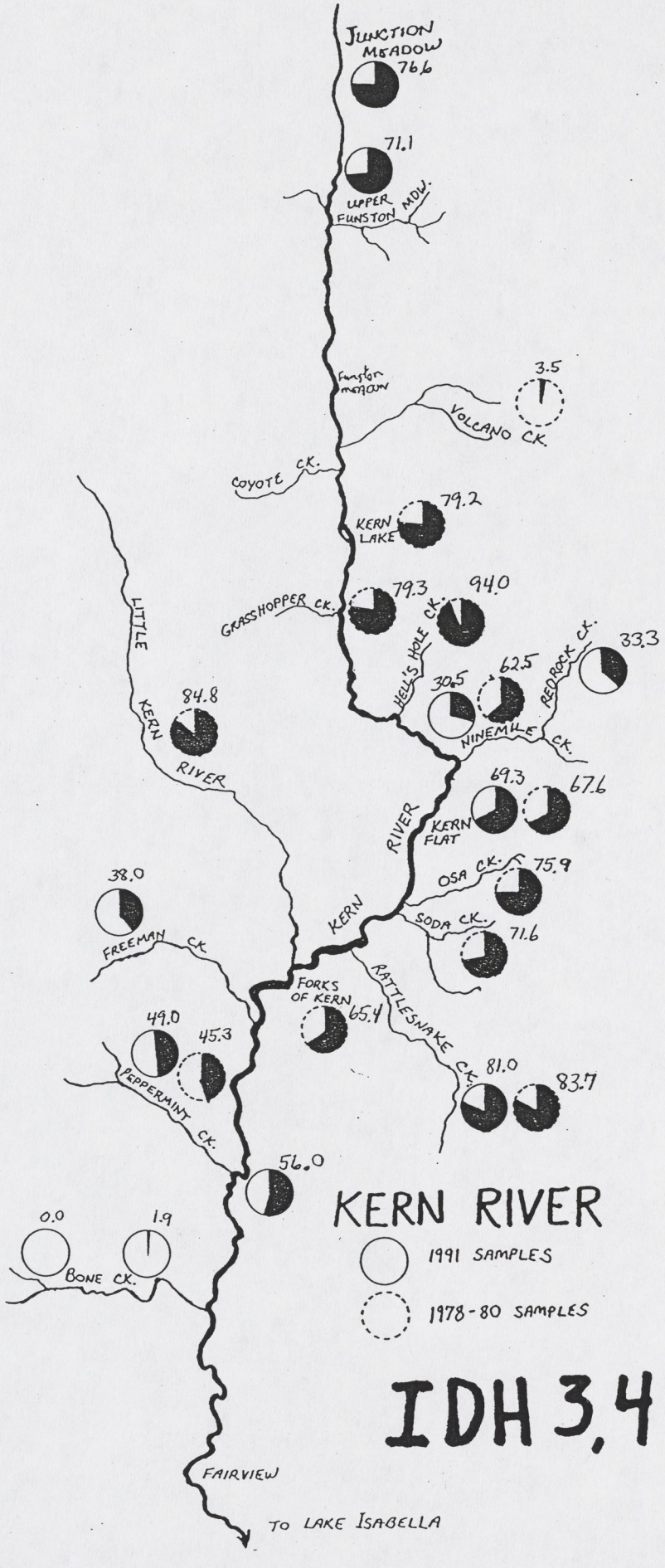
30.8
BONE CK.
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES
FAIRVIEW
TO LAKE ISABELLA

