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

Graham A.E. Gall, Mark J. Bagley
Boyd Bentley And Michael Taylor

Department of Animal Science
University of California, Davis

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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

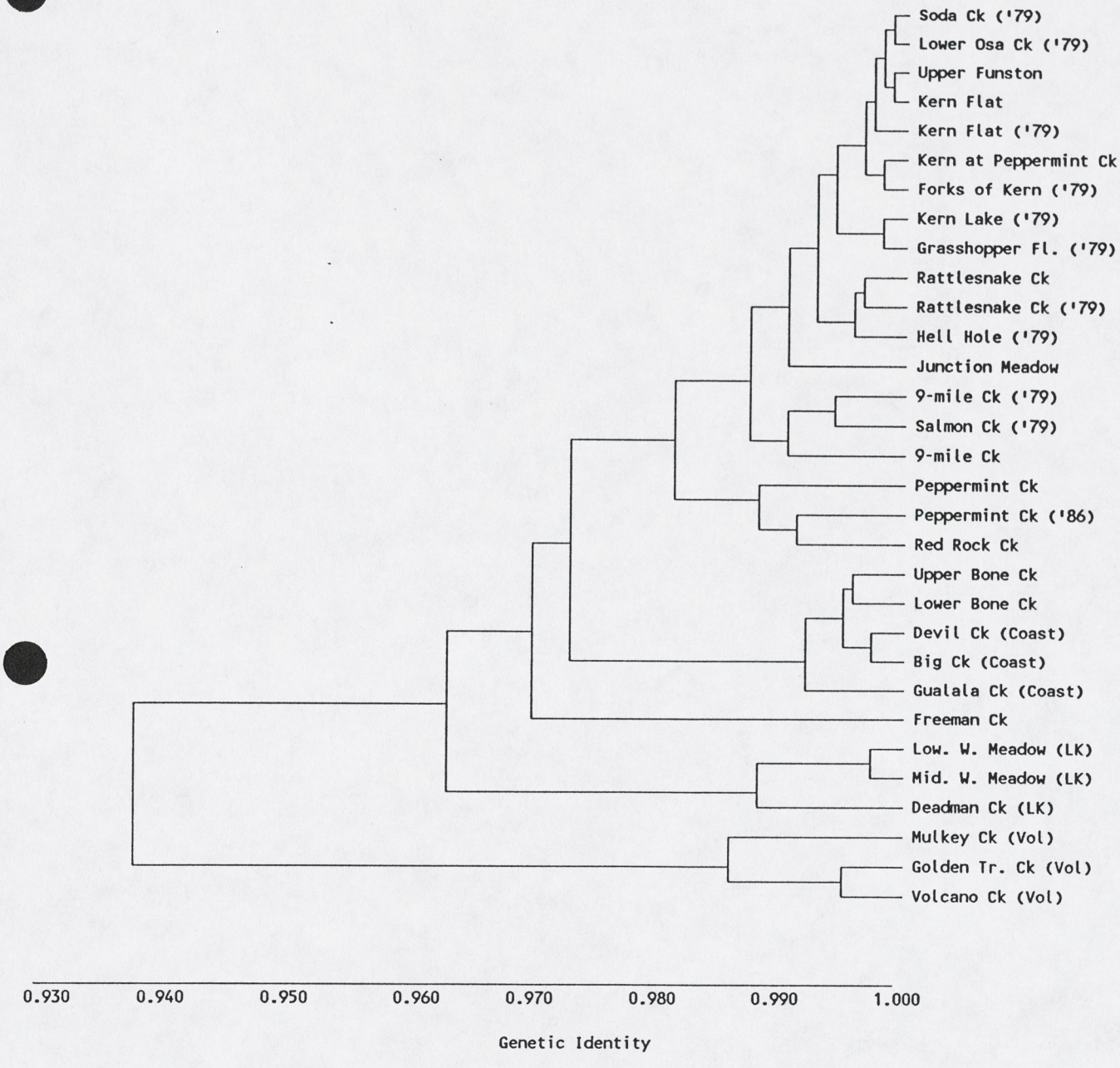


Figure 1. Dendrogram depicting the genetic relationships among rainbow trout sampled from the Kern River system in 1991, Kern River samples collected during earlier studies, and representative populations of Volcano Creek golden trout, Little Kern River golden trout, and coastal rainbow trout.

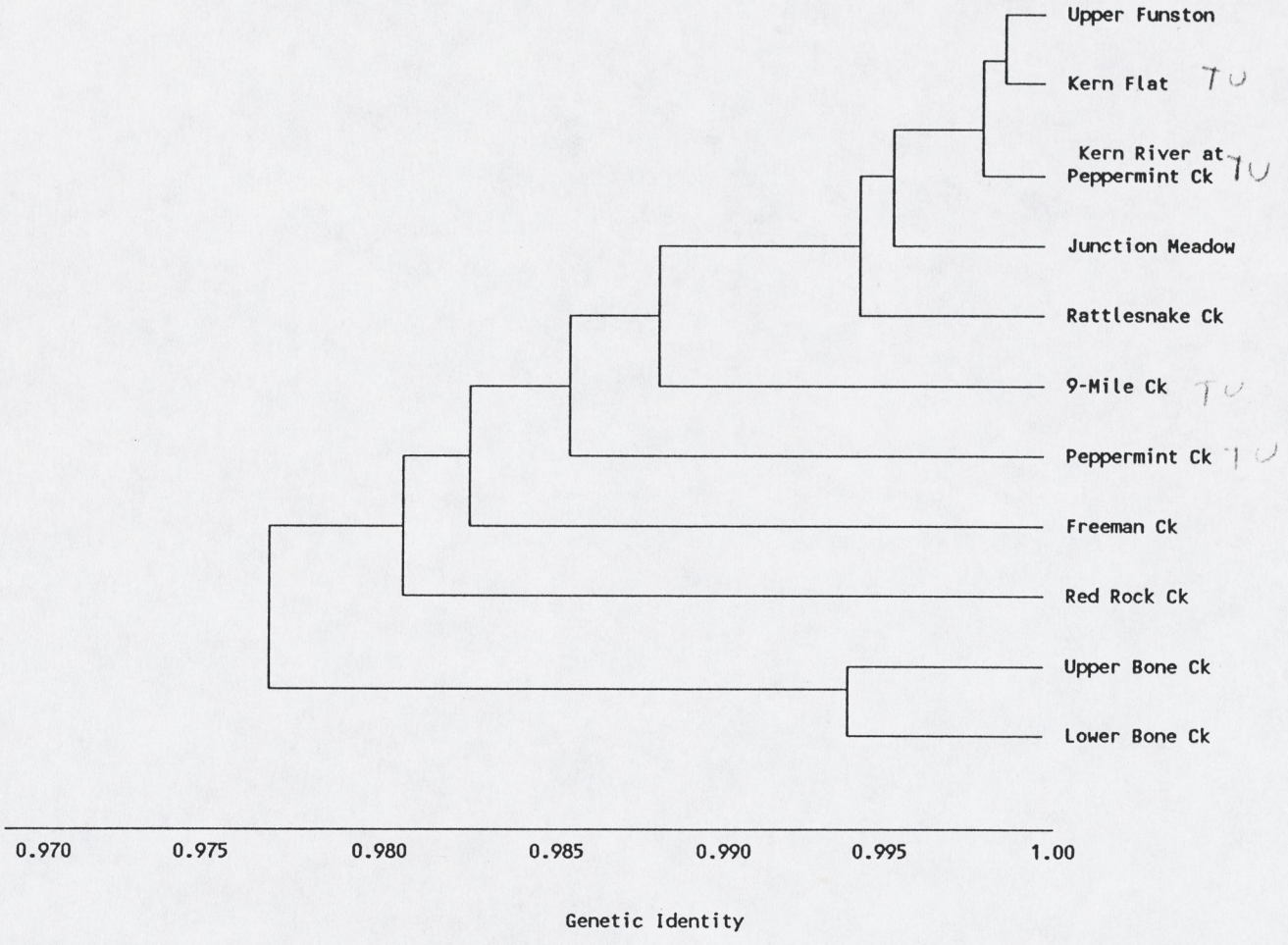
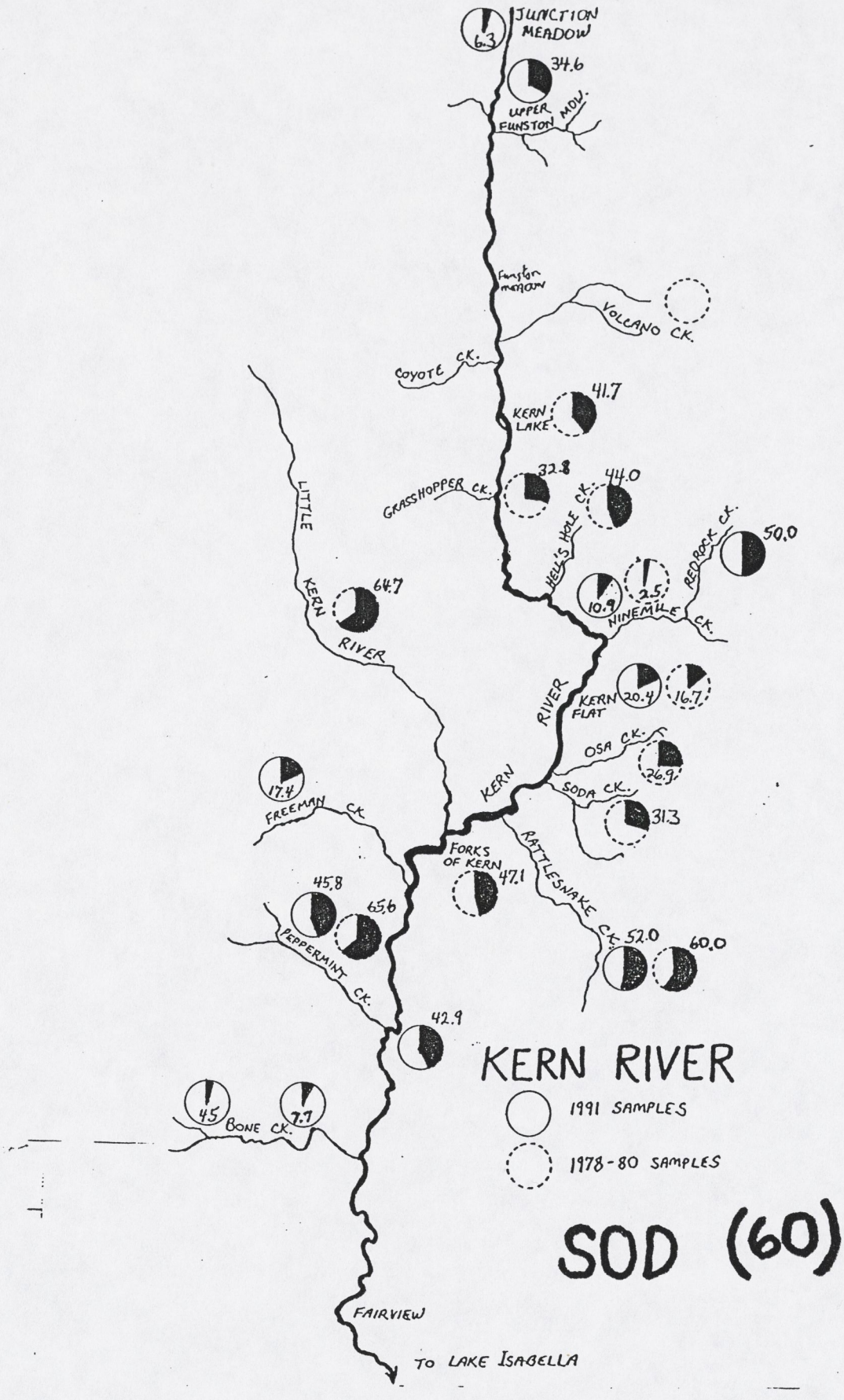
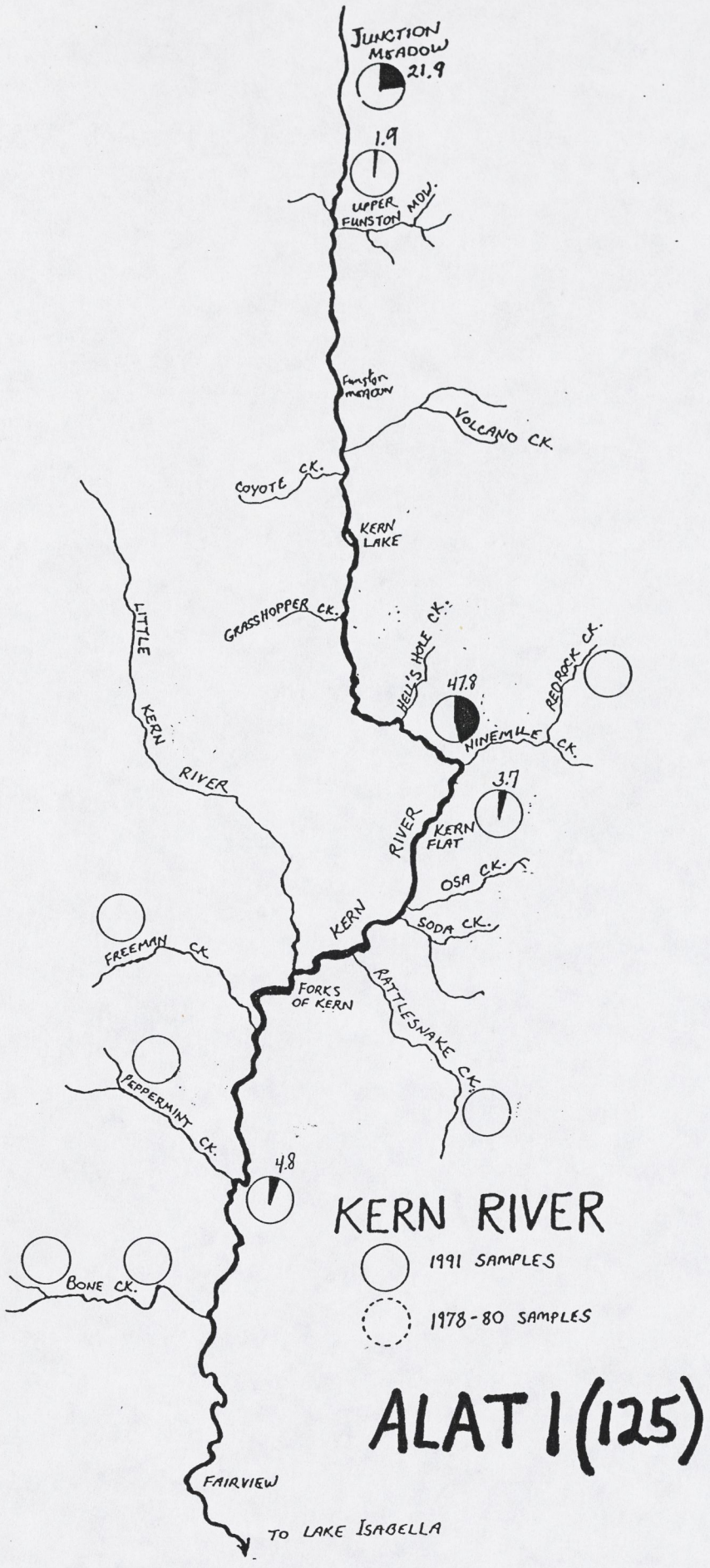


