Effects and Relationships of Stream Hydrology, T.D.S., and Passive CPOM Retention on the Detrital Communities of Three North Central PA Streams

A comparison of an approved trout water to streams with established native trout populations

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Abstract

Three North Central PA streams were studied to compare the differences in streams of marginal environmental quality to those able to sustain naturally reproducing wild trout populations. Data assessment fields included habitat assessments, water chemistry, benthic macroinvertebrates, mycelial biomass, aquatic hyphomycetes, fishes, and a microbial ecology assay. Habitat assessment data provided a hierarchy relating the quantity and quality of available fish habitat. As suspected, the control streams, with natural trout populations, scored higher than the study stream. Comparisons of water chemistry data revealed significance in pH variance, total dissolved solids, conductivity, and total phosphorus. Individual metric score comparisons revealed that the non-native trout stream decreased significantly, compared to its reference site, in community health, balance, and diversity, and in overall environmental quality, at some point during the study period. The most striking differences were seen in percent dominance and taxa diversity. However, mycelial biomass was not significantly different among the sampling sites. An aquatic hyphomycete survey revealed that the non-native trout stream had an overall lower share of reproducing fungi, relative to the total number of fungi in each sample. An electrofishing survey performed at the study site was considered supplemental to the invertebrate data and revealed a similar impairment score. A bacteriological assay was performed a on fish lesion, found during the electrofishing survey. The assay discovered two organisms on the wound, one identified as *Pseudomonas spinosa* and the other belonging to the Vibrionacea family, the latter of which was hypothesized to be the pathogenic organism. It was concluded by the study that further, more detailed analysis was needed in each of these data assessment fields, in order to draw any solid conclusions about the inherent differences found in clean and marginally clean streams. Recommendations were also made for changes in trout fisheries management practices in the study stream, based on both economic principles and this study's broad survey of the marginally clean stream.

Introduction

Trout fishing has often been the subject of controversy among fishermen in Pennsylvania, as it has been throughout the country (Yuskavitch, 1999 A). While many fishermen agree that it is necessary to focus efforts on propagating and maintaining a viable trout fishery within the Commonwealth, it is not the case that all fishermen agree on the methodology employed to achieve this goal (Taylor, 1992).

Both past and present efforts have focused primarily on trout stocking to provide fishermen with the ability to enjoy a greater diversity in their fishing experience. "Artificial propagation in the U.S. dates back to the 1860's and was instituted as a way to repopulate Eastern streams that had experienced significant declines in their wild fish populations from overfishing and human development of the landscape" (Yuskavitch, 1999 A). Fish culture stations, located throughout the state as both private and state-owned entities, are managed intensively to yield maximum size attainment and numbers of several salmonid species for release into both lotic and lentic environments. Hatchery workers also desire and select for more docile fish in breeding, which are easier to handle and grow better, but conversely, this domestication can prove harmful when fish are released into the harsh, wild environment outside the hatchery (White, 1992). Since the goals of these stations center on maximizing biomass, naturally they are subject to overcrowding (White, 1992), along with the adverse effects associated with sustaining high numbers within a confined space. Parasites, diseases, and injuries related to overcrowding pressures are common plagues of these fish hatcheries. "Life in the raceway rarely mimics conditions in the wild. Crowded hatcheries, designed to maximize production and efficiency, are ideally suited for fish diseases and domestication" (Yuskavitch, 1999 A). Add the problem of excluding predators, and these fish culture stations become increasingly more capital intensive with the rising demands of trout enthusiasts. Despite the difficulties and expenses of maintaining fish culture stocks, the Pennsylvania Fish and Boat Commission continues to operate 16 (actually 14

today, according to the 2000 Summary of Laws Handbook) culture stations and provides small fish and guidance to 175 cooperative nurseries owned by sportsmen's groups (Graefe, 1990). Annually the PFBC stocks more than 5 million adult trout from 14 fish culture stations located across the state (PFBC, 2000).

The negative affects hatchery fish thrust on wild or, more accurately, natural populations of trout are all too often unmentioned. Stocked adult fishes outcompete native and wild fishes for food sources due in part to selective breeding and due to behavioral adaptations learned by being raised in a hyperconcentrated environment (White, 1992). The introduction of hatchery-bred fish into environments containing naturally reproducing trout can mimic the same harming effects seen in the introduction of exotic species into new environments. "While competition for food and space between hatchery trout and wild trout of the same species is significant, competition between introduced species and native species can be devastating" (Yuskavitch, 1999 A). These and other reasons make fish stocking seem less sustainable for future fisheries management activities.

A different method of providing fishermen with viable trout fisheries, habitat rehabilitation, however, has been gaining wide-spread acceptance in recent years as a successful way of restoring naturally reproducing trout populations to once productive waters. By improving water quality and employing methods of fluvial geomorphology to allow streams to restore themselves to natural channelization and flow dynamics, recent efforts by environmental groups and consulting firms, along with state and federal agencies, have been able to restore some watersheds back to their natural equilibrium status. Once restored, these waters are able to sustain naturally reproducing trout populations under optimal water quality conditions and greatly improve habitat diversity. These wild trout populations are better able to pass the tests of nature and if protected, are better able to maintain themselves or rebound, where damaged by human activity, once the abuse is halted (White, 1992).

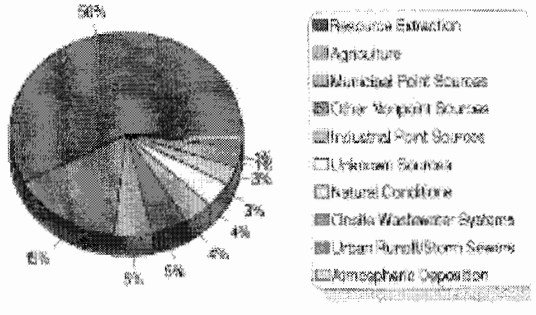
Economically, stream habitat restoration is suspected to be lower in total cost and most certainly less in terms of overall labor requirements than is maintaining fish culture stations, since it is a one-time effort with minimal future interventions, whereas fish culture stocks must be maintained throughout the year, every year, virtually forever. Hatchery management can create short-term fishing, but does not preserve, protect, or improve resources in the long run (White, 1992). A study by Robert J. Behnke (in Taylor, 1992) of Colorado State University points to disastrous economic return on investment from stocking catcheable trout in put-and-take fisheries. This is especially true in comparison to the much lower cost and higher return on investment of managing and protecting wild trout resources. "As Behnke points out, if a catcheable trout costs $3 to hatch, raise and stock and is plucked out of the stream immediately, the state's return on investment is poor. (One must note that only about 60-70 percent of all catchables are actually harvested, and 90 percent of those that avoid the creel cannot survive the rigors of winter streamlife; few will be capable of spawning.)" (Taylor, 1992). "Cultured trout typically live only two or three months after being released from the hatchery, with less than one percent surviving for one year" (Yuskavitch, 1999 A).

The idea behind stream restorations is to work together with, rather than against, the natural processes of stream ecology and hydrology to allow a stream reach to restore itself to a condition more closely resembling its natural equilibrium, whatever the case may be. This natural equilibrium condition is inherently more stable in the long run and more suitable for sustaining naturally reproducing wild trout populations.

What factors may have caused a stream reach to shift away from its natural equilibrium condition into a condition not suitable for sustaining naturally reproducing trout populations? Simply put, any human mediated intervention into the stream or riparian environments of the reach itself or even influences contained within the entire watershed as well. For example, agricultural land-use, particularly cattle

grazing, can degrade a stream by physically injuring aquatic habitats through sedimentation caused by erosion, or through manure, pesticides, and other wastes being directly toxic to fish and other aquatic organisms. This type of environmental degradation is the second leading cause of stream pollution in Pennsylvania [Figure shown below], according to a water pollution report put together by the Pennsylvania Department of Environmental Resources (DER 305-b) in 1994 (Arway, PA Fish &

Figure 1. Major sources of stream pollution in Pennsylvania



Source: 1994 DER 305 (b) Report
Total: 4637 Miles

Boat Commission publication, *year unknown*). These degradations can affect aquatic life in stream reaches miles below, as well as directly at the source of the influence.

Another source of environmental degradation, which has far greater watershed encompassing effects, are past flood control methods attempting to turn the stream into something that looks more like a cattle trough or a water slide at an amusement park. The use of dikes, levees, rip-rap, and other devices aimed at physically controlling a stream's hydraulics actually increases the severity and frequency of future floods, rather than resembling any kind of control mechanism. In some cases, bulldozers would plow down the center of the stream turning it into a featureless channel and constructing huge flood control burms on either side, according to an article appearing in the winter 1997 issue of "Trout" (Rafle, 1997). The article was appropriately titled "A Bulldozer (rather than 'A River') Runs Through It," clearly reflecting the disparity between flood control methods and environmental concerns by replacing a natural concept with man-made heavy machinery.

Stream hydrology is based on a number of factors that interact in a delicate balance. If any one or several of these factors, which is the case in many imperiled watersheds, are disrupted, there are serious adverse affects that inhibit aquatic life in the stream. One in particular is caused by

altering the stream channelization and creates a high width to depth ratio. In other words, the streamflow spreads out across a wider channel base than would naturally occur. In addition to directly affecting wild fish populations, these habitat changes may also affect its carrying capacity. Researchers working with trout populations have collected data that seems to support the notion that changes in stream reach structure and physical habitat do influence fish populations (Isaak and Hubert, 2000).

A high width to depth ratio may be indicated by an increase in the potential for passive retention of coarse particulate organic matter (CPOM). When organic matter greater than 1mm in size is trapped as a function of the "roughness" of a stream (i.e. boulders, large rock, large woody debris, etc.) and there is sufficient flow to otherwise transport the debris further downstream, it is referred to as "active retention". However, when these particles are trapped as a result of there being insufficient flow to keep them moving in the water column or along the streambed, it is referred to as "passive retention" (Lamberti and Gregory, 1996).

CPOM, or plant litter, is a major energy source for stream invertebrates (cf. Stauffer et al. 2000; Suberkropp, 1997), bacteria, and fungi (Allen, 1995), which make up the base of the food chain supporting fish. As allocauthanous materials are introduced into a stream, becoming CPOM, members of the decomposer trophic web immediately colonize them. There is a strong relationship between leaf litter standing crops and secondary productivity, relating to invertebrate abundance, biomass, and production in streams (Wallace et al., 1998). In most streams, processing of allocauthanous inputs from riparian zones far exceed within-stream production (Webster et al., 1995). In-stream allocauthanous processing can be measured across all contributing communities by many different methods. These measures can reflect community health, as well as changes in environmental quality if monitored over extended periods of time. For this study, however, a broad survey of these decomposing and interacting communities will be

conducted, in order to focus attention on the broader issues of approved trout water management in Pennsylvania.

Today, large amounts of money are spent on trout stocking in Pennsylvania streams (some of which have been impaired by environmental degradation) in effort to simulate the conditions found in a once viable resource. That money could be more wisely spent on formulating and implementing watershed improvement plans that would restore the fishery's ability to sustain naturally reproducing trout populations, thereby improving the overall quality and value of the resource itself. When comparing the costs to the benefits of trout stocking, it seems less likely that current budget allocations to trout stocking are capturing the true demands of anglers. In an economic study done on Colorado's fish hatcheries, researchers determined that the cost of producing a hatchery trout caught by an angler is $1.85, while the benefit to the angler ranged from $0.75 to $1.00 (these costs do not include the opportunity costs of providing and maintaining hatchery facilities, which would make the cost of raising one trout even higher than reported) (Yuskavitch, 1999 B). These findings implicate that there is a surplus of funds appropriated to such programs, which could be more efficiently used in other fisheries management activities. Continued implementation of trout stocking practices will only delay the newly resurgent efforts to restore environmental quality to Pennsylvania's watersheds. "Stocking fish is easier than fixing the real problems that keep rivers and streams from reaching their full potential as naturally self-sustaining fisheries" (Yuskavitch, 1999 A). By focusing all current efforts on stream restoration and consolidating appropriated funds devoted to aquatic resources, the rate at which environmental stream conditions are improved could be greatly increased.

A particular method of trout stocking, which is a common practice in Pennsylvania waters, is "put and take" or "cacheable" stocking. This refers to releasing fish of creelable or larger size, intended for quick catch-out (White, 1992). This is often done in waters of marginal environmental quality, where conditions are suitable only for a part of

the year but lethal in others (White, 1992). For example, in warmer waters, or waters that heat up in the summer time in the absence of adequate canopy cover, stocked trout rapidly die off both as a consequence of their inability to find suitable shade cover and due to temperatures that exceed the upper limits of their natural optimum range. Where wild trout are able to physiologically adapt (due in part by evolutionary adaptations) and behaviorally learn to seek out adequate shelter from rising temperatures, stocked trout are unable to do so, or at least do so less efficiently than do wild fish (White, 1992).

Continued efforts to provide a trout fishery in streams of marginal environmental quality are clearly counter productive to preserving, improving, and protecting wild habitat. These efforts are economically inefficient and can actually degrade the quality of aquatic habitats, rather than improve them. Therefore, a decision should be made now, that consolidates efforts on the activity which provides the greatest future benefit for lowest present cost, all else the same. Counting all future generations, habitat restoration becomes the decision that benefits the greatest number of people, since stocking must be continued every year. Furthermore, by focusing on habitat protection, management, and improvement, many marginal waters could become quality waters (Taylor, 1992). As a result, present-day efforts should be concentrated on restoration and preservation activities, in order to more quickly reach the long run goal of long term sustainability in our aquatic resources for future generations to enjoy.

The PA Fish Commission's efforts in recent years, however, have been moving toward protecting Pennsylvania's native trout fisheries. "In 1983 the Commission (PA Fish & Boat Commission) moved into a new era in management of Pennsylvania's trout fisheries. Among the characteristics considered was the presence and abundance of wild trout-- something that had never previously entered into management and stocking decisions. For the first time Pennsylvania had formal management of wild trout, including removal of the very best wild

trout waters from the stocking program" (Graff, 1997). Although these modern day practices are a considerable step in the right direction, some practices continue that are in fact questionable as to how they affect the health of aquatic communities.

This study attempts to investigate the environmental quality differences seen in three Pennsylvania streams of differing fisheries management approaches. The study site for this investigation, Black Hole Creek, is currently classified by the PA Fish & Boat Commission as an "approved trout water". What this essentially means is that the creek is stocked with hatchery fish in advance of an open trout harvest season starting in early spring.

There are two control sites used in this study that reflect differing fisheries management approaches. One, Sugar Camp Run, is a relatively pristine stream that is able to sustain naturally reproducing populations of wild brown and native brook trout with very little influence from trout stocking activities in adjacent downstream waters. A second control site, Big Bear Creek, has been the recent target of an intensive habitat restoration program aimed at preserving the native brook trout population that once flourished there. Big Bear Creek's unique history allows a large section of it to fall under private ownership by a local sportsman's club. The creek in past decades has been impaired by extensive fish stocking management practices and other degrative factors such as dams and access roads. Recent efforts by the sportsman's club, in association with federal and state agencies, have allowed this creek to begin restoring itself back to sustainable equilibrium. Efforts involving trout stocking have since ceased, and now rely on natural processes to rehabilitate this once successful fishery.

This study hypothesizes that Black Hole Creek, a stream of marginal environmental quality, is unfit to be the site of a trout management program, and that other studies, focusing on habitat restoration efforts, will yield greater successes in improving the overall quality of life in an aquatic ecosystem.

Methods

Site Location and Description

The study was conducted on three North Central Pennsylvania freestone streams of similar order, all located within the Susquehanna River Basin. These streams (described below) are shown on the topographic map of Appendix 1. The two control streams are geologically located in the Appalachian Plateau Region and just north of the Fold Belt Region, where the study stream is located.

Study Site 1

The study site is located on Black Hole Creek (BHC), which enters the Susquehanna River near Montgomery, PA. BHC is a second order free-stone stream and is listed as an approved trout water in the PA Fish and Boat Commission's Rules and Regulations Handbook (PFBC, 2000) for the 2000 fishing season. There are no sustainable native or wild trout species living in the stream.

Located upstream of the site are several municipal establishments, a federal prison complex, a golf course, and a facility that manufactures pharmaceuticals. There are also a few small-scale businesses and farms located in the stream's subbasin.

Control Site 1

The first control site, Big Bear Creek (BBC), is located approximately 17 miles north of Montoursville, PA, near the small village of Barbours. BBC is a third order freestone stream, which flows from relatively pristine forested land and is a tributary of the Loyalsock Creek. BBC has recently been the subject of a restoration program, in conjunction with the U.S. Fish and Wildlife Service, the PA Department of Environmental Protection, and the Dunwoody Sportsmen's Club, applying river geomorphology methods described by Rosgen (1996). The project is designed to rechannelize the bulk of the creek's flow and establish adequate habitat for native populations of Brook Trout

in the stream. Historically, the creek has been the site of intense trout stocking by the private hunting club, but now the focus has shifted to preserving the stream's natural game fish, the Brook Trout. Slimy sculpin and clean water invertebrates dominate the forage base in BBC.

Control Site 2

The second control site, Sugar Camp Run (SCR), flows south out of the Tiadaghton State Forest area and empties into Mill Creek, a tributary of the Loyalsock Creek, near Warrensville, PA. This stream is not an "approved trout water", however it may be affected by migrating stocked trout from Mill Creek. SCR is a second order freestone stream of good environmental quality. SCR has established native Brook Trout and wild Brown Trout populations living in the stream, along with a favorable forage base which includes many baitfish and aquatic insect species.

