EFFECTS OF LIBBY DAM, HABITAT, AND AN INVASIVE DIATOM, *DIDYMOSPHENIA GEMINATA*, ON BENTHIC MACROINVERTEBRATE ASSEMBLAGES OF THE KOOTENAI RIVER, MONTANA

Prepared by

Brett D. Marshall, M.Sc.

Prepared for:

Montana Division of Fish, Wildlife and Parks,

FWP Region 1 Headquarters
490 North Meridian Road
Kalispell, MT 59901

April 2007

Version 2.1

- - - CONTENTS - - -

EXECUTIVE SUMMARY

We collected benthic macroinvertebrate and algae samples from five locations below Libby Dam, along with ancillary habitat data. Our goals were: (1) to assess changes in benthic community structure downstream from Libby Dam, (2) to assess the effects of the invasive algae *Didymosphenia geminata* on benthic assemblages and (3) to make recommendations for natural resource management based upon our findings and those of others.

We developed a study design to maximize the potential uses of the data. One of FWP's priorities was to keep the sites and methods consistent with earlier river surveys (Perry et al. 1987, Hauer and Stanford 1997). We also wanted to quantify downstream gradients in community structure below the dam (something that earlier surveys failed to do with their qualitative analysis). In addition, we wanted to use the samples to quantify the effects of *D. geminata*—independent of dam-related station effects. We had a limited budget with which to generate a data set to support all these uses.

We ultimately used a sampling method that used the same basic field methods as earlier studies (Perry et al. 1987, Hauer and Stanford 1997), but used a flow-standardized stratified sampling design. This allowed us to reduce unwanted variation among samples (increasing statistical power and reducing the required sampling effort), and to use a General Linear Modeling (GLM) to adjust benthic response-variable means for the variation related to physical habitat (flow, substrate, algae). Thus the same range of near-substrate flow velocities were sampled at each of the five study sites used by earlier investigators.

We found that Libby Dam continues to exert a major influence on the structure and function of downstream benthic food webs. However, community dissimilarity analysis suggested that the direct influence of Libby Dam was somewhat less intense than indicated by sampling in the 1990's (Hauer and Stanford 1997). This could be due to (1) landscape or climate changes that are unrelated to the dam, to (2) homogenizing habitat quality by *D. geminata* coating the substrata and reducing diversity in community composition, to (3) changing river-flow management, or because (4) we sampled the same flow velocity at all sites (earlier investigators did not control this variable nor did they correlate it with the macroinvertebrates). The reality is probably that a combination of all these factors reduced the effect of dam-related changes in benthic community structure. This does not account for the long-term effects of the dam and reservoir as a potential nutrient sink.

We ran GLM stepwise procedures with all the habitat variables except for epilithic biomass (which was primarily *D. geminata* growth). We found that for most response variables, some

variation could be significantly explained by habitat variables. When we added the average Ash-free-dry-mass (AFDM) of epilithon as a covariate and re-ran the stepwise analysis, we found this term explained more variation in any other habitat variable. After AFDM variation was explained the treatment "SITE" no longer significantly explained variation. This indicates that many aspects of community structure were strongly correlated with the thickness of epilithic biofilms. It is noteworthy that this variable explained more variation than flow—which often accounts for the most spatial variation in community structure within a riffle (e.g., Hart and Fonseca 1995).

Both shredders and scrapers feed upon biofilm-colonized substrata. Therefore we expected that changes in the quantity or quality of biofilms could affect the success (and thereby abundance) of these two functional feeding groups. These taxa should be less common in areas of very low epilithic growth than in areas with moderate growth. In the case of the Kootenai River and the spread of *D. geminata*, biofilms are likely to become sufficiently thick as to interfere with feeding and mobility of these taxa (many of which are clingers). Therefore we performed a nonlinear regression to fit a dose-response curve for the abundance of scrapers and shredders relative to the mass of epilithic biofilms. We identified a threshold level of epilithic biofilms which should not be exceeded to maintain natural ecosystem function. Concentrations of organic material exceeding 8mg/cm^2, completely excluded shredders and dramatically reduced scrapers. The abundance of these important taxa began declining at organic biofilms concentrations between 3-5 mg/cm^2. Ideal production of these important links to higher trophic levels should occur at concentrations less than 5 mg/cm^2.