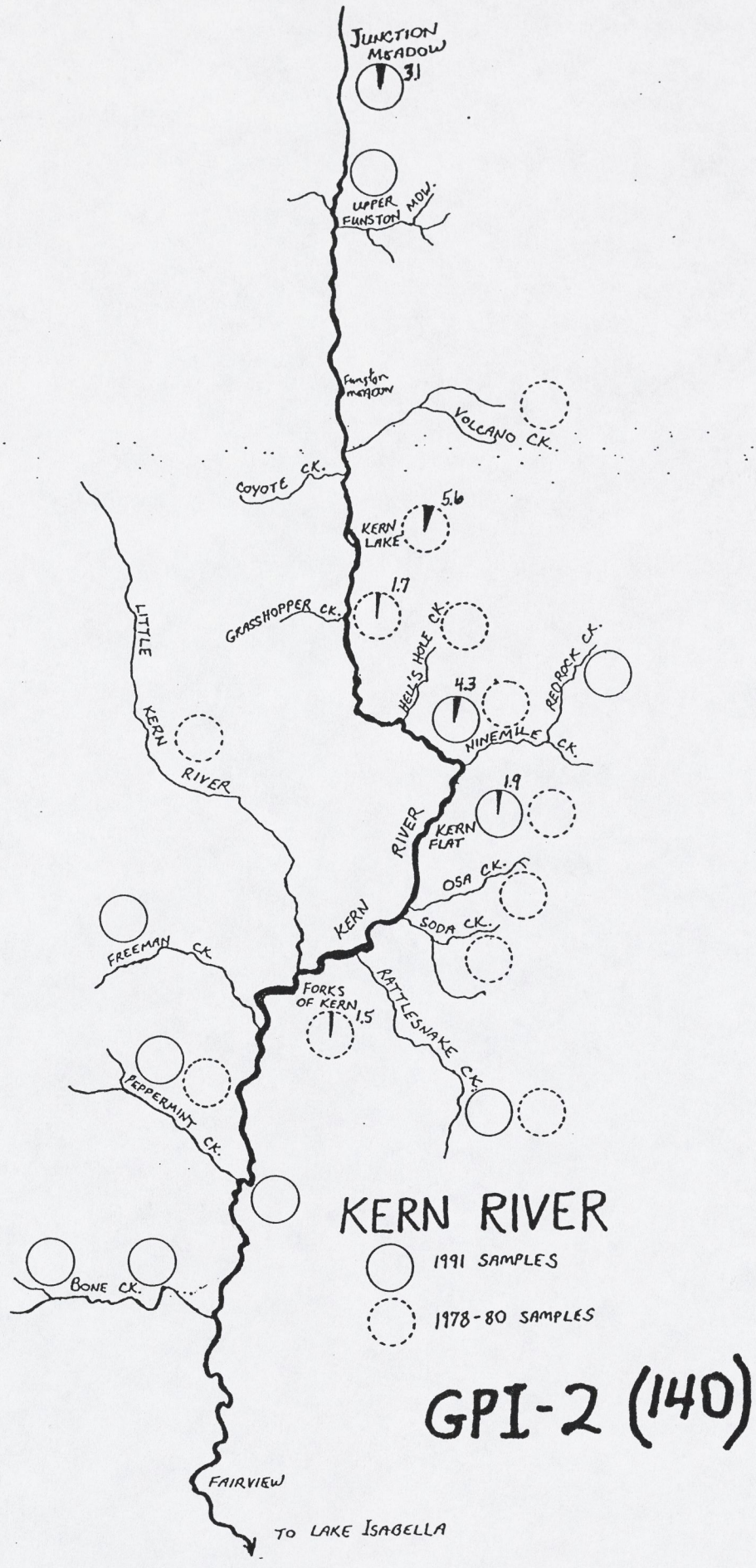
Figure 2. A dendrogram depicting the genetic relationship among samples collected from the Kern River system in 1991.

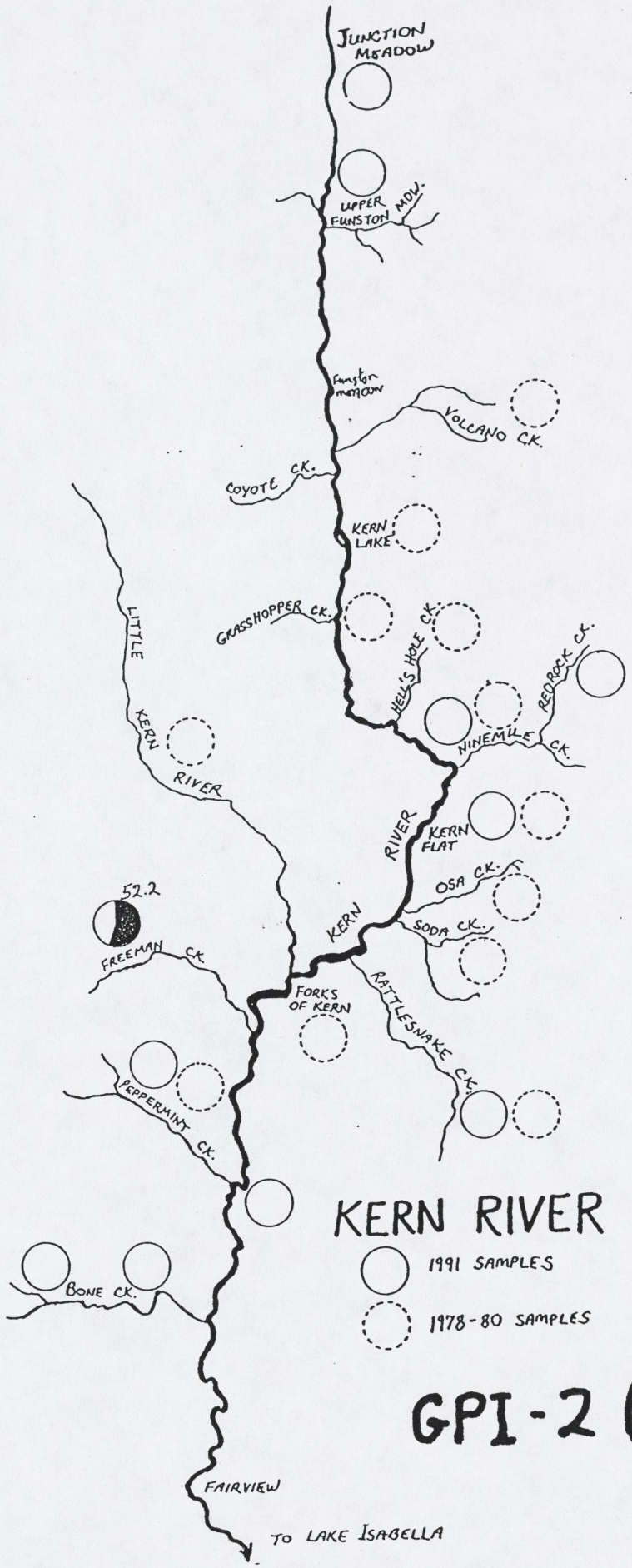
Appendix A

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

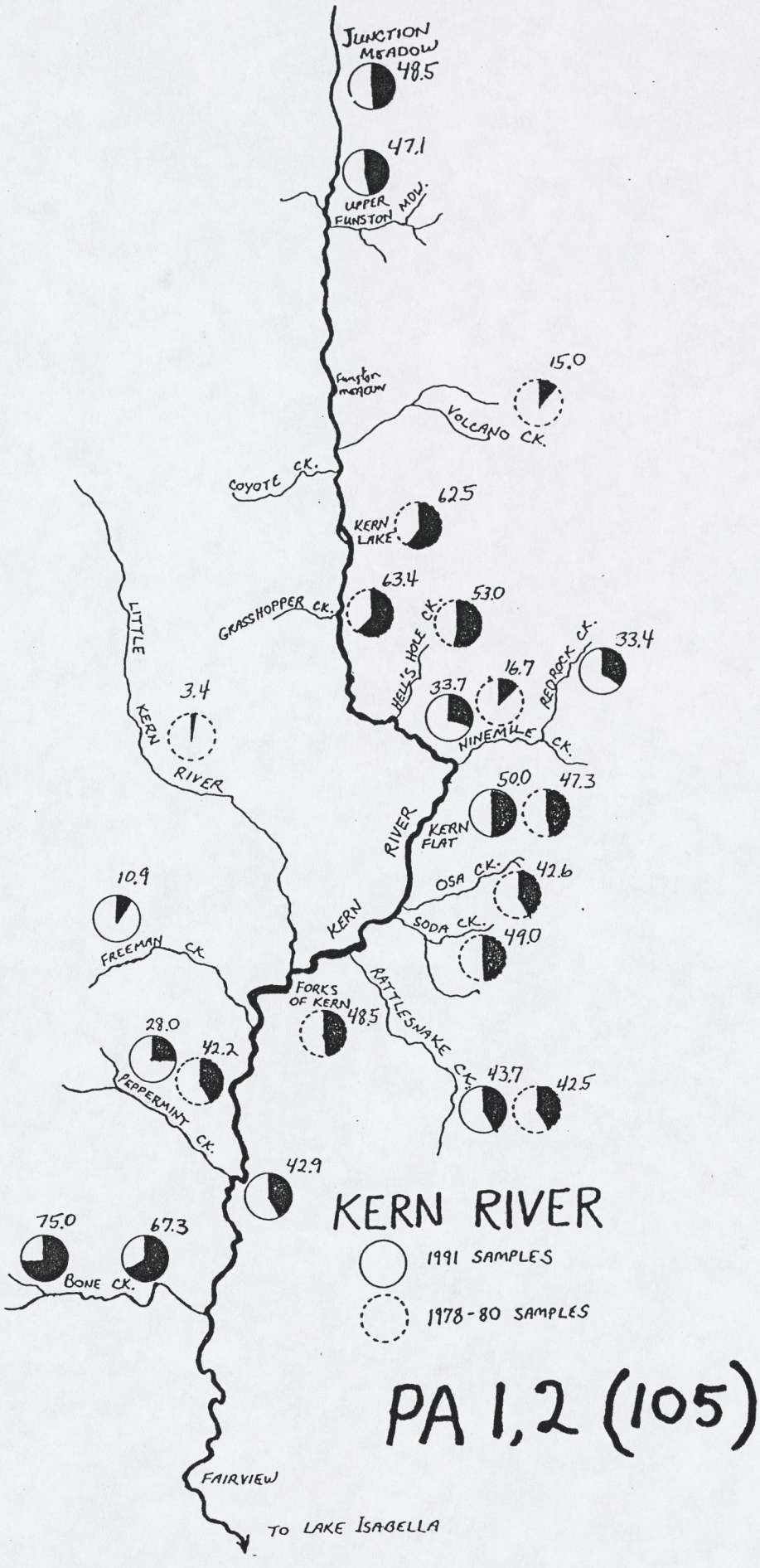

JUNCTION MEADOW
6.3
34.6
UPPER FUNSTON MDW.
Funston meadow
VOLCANO CK.
COYOTE CK.
41.7
KERN LAKE
32.8
GRASSHOPPER CK.
44.0
HELL'S HOLE CK.
50.0
REDROCK CK.
10.9
2.5
NINEMILE CK.
LITTLE KERN RIVER
64.7
KERN RIVER
KERN FLAT
20.4
16.7
OSA CK.
26.9
SODA CK.
31.3
17.4
FREEMAN CK.
KERN
FORK'S OF KERN
47.1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

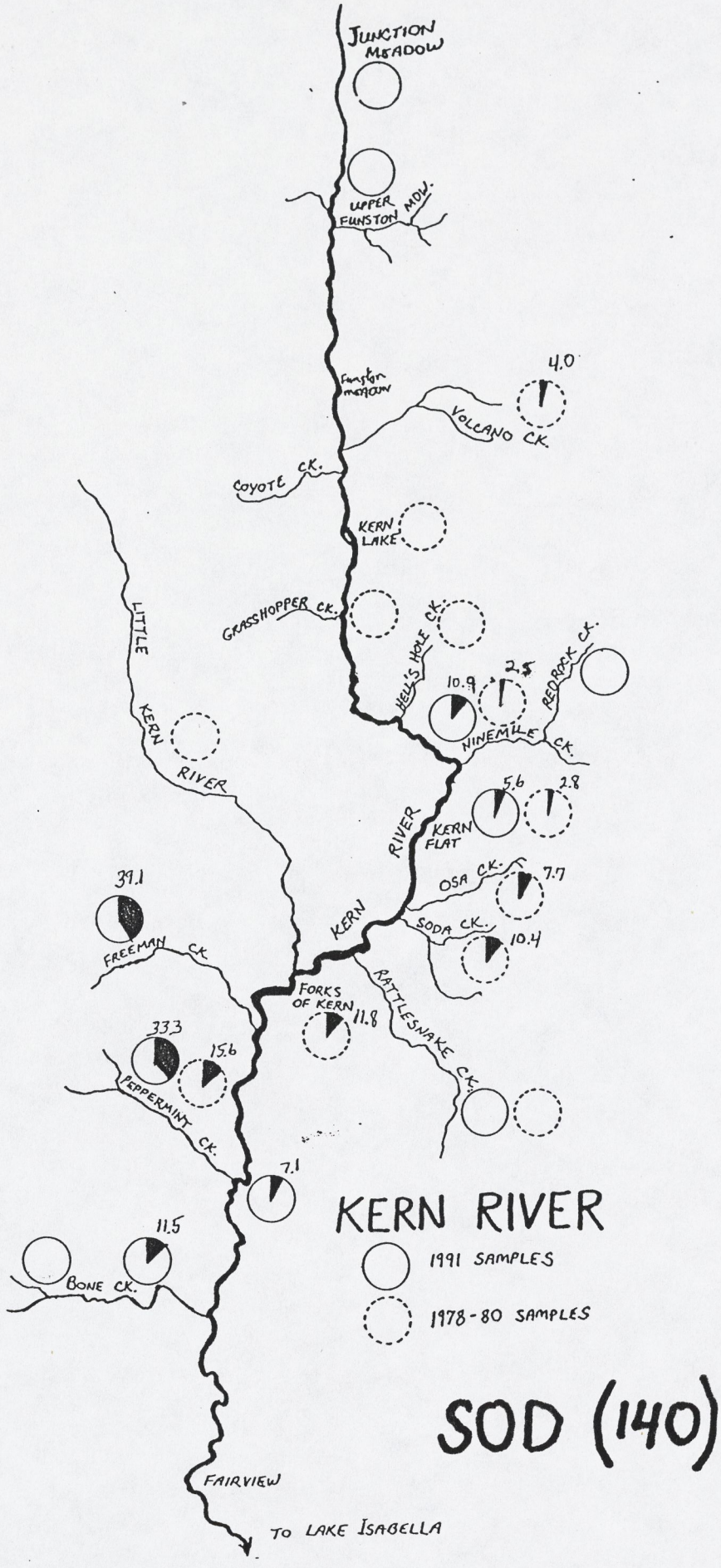

JUNCTION MEADOW
21.9
1.9
UPPER FUNSTON MDW.
Funston meadow
VOLCANO CK.
COYOTE CK.
KERN LAKE
GRASSHOPPER CK.
HELL'S HOLE CK.
REDROCK CK.
47.8
NINEMILE CK.
LITTLE KERN RIVER
3.7
KERN FLAT
KERN RIVER
OSA CK.
SODA CK.
FREEMAN CK.
FORKS OF KERN
RATTLESNAKE CK.
PEPPERMINT CK.
4.8
BONE CK.
KERN RIVER
1991 SAMPLES
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
FORKS OF KERN
1.5
RATTLESNAKE CK.
PEPPERMINT CK.
BONE CK.
FAIRVIEW
TO LAKE ISABELLA
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES
GPI-2 (140)