Habitat Assessment

On-site habitat assessments were performed to examine hydrogeologic properties and similarities between sites. Scores were assigned according to EPA guidelines, as outlined in Plafkin et al. (1989) and recorded on data sheets formulated by the Riverwatch Network (www.riverwatch.org). Habitat parameters included such primary characteristics as bottom substrate, stream velocity or flow, and embeddedness. Secondary habitat parameters included evaluation of velocity/depth regimes, bank/channel alteration, sediment deposition, riffle characteristics, and % bottom exposed. Tertiary habitat parameters included evaluation of bank conditions, bank vegetation, riparian vegetation zone, and overhead canopy cover. Percent similarity was calculated using total habitat scores to compare the study site to each of the reference sites and to compare both reference sites.

Water Chemistry

Water samples were collected from the field using plastic sample bottles that were labeled with date, time, and stream reach. Analyses were performed on-site and in the laboratory to assess the water quality at each site.

Field parameters included pH, conductivity (mV/s), total dissolved solids or TDS (ppm), dissolved oxygen or DO (ppm), and temperature (^{0}C), and were measured using several electronic meters: HACH sens-ion 2, Hanna Instrument's HI-9635, and Yellow Springs Instrument Co.'s YSI – 55.

Laboratory analysis included measuring alkalinity levels by 0.02 N sulfuric acid titration. Other laboratory parameters were measured using a HACH 4000 spectrophotometer and included nitrate, nitrite, reactive (ortho-) phosphorus, total phosphorus, total chlorine, and aluminum, following EPA certified instructions listed in the HACH procedures manual (1997).

Aquatic Macroinvertebrates

The invertebrate community was analyzed by EPA Rapid Bioassessment Protocol III (RBP III), applying genus-level taxonomy to assess each stream's environmental quality. Aquatic macroinvertebrates were collected throughout the experimentation period using kick nets and sampling methods described in Plafkin et. al. (1989).

Samples were transported in plastic containers back to the laboratory and preserved in 10% buffered formalin acetate. The samples were then rinsed with tap water into a number 18-mesh sieve to remove the formalin and any unwanted suspended solids from the sample. The remaining debris was placed into a large pan to be floated in water. Magnesium sulfate was dissolved in warm water and added to the pan to increase the water's density and cause the lighter organic matter, including the invertebrates, to float to the top for easy removal.

The invertebrates were then placed in a grid pan and, using a random numbers system, a 100-organism subsample was collected from the sample. The subsample was identified to genus level using the following dichotomous keys: Lehmkuhl (1979), Merritt and Cummins (1996), Peckarsky et. al. (1990), Stewart and Stark (1988), and Wiggins (1977).

RBP III lab analysis was performed to detect the degree of impairment in the study site, as compared to either control site. The data generated from the assessment was also used to analyze community similarity indices and formulate food web relationships, which could be used to give further insight into the impairment status of the study site, particularly to assess the changes in the shredder community, in response to detritus accumulation from passive hydrologic retention in Black Hole Creek.

Coarse particulate organic matter (CPOM) samples were also collected and analyzed by RBP III. These samples were taken from the artificially constructed leaf packs used in the fungal biomass assay. The leaf packs, before they were treated for ergosterol content analysis, were rinsed with tap water to remove the invertebrates. These samples were then identified to genus level, tallied, and analyzed by RBP using the same standards applied to the kick samples.

Mycelial Biomass

Substrate colonization by eumycotic fungi was measured as relating to amounts of ergosterol compound per sample. Ergosterol was extracted from artificial and natural leaf packs using methods outlined in Newell et al. (1988) and Newell (1992).

Artificial leaf packs were prepared by fastening five leaves to each brick using two rubber bands, one having a label attached showing date, location, and sample number. Maple leaves (*Acer saccharum*) were chosen for this experiment due to their apparent high decomposition rate relative to other temperate species, as reported by Jacobs (1998

unpublished manuscript). Also, green, rather than senescent, leaves were chosen for this experiment due to higher levels of nitrogen and phosphorus (Leff and McArthur, 1990), which have been shown to influence decomposition rates (Taylor et al., 1989).

Leaves were collected preabscission in September and air-dried flat >48 hours, strapped to bricks, and submerged in the stream to incubate. Bricks were then collected at approximately ten-day intervals, two samples per creek per collection date. The samples were placed in a freezer at -4°C until adequate time and lab space became available to complete the ergosterol extraction procedures outlined in Newell et. al. (1988) and Newell (1992). Extracted samples were then analyzed for absorbance at 282 nm using high-pressure liquid chromatography. The resulting peak areas were then plotted against an ergosterol standard curve and the amount of ergosterol in micrograms per sample was derived graphically from matching the peak area to the corresponding standard value represented linearly on the graph.

In late September, once the first sample incubation period had begun and the bricks were submerged on the stream bottom, the ergosterol standard curve was formulated.

Five grams of 95% ergosterol compound was purchased from the Aldrich Chemical Company, Inc. (information about this product can be found online at www.sigma-aldrich.com). Ergosterol is both air and light sensitive, so care in handling and storage was exercised to ensure the compound's purity throughout the experimentation period.

A standard solution was made by dissolving 0.139g erosterol in 100mL HPLC-grade methanol, making a final concentration of 139µg/mL. The standard solution was then saved, kept in a light-retardant bottle, and stored in refrigeration at 4°C to minimize degradation. The standard curve was then derived by injecting ergosterol standard solution in

differing volumes into HPLC and recording the resulting peak areas. These areas were then plotted using a Kaleidoscope computer software program to obtain the desired standard curve line.

The high-pressure liquid chromatography equipment itself was set up with a Waters 991 Photodiode Array Detector, Waters 510 HPLC Pump, and a Millennium software package that included a Quickset instrumentation set up program.

A baseline was initially established and HPLC instrumentation set up by first bleeding HPLC-grade methanol, which was pre-sonicated and degassed, in through the tubing and then running the solvent through the equipment to remove any possible contaminants and residuals from past equipment use. The software recorded and established a baseline value for the methanol solvent, reading at the desired 282nm wavelength.

After the standard solution was run and standard curve established, it was determined that a spike had to be formulated, which would enable the equipment to distinguish different peaks and quantify peak area values. It was determined, after a series of test runs combining varying volumes of ergosterol standard solution and actual samples obtained from the field, that a 20µL spike would be sufficient to achieve this goal. The spike value, which was also run alone, would be subtracted from final sample results to establish the true value of ergosterol obtained from a field sample.

On a predetermined collection date, leaf pack samples were removed from each of the streams and placed in Zip-loc bags for transport. For each sample, ten 13mm discs were cut from the leaves using an aluminum corking device and placed in a round-bottom flask with 25mL HPLC-grade methanol.

The samples were then refluxed for a period of 30 minutes to remove the sterol compounds. 5 mL of a 4% KOH solution was then

added to the flask and refluxed for another 30 minutes to lyse the sterol esters, as explained by Newell (1992). After cooling to room temperature, the samples were then vacuum filtered to remove the leaf discs and any unwanted debris that may have been flocculated into suspension during reflux. Next, the liquid was partitioned into HPLC-grade pentane in a series of three additions, each time placed into a separatory funnel and mixed by inversion to separate the organic and inorganic elements in the solution. To ensure that thorough separation was achieved, a small amount of aqueous sodium chloride was added to the separatory funnel before mixing. The gas produced from this reaction was released from the separatory funnel after each inversion and collected in fume hood to prevent any accidents that may occur from the build up of pressure inside the glassware.

Upon separation, the organic layer containing the desired ergosterol compounds was collected and saved, while the inorganic layers were discarded after each inversion and separation.

The pentane solvent was then evaporated using a rotary evaporator apparatus. Dried samples were then redissolved in HPLC-grade methanol, poured into properly labeled screw-cap vials, wrapped in brown paper to prevent light degradation, and stored in a refrigerator at 4°C to preserve the samples until they could be analyzed.

The samples were analyzed by HPLC method within 48 hours. Each time, before running the actual samples, the methanol solvent was sonicated and degassed and the methanol baseline was re-established. The standard solution was then run again to ensure accuracy and comparability across samples, since the spike value would be removed from the actual sample value when calculating the amount of ergosterol in each sample.

Each sample was run using 80µL of sample solution and 20µL standard solution spike, for a 100µL final injection volume.

Ergosterol eluted consistently between 5.3 and 5.8 minutes and had a distinctive peak shape that was recognizable during each run. Although ergosterol eluted under 6 minutes, each injection was allowed to run for more than 15 minutes to ensure that no trace substance would be left in the column, possibly obscuring future results.

The peak areas obtained from the field samples were finally entered into a Kaleidoscope graphical software program and compared to the standard curve, which was previously derived and placed into the program. After formulation, the amounts of ergosterol in µg per sample were quantified and recorded.

A parallel ergosterol study was conducted using leaves collected from the forest floor to determine whether terrestrial fungi species were contributing to ergosterol compound found in the aquatic samples.

Aquatic Hyphomycetes

Conidial production by aquatic hyphomycetes was measured using methods outlined by Gessner and Chauvet (1994). Leaf samples were collected at random from both Black Hole Creek and Big Bear Creek in February. From each leaf sample, six discs were cut using a 10mm diameter cork borer. The discs were then placed in 10mL filtered stream water and placed in an incubator at 12°C for three days. The disks were then fixed in lactophenol cotton blue, mounted on slides, and stained with 0.01% trypan blue in lactic acid. After 1 minute, the fungi stain was carefully removed from the slides by adding distilled water to one side of the cover slip, while soaking up the stain from the other side using tissue paper. Slides were then examined microscopically to determine fungal community structure and the total number of conidia produced per unit leaf surface.

Fungal species were estimated according to their relative abundance and were placed in eight

classes: 0 = 0% fungi, 1 = 0-1%, 2 = 1-5%, 3 = 5-20%, 4 = 20-40%, 5 = 40-60%, 6 = 60-80%, and 7 = 80-100%. Total numbers of conidia produced per unit leaf surface was estimated from counts in four microscopic fields per leaf disc and divided by the total number of identifiable aquatic hyphomycetes in the same four fields to estimate the class designations for each sample, conferring conidial production per leaf sample. This is a slight variation from the methodology used by Gessner and Chauvet (1994), but the methods used in this study to measure conidial production should be sufficient enough to detect any significant variation in the aquatic fungi communities. Conidia were recognized in comparison to diagrams illustrated in Alexopoulos (1962), Barnett (1960), and Frobisher (1968).

Fish Communities

The fish community in Black Hole Creek was analyzed using RBP IV, as described by Plafkin et. al. (1989). An electrofishing survey was performed in March to give further background as to the study site's impairment status. Conductivity readings were taken in the stream to determine the optimal electric current setting for this survey. A stretch of stream was selected that covered an area 82 meters long by an average of 9 meters wide and contained the entire stream reach used in this study. To conserve time, only one pass was taken through the sample area. This was found to be sufficient in gaining an understanding of the fish community and in further solidifying the invertebrate analysis results.

Fishes were identified in the field using morphology information found in Cooper (1983) and counted in the field for quick release. Most fish were released expediently; however, two fish were kept for pathogenic and parasitologic analysis. A pathogenic assessment was performed, as described below, by infected wound culturing, while parasites were removed from a juvenile creek chub species and

identified using information found in Hugghins (1972) and Meyer (1962).

Microbial Ecology

After performing the electrofishing survey, a yellow bullhead catfish individual was found that had developed a conspicuous lesion on its anterior – ventral side. The sore appeared to be bacterial in origin, rather than a wound from an injury. The fish was transported back to the lab and photographs were taken of the sore. It was determined that the sore should be cultured, as it may provide further insight into the BHC's biological impairment condition.

Swabs of the lesion surface were cultured on TSA media and incubated at 32°C, 22°C, 12°C, and 4°C for bacterial analysis. Visually differing colonies were isolated from the media and streaked for single colonies on new TSA plates. The resulting pure culture isolates were then inoculated onto slants, saved, and stored at 32°C, which was determined as the optimal growth temperature for the organisms.

Biochemical tests were performed and the results used to systematically identify each isolate using methods found in the Bergey's Manual of Determinative Bacteriology (Holt et. al. 1994). Shape and motility were determined microscopically by preparing wet mounts from bacteria inoculated into liquid LB media. A gram stain was performed for each isolate using bacteria from the TSA media. Slants were incubated for approximately 24 hours and used to perform oxidase and catalase tests. The following biochemical tests were also performed to learn more about the metabolism of the unknown isolates: mannitol and glucose utilization indicated by the production of acid and/or gas and lipase exoenzyme detection. Amino acid metabolism was determined by inoculating bacteria onto sulfide-indole-motility agar (SIM agar).

The identity of the isolates was confirmed by performing a 16s ribosomal RNA sequence analysis. The results were researched using the National Center for

Biotechnology Information NCBI website (www.ncbi.nlm.nih.gov) to compare sequences to the national database.

The primer 1 and primer 2 sequences were read from scanned images of the sequencing gels, entered into a DNASTAR computer program, and used to perform a basic BLAST search on the NCBI website. The list of organisms resulting from both primers 1 and 2 sequence BLAST searches were compared to determine the most probably identity of the unknown isolates. This information, combined with information found in the Bergey's Manual of Determinative Bacteriology, was cross-referenced to determine the identified organisms' ecological significance and possible pathogenicity, in connection with the lesion.

Results

Habitat Assessment

Table 1 shows the results of the habitat assessments performed on each of the three sampling sites. The primary habitat characteristics were calculated for each creek and the highest score possible for these categories was 20 points. These categories included percent cobble, velocity, and stream embeddedness. Secondary habitat characteristics were also calculated for each creek comparing these to a high possible score of 10 points and included the following: velocity/depth regimes, bank/channel alteration, sediment deposition, riffle characteristics, percent bottom exposed, bank conditions, bank vegetation, riparian vegetation, and overhead canopy cover. Overall, these characteristics reveal that Big Bear Creek scored higher on average than did Sugar Camp Run, which in turn scored higher than Black Hole Creek, as illustrated by Figure 1, but these scores were not significant ($p=0.219$) at the 0.05 alpha-level by one-way ANOVA analysis. The table also shows the total habitat assessment scores for each site and the percentage each score represents of the total possible score for a single stream reach. These comparisons show the degree of differences between each of the sites (i.e. 128>116>83). Each creek is then compared for similarity to its reference site (i.e. Black Hole Creek to Sugar Camp Run – 71% and to Big Bear Creek – 64%, and Sugar Camp Run to Big Bear Creek – 90%), which also depicts this trend.

The habitat assessment parameters are graphically compared in Figure 1 and show both the similarities and differences between these parameters, among the three sites. The graph also reveals the relative differences among primary and secondary habitat scores for each site.

Water Chemistry

Table 2.1 summarizes the water chemistry data collected for each site throughout the study period, organized by creek. A few of these parameters, considered crucial to the study, are shown in Figure 2.1 for Black Hole Creek (BHC) in log scale format. These include

pH, alkalinity, total dissolved solids (TDS), conductivity, dissolved oxygen (DO), and temperature and demonstrate changes over time. For BHC, pH ranged from as low as 5.52 to as high as 7.40 and the mean pH in BHC was significantly different (p=0.035) than the mean pH found in SCR and BBC at the 0.05 alpha-level (One-way ANOVA). Alkalinity was found to be as low as 1.5 parts per million (ppm) $CaCO_3$ in March and as high as 90 ppm $CaCO_3$ in February and increased around the same time as pH (illustrated by Figure 2.1). This was not significantly different than SCR or BBC (p=0.251, One-way ANOVA). Total dissolved solids (TDS) were found to range from 15.1 to 118.4 ppm and fluctuated in the same pattern as conductivity (Figure 2.1). TDS in BHC were significantly different than in SCR and BBC (p=0.047, One-way ANOVA). Temperature, in BHC, followed a typical pattern by decreasing through the winter months to as low as 2.1^0C, and warming gradually again in the spring (up to 6.3^0C), as shown in Figure 2.1. Conductivity was found to be significantly different (p=0.000) in BHC than in SCR and BBC. DO (p=0.432), aluminum (p=0.315), total chlorine (p=0.158), nitrate nitrogen (p=0.177), nitrite nitrogen (p=0.303), and ortho-phosphorus (p=0.278) were not significantly different between the three sites. Temperature was not significantly different (p=0.849) between the three creeks at the 0.05 alpha-level by one-way ANOVA analysis. Total phosphorus, however, was found to be significantly different between the three sites (p=0.084) by one-way ANOVA at the 0.05 alpha-level.

Table 2.2 reports total dissolved solids (TDS) and conductivity data, comparing each of the three study sites, as they changed over the course of the sampling period. Figures 2.2 and 2.3, in particular, compare changes in TDS and conductivity respectively over time. The results of a one-way ANOVA analysis report significant differences in the mean of values reported in Table 2.2, compared between creeks for both TDS (p=0.027) and conductivity (p=0.000), at the 0.05 alpha-level. This means that both TDS and conductivity was, on average, significantly higher in Black Hole Creek than in either Sugar Camp Run or Big Bear Creek.

Table 2.3 reports the differences in stream pH as they vary across time for each creek. These values are shown in Figure 2.4. The absolute values of changes in stream pH were calculated as means and compared to each other by one-way ANOVA, which revealed that these differences were not significant ($p=0.479$) at the 0.05 alpha-level. Mean differences are as follows for each creek: BHC (0.56 ± 0.58), SCR (0.28 ± 0.13), and BBC (0.33 ± 0.30). Comparing these means and standard deviations reveals that BHC varied greater than BBC, which in turn varied greater than SCR.

Aquatic Macroinvertebrates

A completed list of benthic macroinvertebrates, found at each sampling site, is shown in Table 3.1.