IDH 3,4 (45)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KERN LAKE
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94.0
GRASSHOPPER CK.
HELL'S HOLE CK.
33.3
REDROCK CK.
62.5
30.5
NINEMILE CK.
LITTLE KERN RIVER
84.8
KERN RIVER
69.3
67.6
KERN FLAT
OSA CK.
75.9
38.0
SODA CK.
71.6
KERN
FREEMAN CK.
FORKS OF KERN
65.4
RATTLESNAKE CK.
49.0
45.3
81.0
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56.0
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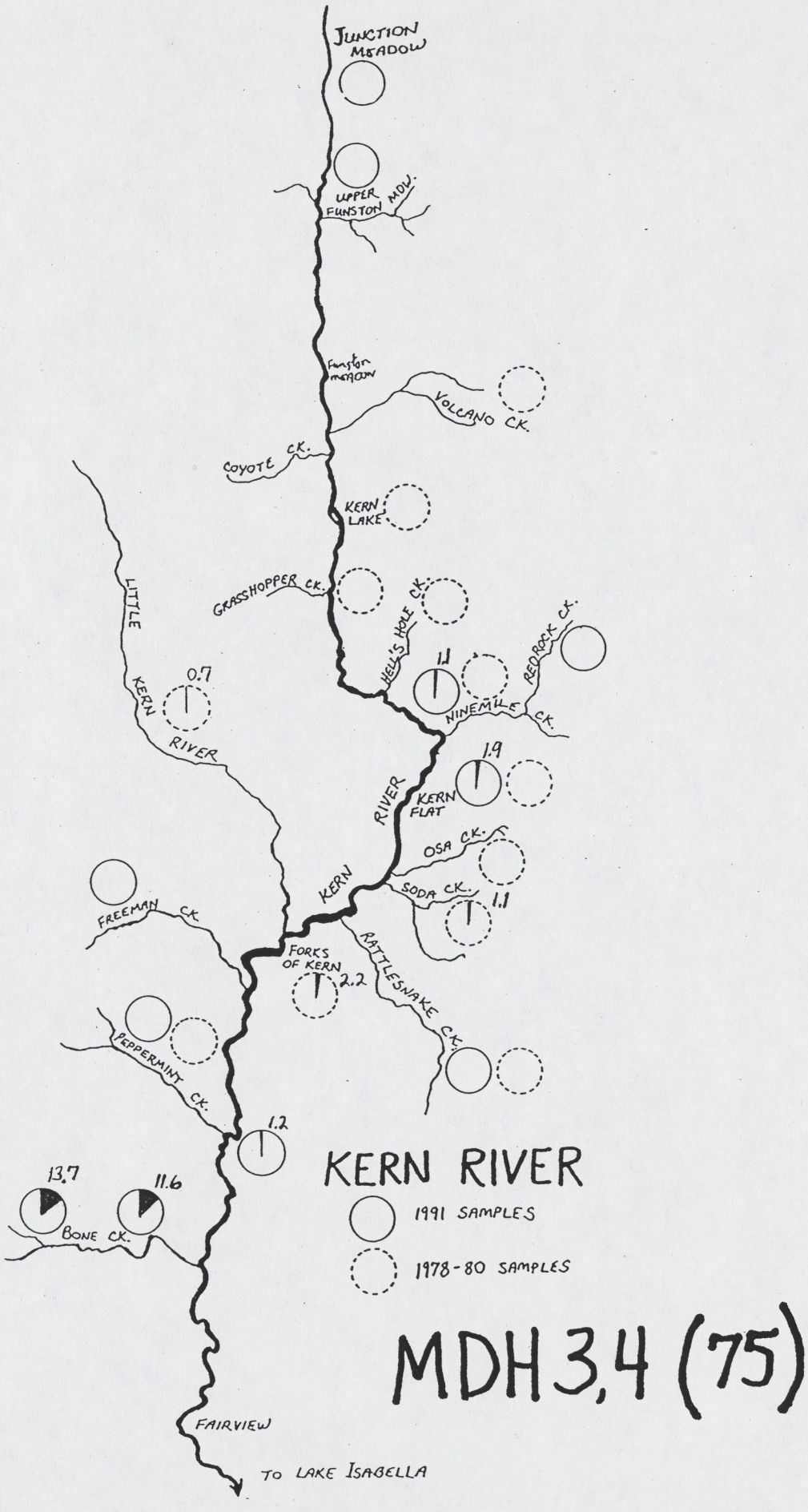
BONE CK.
1978-80 SAMPLES
IDH 3,4 (74)
FAIRVIEW
TO LAKE ISABELLA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GRASSHOPPER CK.
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REDROCK CK.
NINEMILE CK.
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0.7
LITTLE KERN RIVER
KERN RIVER
1.9
KERN FLAT
OSA CK.
SODA CK.
1.1
KERN
FREEMAN CK.
FORKS OF KERN
2.2
RATTLESNAKE CK.
PEPPERMINT CK.
1.2
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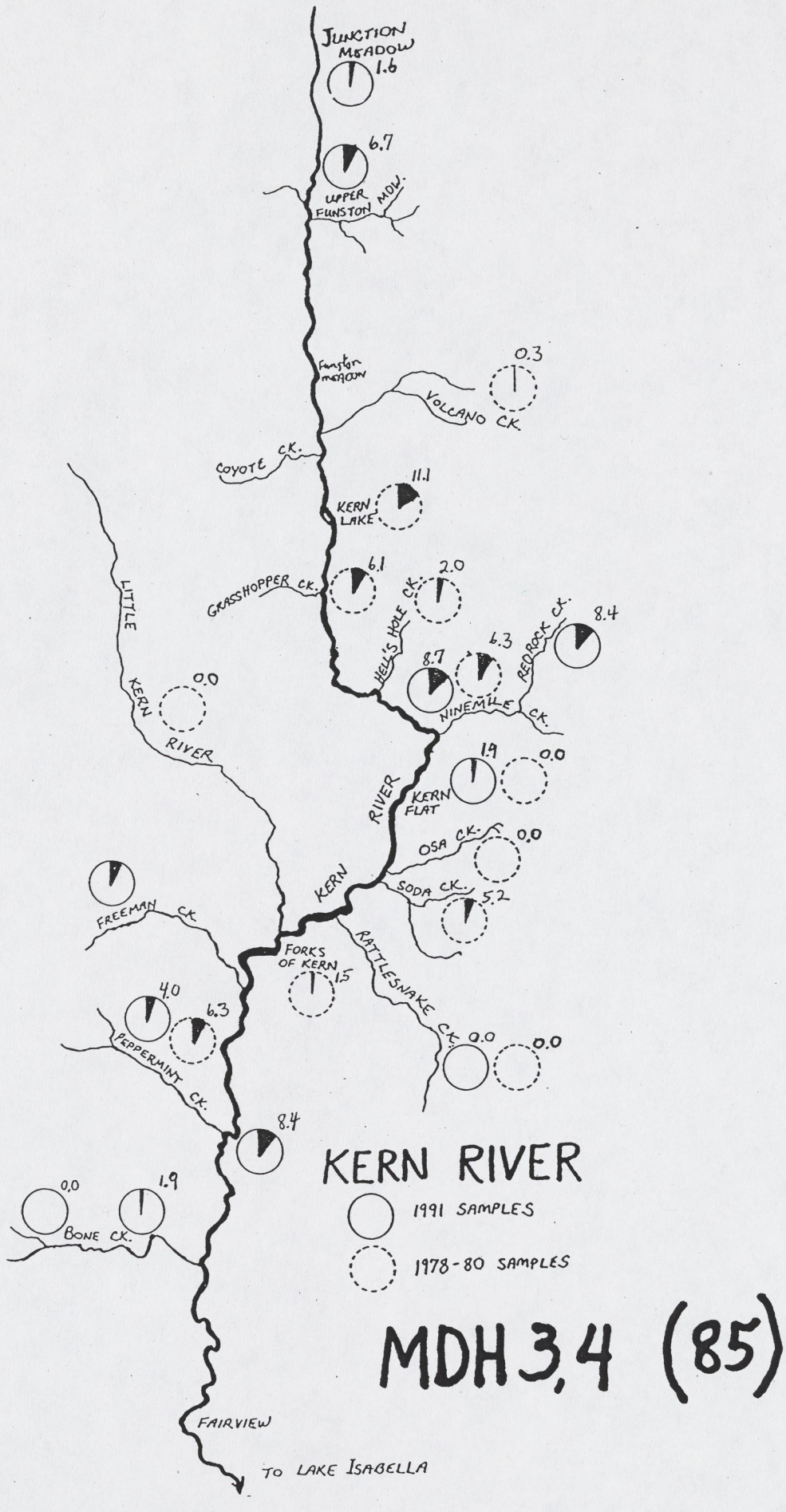
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES
MDH 3,4 (75)
FAIRVIEW
TO LAKE ISABELLA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6.7
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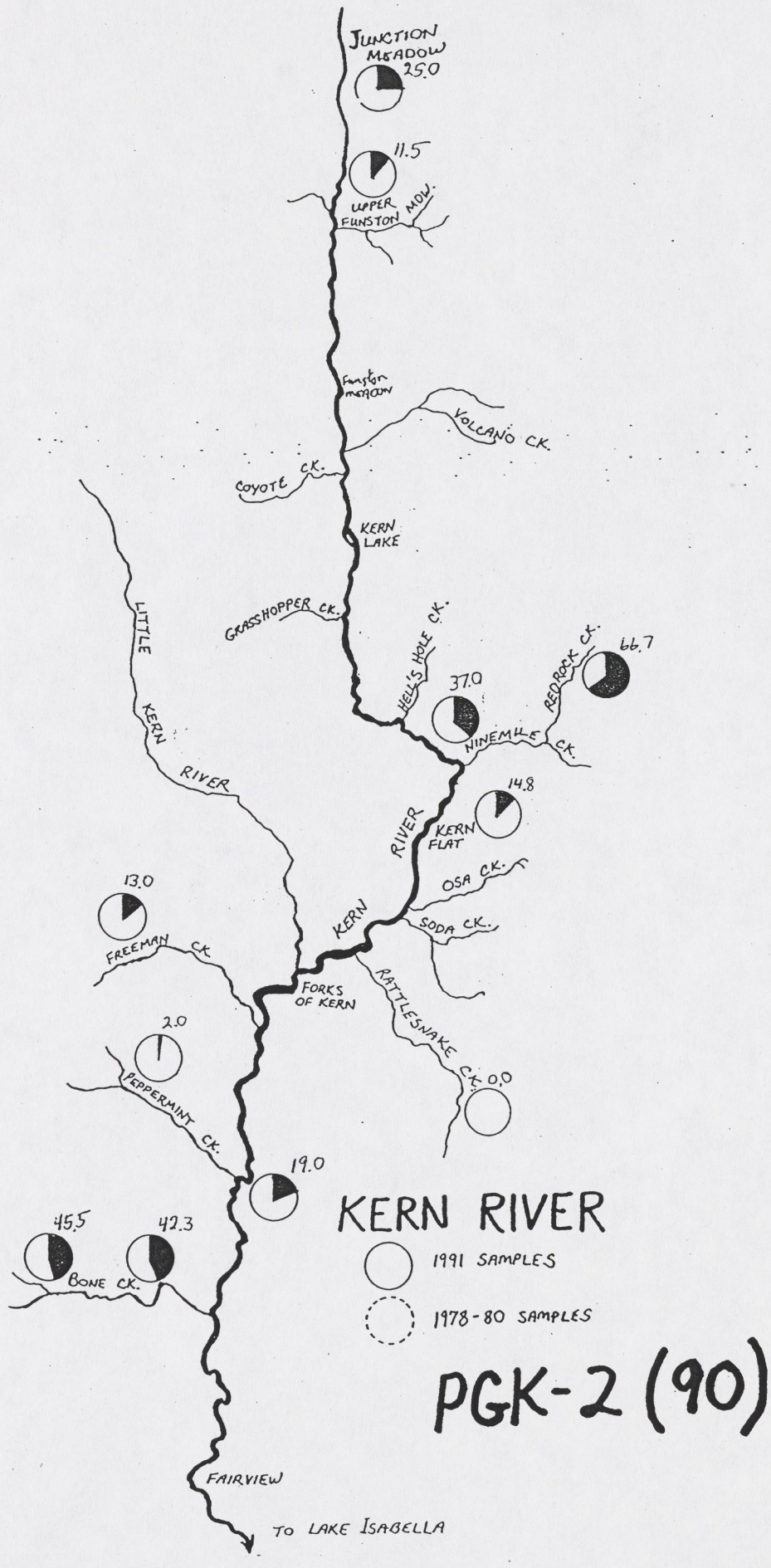
COYOTE CK.
11.1
KERN LAKE
6.1
GRASSHOPPER CK.
2.0
HELL'S HOLE CK.
8.4
REDROCK CK.
6.3
8.7
NINEMILE CK.
LITTLE KERN RIVER
0.0
KERN RIVER
1.9
0.0
KERN FLAT
OSA CK.
0.0
SODA CK.
5.2
KERN
FREEMAN CK.
FORKS OF KERN
1.5
RATTLESNAKE CK.
0.0
0.0
4.0
6.3
PEPPERMINT CK.
8.4
0.0
1.9
BONE CK.
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES
MDH 3,4 (85)
FAIRVIEW
TO LAKE ISABELLA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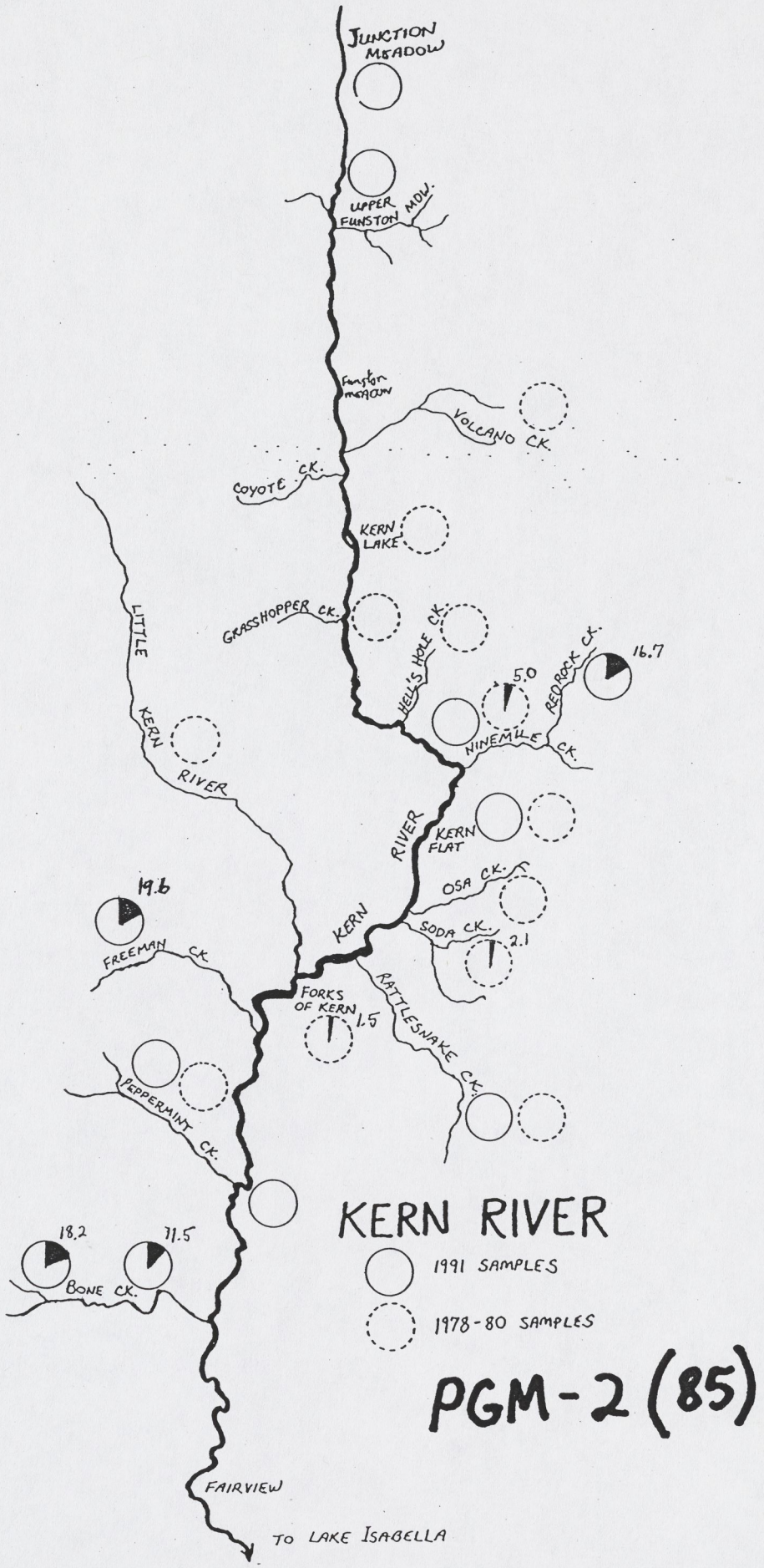
Fungus meadow
VOLCANO CK.
COYOTE CK.
KERN LAKE
GRASSHOPPER CK.
HELL'S HOLE CK.
66.7
REDROCK CK.
37.0
NINEMILE CK.
LITTLE KERN RIVER
14.8
KERN FLAT
KERN RIVER
OSA CK.
SODA CK.
13.0
FREEMAN CK
FORKS OF KERN
RATTLESNAKE CK. 0.0
2.0
PEPPERMINT CK.
19.0
45.5
42.3
BONE CK.
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES
PGK-2 (90)
FAIRVIEW
TO LAKE ISABELLA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COYOTE CK.
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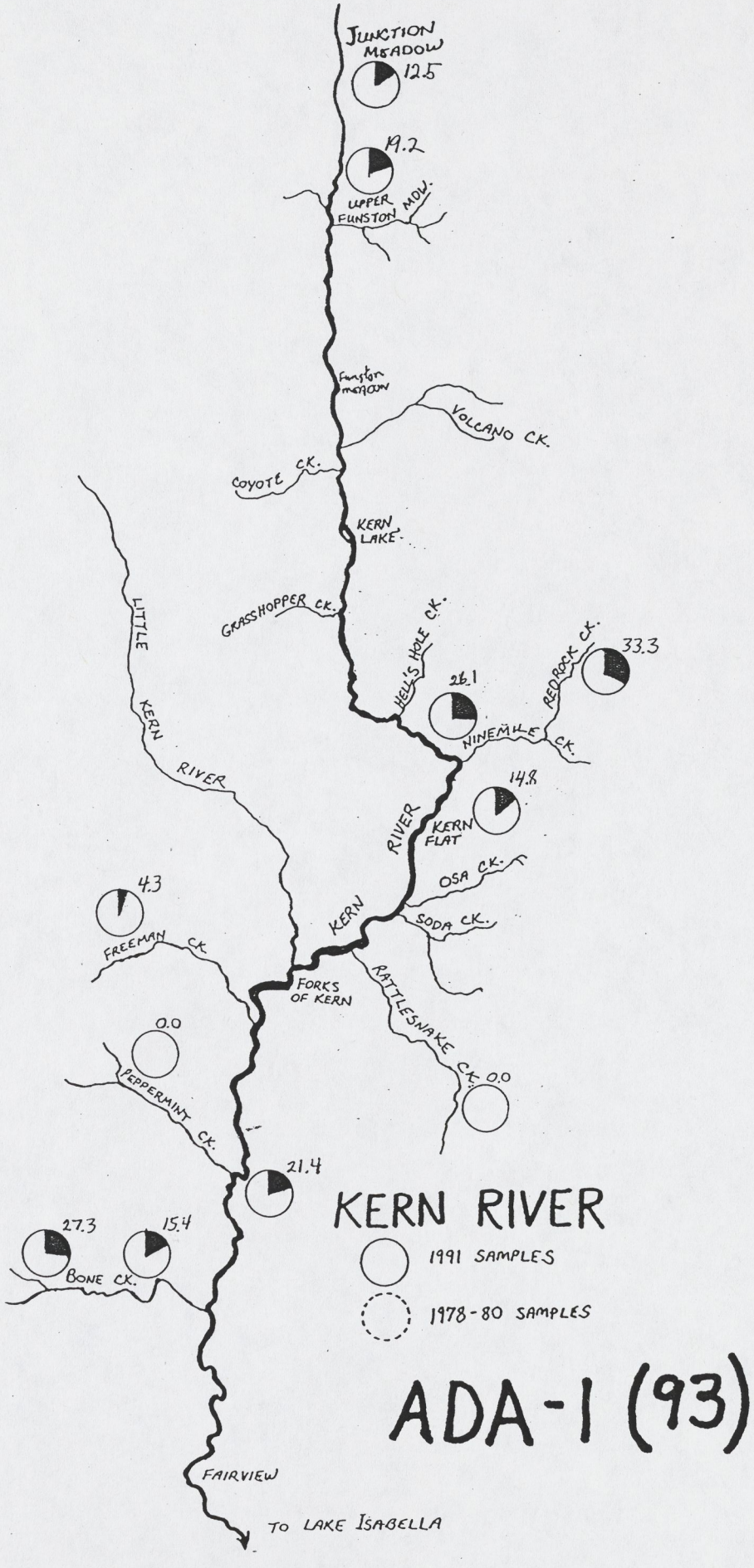
GRASSHOPPER CK.
LITTLE KERN RIVER
HELL'S HOLE CK.
5.0
REDROCK CK.
16.7
NINEMILE CK.
KERN RIVER
KERN FLAT
OSA CK.
SODA CK.
2.1
19.6
FREEMAN CK.
KERN
FORKS OF KERN
1.5
RATTLESNAKE CK.
PEPPERMINT CK.
18.2
11.5
BONE CK.
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES
PGM-2 (85)
FAIRVIEW
TO LAKE ISABELLA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19.2
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MDW.
Funston meadow
VOLCANO CK.
COYOTE CK.
KERN LAKE
GRASSHOPPER CK.
LITTLE KERN RIVER
HELL'S HOLE CK.
26.1
REDROCK CK.
33.3
NINEMILE CK.
14.8
KERN FLAT
KERN RIVER
OSA CK.
SODA CK.
4.3
FREEMAN CK.
FORK'S OF KERN
RATTLESNAKE CK.
0.0
PEPPERMINT CK.
0.0
21.4
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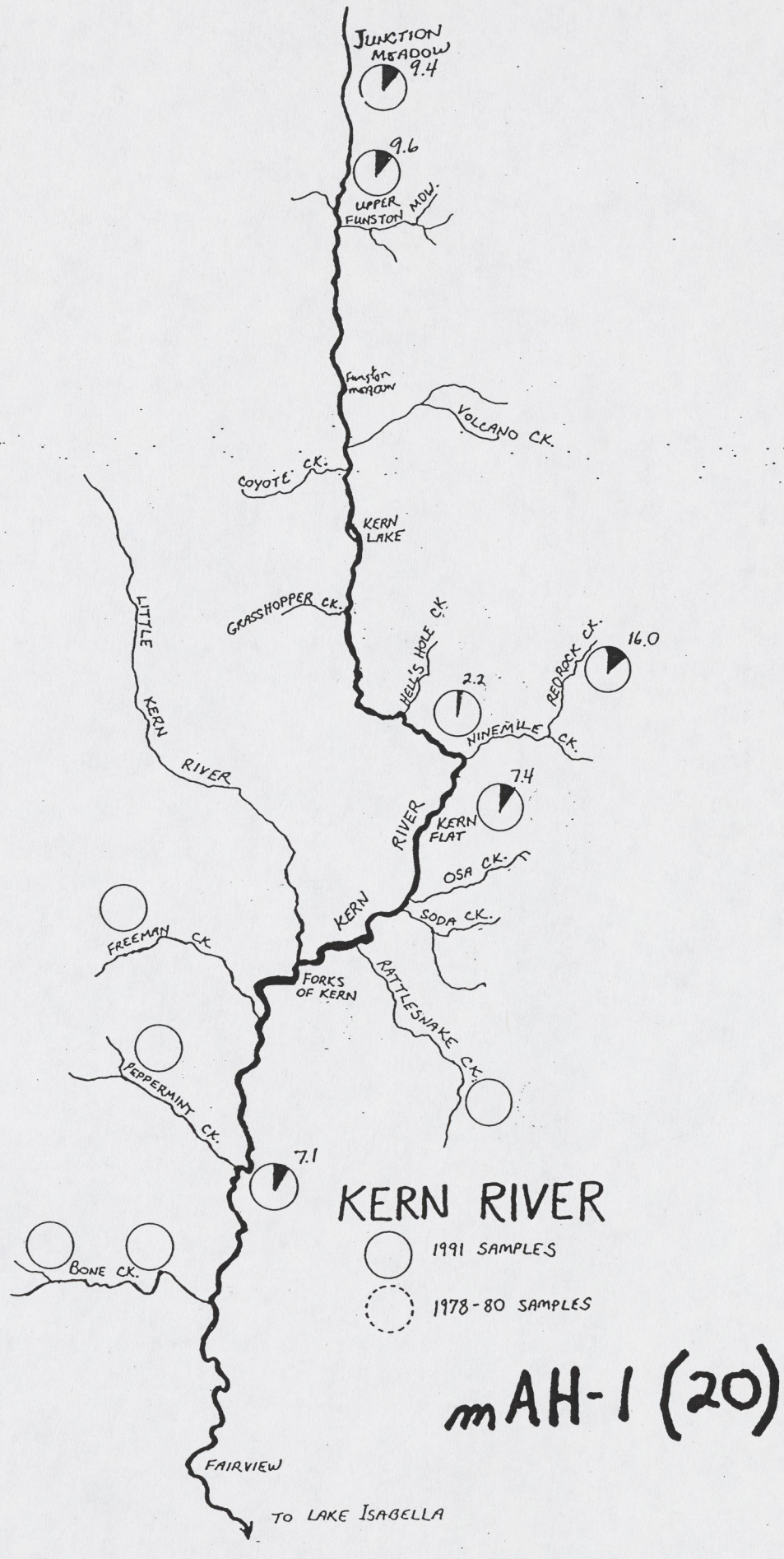
15.4
BONE CK.
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES
ADA-1 (93)
FAIRVIEW
TO LAKE ISABELLA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9.4
9.6
UPPER FUNSTON MDW.
Funston meadow
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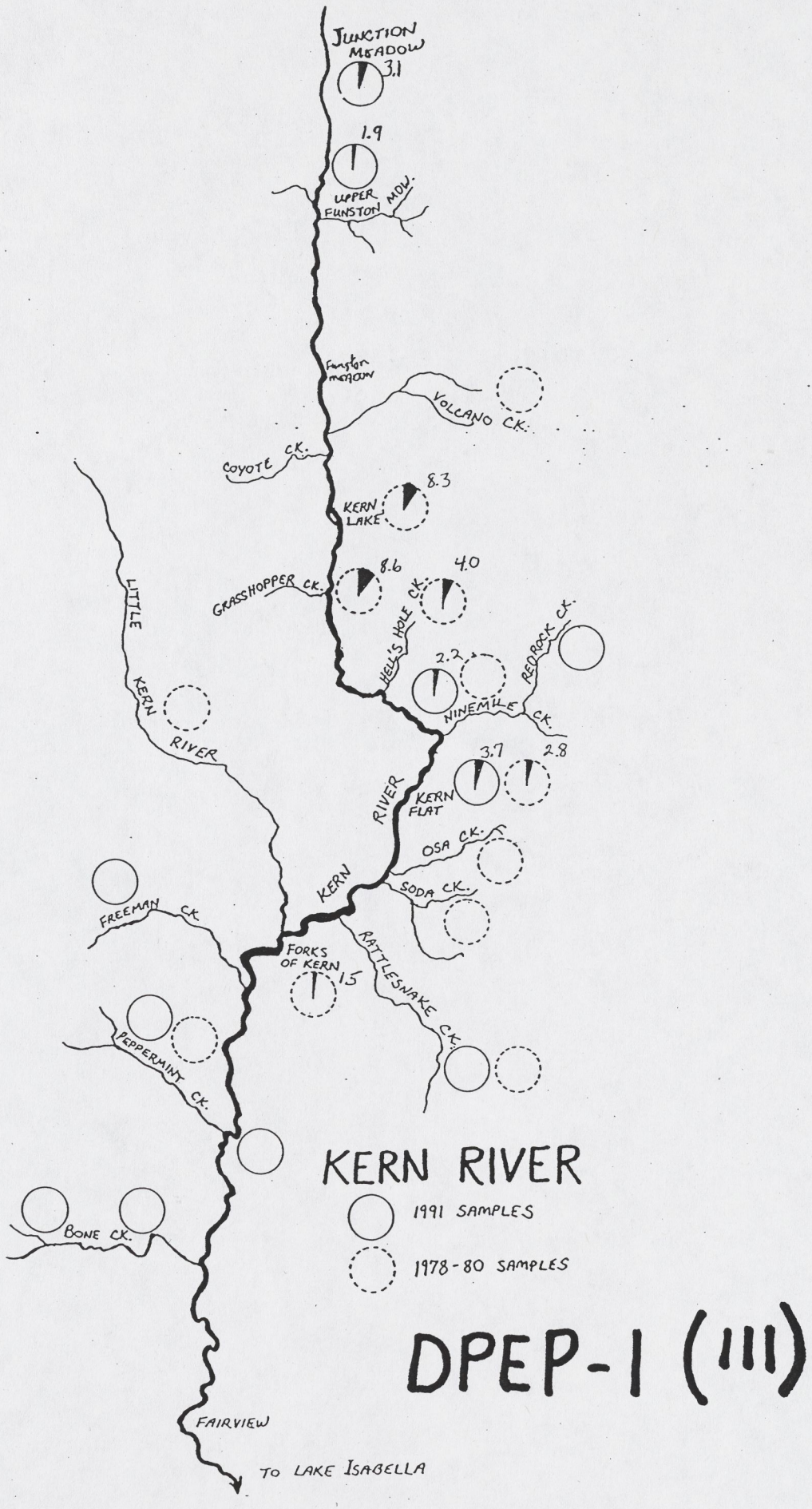
COYOTE CK.
KERN LAKE
GRASSHOPPER CK.
LITTLE KERN RIVER
HELL'S HOLE CK.
2.2
REDROCK CK.
16.0
NINEMILE CK.
7.4
KERN FLAT
RIVER
KERN
OSA CK.
SODA CK.
FREEMAN CK
FORKS OF KERN
RATTLESNAKE CK.
PEPPERMINT CK.
7.1
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES
BONE CK.
FAIRVIEW
TO LAKE ISABELLA

