

Appendix A

Aquatic Benthic Macroinvertebrate Communities of Mammoth and Hot Creeks, California

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Appendix A

Aquatic Benthic Macroinvertebrate Communities of Mammoth and Hot Creeks, California

1.0 INTRODUCTION

Water Right Permit 17332, under which the District may divert water for beneficial uses, requires the District to remain in compliance with Fish and Game Code Sections 5937 and 5946. Specifically, Section 5937 requires “*the owner of any dam to allow sufficient water at all times to pass through a fishway, or over, or around the dam to keep in good condition any fish that may be planted or exist below the dam.*” Additionally, definition of “fish” as specified in Fish and Game Code sections 5937 and 5946 includes aquatic invertebrates, as well as vertebrate fishes. The same standard of “good condition” applies to invertebrates and includes the same standard of ecological health that applies to fish species.

It was suggested by the Mammoth Creek Technical Team during August 2005 that reduced flows in Mammoth Creek resulting from existing bypass flow requirements have reduced water quality and increased substrate consolidation (cementing of gravels and cobbles) and embeddedness (accumulation of fine sediments between gravels and cobbles), thereby reducing habitat availability for benthic macroinvertebrates (BMI) in Mammoth and Hot creeks. Additionally, it was suggested by the Mammoth Creek Technical Team during August 2006 that streambed cleansing (via scouring flows) may result in additional substrate interstitial space availability for colonization of BMIs. Reduced habitat availability could result in BMI communities not remaining in “good condition.”

Available information on BMI communities in Mammoth and Hot Creeks was examined to identify potential trends in BMI community metrics in Mammoth Creek and potential differences BMI communities between Mammoth and Hot creeks. BMI information was obtained from the Draft Mammoth Creek EIR (CH2M Hill 2000), the Hot Creek Hatchery Stressor Identification reports (Jellison *et al.* 2005a, 2005b), and the 2004 Hot Creek Biomonitoring Report published by the California Department of Fish and Game (Harrington 2005). Information from the Draft Mammoth Creek EIR included BMI sampling data obtained during 1992 through 1994 that was analyzed and reported in *Aquatic benthic macroinvertebrate monitoring report* (Vinson 1995). However, original data and analyses provided in Vinson (1995) were not available for use in this evaluation. Information obtained from the Hot Creek Hatchery Stressor Identification reports included a description of a 1993 bioassessment report conducted for the Hot Creek Hatchery and data obtained during 2000 through 2004 BMI monitoring. The CDFG report (Harrington 2005) included additional information regarding the 2004 data described in the Hot Creek Hatchery Stressor Identification reports.

2.0 METHODS

2.1 1992 THROUGH 1994 METHODS – MAMMOTH CREEK

According to CH2M Hill (2000) BMIs were collected from each fish population sample site in Mammoth Creek coincident with the fish population sampling conducted during 1992 through 1994. **Figure A-1** shows the location of fish population and BMI sampling sites during 1992 through 1994. BMI samples were subsequently analyzed using the U.S. Environmental Protection Agency (EPA) Rapid Bioassessment Protocols (Vinson 1995 *in* CH2M Hill 2000).

Several measures of community health were calculated for these three years of data, including measures of richness and diversity. Richness is described by the abundance (numbers) of invertebrates as well as the number of taxa (e.g., species or families). Diversity is a measure of community structure defined by the relationship between the number of distinct taxa and their relative abundance. The indices used to describe and compare the BMI communities during 1992 through 1994 include three measures of richness including: (1) taxa richness; (2) ephemeroptera, plecoptera, and trichoptera (EPT) taxa richness; and (3) family richness (CH2M Hill 2000).

Taxa richness refers to the number of individual taxa, and normally decreases with decreased water quality. Generally, rapid bioassessment protocols utilize genus as the lowest taxonomic level to which individual organisms are identified. However, the taxonomic level utilized by Vinson (1995) was not confirmed for this report. EPT taxa richness refers to the taxa richness within the insect orders ephemeroptera (mayflies), plecoptera (stoneflies), and trichoptera (caddisflies). These orders are considered to be sensitive to water quality, and EPT richness generally decreases with decreasing water quality. Family richness refers to the number of families, which typically decreases with decreasing water quality. In general, low richness (total taxa, EPT taxa, and family) is indicative of degraded water quality affecting the invertebrate community (CH2M Hill 2000).

Other standard measures of diversity calculated for the Mammoth Creek samples include Shannon's H, Simpson's D, and evenness. Shannon's H is widely used in community ecology and is a measure of the average degree of uncertainty in predicting a taxon to which an individual chosen at random will belong. This uncertainty increases as the number of taxa increases and the distribution of taxa becomes more even (the community diversity increases). Simpson's D is defined as the probability of picking two individuals that are of the same group, usually reported as (1-D), such that the index increases with diversity. Evenness is a measure of the distribution of individuals among taxa. Values range from 0-1 and approach zero as a single taxon becomes dominant.

2.2 1993 METHODS – HOT CREEK

The 1993 bioassessment survey (Harrington 1993) was conducted as part of a larger study, which included an assessment of the effectiveness of the Hot Creek Hatchery settling ponds, the impact of untreated discharges from the brood ponds at the Hot Creek Hatchery (Hatchery II), and toxicity tests. A full description of the survey and analysis of the data were not included in either of the available Hot Creek Hatchery Stressor Identification reports (Jellison *et al.* 2005a, 2005b). Specifically, Jellison *et al.* (2005a) stated... "*The 1993 bioassessment should be considered preliminary due to the limited design and small number of individuals counted.*"

However, Jellison *et al.* (2005a) indicated that the 1993 report provided some notable conclusions, and therefore is discussed briefly in this report.

2.3 2000 THROUGH 2004 METHODS

During 2000 through 2004 BMI monitoring in Hot Creek was conducted as required by the Lahontan Regional Water Quality Control Board (LRWQCB) as a condition of CDFG's Hot Creek Hatchery National Pollution Discharge Elimination System (NPDES) permit. Based on the monitoring reports produced after the 2000 through 2004 monitoring efforts, LRWQCB determined that the receiving waters from the Hot Creek Hatchery were impaired and ordered CDFG to determine the cause of impairment. CDFG subsequently entered into an interagency agreement with the University of California to determine the causes of impairment by assembling a team including CDFG biologists, UC scientists, and consultants with combined expertise in limnology, aquatic bioassessment, geomorphology and aquatic toxicology to conduct the stressor identification (SI) employing EPA guidelines (US EPA 2000). The first and second of four stressor identification reports (Jellison *et al.* 2005a, 2005b), which reviewed bioassessment monitoring data, other relevant reports, and available scientific literature, were reviewed to identify potential temporal and spatial trends in BMI communities, and potential differences among sites in Mammoth and Hot creeks.

During 2000, the bioassessment survey methods were modified from the 1993 survey by adding an additional Hot Creek monitoring site immediately upstream of the Hatchery II discharge and increasing the number of organisms identified to the lowest practical taxonomic level (typically genus) from each sample to 300 individual organisms. During 1993, identification of 100 organisms was considered representative of each sample. The macroinvertebrate sampling also was conducted later in the year (26 August 2000) compared to the 1993 sampling (Jellison *et al.* 2005a).

During 2001, two bioassessment monitoring sites were added on the springbrooks immediately below the two major hatchery source springs, AB and CD springs. Additionally, Big Springs was sampled to determine whether it could be used as a reference site. Sampling was conducted during late August (28-29 August 2001) (Jellison *et al.* 2005a).

During 2002 (Harrington 2003), the same six monitoring sites sampled during 2001 were sampled on 10 September 2002. Additionally, physical/habitat dimensions, substrate composition and water column measurements (temperature, D.O., conductivity, and pH) were determined at each monitoring site (Jellison *et al.* 2005a).

For the 2003 annual report (Harrington 2004), CDFG was directed to summarize the bioassessment data from 2000-2003. A detailed description of variation across stations and years for each of the eleven metrics employed is included in the report (Jellison *et al.* 2005a).

Ten sites (**Table A-1**) were sampled on September 7 through 9, 2004 by CDFG staff from the Water Pollution Control Laboratory in Rancho Cordova (CDFG 2004).

Ongoing bioassessment surveys were conducted at the ten sites in Mammoth and Hot creeks, from 2000 through 2004. Two sites (HC-AB and HC-CD) are located in Hot Creek immediately above the hatchery on springbrooks fed by hatchery source springs, and two sites (HC-H3 and HC-H2) are located immediately below the hatchery. HC-H3 samples the benthic community immediately below settling pond 1, while HC-H2 samples Hot Creek below the combined

discharges of all three settling ponds (i.e. ponds 1 and 2, and McBurney pond). Three sites (MC-H1, MC-H2, and MC-H4) are located on Mammoth Creek upstream of the confluence with Hot Creek and three sites (HC-UH7, HC-H7, and HC-H8) are located on Hot Creek below the confluence with Mammoth Creek (Jellison *et al.* 2005a). The Mammoth Creek BMI sampling sites were used as reference samples for comparisons to the Hot Creek sampling sites below the confluence of the two creeks to control for the hydrological influence of the seasonal Mammoth Creek flows and associated sediment loading. Additionally, comparison of the Mammoth Creek BMI data to the data obtained from the sites below the hatchery and below the confluence with Mammoth Creek allow inferences to be drawn about the influence of the hatchery on Hot Creek BMI communities, relative to the influence of Mammoth Creek inflow on Hot Creek communities. **Figure A-2** shows the locations of the 10 BMI sampling sites utilized during 2000 through 2004. **Table A-2** shows the locations and years during which each location was sampled from 2000 through 2004.

2.3.1 Cluster Analysis

Cluster analysis permits an evaluation of community similarity in the taxonomic composition and relative abundance between sites. A Bray-Curtis cluster analysis of BMI data collected during 2004 was presented by Jellison *et al.* (2005a) as a quantitative measure of the differences among sites based on the full data set.

Jellison *et al.* (2005a) performed two cluster analyses using the Bray-Curtis dissimilarity distance measure and Ward clustering algorithm. The first analysis examined the entire suite of 30 BMI samples (10 stations each with three replicates). The natural clustering of the replicate transects at each monitoring site provides a good measure of within versus across site variation. The second analysis was based on a composite (simple sum) sample of the three replicates at each site (Jellison *et al.* 2005a).

2.3.2 Multi-Metric Analysis

In addition to the statistical examination of the individual metrics, Jellison *et al.* (2005a) also examined a simple multi-metric index, which considers the relative differences in each of the individual metrics for bioassessment data collected during 2004. The multi-metric index was calculated by equally weighting each of the 11 individual metrics employed in the bioassessment monitoring reports. Individual metric scores were scaled from the 0 to 10 based on the observed range in each metric (or 10-0 for the 5 metrics which decrease with increasing biotic integrity). The scaled scores of the individual metrics were then summed and multiplied by 10/11 to provide a range from 0 to 100 (Jellison *et al.* 2005a).

2.4 CALIFORNIA STREAM BIOASSESSMENT PROCEDURE METHODS

The California Stream Bioassessment Procedure (CSBP) developed by DFG (Harrington 1996) was used for BMI monitoring during 2000 through 2004. The CSBP is a regional adaptation of the U.S. EPA Rapid Bioassessment Protocols (utilized by Vinson 1995 for conducting the Mammoth Creek monitoring during 1992 through 1994) that has undergone multiple revisions since it was first introduced. However, Jellison *et al.* (2005a) indicated that an early version of the CSBP was used for all sampling events with differences in sampling protocols among years indicated in Sections 2.1 through 2.3, above. The Hot Creek Hatchery bioassessments considered 11 metrics including richness (cumulative taxa richness, cumulative number of EPT

taxa), composition (EPT index, sensitive EPT index, Shannon Diversity, percent Tubificida), tolerance (tolerance value, percentage of organisms in intolerant taxa, percentage of organisms in tolerant taxa, percent composition of the single most dominant taxon), and functional feeding group measures (percent of collector, gatherer, and filterer) (Jellison *et al.* 2005a).

Since the first sampling event conducted as part of the Hot Creek Hatchery BMI monitoring efforts during 1993, all sampling sites were chosen to have similar physical/habitat characteristics to the extent possible. The majority of the sampling sites would be characterized as “runs” with swift velocity, relatively deep, but wadeable channels and bottom substrates consisting of cobble, gravel, and fine sediment. Most of the monitoring locations had low level vegetation growing on the stream banks and no substantial canopy cover. Most monitoring reaches, especially in Hot Creek had vegetation growing on the benthos.

2.4.1 Determining Monitoring Reach Length

The length of each sampling reach was determined in the field based on the extent of similar channel characteristics present at each predetermined monitoring reach. Usually, similar depth and water velocity were the overriding factors determining reach length. After the reach length was determined it was quantified by extending a measuring tape along the edge of the bank. The measuring tape was left in place throughout the sampling event and used to determine the positions of three randomly located transects, along which biological and physical habitat sampling was conducted (CDFG 2004).

2.4.2 Physical/habitat and Ambient Chemical Characteristics

Sampling reach width, depth, and water velocity were determined by averaging three measurements from each of the three randomly chosen transects. The geometric mean substrate particle size was determined by measuring 10 pieces of substrate along five randomly chosen transects. Transects were chosen randomly from all possible one meter intervals on the measuring tape used to determine reach length. Substrate composition was determined through visual observation of the entire monitoring reach. Ambient chemical characteristics were determined using a Yellow Springs Instruments (YSI) Model 85 and pH probe (CDFG 2004).

2.4.3 Biological Sampling

At all monitoring reaches, samples were collected along the three transects for which width, depth, and water velocity data were obtained. Along each transect three locations representing the left margin, right margin, and center of the transect were sampled within a 2 ft² area upstream of the sample location. Benthic organisms were collected at each sample location using a one-foot wide D-frame net with 0.5 mm mesh. The three collections obtained from each transect were combined into a single composited sample representing a 6 ft² area. Sampling of the benthos was performed manually by rubbing cobble and boulder substrates in front of the net followed by “kicking” the upper layers of substrate to dislodge any invertebrates remaining in the substrates. The duration of sampling ranged from 60 to 120 seconds, depending on the amount of boulder and cobble-sized substrates that required rubbing by hand. The composite sample was transferred into a 500 ml wide-mouth plastic jar containing approximately 200 ml of 95% ethanol. Samples were submitted to the Aquatic Bioassessment Laboratory (ABL) in Rancho Cordova along with a Chain of Custody form and stored in the ABL Sample Repository until processing (CDFG 2004).

2.4.4 Laboratory Methodology

Samples were processed at the ABL in Rancho Cordova, California and at the ABL - Chico State University, Chico, California. Sample contents were rinsed through a 0.5 mm mesh sieve to remove excess ethanol during sorting. The sample contents were then evenly distributed on the bottom of a tray divided into 25 cm² grids. BMIs were separated from benthic material with the aid of a stereomicroscope from one randomly selected grid at a time and transferred to vials containing 70% ethanol and 5% glycerol. The process continued until 300 organisms were counted or until all organisms were removed from the tray if it contained fewer than 300 organisms. For subsequent quality control analysis, the material left from the processed grids was transferred into a jar with 70% ethanol and labeled as “remnant” (CDFG 2004).

All organisms were identified to the lowest practical taxonomic level using: Baumann *et al.* (1977); Brown (1972); Edmunds *et al.* (1976); Merritt and Cummins (1995); Pennak (1991); Stewart and Stark (1993); Surdick (1985); Thorp and Covich (1991); Usinger (1963); Weaver (1988); Wiederholm (1983, 1986); Wiggins (1996); and Wold (1974). Organisms in each taxon were saved in individual vials containing 70% ethanol and 5% glycerol and retained as a voucher collection (CDFG 2004).

Taxonomic lists, functional feeding group composition, and the bioassessment metrics (**Table A-3**) were generated for each of the samples using an application developed in Microsoft Excel™ (CDFG 2004).

3.0 RESULTS

3.1 1992 THROUGH 1994 RESULTS – MAMMOTH CREEK

Data obtained from sampling conducted during 1992 through 1994 were first presented in CH2M Hill (2000) and are presented in **Figure A-3**. Total abundance of BMIs was generally higher in the downstream reaches (i.e., reaches D and E). Mayflies (ephemeroptera) and caddisflies (trichoptera) were relatively abundant in all stream reaches. The relative proportion of midges (dipterans) and caddisflies was higher in the downstream reaches. The relative abundance of stoneflies (plecoptera) was lower than that of other species and tended to decrease towards the downstream reaches (CH2M Hill 2000).

The results from Mammoth Creek reported in CH2M Hill (2000) show the same pattern for the richness indices (taxa richness, EPT richness, and family richness), with the 1992 and 1994 indices nearly identical and 1993 slightly lower. For example, the values for EPT richness are 13, 11, and 12 for 1992, 1993, and 1994, respectively, indicating that the number of taxa within these sensitive groups remained constant during those three years. The values of all three measures of richness were reported as typical of mountain streams in the Sierra Nevada and are considered indicative of moderate to good conditions (CH2M Hill 2000).

Results for all diversity indices calculated using data obtained during 1992 through 1994 including Shannon’s H, Simpson’s D, and evenness reportedly showed a slight increase in BMI diversity in Mammoth Creek from 1992 to 1994 (CH2M Hill 2000).

3.2 1993 RESULTS – HOT CREEK

The report describing the 1993 bioassessment survey conducted for the Hot Creek Hatchery monitoring program concluded, “*The biotic condition of Hot Creek below the effluent discharge from Hot Creek Hatchery is severely impaired and the degree of organic pollution improves downstream from the source of the hatchery effluent*” (Jellison *et al.* 2005a).