Table 3.2 shows rapid bio-assessment scores using protocol 3 (RBPIII) for Black Hole Creek, compared to the specific reference site designated in the table for each sampling date. The score is reported as an impairment score relative to the reference score. Black Hole Creek decreased in environmental quality over the sampling period from 66.7 to 25.0 in relation to its reference site. Also reported in the table are community loss indices, Jaccard Coefficients, and the sampling method used to collect invertebrates for each sampling date. The community loss indices and the Jaccard Coefficients did not show trends similar to the impairment score, but each changed similarly in relation to each other (refer to Table 3.2). Community loss increased for the November and January samples (1.00 to 1.50 and 1.78), indicating an increase in dissimilarity from the reference station. The Jaccard Coefficient conversely decreased during this time (0.321 to 0.200 and 0.240), since a decreasing community similarity would result in a decreasing coefficient.

Table 3.3 reports the individual metric scores for Black Hole Creek, calculated by RBPIII method. Total scores are also reported in the table and reflect how the quality of the stream reach changed over the sampling period, as mentioned above for Table 3.2. Figure 3.1 shows these matrices as they change over time. This was done to track individual metric score fluctuations, reflecting community

structure dynamics. The most striking data shown here is the percent dominance comparison to the reference site. A zero value was reported for every sampling date in this category and was significant. Differences between the study site and reference site that are greater than 50% (i.e. a metric score less than 2 or 4, depending on the individual metric) are significant (Plafkin et al., 1989). The following metrics were found to be significantly different by this comparison [Figure 3.1]: total taxa (Nov. and Jan.), FBI (Feb.), ratio scrapers/filtering collectors (Nov., Jan., and Feb.), ratio shredders to total individuals (Jan. and Feb.), ratio EPT/Chironomids (Sept., Jan., and Feb.), percent dominance (Sept. – Feb.), EPT index (Oct. and Nov.), and community loss (Oct. – Feb.).

Table 3.4 reports the actual numbers of each taxa for Black Hole Creek. Also reported are the percent contribution values for the dominant taxa found during each sampling date. Tables 3.5 and 3.6 show the same values for Sugar Camp Run and Big Bear Creek respectively. A one-way ANOVA analysis was performed to compare the mean contribution by dominant taxa in each creek. There was a significant difference ($p=0.007$) in the contribution of taxa between creeks at the .05 alpha-level. Also reported in Tables 3.4, 3.5, and 3.6, are the functional feeding group categories for each taxa, along with a summary reporting the total numbers within these categories. Figures 3.2, 3.3, and 3.4 illustrate these totals as they change over the course of the study. Figure 3.2 shows that the relative contributions of these feeding groups in BHC are more volatile (meaning that any one feeding group is dominating the overall community structure at a given point in time and that these groups rise and fall, relative to each other, with changes in the local environment) than those found in SCR (Figure 3.3) and BBC (Figure 3.4).

Figures 3.5, 3.6, and 3.7 depict the subsample populations for Black Hole Creek (BHC), Sugar Camp Run (SCR), and Big Bear Creek (BBC) respectively. When compared, these graphs show that taxa diversity is higher and that the relative densities of these taxa are spread more evenly across all

taxa in SCR (Figure 3.6) and BBC (Figure 3.7), than is shown for BHC in Figure 3.5.

Mycelial Biomass

Table 4 reports the ergosterol amounts found in each sample for each creek. These values are expressed graphically in Figure 4 and show how the relative biomasses of aquatic leaf-colonizing fungi changed similarly over time in each of the three streams. There was no significant difference in the mean ergosterol amounts between either creek at the 0.05 alpha-level ($p=0.236$), according to a one-way ANOVA analysis of the means.

Aquatic Hyphomycetes

Aquatic hyphomycetes were compared for both Black Hole Creek and Big Bear Creek on two sampling dates and are reported in Table 5. These values are expressed in conidial production classes and correspond to the share of reproducing fungi found at each sampling site as compared to the total number of fungi in the sample. These classes are compared for both BHC and BBC and are shown in Figures 5.1 and 5.2 for each sampling date. The data showed that, on average, Black Hole Creek had smaller share of reproducing fungi present in the samples than did Big Bear Creek for both sampling dates.

Fish Communities

The results of an electrofishing survey performed on Black Hole Creek in March are reported in Table 6. These results report a number of categories including numbers and species caught, age category, parasite and potential pathogen observations, pollution tolerance values (PTV's), functional feeding groups (FFG's), and historic distribution category (native or exotic to the watershed). The number of adult pool species (i.e. Bass, suckers, catfish, trout) was significantly lower than the total number of fishes present ($p=0.02$). Also to be noted is that all bass found in the survey were juveniles of the same age (probably 1 year old) and since this group represented a large share of the total fishes found relative to other groups (9 individuals out of 36; 6 total taxa). A rapid bioassessment protocol 5 (RBPV) impairment score and

interpretation is also reported in the table.

Microbial Ecology

Table 7 reports the results of the unknown microbe identification. There were two different bacteria isolated from the culture. Relevant biochemical tests are also reported in the table, along with a few morphological characteristics. The results of 16S ribosomal RNA sequencing were researched by Dr. Jack Diehl, Professor of Biology at Lycoming College, and coordinated with the results of these biochemical-morphological tests to obtain a positive identity for both unknown organisms. The possible identities for both unknown isolates are reported in Table 7.

Discussion

Habitat Assessment

A habitat assessment was performed to examine hydrologic functions and habitat characteristics for comparison among the three sites. A hierarchy was established from the data that relates the quantity and quality of available fish habitat within the stream. Big Bear Creek (BBC) was found to have more and better available fish habitat than Sugar Camp Run (SCR), which in turn was better than Black Hole Creek (BHC). This can be easily seen in the total score comparisons, percent of possible score comparisons, and the percent similarity comparisons between the sites.

Although the differing means of these scores were found to not be significant, the individual scores do reveal a visually discernable trend. Also the data may be skewed, since BBC scored very low in the bank condition category and the percent bottom exposed category, due to excessive erosion caused by flooding. In its recent past, BBC was subject to devastating floods, made worse by flood control attempts aimed at protecting access roads to the area. One of these attempts can still be seen at the site as rip-rap placed along the bank of the access road. Rip-rap is known to constrict the channel and force water to increase in velocity and erode the streambank even further, both above and below the structure (Rosgen, 1996).

The similarity comparisons show that, overall, SCR was more similar to BBC than to BHC. This points out that, habitat-wise, both SCR and BBC are better suited to support fish than BHC. This is especially true for supporting populations of trout, since trout require a lower temperature range than other fish and canopy cover is much sparser in BHC than in either SCR or BBC. Less canopy cover generally means higher average temperatures. The average temperatures found in this study did not differ significantly among sites. However, due to low sample size and

frequency of sampling, these data may not accurately portray the overall annual temperature ranges found in the creeks.

Water Chemistry

Water chemistry data reflect the same impairment trends as other data suggest (pH variance, TDS, and conductivity were found to be significantly higher in BHC). Conversely, total phosphorus was higher in BBC than in the other two creeks. The reason for this was undeterminable and could be due to the low frequency of sampling in this experiment or possibly even human error.

TDS and conductivity showed similar trends across time in BHC and were significantly higher than in SCR and BBC. This means that there was either higher influence of organic or dissolved metals in BHC than were found in SCR or BBC. This interpretation makes sense, because of the upstream riparian influences present in BHC and not in SCR and BBC. SCR and BBC's headwaters are located in relatively pristine forested land, while BHC's headwaters pass through several municipal areas, a prison complex, a golf course, and a pharmaceutical product producing facility.

BHC was found to vary more greatly in pH over the course of the sampling period than did SCR or BBC. This instability is indicative of riparian influences, which, once again, were not present at the SCR or BBC sites. The pH variance in BHC may have been due to basic or acidic discharges from the pharmaceutical plant, possibly from cleaning solvents such as chromic-sulfuric acid used to clean glassware. More sampling is obviously necessary to obtain any kind of proof to origin of such a pH influence. Also, the sampling must be done both above and below the facility to test for this type of influence. Dichloromethane is another common cleaning solvent used for industrial applications. For this reason, total chlorine was tested for in BHC, but was found to be insignificantly differing compared to SCR and BBC.

Aquatic Macroinvertebrates

RBP impairment scores decreased over the course of the study period for BHC. When looking at individual metric scores, this decrease can be attributed to decreases in the ratio of scrapers to filtering collectors and the ratio of Ephemeroptera, Trichoptera, and Plecoptera (EPT) to chironomids. Also to be noted is that the Modified Hilsenhoff Family Biotic Index (FBI) and the community loss index decreased in relation to SCR in February.

Neither community loss indices nor Jaccard Coefficients showed trends similar to the impairment score, but each changed similarly in relation to each other. Community loss increased for the November and January samples, indicating an increase in dissimilarity from the reference station. The Jaccard Coefficient conversely decreased during this time, since a decreasing community similarity would result in a decreasing coefficient. This is not surprising, since they are mathematically formulated in such a way as to reflect this difference, and they use some of the same data. For example, both formulae use the number of species common to both samples being compared and can be found in Plafkin et al (1989). The Jaccard coefficient, however, is a measure of the presence or absence of similar taxon, while the community loss index reports changes in the total number of taxa relative to the reference station (Plafkin et al., 1989).

Individual metric scores for the RBP's performed for BHC reflect changes comparable to the reference site (Plafkin et al., 1989). Since these streams are located in close proximity to each other and are subject to the same atmospheric condition (i.e. acid rain events), changes occurring in the metric scores are due to internal factors affecting BHC's environmental quality. Differences between the study site and reference site that are greater than 50% (i.e. a metric score less than 2 or 4, depending on the

individual metric) are significant (Plafkin et al., 1989).

Total number of taxa was significantly lower in the November and January samples. Since this score reflects community health, and since community health generally increases with increasing water quality, habitat diversity, or habitat suitability (Plafkin et al., 1989), BHC decreased in community health during these times.

For the Modified Hilsenhoff FBI, a metric score of zero is significant, so there were no samples differing significantly from the reference site, but the change seen in the February sample should be equally noted. This change means that both sites decreased in quality at this time, but decreased in different proportions, reflecting the notion that SCR's aquatic communities are more stable than BHC's. This ambiguous decrease, however, could have been due to differing, simultaneously occurring, and internal influences, but is less likely than the above-proposed notion.

The ratio of scrapers to filtering collectors was significantly different (<4) from November through February. This metric reflects the riffle/run community food base and provides insight into the nature of potential disturbance factors (Plafkin et al., 1989). This is explained by the huge increase in the filtering collector community in November, or more specifically an individual filter-feeding taxa (*Prosimulium sp.*). At the same time, a significant decrease in the ratio of shredders to total individuals in January and February would correspond to this change, due to niche occupation. The total population of shredders, specifically *Taeniopteryx sp.*, decreased phenomenally in response to the increase in Prosimuliums. There was no significant difference found in the ratio of shredders to total in November, however, possibly due to a lag effect in niche occupation interactions.

The ratio of EPT to Chironomids was significantly different in September and again in

January and February. These indicator groups reflect community balance and even distributions among these groups, with substantial representation in the sensitive groups (E,P, and T), reflect good community balance (Plafkin et al., 1989). This would mean that BHC's invertebrate communities were imbalanced during these months. However, it is very likely that a random subsample could miss individuals, and since this metric is calculated using numbers of individuals, it was proposed that a subsampling is not the best measure of this metric. Also, since Chironomid numbers are placed in the denominator of the formula used to calculate this metric and since Chironomids are miniature in size compared to other aquatic insects, these individuals will be easily missed during the floating of the sample. Chironomids frequently stick to leaves and matted algae, making them hard to extract or even find during examinations of the sample. Therefore, significance of this metric was not considered a good reflection of community imbalance in BHC.

The percent dominance metric was found to be very significant throughout the study period. This, as explained before, was due primarily to the population "booms" and "busts" seen for *Taeniopteryx sp.* and *Prosimulium sp.* These phenomena can be simply explained as being linked to the passive retention problems (resulting from a high width to depth ratio) in BHC. In the autumn of the year, when huge quantities of abscised leaves fall into or are washed into the creek, in-stream, retained debris increases greatly. This influence into a stream with a high width to depth ratio becomes trapped and the natural hydrology of the stream is unable to flush the debris downstream (Hauer and Lamberti, 1996). An accumulation of debris, and subsequently a huge increase in available food for shredders initially, leads to a boom in the population of dominant shredders (*Taeniopteryx sp.*). Conversely, after this leaf litter is processed and the surplus food

supply for shredders exhausted, other feeding groups are able to return to normal population levels. However, the *Prosimulium* boom in January indicates that there were other factors in the stream influencing community dynamics. Since Prosimuliums are filter feeders, it was concluded that some type of increase in organic influence in BHC could have allowed for this event. This correlates with the significant increases in total dissolved solids (TDS) and conductivity that occurred at this time. However, due to the infrequency of nutrient sampling and minimal inorganic compound sampling, the specific factor allowing for this boom can not be identified. This increase in Prosimuliums could be due to behavioral characteristics, but more sampling is needed to establish this factor in this particular creek.

The EPT index was found to be significantly different in October and November. This metric generally increases with increasing water quality (Plafkin et al., 1989). The total number of distinct taxa within the Ephemeroptera, Plecoptera, and Trichoptera groups decreased for BHC in relation to the reference sites for these samples. Since these orders are typically pollution sensitive (Plafkin et al., 1989), this may provide evidence of a pollution event occurring in BHC during these times, but more intensive sampling is needed to show a strong correlation to this effect.

The community loss metric measures community dissimilarity between the study site and the reference site (Plafkin et al., 1989), and was found to be significantly different from October to February. This index is normally used to detect species lost in the same stream through different reaches (Plafkin et al., 1989). However, since this study compared single reaches between different streams, this metric was not considered to be a good measure of community loss *per se*, but a measure of dissimilarity between the BHC invertebrate community and the reference site's invertebrate community. The community dissimilarity found is not

surprising, since environmental quality and habitat quality were found to differ among these streams.

Functional feeding group changes were examined and interpreted through graphical displays. These graphs clearly depict volatility differences in the abundance of these feeding groups, which is also reflected by the significant differences found in percent dominance among these sites. The shredder and filtering collector increases found in BHC are also represented in these graphs and reflect differences in community balance among the three creeks. In SCR and BBC, the populations of each feeding group are represented relatively evenly through the sampling period, while these groups are distributed less evenly as a share of total population in BHC through the sampling period. The large increases in predator and both collector communities in SCR can be explained as a predator-prey or behavioral interaction, rather than a niche occupation interaction (crowding out), as found in BHC. This is because, as the number of invertebrate predators increase, the number of prey (gathering and filtering collectors) will decrease, while shredder, scraper, and collector groups can only interact in a way that affects niche occupation. Therefore, the community imbalance is significant in BHC, as compared to SCR and BBC. The community imbalance in BHC can be explained as function of passive CPOM retention, as previously mentioned.

Taxa diversity and percent dominance differences are best shown graphically, by plotting the subsample populations of each taxa on a line graph and comparing spacial differences among each line. It is clearly shown by these graphs [Figures 3.5, 3.6, and 3.7] that diversity in BHC was lower than in SCR, which in turn was lower than in BBC. Also shown by these graphs, are the percent dominance differences, seen as the number of a single taxa's population appearing spacially well above all other populations on the graph. These differences were interpreted as

measure of poor community balance in BHC, relative to SCR and BBC.

Mycelial Biomass

Ergosterol is not a vascular plant sterol, so its detection in decaying litter is an indication of microbial colonization and is particularly useful as a fungal-index molecule, relating to mycelial biomass (Newell, 1992). In addition, abscised leaves found terrestrially were examined for the presence of ergosterol. There was no ergosterol found in these leaves, showing that ergosterol found in stream incubated leaves for this study are due to aquatic fungi.

Similar changes were detected over time for fungal biomass in each creek. However, these trends do not indicate any significant changes in biomass, due to the infrequency of sampling.

There was no significant difference found in the amounts of ergosterol between sites. Data collections were not repeated enough times, however, to draw upon any conclusions as to the relative amounts of fungi found in these creeks.

Aquatic Hyphomycetes

The share of reproducing aquatic fungi were measured in BHC and BBC in February and compared. BHC was found to have a lower share of reproducing fungi than BBC, seen by a higher number of slides designated to lower production classes. The only conclusions drawn from this disparity were that fugal community growth may have been inhibited by water quality factors, relative to BBC, and that further sampling in this area is needed in order to show any significant data. Also, future studies of reproducing aquatic hyphomycetes may desire to combine mycelial biomass results with these results, in order to show correlation between these data. This may allow for better conclusions to be drawn about water quality differences in these streams.

Fish Communities

An electrofishing survey on BHC was performed to obtain data

supplemental to invertebrate data for biological impairment conditions. A total score of 36 was found for the RBP analysis, which corresponds to a "fair" impairment category rating. This score, however, was interpreted as "poor", due to frequency of parasites and pathogenic lesions present on many of the fishes found in the survey. The parasites were found on every creek chub, and were identified as the cysts of a trematode worm, *Uvulifer ambloblites*, a common parasite to these fish, characterized by "black spots" found on the epithelia of fishes (cf. Hugghins, 1972 and Meyer, 1954).

The number of adult pools species were found to be significantly lower than the total number of fishes found in the survey. This finding does not suggest any implications for water quality conditions, but when looking at the age category of a particular taxa, does imply a recent impact on water quality in the stream.

The number of smallmouth bass represented a large share of the total number of fishes found, relative to the total number of taxa present. Since these bass were all juvenile and of similar age (approximately 1 year old), it was concluded that these findings may imply that the adult bass population was either killed off or forced out of the stream reach by external pressures. Some external pressures may include road construction or resource extraction occurring close to the stream reach. There was extensive bridge reconstruction done just below the study site on BHC in November following damage caused by flooding in October, but since the electrofishing survey was performed in March, it was determined that fish should have returned to normal behavioral activities by this time.