mAH-1 (20)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3.1
1.9
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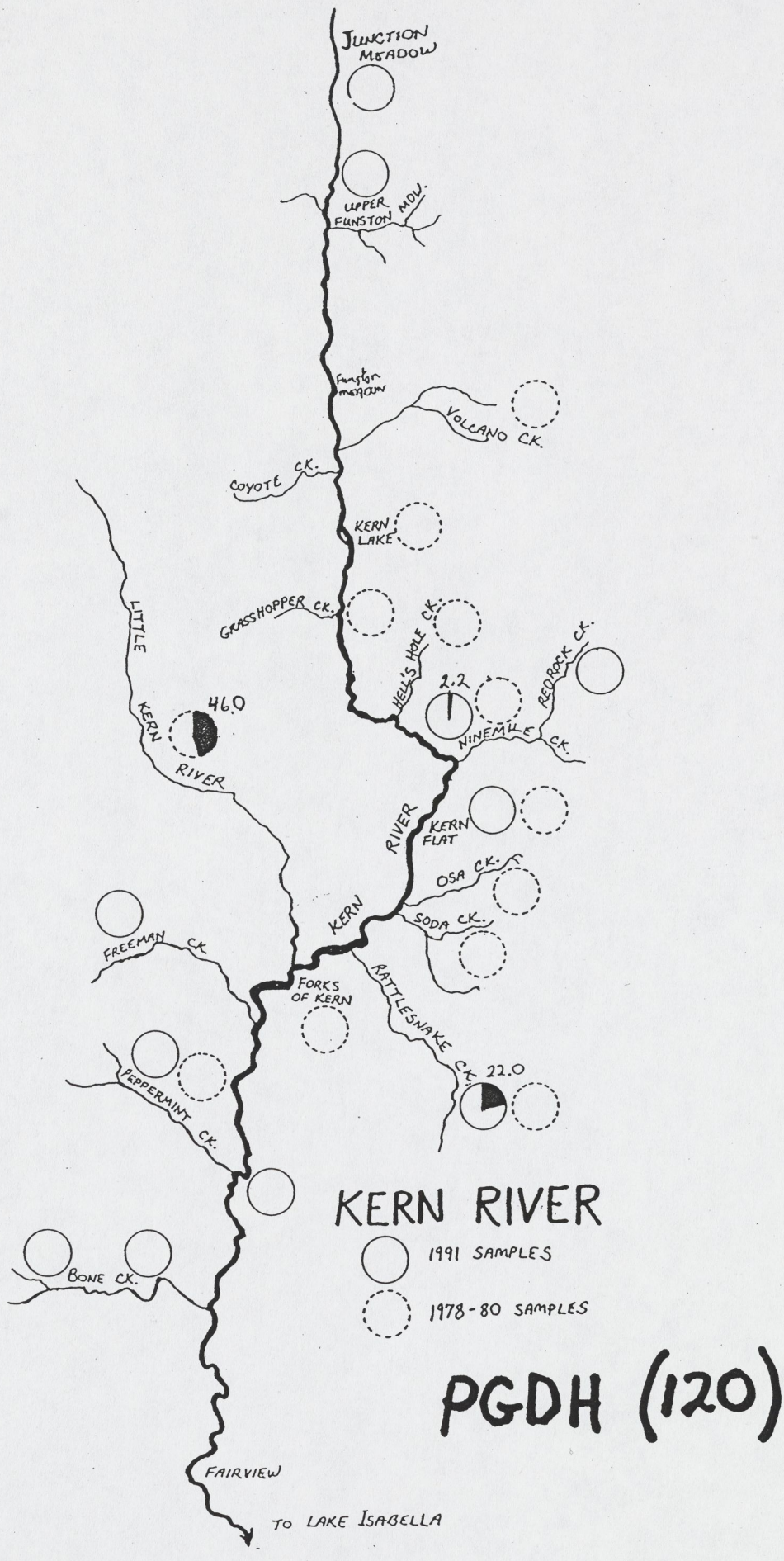
Funston meadow
VOLCANO CK.
COYOTE CK.
8.3
KERN LAKE
8.6
4.0
GRASSHOPPER CK.
HELL'S HOLE CK.
REDROCK CK.
2.2
NINEMILE CK.
LITTLE KERN RIVER
3.7
2.8
KERN FLAT
KERN RIVER
OSA CK.
SODA CK.
KERN
FREEMAN CK.
FORKS OF KERN
1.5
RATTLESNAKE CK.
PEPPERMINT CK.
BONE CK.
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES
DPEP-1 (III)
FAIRVIEW
TO LAKE ISABELLA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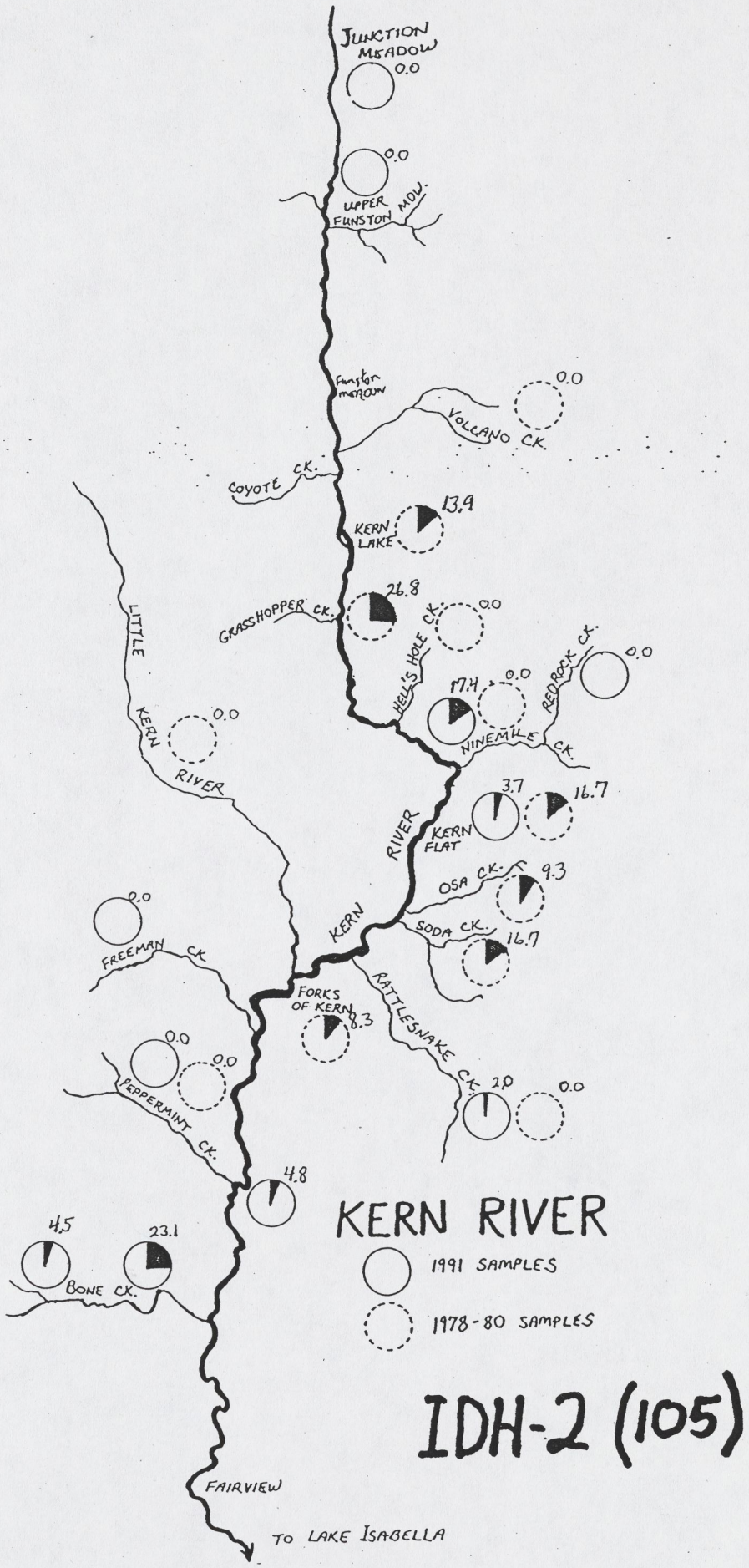
KERN LAKE
GRASSHOPPER CK.
LITTLE KERN RIVER
HELL'S HOLE CK.
REDROCK CK.
2.2
NINEMILE CK.
46.0
KERN RIVER
KERN FLAT
OSA CK.
SODA CK.
KERN RIVER
FREEMAN CK
FORKS OF KERN
RATTLESNAKE CK.
22.0
PEPPERMINT CK.
BONE CK.
FAIRVIEW
TO LAKE ISABELLA
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES
PGDH (120)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0.0
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Funston meadow
0.0
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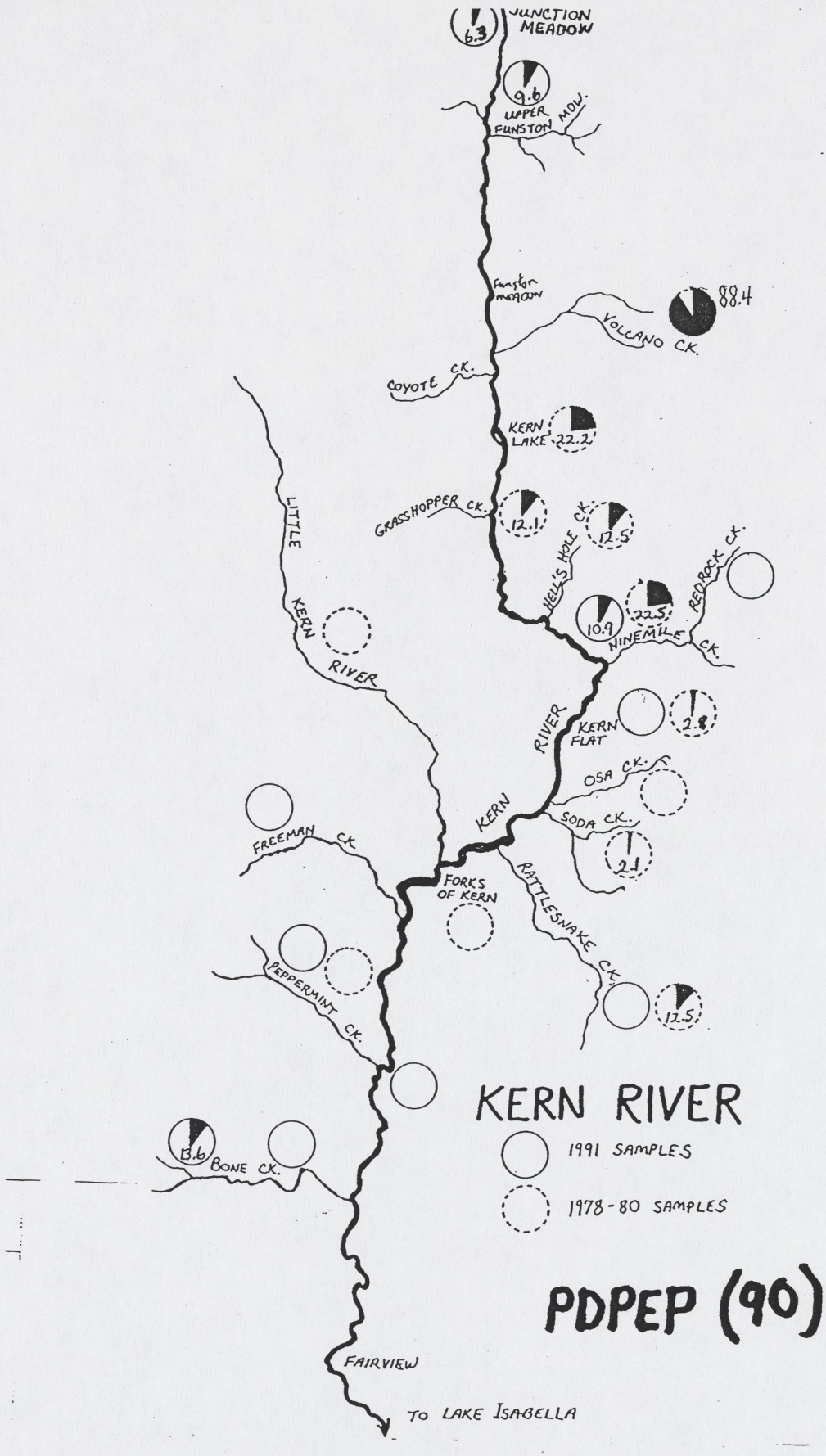
COYOTE CK.
13.9
KERN LAKE
26.8
GRASSHOPPER CK.
0.0
HELLS HOLE CK.
17.4
0.0
REDROCK CK.
0.0
NINEMILE CK.
LITTLE KERN RIVER
0.0
KERN RIVER
3.7
16.7
KERN FLAT
OSA CK.
9.3
SODA CK.
16.7
0.0
FREEMAN CK.
KERN
FORKS OF KERN
8.3
RATTLESNAKE CK.
2.0
0.0
0.0
0.0
PEPPERMINT CK.
4.8
4.5
23.1
BONE CK.
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES
IDH-2 (105)
FAIRVIEW
TO LAKE ISABELLA


JUNCTION MEADOW
6.3
9.6
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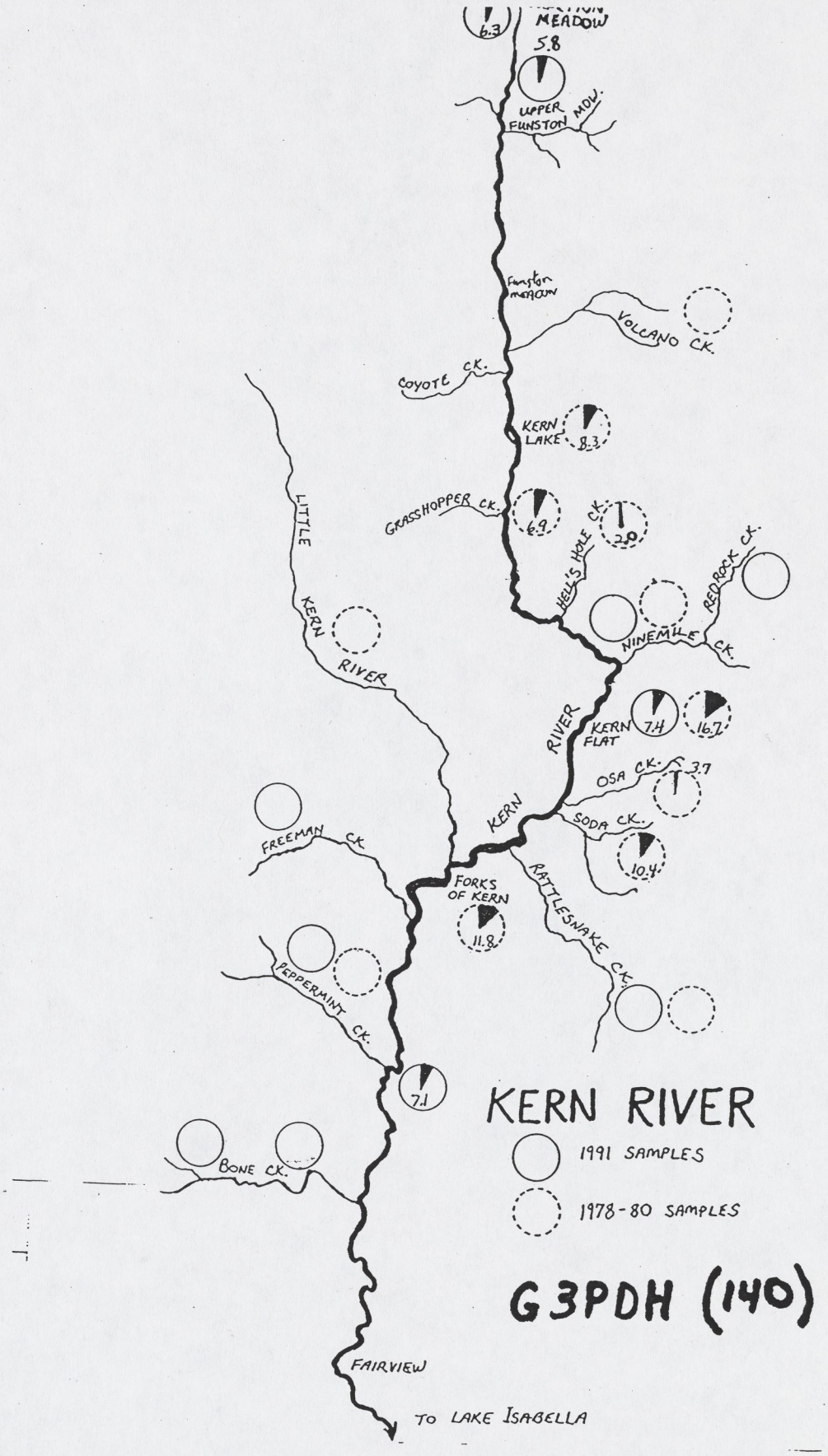
Funston meadow
88.4
VOLCANO CK.
COYOTE CK.
KERN LAKE 22.2
GRASSHOPPER CK.
12.1
HELL'S HOLE CK.
12.5
REDROCK CK.
22.5
10.9
NINEMILE CK.
LITTLE KERN RIVER
KERN RIVER
KERN FLAT
2.8
OSA CK.
SODA CK.
2.1
FREEMAN CK.
KERN
FORKS OF KERN
RATTLESNAKE CK.
12.5
PEPPERMINT CK.
13.6
BONE CK.
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES
PDPEP (90)
FAIRVIEW
TO LAKE ISABELLA