Jellison *et al.* (2005a) indicated that the 1993 bioassessment survey conducted for the Hot Creek Hatchery monitoring program should be considered preliminary due to the limited study design and small number of individuals counted. However, Jellison *et al.* (2005a) also noted that the following observations warranted mention:

- The samples were collected during early summer (14 June 1993) during a relatively wet (114 % of 1933-1996 average) year, a period in which significant flushing flows would have occurred immediately prior to sampling at three of the four survey sites.
- The degraded condition of Hot Creek, relative to Mammoth Creek, extended far downstream of the hatchery to lower Hot Creek Ranch where the monitoring site was chosen to represent “best” conditions. This was true for all 6 metrics analyzed.
- There was little change in any of the metrics between the monitoring sites immediately below Hatchery II and downstream on Hot Creek Ranch.
- The IAI index (ratio of the numbers of EPT to chironomids and annelids) showed the most marked changes across the four monitoring sites (Jellison *et al.* 2005a).

3.3 2000 THROUGH 2004 RESULTS

During the Hot Creek Hatchery stressor identification process, several analyses were conducted on data collected during 2000 through 2004. Results of the review of annual BMI monitoring reports, enumeration and calculation of selected biometrics, and the results of analysis of taxon-specific differences among years are presented below.

3.3.1 Review of 2000-2004 Hot Creek Hatchery Bioassessment Reports

2000 Monitoring Report

During 2000, 18 different metrics were evaluated instead of the six metrics evaluated during 1993. Jellison *et al.* (2005a) reported that few differences among stations were statistically significant ($\alpha = 0.05$). However, most metrics suggested the Hot Creek monitoring stations were degraded compared to the Mammoth Creek station (during 2000 only one Mammoth Creek site was sampled). The EPT metrics (EPT taxa, EPT Index, Sensitive EPT Index) were the exceptions with higher values often observed below the hatchery (Jellison *et al.* 2005a). Additionally, Jellison *et al.* (2005a) noted that the 2000 annual monitoring report (Harrington 2001) concluded that:

- There is organic enrichment downstream of the Hot Creek Hatchery and the problem is evident throughout the sampled reach.

- Some metrics including percent collector-filterers and percent tubificidae were stronger indicators than others of organic richness in the sites below the hatchery. Toxic contamination was an unlikely cause of the increased richness because richness and composition metrics yielded mixed results and there were more sensitive organisms in Hot Creek, relative to Mammoth Creek.
- Mammoth Creek is not a suitable control site to judge Hot Creek conditions (Jellison *et al.* 2005a).

2001 Monitoring Report

Jellison *et al.* (2005a) noted that the 2001 report (Harrington 2002) concluded that:

- The percent tubificidae and percent collector-filterer metrics at springbrook monitoring sites (HC-AB and HC-CD) were high, and using them as controls indicates the hatchery has a smaller impact on Hot Creek than previously indicated.
- The benthic community at Big Springs differed significantly from that at Mammoth Creek or the Hot Creek springbrooks and thus cannot provide an appropriate control.
- Most of the biological metric values showed minor increases or decreases between the 2000 and 2001 sampling events. However, there are still obvious signals showing an influence of organic enrichment downstream of the Hatchery.
- The relatively high sensitive EPT index values observed for Hot Creek samples below the hatchery during 2000 may have been anomalous and were lower for 2001 samples.
- Statistical comparisons of metrics across sites were not performed because the assumptions of the experimental design could not be adequately met in an uncontrolled natural setting such as Hot Creek (Jellison *et al.* 2005a).

2002 Monitoring Report

Jellison *et al.* (2005a) indicated that, generally, the variation in the suite of metrics analyzed during 2002 was similar to the variation observed during previous years with the exception of the metric percent tubificida. Additionally, Jellison *et al.* (2005a) noted:

- Percent tubificida declined markedly at Hot Creek monitoring sites below the hatchery, relative to 2001.
- Percent collector-filterers also declined at these sites, although less markedly.
- The report concludes that the decreases in percent tubificida and percent collector-filterers indicated a decrease in the influence of organic enrichment downstream of the hatchery (Jellison *et al.* 2005a).

2003 Results

For the 2003 annual report (Harrington 2004), DFG summarized the bioassessment data from 2000 through 2003. A detailed description of variation across stations and years for each of the eleven metrics employed was included in the report. Jellison *et al.* (2005a) noted:

- Generally, the biological metrics indicated Mammoth Creek was in better condition than Hot Creek sites.
- The Mammoth Creek site contained pool/riffle sequences typical of a higher gradient channel and had coarse substrate compared to Hot Creek sites which contained run habitat and fine substrates.
- Using AB and CD springbrook sites as reference sites led to 7 of 11 biological metrics indicating more degraded conditions immediately below the hatchery and only 5 of 11 metrics indicating more degraded conditions at the furthest downstream sites (H7 and H8) with the remaining 6 indicating improvement (Jellison *et al.* 2005a).

2004 Monitoring Report

Jellison *et al.* (2005a) indicated that the 2004 data (Harrington 2005) generally were similar to those collected during 2000 through 2003 and CDFG concluded:

- For the 11 biological metrics used to indicate responses to impairment, only 2 met the expectation for the above and below hatchery comparison using the AB and CD spring supply channels for a control and only 4 met this expectation for the Mammoth Creek and Hot Creek comparison. However, as in years past, the number of tubificid worms increased downstream of the hatchery discharge.

3.3.2 Physical Habitat Characteristics

Physical habitat characteristics were recorded during all sampling events during all years. However, no analysis was conducted on physical habitat data within or among years, in part because physical habitat was reported as similar among years (Jellison *et al.* 2005) and physical habitat data obtained prior to the 2004 sampling event are not readily available. Therefore, physical habitat data are not presented in this report. However, the 2004 physical habitat data are available in Jellison *et al.* (2005a) and Harrington (2005).

3.3.3 Summary of Benthic Macroinvertebrate Metrics from 2000 through 2004

BMI sampling sites were not consistent among all years because sites were added as deemed necessary and appropriate during the monitoring program. Table A-2 shows the locations and years during which each location was sampled from 2000 through 2004.

Cumulative taxonomic richness values varied from 16 to 45 across all sites and years and was lower at the springbrook sites upstream of the Hot Creek Hatchery (HC-AB and HC-CD) and immediately below the hatchery (HC-H2 and HC-H3), compared to Mammoth Creek and Hot Creek sites below the confluence with Mammoth Creek (**Figure A-4**). The year-to-year variation in this metric was highest at the Mammoth Creek sites and ranged from 27 to 44 at MC-H4. The 4-year mean value, 20, at the shallower springbrook site (HC-CD) was

significantly less (t-test, $p < 0.02$) than the 4-year mean value at the deeper springbrook site (HC-AB) but neither was significantly different from the Mammoth Creek site (MC-H4) due to the high inter-year variation observed at that Mammoth Creek site. The taxonomic richness of HC-H3 was significantly less than HC-AB but not HC-CD. The 5-year mean taxonomic richness decreased from 36 at MC-H4 to 32.2 at HC-H7 and 30.8 at HC-H8 but this decrease was not statistically significant ($p > 0.05$) (Jellison *et al.* 2005a).

The cumulative EPT taxonomic richness metric shows similar patterns among stations except that Hot Creek monitoring sites below the confluence with Mammoth Creek are intermediate between those of Mammoth Creek and the springbrook monitoring stations (**Figure A-5**). The comparisons between Mammoth Creek sampling sites and Hot Creek sampling sites below the confluence with Mammoth Creek were statistically significant (Jellison *et al.* 2005a). The Mammoth Creek sites generally were richer than the springbrook or other Hot Creek sites.

The EPT Index is the percentage of organisms in ephemeroptera, plecoptera, or trichoptera taxa (**Figure A-6**). As with most measures of relative abundance, this index varied more among years than the richness metrics and is thus less well-suited to detect differences among stations. The EPT Index is expected to decrease due to impairment. Indices at the Hot Creek stations below the confluence with Mammoth Creek were generally higher (overall mean, 17.6 %) than the indices at the Mammoth Creek stations (mean 9.9 %) (Jellison *et al.* 2005a).

The sensitive EPT index is the percentage of organisms that are in the ephemeroptera, plecoptera, or trichoptera orders that are considered sensitive to impairment (i.e., tolerance values less than 3). The sensitive EPT index is expected to decrease in response to impairment. As with the EPT Index, the sensitive EPT Index was highly variable (within stations among years, and among stations) (**Figure A-7**). This metric was significantly lower at the Mammoth Creek monitoring stations than all the other stations. However, there was no significant difference among the other stations (Jellison *et al.* 2005a).

The Shannon Diversity index, a general measure of sample diversity, is expected to decrease in response to impairment. Shannon Diversity ranged from 1.1 to 2.6 among all stations and all years (**Figure A-8**). Values were low at the HC-CD monitoring site for three of the four years, but a high value at this site during 2001 made differences between this site and others statistically insignificant ($p > 0.05$). The Shannon Diversity at the settling pond outflow site (HC-H3) was significantly less than the Shannon Diversity at the AB springbrook (HC-AB), Mammoth Creek (MC-H4) and lower Hot Creek (HC-H7, HC-H8) sites (Jellison *et al.* 2005a).

The percent of organisms in the family tubificidae is expected to increase in response to impairment, and specifically organic enrichment, and varied from 1 percent to 57 percent among sites and years (**Figure A-9**). This metric was lowest at Mammoth Creek monitoring sites, intermediate at the hatchery springbrook sites, and high at all Hot Creek sites below the hatchery, including below the confluence with Mammoth Creek. This metric at the Mammoth Creek monitoring site (MC-H4) was significantly lower ($p < 0.05$) than the percent tubificidae at all Hot Creek sites except the CD springbrook site (HC-CD) (Jellison *et al.* 2005a).

The mean tolerance value is a calculated value between 0 and 10, weighted for the abundance of individuals designated as pollution tolerant (higher values) or intolerant (lower values). The mean tolerance value among sites and years ranged from 5.2 to 8 among Hot Creek stations with a mean of 6.5, which indicates a benthic community dominated by organisms with a relatively high tolerance (**Figure A-10**). While there were no significant differences in values among Hot

Creek stations, the values at the Mammoth Creek monitoring stations were lower than the values at all the other stations, with a 5-year mean of 5.1 at station MC-H4 (Jellison *et al.* 2005a).

The multi-year mean percent intolerant organisms ranged from 1.25 percent at the CD springbrook site to 4.25 percent at the AB springbrook site, with values on Mammoth Creek and Hot Creek below the confluence with Mammoth Creek intermediate to these values (**Figure A-11**). Intolerant taxa were completely absent at Hot Creek sites immediately below the settling ponds (HC-H3) and hatchery discharge (HC-H2) (Jellison *et al.* 2005a).

The multi-year mean percentage of organisms in tolerant taxa (Tolerance Value [TV] 8 to 10) was highly variable (**Figure A-12**). The percentage of tolerant taxa at Mammoth Creek (MC-H4) was lower than the percentage of tolerant taxa all other Hot Creek sites (Jellison *et al.* 2005a).

The multi-year mean value for the metric of the percentage of dominant taxa (i.e., percent composition of the single most dominant taxon) ranged from 19 percent to 27 percent for Hot Creek sites above the confluence with Mammoth Creek to 31 percent to 44 percent below the confluence (**Figure A-13**). The Mammoth Creek site (MC-H4) had a mean value of 36 percent. Thus, while this metric increased from the springbrooks to the most downstream sites, the Mammoth Creek monitoring site had values similar to those below the confluence with Hot Creek (Jellison *et al.* 2005a).

Functional feeding group metrics differ in their responses to impairment. The percentage of organisms in the collector-gatherer group is expected to increase in response to impairment. The percentage (multi-year mean = 46.6 percent) of collector-gatherers at the Mammoth Creek monitoring site was lower than the percentage at all other Hot Creek monitoring sites (multi-year means ranged from 59 percent to 74 percent) except for the settling pond outflow (HC-H3), which had a similar percentage (mean = 51) (**Figure A-14**) (Jellison *et al.* 2005a).

3.3.4 Analysis of taxon-specific differences among sites

Bioassessment metrics are utilized to condense and summarize species changes occurring in complex benthic communities. In some cases a taxon-by-taxon analysis of observed changes may provide more useful or additional information. Jellison *et al.* (2005a) analyzed changes in abundance of individual taxa for all taxa that showed a “marked difference” between Mammoth Creek and Hot Creek sample sites, and which constituted a “significant proportion (>5%) of sampled individuals.” The results of the analysis conducted by Jellison *et al.* (2005a) are presented below.

Coleoptera

Riffle beetles feed on periphyton and reportedly are somewhat tolerant of fine sediment (Relyea *et al.* 2000). Both larvae and adults are found in streams and typically inhabit shallow, rocky, turbulent areas (riffles). Riffle beetles in the genus *Optioservus* (TV = 4), were abundant at all three Mammoth Creek sites, constituting 25 to 60 percent of the total abundance (**Figure A-15**, from Figure 17 in Jellison *et al.* (2005a)). They also were present, but were substantially less abundant at both springbrook sites (HC-AB and HC-CD). *Optioservus* was completely absent at Hot Creek sites immediately below the hatchery and only in low abundances at Hot Creek sites below the confluence with Mammoth Creek (Jellison *et al.* 2005a).

Riffle beetles in the genus *Zaitzevia* (TV = 4) were present in lower abundances than *Optioservus* at the Mammoth Creek sites but were completely absent from all Hot Creek sites including monitoring sites on the springbrooks (**Figure A-16**, from Figure 18 in Jellison *et al.* (2005a)).

Diptera

Black flies, *Simulium sp.* (TV = 6), were present in low (0 to 3%) relative abundance at all stations except Hot Creek below the confluence with Mammoth Creek (**Figure A-17**, from Figure 21 in Jellison *et al.* (2005a)). At HC-H7 their relative abundances ranged from 3 to 28 percent over the five years sampled (Jellison *et al.* 2005a).

Ephemeroptera

The moderately sensitive mayfly, *Fallceon quilleri* (TV = 4), was present in low (1.5-4.5%) relative abundance at Mammoth Creek sites and Hot Creek sites below the confluence with Mammoth Creek but mostly absent from other Hot Creek sites (**Figure A-18**, from Figure 22 in Jellison *et al.* (2005a)).

Trichoptera

Sensitive caddisflies in the genus *Brachycentrus* (TV = 1) were present in low relative abundance in Mammoth Creek and increased slightly in lower Hot Creek (**Figure A-19**, from Figure 23 in Jellison *et al.* (2005a)). They were not observed at any Hot Creek sites immediately above or below the hatchery. Individuals in the Hot Creek samples likely were *B. echo* (D. Herbst, pers. comm. in Jellison *et al.* 2005a), which display a greater tolerance to sediment and warm temperatures than its congeners *B. americanus* and *B. occidentalis* (Jellison *et al.* 2005a).

Micro-caddisflies in the genera *Ochrotrichia* (TV = 4) and *Oxyethira* (TV = 3) were mostly absent from Mammoth Creek (**Figures A-20** and **A-21**, from Figures 24 and 25 in Jellison *et al.* (2005a)). Relative abundance of *Ochrotrichia sp.* increased slightly below McBurney Pond and further at the lower Hot Creek sites. Relative abundance of *Oxyethira sp.* increased across the hatchery and remained relatively high at the downstream Hot Creek sites compared to Mammoth Creek (Jellison *et al.* 2005a).

Podocopida

Ostrocods in the family Cyprididae (TV = 8) were moderately abundant at the springbrook monitoring stations and immediately below the hatchery (**Figure A-22**, from Figure 28 in Jellison *et al.* (2005a)). Their relative abundance was substantially lower in Mammoth Creek and in Hot Creek below the confluence (Jellison *et al.* 2005a).

Tubificida

Worms in the Tubificidae family (TV = 10) were present in moderate (1 to 18 percent) but variable relative abundances at the springbrook monitoring sites, increased across the hatchery, and increased in Hot Creek downstream of the confluence with Mammoth Creek where they constituted between 18 and 57 percent of the total number of organisms (**Figure A-23**, from Figure 29 in Jellison *et al.* (2005a)). Tubificid worms were least abundant at the Mammoth Creek sites. These oligochaetes are classic indicators of organic enrichment and sediments in

streams. Naididae (TV = 10) were less abundant (0 to 10 percent) and variable across the stations (**Figure A-25**, from Figure 30 in Jellison *et al.* (2005a)).

Planarids

Planarid flatworms (Turbellaria: Planariidae, TV = 4) showed a similar increase immediately below the hatchery and moderately high relative abundance at the lower Hot Creek stations (**Figure A-25**, from Figure 31 in Jellison *et al.* (2005a)).

3.4 CLUSTER ANALYSIS OF 2004 BIOASSESSMENT DATA

Generalized metrics greatly simplify the information contained in BMI samples, but in so doing lose information. The taxon-specific analysis suggests there are significant differences among the monitoring sites that require additional analysis that are not fully captured in the analysis of metrics (Jellison *et al.* 2005a).

Cluster analysis permits an evaluation of community similarity in the taxonomic composition and relative abundance between sites. A Bray-Curtis cluster analysis of BMI data collected during 2004 was presented by Jellison *et al.* (2005a) as a quantitative measure of the differences among sites based on the full data set.

Jellison *et al.* (2005a) performed two cluster analyses using the Bray-Curtis dissimilarity distance measure and Ward clustering algorithm. The first analysis examined the entire suite of 30 BMI samples (10 stations each with three replicates). The natural clustering of the replicate transects at each monitoring site provides a good measure of within versus across site variation. The second analysis was based on a composite (simple sum) sample of the three replicates at each site (Jellison *et al.* 2005a).