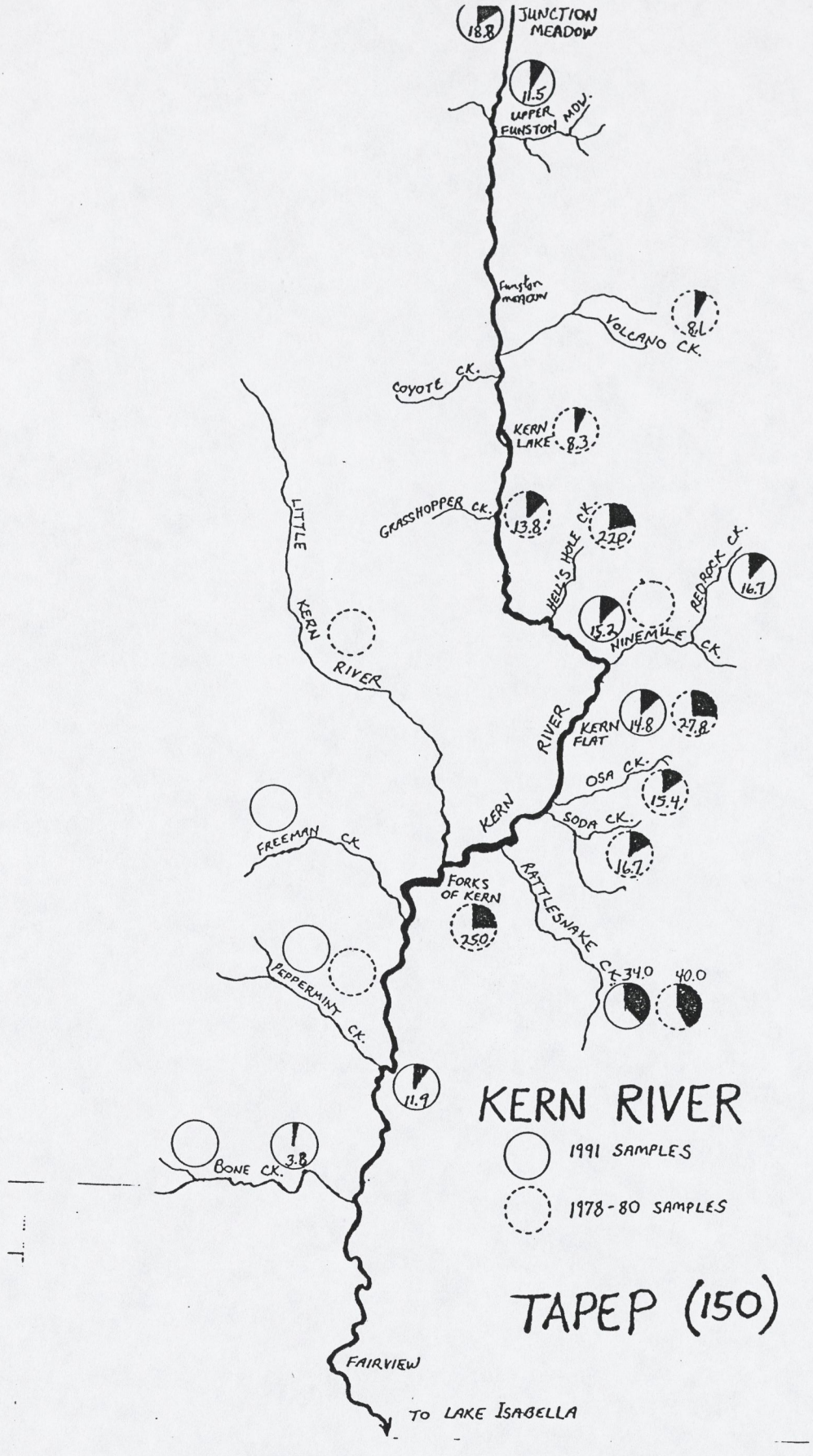

JUNCTION MEADOW
UPPER FUNSTON MDW.
Funston meadow
VOLCANO CK.
COYOTE CK.
KERN LAKE
GRASSHOPPER CK.
HELL'S HOLE CK.
REDROCK CK.
NINEMILE CK.
LITTLE KERN RIVER
KERN RIVER
KERN FLAT
OSA CK.
SODA CK.
52.2
FREEMAN CK
KERN
FORKS OF KERN
RATTLESNAKE CK.
PEPPERMINT CK.
BONE CK.
FAIRVIEW
TO LAKE ISABELLA
KERN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

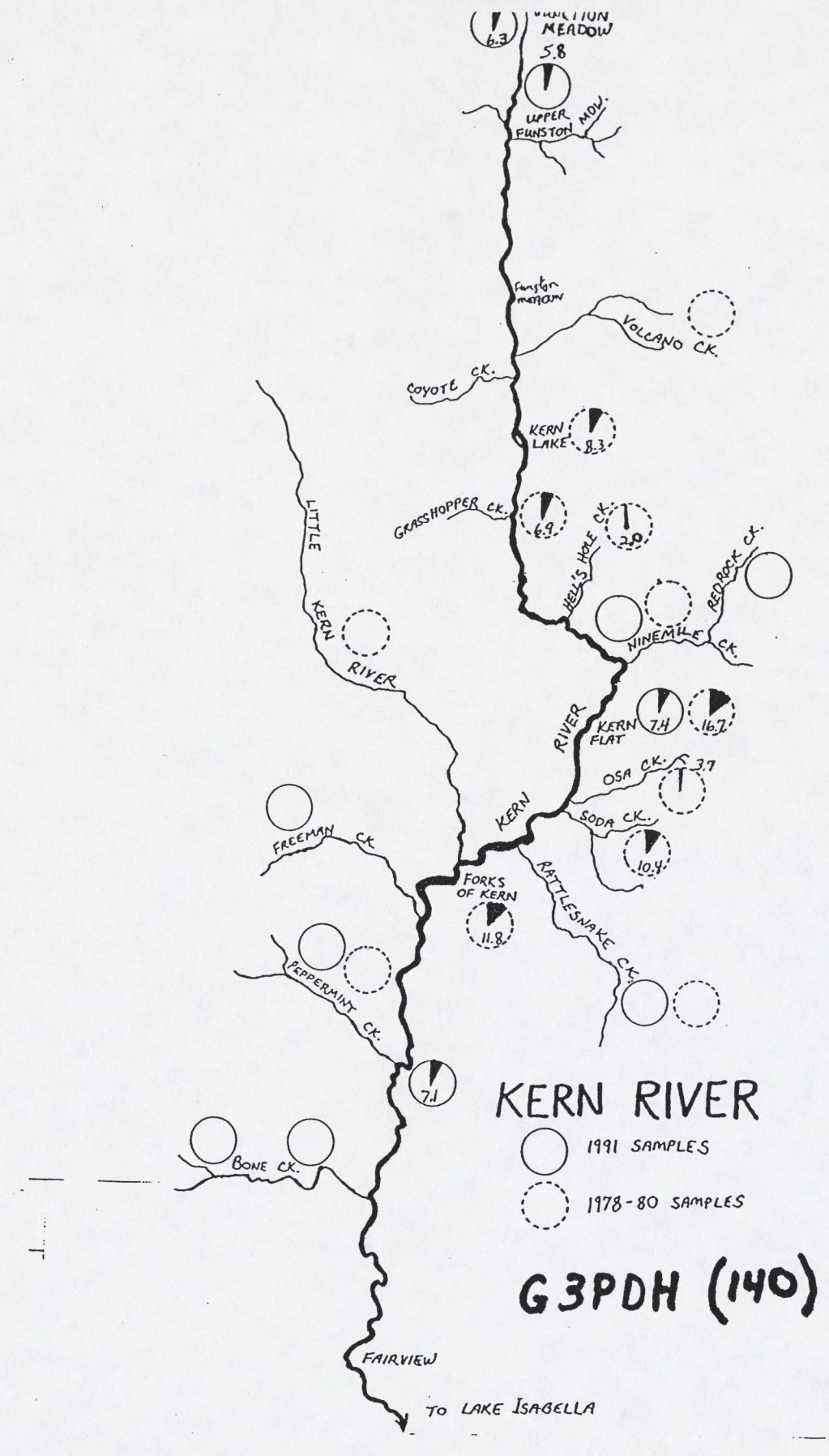
GPI-2 (46)

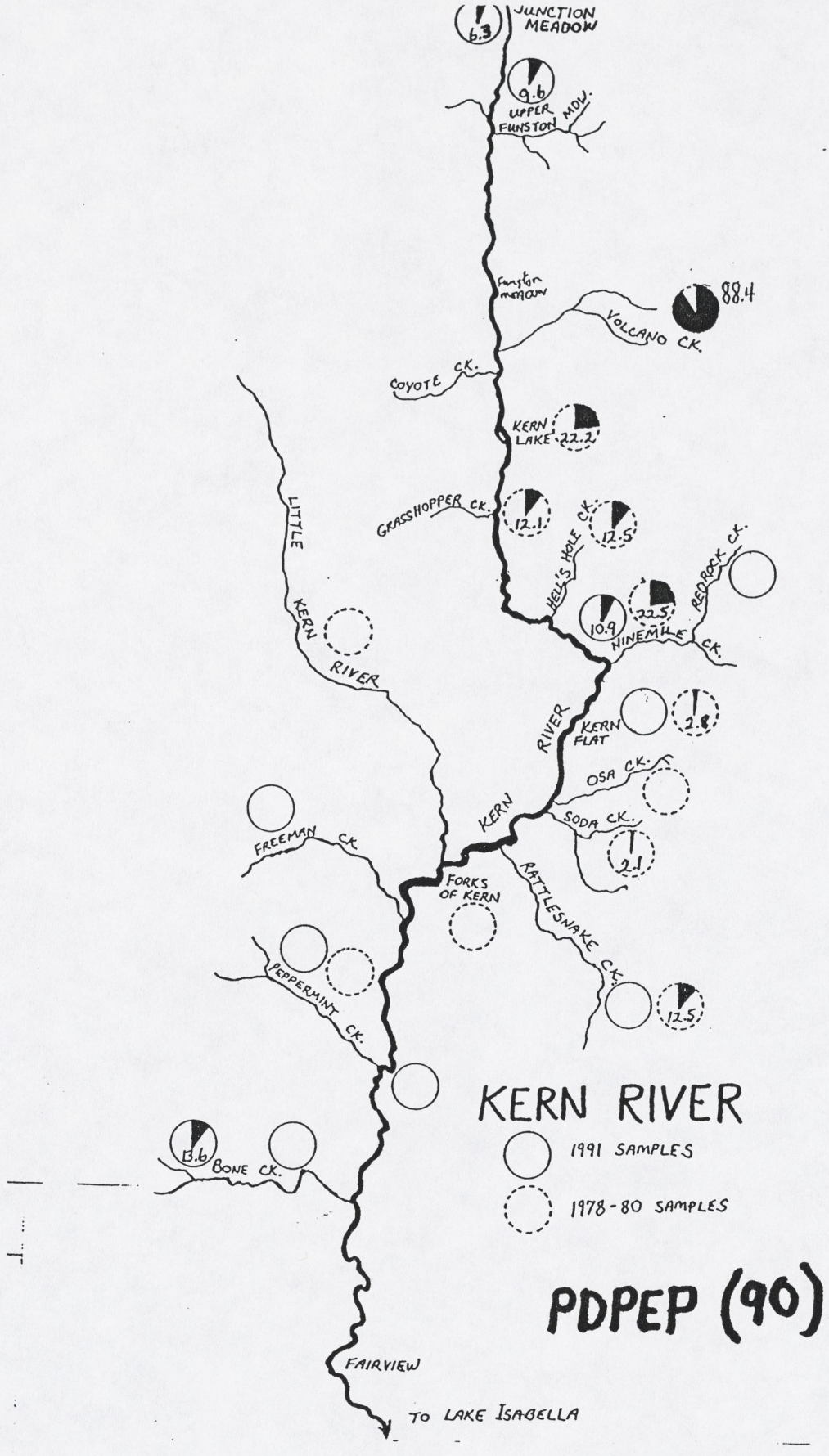

JUNCTION MEADOW
48.5
47.1
UPPER FUNSTON MDW.
Funston meadow
15.0
VOLCANO CK.
COYOTE CK.
62.5
KERN LAKE
63.4
GRASSHOPPER CK.
53.0
HELL'S HOLE CK.
33.4
REDROCK CK.
16.7
33.7
NINEMILE CK.
LITTLE KERN RIVER
3.4
KERN RIVER
50.0
47.3
KERN FLAT
OSA CK.
42.6
SODA CK.
49.0
10.9
FREEMAN CK.
FORKS OF KERN
48.5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