6.3
MEADOW
5.8
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Funston meadow
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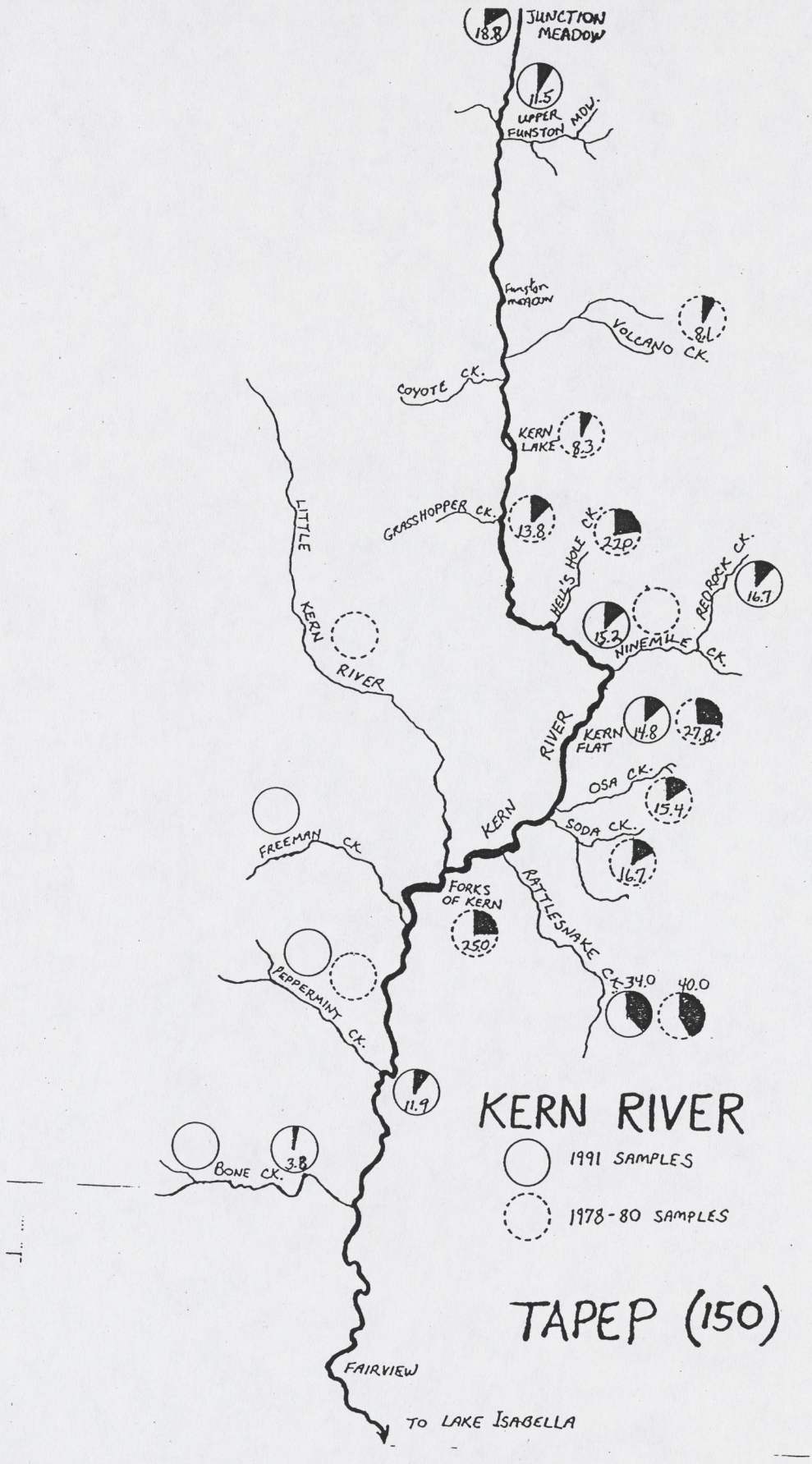
COYOTE CK.
KERN LAKE
8.3
GRASSHOPPER CK.
6.9
HELL'S HOLE CK.
2.0
REDROCK CK.
NINEMILE CK.
LITTLE KERN RIVER
KERN RIVER
KERN FLAT
7.4
16.7
OSA CK.
3.7
SODA CK.
10.4
FREEMAN CK.
KERN
FORKS OF KERN
11.8
RATTLESNAKE CK.
PEPPERMINT CK.
7.1
BONE CK.
FAIRVIEW
TO LAKE ISABELLA
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES
G3PDH (140)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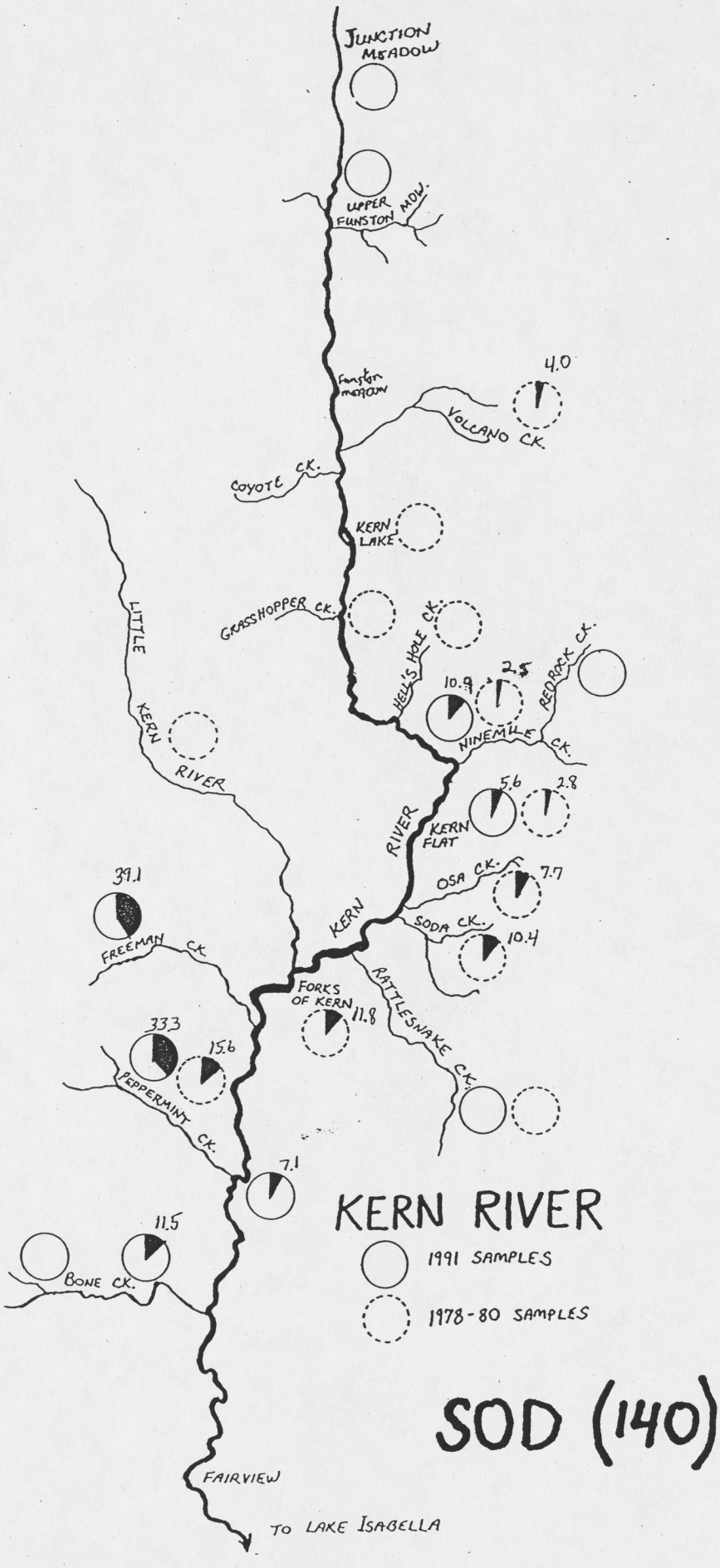
COYOTE CK.
KERN LAKE
8.3
GRASSHOPPER CK.
13.8
HELL'S HOLE CK.
22.0
REDROCK CK.
16.7
15.2
NINEMILE CK.
LITTLE KERN RIVER
KERN RIVER
KERN FLAT
14.8
27.8
OSA CK.
15.4
SODA CK.
16.7
FREEMAN CK.
KERN
FORKS OF KERN
25.0
RATTLESNAKE CK.
34.0
40.0
PEPPERMINT CK.
11.9
BONE CK.
3.8
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES
TAPEP (150)
FAIRVIEW
TO LAKE ISABELLA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Funston meadow
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COYOTE CK.
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GRASSHOPPER CK.
HELL'S HOLE CK.
REDROCK CK.
10.9
2.5
NINEMILE CK.
LITTLE KERN RIVER
KERN RIVER
5.6
2.8
KERN FLAT
OSA CK.
7.7
SODA CK.
10.4
39.1
FREEMAN CK.
KERN
FORKS OF KERN
11.8
RATTLESNAKE CK.
33.3
15.6
PEPPERMINT CK.
7.1
11.5
BONE CK.
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES
SOD (140)
FAIRVIEW
TO LAKE ISABELLA

GENETIC ANALYSIS OF THREATENED TROUT:

LITTLE KERN GOLDEN TROUT

INDEPENDENCE LAKE CUTTHROAT TROUT

David H. Mirman, Mark J. Bagley
Supawadee Poompuang, Yvonne Kong, and
Graham A.E. Gall

Department of Animal Science
University of California, Davis

Report to

California Department of Fish and Game
Threatened Trout Committee

September 14, 1992

EXECUTIVE SUMMARY

Fourteen samples collected in 1992 from seven tributaries in the Little Kern basin were the subject of this study. Genetic variation was detected using standard electrophoretic techniques. Overall genetic variability as measured by estimated average heterozygosities and percent polymorphic loci were very low, but consistant with previously sampled LKGT populations (Berg, 1987). This low diversity suggests small population sizes and/or recent bottleneck event(s). All fourteen samples of LKGT were genetically very similar to each other. They clustered tightly with LKGT from previous studies but separately from representative KRRT, VCGT and hatchery rainbow trout. There was no evidence of outside influence for thirteen of the fourteen samples. The Little Kern R. at Shotgun Creek population sample showed low frequencies of two unusual alleles. These alleles may have originated in hatchery rainbow trout or KRRT, in which case the introgression would have been one or more generations in the past. They were unlikely to have originated in the Coyote Creek stock used to seed the Little Kern at Shotgun Creek area. The alleles may have originated in survivors of the chemical treatments, but even if this were so the frequencies observed were surprisingly large. The sample size of 15 fish makes more definitive conclusions about the origins of these unusual alleles unwarranted.

INTRODUCTION

Three forms of rainbow trout are generally recognized in the Kern River basin. These are: the Little Kern Golden Trout (Oncorhynchus mykiss whitei, abbreviated LKGT), the Kern River Rainbow Trout (O. mykiss gilberti, abbreviated KRRT), and the Volcano Creek Golden Trout (O. mykiss aquabonita, abbreviated VCGT).

This study is a follow up to previous studies in the region, including a study of KRRT carried out in 1991 (referred to subsequently as Gall et al., 1991). The primary purpose of the present study was to genetically analyze fourteen samples of LKGT collected in 1992 and attempt to determine if there had been any introgression from rainbow trout.

MATERIALS AND METHODS

POPULATIONS

Fourteen Little Kern R. basin samples from seven tributaries were the subject of this study. These were (with sample size):

Clicks Creek (n=20)
Deep Creek (n=18)
Little Kern River at Shotgun Creek (n=15)
Lower Lion Creek (n=4)
Middle Lion Creek (n=6)
Upper Lion Creek (n=5)
Lower Rifle Creek (n=10)
Middle Rifle Creek (n=9)
Upper Rifle Creek (n=6)
Shotgun Creek below lowest barrier (n=9)
Shotgun Creek below Pistol Creek (n=14)
Lower Tamarack Creek (n=7)
Middle Tamarack Creek (n=18)
Upper Tamarack Creek (n=9)

In addition to these samples collected in 1992, data from six LKGT populations collected between 1978 and 1981 were available in Berg (1987). These were (with sample sizes and dates of collection):

Coyote Creek (n=64 1981)
Little Kern R. at Broder's cabin (n=16 1978-80)
Lower Wet Meadows Creek (n=24 1978-80)
Middle Wet Meadows Creek (n=21 1978-1980)
Deadman Creek (n=14 1978-1980)
Sheep Creek (n=17 1978-1980)

Data from representative samples of three other rainbow trout lineages were also considered relevant. These included:

Kern River rainbow trout (Gall et al., 1991)
- Junction Meadows (n=16 1991)
- Kern River at Kern Flat (n=27 1991)
- Kern River at Peppermint Creek (n=27 1991)
- Rattlesnake Creek at Bonita Flat (n=25 1991)

Volcano Creek golden trout (Berg, 1987)

Mulkey Creek (n=31 1981)
Golden Trout Creek, Stringer (n=16 1978-1980)
Volcano Creek (n=19 1978-80)

Hatchery rainbow trout (Gall et al., 1981)
Hatchery rainbow Shasta (n=28 1978-80)
Hatchery rainbow Pit R. (n=45 1978-80)
Hatchery rainbow Davis (n=12 1978-80)

METHODS OF ANALYSIS

Genetic variation was detected using standard starch-gel electrophoretic techniques as previously described in Berg and Gall (1988), and in Bartley and Gall (1990). Proteins were examined in extracts of five tissues: blood, eye, heart, liver and muscle. Initially 86 loci were used in the analysis. Nine of the loci were excluded from the analysis because interpretation of the zymograms was judged unreliable. The nine loci excluded were: AAT-1,2, ALAT-1, α-GLU, β-GUS, EST-6,7, IDDH-1, and IDDH-2. Allele frequencies were calculated for each of the remaining 77 loci. Analysis of overall genetic diversity of the fourteen samples, assessement of possible introgression, and genetic relationships among the fourteen samples depicted in Figure 1 were all based on these 77 loci. For comparisons with previous studies, only those loci common to all studies could be used. Thus, the genetic relationships between LKGT and other rainbow trout groups depicted in Figure 2 are based on the following 23 loci:

ADH; CK-1; DPEP-1; G3PDH-1; GPI-1; GPI-2; GPI-3; IDH-2; IDH-3,4; LDH-3,4; MDH-3,4; MDHP-1,2; PA-1,2; PDPEP-1; PGDH; PGM-1; SOD-1, and TAPEP

Genetic identities were estimated using Nei's genetic identity statistic I (Nei 1972). Dendrograms were drawn

using the UPGMA (Unweighted Pair-wise Group Mean Average) method. Technical comments on two of the loci included in the analysis are found in Appendix 1.

RESULTS AND DISCUSSION

OVERALL GENETIC DIVERSITY

The following loci were monomorphic in all fourteen samples:

AAT-3; AAT-4; mAAT-1; mAAT-2; mAAT-3; ACP-1 ACP-2; ADA-1; ADA-2; ADH; mAH-4; ALAT-2; β-GALA-1; CK-1; CK-2; CK-3; CK-4; CK-5; DPEP-1; EST-D; FBALD-3 FBALD-4; G3PDH-1; G3PDH-2; G3PDH-3; G3PDH-4; GAPDH-1 GAPDH-2; GAPDH-5; GAPDH-6; GPI-1; GPI-2; GPI-3; GR; HAGH; IDH-1; IDH-2; LDH-1; LDH-2; LDH-3; MDHP-1; MDHP-2; MDHP-3,4; MDH-1,2; MDH-3,4; mMDH-1; mMDH-2; MPI; PDPEP-1; PEPLT; PGK-1; PGM-1; PGM-2; TAPEP; TPI-1; TPI-2; TPI-3; and TPI-4

The following loci were polymorphic in at least one sample:

ACRO-2; mAH-1; mAH-2; AH-1; DPEP-2; IDH-3,4; LDH-4; PA-1,2; PDPEP-2; PGDH; PGK-2; PGM-3,4; and SOD-1

Table 1 shows the allele frequencies for each polymorphic locus. Sixteen loci were polymorphic, corresponding to 21 percent polymorphic loci. Table 2 gives estimated average heterozygosities for each of the fourteen samples. Heterozygosities ranged from 0.022 (Deep Creek) to 0.052 (Little Kern R. at Shotgun Creek). Both the proportion of polymorphic loci and estimated average heterozygosities are low compared to rainbow trout populations in general but consistant with previous findings for LKGT. Berg reported 21 percent polymorphic loci with an average heterozygosity of 0.058 for six LKGT populations and 35 percent polymorphic

loci with an average heterozygosity of 0.10 for seven KRRT populations (Berg, 1987). The low levels of overall genetic diversity suggest small population sizes (presumably implying limited spatial migration) and/or population bottlenecks (temporary but acute reduction in population size) in the recent evolution of LKGT.

OVERVIEW OF RELATIONSHIPS

Relationships among the fourteen samples can be seen in the dendrogram in Figure 1. The most significant result is the very high level of similarity of these populations to each other. In Gall et al., (1991) the genetic identity (I) of the most divergent pair of populations among KRRT was 0.971. The genetic identity (I) of the most divergent pair among these fourteen LKGT populations is 0.985, and most pairs are more similar than 0.990. This is presumably due in large part to the overall lack of genetic diversity of these populations as discussed above. Given the high degree of genetic similarity among sampled populations, the relationships indicated in Figure 1 should be viewed with approriate caution. The Lion Creek and Rifle Creek populations had very small sample sizes and their relationships should be viewed as particularly uncertain.

These caveats aside, the genetic relationships evident in Figure 1 appear largely consistent with geographic relationships, and with known sources of stocks. Clicks Creek clustered with Deep Creek, both of which were seeded

with fish from Fish Creek (Dan Christenson, personnel communication). Populations in and near Shotgun Creek clustered together, and the three Rifle Creek populations clustered together (all of which were stocked from Coyote Creek). These eight populations formed a large cluster. The three Tamarack Creek populations and the three Lion Creek populations formed a second large cluster. Tamarack Creek was stocked from Willow Creek; Lion Creek was stocked from Sheep Creek (Dan Christenson, personnel communication). As Sheep Creek is a tributary of Willow Creek, the Tamarack/Lion clustering is again consistant with the geography and origin of these populations.

While all of these samples were genetically similar, within-creek samples were particularly similar to each other. On this basis, the lower Lion Creek, middle Lion Creek, and upper Lion Creek samples were pooled to form a Lion Creek sample (n=15); the lower Rifle Creek, middle Rifle Creek, and upper Rifle Creek samples were pooled to form a Rifle Creek sample (n=25); and the lower Tamarack Creek, middle Tamarack Creek and upper Tamarack Creek samples were pooled to form a Tamarack Creek sample (n=36). This reduced the effective number of populations of LKGT collected in 1992 from fourteen to eight. The increase in sample sizes afforded by this pooling was expected to give sample allele frequencies which better reflected actual population allele frequencies.

Genetic relationships among these eight populations, six previously sampled LKGT populations, and representative

samples of KRRT, VCGT, and hatchery rainbow trout are shown in the dendrogram in Figure 2.

The fourteen LKGT populations (eight from 1992 and six from 1978-81) group together tightly. The four KRRT samples form a second tight cluster. Distances between LKGT sampled populations and KRRT sampled populations were around 0.945. The three VCGT sampled populations clustered together, at a distance of approximately 0.937 from both LKGT or KRRT samples. Pit River hatchery and Shasta hatchery stocks grouped together, at a distance of approximately 0.969 from the VCGT group. The Davis strain hatchery sample did not group with the other two hatchery populations. It appears as an outlier at a distance of 0.958 from the KRRT group. It should be emphasized that none of the LKGT samples grouped with non-LKGT samples.

ASSESSMENT OF POSSIBLE INTROGRESSION

Results: Presence of alleles IDH-2 (105), LDH-3 (80), MDH-3,4 (75), and PGM-2 (85) are considered highly inferential of hatchery rainbow trout genetic influence. None of these alleles were present in any of the samples analyzed in this study. IDH-3,4 (45) and SOD-1 (100) alleles are also characteristic of hatchery rainbow trout. They were found at frequencies of 0.02 (i.e. one heterozygous fish) and 0.07 (i.e. two heterozygous fish) respectively in the Little Kern River at Shotgun Creek sample. Neither of these alleles were found in any of the other population samples.

Alleles DPEP-2 (107), IDH-3,4 (74), PA-1,2 (100), PGDH (105) and SOD-1 (32) are considered diagnostic for LKGT. DPEP-2 (107) was found in six of the eight samples. IDH-3,4 (74) was found at moderate to high frequencies in all samples (ranging from 0.60 in Rifle Creek to 0.98 in Clicks Creek). PA-1,2 (100) was found in high frequency in all samples (ranging from 0.72 in Shotgun Creek below the lowest barrier to 1.00 in Clicks Creek, and Deep Creek). PGDH (105) was found in five of the eight samples. SOD-1 (32) was found at high frequency in all samples (ranging from 0.57 in Little Kern River at Shotgun Creek to 1.00 in Lion Creek).

The following loci are considered diagnostic for Kern River Rainbow Trout (KRRT): IDH-3,4 (74), MDH-3,4 (85), PA-1,2 (105), and SOD-1 (32). IDH-3,4 (74) was found at moderate to high frequency in all samples. PA-1,2 (105) was found at moderate frequency in two samples: Rifle Creek at 0.17, and Shotgun Creek below lowest barrier at 0.28. MDH-3,4 (85) was absent from all samples. SOD-1 (32) was found at moderate to high frequency in all samples.

Alleles IDH-3,4 (100) and PDPEP-2 (86) are both diagnostic for VCGT. IDH-3,4 (100) was found at low to moderate frequencies in all populations (from 0.03 in Clicks Creek to 0.40 in Lion Creek). PDPEP-2 (86) was found at 0.02 in Rifle Creek. It was absent from the other seven populations.