The cluster analysis indicates that differences among sites are large, relative to within site variation because most replicate transects cluster closely together (**Figures A-26 and A-27**). The most notable exception is the 3rd transect from Hot Creek below McBurney Pond (HC-H2 Transect 3) which was much more similar to the Mammoth Creek samples than other Hot Creek samples. Samples from the most upstream Mammoth Creek monitoring site (MC-H1) cluster closely together and are most similar to the two other Mammoth Creek sites (MC-H2 and MC-H4). Samples from the two springbrook monitoring sites (HC-AB and HC-CD) are tightly clustered in two different groups. HC-CD springbrook clusters with the Mammoth Creek sites, while HC-AB springbrook is more similar to the other Hot Creek sites (Jellison *et al.* 2005a).

Cluster analysis on the composite samples reveals similar affinities. The most similar sites are the three Mammoth Creek monitoring sites (MC-H1, MC-H2, and MC-H4). The HC-CD springbrook site is more closely related to the Mammoth Creek samples than to the HC-AB springbrook site or other Hot Creek samples. The HC-AB springbrook site is most similar to the site immediately below the hatchery (HC-H3) although not as similar as the Mammoth Creek sites are to each other. Additionally, the HC-AB springbrook sites are not as similar to the HC-H3 sites as the downstream Hot Creek sites are to each other (HC-UH7, HC-H7, and HC-H8). The downstream Hot Creek sites (HC-H7 and HC-H8) are very similar and next most similar to the Hot Creek site immediately upstream (HC-UH7), followed by those below the Hatchery (HC-H2 and HC-H3) (Jellison *et al.* 2005a).

The only difference in clustering that arises from using the composite samples as opposed to the individual transects is that the two lowest Hot Creek samples (HC-H7 and HC-H8) are most closely clustered with the site immediately upstream (HC-UH7) rather than the Hot Creek site below the hatchery but immediately above the confluence with Mammoth Creek (HC-H2) (Jellison *et al.* 2005a).

3.5 MULTI METRIC ANALYSIS ON 2004 BIOASSESSMENT DATA

In addition to differences in BMI communities identified by cluster analyses, there are also differences in individual metrics between Mammoth Creek sites and those on Hot Creek below the confluence with Mammoth Creek. An equal weighting of the 11 metrics would suggest that while biotic integrity is low in the springbrooks (HC-AB and HC-CD) and immediately downstream of the hatchery (HC-H2 and HC-H3), it does not change across either of the two comparisons (i.e. springbrooks versus immediately below the hatchery, and Mammoth Creek versus Hot Creek below the confluence).

Figure A-28, obtained from Jellison *et al.* (2005a) presents the multi-metric analysis to allow a summary comparison of all the various metrics across stations, but Jellison *et al.* (2005a) cautions the reader against attaching unwarranted significance to the overall score.

4.0 DISCUSSION

Reference conditions based on benthic macroinvertebrates have not been established for the Lahontan region. Therefore, until reference conditions and biocriteria are established, investigators must use best professional judgment to evaluate the extent to which observed impacts represent significant impairments of specific aquatic life beneficial use categories (Jellison *et al.* 2005a). As such, the available information and BMI data obtained from CH2M Hill (2000), Jellison *et al.* (2005a), (Jellison *et al.* 2005b), and Harrington (2005) were used to identify potential causes of relationships and differences in BMI metrics, taxa, and taxa clustering identified above.

4.1 1992 THROUGH 1994 DISCUSSION – MAMMOTH CREEK

CH2M Hill (2000) reported that the aquatic invertebrate sampling conducted from 1992 to 1994 suggests that the aquatic invertebrate community is relatively healthy, being composed of a relatively large number of taxa (around 20), representing a number of different families (around 15), and with good representation of the more sensitive taxa within the EPT orders. Standard diversity measures indicate that the ecological health of the benthic invertebrate fauna did not change significantly between 1992 and 1994 (Vinson 1995 *in* CH2M Hill 2000).

4.2 2000 THROUGH 2004 DISCUSSION

BMI sampling conducted during 2000 through 2004 as part of the Hot Creek Hatchery biomonitoring efforts associated with the hatchery's NPDES permit attempted to use the springs upstream from the hatchery and the sites in Mammoth Creek as reference sample sets for testing the differences between BMI communities immediately below the hatchery and on Hot Creek below the confluence with Mammoth Creek (Jellison *et al.* 2005a). However, Jellison *et al.*

(2005a) also indicated that the utility of the upstream hatchery spring data sets was unknown due to the potential influence of the springs in the BMI community.

Examination of the data indicates that, for the purposes of this report, the springbrook sampling sites (HC-AB and HC-CD) data do not provide for meaningful comparisons. Specifically, cluster analysis indicates that the BMI community at springbrook site HC-CDS is most similar to the Mammoth Creek sites, while the BMI community at springbrook site HC-ABS is most similar to one of the sites immediately downstream of the hatchery. While the data from the springbrook sites may be useful for Hot Creek Hatchery stressor identification purposes, the data are not further discussed with respect to the determination of the condition of fish food availability in Mammoth Creek.

According to Jellison *et al.* (2005a), high year-to-year and spatial variability often make it necessary to consider multiple years of bioassessment data (e.g., de Vlaming *et al.* 2004). The five years of available Hot Creek Hatchery bioassessment data provided information on small-scale spatial and year-to-year variability of the benthic macroinvertebrate community in Hot and Mammoth creeks in the vicinity of the hatchery. The presence of statistically significant differences in the 2000 through 2004 data set was examined by performing multi-year pair-wise t-tests between pairs of stations. Because the power of this statistic is so low with small sample sizes (i.e., the relatively few years of data), and the region is characterized by high environmental variability, the absence of a significant effect cannot be interpreted as indicating that no difference among sites exists. However, many statistically significant differences are present. Causal interpretation of these observed differences will likely require a full characterization of causes (Jellison *et al.* 2005a).

4.2.1 Discussion of Metric Differences

Differences in BMI metrics among sites (exclusive of HC-AB and HC-CD) and interpretation of the differences are summarized below:

- Taxonomic richness generally is higher in Mammoth Creek and Hot Creek below the confluence with Mammoth Creek than in Hot Creek upstream of Mammoth Creek, indicating that Mammoth Creek and Hot Creek below the confluence have similar biotic integrities and that the BMI community in Hot Creek upstream of Mammoth Creek may be influenced by factors not associated with Mammoth Creek and may normalize under the influence of Mammoth Creek inflow.
- EPT taxa richness generally is higher in Mammoth Creek than in Hot Creek upstream of the confluence and slightly higher than downstream of the confluence, indicating that Mammoth Creek has higher biotic integrity than Hot Creek.
- The EPT index (percentage of EPT taxa at a site) generally is lower in Mammoth Creek than in Hot Creek upstream and downstream of the confluence with Mammoth Creek, indicating that Hot Creek has higher biotic integrity than Mammoth Creek.
- The sensitive EPT index (percentage of sensitive EPT taxa at a site) generally is lower in Mammoth Creek than in Hot Creek downstream of the confluence with Mammoth Creek, indicating that Hot Creek downstream of the confluence with

Mammoth Creek has higher biotic integrity than Mammoth Creek. The high degree of variability in the sensitive EPT index in Hot Creek upstream of the confluence with Mammoth Creek make interpretation of this metric difficult between those sites.

- Shannon Diversity generally is similar in Mammoth Creek and in Hot Creek below the confluence with Mammoth Creek and generally is higher than in Hot Creek above the confluence with Mammoth Creek, indicating that the BMI community in Hot Creek upstream of Mammoth Creek may be influenced by factors not associated with Mammoth Creek and may normalize under the influence of Mammoth Creek inflow.
- The percent tubificidae metric is substantially lower in Mammoth Creek than in Hot Creek, indicating that Mammoth Creek has higher biotic integrity than Hot Creek.
- The mean tolerance value metric is higher in Hot Creek than in Mammoth Creek, indicating that Hot Creek is inhabited by a BMI community that is dominated by tolerant organisms. BMI communities dominated by tolerant organisms generally indicate impaired conditions.
- The percentage of intolerant organisms is higher in Mammoth Creek and Hot Creek below the confluence with Mammoth Creek than in Hot Creek upstream of Mammoth Creek, indicating that the BMI community in Hot Creek upstream of Mammoth Creek may be influenced by factors not associated with Mammoth Creek and may normalize under the influence of Mammoth Creek inflow. However, during 2004 the percentage of intolerant organisms in Hot Creek downstream of Mammoth Creek was substantially higher than in Mammoth Creek. Because the dramatic difference occurred during only one year, it is inappropriate to speculate on the meaning of the difference.
- The multi-year mean percentage of organisms in tolerant taxa was highly variable. However, the percentage of tolerant organisms generally was lower at Mammoth Creek (MC-H4) than all Hot Creek sites, indicating that Mammoth Creek had higher biotic integrity than Hot Creek.
- The multi-year mean value for the percentage of dominant taxa metric generally was lower in Hot Creek upstream of the confluence with Mammoth Creek than those sites in Mammoth Creek and in Hot Creek downstream of Mammoth Creek. The percentage of dominant taxa was similar in Mammoth Creek and the downstream Hot Creek sites, indicating that the BMI community in Hot Creek upstream of Mammoth Creek may be influenced by factors not associated with Mammoth Creek and may normalize under the influence of Mammoth Creek inflow.
- The percentage of collector-gatherers in Mammoth Creek generally was lower than in any of the Hot Creek sites, indicating that Mammoth Creek had higher biotic integrity than Hot Creek. However, the percentage of collector-gatherers generally was highest in Hot Creek upstream of Mammoth Creek, indicating that the BMI community in Hot Creek upstream of Mammoth Creek may be

influenced by factors not associated with Mammoth Creek and may normalize under the influence of Mammoth Creek inflow.

In addition to the inferences drawn from the individual BMI metrics for this report, Jellison *et al.* (2005a) provided additional suggestions derived from BMI metric analysis associated with Hot Creek Hatchery stressor identification.

- The higher values of the EPT index immediately below the hatchery and on Hot Creek below the confluence with Mammoth Creek suggest no impact or even a positive influence of the hatchery on biotic integrity (Jellison *et al.* 2005a).

Summary of taxon-specific differences among sites

Despite the inconclusiveness of the metrics analysis, taxon-specific changes in community dominants (riffle beetles, flatworms, and tubificids) strongly indicate altered community structure below the hatchery (Jellison *et al.* 2005a). Specifically, Jellison *et al.* (2005a) reported that there were a number of notable taxon-specific differences among the various biomonitoring sites. In several cases, there were major shifts in the dominant taxa in a community including:

- The riffle beetles, *Optioservus sp.* and *Zaitzevia sp.* constituted a higher percentage of the benthic community at Mammoth Creek sites (substantially higher for *Optioservus sp.*) but was mostly absent below the confluence with Hot Creek. The genera also were completely absent immediately below the hatchery (Jellison *et al.* 2005a). High percentages of riffle beetles generally indicate low impairment, suggesting that conditions in Mammoth Creek are somewhat better than those in Hot Creek.
- Black flies in the genus *Simulium* have moderately high tolerance value (6) and were generally higher in Hot Creek downstream of Mammoth Creek than in any other sampling sites, indicating that Mammoth Creek exhibited better conditions than the downstream sites in Hot Creek.
- Mayfly and caddisfly abundance generally was higher in Mammoth Creek and Hot Creek downstream of the confluence with Mammoth Creek, indicating that conditions in Mammoth Creek were better than those in Hot Creek upstream of the confluence with Mammoth Creek.
- Micro-caddisfly genera *Ochrotrichia* and *Oxyethria* abundance was variable among sites, but generally was higher in Hot Creek than in Mammoth Creek, indicating better conditions in Hot Creek than in Mammoth Creek. The micro-caddisflies (family hydroptilidae) are moderately tolerant of stress. This family of caddisflies has the unusual feeding habit of piercing the cells of filamentous algae to obtain nutrition, and may be indicators of an increase in the relative abundance of filamentous algae in Hot Creek, relative to Mammoth Creek (Jellison *et al.* 2005a).
- Ostracods in the family cyprididae generally were present in higher relative abundance in Hot Creek upstream of Mammoth Creek than in Mammoth Creek or at the downstream sites in Hot Creek, indicating that the BMI community in Hot Creek upstream of Mammoth Creek may be influenced by factors not associated

with Mammoth Creek and may normalize under the influence of Mammoth Creek inflow.

- Intolerant oligochaete worms in the family tubificidae generally constituted a relatively minor portion of the Mammoth Creek site BMI communities and increased markedly in upstream and downstream Hot Creek samples, indicating that the BMI community in Hot Creek upstream of Mammoth Creek may be influenced by factors not associated with Mammoth Creek. Increases in tubificids or other oligochaetes below trout farms have been noted elsewhere and been determined to have been caused by hatchery effluents (VDEQ 2002, NC DENR 2002, Carmago 1992, and Loch *et al.* 1996 in Jellison *et al.* 2005a).
- Naididae (TV = 10) also increased from Mammoth Creek to the downstream Hot Creek sites, indicating that conditions in Mammoth Creek were better than those in Hot Creek downstream of the confluence with Mammoth creek.
- As with tubificids, planarid flatworms (TV = 4) also increased from Mammoth Creek to the downstream Hot Creek sites, indicating that the BMI community in Hot Creek may be influenced by factors not associated with Mammoth Creek.

4.2.2 Multi-year statistical comparisons of site metrics

In general, BMIs respond to abiotic and biotic environmental variables over a range of spatial and temporal scales. Additionally, no single metric adequately captures the responses of these complex biological communities. The observed differences in the twelve metrics considered were summarized by enumerating their support for or against seven general trends identified by Jellison *et al.* (2005a). However, because many of the trends do not relate to Mammoth Creek, they were dismissed from further discussion. Three trends related to the determination of Mammoth Creek fish food condition and are expressed as the following hypotheses (Jellison *et al.* 2005a):

- 1) Hot Creek below the confluence with Mammoth Creek has lower biotic integrity than Mammoth Creek (MC-H4 > HC-H7).
- 2) Hatchery springbrook sites (HC-AB and HC- CD) have lower biotic integrity than Mammoth Creek (MC-H4).
- 3) The biotic integrity of Hot Creek increases below the confluence with Mammoth Creek (HC-H7 > HC-H2, HC-H3).

The three trends are summarized from Jellison *et al.* (2005a) in **Table A-4** with respect to each of the 11 metrics examined from data collected during 2000 through 2004.

Four of the twelve metrics strongly support ($p < 0.05$) the hypothesis that Hot Creek below the confluence with Mammoth Creek has lower biotic integrity than Mammoth Creek, while two give the opposite result and the other six metrics differ by too little or are too variable to provide evidence one way or the other. Six of twelve metrics strongly support the hypothesis that the hatchery springbrook reaches (monitoring sites HC-AB and HC-CD) have lower biotic integrity than Mammoth Creek (MC-H4). However, 3 and 2 metrics, respectively, significantly differed in the opposite direction to that hypothesized (HC-AB < MC-H4; and HC-CD < MC-H4). Metrics varied in both directions between Hot Creek above (H3 and H2) and below (H7) the

confluence with Mammoth Creek. However, twice as many metrics indicate an increase in biotic integrity below the confluence with Mammoth Creek (Jellison *et al.* 2005a).

In summary, evaluation of the metrics across all the years provides support for all three hypotheses related to Mammoth Creek presented by Jellison *et al.* (2005a).

4.2.3 Cluster analysis on 2004 bioassessment data

The clustering clearly indicates that the Mammoth Creek sites (and CD springbrook) are different from all the Hot Creek sites. Specifically, the cluster analysis indicates there are significant differences in the benthic macroinvertebrate communities sampled in Hot Creek above and below the confluence with Mammoth Creek. Multi-year across-site comparisons of the 11 BMI metrics included in the 2000 through 2004 annual NPDES monitoring reports suggest decreased biotic integrity below the hatchery, but are inconclusive due to contradictory signals from several metrics. In the absence of a robust and regionally tested Index of Biotic Integrity (IBI), it is problematic to assign weight to individual metrics. A regional index of biotic integrity employing 12 to 15 carefully tested BMI metrics is currently being developed but has not been sufficiently tested to know whether it can be usefully applied to this situation (Jellison *et al.* 2005a).

4.2.4 Multi-metric index

As noted in the previous sections, the springbrook communities are markedly different than other sites. There also are distinct differences in individual metrics between Mammoth Creek sites and those on Hot Creek below the confluence with Mammoth Creek. While the abundant tubificids at the lower sites yield a low score for this metric and decrease the multi-metric score, this is compensated for by increases in the scores of percent of sensitive EPT taxa, EPT index, and percent intolerant taxa (Jellison *et al.* 2005a). Thus, the multi-metric index highlights the fact that several of the metrics gave counter indications with respect to changes in biotic integrity and indicates the difficulty in interpreting these metrics (Jellison *et al.* 2005a).

Overall, the multi-metric analysis indicates that biotic integrity in Mammoth Creek is better than that of Hot Creek below the hatchery (i.e., upstream of Mammoth Creek) and is similar to that of Hot Creek below the confluence with Mammoth Creek. However, because the multi metric analysis was performed using data from one year, the results should be interpreted carefully. Specifically, the high degree of variability in many of the individual metrics utilized in the multi-metric analysis could affect the overall multi-metric index score at any individual site, thereby potentially skewing the results of the analysis.

5.0 CONCLUSIONS

The previously described CDFG interpretation of maintaining “good condition” addresses the macroinvertebrate community, and the term “fish” as defined in California Fish and Game Code Sections 5937 and 5946 includes both vertebrate and invertebrate aquatic life.