The factors contributing to population explosions (blooms) of *D. geminata* are not understood. The phenomenon began occurring all over the northern hemisphere in the late 1980's and coincided with dramatic reductions in stratospheric ozone levels and marked increases in the amount of ultra-violet radiation reaching the earth's surface. Controlled field and laboratory investigations should provide greater insight into the factors contributing to the growth of *D. geminata*—and help determine which factors can be controlled and which cannot.

The abundance of oligochaete worms was strongly correlated with *D. geminata* concentrations, and could increase the exposure risk of some fishes to the parasite *Myxobolus cerebralis* – the organism responsible for whirling disease.

ACKNOWLEDGEMENTS

Many people assisted this project along the way. First I gratefully thank the Montana Department of Fish, Wildlife and Parks (FWP), especially Jim Dunnigan, for funding and initiating this study. The field and laboratory aspects of the study were conducted by EcoAnalysts, Inc. (EAI). Some of the EAI members that specifically helped the project included: Gary Lester, Scott Lindstrom, Lisa Anderson, Shanda McGraw, Matt Hill, Matt Hall, The Sorting Laboratory, The Taxonomy Team, David Richards, Tristan Arrington, Tiffany Chandler, Monika Wigert, and Julia Eichman. The study was improved by the comments of people from many other organizations, including but not limited to: Ryan Sylvester (FWP) Charlie Holderman (KToI), Greg Hoffman (USACE). When I use the word, "we" in this report, I reflect the efforts of all those that contributed to the success of this study. Thank you very much.

Suggested citation:

Marshall, B. D. 2007. Effects of Libby Dam, Habitat, and an Invasive Diatom, *Didymosphenia geminata,* on benthic macroinvertebrate assemblages of the Kootenai River, Montana. Prepared for the Montana Department of Fish Wildlife and Parks, Kalispell Montana.

1. INTRODUCTION

1.1. Background

The completion of Libby Dam and the creation of the 109-mile Libby Reservoir in 1972 changed much of the Kootenai River ecology. The filling of Libby Reservoir inundated and eliminated 109 miles of the main-stem Kootenai River and 40 miles of critical low-gradient tributary habitat. This conversion of a large segment of the Kootenai River from a lotic to a lentic environment also changed the aquatic community. The construction and operation of Libby Dam also substantially altered the ecology of the riverine environment within the Kootenai River downstream of Libby Dam.

The authorized purpose of Libby Dam is to provide power (91.5%), flood control (8.3%), navigation and various other benefits (0.2%; Storm et al. 1982). These uses come at the expense of the normative hydrograph. Since the construction and operation of Libby Dam, the hydrograph has substantially changed, with the two largest differences being increases in daily flow fluctuation and in seasonal discharge (Figure 1). Hydropower-related discharge variations in the Kootenai River have resulted in a wider zone of water fluctuation, or varial zone, which has subsequently become biologically unproductive. Research has shown that normal vegetated variable zones are significantly impacted when abnormal fluctuating water levels and flows produce a highly altered riparian zone (Mack et al. 1990, Mackey et al. 1987, Suchomel 1994).

Reduction in the Kootenai River's natural spring freshets due to flood control has eliminated much of the hydraulic energy needed to maintain the river channel and to periodically re-sort river gravels. A lack of flushing flows has caused sediment buildup in the river cobbles which are important for insect production, fish food availability, and security cover. In addition, large numbers of sessile aquatic insects in the varial zone are stranded when river discharge and stage have large, daily fluctuations. The reduction in magnitude of spring flows has caused increased embeddedness of substrates, resulting in a loss of interstitial spaces in cobble and gravel substrates and, in turn, a loss of habitat for algal colonization, an overall reduction in species diversity and in standing crop. Notably, benthic macroinvertebrate densities are one of the most important factors influencing growth and density of trout in the Kootenai River (May and Huston 1983).

The first detailed ecological studies of the macroinvertebrate community of the Kootenai River after the construction of Libby Dam occurred in the 1980's (Perry and Huston 1983; Perry 1984; Perry and Perry 1986; Perry et al. 1986). Approximately one decade later, Hauer and Stanford (1997) launched a second investigation of which the primary purposes were 1) to determine the effect of seasonal variation in hydropower operations on the zoobenthos of the Kootenai River, and 2) to directly compare, where possible, changes in the benthic species diversity and density that may have occurred between the earlier study and conditions that occurred during the 1994/1995 period. The scientific rationale for Hauer and Stanford's study was based on the need to describe the benthic community under quasi-equilibrium conditions that differed from conditions in the 1980's.