Discussion: DPEP-2 (107) has previously been found at

frequencies of 0.04 to 0.31 in LKGT populations (Berg 1987). In the present study two populations did not express the allele (absent from Clicks Creek, and Deep Creek). In those samples expressing the 107 allele, frequencies ranged from 0.07 in Rifle Creek to as high as 0.43 in Little Kern at Shotgun Creek. Frequencies of this allele seem particularly variable (e.g. 0.50 in lower Tamarack Creek vs. 0.05 in middle Tamarack Creek and 0.00 in upper Tamarack Creek). On the other hand, it is not found at all in hatchery rainbow trout (Berg 1987) and has a very limited distribution among KRRT. This suggests its presence is a good indicator of LKGT, but its absence is not informative.

IDH-2 (105) is generally found at moderate to high frequency in hatchery populations (0.46-0.80 Gall et al., 1981). Most KRRT have this allele in low frequency (Berg 1987, Gall et al., 1991). None of the LKGT populations analysed in Berg 1987 (six populations) expressed this allele and none of the eight populations in this study express it. Although it has not previously been used as diagnostic of LKGT, the absence of IDH-2 (105) might be at least inferential for LKGT.

Allele IDH-3,4 (74) has previously been found at high frequencies in LKGT (0.70-1.00 Berg 1987), at moderate frequencies in KRRT (0.02-0.80 Gall et al., 1991) and low frequencies in hatchery rainbow trout (0.00-0.33 Gall et al., 1981). The highest frequency of IDH-3,4 (74) in the 1991 study of KRRT (Gall et al., 1991) was 0.81 with most samples

considerably lower than this (0.30-0.50). The lowest frequencies in this study are 0.60 at Lion Creek, and 0.64 at Shotgun Creek below lowest barrier. The other samples had frequencies greater than 0.70.

At the same locus, IDH-3,4 (45) is considered inferential of rainbow trout introgression. It is found in KRRT, but was not found in the six populations of LKGT sampled between 1978 and 1981 (Berg, 1987). IDH-3,4 (45) was found at a frequency of 0.02 in the Little Kern at Shotgun sample, but was not found in any of the other samples. IDH-3,4 (100), which is nearly fixed in VCGT, was not found at a frequency higher than 0.40 (in Lion Creek). While the highest frequency of the 100 allele in LKGT reported by Berg (1987) was 0.30, the lowest reported for VCGT was 0.92. Although the ranges expected of the frequency of the IDH-3,4 (74) allele in LKGT and KRRT overlap, the data for this allele, taken in combination with the absence of IDH-3,4 (45) and moderate to low frequencies of IDH-3,4 (100) are consistant with LKGT origin of seven of the eight samples. Only the presence of the IDH-3,4 (45) allele in the Little Kern at Shotgun population is aberrant. As this population also had an abberrant SOD-1 allele, it will be analysed in more detail below.

MDH-3,4 (85) is a rare allele whose presence at any frequency is diagnostic of KRRT, although not all KRRT populations have this allele. It had not previously been found in LKGT (Gall et al., 1981, Berg, 1987) and was not

found in this study.

Allele PA-1,2 (100) has previously been reported to exist at frequencies of 0.80-1.00 in LKGT (Berg, 1987, Gall et al., 1981). It is found at more moderate frequencies of 0.30 to 0.50 in KRRT. In this study it was found at 0.72 in Shotgun Creek below the lowest barrier, 0.82 in Rifle Creek, and 0.90-1.00 in the other six samples. The alternative allele, PA-1,2 (105) is found at moderate or high frequency in KRRT (0.30-1.00 Berg, 1987, Gall et al., 1991). It is generally at low frequencies (less than 0.20) or absent in KRGT populations (Berg, 1987). PA-1,2 (105) was absent from two samples (Clicks Creek, and Deep Creek), at moderate frequency in two samples (Rifle Creek at 0.17, and Shotgun Creek below lowest barrier at 0.28) and at low frequencies in the other four samples (see Table 1). As in the case of the IDH-3,4 locus, there is some overlap in the range of allele frequencies expected of LKGT and of KRRT, but the data from this locus do not suggest introgression.

PDPEP-2 (86) is an excellent marker for VCGT (0.85-0.98 in Berg, 1987). It is generally not found in LKGT, although it may occasionally be found at low frequency (0.07 in Coyote Creek, 0.02 in lower Wet Meadows Creek and 0.03 in lower Deadman Creek, Berg, 1987). It was found at a frequency of 0.02 in Rifle Creek and was not found in the other seven populations.

PGDH (105) is a rare allele whose presence, at any frequency, has been considered diagnostic for LKGT. It has

generally not been found outside of the Little Kern basin; however, recently it has been found in some KRRT populations (Rattlesnake Creek at 0.22 and Kern River at Kern Flats 0.02 Gall et al., 1991). Its presence must therefore be considered highly inferential rather than completely diagnostic. It was present in five of the eight populations (see Table 1). It was absent from Deep Creek, Lion Creek, and Tamarack Creek.

Allele SOD-1 (32) is found at very high frequencies in LKGT (0.7-1.00, one sample at 0.34, Berg, 1987), and at moderate frequencies in KRRT (0.10-0.52 Gall et al., 1991, Berg 1987, Gall et al., 1981). It is not generally found in hatchery rainbow trout (Gall et al., 1981). The highest frequency of SOD-1 (32) found in last year's study of KRRT was 0.52 (Gall et al., 1991). The lowest frequencies found in this study were: Little Kern R. at Shotgun Creek at 0.57, Shotgun Creek below Pistol Creek at 0.57, Clicks Creek at 0.58, and Shotgun Creek below lowest barrier at 0.61. The other samples range from 0.75-1.00 (fixed in Lion Creek).

Another allele at this locus, SOD-1 (100) is considered inferential of rainbow trout influence. It was not found in the six populations of LKGT sampled between 1978 and 1981 (Berg, 1987). It was not found in seven of eight samples in the present study; however, it was present in the Little Kern at Shotgun sample at a frequency of 0.07. This is suggestive of introgression. Both KRRT and hatchery rainbow trout have this allele at high frequency (Gall et al., 1981, Gall et

al., 1991).

LITTLE KERN AT SHOTGUN CREEK SAMPLE

The Little Kern at Shotgun Creek (abbreviated LKS) sample has alleles unexpected in LKGT at two of six loci considered inferential for rainbow trout. Two fish were heterozygous for SOD-1 (100). One of these two fish was also heterozygous for IDH-3,4 (45). Neither of these alleles were observed in the six populations of LKGT sampled in 1978-81 (Berg, 1987). Both of these alleles are present in KRRT. Furthermore, one of the two fish carrying the SOD-1 (100) allele was the only fish in the sample to carry a PA-1,2 (105) allele. This allele is relatively common in both KRRT and hatchery rainbow trout, but exists only at low frequency in LKGT. The data at these three loci raise the possibility of introgression from either hatchery rainbow trout or KRRT. If this were the case, it would most likely be an introgression one or more generations in the past, otherwise the diagnostic alleles would occur at more loci and in higher freqency.

There are two possible LKGT sources of these alleles. First, they might have occured naturally in the population used to seed the LKS population, but not have been detected in previous samples. Second, they might be descendants of chemical treatment survivors.

Fish from Coyote Creek were used as progenitors of the LKS population (Dan Christenson, personnel communication).

We can estimate the probability that these alleles were present at low frequency in Coyote Creek but were not observed due to a combination of their rarity and chance in sampling. For the sake of these calculations, we assume all samples are true simple random samples. Forty fish were sampled from Coyote Creek between 1974 and 1976, and 64 more were taken in 1981. Neither of these alleles were seen in either population (Smith, 1981, and Berg, 1987 respectively). From this we can be 95% confident that the frequency of SOD-1 (100) in Coyote Creek was less than 0.014, and that the frequency of IDH-3,4 (45) was less than 0.007. While we can be certain that these alleles were not present in the Coyote creek population at the higher frequencies observed in the LKS population (probability less than 0.001) we cannot rule out their existance entirely.

Smith (1981) has extensive data on LKGT populations collected between 1974 and 1976, before the chemical treatment of the Little Kern River at Shotgun Creek area. This treatment was intended to remove stocks which were classified as introgressed from rainbow trout. Although Smith's (1981) study included only 12 loci and data from the study could not be included in the comparative study shown in Figure 2, data on both SOD-1 and IDH-3,4 were available. Table 3 gives the allele frequencies for SOD-1 (100) and IDH-3,4 (45) for the five samples geographically nearest to LKS. Both of these alleles were present in the area, but at very low frequencies (less than 0.04). The highest frequency of

IDH-3,4 (45) near the sample site was 0.03 and the highest frequency out of all 32 populations sampled in the basin was 0.08 at North Fork Clicks Creek. The highest frequency of SOD-1 (100) near the sample LKS site was 0.02 and the highest frequency in the basin was 0.34, also at North Fork Clicks Creek. Thus, it is possible that chemical treatment survivors carried these alleles and that our sample includes their descendants. However, in light of the low frequencies mentioned above and the fact that the chemical treatment surely killed off a portion of the fish present, an IDH-3,4 (45) frequency of 0.07 is still surprisingly high.

There is additional information to be gained from the fact that one fish expressed both of these unusual alleles. If both of these alleles were naturally occuring at the frequencies observed in our sample (i.e. our sample mean equals the true mean), there would only be a 10% probability we would have observed both alleles in one fish in our 15 fish sample. In making this calculation we assume that the two loci are independant (not linked) and that our sample is a true simple random sample of the population. This argument applies equally against both possible LKGT origins of these alleles (naturally occuring at low frequency in Coyote Creek, and chemical treatment survivors). It would not apply to fish descended from populations which had these alleles at high frequency (i.e. non-LKGT populations). As ten percent is not unreasonably small and the alleles must have originated somewhere, this argument is far from conclusive.

CONCLUSIONS

Seven of the eight samples did not show any alleles suggestive of outside rainbow trout influence. While not every sample shows every allele diagnostic of LKGT, taken as a whole the data suggest very strongly that these samples have been taken from LKGT with no detectable admixture from outside sources. It should be noted that the samples which are slightly aberrant are not the same from locus to locus. For example, DPEP-2 (107) was not found in Clicks Creek, but the sample was fixed for PA-1,2 (100), had PGDH (105), and was within the normal range of frequencies for LKGT at the other diagnostic loci. Thus, there is no evidence to suggest introgression in seven of the eight populations.

Two alleles associated with both hatchery rainbow trout and with KRRT were found in the the eighth population - Little Kern River at Shotgun Creek. As these were both at low frequency and all other diagnostic alleles were within the frequecny ranges expected of LKGT, any introgression that may have occured must have been one or more generations in the past. The Coyote Creek stock which was used to seed the Little Kern at Shotgun population was an unlikely source of these alleles. Survivors of the chemical treatment are a more likely source than the Coyote Creek fish, but even under this scenario, the observed allele frequencies were surprisingly high. The sample size of fifteen fish makes more definitive statements about rare alleles difficult.

INDEPENDENCE LAKE CUTTHROAT TROUT

The 32 fish from Indendence Lake were homozygous for cutthroat alleles at all diagnostic loci. These loci included ADA-2, CK-2, DPEP-1, MDH-2 and MDHP 3,4. The fish were also homozygous for the common cutthroat allele at the inferential loci AAT-4, ACP-1, ACP-2, mAH-5, TAPEP-1, PA-1,2, PGK-2, and SOD-1. Thus we are confident that the cutthroat trout examined were not introgressed with rainbow trout.

The population is far from monomorphic, however. High levels of variability were observed at IDDH-1, IDDH-2, PGM-3,4, and mAH-2. Lower levels of variability were observed at AAT-1,2, AH-1, GPI-3, IDH-3,4, MDH-3,4, PGDH and ACRO-2. We once considered high levels of heterozygosity at IDDH-1 and IDDH-2 to be suggestive of rainbow trout introgression. However, the present analysis for the Independence Lake population and the analysis performed last year for Heenan Lake cutthroat trout (Gall et al., 1991) indicate that this clearly is not true, at least for Lahonton cutthroat trout.

Allele frequencies at polymorphic loci are presented separately for adults and juveniles in Table 4. Sample sizes were 13 and 19 individuals respectively. There were slight differences in allele frequencies between the two groups; however, at most loci this could be atributed to sampling error due to small sample sizes. An exception was IDDH-1 which was nearly invariant in juveniles yet was highly

polymorphic in adults. However, IDDH is a notoriously difficult system to analyze and were are reluctant to draw conclusions from this single locus.

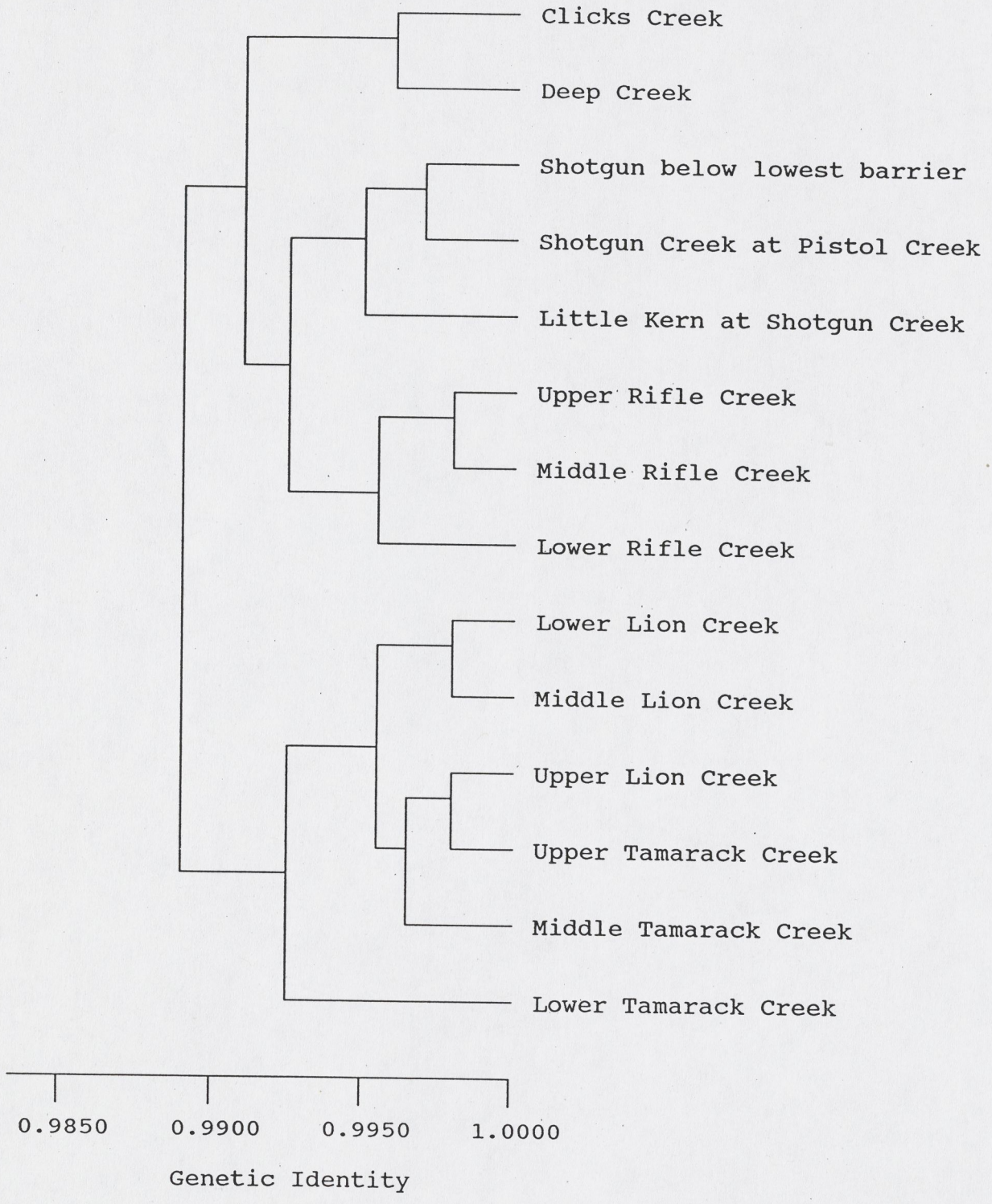

Figure 1. Dendrogram depicting genetic relationships among rainbow trout sampled from the Little Kern River system in 1992. Based on 77 loci.

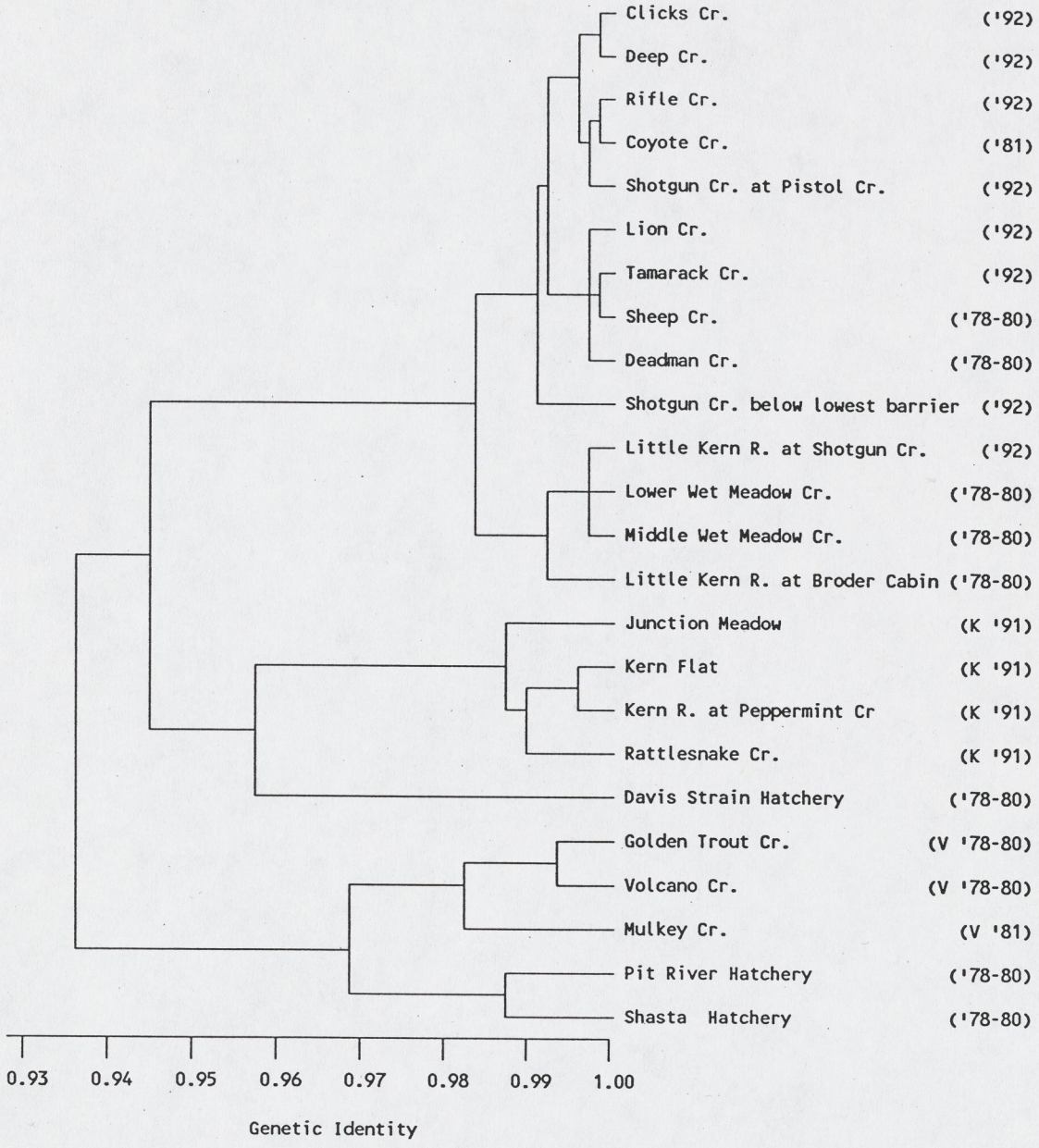

Figure 2. Dendrogram depicting genetic relationships among populations based on 23 loci. Eight samples from the Little Kern basin collected in 1992 are compared to representative samples from Little Kern golden trout, Volcano Creek golden trout (V), Kern River rainbow trout (K), and hatchery rainbow trout (collected in the years indicated).