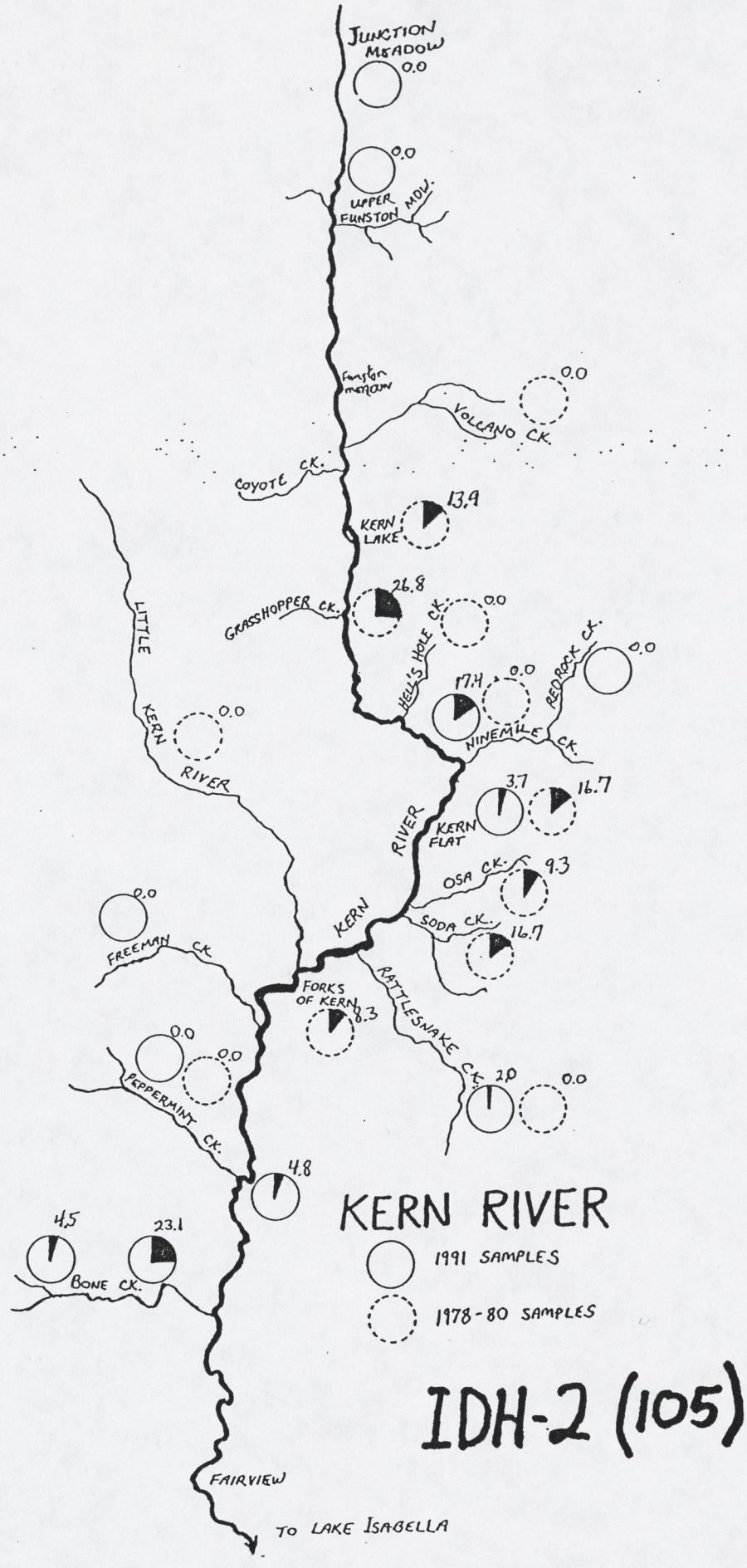

JUNCTION MEADOW
UPPER FUNSTON MDW.
Funston meadow
4.0
VOLCANO CK.
COYOTE CK.
KERN LAKE
GRASSHOPPER CK.
HELL'S HOLE CK.
10.9
2.5
REDROCK CK.
NINEMILE CK.
LITTLE KERN RIVER
KERN RIVER
5.6
2.8
KERN FLAT
OSA CK.
7.7
SODA CK.
10.4
39.1
FREEMAN CK.
KERN
RATTLESNAKE CK.
FORKS OF KERN
11.8
33.3
15.6
PEPPERMINT CK.
7.1
11.5
BONE CK.
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES
FAIRVIEW
TO LAKE ISABELLA

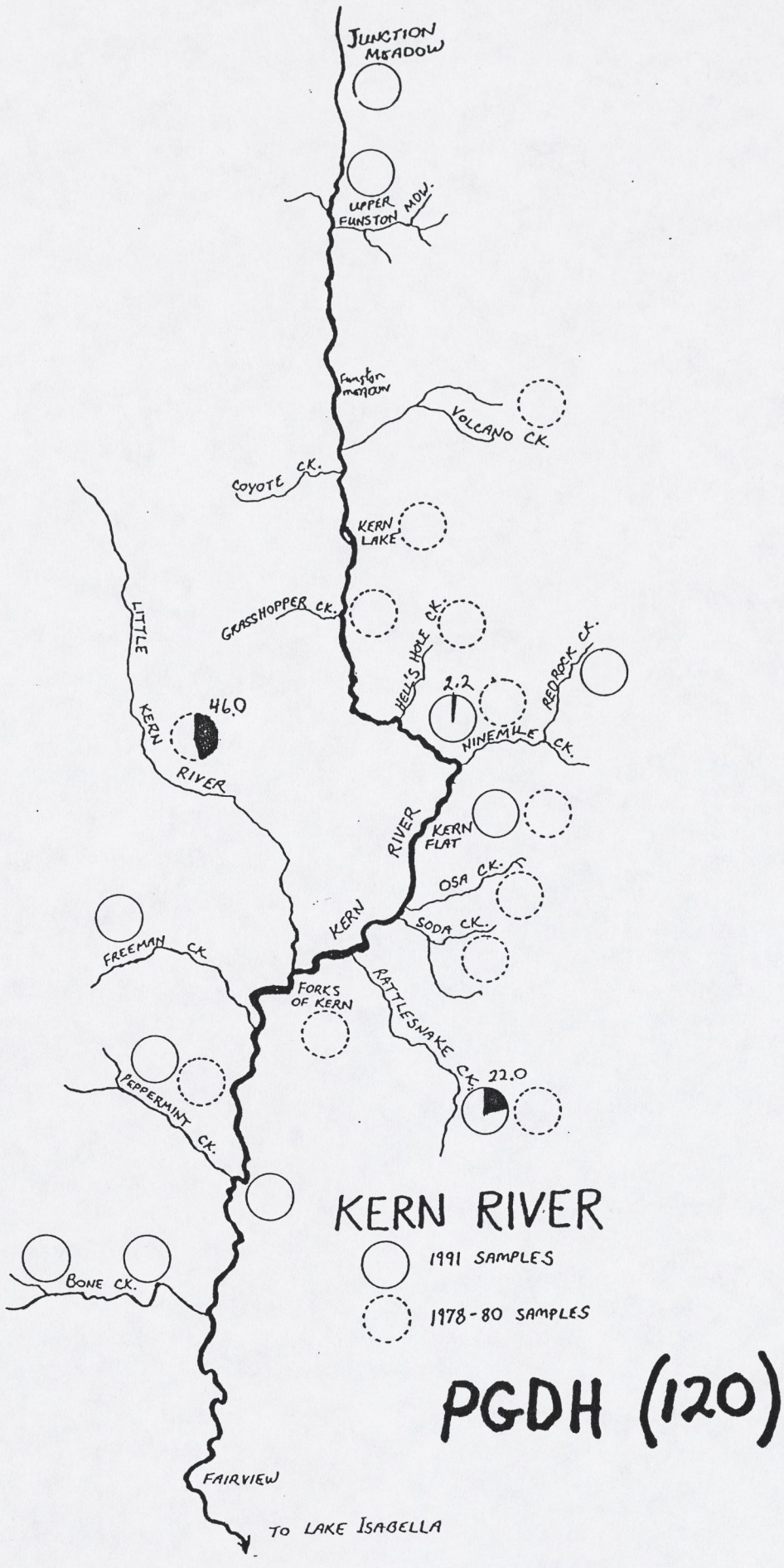
SOD (140)


18.8
JUNCTION MEADOW
11.5
UPPER FUNSTON MDW.
Funston meadow
VOLCANO CK.
8.6
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


6.3
MEADOW
5.8
UPPER FUNSTON MDW.
Funston meadow
VOLCANO CK.
COYOTE CK.
KERN LAKE
8.3
GRASSHOPPER CK.
6.9
HELL'S HOLE CK.
2.9
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
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)


6.3
JUNCTION MEADOW
9.6
UPPER FUNSTON
MDW.
Funston meadow
88.4
VOLCANO CK.
COYOTE CK.
KERN LAKE
22.2
GRASSHOPPER CK.
12.1
HELL'S HOLE CK.
12.5
REDROCK CK.
10.9
22.5
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


JUNCTION MEADOW
0.0
0.0
UPPER FUNSTON MDW.
Fungus meadow
0.0
VOLCANO CK.
COYOTE CK.
13.9
KERN LAKE
26.8
GRASSHOPPER CK.
0.0
HELL'S HOLE CK.
17.4
0.0
NINEMILE CK.
REDROCK CK.
0.0
LITTLE KERN RIVER
0.0
3.7
16.7
KERN FLAT
KERN RIVER
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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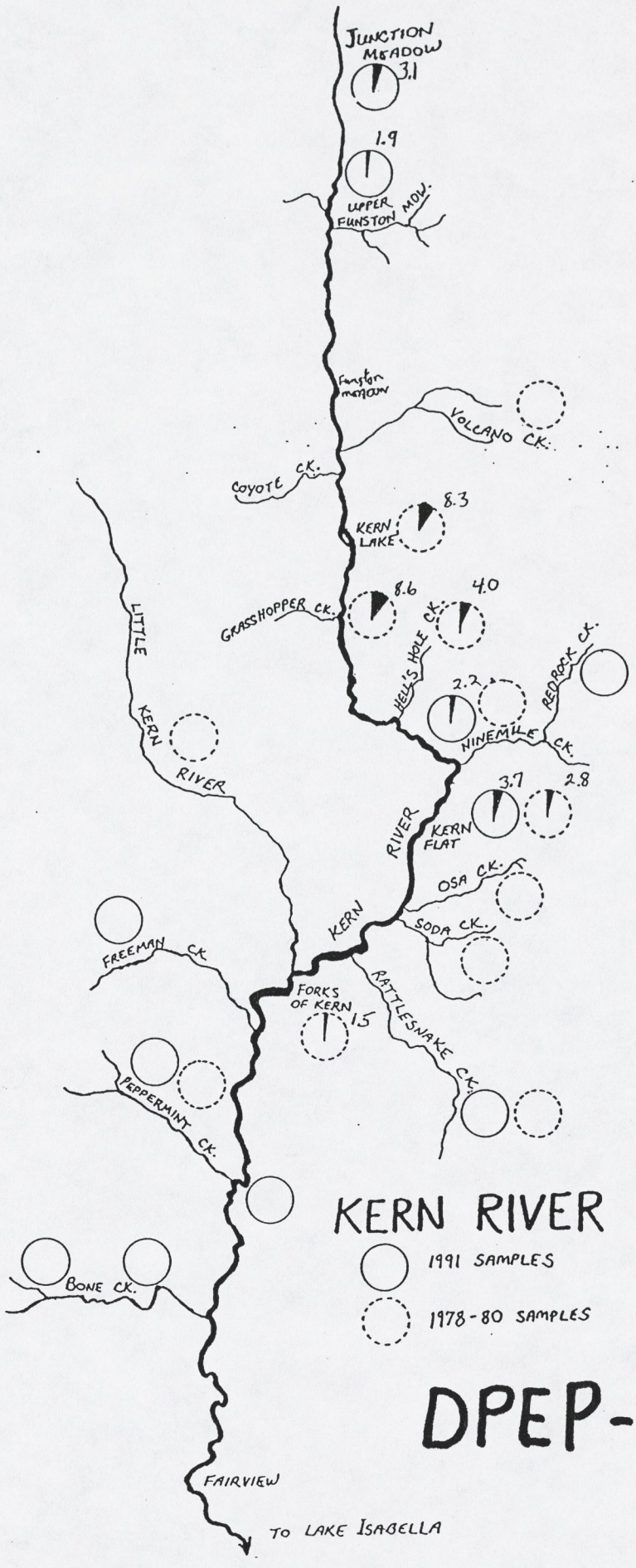
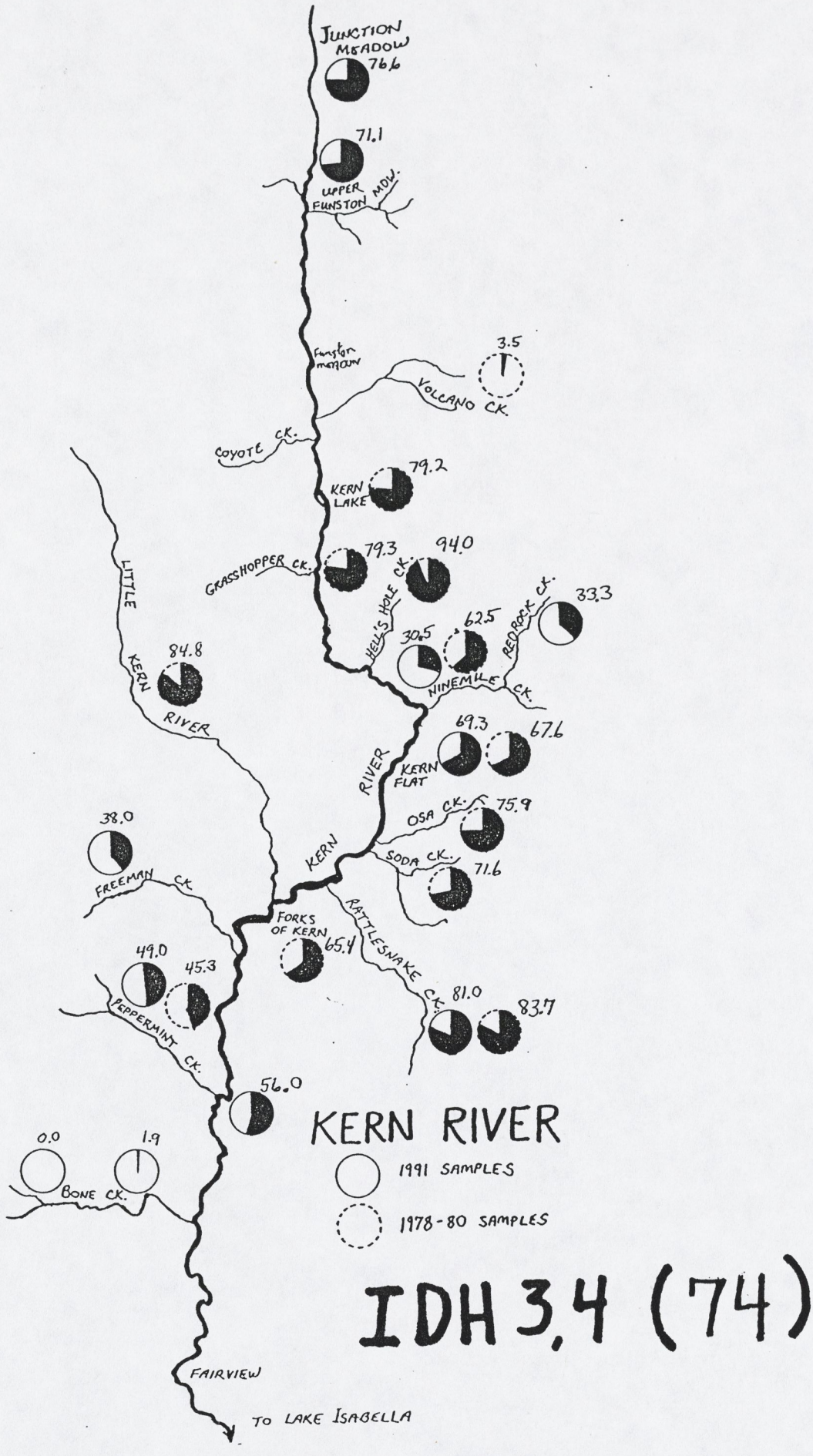
UPPER FUNSTON MDW.
VOLCANO CK.
COYOTE CK.
KERN LAKE
GRASSHOPPER CK.
HELL'S HOLE CK.
REDROCK CK.
2.2
NINEMILE CK.
LITTLE KERN RIVER
46.0
KERN RIVER
KERN FLAT
OSA CK.
SODA CK.
FREEMAN CK
FORKS OF KERN
RATTLESNAKE CK.
22.0
PEPPERMINT CK.
BONE CK.
FAIRVIEW
TO LAKE ISABELLA
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES

PGDH (120)


JUNCTION MEADOW
3.1
1.9
UPPER FUNSTON MDW.
Funston meadow
VOLCANO CK.
COYOTE CK.
8.3
KERN LAKE
8.6
4.0
GRASSHOPPER CK.
HELL'S HOLE CK.
LITTLE KERN RIVER
2.2
REDROCK CK.
NINEMILE CK.
3.7
2.8
KERN FLAT
KERN RIVER
OSA CK.
SODA CK.
FREEMAN CK
FORKS OF KERN
1.5
RATTLESNAKE CK.
PEPPERMINT CK.
BONE CK.
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES
FAIRVIEW
TO LAKE ISABELLA

DPEP-1 (III)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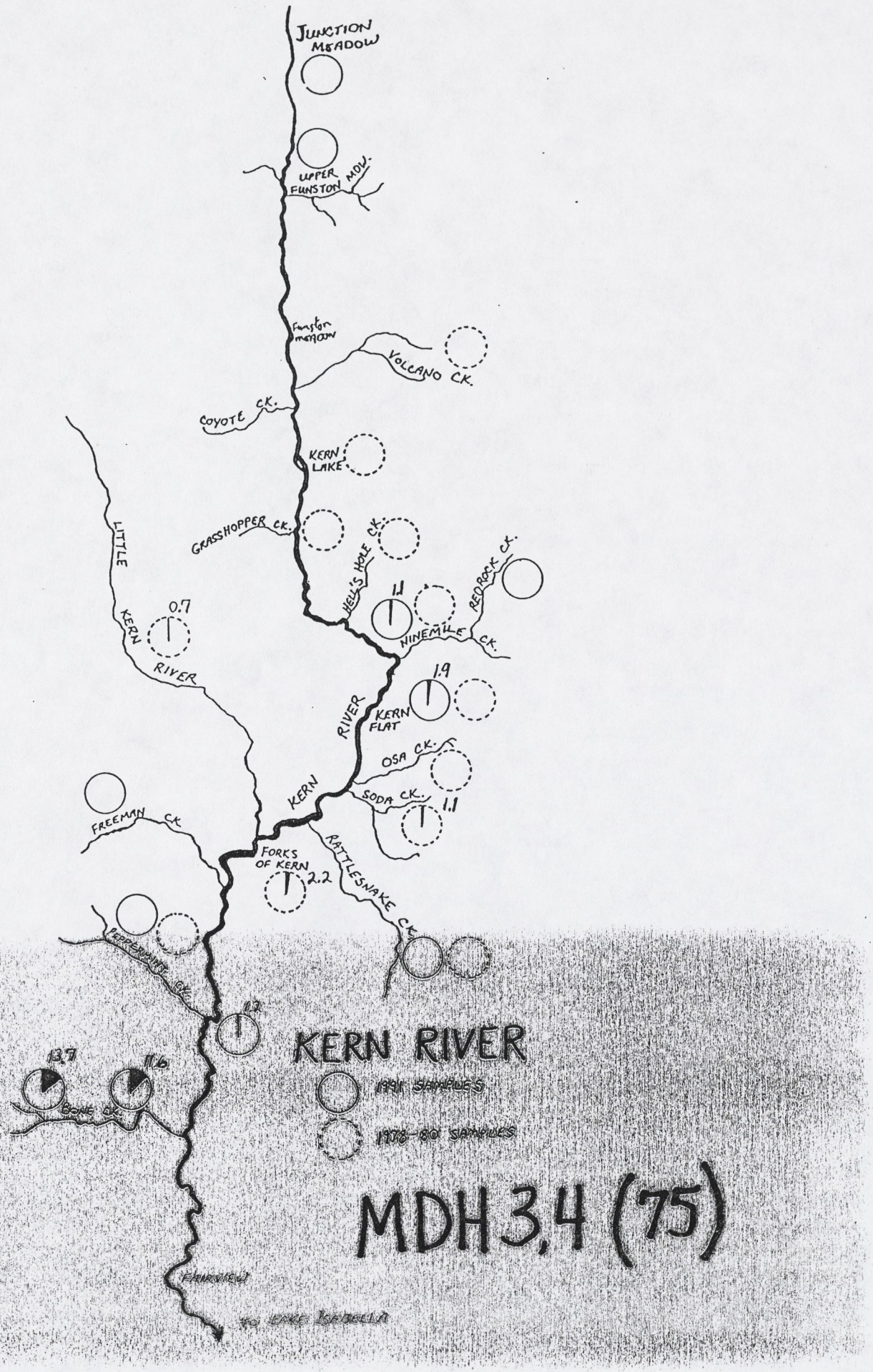
76.6
71.1
UPPER FUNSTON MDW.
Funston meadow
3.5
VOLCANO CK.
COYOTE CK.
79.2
KERN LAKE
79.3
94.0
GRASSHOPPER CK.
KELLY'S HOLE CK.
REDROCK CK.
33.3
62.5
30.5
NINEMILE CK.
LITTLE KERN RIVER
84.8
KERN RIVER
69.3
67.6
KERN FLAT
OSA CK.
75.9
38.0
SODA CK.
71.6
FREEMAN CK.
KERN
FORKS OF KERN
65.4
RATTLESNAKE CK.
49.0
45.3
81.0
83.7
PEPPERMINT CK.
56.0
KERN RIVER
0.0
1.9
1991 SAMPLES
BONE CK.
1978-80 SAMPLES
IDH 3,4 (74)
FAIRVIEW
TO LAKE ISABELLA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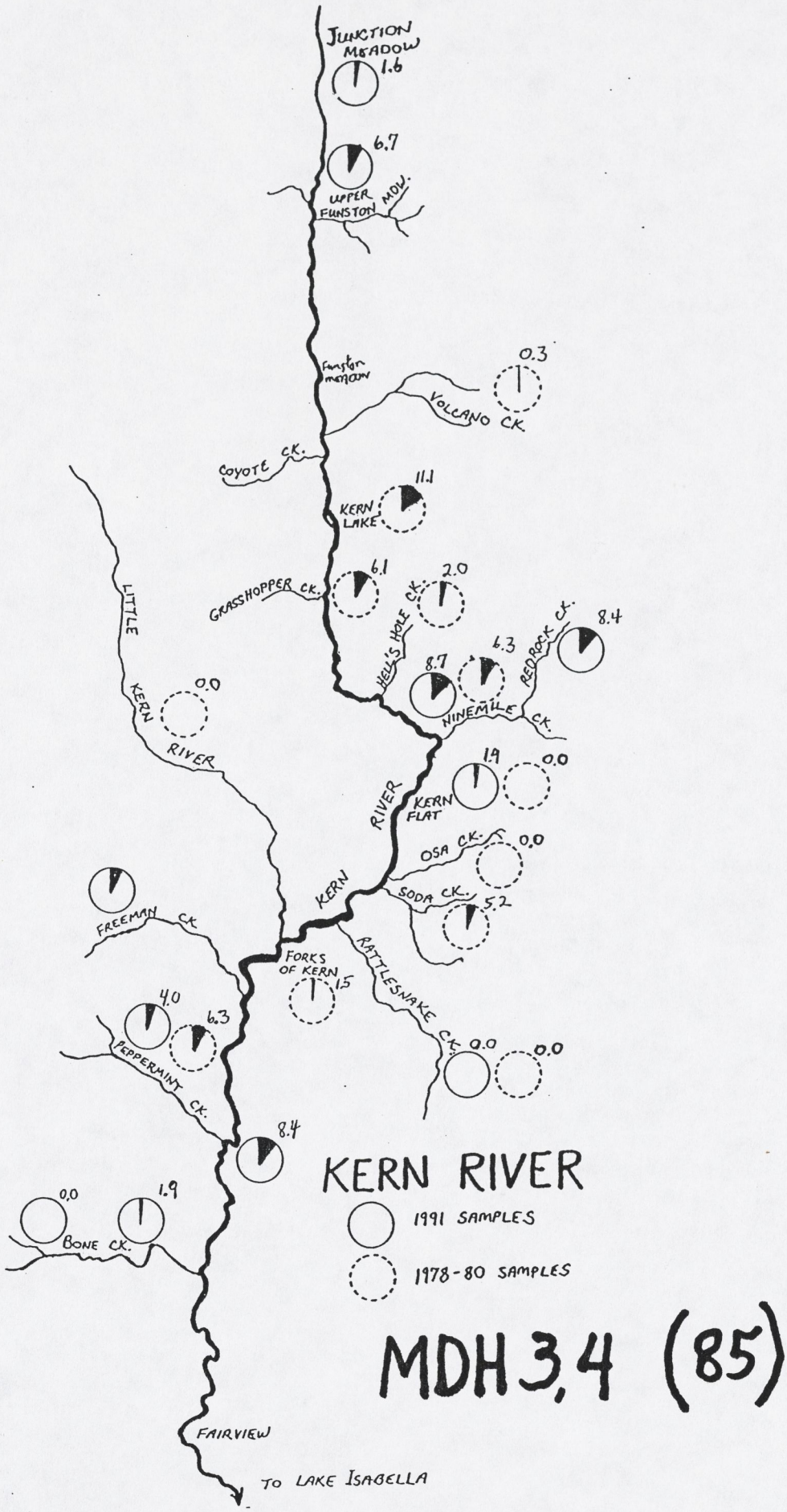
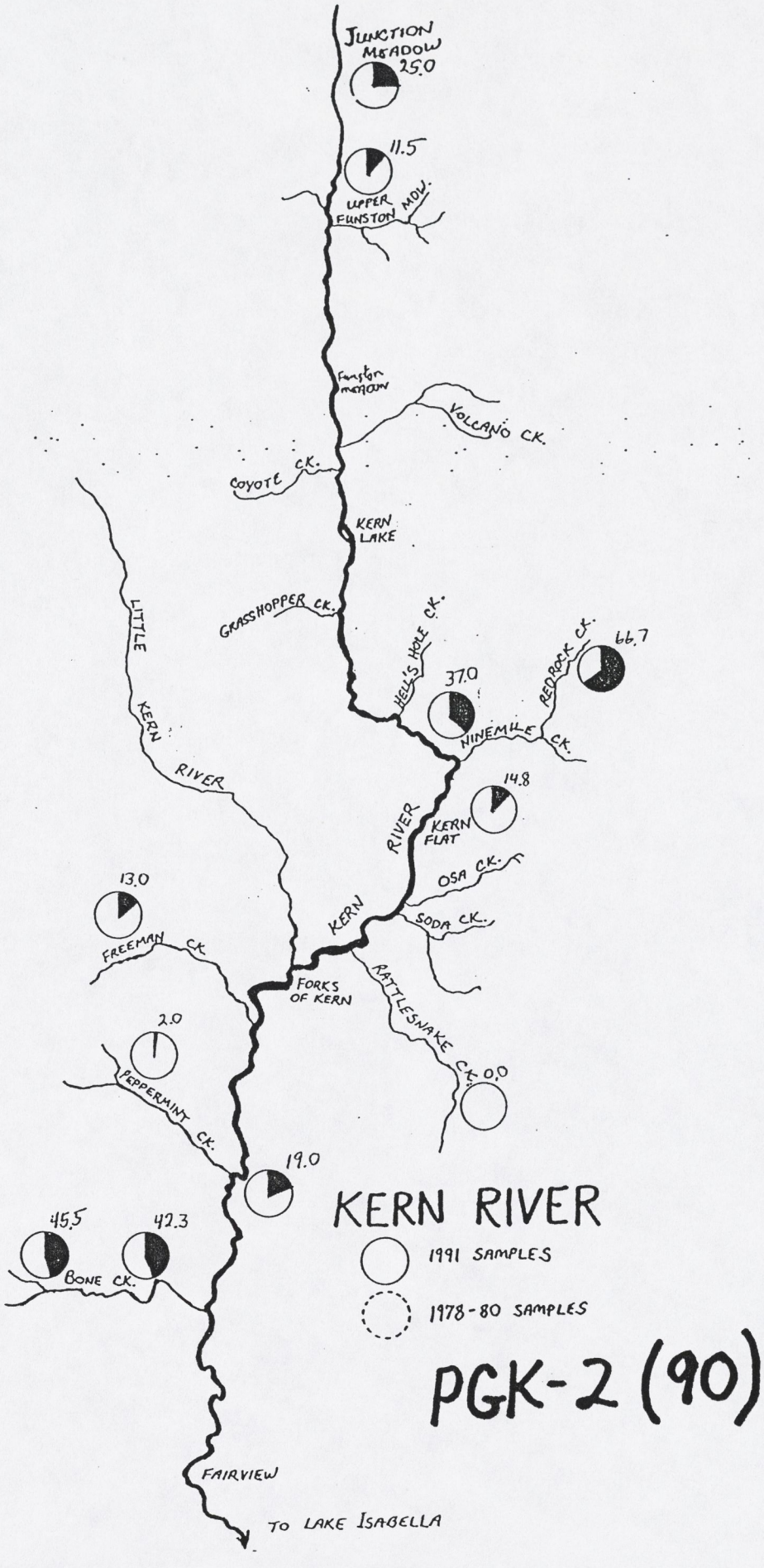
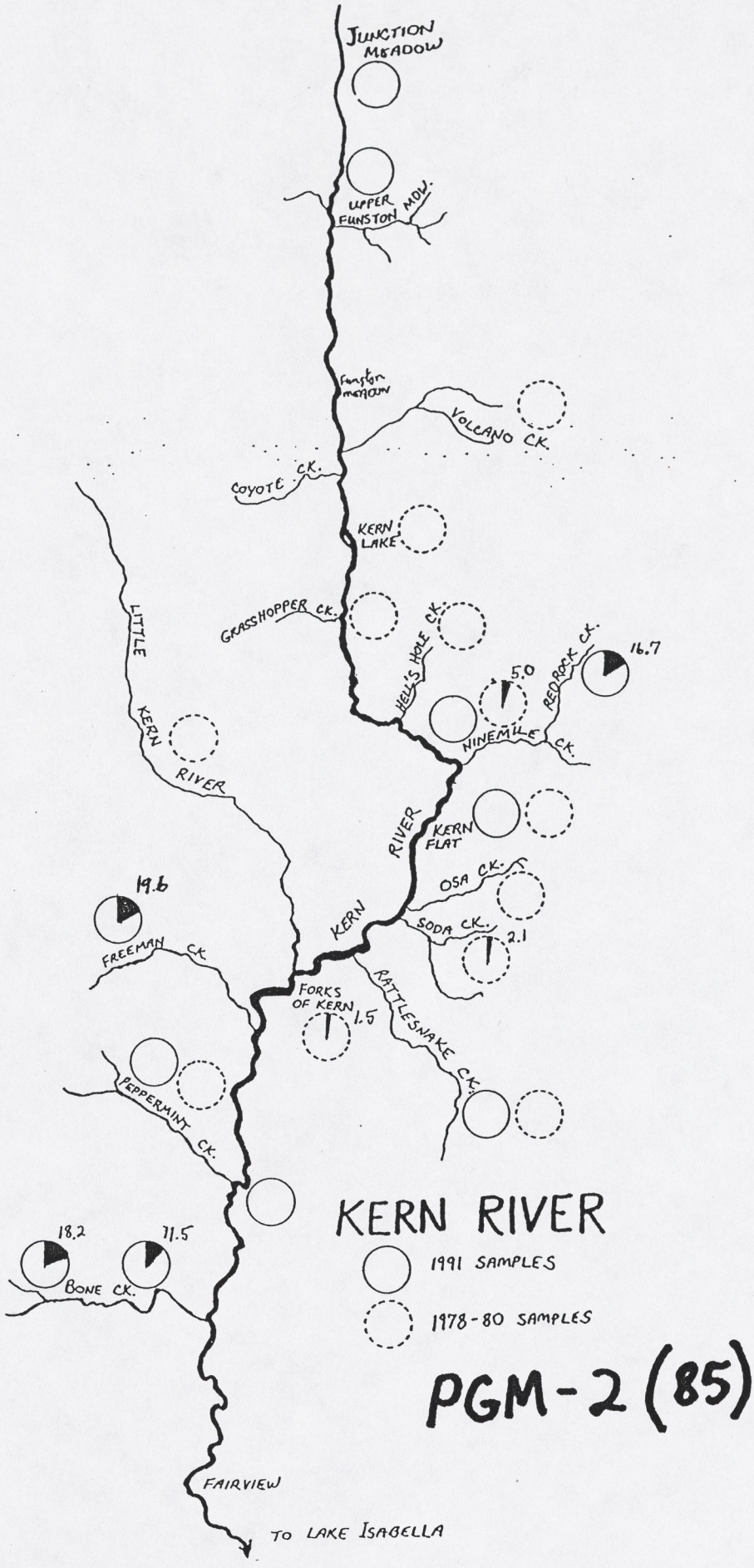
UPPER FUNSTON MDW.
Funston meadow
VOLCANO CK.
COYOTE CK.
KERN LAKE
GRASSHOPPER CK.
HELL'S HOLE CK.
REDROCK CK.
NINEMILE CK.
LITTLE KERN RIVER
0.7
1.1
1.9
KERN RIVER
KERN FLAT
OSA CK.
SODA CK.
1.1
FREEMAN CK.
KERN
FORKS OF KERN
2.2
RATTLESNAKE CK.
PEPPERMINT CK.
1.7
13.7
11.6
BOAT CK.
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES
MDH 3,4 (75)
FAIRVIEW
TO LAKE ISABELLA