CH2M Hill (2000) reported that the aquatic invertebrate sampling conducted from 1992 to 1994 suggests that the aquatic invertebrate community is relatively healthy, being composed of a relatively large number of taxa (around 20), representing a number of different families (around

15), and with good representation of the more sensitive taxa within the EPT orders. Standard diversity measures indicate that the ecological health of the benthic invertebrate fauna did not change significantly between 1992 and 1994 (Vinson 1995 *in* CH2M Hill 2000).

Subsequent BMI sampling was conducted as a requirement of the Hot Creek Hatchery NPDES permit. Sampling was conducted at one Mammoth Creek site upstream of the confluence with Hot Creek and at multiple sites in Hot Creek from 2000 through 2004. Two additional sites on Mammoth Creek were sampled during 2004 only.

Examination of individual metrics from all years provides variable results. Specifically, some metrics indicate that Mammoth Creek has better conditions than Hot Creek in general, while others indicate better conditions in Hot Creek. Additionally, some metrics display high degrees of spatial and/or temporal variability, while others are consistent among years and sites. However, a statistical examination of the data indicate that four of the twelve metrics examined strongly support ($p < 0.05$) the hypothesis that Hot Creek below the confluence with Mammoth Creek has lower biotic integrity than Mammoth Creek, while two give the opposite result and the other six metrics differ by too little or are too variable to provide evidence one way or the other. Six of twelve metrics strongly support the hypothesis that the Hot Creek Hatchery springbrook inflows have lower biotic integrity than Mammoth Creek. Metrics varied in both directions between Hot Creek above and below the confluence with Mammoth Creek. However, twice as many metrics indicate an increase in biotic integrity below the confluence with Mammoth Creek (Jellison *et al.* 2005a).

A multi-metric evaluation of data obtained from 2004 sampling events indicated that biotic integrity in Mammoth Creek is better than that of Hot Creek below the hatchery (i.e., upstream of Mammoth Creek) and is similar to that of Hot Creek below the confluence with Mammoth Creek. However, because the multi-metric analysis was performed using data from one year, the results should be interpreted carefully. Specifically, the high degree of variability in many of the individual metrics utilized in the multi-metric analysis could affect the overall multi-metric index score at any individual site, thereby potentially skewing the results of the analysis.

Overall, analysis of available data indicate that, over time and among sites, Mammoth Creek benthic macroinvertebrates are in good condition in comparison to available data obtained from a similar stream.

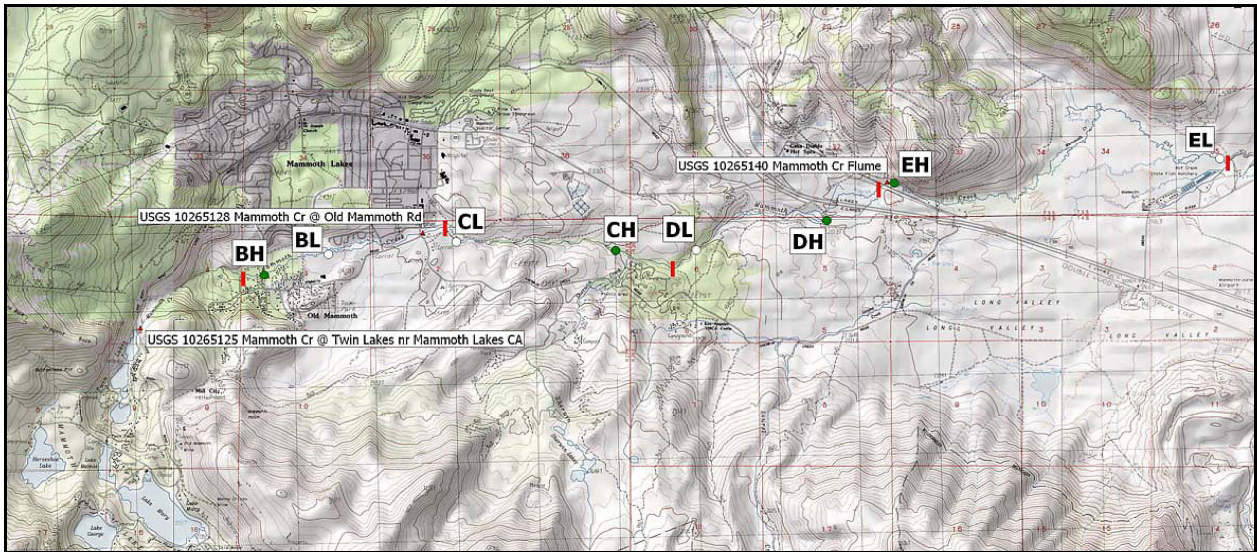


Figure A-1. BMI sample collection locations in Mammoth Creek during 1992 through 1994.

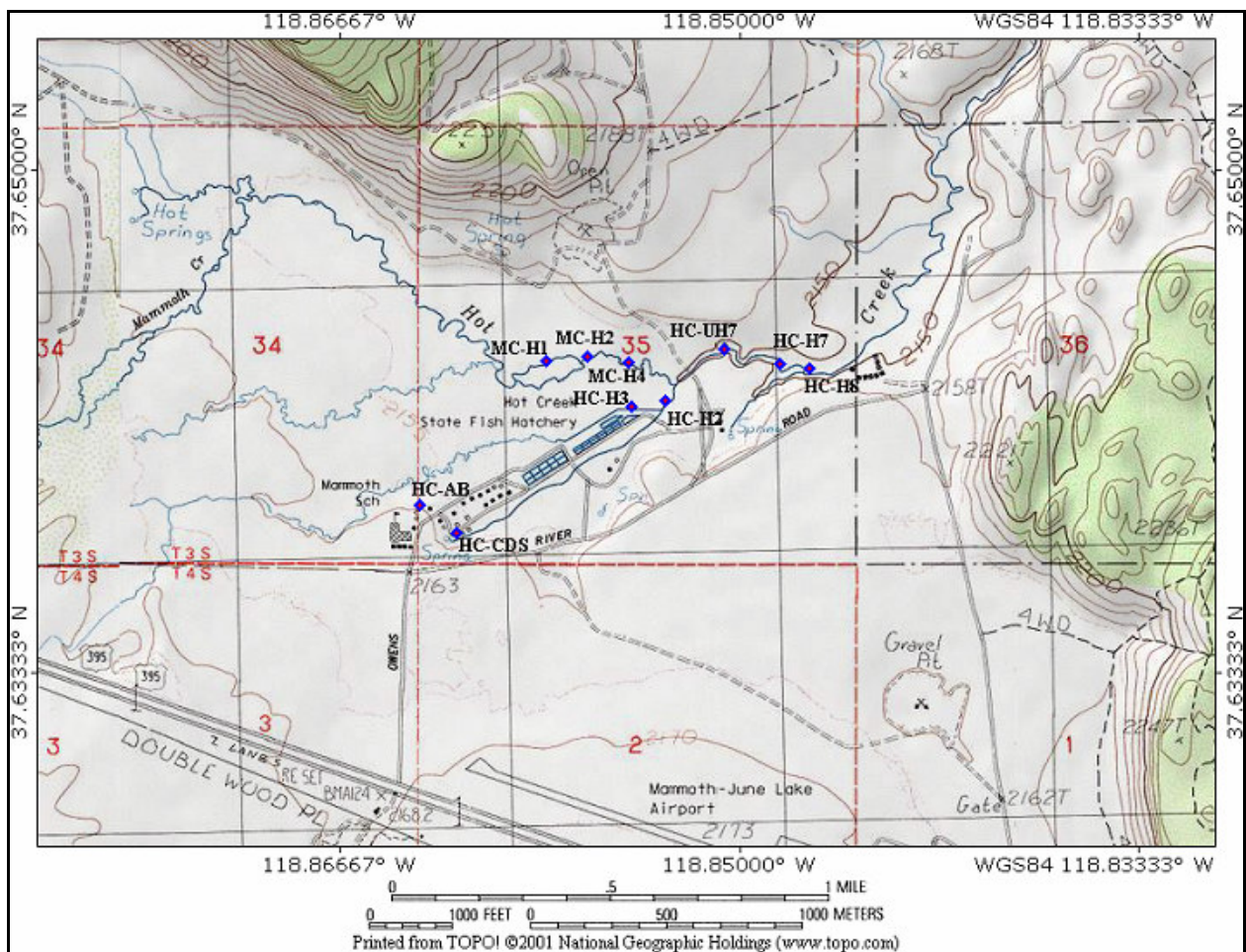


Figure A-2. BMI sample collection locations in Mammoth and Hot Creeks during 2000 through 2004 (from Jellison *et al.* 2005a).

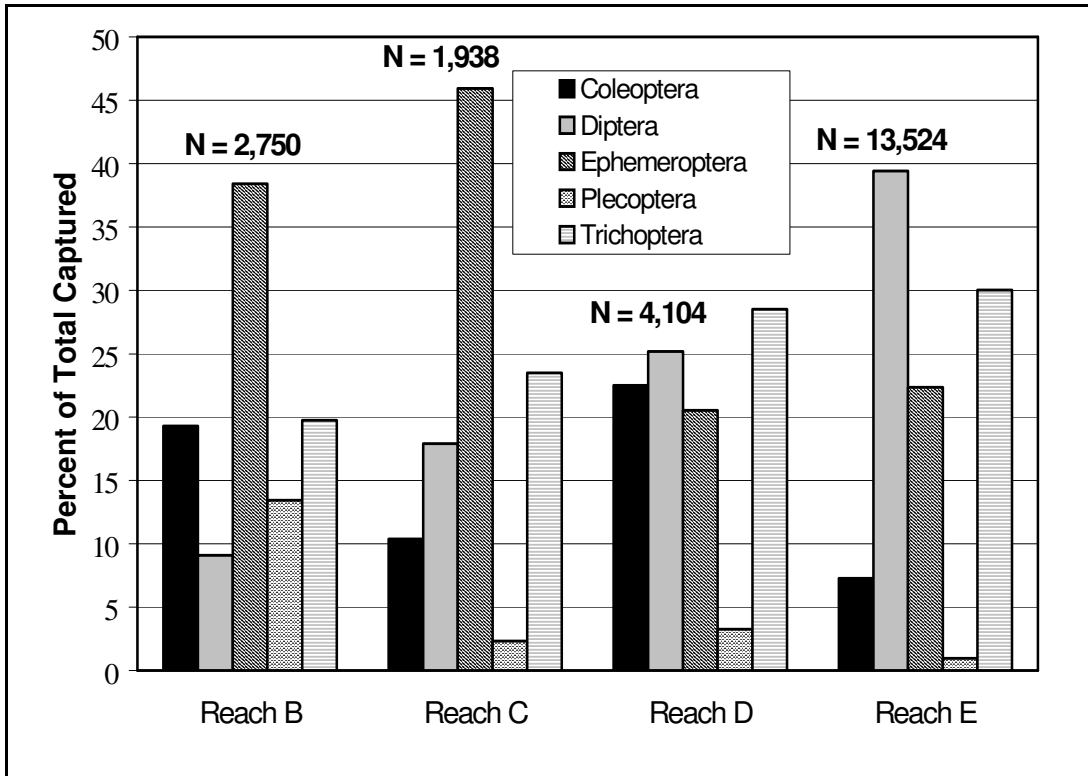


Figure A-3. Relative abundance of *Coleoptera* (beetles), *Diptera* (flies, gnats, midges, and mosquitoes), *Ephemeroptera* (mayflies), *Plecoptera* (stoneflies), and *Trichoptera* (caddisflies) in Reaches B through E of Mammoth Creek. Data are compiled from Vinson (1995) and represent the actual number of organisms collected (from CH2M Hill 2000).

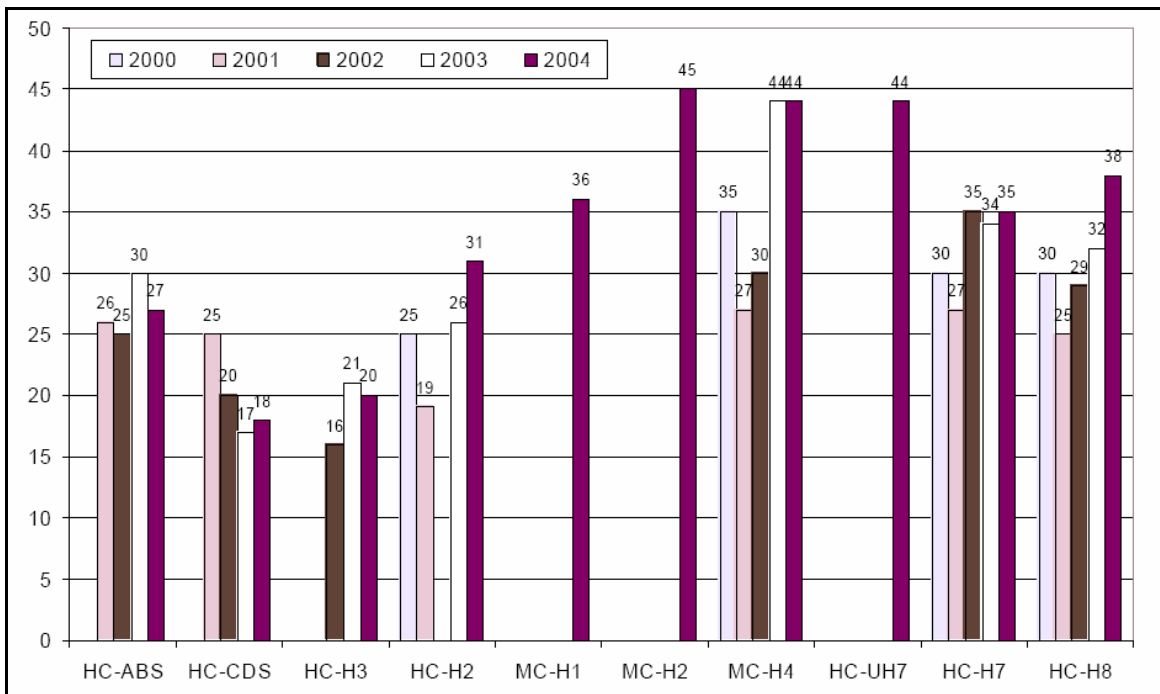


Figure A-4. Cumulative taxonomic richness in Mammoth Creek and Hot Creek during 2000 through 2004 (from Jellison *et al.* 2005a).

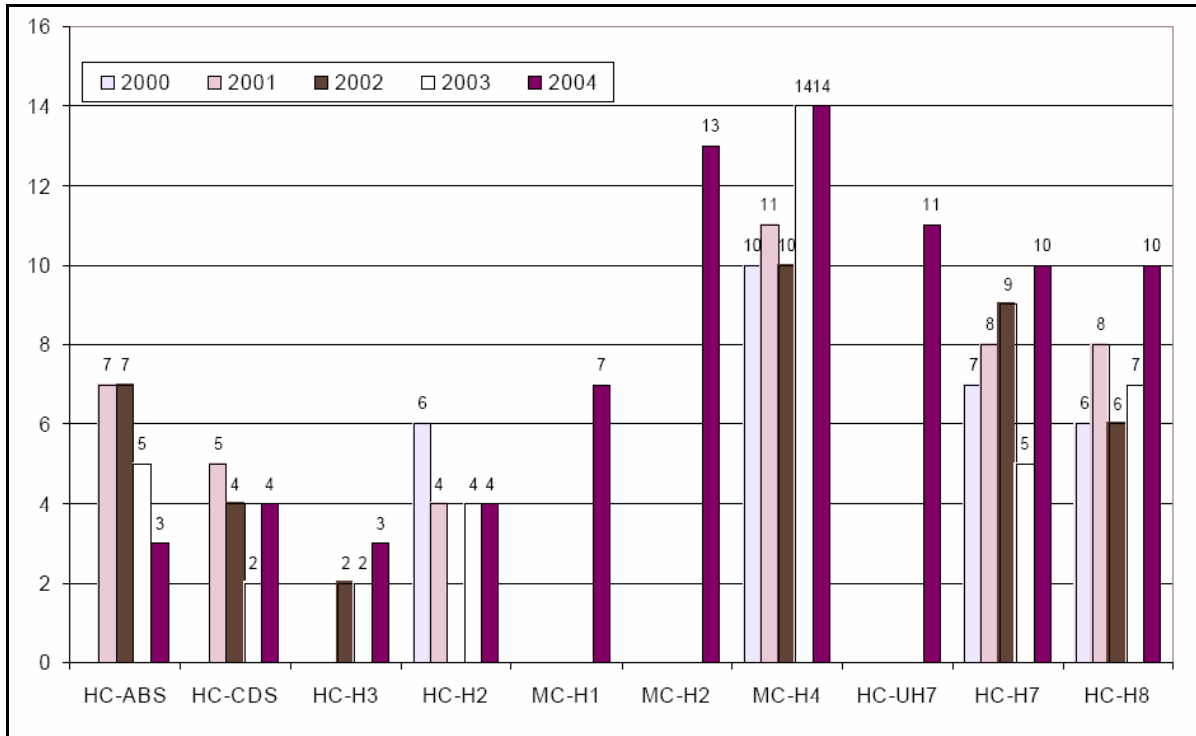


Figure A-5. Cumulative EPT taxonomic richness in Mammoth Creek and Hot Creek during 2000 through 2004 (from Jellison *et al.* 2005a).