Table 1. Allele frequencies for 1992 samples of Little Kern golden trout. Data for 16 polymorphic loci shown (frequencies may not add to 1.00 due to rounding).

		Click	Deep	L.K Shtgn	Lion	Rifle	Shtgn lowest	Shotgn Pistol	Tmrk
ACRO-2	100	1.00	1.00	0.93	0.47	0.62	0.72	0.82	0.19
	95	----	----	0.07	0.53	0.38	0.28	0.18	0.81
AH-1	100	1.00	0.94	1.00	1.00	1.00	1.00	1.00	1.00
	69	----	0.06	----	----	----	----	----	----
mAH-1	100	1.00	1.00	1.00	0.57	0.52	0.89	0.57	0.53
	30	----	----	----	0.01	0.28	----	0.21	0.03
	20	----	----	----	0.40	0.20	0.11	0.21	0.42
mAH-2	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99
	60	----	----	----	----	----	----	----	0.01
DPEP-2	107	----	----	0.43	0.07	0.16	0.22	0.11	0.13
	100	1.00	1.00	0.57	0.93	0.84	0.78	0.89	0.87
IDH-3,4	100	0.02	0.08	0.22	0.40	0.12	0.36	0.27	0.22
	74	0.98	0.92	0.77	0.60	0.88	0.64	0.73	0.78
	45	----	----	0.02	----	----	----	----	----
LDH-4	100	0.95	0.94	1.00	1.00	1.00	1.00	1.00	1.00
	72	0.05	0.06	----	----	----	----	----	----
PA-1,2	105	----	----	0.05	0.05	0.17	0.28	0.04	0.08
	100	1.00	1.00	0.95	0.95	0.83	0.72	0.96	0.92
PDPEP-2	100	1.00	1.00	1.00	1.00	0.98	1.00	1.00	1.00
	86	----	----	----	----	0.02	----	----	----
PGDH	105	0.08	----	0.39	----	0.06	0.11	0.11	----
	100	0.92	1.00	0.61	1.00	0.94	0.89	0.89	1.00
PGK-2	100	1.00	1.00	0.64	0.93	0.94	0.72	0.93	0.91
	90	----	----	0.36	0.07	0.06	0.28	0.07	0.09
PGM-3,4	115	----	----	----	----	0.09	0.06	0.05	----
	110	0.28	0.56	0.56	0.35	0.47	0.53	0.64	0.27
	105	0.44	0.42	0.42	0.65	0.44	0.42	0.31	0.48
	100	0.28	0.03	0.02	----	----	----	----	0.14
SOD-1	100	----	----	0.07	----	----	----	----	----
	71	0.43	0.25	0.37	----	0.24	0.39	0.43	0.10
	32	0.57	0.75	0.57	1.00	0.76	0.61	0.57	0.90

Table 2. Estimated Average Heterozygosities for each population collected in 1992, based on 77 loci.

Population	Average Heterozygosity
Clicks Cr.	0.030
Deep Cr.	0.022
L Kern R. Shotgun Cr.	0.052
lower Lion Cr.	0.031
middle Lion Cr.	0.030
upper Lion Cr.	0.031
lower Rifle Cr.	0.048
middle Rifle Cr.	0.045
upper Rifle Cr.	0.044
Shotgun Cr. blw lowest	0.051
Shotgun Cr. blw Pistol	0.043
lower Tamarack Cr.	0.032
middle Tamarack Cr.	0.042
upper Tamarack Cr.	0.035

Table 3. SOD-1 (100) and IDH-3,4 (45) allele frequencies for select LKGT populations sampled 1974-1976 (with sample sizes).

Population	SOD-1 (100)	IDH-3,4 (45)
L Kern R. above Broder' Cabin (n=37)	0.00	0.01
L Kern R. below Broder's Cabin (n=39)	0.01	0.03
L Kern R. at Wet Meadows Cr. (n=33)	0.02	0.01
L Kern R. at Rifle Cr. (n=41)	0.00	0.01
lower Shotgun Cr. (n=31)	0.00	0.00

Table 4. Allele frequencies for 15 polymorphic loci in Independence Lake Lahonton cutthroat trout (frequencies may not add to 1.00 due to rounding).

		Adults	Juveniles	Total Population
AAT-1,2	100	0.96	0.93	0.94
	90	0.04	0.07	0.06
ACRO-2	100	0.92	0.92	0.92
	95	0.08	0.08	0.08
AH-1	110	----	0.08	0.05
	100	1.00	0.92	0.95
mAH-2	100	0.04	0.16	0.11
	60	0.96	0.84	0.89
GPI-3	115	0.15	0.03	0.07
	100	0.85	0.97	0.93
IDDH-1	547	----	0.03	0.02
	307	0.73	0.97	0.88
	100	0.27	----	0.11
IDDH-2	168	0.44	0.61	0.53
	100	0.56	0.39	0.47
IDH-3,4	126	0.13	0.05	0.09
	100	0.37	0.45	0.41
	88	0.50	0.50	0.50
MDH-3,4	100	1.00	0.96	0.98
	75	----	0.04	0.02
PGDH	100	1.00	0.95	0.97
	80	----	0.05	0.03
PGM-3,4	115	0.67	0.70	0.69
	105	0.33	0.30	0.31

REFERENCES

Bartley, Devin M., and Gall, Graham A.E. 1990. Genetic Structure and Gene Flow in Chinook Salmon Populations of California. Trans. Amer. Fish. Soc. 119:55-71.

Berg, William J. 1987. Evolutionary Genetics of Rainbow Trout Parasalmo gairderii. Ph. D. Dissertation, Univ. of Calif., Davis, CA.

Berg, William, J., and Gall, Graham A.E. 1988. Gene Flow and Genetic Differentiation Among California Coastal Rainbow Trout Populations. Can J. Fish and Aq. Sci. 45:122-132.

Gall, Graham A.E., Bagley, Mark J., Bentley, Boyd, and Taylor, Michael. 1991. Genetic Analysis of Kern River Rainbow Trout Populations with a Note on Two Cutthroat Populations. Rpt., Threatened Trout Comm., Calif. Dept. Fish and Game.

Gall, Graham A.E., Bannon, Michael, Smith, Randolph C., and Bently, Boyd. 1981. Native Trout of California. Prog. Rpt., Threatened Trout Comm., Calif. Dept. Fish and Game.

Nei, M. 1972. Genetic Distance Between Populations. Am. Nat. 106:283-292.

Smith, R.C. 1981. Biochemical - Genetic and Meristic Analysis of populations of Little Kern River basin Golden Trout. Ph. D. Dissertation, Univ. of Calif., Davis, CA.

APPENDIX ONE: Technical notes on ACRO-2 and mAH-1

A monomeric allele system was discovered in this study which was highly polymorphic. We have named the system ACRO-2 because it showed up as a fast-migrating acromatic band. The system was observed on a 11.4% starch gel made with TBCL-8.0 buffer (0.03M Tris, 0.064M Citrate, pH 8.0). The gel was run using a TBCL-8.2 buffer (0.06M Lithium Hydroxide, 0.30M Boric acid). The protein appears with standard 1.25% MTT, 2.5% PMS stains. The ACRO-2 system was observed in heart tissue, but not in liver, muscle or retina tissue. The locus is polymorphic both in Little Kern golden trout and in Independence Lake cutthroat trout. Analysis of past smaples has revealed polymorphisms at ACRO-2 in Kern River rainbow trout as well. The distribution of allele frequencies ranged from 1.00 for the ACRO-2 (100) allele in Deep Creek to 0.81 for the ACRO-2 (95) allele in Tamarack Creek. Considering the low level of diversity encountered at other loci in the region, we highly recommend inclusion of this system in futrue analyses.

We observed bands under an AH stain which could not be positively classified with any of the known AH loci. These bands migrated very slowly, and at least superficially appear to be associated with mAH-1. Two varieties were observed: one which produced a single band, and one which produced a double band. The bands were highly population specific, and were scored as alleles of the mAH-1 locus. While the true genetic nature of these bands is unclear, their genetic

origin is not in doubt and they were therefore included in analyses of genetic identity.

GENETIC ANALYSIS OF THREATENED CALIFORNIA TROUT

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Report to

California Department of Fish and Game
Threatened Trout Committee

June, 1993

INTRODUCTION

Three forms of rainbow trout are generally recognized in the Kern River basin. These are: the Little Kern golden trout (Oncorhynchus mykiss whitei, abbreviated LKGT), the Kern River rainbow trout (O. mykiss gilberti, abbreviated KRRT), and the Volcano Creek golden trout (O. mykiss aquabonita, abbreviated VCGT). This report summarized results of progress as a continuation of studies on the trout of the region. Earlier reports summarized a study of KRRT carried out in 1991 (referred to subsequently as Gall et. al. 1991) and a study of LKGT carried out in 1992 (referred to subsequently as Mirman et. al. 1992). The primary purpose of the present study was to genetically analyze thirteen samples of LKGT and four samples of KRRT and attempt to determine if there had been any introgression from outside sources. One sample of McCloud redband trout and two samples of cutthroat trout collected in 1992 also were analyzed for possible introgression.

POPULATIONS (with sample size, n)

Little Kern River basin samples:

Little Kern River sample "C" (n=5)
Little Kern River sample "B" (n=19)
Little Kern River sample "A" (n=18)
Little Kern River kilometer 0.6 (n=9)
Little Kern River kilometer 1.0 (n=11)
Little Kern River kilometer 2.3 (n=4)
Little Kern River kilometer 2.7-3.1 (n=6)
Little Kern River kilometer 3.2-4.1 (n=8)
Little Kern River at Shotgun Creek (n=8)
Shotgun Creek at Pistol Creek (n=6)
Pecks Canyon Creek (n=40)
Lower Wet Meadows Creek (n=11)
Upper Wet Meadows Creek (n=14)

Kern River basin samples:

Main branch Upper Durwood Creek (n=12)
North branch Upper Durwood Creek (n=7)
South branch Upper Durwood Creek (n=10)
Upper Osa Creek (n=19)

McCloud redband sample:

Trout Creek (n=40)

Cutthroat trout samples:

Four Mile Canyon Creek (n=25)
Silver King Creek (n=28).

Data from previous studies used for comparative analyses were (with sample sizes and year of collection):

Little Kern golden trout:

Shotgun Creek below Pistol Creek (n=14 1992)
Shotgun Creek below lowest barrier (n=9 1992)
Little Kern River at Shotgun Creek (n=15 1992)
Little Kern River at Broder's cabin (n=16 1978-80)
Lower Wet Meadows Creek (n=24 1978-80)
Middle Wet Meadows Creek (n=21 1978-80)
Coyote Creek (n=64 1981)

Kern River rainbow trout:

Junction Meadows (n=16 1991)
Kern River at Kern Flat (n=27 1991)
Kern River at Peppermint Creek (n=27 1991)
Rattlesnake Creek at Bonita Flat(n=25 1991)
Lower Osa Creek (n=27 1978-80)

Volcano creek golden trout:

Volcano Creek (n=19 1978-80)

Hatchery rainbow trout:

Davis strain (n=12 1978-80)
Pit River strain (n=45 1978-80)
Shasta strain (n=28 1978-80)

METHODS OF ANALYSIS

Genetic variation was detected using standard starch-gel electrophoretic techniques as previously described in Berg and Gall (1988) and in Bartley and Gall (1990). Proteins were examined in extracts of five tissues: blood, eye, heart, liver and muscle. Initially 74 loci were used in the analysis. Nine of the loci were excluded from the analysis because interpretation of the zymograms was judged unreliable. The nine loci excluded were: mAH-1, ALAT-1, α-GLU, GR, MDHP-1,2, MDHP-3, and PGM-3,4. Allele frequencies were calculated for each of the remaining 65 loci. Analysis of overall genetic diversity of the twenty samples, assessment of possible introgression, and genetic relationships among the twenty samples were all based on these 65 loci. For comparisons with previous studies, only those loci common to all studies could be used. Thus, the genetic relationships between LKGT and KRRT sampled in 1992 and other rainbow trout groups were based on the following 20 loci:

ADH; CK-1; DPEP-1; G3PDH-1; GPI-1; GPI-2; GPI-3; IDH-2; IDH-3,4; LDH-3; LDH-4; MDH-3,4; PA-1,2; PDPEP; PGDH; SOD-1; and TAPEP

Genetic identities were estimated using Nei's genetic identity statistic I (Nei, 1972). Dendrograms were drawn using the UPGMA (Unweighted Pair-wise Group Mean Average) method.

RESULTS AND DISCUSSION

Overall Genetic Diversity

The following 31 loci were monomorphic in all samples:

AAT-1, AAT-2, AAT-3, mAAT-1, mAAT-2, mAAT-3, ADA-2, ADH, AH-2, ALAT-2, CK-2, CK-5, G3PDH-1, G3PDH-2, GPI-1, GPI-2, GPI-3, IDDH-1, IDDH-2, IDH-1, LDH-1, LDH-2, LDH-3, MPI, PEPLT, PGK-1, PGM-2, TPI-1, TPI-2, TPI-3, and TPI-4.

The follow 34 loci were polymorphic in at least one sample:

AAT-4, ACRO-2, ADA-1, mAH-2, mAH-4, CK-1, CK-3, CK-4, DPEP-1, DPEP-2, EST-6,7, EST-D, G3PDH-4, IDH-2, IDH-3,4, LDH-4, LDH-5, MDHP-4, MDH-1,2, MDH-3,4, mMDH-1, mMDH-2, PA-1,2, PDPEP-2, PGDH, PGK-2, PGM-1, SOD-1, and TAPEP.

Table 1 shows the allele frequencies for each polymorphic locus for the Little Kern golden trout samples. Table 2 shows the allele frequencies for each polymorphic locus for the Kern River rainbow trout, cutthroat trout, and redband trout samples. Table 3 gives the estimated average heterozygosities for each sample. Heterozygosities of LKGT ranged from 0.027 (Shotgun Creek at Pistol Creek) to 0.065 (Peck's Canyon Creek). Heterozygosities of KRRT ranged from 0.043 (Osa Creek) to 0.073 (North Durwood Creek). Four Mile Creek cutthroat and Silver King Creek cutthroat had estimated heterozygosities of 0.009 and 0.086, respectively. These values are consistent with estimated heterozygosities obtained from previous studies (Berg, 1987, Mirman et. al. 1992).

OVERVIEW OF RELATIONSHIPS

Genetic relationships among the twenty samples are shown in Figure 1. The two cutthroat trout samples, Four Mile Creek and Silver King Creek, were separated from the rainbow trout samples at a genetic identity of 0.80. Three clusters can be distinguished within the rainbow trout samples. The redband rainbow trout sample from Trout Creek formed a cluster by itself, at a genetic identity of 0.92. The four samples of KRRT formed a second group, at a genetic identity of 0.96. The final cluster consisted of the LKGT samples with a genetic identity of 0.98. Within the four KRRT samples the three Durwood Creek samples clustered closely together, while the Osa Creek sample was not much farther from the LKGT cluster than it was from the three Durwood Creek samples. Due to the high degree of genetic similarity among the LKGT samples, relationships within this cluster should be viewed with appropriate caution. Although all thirteen LKGT samples are similar to each other, three clusters may be recognized. The first consists of the three Little Kern River samples labelled "A", "B", and "C" as well as the two Wet Meadows Creek samples. The two Wet Meadows Creek samples were more similar to each other than to the Little Kern River samples. The second group consists of the five Little Kern River samples taken from kilometer markers 0.6-4.1, Little Kern River at Shotgun Creek, and Shotgun Creek at Pistol Creek. Finally, Peck's Canyon Creek formed a cluster by itself.

Figure 2 shows genetic relationships between samples collected in 1992 and relevant samples previously analyzed including LKGT, KRRT, hatchery rainbow trout, and Volcano Creek Golden Trout. With the exception of Peck's Canyon Creek, LKGT samples collected in 1992 clustered closely with previous samples of LKGT. Samples from Wet Meadows Creek taken in 1992 clustered closely with samples from Wet Meadows Creek taken from 1978-1980.

In addition to the large cluster of LKGT, three other clusters can be recognized. The first of these consists of four samples of KRRT collected in 1991. The second consists of the Durwood Creek samples taken in 1992, as well as the Pecks Canyon Creek sample and a sample of Volcano Creek golden trout collected in 1978-1980. This clustering does not necessarily imply Volcano Creek golden trout influence in the samples from Durwood Creek and Pecks Canyon Creek. For example, Volcano Creek golden trout have the allele PDPEP(90) at an average frequency of 0.92 (Berg, 1987) and this allele was not found at all in the Durwood Creek samples and was found in Peck's Canyon Creek at a frequency of only 0.02. On the other hand, VCGT have a high frequency of IDH-3,4(100), and this allele was found in the Durwood Creek and Pecks Canyon Creek samples at high frequency, contributing to the clustering seen in Figure 2. This allele is also commonly found in KRRT (albeit at lower frequencies).