JUNCTION MEADOW
1.6
6.7
UPPER FUNSTON MDW.
Funston meadow
0.3
VOLCANO CK.
COYOTE CK.
11.1
KERN LAKE
6.1
GRASSHOPPER CK.
2.0
HELL'S HOLE CK.
8.4
REDROCK CK.
8.7
6.3
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


JUNCTION MEADOW
25.0
11.5
UPPER FUNSTON MDW.
Funston meadow
VOLCANO CK.
COYOTE CK.
KERN LAKE
GRASSHOPPER CK.
LITTLE KERN RIVER
HELL'S HOLE CK.
37.0
REDROCK CK.
66.7
NINEMILE CK.
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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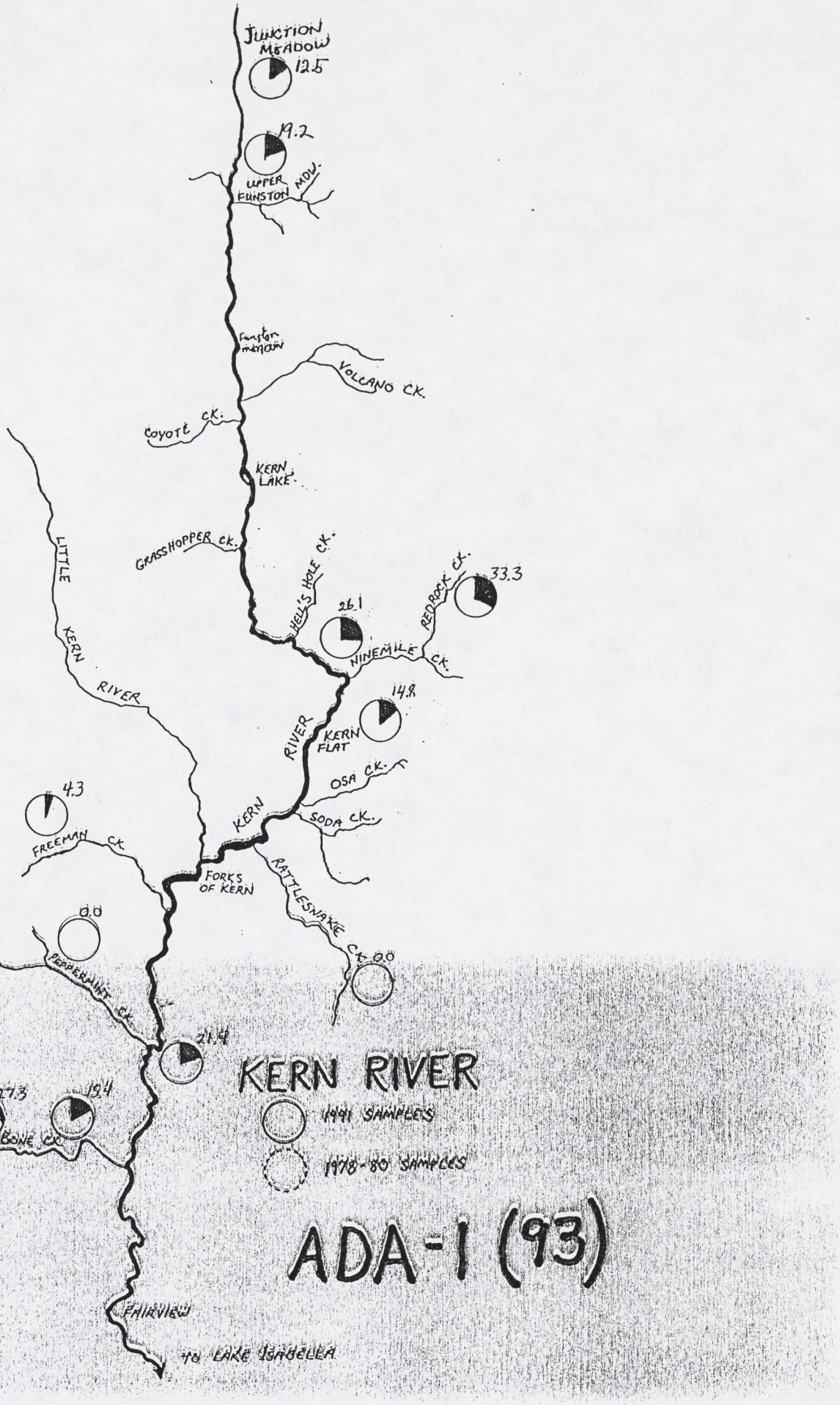
Funston meadow
VOLCANO CK.
COYOTE CK.
KERN LAKE
GRASSHOPPER CK.
HELL'S HOLE CK.
REDROCK CK.
16.7
5.0
NINEMILE CK.
LITTLE KERN RIVER
KERN RIVER
KERN FLAT
OSA CK.
SODA CK.
2.1
19.6
FREEMAN CK.
KERN
FORKS OF KERN
1.5
RATTLESNAKE CK.
PEPPERMINT CK.
18.2
11.5
BONE CK.
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES
PGM-2 (85)
FAIRVIEW
TO LAKE ISABELLA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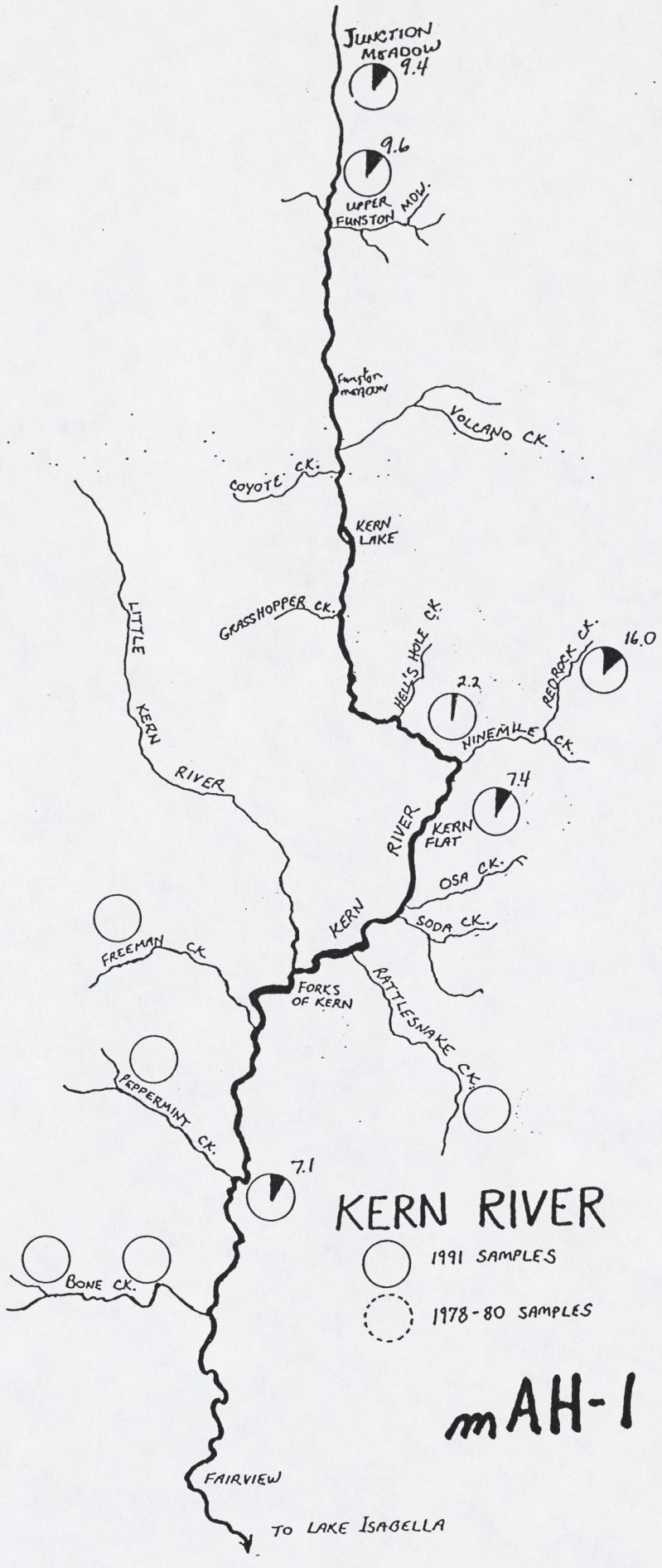
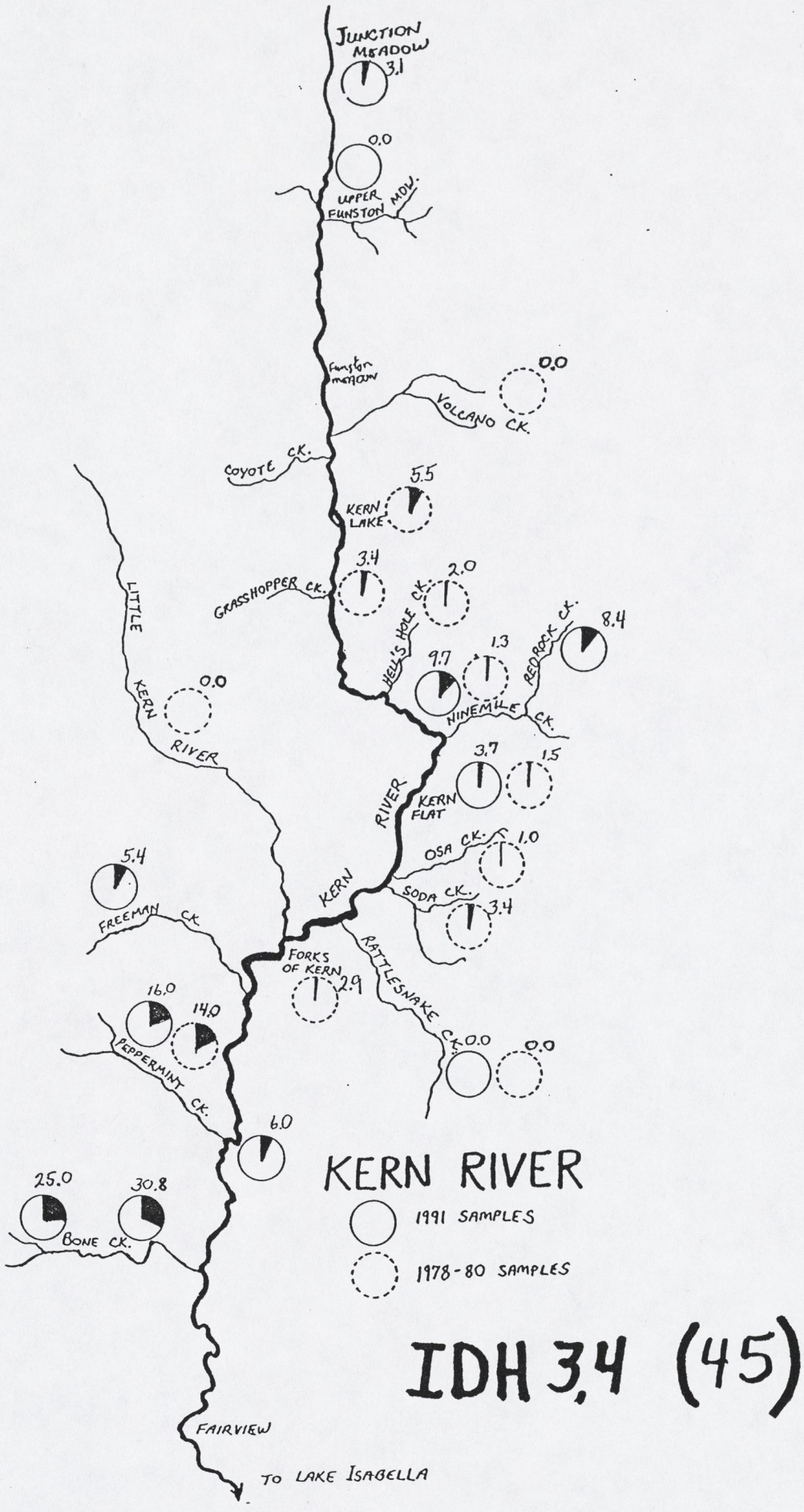
12.5
19.2
UPPER FUNSTON MDW.
Funston meadow
VOLCANO CK.
COYOTE CK.
KERN LAKE
GRASSHOPPER CK.
LITTLE KERN RIVER
HELL'S HOLE CK.
26.1
NINEMILE CK.
REDROCK CK.
33.3
14.8
KERN FLAT
KERN RIVER
OSA CK.
SODA CK.
4.3
FREEMAN CK
FORKS OF KERN
RATTLESNAKE CK.
0.0
0.0
PEPPERMINT CK.
21.4
27.3
19.4
BONE CK.
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES
ADA-1 (93)
FAIRVIEW
TO LAKE ISABELLA