Microbial Ecology

Two bacterial species were cultured from the open sore discovered on a yellow bullhead catfish during the electrofishing survey. One species was positively identified as *Pseudomonas spinosa*, a Pseudomonad commonly found to

inhabit freshwater environments (Holt, 1994). The other species found was not positively identified, due to inconclusive 16s rRNA sequencing results. From the biochemical testing, however, it was hypothesized that the infectious species belonged to the Vibrionaceae family and either the Vibrio or Aeromonas genera. Since little was known about the ecology of the Pseudomonad species and there are many clinical species of Vibrio and Aeromonas that are known to be infectious (Holt, 1994), it was assumed that the wound infection on the fish was caused by this species.

Conclusion

Much further and more intensive study is need on Black Hole Creek, in order to make any solid conclusions or implications for fisheries management in this stream. However, from this preliminary study, it can be established that BHC is of marginal environmental quality, as compared to Sugar Camp Run and Big Bear Creek. It is obvious, from the data found in this study and from current fisheries management practices on the stream, that BHC is currently unable to support naturally reproducing populations of wild trout. Perhaps stream clean-up efforts and fluvial geomorphologic restoration could allow this stream to reach wild trout population-sustaining capabilities.

Efforts to clean up BHC are not foreseeable in the near future, since current stream restoration efforts are being implemented on streams with already established native trout populations. Furthermore, funding for these programs are scarce and stream habitat restoration is a relatively new practice without widespread implementation in Pennsylvania waters.

Though funding for stream restoration activities seems to be scarce, one should not conclude that funds are not available or could not be made available. Changes in current PFBC fisheries management could free up funds to support these activities. Inefficiencies were found by economic studies done in

Colorado that report surplus amounts of money are currently spent on trout stocking efforts, relative to anglers' willingness to pay for cacheable trout fishing. If a similar economic study were to be performed in Pennsylvania and similar findings discovered, then it could be concluded that Pennsylvania does spend too much money on these activities, relative to amounts spent on stream habitat restoration. Above all this is the obstacle of educating the public on the degenerative effects of "put and take" trout stocking and reworking long-standing fisheries management policies.

In conclusion, fisheries management practices must be examined more closely for inefficiencies. Also, more intensive stream sampling is needed to more accurately classify waters of marginal environmental quality from waters managed as trout fisheries. BHC would be better suited for supporting non-game fish species and warm water game species, rather than trout, in its current condition. For this reason, and for possible economic reasons, it was recommended that BHC should not be managed as a "put and take" fishery, when money spent on this activity could be better spent on restoring this stream for sustaining naturally reproducing trout populations.

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Appendix


CASCADE
LEWIS
GAMBLE
Jacoby Mountain
TIADAGHTON STATE FOREST
PLUNKETTS
SCR
BBC
HEPBURN
ELDRED
ALLEGHENY
UPPER FAIRFIELD
MILL CREEK
LOYALSOCK
OLD LYCOMING
FAIRFIELD
MUNCY
Williamsport
Montoursville
South Williamsport
Sylvan Dell
Duboistown
BALD EAGLE MOUNTAIN
TIADAGHTON STATE FOREST
CLINTON
ARMSTRONG
BHC
Maple Hill
Montgomery
BRADY
WHITE DEER RIDGE
DELAWARE
LYCOMING CO
UNION CO
NORTHUMBERLAND CO
WASHINGTON
SOUTH WHITE DEER RIDGE
GREGG
Muncy
Allenwood
Spring Garden

TABLE 1: Habitat Assesment Scores

	Primary Habitat Characteristics			Secondary Habitat Characteristics								
Creek	% Cobble	Velocity	Embedded-ness	Velocity/depth regimes	Bank/channel alteration	Sediment deposition	Riffle char-acteristics	% Bottom exposed	Bank conditions	Bank vegetation	Riparian vegetation	Overhead canopy
Black Hole Creek	17	11	8	5	7	2	5	10	2	3	10	3
Sugar Camp Run	20	20	12	10	5	4	8	10	5	5	10	7
Big Bear Creek	18	20	20	7	10	10	10	4	1	9	10	9
possible score:	*20*	*20*	*20*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*

	Total Score Comparisons	
Creek	Site score	% of possible score
Black Hole Creek	83	55
Sugar Camp Run	116	77
Big Bear Creek	128	85
possible score:	*150*	*100*

Similarity Comparisons		
% similarity BHC/SCR	% similarity BHC/BBC	% similarity SCR/BBC
71	64	90

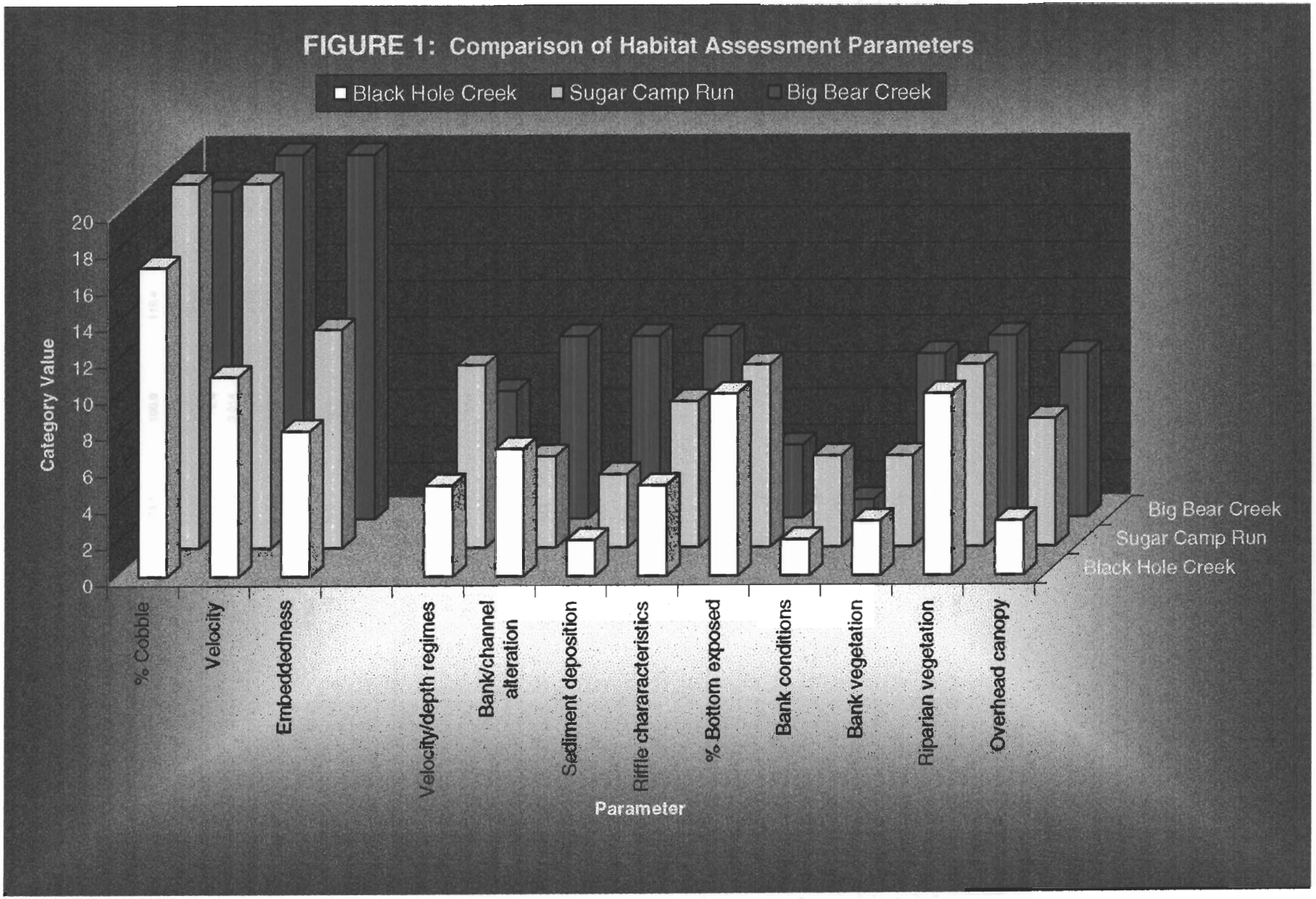


FIGURE 1: Comparison of Habitat Assessment Parameters
Black Hole Creek
Sugar Camp Run
Big Bear Creek
Category Value
0
2
4
6
8
10
12
14
16
18
20
% Cobble
Velocity
Embeddedness
Velocity/depth regimes
Bank/channel alteration
Sediment deposition
Riffle chararacteristics
% Bottom exposed
Bank conditions
Bank vegetation
Riparian vegetation
Overhead canopy
Parameter
Big Bear Creek
Sugar Camp Run
Black Hole Creek

TABLE 2.1: Water Chemistry Data

Black Hole Creek	Sampling date							
Parameter	10/5/99	1/15/00	2/10/00	2/17/00	2/24/00	3/8/00	3/21/00	3/30/00
pH	5.52	5.80	7.40	6.22	6.50	6.33	6.44	6.14
Alkalinity (ppm CaCO3)	21.0	13.0	90.0	11.0	10.5	2.1	1.5	4.5
TDS (ppm)	31.6	29.8	74.1	100.9	118.4	34.7	52.8	15.1
Conductivity (mv/s)	73.0	60.8	92.3	138.7	170.0	74.1	74.6	20.9
DO (ppm)	10.12	17.65	16.40	16.50	15.34	15.09	12.75	13.02
Temp. (^{0}C)	12.2	2.2	2.1	2.4	3.8	4.2	6.3	6.3
Aluminum (mg/L)	0.013	0.006	0.005	0.014	0.012	N/A	0.008	0.004
Total Chlorine (mg/L)	0.04	0.02	0.05	0.03	0.03	N/A	0.02	0.01
Nitrate (mg/L)	1.7	1.9	0.2	0.7	0.7	N/A	0.5	1.1
Nitrite (mg/L)	0.20	0.68	N/A	N/A	N/A	N/A	N/A	N/A
Ortho-P (mg/L)	0.028	0.096	0.04	0.071	0.088	N/A	0.090	0.031
Total-P (mg/L)	0.8	1.0	N/A	N/A	N/A	N/A	N/A	N/A

Sugar Camp Run	Sampling date							
Parameter	10/5/99	1/15/00	2/10/00	2/17/00	2/24/00	3/8/00	3/21/00	3/30/00
pH	6.13	6.50	6.23	6.12	6.56	6.34	N/A	N/A
Alkalinity (ppm CaCO3)	10.0	8.0	8.5	11.3	13.0	12.5	N/A	N/A
TDS (ppm)	8.3	8.9	12.9	15.6	14.5	13.3	N/A	N/A
Conductivity (mv/s)	30.4	17.9	24.6	31.3	19.3	16.8	N/A	N/A
DO (ppm)	10.84	18.34	17.60	14.30	16.40	18.10	N/A	N/A
Temp. (^{0}C)	10.7	1.4	1.2	1.9	3.1	5.3	N/A	N/A
Aluminum (mg/L)	0.000	0.004	N/A	N/A	N/A	N/A	N/A	N/A
Total Chlorine (mg/L)	0.02	0.02	N/A	N/A	N/A	N/A	N/A	N/A
Nitrate (mg/L)	2.1	2.0	N/A	N/A	N/A	N/A	N/A	N/A
Nitrite (mg/L)	0.80	0.79	N/A	N/A	N/A	N/A	N/A	N/A
Ortho-P (mg/L)	0.038	0.080	N/A	N/A	N/A	N/A	N/A	N/A
Total-P (mg/L)	0.45	1.00	N/A	N/A	N/A	N/A	N/A	N/A

Big Bear Creek	Sampling date							
Parameter	10/5/99	1/15/00	2/10/00	2/17/00	2/24/00	3/8/00	3/21/00	3/30/00
pH	6.15	5.44	5.4	5.98	5.78	5.67	N/A	N/A
Alkalinity (ppm CaCO3)	3.2	0	0	6.2	0	0	N/A	N/A
TDS (ppm)	4.6	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Conductivity (mv/s)	12.1	0	0	0	N/A	2.8	N/A	N/A
DO (ppm)	10.89	15.30	15.82	N/A	N/A	13.2	N/A	N/A
Temp. (^{0}C)	9.9	4.2	0.9	N/A	N/A	4.9	N/A	N/A
Aluminum (mg/L)	0.019	0.009	0	0.003	N/A	0.01	N/A	N/A
Total Chlorine (mg/L)	0.00	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Nitrate (mg/L)	1.0	1.9	1.9	N/A	N/A	0.2	N/A	N/A
Nitrite (mg/L)	0.20	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Ortho-P (mg/L)	0.025	0.100	0.237	N/A	N/A	0.11	N/A	N/A
Total-P (mg/L)	0.48	0.40	0.38	N/A	N/A	0.5	N/A	N/A

TABLE 2.2: Total Dissolved Solids (TDS) and Conductivity

TDS

	10/5/99	1/15/00	2/10/00	2/17/00	2/24/00	3/8/00	3/21/00	3/30/00
Black Hole Creek	31.6	29.8	74.1	100.9	118.4	34.7	52.8	15.1
Sugar Camp Run	8.3	8.9	12.9	15.6	14.5	13.3	N/A	N/A
Big Bear Creek	4.6	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Conductivity

	10/5/99	1/15/00	2/10/00	2/17/00	2/24/00	3/8/00	3/21/00	3/30/00
Black Hole Creek	73.0	60.8	92.3	138.7	170.0	74.1	74.6	20.9
Sugar Camp Run	30.4	17.9	24.6	31.3	19.3	16.8	N/A	N/A
Big Bear Creek	12.1	0	0	0	N/A	2.8	N/A	N/A

TABLE 2.3: Stream pH

	10/5/99	1/15/00	2/10/00	2/17/00	2/24/00	3/8/00	3/21/00	3/30/00
Black Hole Creek	5.52	5.80	7.40	6.22	6.50	6.33	6.44	6.14
Sugar Camp Run	6.13	6.50	6.23	6.12	6.56	6.34	N/A	N/A
Big Bear Creek	6.15	5.44	5.4	5.98	5.78	5.67	N/A	N/A

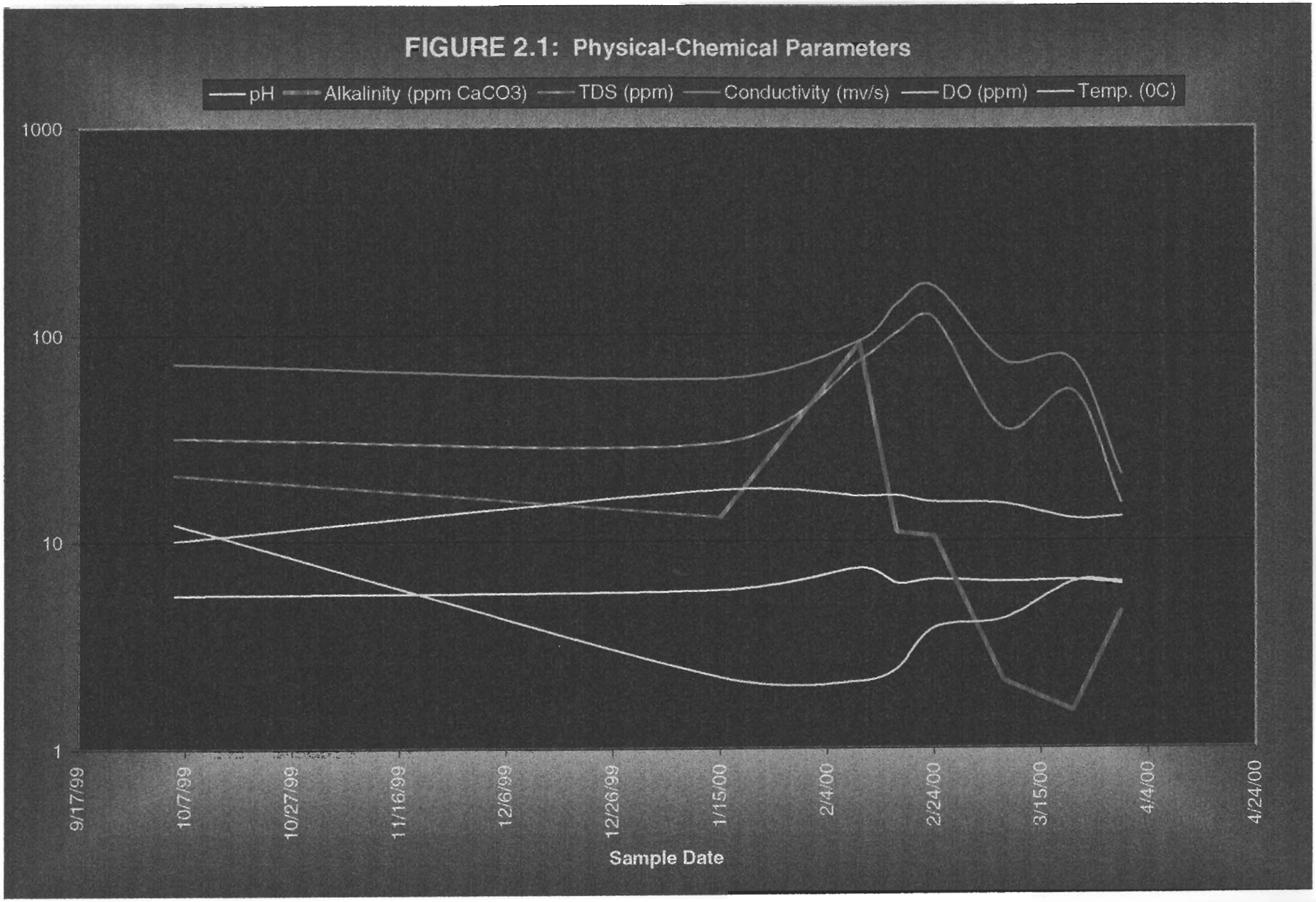


FIGURE 2.1: Physical-Chemical Parameters
pH
Alkalinity (ppm CaCO3)
TDS (ppm)
Conductivity (mv/s)
DO (ppm)
Temp. (0C)
1000
100
10
1
9/17/99
10/7/99
10/27/99
11/16/99
12/6/99
12/26/99
1/15/00
2/4/00
2/24/00
3/15/00
4/4/00
4/24/00
Sample Date