The third cluster consisted of three representative hatchery samples. They clustered at some distance from the other groups (0.88). While some samples of wild fish expressed low frequencies of some alleles typical of hatchery rainbow trout, none of these alleles occurred at a frequency high enough to cause them to cluster with the hatchery samples.

Surprisingly, the sample from Osa Creek taken in 1992 and the previous sample of Osa Creek from 1978-1980 not only clustered at some distance from the other samples in the analysis, but at a distance of 0.89 from each other. This was not due to the influence of one or two aberrant alleles, but to significant differences at a number of loci. The 1978 sample had alleles at G3PDH, IDH-3,4, and TAPEP that were not found in the 1992 sample. The 1992 sample had an allele at PDPEP that was not found in the 1978 sample; furthermore, even when alleles were shared their frequencies were different at a number of loci (including IDH-3,4, MDH-3,4, PA-1,2, and SOD-1). Both samples have allele frequencies consistent with

KRRT, but the differences between them suggest these samples were from different populations, perhaps due to sampling at different locations.

POSSIBLE INTROGRESSION

Little Kern Golden Trout samples:

The presence of alleles IDH-2(105), LDH-3(80), MDH-3,4(75), and PGM-2(85) are considered highly inferential of rainbow trout influence. None of these alleles were present in any of the LKGT samples analyzed in this study. IDH-3,4(45) and SOD-1(100), also characteristic of rainbow trout, were found in seven populations but at low frequency (Table 4).

Alleles DPEP-2(107), IDH-3,4(74), PA-1,2(100), PGDH(105), and SOD-1(32) are considered diagnostic for LKGT. DPEP-2(107) was found in ten of the thirteen LKGT samples. IDH-3,4(74) was found at a frequency of 0.32 in Peck's Canyon Creek, and at moderate to high frequencies in all other samples (ranging from 0.54 in Little Kern River kilometer 2.1 to 0.86 in Little Kern River "B"). PA-1,2(100) was found at moderate to high frequencies in all samples (ranging from 0.48 in Shotgun Creek to 1.00 in Little Kern River "C", and the two Wet Meadows Creek samples). PGDH(105) was found in eleven of the thirteen samples. SOD-1(32) was found at a frequency of 0.15 in Peck's Canyon Creek and at moderate to high frequencies in all other samples (ranging from 0.39 at Little Kern River kilometer 0.6 to 0.77 in upper Wet Meadows Creek).

The alleles IDH-3,4(74) and SOD-1(32) are helpful in differentiating LKGT from KRRT as they are generally found at lower frequencies in KRRT than in LKGT. Both were found at moderate or high frequencies in all LKGT samples, except Peck's Canyon Creek, as mentioned earlier. PA-1,2(105) and MDH-3,4(85) are also diagnostic of KRRT. PA-1,2(105) was found in ten of the thirteen samples, at frequencies ranging from 0.03 in the Little Kern

River "B" sample to 0.52 in Shotgun Creek at Pistol Creek sample. MDH-3,4(85) was found in Peck's Canyon Creek at a frequency of 0.13. It was not found any other LKGT samples.

Allele IDH-3,4(100) occurs at high frequency in coastal rainbow trout and is nearly fixed in VCGT. It was found at low to moderate frequencies in all thirteen samples (ranging from 0.13 in Little Kern River "A" to 0.61 in Peck's Canyon Creek). PDPEP(86) is commonly found at frequencies greater than 0.90 in VCGT but only at very low frequencies in other Kern basin trout (Berg, 1987). PDPEP(86) was found at a frequency of 0.02 in Peck's Canyon Creek. It was not found in any other LKGT sample.

Based on the observed allele frequencies, the following samples showed no evidence of introgression: Little Kern River "C", Little Kern River "B", Little Kern River kilometer 1.0, Shotgun Creek at Pistol Creek, lower Wet Meadows Creek, and Upper Wet Meadows Creek. Each of these populations had high frequencies of the alleles IDH-3,4(74), and SOD-1(32), which tends to be the characteristic of LKGT. Shotgun Creek has a 0.48 frequency of PA-1,2(100) and each of the other five had this allele at high frequency. Each sample except Shotgun Creek had the allele DPEP-2(107) at low frequency, a rare allele whose presence at any frequency is considered diagnostic for LKGT. However, not all LKGT populations have this allele (Berg, 1987, Mirman et. al. 1992). PGDH(105) is another rare allele considered diagnostic for LKGT (Berg, 1987). Four of these six populations had this allele (absent from Little Kern River mile 1.0 and Shotgun Creek). None of the alleles diagnostic for coastal rainbow trout were found in these samples. On the basis of diagnostic loci, these six samples appear to be taken from pure LKGT populations (but see following discussion about Little Kern River kilometer 1.0).

Six populations had allele frequencies typical of LKGT at most loci but had low frequencies of aberrant alleles at IDH-3,4 and/or SOD-1. These were: Little Kern River "A",

Little Kern River kilometer 0.6, Little Kern River kilometer 2.3, Little Kern River kilometer 2.7, Little Kern River kilometer 3.2, and Little Kern River at Shotgun Creek. Frequencies of the aberrant alleles are summarized in Table 4. These are the same two alleles found in a previous sample from Little Kern River at Shotgun Creek (Mirman et. al. 1992). It is possible for a sample which originated in a population with low frequencies of these alleles to be devoid of these alleles by sampling error. For example, although the Little Kern River mile 1.0 sample and the Little Kern River mile 2.7 samples did not contain either of these alleles, the presence of IDH-3,4(45) in the other samples listed in Table 4 raises the possibility that IDH-3,4(45) is in these samples but was not found due to sampling error. Assuming a true IDH-3,4(45) frequency of 0.023 constant through all seven populations (this is the average of the seven sample frequencies), simply with random sampling and random mating, there would be approximately a 0.90 probability of not finding the allele in two or more of the seven populations sampled. For SOD-1(100), under the same assumptions there would be approximately a 0.56 probability of not finding the allele in five or more of the seven populations. The low frequencies of these alleles and the small sample sizes both contribute to making these probabilities large. The presence of rare alleles in a number of geographically continuous sampling sites suggests a common origin for these alleles.

The sample from Peck's Canyon Creek had alleles more typical of rainbow trout as follows: IDH-3,4(45) at a frequency of 0.07, SOD-1(100) at a frequency of 0.18, and MDH-3,4(85) at a frequency of 0.13. MDH-3,4(85) has not previously been found in LKGT, and this was the only sample in this study to have it. An allele characteristic of VCGT, PDPEP(86), was found at a frequency of 0.02. This allele has been found in both LKGT and KRRT at low frequencies (Berg, 1987). The sample had low frequencies of alleles typically found in LKGT as follows: IDH-3,4(74) at a frequency of 0.32, PGDH(105) at a frequency of 0.05, DPEP-

2(107) at a frequency of 0.16, and SOD-1(32) at a frequency of 0.15. While the PGDH and DPEP alleles may be found at frequencies this low or lower in LKGT, the IDH-3,4(74) and SOD-1(32) alleles are generally found at much higher frequencies in LKGT (Berg, 1987, Mirman et. al. 1992). Overall, the data suggest the Peck's Canyon Creek sample was obtained from an introgressed population. Examination of genotypes of individual fish in this sample suggests this hybridization happened at least several generations in the past. For example, two fish display both PGDH(105) and MDH-3,4(45), and four other fish display both DPEP(107) and MDH-3,4(45), but only one fish displays both PGDH(105) and DPEP(107). If the hybridization had been recent, more fish displaying both LKGT alleles would be expected, and less fish would be expected to display one each of the LKGT and rainbow alleles. The observed allele frequencies at these loci suggest several generations of random mating. The data at other loci are consistent with these conclusions.

Kern River rainbow trout samples:

The following alleles are typical of KRRT: IDH-2(105), IDH-3,4(74), MDH-3,4(85), PA-1,2(105), PDPEP(90), SOD-1(32), and TAPEP(124). IDH-2(105) was found in North Durwood Creek at a frequency of 0.06 and in South Durwood Creek at a frequency of 0.10. IDH-3,4(74) was found at moderate frequencies in each of the four KRRT samples. PA-1,2(105) was found at a frequency of 0.65 in South Durwood Creek and at high frequency in the other three samples. TAPEP(124) was found in each of the Durwood Creek samples at a frequency of 0.10-0.11. The alleles MDH-3,4(85), and PDPEP(90) were not found in these four samples.

Alleles IDH-2(105), PDPEP(90) and TAPEP(124) are rare alleles whose presence is diagnostic of KRRT but whose absence is not informative. Allele IDH-3,4(74) is generally

found at higher frequency than observed in the three Durwood Creek samples (Gall et. al., 1991, Berg 1987). IDH-3,4(45), considered indicative of hatchery trout influence, was found in both middle Durwood Creek (at a frequency of 0.10) and in South Durwood Creek (at a frequency of 0.02). The Durwood Creek samples also displayed SOD-1(100) at relatively high frequency, an allele typically found at very low frequencies in KRRT and at higher frequencies in hatchery rainbow trout. The upper Osa Creek sample did not display either of these alleles.

Previous studies of KRRT show great variation in the genetic makeup of KRRT from one stream to another (Gall et. al., 1991) making assessment of introgression more difficult than with LKGT. The Durwood Creek samples show some evidence suggestive of hatchery trout influence while the upper Osa Creek sample shows no evidence of outside influence. However, there is no clear baseline of "pure" KRRT genetic makeup for comparison as there is with LKGT. Previous studies indicate that KRRT may typically possess more coastal rainbow alleles than other trout in the Kern basin (Berg, 1987, Gall et. al. 1991).

Redband Trout sample:

The following alleles are used in differentiating redband trout from rainbow trout: GPI-1(183), IDH-3,4(45), LDH-4(72), MDH-3,4(100), PA-1,2(105), SOD-1(100), and TAPEP(60). Alleles GPI-1(183) and LDH-4(72) were not found in the Trout Creek sample, while the other diagnostic alleles were found at the frequencies indicated in Table 1.

A high frequency of LDH-4(72) is diagnostic for inland redband trout. This allele was not found in the Trout Creek sample. High frequencies of GPI-1(150), SOD-1(100) and TAPEP(60) are characteristic of Goose Lake Redband trout (Berg, 1987). The Trout Creek sample had only a 0.23 frequency of SOD-1(100), only a 0.10 frequency of TAPEP(100) and did not have the GPI-1(150) allele. Both inland redband trout and Goose Lake redband trout

typically have a low frequency of IDH-3,4(45) and a very high frequency of MDH-3,4(100) (Berg, 1987). The Trout Creek sample had a 0.50 frequency of IDH-3,4(45) and a 0.45 frequency of MDH-3,4(100). These frequencies, as well as those above, are typical of McCloud River type redband trout. The Trout Creek sample appears to be from a population of McCloud River type redband trout.

Cutthroat Trout samples:

The following alleles are used to differentiate cutthroat trout from rainbow trout: AAT-4(120), mAH-2(60), mAH-4(119), ADA-2(115), CK-2(85), DPEP-1(111), MDH-2(130), MDHP-3,4(116), and TAPEP(124). The Four Mile Canyon Creek sample was fixed for each of these alleles. The Silver King Creek sample had MDH-2(130) at a frequency of 0.41, and each of the other diagnostic cutthroat alleles at frequencies of 0.75-0.82.

The Four Mile Canyon Creek sample had been sampled previously (Mirman, 1992). As reported previously, there was no evidence of introgression with rainbow trout; Four Mile Canyon Creek appears to contain pure cutthroat trout. Both samples had very low estimated average heterozygosity. The only loci showing any polymorphism in this sample were EST-6 and EST-7. Low heterozygosities generally indicate either a small breeding population or a recent bottleneck in population size. However, this is not necessarily cause for alarm as cutthroat trout have often been found to have heterozygosities this low or even lower (Bartley and Gall, 1989).

The Silver King Creek sample showed alleles indicative of rainbow trout influence at every diagnostic locus. At most of these loci the frequency of rainbow alleles was between 0.18 and 0.25. Examination of genotypes of individual fish suggested eighteen of the twenty-eight fish were pure cutthroat trout, two fish were pure rainbow trout, and eight fish were

hybrids. None of the eight presumptive hybrids was a first generation hybrid; five fish were of primarily cutthroat origin displaying rainbow alleles at from one to five of the nine diagnostic loci. The other three hybrids were of primarily rainbow trout origin, and were heterozygous for the cutthroat allele at either one or two diagnostic loci. Although there were no first generation hybrids, these data suggest recent or ongoing hybridization.

CONCLUSIONS

Of the twenty samples collected in 1992, thirteen samples were from the Little Kern basin, four samples were from the Kern River basin, two samples were cutthroat trout from the Silver King basin, and one sample was a presumptive redband trout from Trout Creek in the McCloud River basin. Genetic variation was detected using standard electrophoretic techniques. Overall levels of genetic diversity were consistent with previous findings for each group of trout.

Of the Little Kern River basin samples, the two samples from Wet Meadows Creek, the Shotgun Creek at Pistol Creek sample, the Little Kern River sample "C" and the Little Kern River sample "B" showed no evidence of introgression and were found to be pure Little Kern Golden Trout. Peck's Canyon Creek golden trout showed clear evidence of introgression with rainbow trout at least several generations in the past. Little Kern River samples "A", kilometer 0.6, kilometer 2.3, kilometer 2.7, kilometer 3.2 and the sample taken at Shotgun Creek had one or both of the rainbow trout alleles found in a previous sample of Little Kern River at Shotgun Creek. The Little Kern River sample at kilometer 1.0 showed no direct evidence of introgression but may also have one or both of these alleles.

Kern River Rainbow trout from Osa Creek showed no evidence of introgression; however, they were surprisingly different from a previous sample of Osa Creek trout. Kern

River Rainbow trout from Durwood Creek have some alleles typical of hatchery rainbow trout, but KRRT are known to vary greatly from location to location and these observed allele frequencies could be typical of Kern River rainbow trout. Four Mile Creek cutthroat trout were found to be pure. Silver King Creek contained a mixture of pure cutthroat trout, pure rainbow trout, and hybrids. The Trout Creek sample was found to be a McCloud River redband Trout.

REFERENCES

Bartley, Devin M., and Gall, Graham, A.E. 1989. Biochemical Genetic Analysis of Native Trout in Nevada: Report on Populations Collected from 1976-1988. Rept. Nevada Dept. Wildlife.

Bartley, Devin M., and Gall, Graham, A.E. 1990. Genetic Structure and Gene Flow in Chinook Salmon Populations of California. Trans. Amer. Fish. Soc. 119:55-71.

Berg, William J. 1987. Evolutionary Genetics of Rainbow Trout Parasalmo gairderii. Ph. D. Dissertation, Univ. of Calif., Davis, CA.

Berg, William J., and Gall Graham A.E. 1988. Gene Flow and Genetic Differentiation Among California Coastal Rainbow Trout Populations. Can. J. Fish. and Aq. Sci. 45:122-132.

Gall, Graham A.E., Bagley, Mark J., Bentley, Boyd, and Taylor Michael. 1991. Genetic Analysis of Kern River Rainbow Trout Populations with a Note on Two Cutthroat Populations. Rpt. Threatened Trout Comm., Calif. Dept. Fish and Game.

Mirman, David H., Bagley, Mark J., Poompuang, Supawadee, Kong, Yvonne, and Gall, Graham A.E. 1992. Genetic Analysis of Threatened Trout: Little Kern Golden Trout, Independance Lake Cutthroat Trout. Rpt. Threatened Trout Comm., Calif. Dept. Fish and Game.

Nei, M. 1972. Genetic Distance Between Populations. Am. Nat. 106:283-292.

Smith, R.C. 1981. Biochemical-Genetic and Meristic Analysis of populations of Little Kern River basin Golden Trout. Ph.D. Dissertation, Univ. of Calif., Davis, CA.

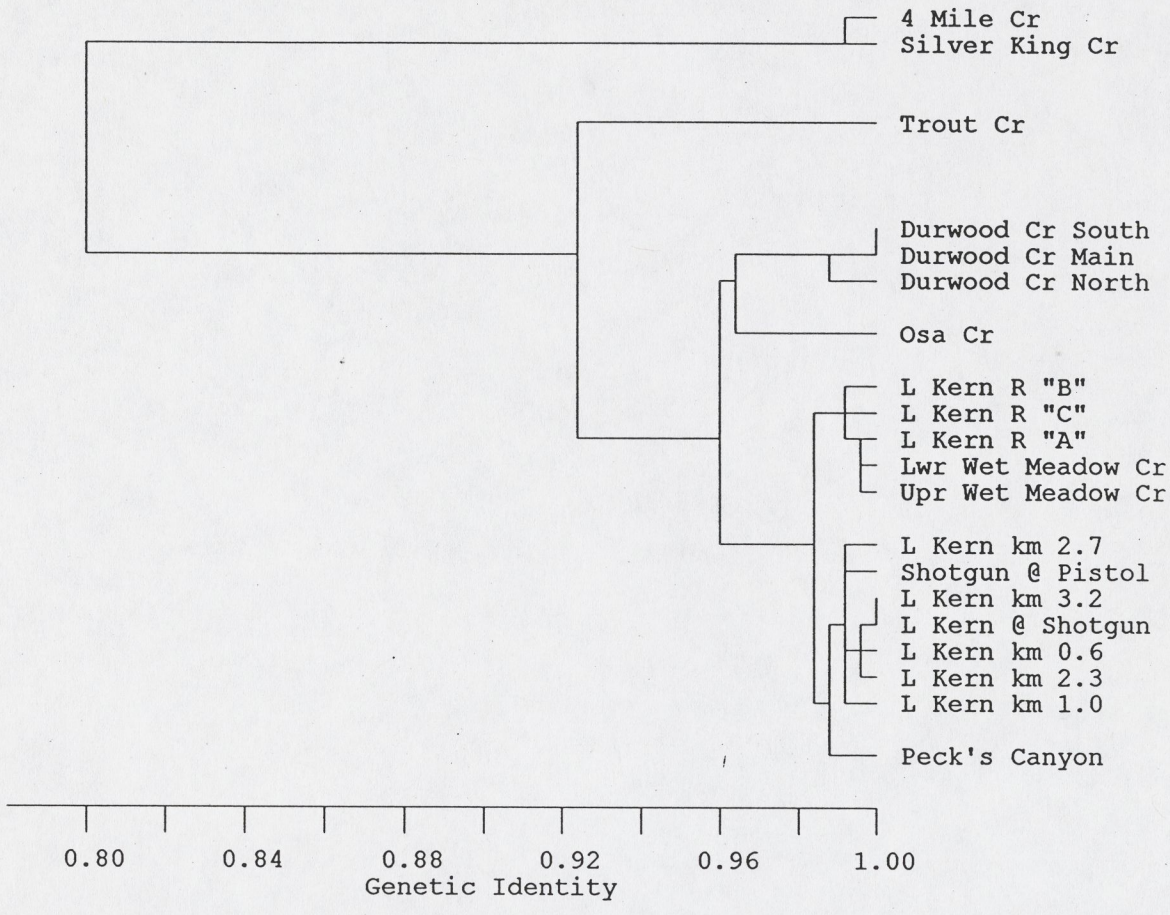

Figure 1. Dendrogram depicting genetic relationships among trout sampled from the Little Kern River system in 1992. Based on 66 loci.