JUNCTION MEADOW 9.4
9.6
UPPER FUNSTON MDW.
Funston meadow
VOLCANO CK.
COYOTE CK.
KERN LAKE
GRASSHOPPER CK.
HELL'S HOLE CK.
2.2
REDROCK CK.
16.0
NINEMILE CK.
LITTLE KERN RIVER
KERN RIVER
7.4
KERN FLAT
OSA CK.
SODA CK.
KERN
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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0.0
UPPER FUNSTON MDW.
Funston meadow
0.0
VOLCANO CK.
COYOTE CK.
5.5
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
0.0
KERN RIVER
3.7
1.5
KERN FLAT
OSA CK.
1.0
SODA CK.
3.4
5.4
FREEMAN CK.
KERN
FORKS OF KERN
2.9
RATTLESNAKE CK.
0.0
0.0
16.0
14.0
PEPPERMINT CK.
6.0
25.0
30.8
BONE CK.
KERN RIVER
1991 SAMPLES
1978-80 SAMPLES
IDH 3,4 (45)
FAIRVIEW
TO LAKE ISABELLA

2-22-93

Robert Behnke
Dept of Fisheries and Wildlife Biology
Colorado State University
Fort Collins, CO 80512

Dear Mr. Behnke,

Members of the South Coast Chapter of Trout Unlimited have been aggressively campaigning for regulation and management changes on the Kern River. After being turned down two years ago by our Dept. of Fish and Game, we have been busy collecting the data they would require to consider and changes. Enclosed is some of the that data collected. CAl DFG was in charge of the genetics report, TU paid Dr. Terry Roelofs of Humbolt University to age scales we collected. Some of this data according to the DFG contradicts your autumn "Trout" article. They have taken the position that the Kern River gilberti, is wide spread and a healthy population. From my own personal observations I disagree with them.

— holds up in court.

Mr. Behnke, I was hoping you might possible take the time from you schedule to review these findings, and perhaps offer some guidance to my chapters efforts to better the Kern River Fishery. There is more genetic findings, from collection we did this last year, but we have not been able to obtain these from DFG. If your are interested I will send this as soon as it is available.

Sincerely,

Rodger Lowery
Fisheries and Conservation Chairman, South Coast TU

20222 Spruce Ave.
Santa Ana Hts. CA 92707
714-756-9367

N = 84 sample upper Kern River 84

77 scaler

I+ II+ = 91%

I+ - 52% 9'-10

II+ 39% —

III+ 8% — 12"

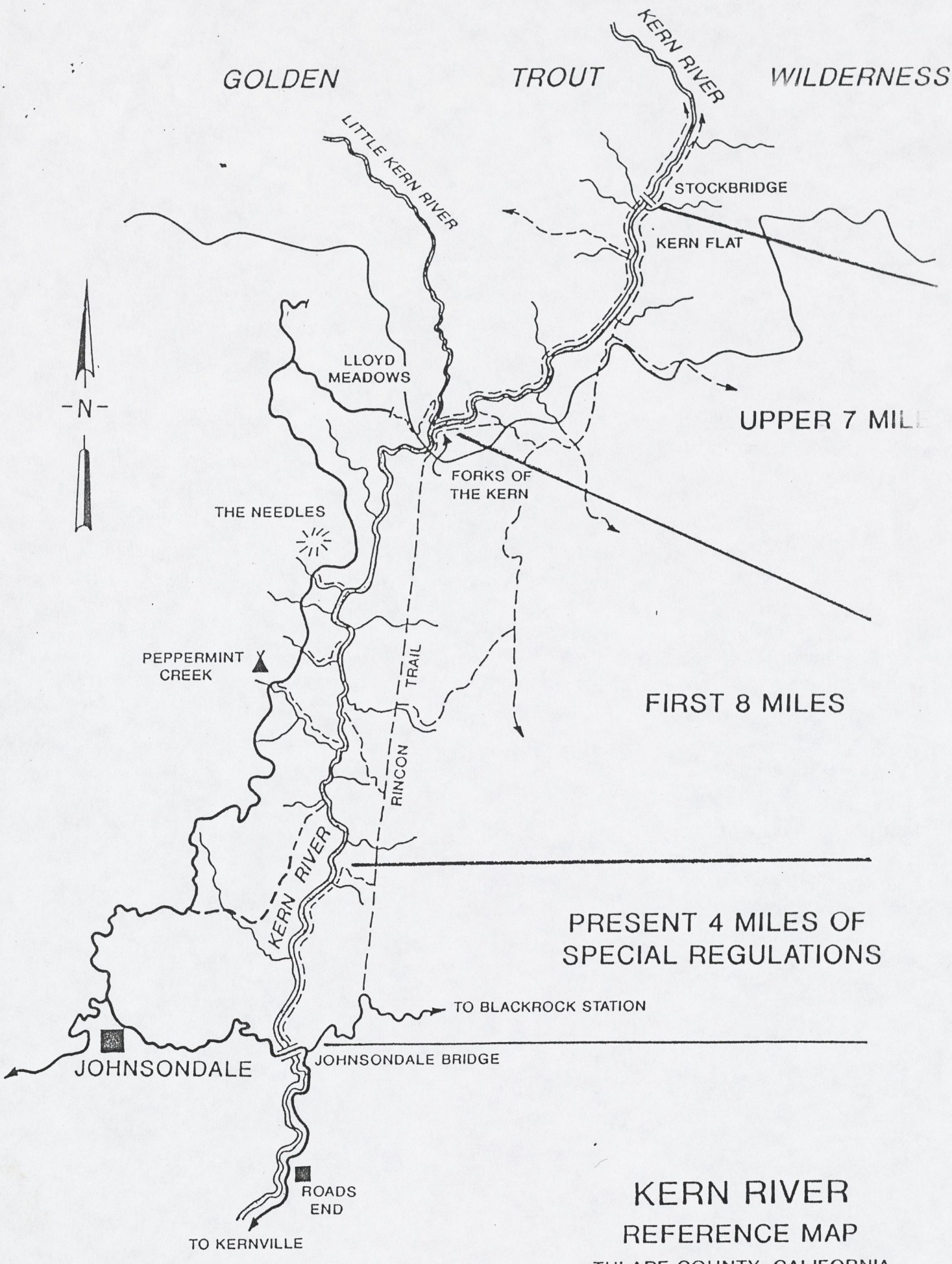
IV+ - 1% — 14"

high mortality
age class
- recruiting -

no-kill section

II+ 9-10' every

3rd v. good spawning 12-14"



KERN RIVER
REFERENCE MAP
TULARE COUNTY, CALIFORNIA

Review Draft

Scale Analysis of Upper Kern River Rainbow Trout (Oncorhynchus mykiss) Sampled by Trout Unlimited in the Fall of 1991.

Submitted to Dr. Terry Roelofs, Humboldt State University

by
Ross N. Taylor
on
November 24, 1992

INTRODUCTION

Information collected about the age and growth of fish is often useful in managing fisheries. Growth rates reveal the general condition of a fish population and this information can be valuable in comparing rates from previous years within a single watershed or comparing rates between similar watersheds. Growth rates can determine if a population of interest is relatively slow or fast growing, which in turn, can determine the types of management techniques to employ to improve the fishery. These techniques may include thinning or augmenting the population.

Age determinations in fish are based on the seasonal changes in growth rates that cause growth marks or checks to form on various hard body parts including scales, otoliths and fin rays or spines. The annual marks are referred to as year marks, annual marks, annual rings or annuali. In temperate areas there is usually a period of very slow or no growth during the winter and early spring. Generally the stronger the seasonal variations, the more obvious the annual marks. When reading scales the period of slow or no growth is revealed as a tightening of the circular rings (circuli) of the scale. In contrast, age determination of species from tropical regions is more difficult due either to steadier growth rates year round or growth checks due to environmental influences such as food supply, population densities, or periods of drought (Lowe, 1964 and Kanal, 1969 in Tesch, 1971).

The sampling of fish for age and growth analysis has some inherent problems one should be aware of. Habitat preferences and individual behavior may vary with fish size and time of year. Faster growing fish may be located in different places than slower growing members of the same population. Size variations within a year class can distort results if sampling technique is size selective. Unfortunately, most sampling techniques are size selective. For example, electroshocking may be biased toward larger fish because their larger size creates a stronger field of attraction to the electrical current. Conversely, a sampling regime by hook and line might be biased toward catching less wary (usually smaller and younger) individuals of a population.

This report analyses the age and growth of rainbow trout (Oncorhynchus mykiss) sampled from the upper Kern River during the fall of 1991. This report will hopefully aid Trout Unlimited and concerned biologists in better management of this population.

METHODS AND MATERIALS

Scale samples were collected from 84 rainbow trout for age determination by Trout Unlimited members on the upper Kern River "no kill" section between October 10, 1991 and December 12,1991. The samples were obtained from the area between the dorsal fin and lateral line of each fish with a pocket knife. Lengths of fish were measured to the nearest quarter inch.

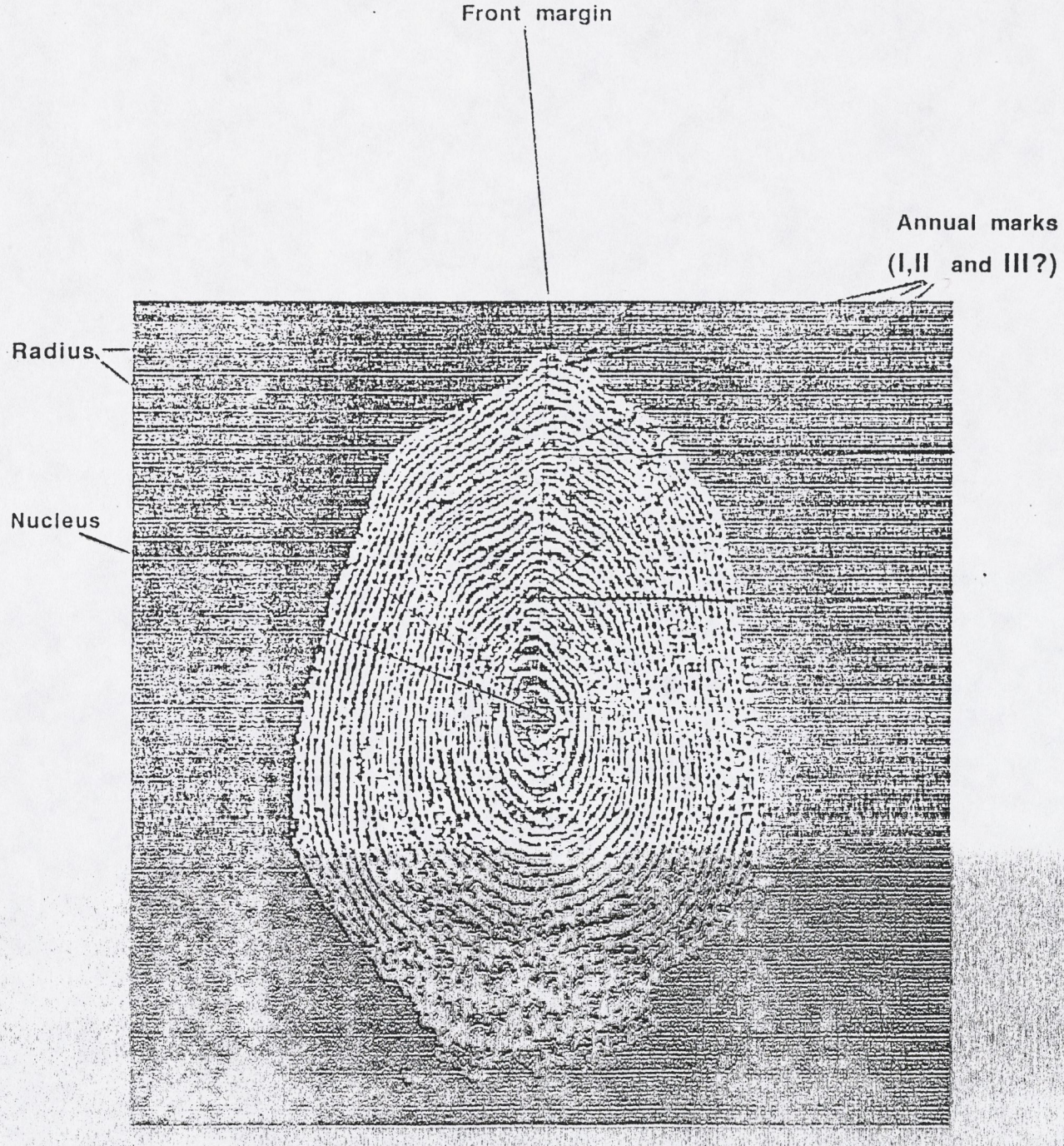
Mounting of scales employed the following steps:

1) Scales from a sample were removed from the coin envelope and soaked in soapy water for several minutes to loosen the scales and remove dirt particles and slime.
2) Scales were rinsed in water to remove soap film.
3) Eight to 12 scales from the sample were placed on a microscope slide and examined with a dissecting microscope.
4) A second slide was used as a cover slip if at least two readable scales were detected. If only regenerated scales were found, additional scales were examined until ones of readable quality were located.
5) Tape was used to secure the two microscope slides and the mounted sample was then air dried.

The scales were examined with a microfiche reader set at 46.2x magnification at the California Department of Fish and Game office in Arcata. The radius of a scale was first measured, which is the distance from the mid-point of the scale nucleus to the middle of the front margin (Figure 1). Age determination was based on counting the annual marks or checks present on a scale (areas of cutting over or constriction of the circuli) (Figure 1). Measurements from the mid point of the scale nucleus to the outer edge of each annulus were recorded. All measurements were taken in centimeters by placing a clear plastic ruler over the projected image of the scale. Since the method of back calculating lengths at earlier ages only requires relative measurements, the scale readings were not corrected for the 46.2x magnification prior to back calculation.

Before performing back calculations of length it is necessary to determine the actual relationship between the growth of some dimension of the scale and the length of the fish. This was accomplished by plotting the fork lengths of all fish sampled against their corresponding scale radii with a computer graphing program. The resulting relationship then determines the formula used for performing back calculations. Possible relationships include: 1) linear and passing through the orgin; 2) linear, but passing through the y-axis; 3) curved (slope increasing); or 4) S-shaped (slope at first increasing, then decreasing) (Tesch, 1971). Back calculations were

Figure 1. An optical pattern recognition (O.P.R.) created image of a scale from a 24.1 cm upper Kern River rainbow trout sampled on Nov. 11, 1991.



performed on an Excel spreadsheet using the appropriate formula. All rainbow trout fork lengths were converted from inches to centimeters prior to back calculations.