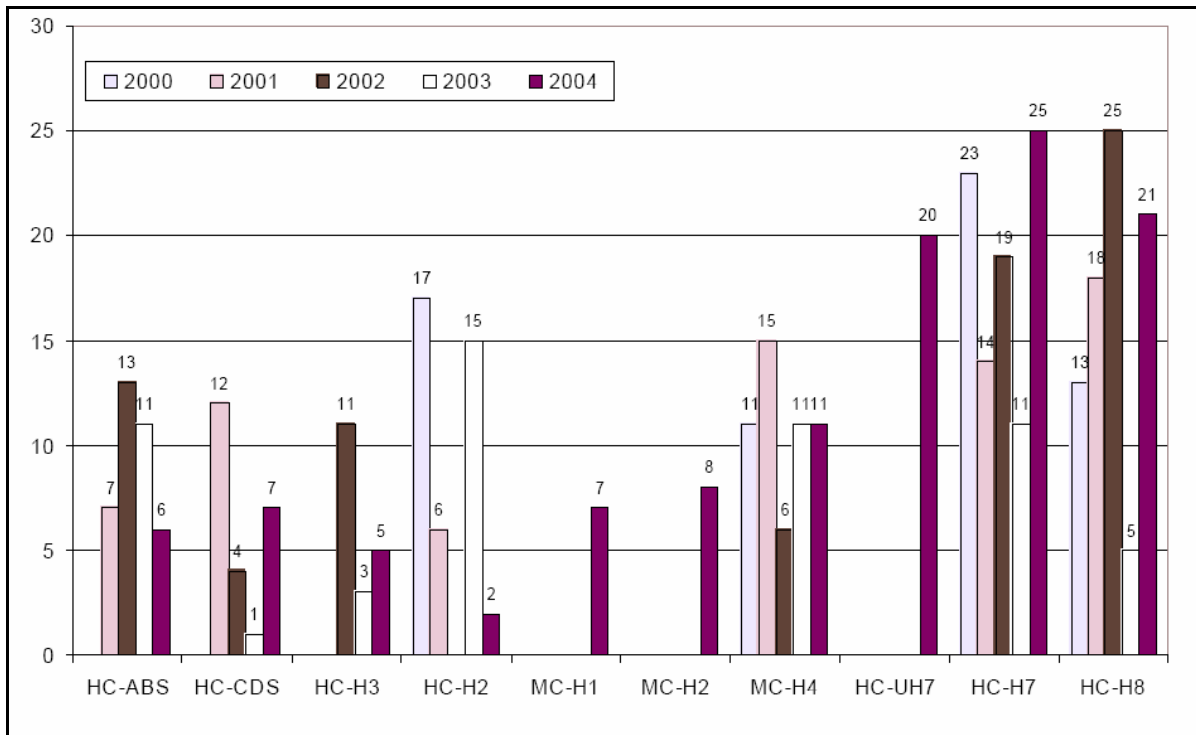


Figure A-6. EPT Index (percentage of EPT taxa) in Mammoth Creek and Hot Creek during 2000 through 2004 (from Jellison *et al.* 2005a).

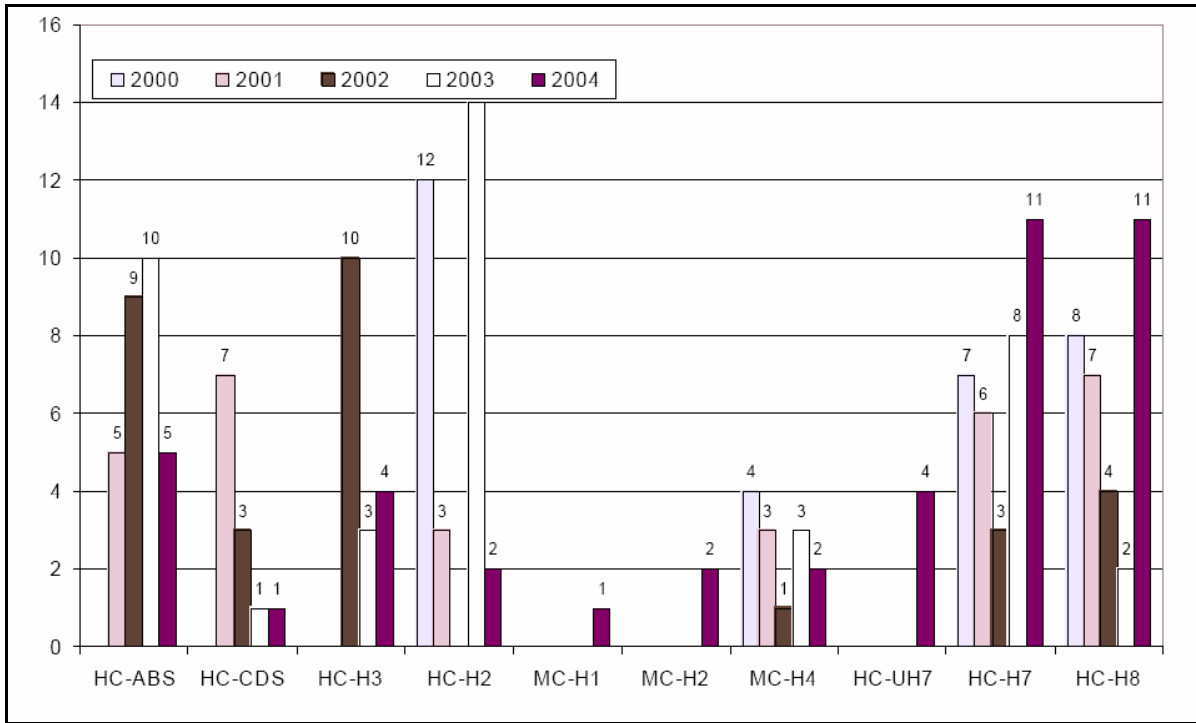


Figure A-7. Sensitive EPT index (percentage of sensitive EPT taxa) in Mammoth Creek and Hot Creek during 2000 through 2004 (from Jellison *et al.* 2005a).

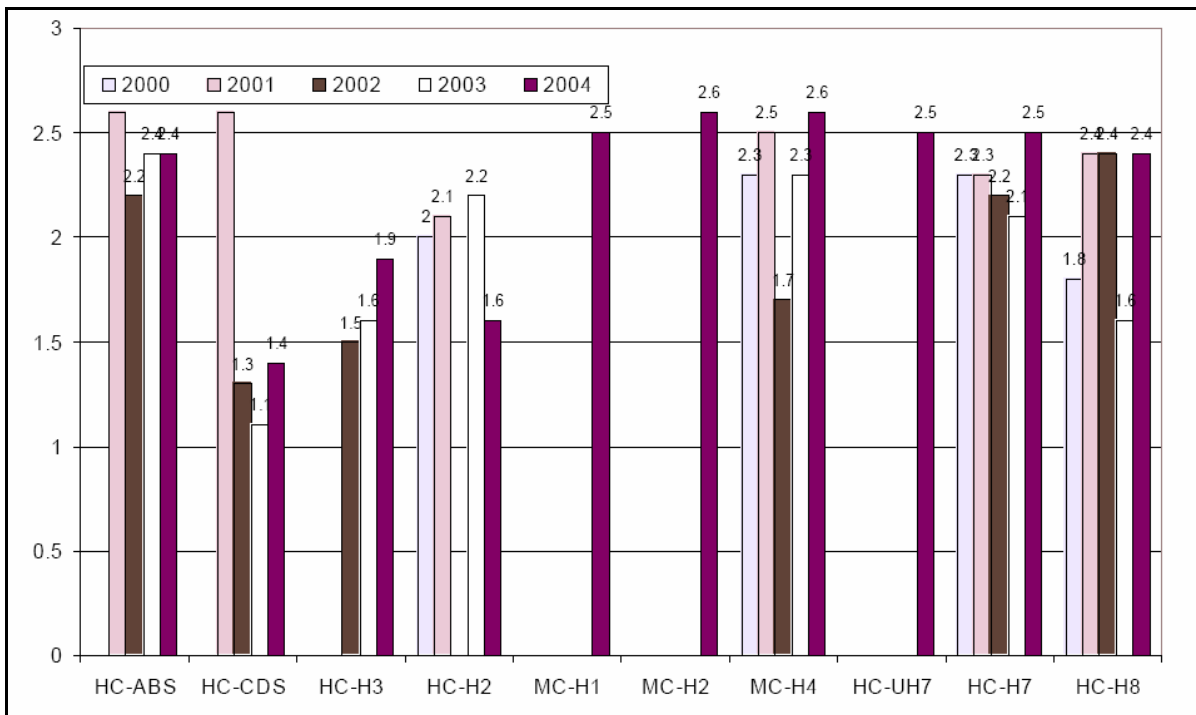


Figure A-8. Shannon diversity index values in Mammoth Creek and Hot Creek during 2000 through 2004 (from Jellison *et al.* 2005a).

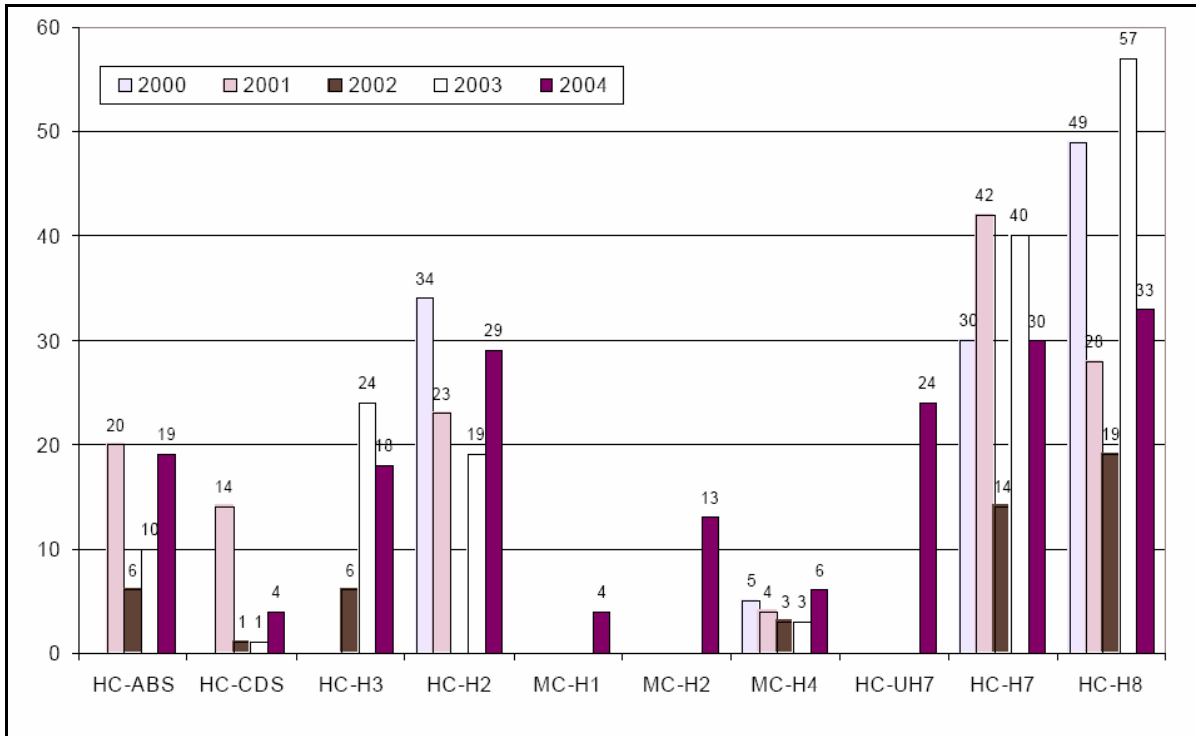


Figure A-9. Percent of organisms in the family Tubificidae in Mammoth Creek and Hot Creek during 2000 through 2004 (from Jellison *et al.* 2005a).

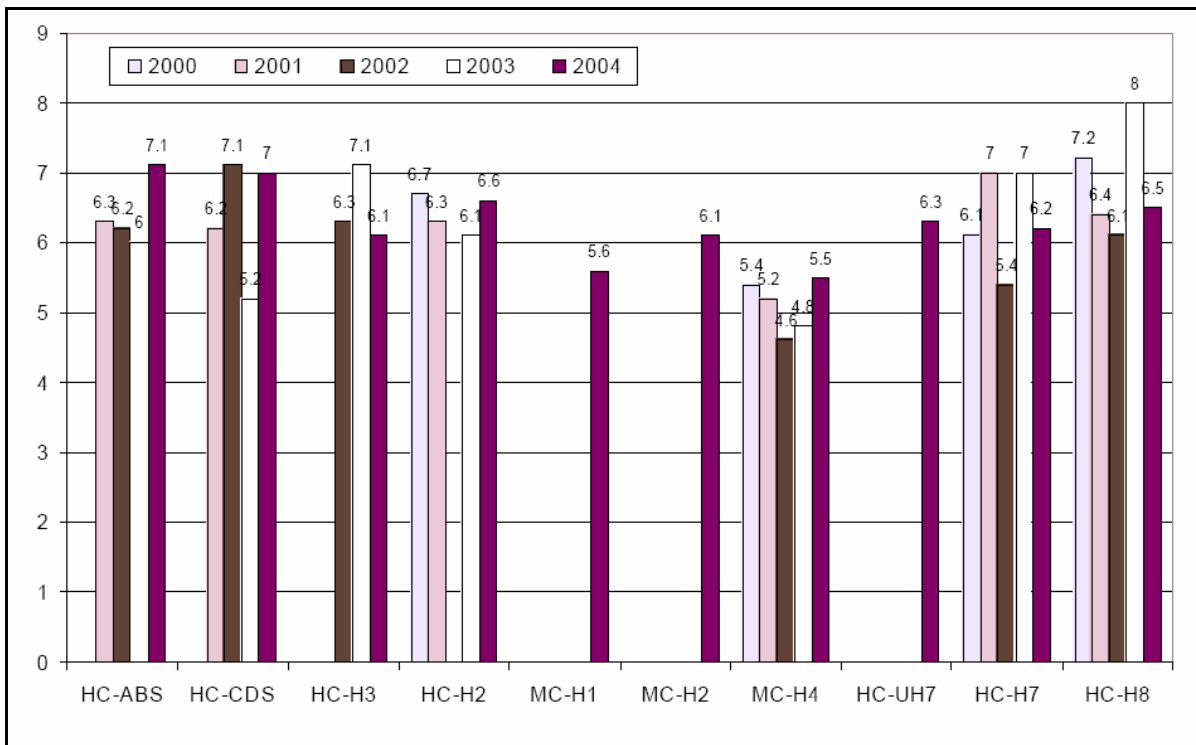


Figure A-10. Mean BMI tolerance values in Mammoth Creek and Hot Creek during 2000 through 2004 (from Jellison *et al.* 2005a).

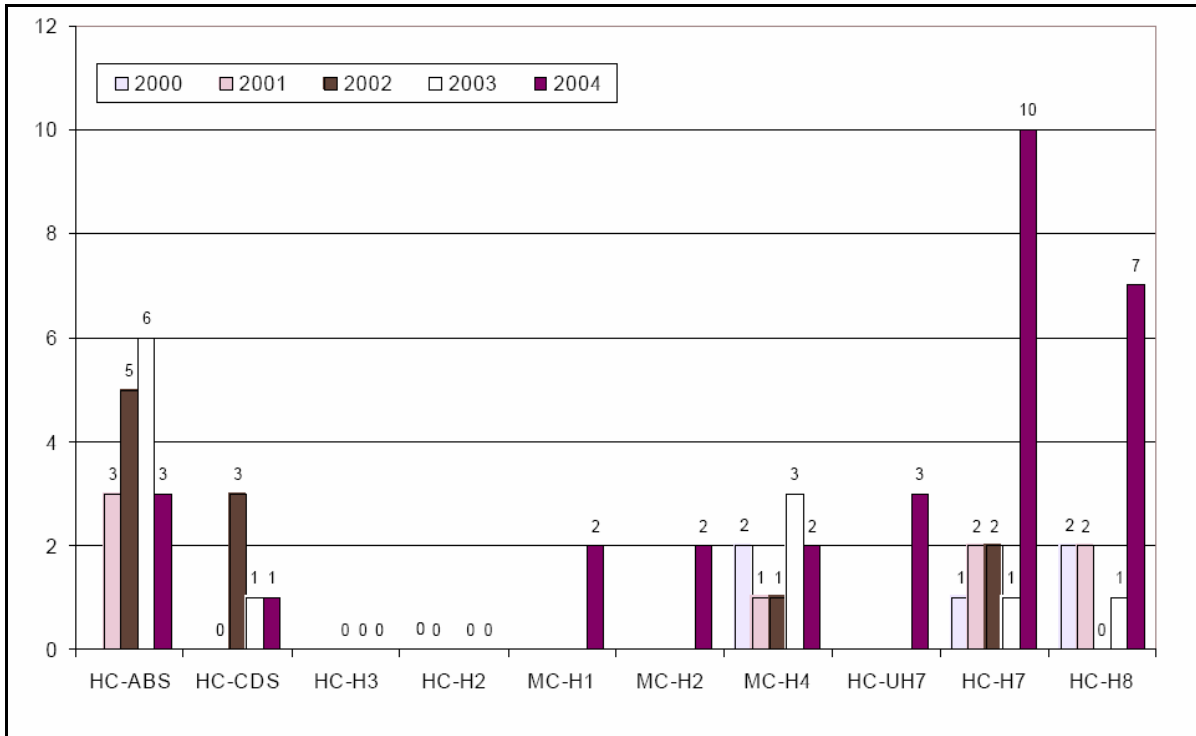


Figure A-11. Percentage of intolerant BMI tax in Mammoth Creek and Hot Creek during 2000 through 2004 (from Jellison *et al.* 2005a).