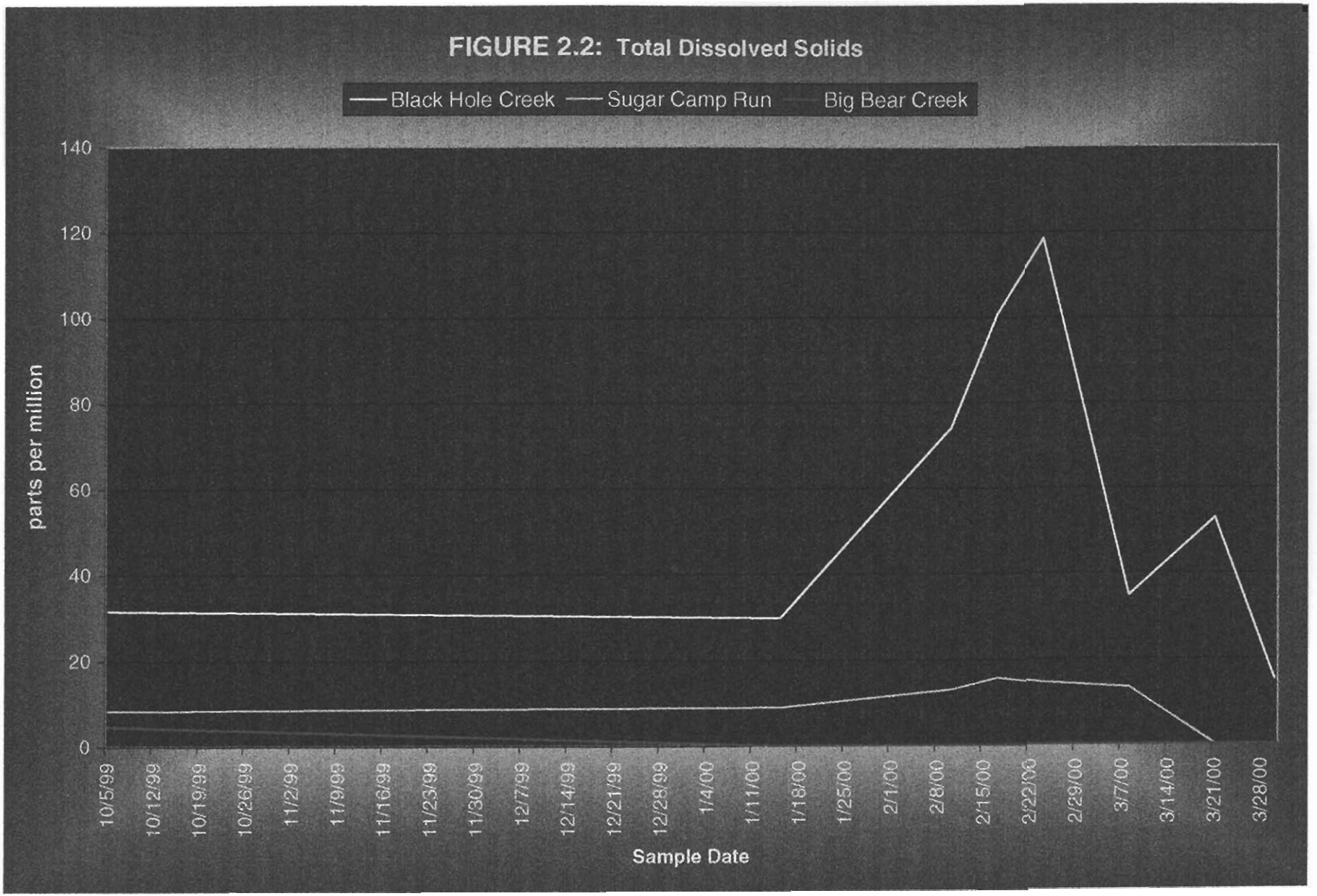
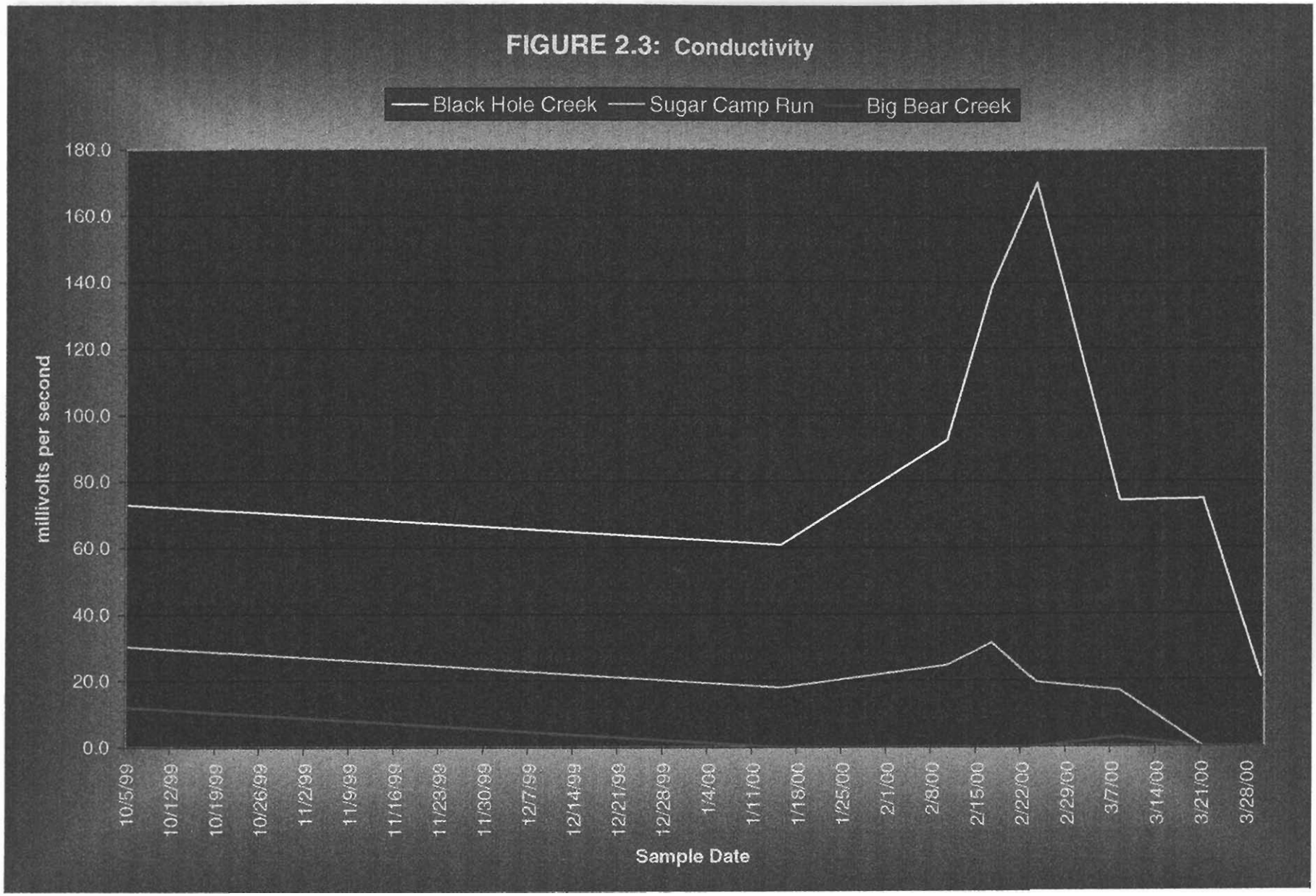


FIGURE 2.2: Total Dissolved Solids
Black Hole Creek
Sugar Camp Run
Big Bear Creek
parts per million
140
120
100
80
60
40
20
0
10/5/99
10/12/99
10/19/99
10/26/99
11/2/99
11/9/99
11/16/99
11/23/99
11/30/99
12/7/99
12/14/99
12/21/99
12/28/99
1/4/00
1/11/00
1/18/00
1/25/00
2/1/00
2/8/00
2/15/00
2/22/00
2/29/00
3/7/00
3/14/00
3/21/00
3/28/00
Sample Date

FIGURE 2.3: Conductivity



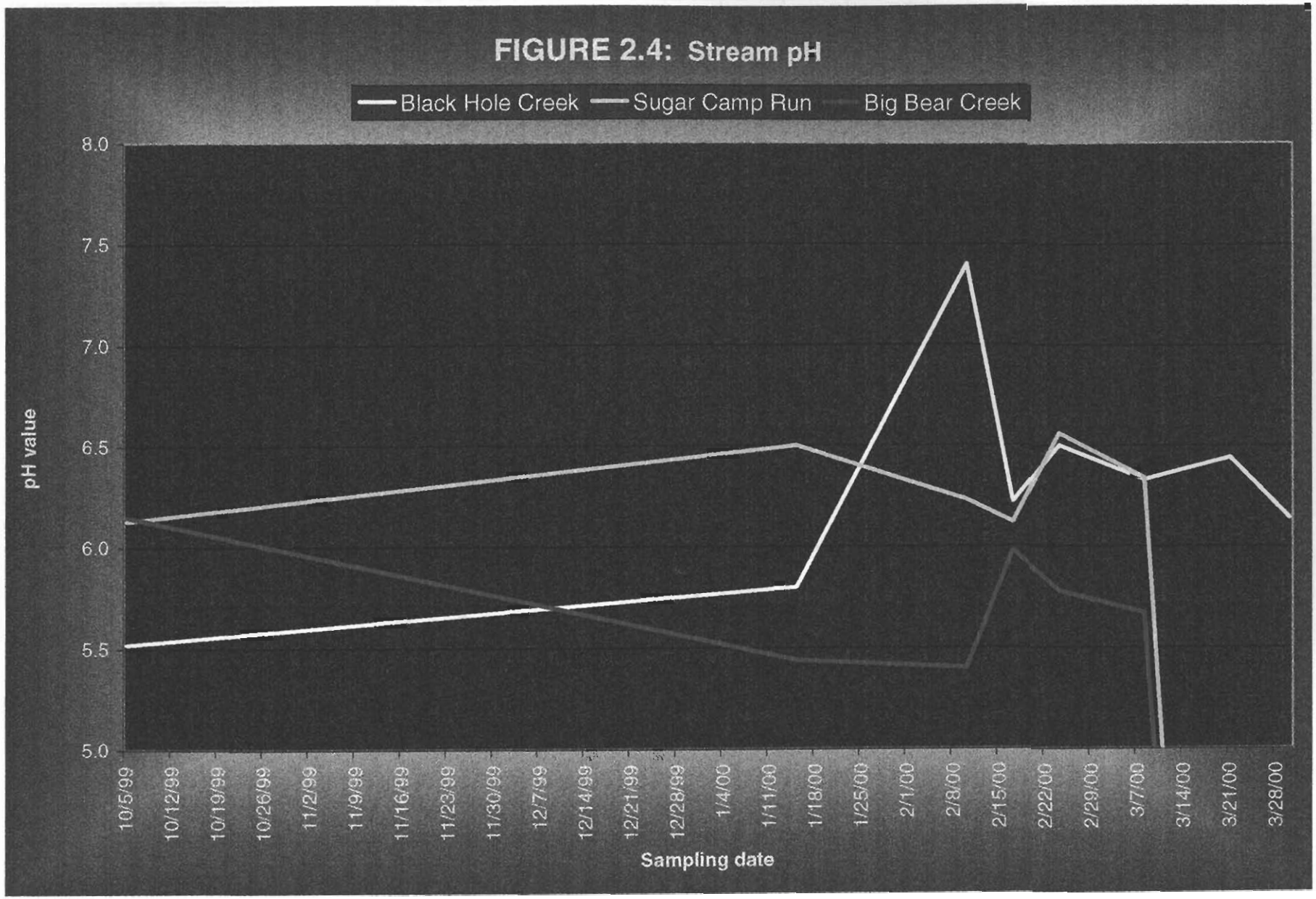


FIGURE 2.4: Stream pH
Black Hole Creek
Sugar Camp Run
Big Bear Creek
8.0
7.5
7.0
6.5
6.0
5.5
5.0
pH value
10/5/99
10/12/99
10/19/99
10/26/99
11/2/99
11/9/99
11/16/99
11/23/99
11/30/99
12/7/99
12/14/99
12/21/99
12/28/99
1/4/00
1/11/00
1/18/00
1/25/00
2/1/00
2/8/00
2/15/00
2/22/00
2/29/00
3/7/00
3/14/00
3/21/00
3/28/00
Sampling date

TABLE 3.1 Aquatic Macroinvertebrates

			Black Hole Creek					Sugar Camp Run				Big Bear Creek					
			kick	kick	CPOM	kick	kick	kick	kick	CPOM	kick	kick	kick	kick	kick	kick	kick
			9/18/99	10/10/99	11/5/99	1/15/99	2/24/99	9/18/99	10/10/99	11/5/99	1/15/99	9/18/99	10/10/99	11/5/99	1/15/99	2/24/99	3/21/00
Nematoda		nematode sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oligocheata		oligocheate sp.	3	3	0	3	0	1	1	0	0	1	0	0	0	0	0
Plecoptera	_Capniidae	paracapnia	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0
	_Chloroperlidae	suwallia	0	0	0	0	0	2	2	0	5	0	0	0	0	1	0
		sweltsa	0	0	0	0	0	0	0	1	0	0	0	0	0	20	0
	_Leuctridae	leuctra	0	0	0	0	1	0	0	0	8	12	21	16	8	3	14
	_Peltoperlidae	peltoperla	0	0	0	0	0	0	0	1	1	1	1	1	2	2	0
	_Perlidae	paragnetina	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0
	_Perlodidae	isogenoides	0	0	0	0	0	0	0	0	0	0	0	0	8	2	3
		isoperla	4	1	0	0	1	2	5	15	4	0	0	0	1	3	3
	_Taeniopterygidae	taeniopteryx	48	51	39	8	1	5	6	3	1	0	0	0	0	0	0
	_Pteronarcidae	pteronarcys	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Ephemeroptera	_Baetidae	baetis	0	0	0	0	0	16	7	1	20	30	1	1	6	3	10
		acentrella	0	0	0	0	0	1	3	0	0	0	0	0	0	0	0
	_Ephemerellidae	drunella	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
		ephemerella	0	3	0	3	6	0	0	0	7	0	3	7	9	5	13
		serratella	0	0	0	0	0	5	5	0	0	0	0	0	0	0	0
	_Ephemeridae	ephemera	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0
	_Heptageniidae	epeorus	0	0	0	0	0	3	3	0	6	3	3	15	24	33	18
		heptagenia	0	0	0	0	0	0	0	0	0	1	0	2	4	5	4
		stenacron	0	0	0	0	0	6	4	1	0	0	0	0	0	0	0
		stenonema	4	3	1	2	2	0	0	0	6	10	11	7	0	1	0
	_Oliginuridae	isonychia	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
	_Leptophlebiidae	chloroterpes	0	1	0	1	0	11	10	0	0	0	0	0	0	0	0
		paraleptophlebia	0	0	0	0	0	0	0	0	4	5	11	6	11	10	16
Megaloptera	_Corydalidae	nigronia	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Tricoptera	_Brachycentridae	brachycentrus	1	1	0	0	0	1	1	0	0	1	0	2	0	5	0
	_Hydropsychidae	cheumatopsychidae	8	9	0	0	2	1	4	0	2	0	0	0	0	0	0
		diplectrona	0	0	0	0	0	8	5	0	3	0	0	0	0	0	0
		hydropsyche	0	0	0	0	0	5	5	0	4	18	20	9	5	0	2
	_Lepidostomatidae	lepidostoma	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0
	_Leptoceridae	setodes	0	0	0	0	0	0	0	0	0	0	12	7	10	0	1
	_Philopotamiidae	chimarra	12	10	0	0	5	0	0	0	0	0	0	0	0	0	0
		dolophilodes	0	0	0	0	0	0	0	0	2	4	9	4	2	1	6
	_Polycentropidae	neureclipsis	0	0	0	0	0	11	10	0	0	0	0	0	0	0	0
	_Phryganeidae	phryganeidae sp.	0	0	0	0	0	0	0	0	0	1	2	1	1	0	0
	_Rhyacophilidae	rhyacophila	0	0	0	0	0	2	2	0	2	0	0	0	0	4	0
Coleoptera	_Elmidae	promoresia	16	14	0	4	2	1	1	0	0	0	0	0	0	0	0
		optioservus	0	0	0	0	0	0	0	0	0	0	2	2	1	0	0
		stenelmis	15	15	0	3	1	2	3	0	1	0	0	0	0	0	0
	_Psephenidae	psephenus	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Diptera	_Athericidae	atherix	1	1	0	0	0	0	0	0	1	18	1	19	2	5	6
	_Chironomidae	chironomid sp.	3	3	2	11	9	29	26	1	27	11	4	6	8	1	8
	_Simuliidae	prosimulium	0	0	0	105	111	0	0	0	11	0	0	0	5	2	1
		simulium	1	1	2	0	1	0	0	0	0	0	0	0	0	0	0
	_Tipulidae	hexatoma	1	1	0	0	1	1	1	0	0	0	0	0	0	0	0
		tipula	0	0	0	0	0	0	0	0	0	0	4	1	2	0	3