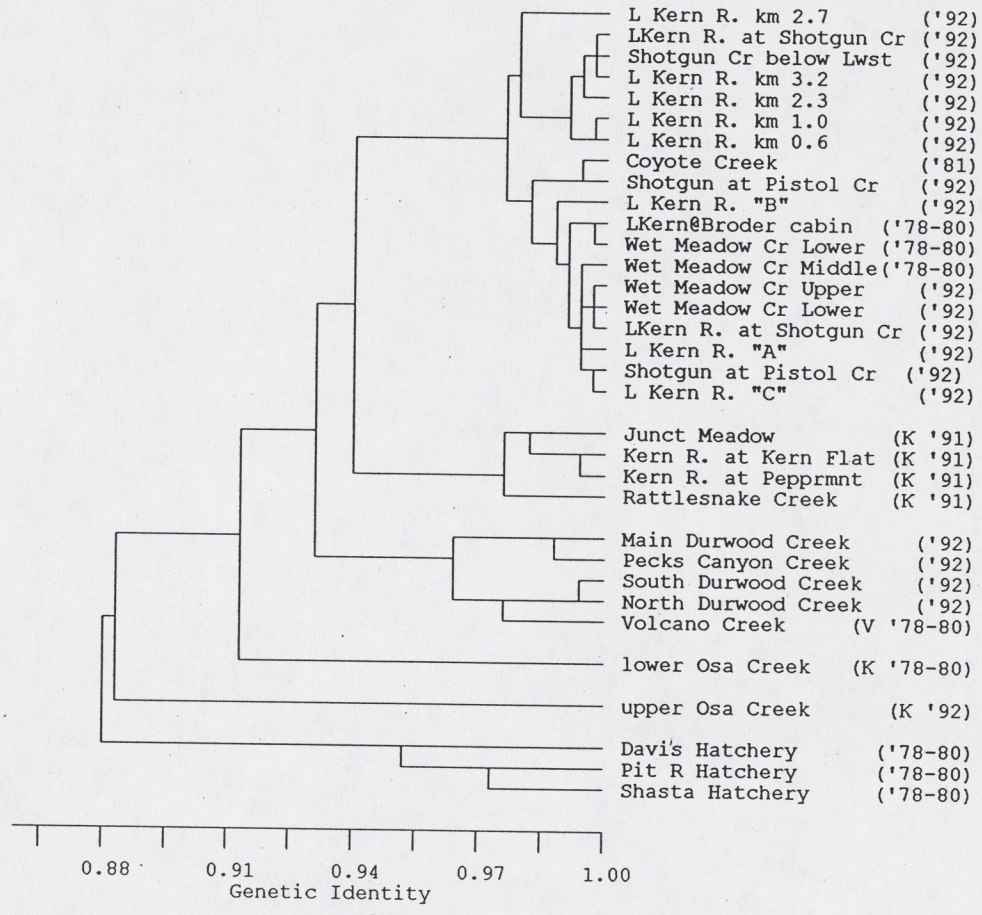

Figure 2. Dendrogram depicting genetic relationships among populations based on 20 loci. Seventeen samples collected from the Kern River basin in 1992 are compared to representative samples from Little Kern Golden Trout, Volcano Creek golden trout (V), Kern River rainbow trout (K) and hatchery rainbow trout (collected in the years indicated).

Table 1. Allele frequencies for 1992 samples of Little Kern golden trout, Little Kern River samples. Data for 25 polymorphic loci shown (frequencies may not add to 1.00 due to rounding).

Locus	Allele	LK C	LK B	LK A	LK 0.6	LK 1.0	LK 2.3	LK 2.7	LK 3.2
AAT-4	120	----	0.03	----	----	----	----	----	----
	100	1.00	0.97	1.00	1.00	1.00	1.00	1.00	1.00
ACRO-2	100	0.40	0.47	0.61	0.78	0.95	0.63	0.83	0.75
	95	0.60	0.53	0.39	0.22	0.05	0.37	0.17	0.25
ADA-1	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	93	----	----	----	----	----	----	----	----
AH-1	110	----	----	----	----	----	0.12	----	----
	100	1.00	1.00	1.00	1.00	1.00	0.88	1.00	1.00
	84	----	----	----	----	----	----	----	----
mAH-4	119	----	----	----	----	----	----	----	----
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
CK-3	100	1.00	1.00	1.00	1.00	1.00	0.75	0.92	1.00
	85	----	----	----	----	----	0.25	0.08	----
DPEP-1	111	----	----	----	----	----	----	----	----
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
DPEP-2	107	0.10	0.50	0.28	0.28	0.18	----	----	0.06
	100	0.90	0.50	0.72	0.72	0.82	1.00	1.00	0.94
EST-6,7	103	0.15	----	0.04	----	----	----	----	----
	100	0.65	0.70	0.74	0.85	0.68	0.94	0.88	0.92
	97	0.20	0.30	0.22	0.15	0.32	0.06	0.12	0.08
EST-D	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	95	----	----	----	----	----	----	----	----
G3PDH-4	100	1.00	1.00	1.00	0.94	1.00	1.00	1.00	1.00
	81	----	----	----	0.06	----	----	----	----
IDH-3,4	126	----	----	----	----	----	----	----	0.03
	100	0.15	0.14	0.13	0.22	0.34	0.38	0.46	0.38
	74	0.85	0.86	0.85	0.75	0.66	0.56	0.54	0.56
	45	----	----	0.03	0.03	----	0.06	----	0.03

Table 1. (cont.) Allele frequencies for 1992 samples of Little Kern golden trout, Little Kern River samples. Data for 25 polymorphic loci shown (frequencies may not add to 1.00 due to rounding).

Locus	Allele	LK C	LK B	LK A	K 0.6	LK 1.0	LK 2.3	LK 2.7	LK 3.2
LDH-4	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.94
	72	----	----	----	----	----	----	----	0.06
MDHP-4	105	0.10	----	----	0.06	----	----	----	----
	100	0.90	1.00	1.00	0.94	1.00	1.00	1.00	1.00
MDH-3,4	119	----	----	----	----	----	----	----	----
	100	1.00	0.82	1.00	1.00	1.00	1.00	1.00	1.00
	95	----	0.18	----	----	----	----	----	----
	85	----	----	----	----	----	----	----	----
mMDH-2	244	----	----	----	----	----	----	----	----
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	80	----	----	----	----	----	----	----	----
PA-1,2	105	1.00	0.97	0.83	0.73	0.66	0.81	0.84	0.87
	100	----	0.03	0.17	0.27	0.34	0.19	0.16	0.13
PGDH	105	0.20	0.50	0.36	0.11	----	0.13	0.67	0.13
	100	0.80	0.50	0.64	0.89	1.00	0.87	0.33	0.87
PGK-2	120	----	----	----	----	----	----	----	----
	100	0.50	0.18	0.44	0.50	0.91	0.75	0.83	0.87
	90	0.50	0.82	0.56	0.50	0.09	0.25	0.17	0.13
SOD-1	100	----	----	----	----	----	----	0.08	0.13
	60	0.40	0.40	0.47	0.61	0.55	0.38	0.42	0.44
	32	0.60	0.60	0.53	0.39	0.45	0.62	0.50	0.44
TAPEP	124	----	----	----	----	----	0.13	----	----
	100	1.00	1.00	1.00	1.00	1.00	0.87	1.00	1.00

Table 1. (cont.) Allele frequencies for 1992 samples of Little Kern golden trout, remaining samples. Data for 25 polymorphic loci shown (frequencies may not add to 1.00 due to rounding).

Locus	Allele	LKSht	ShtPstl	Pecks	LW Mead	UW Mead
AAT-4	120	----	----	----	----	----
	100	1.00	1.00	1.00	1.00	1.00
ACRO-2	100	0.81	1.00	0.86	0.54	0.53
	95	0.19	----	0.14	0.46	0.47
ADA-1	100	1.00	1.00	0.96	1.00	1.00
	93	----	----	0.04	----	----
AH-1	110	----	----	----	----	----
	100	1.00	1.00	0.94	1.00	1.00
	84	----	----	0.06	----	----
mAH-4	119	----	----	0.01	----	----
	100	1.00	1.00	0.99	1.00	1.00
CK-3	100	1.00	1.00	1.00	1.00	1.00
	86	----	----	----	----	----
DPEP-1	111	----	----	0.04	----	----
	100	1.00	1.00	0.96	1.00	1.00
DPEP-2	107	0.19	----	0.16	0.36	0.40
	100	0.81	1.00	0.84	0.64	0.60
EST-6,7	103		----	0.02	----	----
	100	n.d.	1.00	0.93	0.80	0.62
	97		----	0.04	0.20	0.38
EST-D	100	0.94	1.00	1.00	1.00	1.00
	95	0.06	----	----	----	----
G3PDH-4	100	1.00	1.00	0.94	1.00	1.00
	81	----	----	0.06	----	----
IDH-3,4	126	----	----	----	----	----
	100	0.34	0.21	0.61	0.16	0.17
	74	0.63	0.79	0.32	0.84	0.83
	45	0.03	----	0.07	----	----

Table 1. (cont.) Allele frequencies for 1992 samples of Little Kern golden trout, remaining samples. Data for 25 polymorphic loci shown (frequencies may not add to 1.00 due to rounding).

Locus	Allele	LKSht	ShtPstl	Pecks	LW Mead	UW Mead
LDH-4	100	1.00	1.00	1.00	1.00	1.00
	72	----	----	----	----	----
MDHP-4	105	----	----	----	----	----
	100	1.00	1.00	1.00	1.00	1.00
MDH-3,4	119	----	----	0.01	----	----
	100	1.00	1.00	0.87	1.00	1.00
	95	----	----	----	----	----
	85	----	----	0.13	----	----
mMDH-2	244	----	----	0.06	0.04	0.20
	100	1.00	1.00	0.90	0.96	0.80
	80	----	----	0.04	----	----
PA-1,2	105	0.91	0.48	0.80	1.00	1.00
	100	0.09	0.52	0.20	----	----
PGDH	105	0.25	----	0.05	0.45	0.43
	100	0.75	1.00	0.95	0.55	0.57
PGK-2	120	----	----	0.13	----	----
	100	0.69	0.92	0.40	0.23	0.40
	90	0.31	0.08	0.48	0.77	0.60
SOD-1	100	----	----	0.18	----	----
	60	0.56	0.25	0.68	0.41	0.23
	32	0.44	0.75	0.15	0.59	0.77
TAPEP	124	----	----	0.96	----	----
	100	1.00	1.00	0.04	1.00	1.00

Table 2. Allele frequencies for 1992 samples of Kern River Rainbow trout, Trout Creek Redband Trout, and Cutthroat trout. Data for 35 polymorphic loci shown (frequencies may not add to 1.00 due to rounding).

Locus	Allele	UDurM	UDurN	UDurS	OsaCr	Trout	4Mile	Silver
AAT-4	120	----	----	----	----	----	1.00	0.79
	100	1.00	1.00	1.00	1.00	1.00	----	0.21
ACRO-2	100	0.35	0.69	0.35	0.34	1.00	1.00	0.98
	95	0.65	0.31	0.65	0.66	----	----	0.02
ADA-1	100	0.75	0.72	0.85	1.00	0.91	1.00	0.96
	93	0.25	0.28	0.15	----	0.09	----	0.04
ADA-2	115	----	----	----	----	----	1.00	0.77
	100	1.00	1.00	1.00	1.00	1.00	----	0.23
mAH-2	100	1.00	1.00	1.00	1.00	1.00	----	0.21
	60	----	----	----	----	----	1.00	0.79
mAH-4	119	0.25	0.11	0.15	0.08	----	1.00	0.75
	100	0.75	0.89	0.85	0.92	1.00	----	0.25
CK-1	100	0.85	0.89	0.90	1.00	1.00	1.00	1.00
	70	0.15	0.11	0.10	----	----	----	----
CK-2	100	1.00	1.00	1.00	1.00	1.00	----	0.18
	85	----	----	----	----	----	1.00	0.82
CK-3	105	0.05	0.29	0.15	----	----	----	----
	100	0.95	0.71	0.85	1.00	1.00	1.00	1.00
CK-4	105	----	----	----	----	----	----	0.32
	100	1.00	1.00	1.00	1.00	1.00	1.00	0.68
DPEP-1	111	----	----	----	----	----	1.00	0.80
	100	1.00	1.00	1.00	1.00	1.00	----	0.20
EST-6,7	103	0.03	----	----	0.02	0.01	0.04	0.05
	100	0.90	0.83	0.78	0.93	0.59	0.77	0.69
	97	0.08	0.17	0.23	0.04	0.40	0.19	0.27
G3PDH-4	100	0.15	0.11	0.15	0.84	0.97	1.00	0.88
	81	0.85	0.89	0.85	0.16	0.03	----	0.13

Table 2. (cont.) Allele frequencies for 1992 samples of Kern River Rainbow Trout, Trout Creek Redband Trout, and Cutthroat trout. Data for 35 polymorphic loci shown (frequencies may not add to 1.00 due to rounding).

Locus	Allele	UDurM	UDurN	UDurS	OsaCr	Trout	4Mile	Silver
IDDH-1	307	----	----	----	----	----	1.00	0.82
	100	1.00	1.00	1.00	1.00	1.00	----	0.18
IDDH-2	100	1.00	1.00	1.00	1.00	1.00	----	0.20
	33	----	----	----	----	----	1.00	0.80
IDH-2	105	----	0.06	0.10	----	----	----	----
	100	1.00	0.94	0.90	1.00	1.00	1.00	1.00
IDH-3,4	124	0.05	0.14	0.13	----	----	----	----
	100	0.50	0.72	0.58	0.66	0.50	0.50	0.59
	88	----	----	----	----	----	0.50	0.40
	74	0.35	0.14	0.28	0.34	----	----	----
	45	0.10	----	0.03	----	0.50	----	0.01
LDH-4	100	0.95	1.00	0.90	1.00	1.00	1.00	1.00
	72	0.05	----	0.10	----	----	----	----
LDH-5	100	0.95	0.72	0.80	1.00	1.00	1.00	1.00
	97	0.05	0.28	0.20	----	----	----	----
MDHP-4	105	----	----	----	----	----	1.00	0.82
	100	1.00	1.00	1.00	1.00	1.00	----	0.18
MDH-1,2	130	----	----	----	----	----	0.50	0.41
	100	1.00	1.00	1.00	1.00	1.00	0.50	0.59
	42	----	----	----	----	----	----	----
MDH-3,4	119	----	0.03	----	----	----	----	----
	100	1.00	0.94	0.90	0.70	0.45	1.00	0.95
	95	----	----	----	0.30	0.49	----	----
	85	----	----	----	----	0.06	----	0.05
	75	----	0.03	0.10	----	----	----	----
mMDH-1	-150	----	----	----	----	----	----	0.02
	-100	1.00	1.00	1.00	1.00	1.00	1.00	0.98
mMDH-2	244	----	----	0.05	0.03	----	----	----
	100	1.00	1.00	0.95	0.97	1.00	1.00	1.00
PA-1,2	105	0.95	0.62	1.00	0.98	0.59	----	0.41
	100	0.05	0.38	----	0.02	0.41	1.00	0.59

Table 2. (cont.) Allele frequencies for 1992 samples of Kern River Rainbow Trout, Trout Creek Redband Trout, and Cutthroat trout. Data for 35 polymorphic loci shown (frequencies may not add to 1.00 due to rounding).

Locus	Allele	UDurN	NDurM	UDurS	OsaCr	Trout	4Mile	Silver
PDPEP-2	100	1.00	1.00	1.00	1.00	1.00	1.00	0.98
	86	----	----	----	----	----	----	0.02
PGK-2	100	1.00	1.00	1.00	0.92	0.18	----	0.05
	90	----	----	----	0.08	0.82	1.00	0.95
PGM-1	100	1.00	1.00	1.00	0.90	1.00	1.00	1.00
	-100	----	----	----	0.10	----	----	----
SOD-1	100	0.25	0.11	0.05	----	0.23	----	----
	60	0.75	0.89	0.95	0.95	0.77	1.00	1.00
	32	----	----	----	0.05	----	----	----
TAPEP	124	0.10	0.11	0.10	----	0.10	1.00	0.77
	100	0.90	0.89	0.90	1.00	0.90	----	0.23

Table 3. Estimated Average Heterozygosities for each population, based on 66 loci.

Population	Estimated Average Heterozygosity
Durwood Creek main	0.059
Durwood Creek North	0.073
Durwood Creek South	0.070
Four Mile Canyon Creek	0.009
L Kern R. "C"	0.051
L Kern R. "B"	0.059
L Kern R. "A"	0.058
L Kern R. mile 0.6	0.057
L Kern R. mile 1.0	0.041
L Kern R. mile 2.3	0.063
L Kern R. mile 2.7	0.047
L Kern R. mile 3.2	0.049
L Kern R. at Shotgun Cr	0.046
Osa Creek top	0.043
Peck's Canyon Creek	0.065
Shotgun Cr at Pistol Cr	0.027
Silver King Creek	0.086
Trout Creek	0.051
Wet Meadow Creek lower	0.052
Wet Meadow Creek upper	0.059

Table 4. IDH-3,4(45) and SOD-1(100) allele frequencies for LKGT populations sampled 1992.

Population	frequency IDH-3,4(45)	frequency SOD-1(100)
L. Kern R. "A"	0.03	0.00
L. Kern R. 0.6	0.03	0.00
L. Kern R. 2.3	0.06	0.00
L. Kern R. 2.7	0.00	0.08
L. Kern R. 3.2	0.03	0.13
L. Kern at Shotgun	0.03	0.00
Peck's Canyon Cr	0.07	0.18