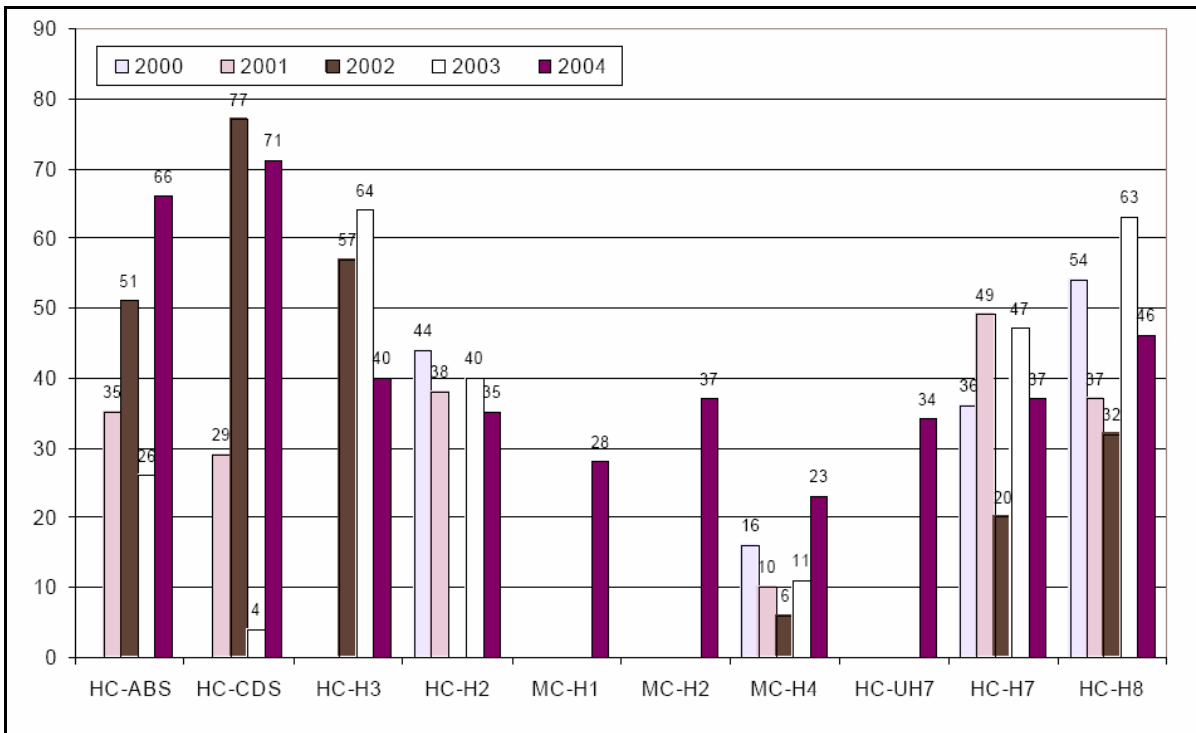


Figure A-12. Percentage of tolerant BMI tax in Mammoth Creek and Hot Creek during 2000 through 2004 (from Jellison *et al.* 2005a).

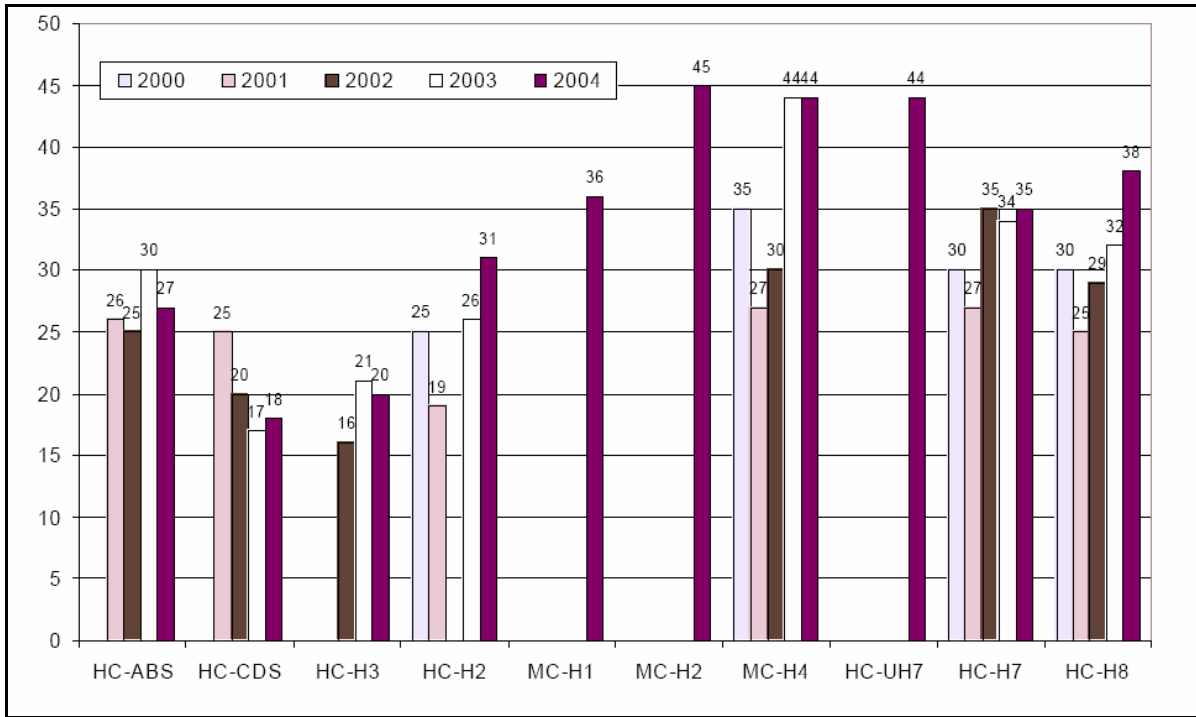


Figure A-13. Percentage of dominant BMI tax in Mammoth Creek and Hot Creek during 2000 through 2004 (from Jellison *et al.* 2005a).

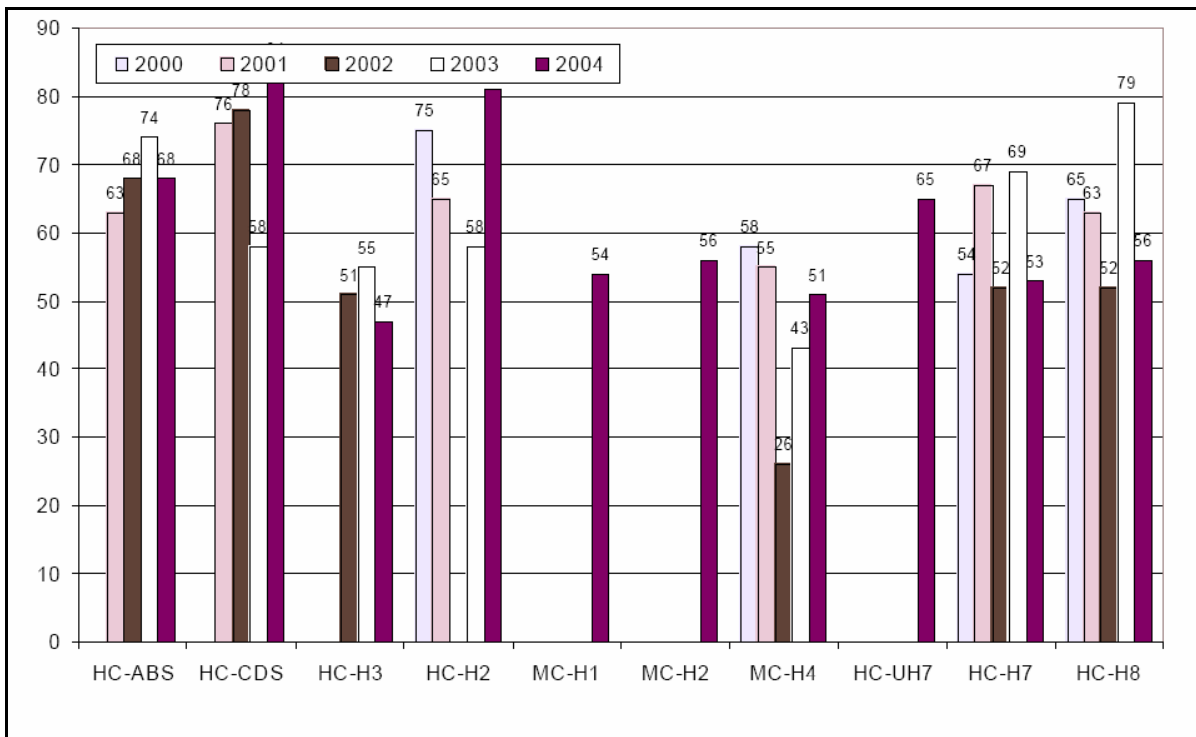


Figure A-14. Percentage of collector-gatherers in Mammoth Creek and Hot Creek during 2000 through 2004 (from Jellison *et al.* 2005a).

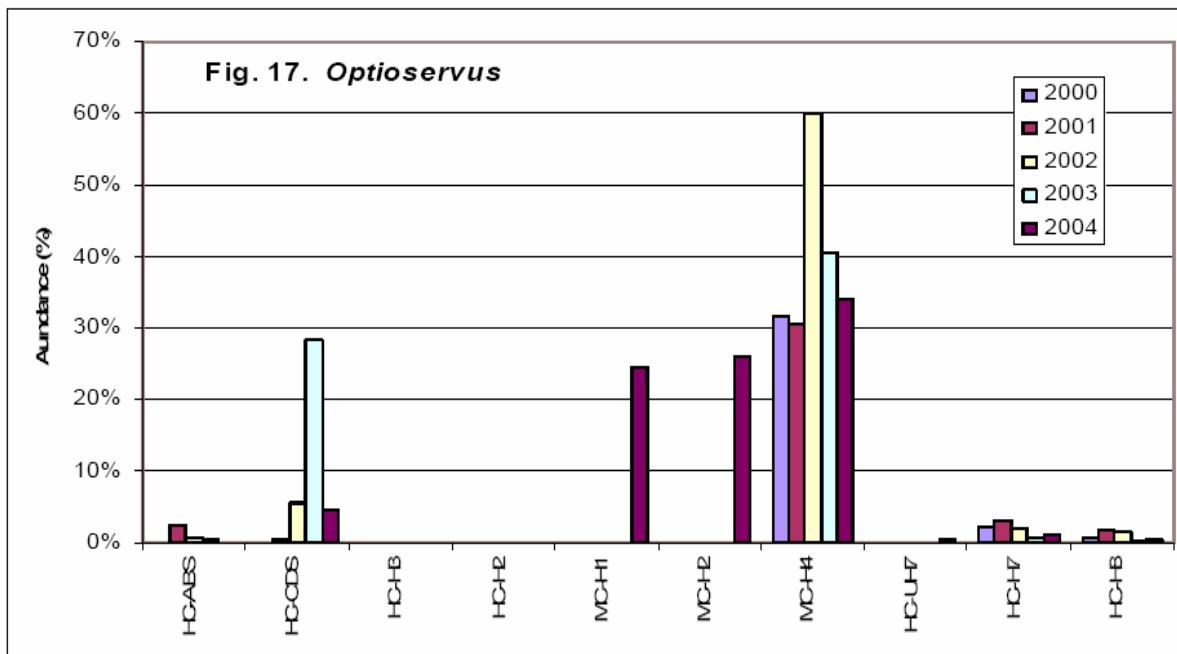


Figure A-15. Relative abundance (%) of riffle beetles (*Optioservus sp.*) in Mammoth Creek and Hot Creek during 2000 through 2004 (from Jellison *et al.* 2005a).

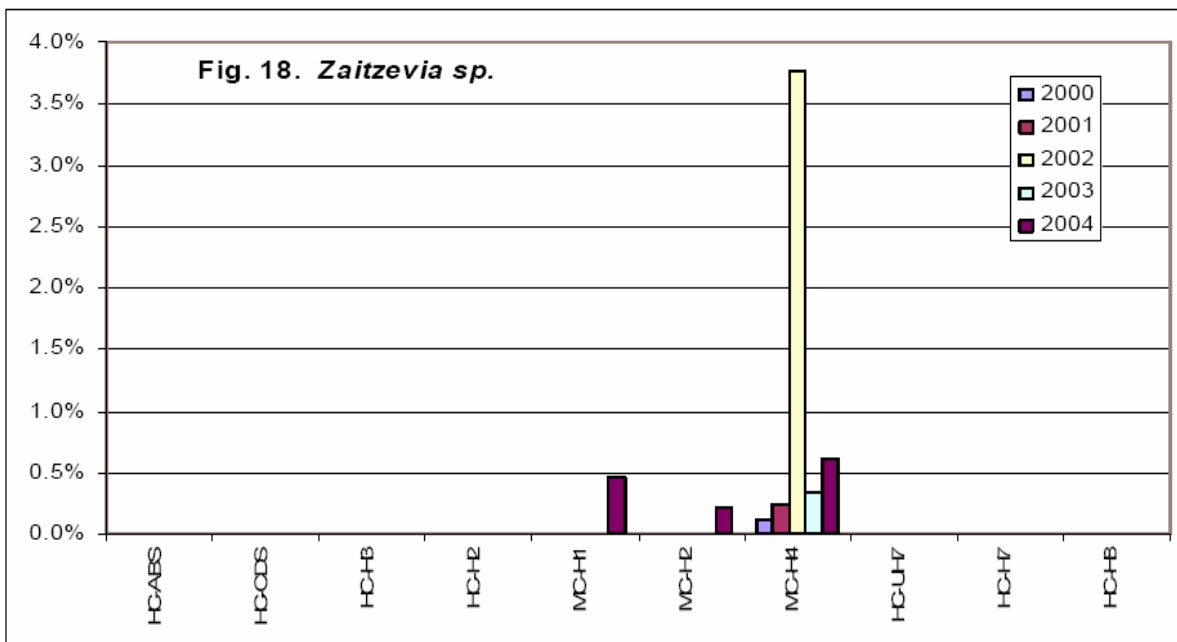


Figure A-16. Relative abundance (%) of riffle beetles (*Zaitzevia sp.*) in Mammoth Creek and Hot Creek during 2000 through 2004 (from Jellison *et al.* 2005a).

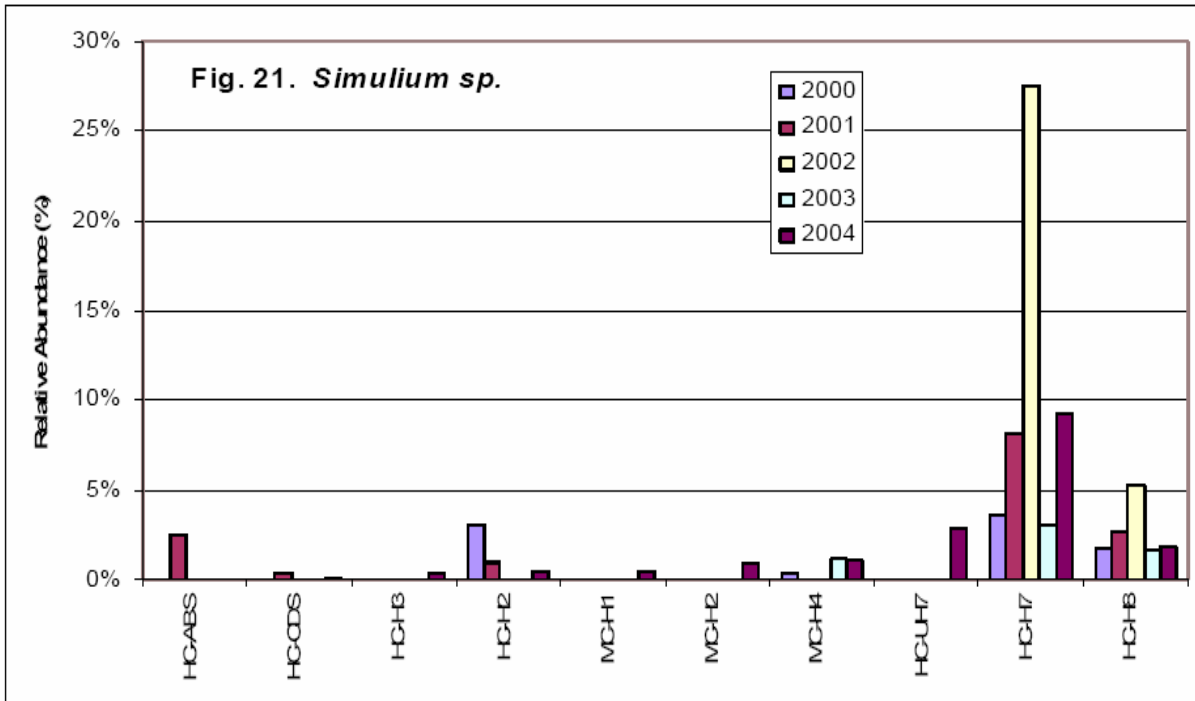


Figure A-17. Relative abundance (%) of black flies (*Simulium sp.*) in Mammoth Creek and Hot Creek during 2000 through 2004 (from Jellison *et al.* 2005a).