TABLE 3.2: Macroinvertebrate Analyses

Rapid Bioassessment Protocol 3 - genus level taxonomy

Sample date	Impairment score	Reference Site	Community Loss Index	Jaccard Coefficient	Sample Method
9/18/99	66.7	Sugar Camp R.	1.00	0.321	Kick
10/10/99	62.5	Sugar Camp R.	1.00	0.321	Kick
11/5/99	52.4	Sugar Camp R.	1.50	0.200	CPOM
1/15/00	37.5	Big Bear Creek	1.78	0.240	Kick
2/24/00	25.0	Sugar Camp R.	1.00	0.231	Kick

TABLE 3.3 Macroinvertebrate RBP-metric Analyses

RBP 3_gen - Metric Dynamics for Black Hole Creek

	metric 1	metric 2	metric 3	metric 4	metric 5	metric 6	metric 7	metric 8	**total score**
Sample date	Total Taxa	F.B.I.	Rat-SC/FC	Rat-Sh/tot	EPT/Chir	% Dom.	EPT index	Com. loss	
9/18/99	4	6	6	6	0	0	4	6	**66.7**
10/10/99	4	6	6	6	6	0	0	4	**66.7**
11/5/99	2	6	N/A	6	6	0	0	2	**52.4**
1/15/00	2	6	2	2	0	0	2	4	**37.5**
2/24/00	4	2	0	0	0	0	4	2	**25**

TABLE 3.4: Distribution and % Dominance of Invertebrate Genera in Black Hole Creek

		Black Hole Creek				
		kick	kick	CPOM	kick	kick
F.F.G.		9/18/99	10/10/99	11/5/99	1/15/00	2/24/00
other	oligocheate sp.	3	3	0	3	0
SH	leuctra	0	0	0	0	1
P	isoperla	4	1	0	0	1
SH	taeniopteryx	48	51	39	8	1
CG	ephemerella	0	3	0	3	6
SC	stenonema	4	3	1	2	2
CG	chloroterpes	0	1	0	1	0
FC	brachycentrus	1	1	0	0	0
FC	cheumatopsychidae	8	9	0	0	2
FC	chimarra	12	10	0	0	5
SC	promoresia	16	14	0	4	2
SC	stenelmis	15	15	0	3	1
SC	psephenus	1	1	0	0	0
P	atherix	1	1	0	0	0
CG	chironomid sp.	3	3	2	11	9
FC	prosimulium	0	0	0	105	111
FC	simulium	1	1	2	0	1
P	hexatoma	1	1	0	0	1
		118	118	44	140	143
		41%	43%	89%	75%	78%
		taeniopteryx	taeniopteryx	taeniopteryx	prosimulium	prosimulium

Functional feeding group (F.F.G.) totals:

	9/18/99	10/10/99	11/5/99	1/15/00	2/24/00
Predators (P)	6	3	0	0	2
Shredders (SH)	48	51	39	8	2
Scrapers (SC)	36	33	1	9	5
Filtering Coll. (FC)	22	21	2	105	119
Gathering Coll. (CG)	3	7	2	15	15
Other	3	3	0	3	0

TABLE 3.5: Distribution and % Dominance of Invertebrate Genera in Sugar Camp Run

		Sugar Camp Run			
		kick	kick	CPOM	kick
F.F.G		9/18/99	10/10/99	11/5/99	1/15/00
other	oligocheate sp.	1	1	0	0
SH	paracapnia	0	0	0	9
P	suwallia	2	2	0	5
P	sweltsa	0	0	1	0
SH	leuctra	0	0	0	8
SH	peltoperla	0	0	1	1
P	paragnetina	2	2	0	0
P	isoperla	2	5	15	4
SH	taeniopteryx	5	6	3	1
CG	baetis	16	7	1	20
CG	acentrella	1	3	0	0
SC	drunella	0	0	1	0
CG	ephemerella	0	0	0	7
CG	serratella	5	5	0	0
CG	ephemera	2	2	0	0
CG	epeorus	3	3	0	6
CG	stenacron	6	4	1	0
SC	stenonema	0	0	0	6
FC	isonychia	1	1	0	0
CG	chloroterpes	11	10	0	0
CG	paraleptophlebia	0	0	0	4
P	nigronia	0	0	0	1
FC	brachycentrus	1	1	0	0
FC	cheumatopsychidae	1	4	0	2
FC	diplectrona	8	5	0	3
FC	hydropsyche	5	5	0	4
SH	lepidostoma	0	0	0	1
FC	dolophilodes	0	0	0	2
FC	neureclipsis	11	10	0	0
P	rhyacophila	2	2	0	2
SC	promoresia	1	1	0	0
SC	stenelmis	2	3	0	1
P	atherix	0	0	0	1
CG	chironomid sp.	29	26	1	27
FC	prosimulium	0	0	0	11
P	hexatoma	1	1	0	0
		118	109	24	126
		25% chironomid sp.	24% chironomid sp.	63% isoperla	21% chironomid sp.

Functional feeding group (F.F.G.) totals:

	9/18/99	10/10/99	11/5/99	1/15/00
Predators (P)	9	12	16	13
Shredders (SH)	5	6	4	20
Scrapers (SC)	3	4	1	7
Filtering Coll. (FC)	27	26	0	22
Gathering Coll. (CG)	73	60	3	64
Other	1	1	0	0

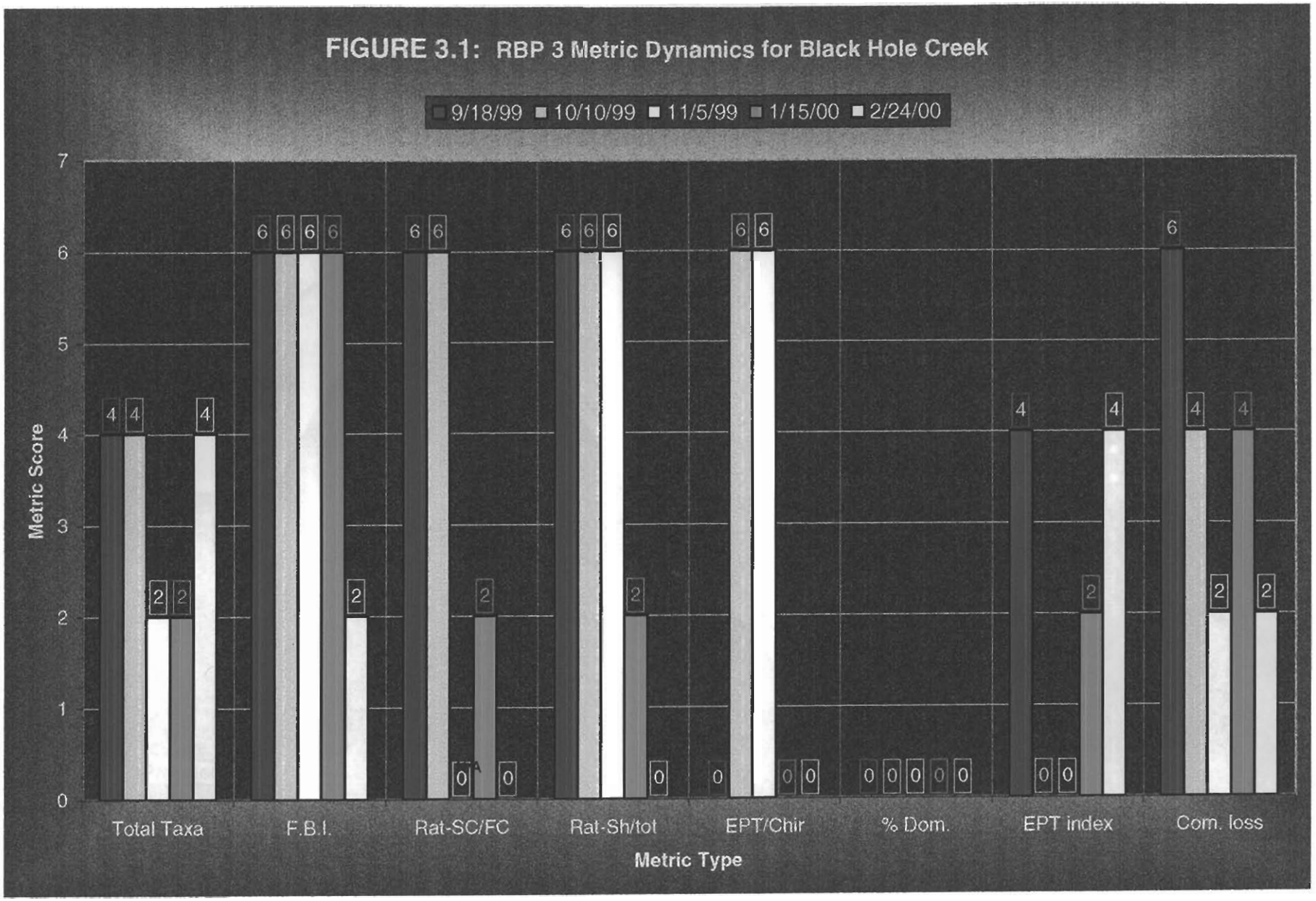
TABLE 3.6: Distribution and % Dominance of Invertebrate Genera in Big Bear Creek

		Big Bear Creek				
		kick	kick	kick	kick	kick
F.F.G.		9/18/99	10/10/99	11/5/99	1/15/00	2/24/00
other	oligocheate sp.	1	0	0	0	0
P	suwallia	0	0	0	0	1
P	sweltsa	0	0	0	0	20
SH	leuctra	12	21	16	8	3
SH	peltoperla	1	1	1	2	2
P	isogenoides	0	0	0	8	2
P	isoperla	0	0	0	1	3
SH	pteronarcys	0	0	0	1	0
CG	baetis	30	1	1	6	3
CG	ephemerella	0	3	7	9	5
CG	epeorus	3	3	15	24	33
SC	heptagenia	1	0	2	4	5
SC	stenonema	10	11	7	0	1
CG	paraleptophlebia	5	11	6	11	10
FC	brachycentrus	1	0	2	0	5
FC	hydropsyche	18	20	9	5	0
SH	lepidostoma	0	0	0	0	2
CG	setodes	0	12	7	10	0
FC	dolophilodes	4	9	4	2	1
SH	phryganeidae sp.	1	2	1	1	0
P	rhyacophila	0	0	0	0	4
SC	optioservus	0	2	2	1	0
P	atherix	18	1	19	2	5
CG	chironomid sp.	11	4	6	8	1
FC	prosimulium	0	0	0	5	2
SH	tipula	0	4	1	2	0
		116	105	106	110	108
		26% baetis	20% leuctra	18% atherix	22% epeorus	31% epeorus

Functional feeding group (F.F.G.) totals:

	9/18/99	10/10/99	11/5/99	1/15/00	2/24/00
Predators (P)	18	1	19	11	35
Shredders (SH)	14	28	19	14	7
Scrapers (SC)	11	13	11	5	6
Filtering Coll. (FC)	23	29	15	12	8
Gathering Coll. (CG)	49	34	42	68	52
Other	1	0	0	0	0

FIGURE 3.1: RBP 3 Metric Dynamics for Black Hole Creek



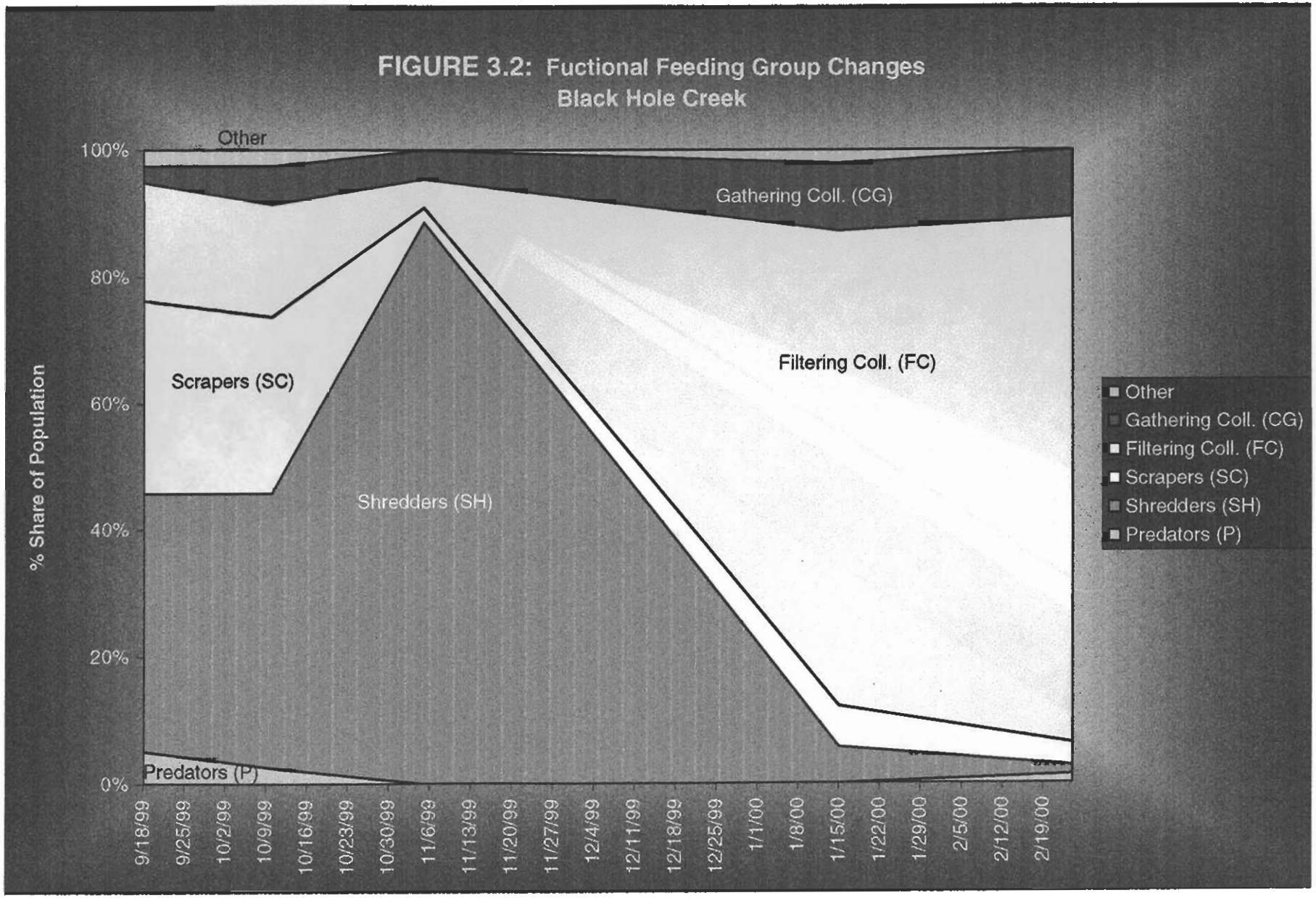


FIGURE 3.2: Fuctional Feeding Group Changes
Black Hole Creek
% Share of Population
100%
80%
60%
40%
20%
0%
Other
Gathering Coll. (CG)
Filtering Coll. (FC)
Scrapers (SC)
Shredders (SH)
Predators (P)
9/18/99
9/25/99
10/2/99
10/9/99
10/16/99
10/23/99
10/30/99
11/6/99
11/13/99
11/20/99
11/27/99
12/4/99
12/11/99
12/18/99
12/25/99
1/1/00
1/8/00
1/15/00
1/22/00
1/29/00
2/5/00
2/12/00
2/19/00
Other
Gathering Coll. (CG)
Filtering Coll. (FC)
Scrapers (SC)
Shredders (SH)
Predators (P)

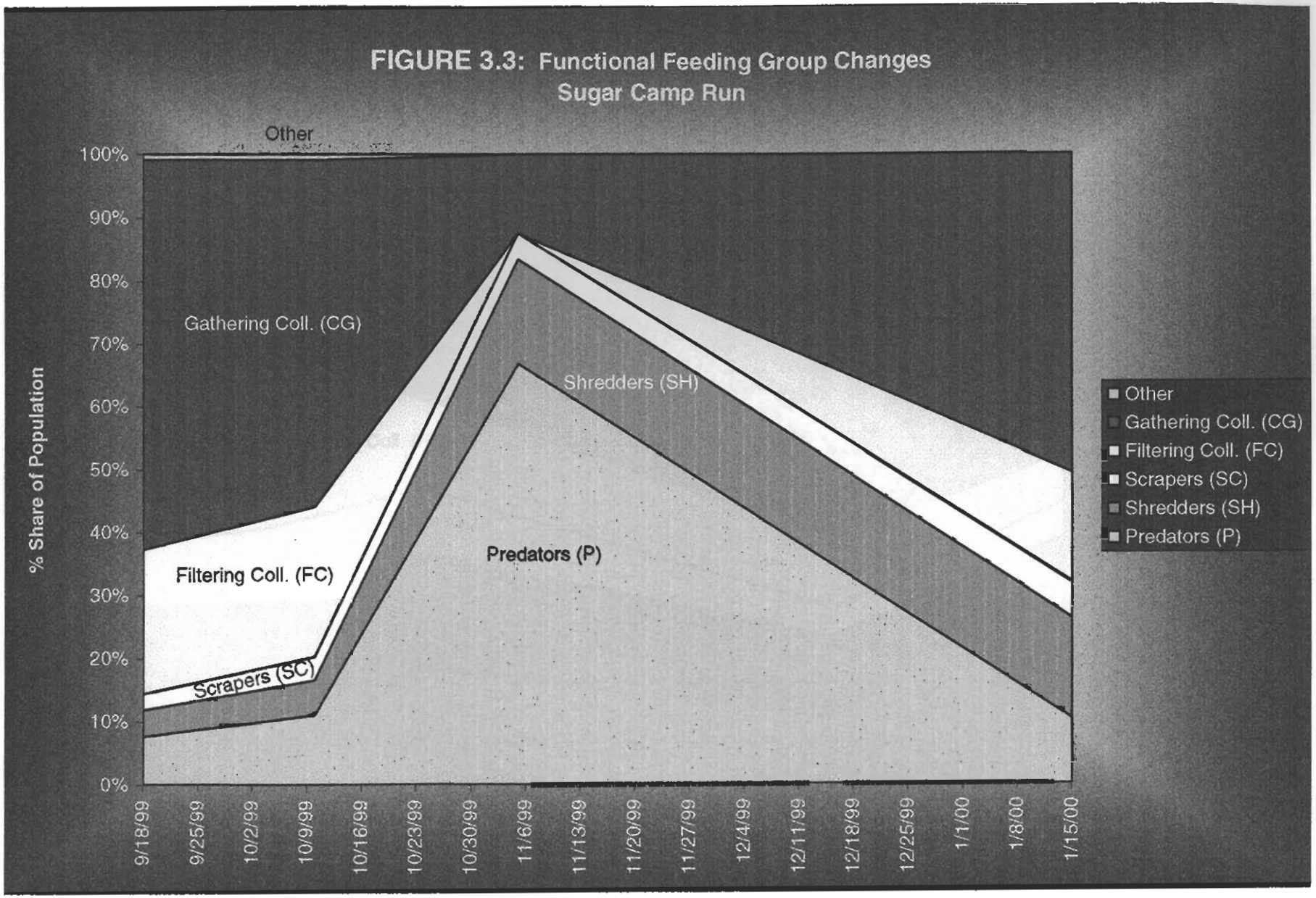


FIGURE 3.3: Functional Feeding Group Changes
Sugar Camp Run
Other
Gathering Coll. (CG)
Filtering Coll. (FC)
Scrapers (SC)
Shredders (SH)
Predators (P)
% Share of Population
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
9/18/99
9/25/99
10/2/99
10/9/99
10/16/99
10/23/99
10/30/99
11/6/99
11/13/99
11/20/99
11/27/99
12/4/99
12/11/99
12/18/99
12/25/99
1/1/00
1/8/00
1/15/00
Other
Gathering Coll. (CG)
Filtering Coll. (FC)
Scrapers (SC)
Shredders (SH)
Predators (P)