RESULTS

Out of the original 84 scales samples only 77 contained readable scales. Seven samples were composed entirely of regenerated scales, a common occurrence in salmonid scales. For instance, in some brown trout populations, studies have revealed up to 73 percent of scales from two year olds were rengenerated (Tesch, 1971). Ages of the Kern River rainbow trout ranged from 1+ to 4+ years. The 1+ and 2+ age classes included the majority (90.9%) of the trout scales examined (Table1). All scale measurements and back calculated lengths are presented in tabular form (Appendix I).

Table 1. Frequencies of age classes for 77 rainbow trout sampled from the upper Kern River, fall 1991.

Age	Frequency	Percentage of sample
1+	40	51.9%
2+	30	39.0%
3+	6	7.8%
4+	1	1.3%

The plot of fork lengths versus scale radii resulted in a straight line that passed through the y-axis at 9.4903 centimeters (Figure 2). Thus the appropriate formula used to back calculate lengths was:

$$L_n - C = S_n / S\ (L - C)$$

where:

L_n = length of fish when annulus "n" was formed.

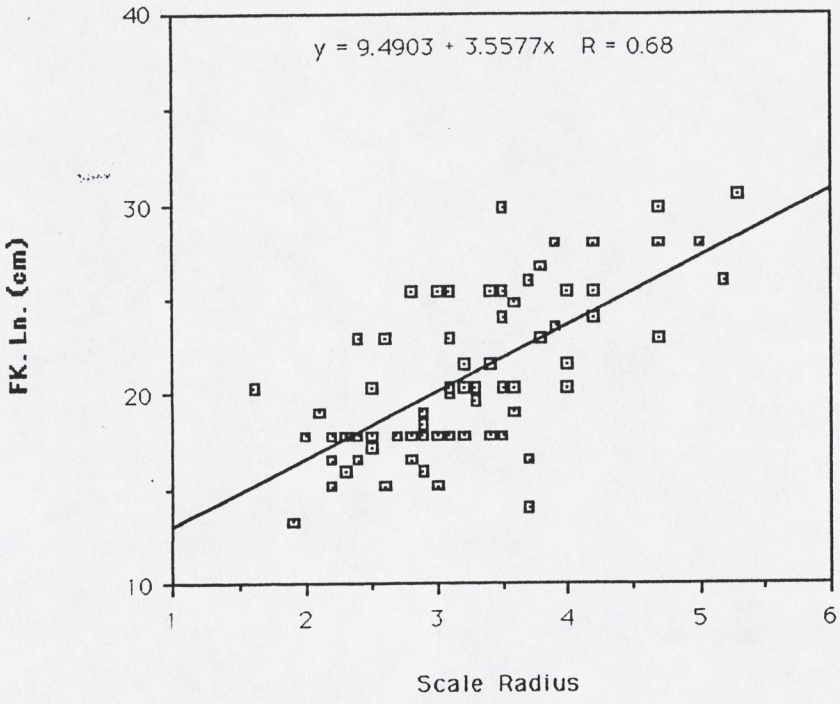
L = length of fish at time scale sample was obtained.

S_n = radius of annulus "n" (at length "L_n").

S = total scale radius.

C = y-intercept of regression line.

Figure 2. Fork lengths (cm) vs. scale radii (cm @ 46.2x) of upper Kern River rainbow trout sampled in fall, 1991.



The back calculated fork lengths at Age 1 ranged from 11.7 cm to 19.45 cm with average of 14.31 cm. The back calculated fork lengths at Age 2 ranged from 16.08 cm to 26.32 cm with an average of 20.47 cm. At Age 3 the back calculated fork lengths ranged from 20.43 cm to 28.12 cm with an average of 25.16 cm. The single fish aged as a 4+ year old was not used in the back calculation because the only

readable scale sample revealed spawning checks which lead to difficulties in determining the number and exact location of annual checks. The resportion of scale margins during spawning causes severe cutting-over and scarring of the circuli that interferes with the determination of the number and placement of annual checks.

A frequency histogram was also created with the fork length data to determine if the age classes were distinguishable by another methodology (Figure 3). Distinct peaks are only noticeable for the Age 1 and Age 2 year classes because insufficient data existed for Age 3 and Age 4 year classes.

DISCUSSION

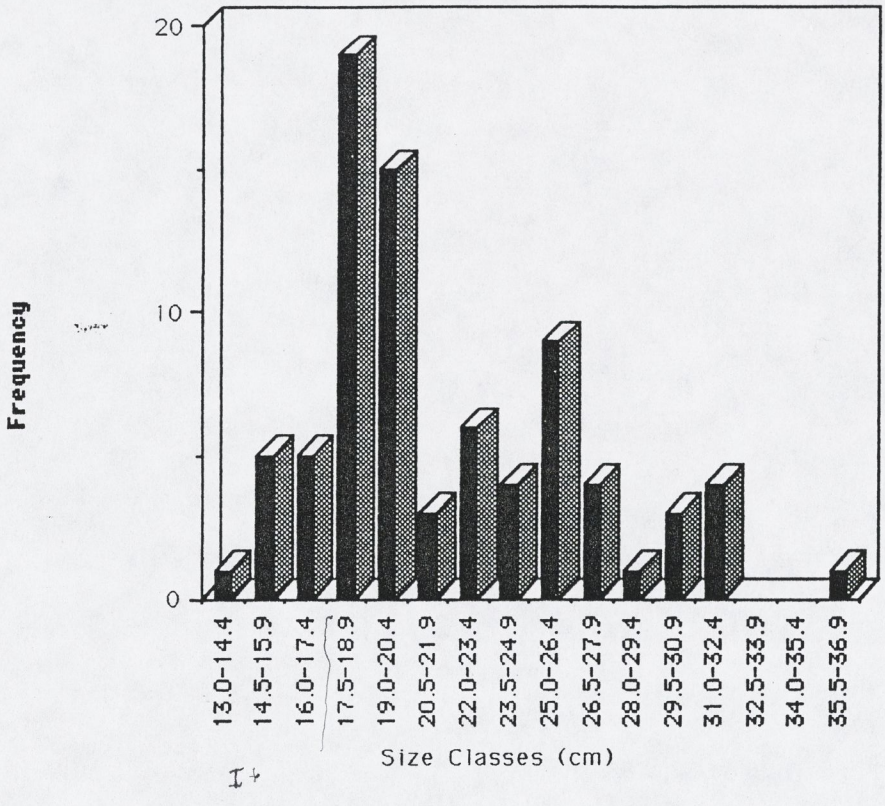
The scales sampled for this study allow for an estimate of the lengths at Age 1 and Age 2, thus an estimate of the growth rates of upper Kern River rainbow trout during their first two years of life. However, the lack of scale samples from older fish allows for limited insights of the growth rates in this portion of the upper Kern River rainbow trout population.

The growth rates of the upper Kern River rainbows sampled are comparable to the results of similar mid-elevation, in-river rainbow trout populations. Purkett (1951) reported the following back calculated lenghts at age for rainbow trout from the West Gallatin River in Montana sampled at mid elevations (4000-5000 feet): Age 1 - 3.4" ; Age 2 - 7.4" ; Age 3 - 11.5" and Age 4 - 14.5".

In concluding, several changes in sampling methodology are recommended for future studies. Primarily, scale samples should be obtained from larger (thus older) members of the population. Sampling techniques should not be limited to hook and line efforts that are biased toward younger fish. Secondly, accurate fork length measurements should be determined of all fish sampled. Most of the recorded lengths were to the whole inch, suggesting rounding or estimating by Trout Unlimited samplers. Lengths of fish in the size range sampled should be determined to the nearest millimeter with a small measuring board.

Finally, collect scale samples throughout the fishing season (May - November) instead of primarily two months in the fall. The late fall sampling period lead to some initial difficulty in the age determination process. Late fall appears to be the period when the upper Kern River rainbows are starting to lay down an annual check on the outer edge of their scales. An earlier sampling period would ease in the determination of annual checks.

Figure 3. Frequency distribution of fork lengths (cm) of upper Kern River rainbow trout sampled in fall, 1991.



LITERATURE CITED

Purkett, C.A. 1951. Growth rates of trout in relation to elevation and temperature. Trans., Amer. Fish. Soc. (80), 251-259.

Tesch, F.M. 1971. Age and growth. In: Methods for the Assessment of Fish Production in Fresh Water. (ed. by W.E. Ricker) Blackwell Scientific Pub. Oxford and Edinburgh. 98 -130.

Appendix I. Data Set for Kern River Rainbow Trout Scales

ID#	FL(cm)	Age	Fo. to OE	OE to I	Fo. to I	I to II	II to III	FL Age 1	FL Age 2	FL Age 3
1	20.30	1+	1.60	0.50	1.10			16.92		
2	17.80	1+	2.50	1.20	1.30			13.81		
3	17.80	1+	2.30	0.90	1.40			14.55		
4	25.40	2+	3.10	2.10	1.00	1.30		14.62	21.29	
5	17.80	1+	3.00	1.60	1.40			13.37		
6	17.80	1+	2.00	0.90	1.10			14.06		
7	17.80	1+	2.30	1.00	1.30			14.19		
8	17.80	1+	3.20	1.70	1.50			13.39		
9	15.20	1+	2.60	1.40	1.20			12.13		
10	22.90	2+	2.60	1.50	1.10	0.70		15.16	18.77	
11	19.00	1+	2.90	1.50	1.40			14.08		
12	20.30	1+	2.50	1.10	1.40			15.54		
13	20.30	1+	2.50	0.80	1.70			16.84		
14	27.90	2+	3.90	2.00	1.90	1.20		18.46	24.12	
15	30.50	3+	5.30	3.70	1.60	1.50	1.60	15.83	21.78	28.12
16	17.80	1+	3.10	1.40	1.70			14.05		
17	17.80	1+	3.20	1.60	1.60			13.65		
18	23.50	2+	3.90	2.20	1.70	1.30		15.60	20.27	
19	24.10	2+	4.20	2.80	1.40	1.60		14.36	19.93	
20	24.80	2+	3.60	2.70	0.90	1.90		13.32	21.40	
21	15.20	1+	3.00	0.90	2.10			13.49		
22	22.90	2+	3.10	1.10	2.00	0.80		18.14	21.60	
23	25.40	2+	3.00	1.70	1.30	0.90		16.38	21.16	
24	25.40	2+	2.80	1.60	1.20	0.80		16.31	20.85	
25	25.40	2+	3.50	2.10	1.40	1.40		15.85	22.22	
26	25.40	2+	3.40	1.90	1.50	1.30		16.51	22.59	
27	20.00	2+	3.10	2.00	1.10	1.00		13.22	16.61	
28	17.80	1+	2.50	1.30	1.20			13.48		
29	19.00	1+	2.10	0.80	1.30			15.38		
30	22.90	2+	2.40	1.50	0.90	0.70		14.52	18.43	
31	14.00	1+	3.70	1.60	2.10			12.05		
32	20.30	1+	3.10	1.60	1.50			14.72		
33	25.40	2+	4.00	2.20	1.80	1.30		16.65	21.82	
34	28.00	3+	5.00	3.90	1.10	1.70	1.50	13.56	19.86	25.41
35	26.70	2+	3.80	1.60	2.20	1.20		19.45	24.89	
36	21.60	2+	3.20	1.90	1.30	1.30		14.41	19.33	
37	18.40	1+	2.90	1.10	1.80			15.02		
38	20.30	1+	4.00	1.20	2.80			17.06		
39	27.90	2+	4.20	2.90	1.30	1.40		15.19	21.33	
40	26.00	2+	3.70	2.10	1.60	1.30		16.63	22.43	
41	17.80	1+	2.40	0.60	1.80			15.72		
42	16.50	1+	2.80	1.30	1.50			13.25		
43	17.10	1+	2.50	1.30	1.20			13.14		
44	21.60	2+	4.00	2.30	1.70	1.60		14.64	19.48	
45	20.30	1+	2.50	0.60	1.90			17.71		
46	15.90	1+	2.30	1.00	1.30			13.11		
47	17.80	1+	3.50	1.60	1.90			14.00		
48	22.90	2+	4.70	2.50	2.20	1.50		15.77	20.05	
49	15.90	1+	2.90	1.10	1.80			13.47		
50	17.80	1+	3.40	1.80	1.60			13.40		
51	16.50	1+	2.40	1.50	0.90			12.12		
52	29.80	3+	4.70	3.30	1.40	1.70	1.20	15.54	22.89	28.07
53	17.80	1+	2.20	1.10	1.10			13.65		
54	16.50	1+	3.70	1.40	2.30			13.85		
55	17.80	1+	2.80	1.50	1.30			13.35		
56	17.80	1+	2.40	0.80	1.60			15.03		
57	17.80	1+	2.50	1.30	1.20			13.48		
58	16.50	1+	2.20	1.00	1.20			13.31		
59	17.80	2+	2.90	1.70	1.20	1.10		12.93	16.08	
60	17.80	1+	2.70	1.30	1.40			13.80		

Appendix I. Data Set for Kern River Rainbow Trout Scales

ID#	FL(cm)	Age	Fo. to OE	OE to I	Fo. to I	I to II	II to III	FL Age 1	FL Age 2	FL Age 3
61	20.30	2+	3.20	1.60	1.60	1.10		14.90	18.61	
62	21.60	2+	3.40	2.00	1.40	0.90		14.48	17.68	
63	20.30	2+	3.60	2.10	1.50	1.20		13.99	17.60	
64	19.70	1+	3.30	1.50	1.80			15.06		
65	19.00	1+	3.60	1.80	1.80			14.25		
66	26.00	3+	5.20	3.80	1.40	2.00	1.10	13.94	20.29	23.78
67	20.30	2+	3.30	1.50	1.80	1.00		15.39	18.66	
68	15.20	1+	2.20	0.90	1.30			12.86		
69	20.30	2+	3.50	1.90	1.60	1.20		14.43	18.14	
70	24.10	2+	3.50	2.40	1.10	1.50		14.08	20.34	
71	27.90	3+	4.70	3.30	1.40	1.40	1.00	14.97	20.46	
72	22.90	2+	3.10	1.90	1.20	1.70		14.68	22.03	
73	29.80	2+	3.50	2.30	1.20	1.70		16.45	26.32	
74	25.40	2+	4.20	2.70	1.50	1.40		15.17	20.48	
75	22.90	3+	3.80	2.50	1.30	0.90	0.90	14.08	17.25	20.43
76	13.30	1+	1.90	0.80	1.10			11.70		
							Sum=	1088		
					Average FL a		Age 1=	14.31		
								Sum=	737	
						Average FL a		Age 2 =	20.47	
									Sum=	125.81
							Average FL at Age 3 =			25.16

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Penn
allozymes
etc.

FROM: Rodger Lowery - T.U.
20222 Spruce Ave
Santa Ana Hts, CA. 92707

RETURN POSTAGE GUARANTEED

TO: Robert Behnke
Dept Fisheries & Wildlife Biology
Colorado State University
Fort Collins, CO
80512