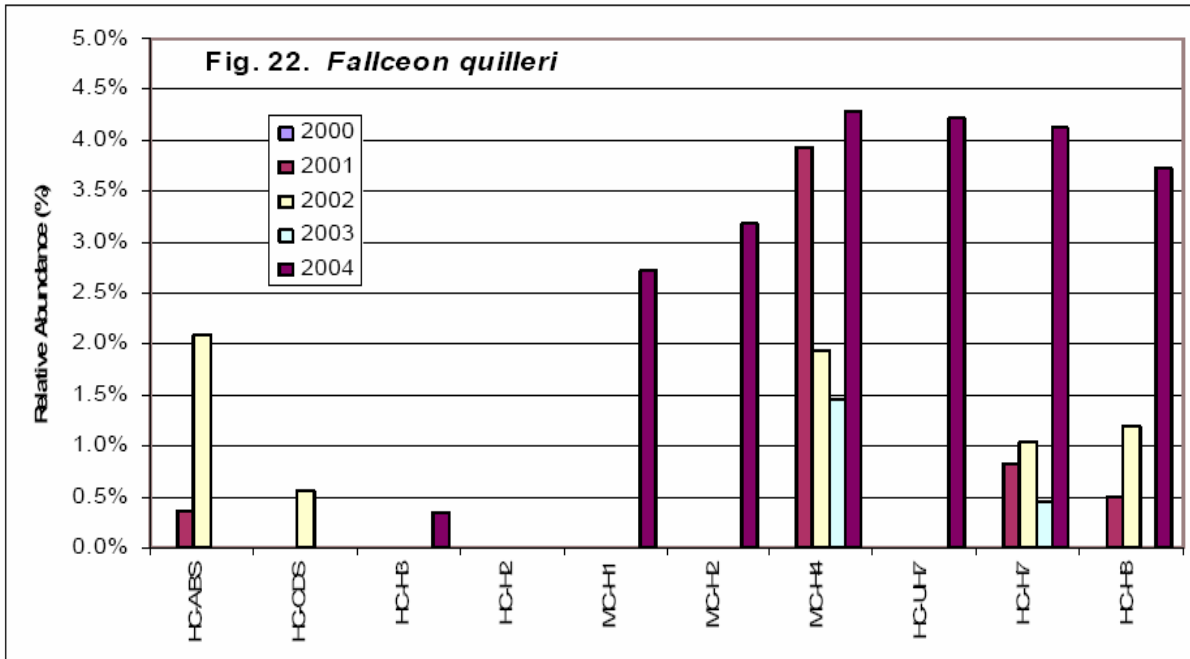


Figure A-18. Relative abundance (%) of the mayfly *Fallceon quilleri* in Mammoth Creek and Hot Creek during 2000 through 2004 (from Jellison *et al.* 2005a).

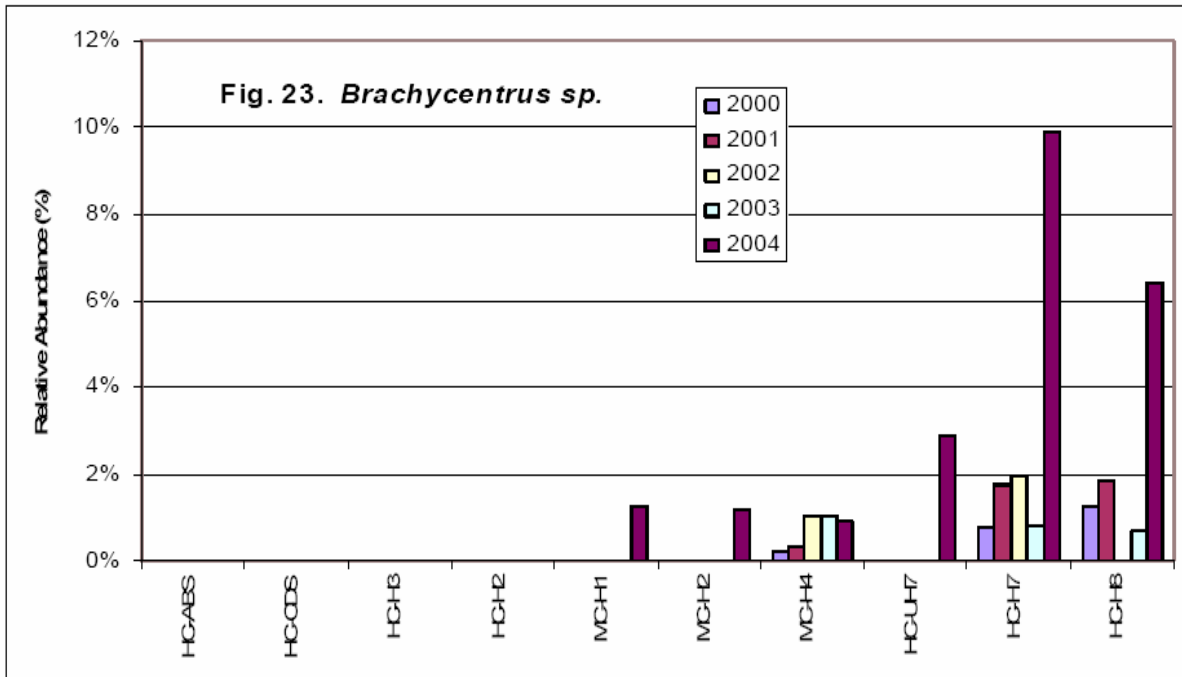


Figure A-19. Relative abundance (%) of caddisflies in the genus *Brachycentrus* in Mammoth Creek and Hot Creek during 2000 through 2004 (from Jellison *et al.* 2005a).

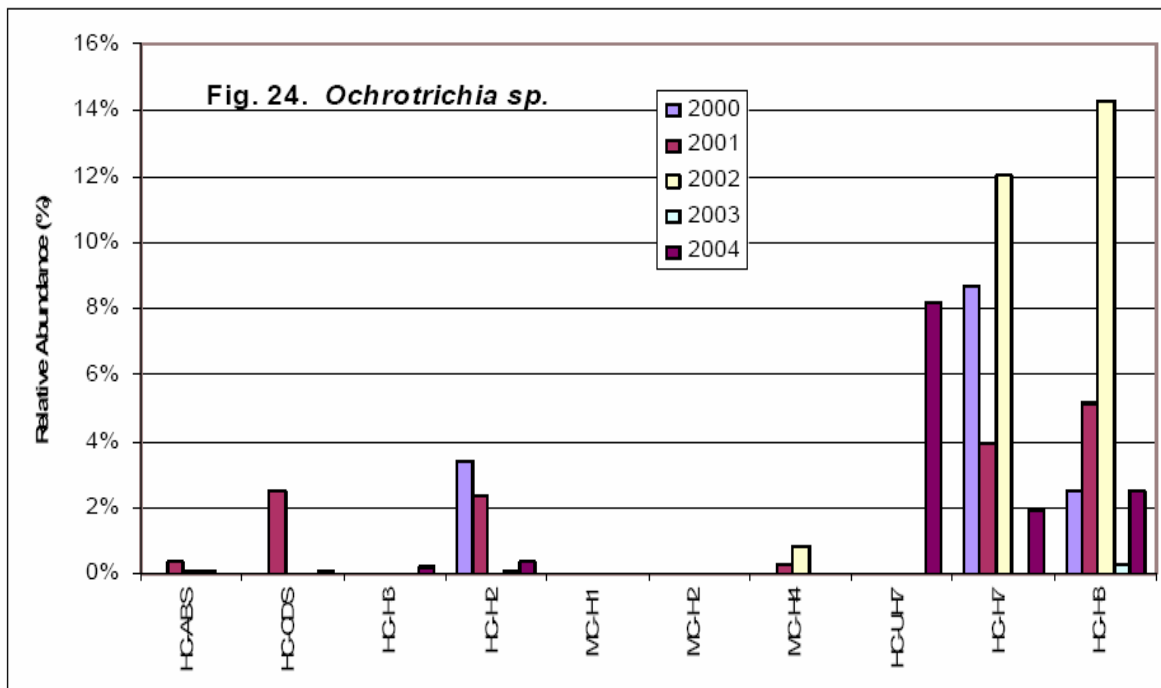


Figure A-20. Relative abundance (%) of micro-caddisflies in the genus *Ochrotrichia* in Mammoth Creek and Hot Creek during 2000 through 2004 (from Jellison *et al.* 2005a).

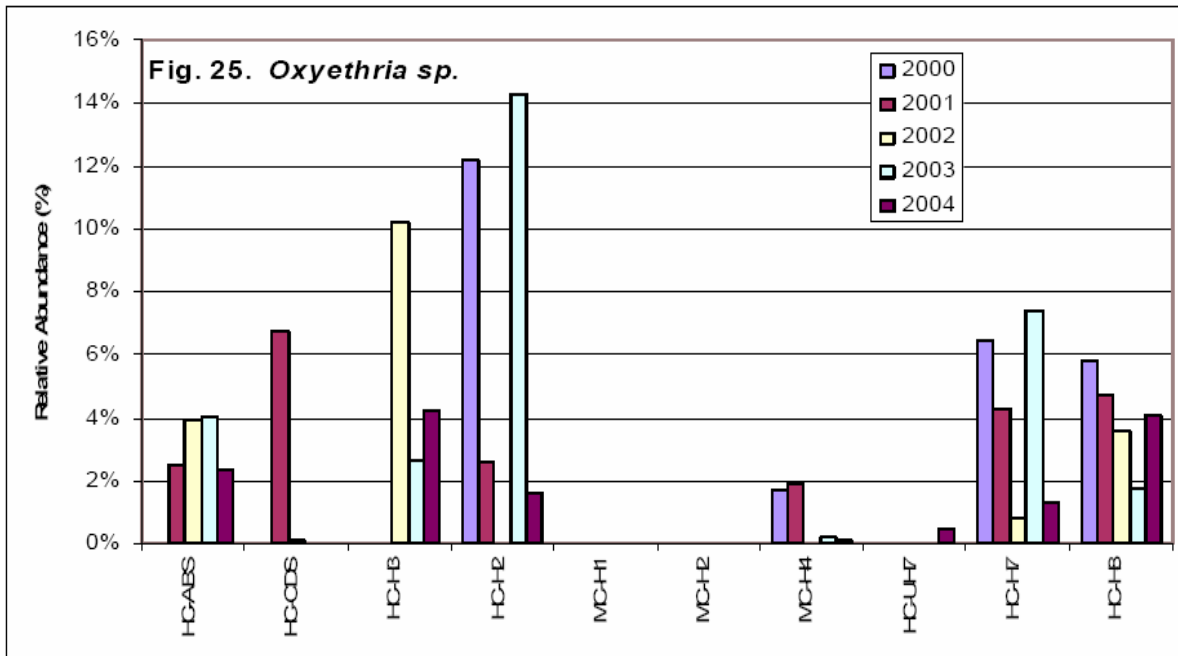


Figure A-21. Relative abundance (%) of micro-caddisflies in the genus *Oxyethria* in Mammoth Creek and Hot Creek during 2000 through 2004 (from Jellison *et al.* 2005a).

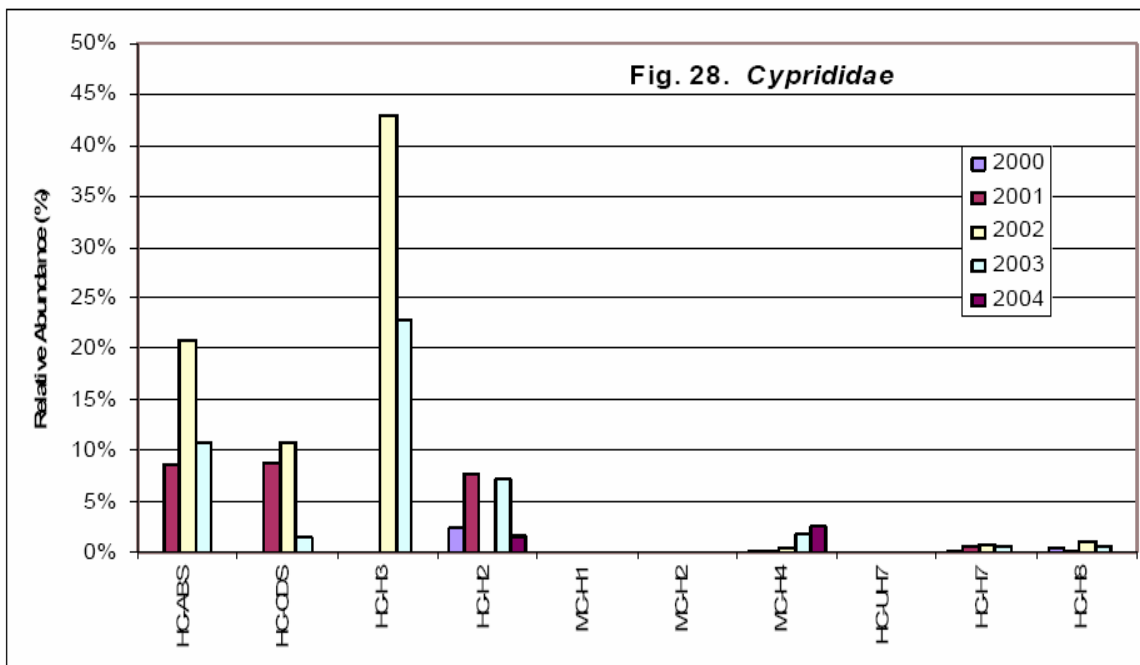


Figure A-22. Relative abundance (%) of ostracods in the genus *Cyprididae* in Mammoth Creek and Hot Creek during 2000 through 2004 (from Jellison *et al.* 2005a).

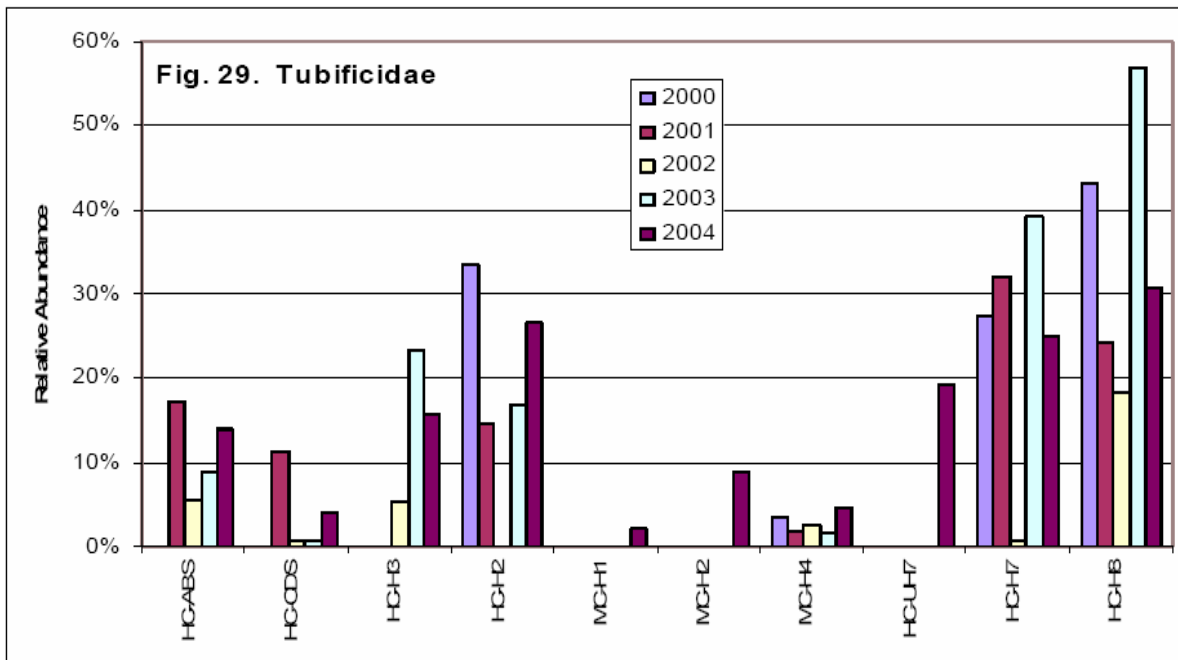


Figure A-23. Relative abundance (%) of tubificid worms in the family Tubificidae Mammoth Creek and Hot Creek during 2000 through 2004 (from Jellison *et al.* 2005a).

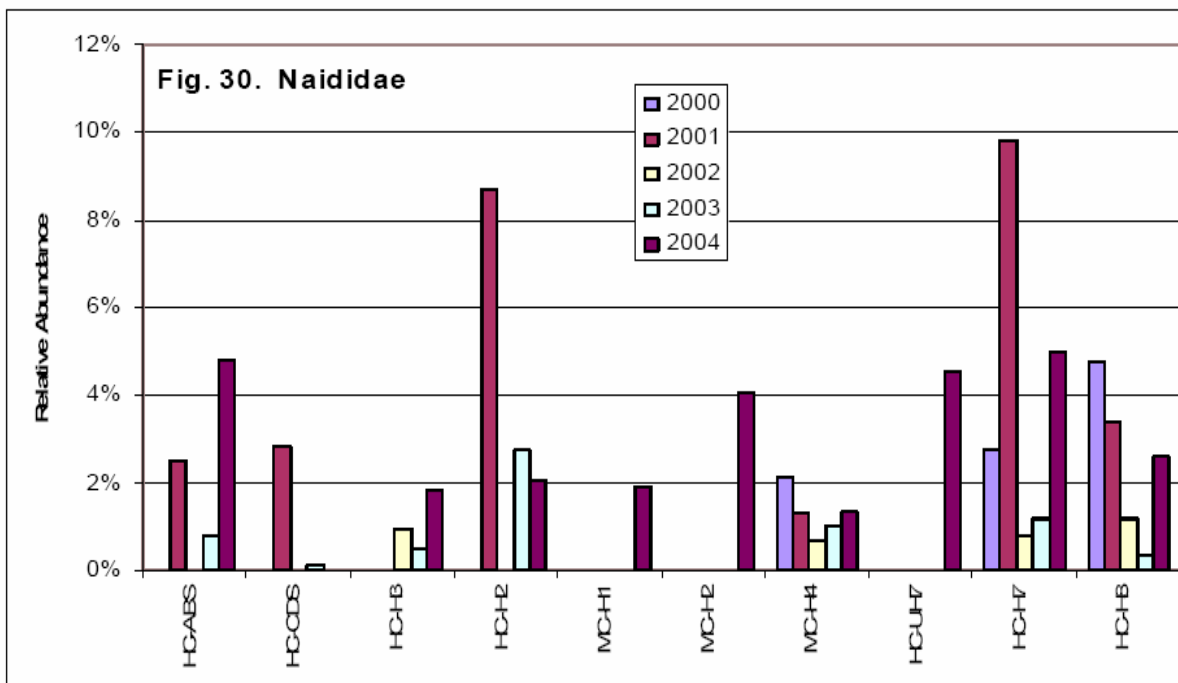


Figure A-24. Relative abundance (%) of tubificid worms in the family Naididae in Mammoth Creek and Hot Creek During 2000 Through 2004 (from Jellison *et al.* 2005a).