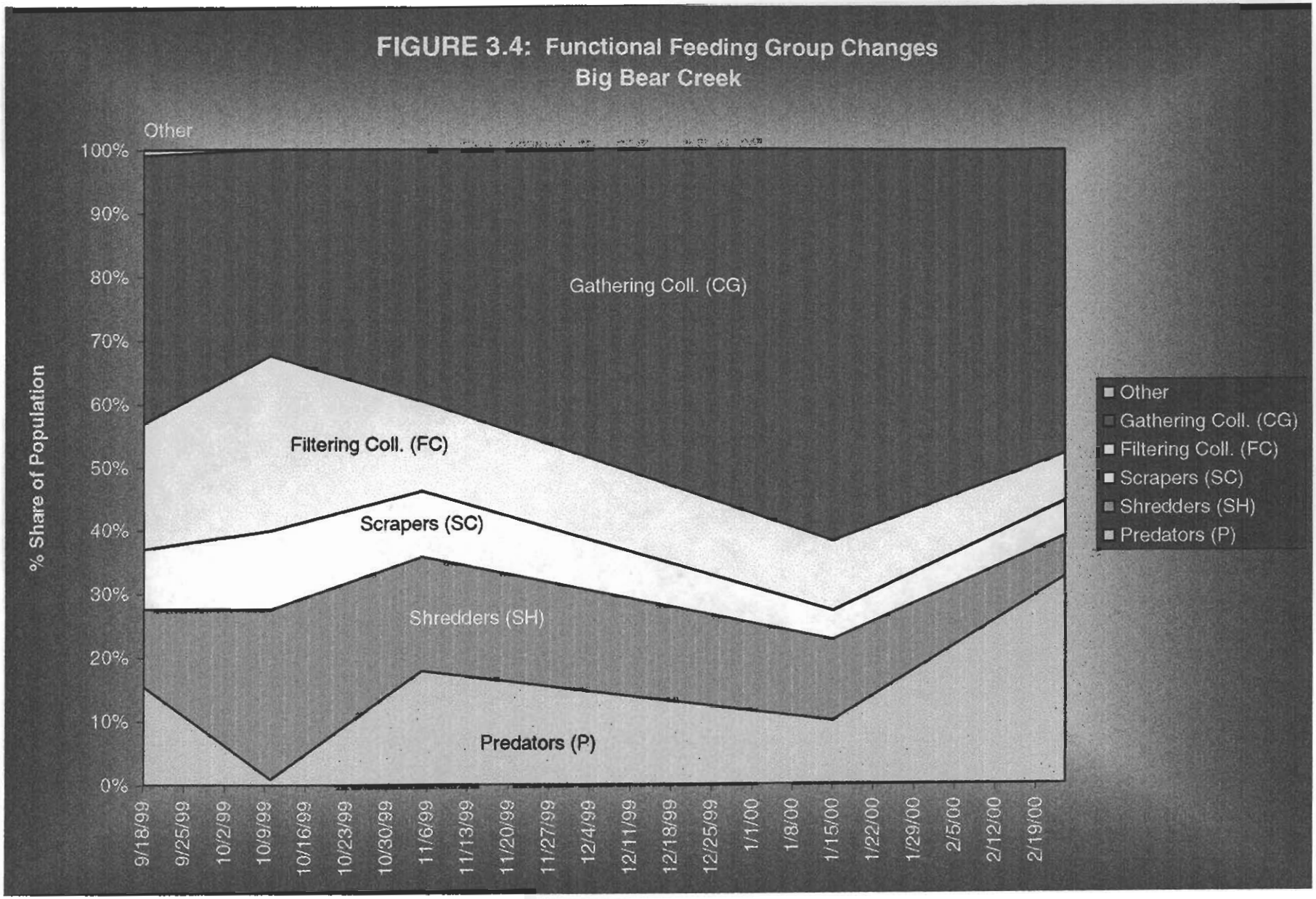


FIGURE 3.4: Functional Feeding Group Changes
Big Bear Creek
% Share of Population
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Other
Gathering Coll. (CG)
Filtering Coll. (FC)
Scrapers (SC)
Shredders (SH)
Predators (P)
9/18/99
9/25/99
10/2/99
10/9/99
10/16/99
10/23/99
10/30/99
11/6/99
11/13/99
11/20/99
11/27/99
12/4/99
12/11/99
12/18/99
12/25/99
1/1/00
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1/15/00
1/22/00
1/29/00
2/5/00
2/12/00
2/19/00

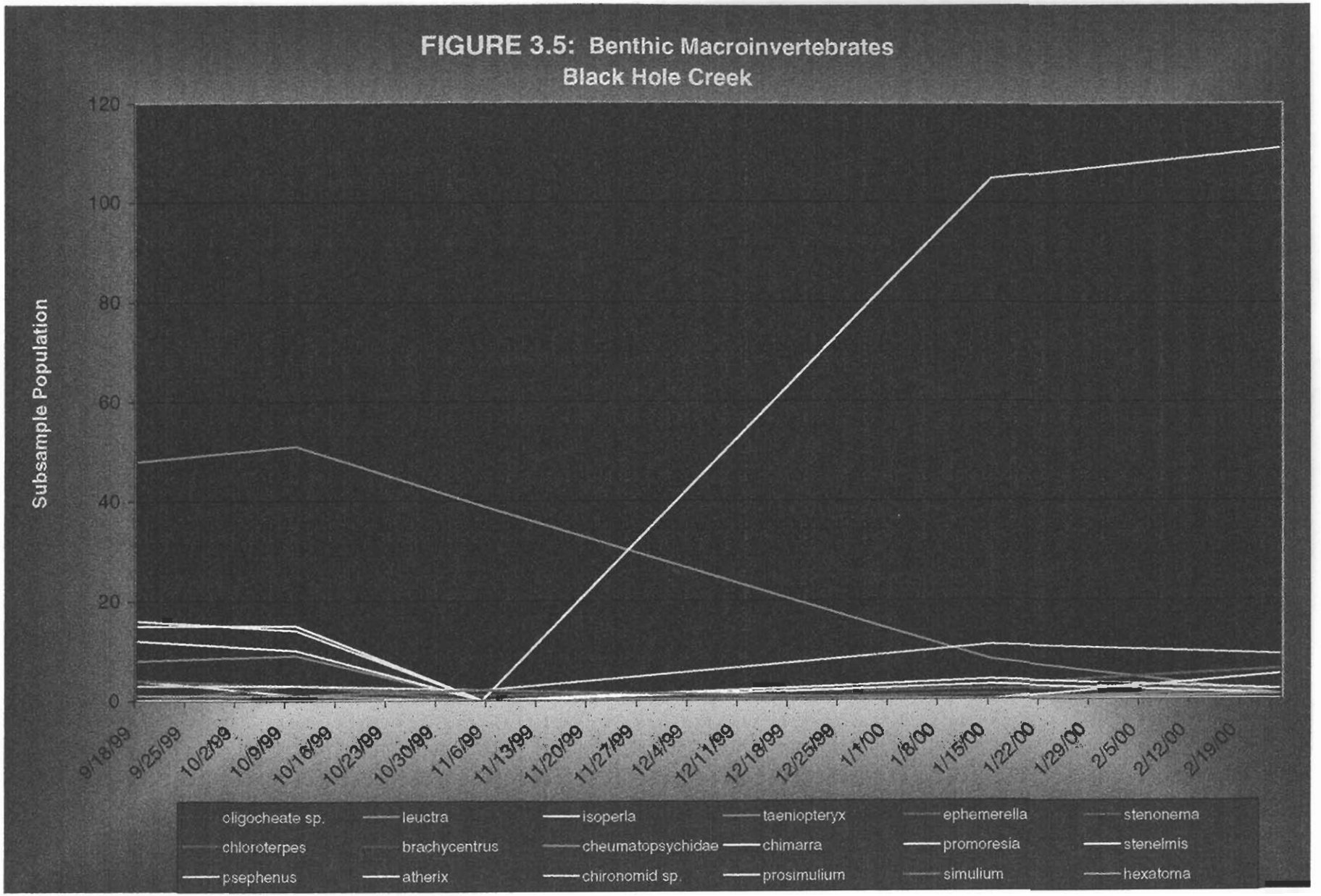
FIGURE 3.5: Benthic Macroinvertebrates
Black Hole Creek
Subsample Population
120
100
80
60
40
20
0
9/18/99
9/25/99
10/2/99
10/9/99
10/16/99
10/23/99
10/30/99
11/6/99
11/13/99
11/20/99
11/27/99
12/4/99
12/11/99
12/18/99
12/25/99
1/1/00
1/8/00
1/15/00
1/22/00
1/29/00
2/5/00
2/12/00
2/19/00
oligocheate sp.
leuctra
isoperla
taeniopteryx
ephemerella
stenonema
chloroterpes
brachycentrus
cheumatopsychidae
chimarra
promoresia
stenelmis
psephenus
atherix
chironomid sp.
prosimulium
simulium
hexatoma

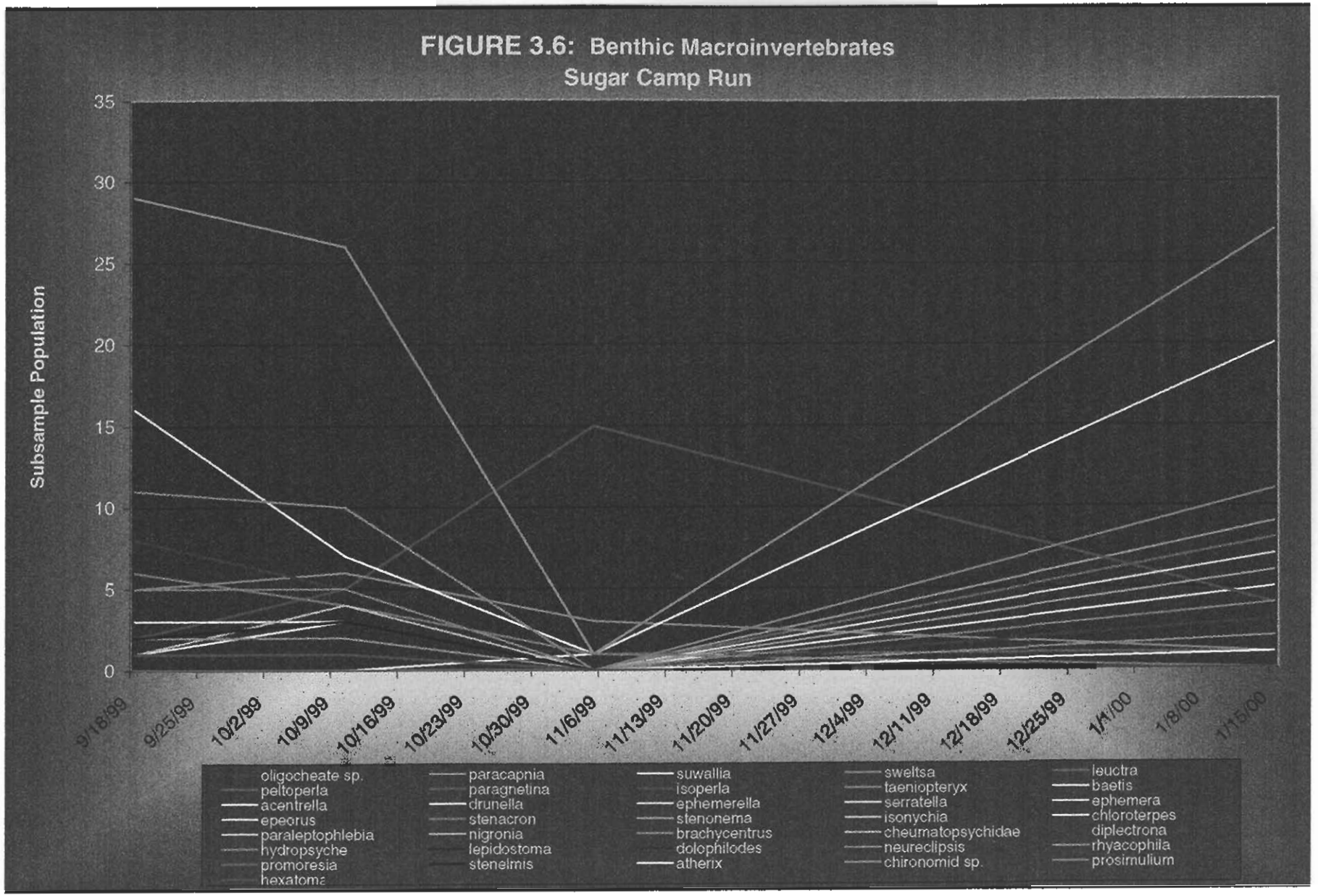
FIGURE 3.6: Benthic Macroinvertebrates
Sugar Camp Run
Subsample Population
35
30
25
20
15
10
5
0
9/18/99
9/25/99
10/2/99
10/9/99
10/16/99
10/23/99
10/30/99
11/6/99
11/13/99
11/20/99
11/27/99
12/4/99
12/11/99
12/18/99
12/25/99
1/1/00
1/8/00
1/15/00
oligocheate sp.
peltoperla
acentrella
epeorus
paraleptophlebia
hydropsyche
promoresia
hexatoma
paracapnia
paragnetina
drunella
stenacron
nigronia
lepidostoma
stenelmis
suwallia
isoperla
ephemerella
stenonema
brachycentrus
dolophilodes
atherix
sweltsa
taeniopteryx
serratella
isonychia
cheumatopsychidae
neureclipsis
chironomid sp.
leuctra
baetis
ephemera
chloroterpes
diplectrona
rhyacophila
prosimulium