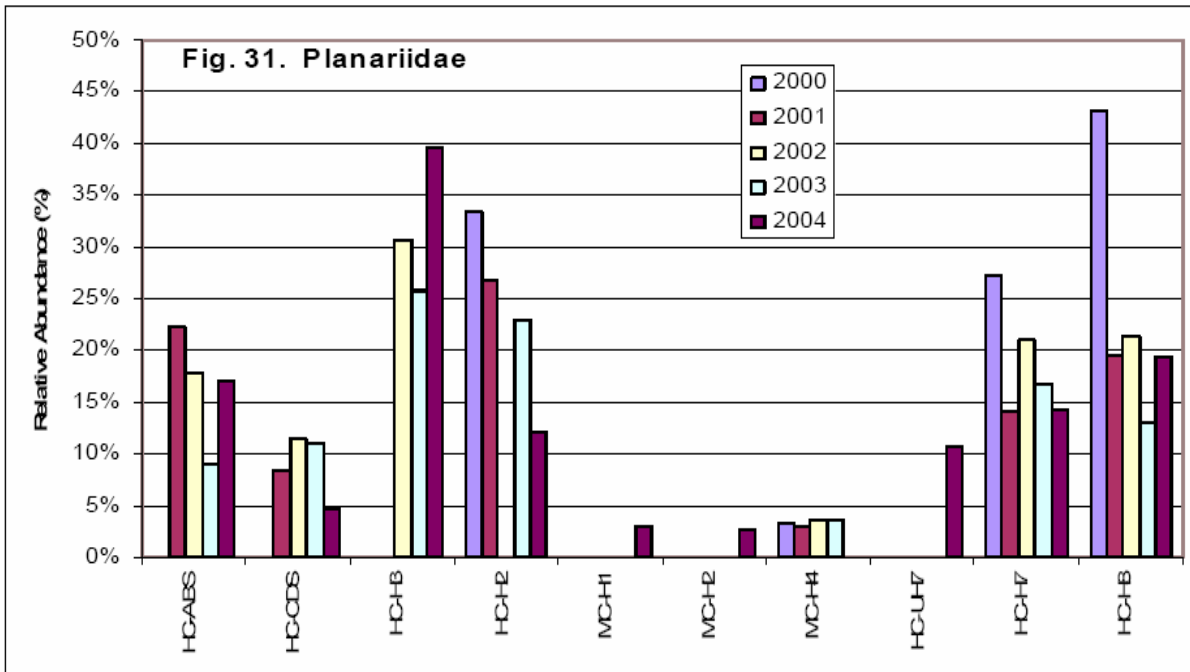


Figure A-25. Relative abundance (%) of planarid flatworms in Mammoth Creek and Hot Creek during 2000 through 2004 (from Jellison *et al.* 2005a).

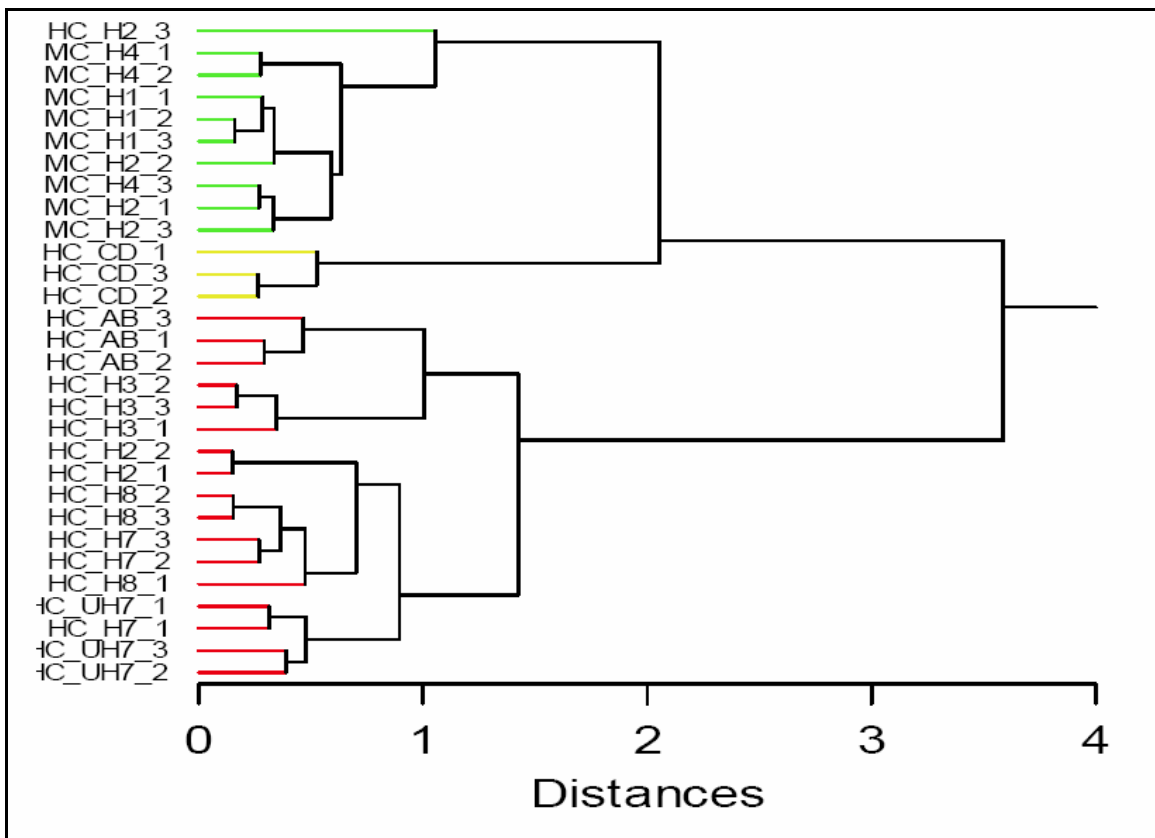


Figure A-26. Cluster analysis of 2004 BMI relative abundance data obtained from all transects (from Jellison *et al.* 2005a).

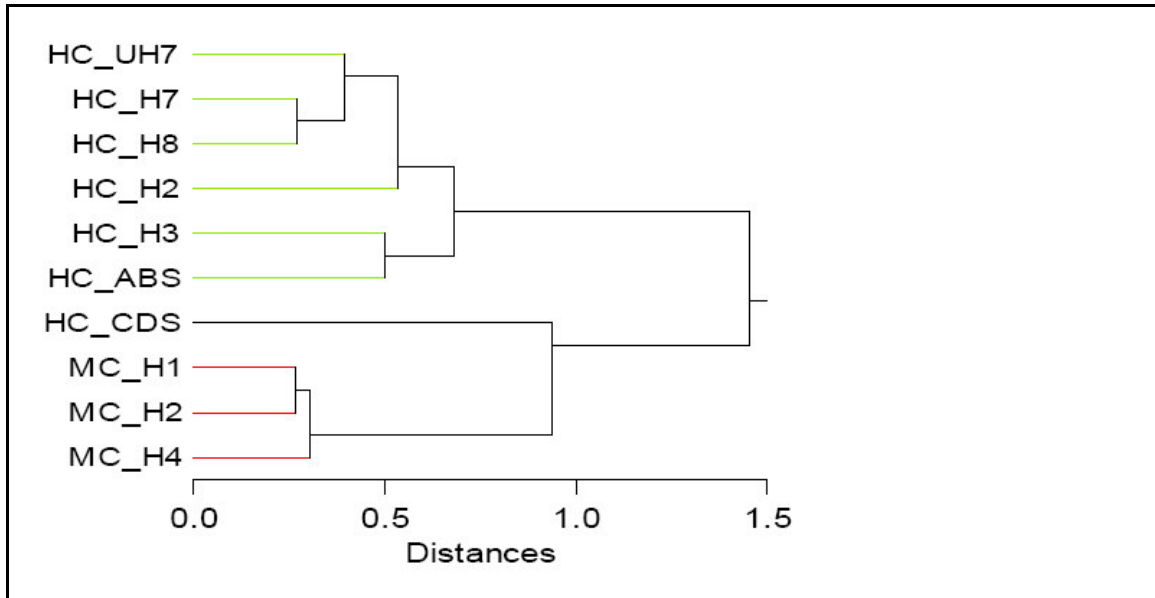


Figure A-27. Cluster analysis of 2004 BMI relative abundance data with replicate transects averaged (from Jellison *et al.* 2005a).

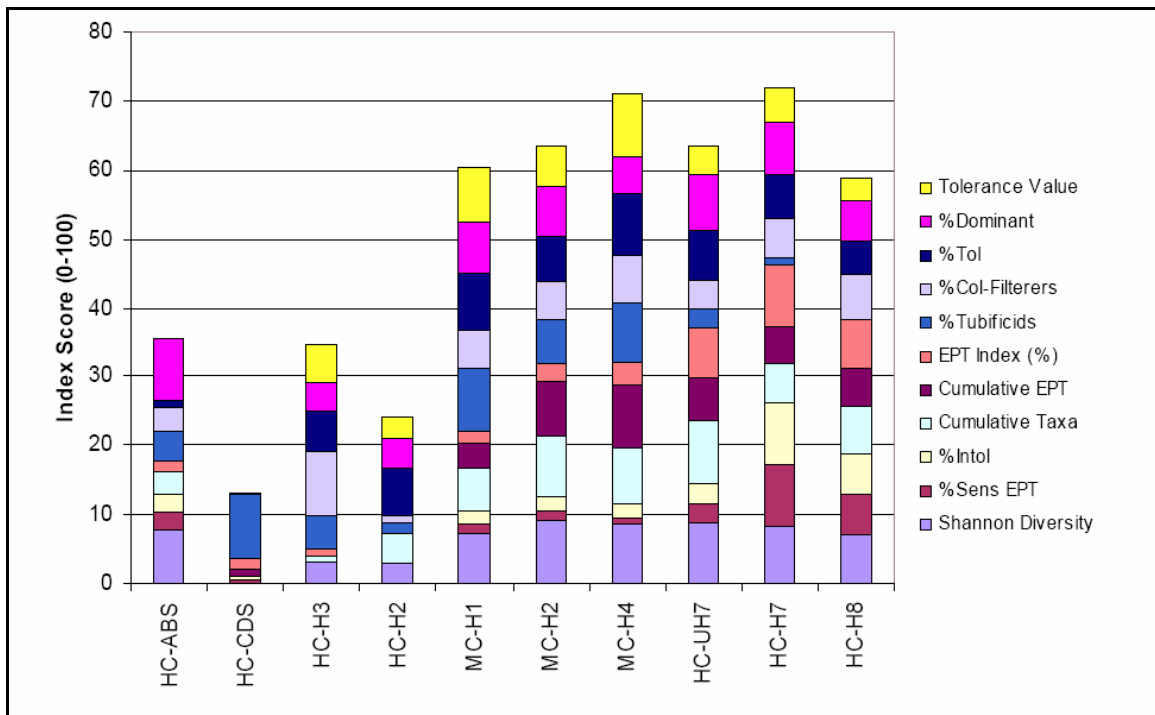


Figure A-28. Hot Creek multi-metric index (from Jellison *et al.* 2005a).

Table A-1. Sampling site codes, GPS coordinates, and site descriptions for the ten monitoring stations where biological and physical/habitat assessments were conducted during 2000 through 2004 (from CDFG 2004).

Sampling Site Codes	GPS Coordinates	Site Description
Sites Used in Above Hatchery and Below Hatchery Comparison		
HC-ABS	N 37° 38' 20.2" W 118° 51' 44.8"	AB Spring upstream of the Hatchery
HC-CDS	N 37° 38' 16.9" W 118° 51' 39.3"	CD Spring upstream of the Hatchery
HC-H3	N 37° 38' 32.1" W 118° 51' 13.0"	Hot Creek below Settling Pond Outflow
HC-H2	N 37° 38' 32.8" W 118° 51' 08.0"	Hot Creek below Hatchery
Sites Used in Mammoth Creek and Below Hatchery Comparison		
MC-H1	N 37° 38' 37.5" W 118° 51' 25.7"	Mammoth Creek above MC-H2
MC-H2	N 37° 38' 38.0" W 118° 51' 19.7"	Mammoth Creek above MC-H4
MC-H4	N 37° 38' 37.4" W 118° 51' 13.4"	Mammoth Creek near confluence with Hot Creek
HC-UH7	N 37° 38' 38.9" W 118° 50' 59.0"	Hot Creek above HC-H7
HC-H7	N 37° 38' 37.1" W 118° 50' 50.8"	Hot Creek above Brood Pond Discharge
HC-H8	N 37° 38' 36.6" W 118° 50' 46.2"	Hot Creek below Brood Pond Discharge

Table A-2. BMI sampling sites in Mammoth Creek and Hot Creek during 2000 through 2004 (from Jellison *et al.* 2005a).

Site Code	Site Description	Years Sampled				
		2000	2001	2002	2003	2004
HC-ABS	AB springbrook upstream of the Hatchery		X	X	X	X
HC-CDS	CD springbrook upstream of the Hatchery		X	X	X	X
HC-H3	Hot Creek below Settling Pond#1 Outflow			X	X	X
HC-H2	Hot Creek below hatchery and settling ponds	X	X		X	X
MC-H1	Mammoth Creek above MC-H2					X
MC-H2	Mammoth Creek above MC-H4					X
MC-H4	Mammoth Creek near confluence with Hot Creek	X	X	X	X	X
HC-UH7	Hot Creek immediately below confluence with Mammoth Creek					X
HC-H7	Hot Creek above Hatchery II Discharge	X	X	X	X	X
HC-H8	Hot Creek below Hatchery II Discharge	X	X	X	X	X

Table A-3. Bioassessment metrics used to describe characteristics of the benthic macroinvertebrate community for the ten monitoring stations where biological and physical/habitat assessments were conducted.

BMI Metric	Description	Response to Impairment
Richness Measures		
Taxa Richness	Total number of individual taxa	Decrease
EPT Taxa	Number of taxa in the Ephemeroptera (mayfly), Plecoptera (stonefly) and Trichoptera (caddisfly) insect orders	Decrease
Composition Measures		
EPT Index (%)	Percent composition of mayfly, stonefly and caddisfly larvae	Decrease
Sensitive EPT Index (%)	Percent composition of mayfly, stonefly and caddisfly larvae with tolerance values between 0 and 3	Decrease
Shannon Diversity Index	General measure of sample diversity that incorporates richness and evenness (Shannon and Weaver 1963)	Decrease
Percent Tubificida	Percent composition of the tolerant oligochaet order Tubificida	Increase
Tolerance/Intolerance Measures		
Tolerance Value	Value between 0 and 10 weighted for abundance of individuals designated as pollution tolerant (higher values) or intolerant (lower values)	Increase
Percent Intolerant Organisms	Percent of organisms in sample that are highly intolerant to impairment as indicated by a tolerance value of 0, 1 or 2	Decrease
Percent Tolerant Organisms	Percent of organisms in sample that are highly tolerant to impairment as indicated by a tolerance value of 8, 9 or 10	Increase
Percent Dominant Taxa	Percent composition of the single most abundant taxon	Increase
Functional Feeding Groups (FFG)		
Percent Collectors	Percent of macrobenthos that collect or gather fine particulate matter	Increase
Percent Filterers	Percent of macrobenthos that filter fine particulate matter	Increase
Percent Grazers	Percent of macrobenthos that graze upon periphyton	Variable
Percent Predators	Percent of macrobenthos that feed on other organisms	Variable
Percent Shredders	Percent of macrobenthos that shreds coarse particulate matter	Decrease

Table A- 4. Summary of 11 metrics and whether they support/refute the trends noted in the bioassessment data (from Jellison *et al.* 2005a).

Hypothesis	1	2		3	
Biotic Integrity	H4 > H7	H4 > AB	H4 > CD	H7 > H3	H7 > H2
Cumulative Taxa	=	++	++	++	++
Cumulative EPT	++	++	++	++	++
EPT Index	--	=	++	+	=
Sensitive EPT Index	--	--	=	=	=
Shannon Diversity	=	+	+	++	=
Percent Tubificida	++	++	=	--	=
Tolerance Value	++	++	++	=	=
%Intolerant (0-2)	=	--	=	=	=
%Tolerant (8-10)	++	++	++	+	=
%Dominant	=	--	--	--	=
%Collector-	=	=	-	=	-
Filterer					
%Collector-Gatherers	=	++	++	=	=
++ Strong support in favor indicated by statistically significant difference (p<0.05) + Support in favor indicated in majority of year to year comparisons and means differ by more than 1 Std. Dev. = Variable, does not support or refute hypothesis - Weak refutation of hypothesis indicated by majority of year to year comparisons and means which differ by more than 1 Std. Dev. -- Strong refutation of hypothesis indicated by statistically significant difference opposite the hypothesized one > Weak decrease (more than 1 SD but p > 0.05) in biotic integrity (Jellison <i>et al.</i> 2005a)					

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