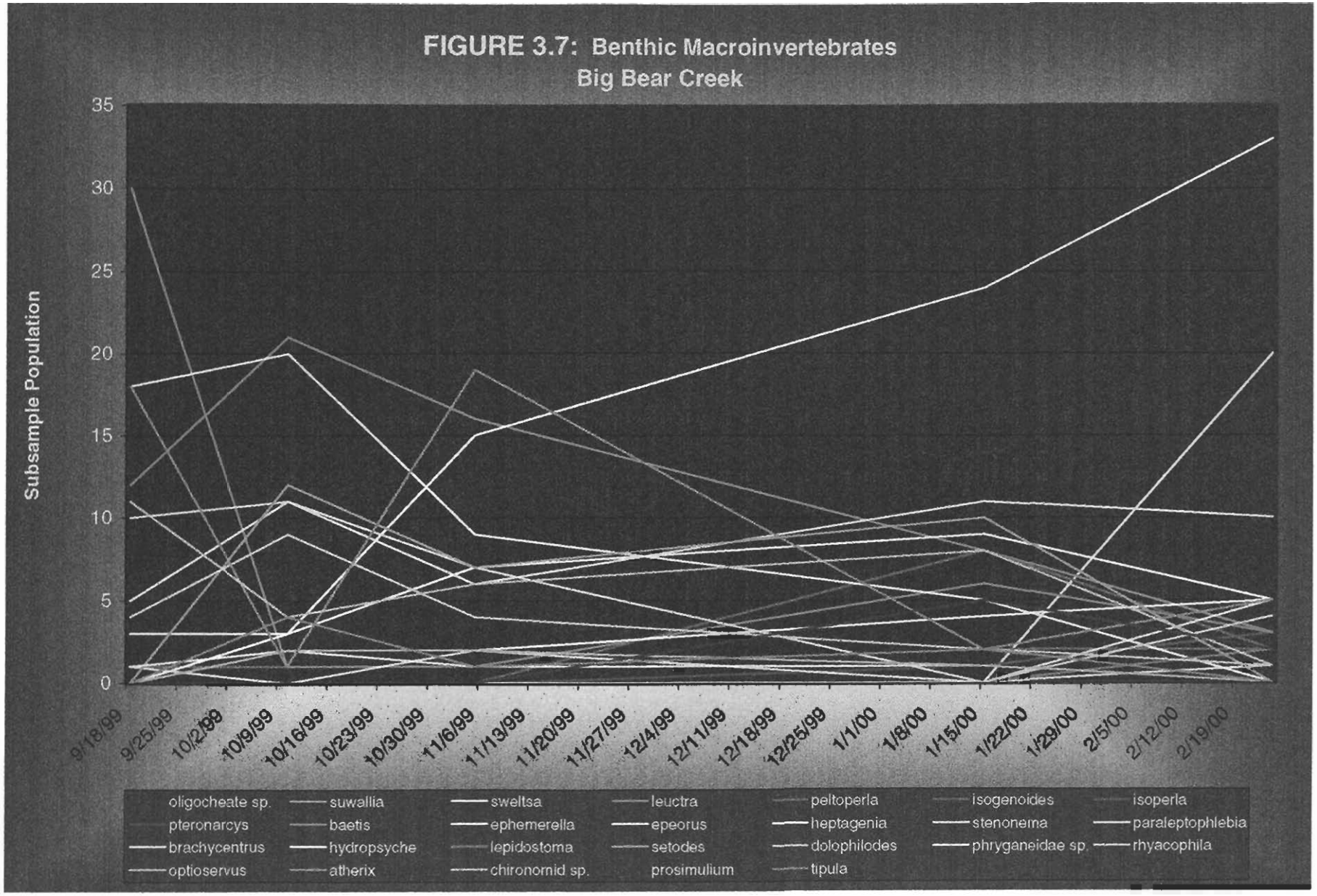
FIGURE 3.7: Benthic Macroinvertebrates
Big Bear Creek
Subsample Population
35
30
25
20
15
10
5
0
9/18/99
9/25/99
10/2/99
10/9/99
10/16/99
10/23/99
10/30/99
11/6/99
11/13/99
11/20/99
11/27/99
12/4/99
12/11/99
12/18/99
12/25/99
1/1/00
1/8/00
1/15/00
1/22/00
1/29/00
2/5/00
2/12/00
2/19/00
oligocheate sp.
suwallia
sweltsa
leuctra
peltoperla
isogenoides
isoperla
pteronarcys
baetis
ephemerella
epeorus
heptagenia
stenonema
paraleptophlebia
brachycentrus
hydropsyche
lepidostoma
setodes
dolophilodes
phryganeidae sp.
rhyacophila
optioservus
atherix
chironomid sp.
prosimulium
tipula

TABLE 4: Mycelial Biomass (ergosterol quantified comparison)

	Sample Date			
Creek	10/5/99	10/22/99	11/4/99	11/24/99
Black Hole Creek	22	15	22	15
Sugar Camp Run	31	6.5	10	9.6
Big Bear Creek	9.6	1.2	11	13
			Ergosterol	(ug/sample)

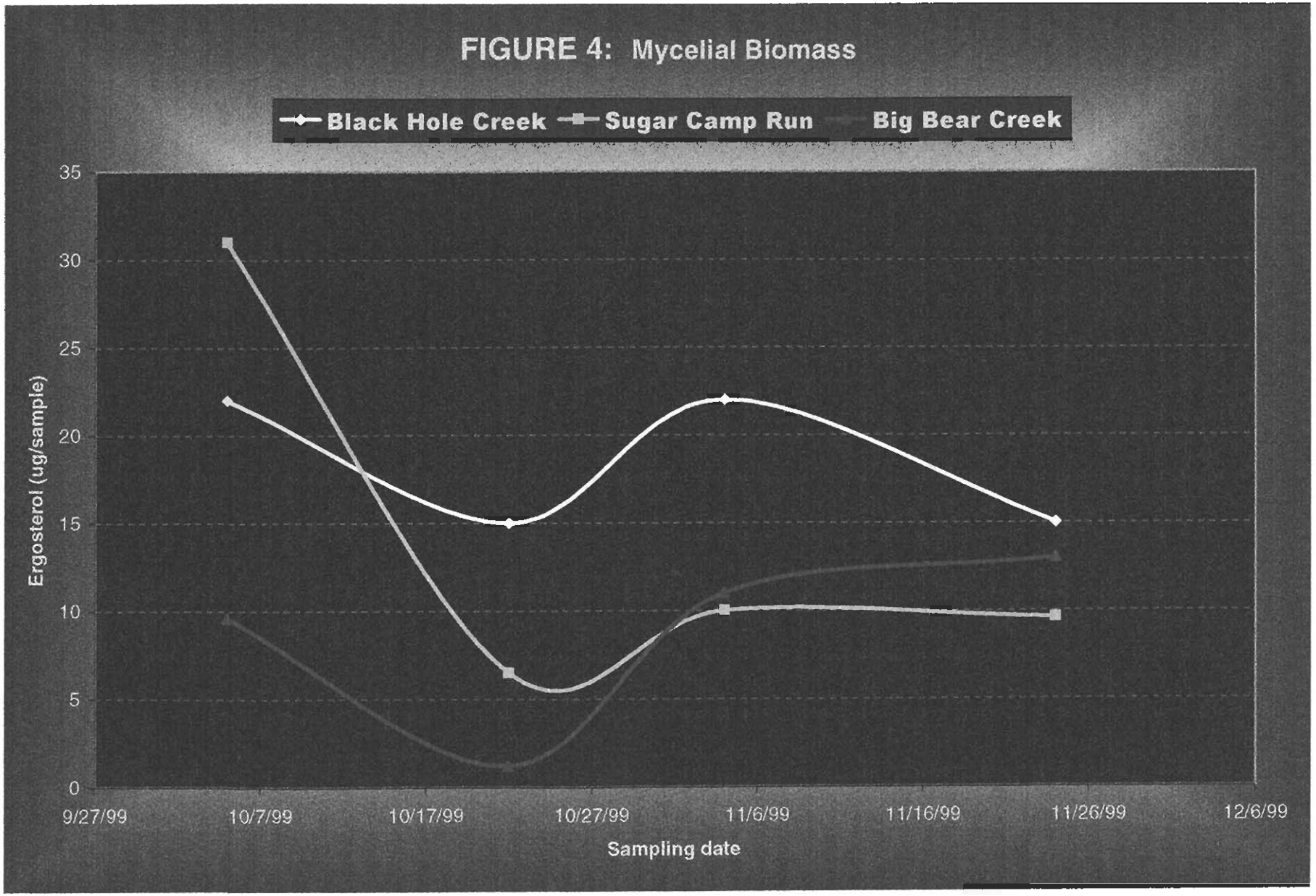
FIGURE 4: Mycelial Biomass
Black Hole Creek
Sugar Camp Run
Big Bear Creek
35
30
25
20
15
10
5
0
Ergosterol (ug/sample)
9/27/99
10/7/99
10/17/99
10/27/99
11/6/99
11/16/99
11/26/99
12/6/99
Sampling date

TABLE 5: Aquatic Hyphomycetes

Conidial Production Estimates

slides similar in production class designation...

		class 0	class 1	class 2	class 3	class 4	class 5	class 6	class 7
Black Hole Creek	2/10/00	0	4	1	1	0	0	0	0
Big Bear Creek	2/10/00	0	1	1	2	2	0	0	0
		class 0	class 1	class 2	class 3	class 4	class 5	class 6	class 7
Black Hole Creek	2/17/00	0	3	2	1	0	0	0	0
Big Bear Creek	2/17/00	0	1	2	2	1	0	0	0

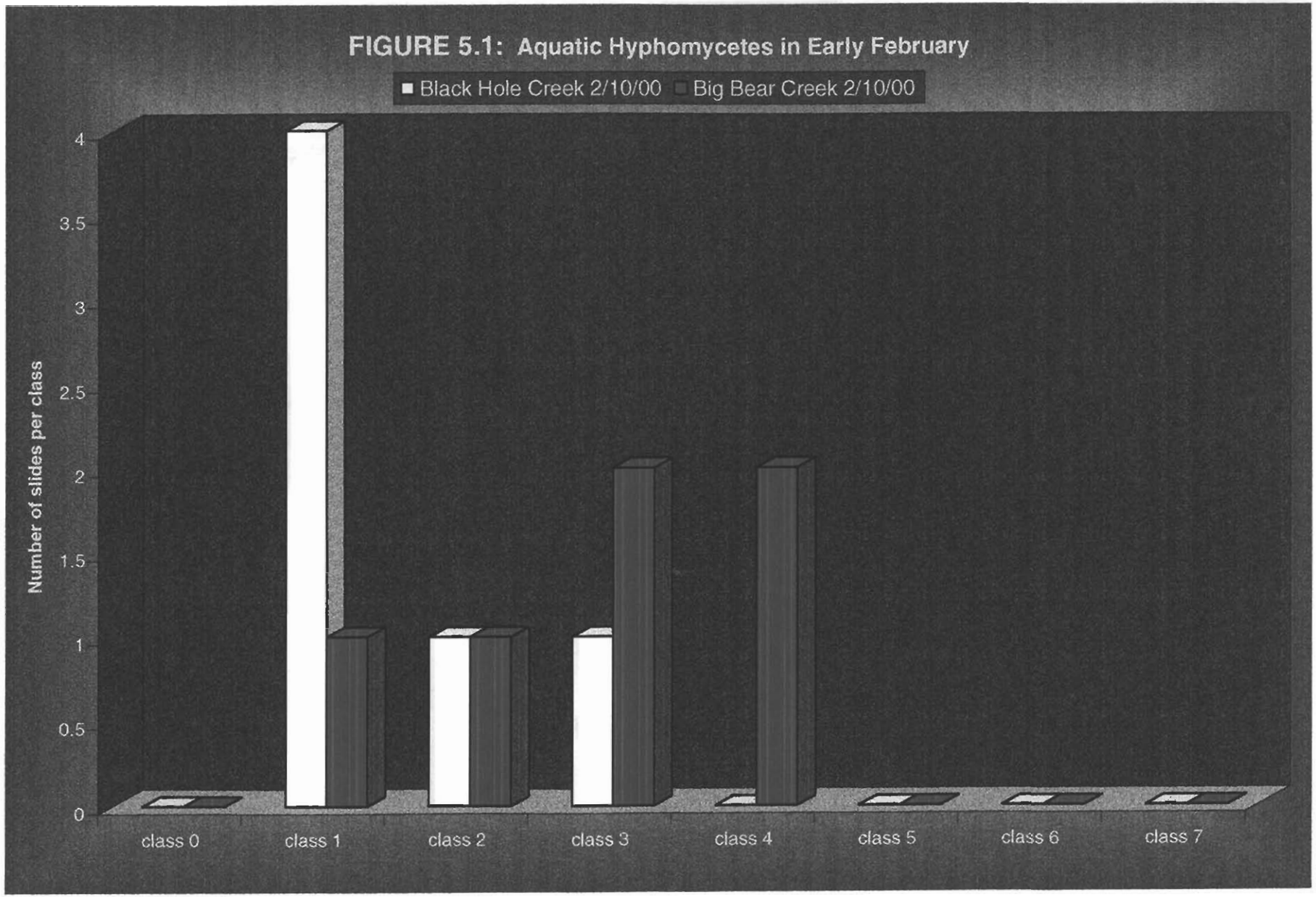
FIGURE 5.1: Aquatic Hyphomycetes in Early February
Black Hole Creek 2/10/00
Big Bear Creek 2/10/00
Number of slides per class
4
3.5
3
2.5
2
1.5
1
0.5
0
class 0
class 1
class 2
class 3
class 4
class 5
class 6
class 7

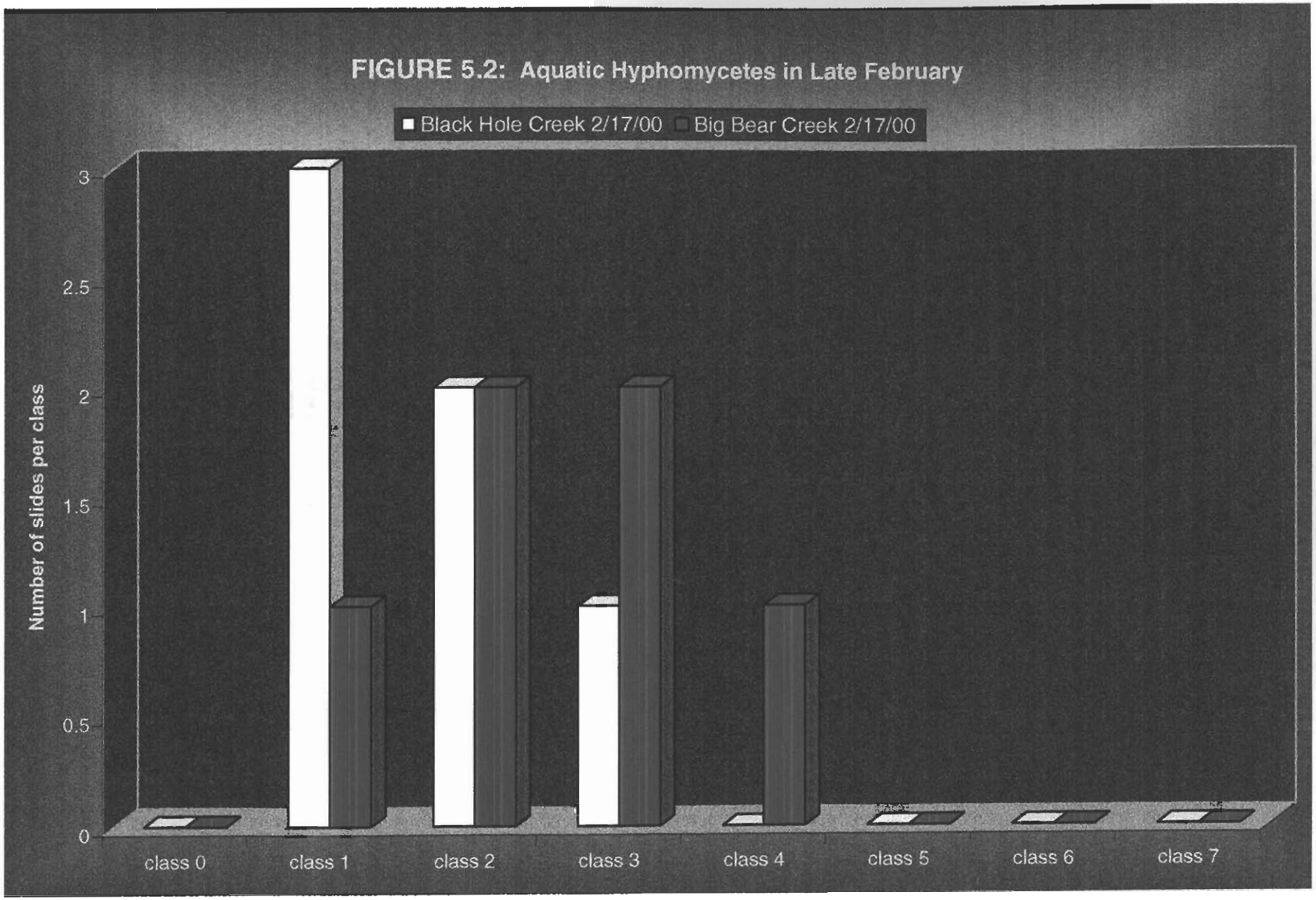
FIGURE 5.2: Aquatic Hyphomycetes in Late February
Black Hole Creek 2/17/00
Big Bear Creek 2/17/00
Number of slides per class
3
2.5
2
1.5
1
0.5
0
class 0
class 1
class 2
class 3
class 4
class 5
class 6
class 7

TABLE 6: Fish Community

Electrofishing Survey on Black Hole Creek

Number indv.	Species	Age grouping	Observations	P.T.V.	F.F.G	Native/Exotic
1	Blacknose Dace	Adult	Clean -- release	Tol	Gen	Native
1	Bullhead Catfish	Adult	Lg.bloody lesions, parasites -- capture	Tol	Ins	Native
3	Creek Chub*	Adult	2 w/ parasites -- capture 1 w/ parasites	Tol	Gen	Native
11	Creek Chub*	Juv.	some w/ parasites -- release all	Tol	Gen	Native
1	Slimy Sculpin*	Adult	Clean -- release	IM	Ins	Native
2	Slimy Sculpin*	Juv.	Clean -- release all	IM	Ins	Native
9	Smallmouth Bass	Juv.	Color var. among indv. -- release all	IM	Ins	Native
4	White Sucker	Adult	2 w/ parasites, 2 clean -- release all	Tol	Omn	Native

Total # species = 6

RBP 5 score = 36

interpreted as "poor" rating because of the general presence of parasites and possible baterial pathogens

TABLE 7: Microbial Ecology

	two bacterial species isolated from lesion	
Biochemical and Morphological Testing --	Species 1	Species 2
1 Shape	rod	rod
2 Motility (wet mount)	+	+slow
3 Gram reaction	negative	negative
4 Oxidase	+	+
5 Catalase	+	+
6 Mannitol Acid/Gas production	(+/+)	(-/-)
7 Glucose Acid/Gas production	(+/+)	(+weak/-)
8 Lipase	+	+slow
9 H_2S production	-	-
10 Indole	+	-
11 Motility (agar stab)	+	-
12 Temperature growth (33^0C)	+	+slow

Proposed Identidy--

*Species 1: Vibrio sp. or Aeromonas sp.

Species 2: *Pseudomonas spinosa*

* Species 1 rRNA sequence results were inconclusive. A hypothesis was formulated, indicating either the Vibrio or Aeromonas genus as the identity of Species 1 based soley on the biochemical and morphological data.

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