Flow regimes during the 1980's and 1990's below Libby Dam also differed substantially. Hauer and Stanford (1997) observed several substantial changes in species composition, distribution and abundance when compared to the information gathered in the 1980's (Perry and Huston 1983; Perry 1984; Perry and Perry 1986; Perry et al. 1986). Hauer and Stanford (1997) also concluded that the varial zone was substantially impacted by daily flow fluctuations and recommended a reduction in daily discharge variation from Libby Dam.

1.2. Scientific Rationale for Additional Research below Libby Dam

Since the Hauer and Stanford (1997) study on the Kootenai River almost a decade ago, the physical and biological conditions have changed substantially below Libby Dam. Perhaps the most obvious change has been an alteration in flow-regime management, which has certainly affected the biota.

Load following is the release of dam water through the turbines for a short duration with flow ranging from a low cubic feet per second (cfs) to 25,000 cfs, which is near-maximum powerhouse capability. This was a common practice in the late 1970's and early 1980's when it was common to see load following from a minimum to maximum powerhouse capacity on a daily basis, and in some instances, twice each day. This activity is generally a response to power demand and marketing. Through the 1980's and early 1990's similar operations occurred, although it was primarily limited to ramping up and back down a single time during a 24-hour period, from November to March.

Daily load following during the summer months has not occurred as frequently after the 1994 listing of the Kootenai River White Sturgeon as Endangered. This follows the steady flow requirement for white sturgeon egg incubation in the Biological Opinion. However, daily load following did occur during the periods of January, February, March, and December, 1998, and again in November, 1999. Daily load following also continued during the winter months of 2000—January through March—as well as in December, 2000, in response to a power emergency.

The practice of daily load following also became less prevalent after the 1998 listing of Bull Trout as Endangered. The associated ramping rates were contained within the1999 U.S. Army Corps of Engineers Biological Assessment and they have not occurred at Libby Dam since December 2000. Figures 2 and 3 illustrate the reduction in Libby Dam's daily discharge variation--a result of changes in river operation since the mid-1990's.

Given the trend of decreased daily discharge variability at Libby Dam, and assuming that other biological and physical variables were held constant through time, one might predict that both secondary and tertiary production may have increased in the Kootenai River below Libby Dam during the period of 1995-2004. Montana FWP has a long-term fish population-monitoring site on the main-stem Kootenai River (Flower/Pipe Section) near Libby, MT.

Synder (2001), and Synder and Minshall (1996), were the first investigators to evaluate primary production in the Kootenai River after the construction of Libby Dam. They reported low levels of benthic chlorophyll production at sites below the dam and they classified the regulated reaches of the Kootenai River below Libby Dam as oligotrophic to ultra-oligotrophic. Ongoing investigations of the Kootenai River's productivity levels, being conducted by the Kootenai Tribe of Idaho (Holderman and Hardy 2004) generally concur with Snyder and Minshall's original assessment. However, within the previous several years, the diatom *Didymosphenia geminata* has become very prolific throughout the Kootenai River below Libby Dam.

Holderman and Hardy (2004) found that in 2001 and 2002 *Didymosphenia geminata* dominated the biomass of all their seven sampling locations. These locations ranged from river mile 78 to 218 on the Kootenai River. In the last 3 years, this stalked diatom has increased to nuisance levels. Unattached and free drifting mats of the diatom stalks are commonplace in the Kootenai River, especially after a period of increased discharge

from Libby Dam. The impact of *Didymosphenia geminata's* proliferation on insect production and community composition is not known.

1.3. Specific goals of this study.

The RFP for this project specifically requested us to address three primary tasks. First, to describe the differences among the communities. Second, to describe the effects of *D. geminata* on the benthos. Third, to provide a summary of information regarding *D. geminata* research through the year 2006. We made our results comparable with the earlier studies by examining the abundance of dominant taxa reported by Hauer and Stanford (1997) and by examining how a similarity gradient changed in earlier studies and our study.

2. METHODS

Our study began with several goals. We wanted to compare our findings with the findings of Hauer and Stanford (1997) as well as assess the changes in the benthic fauna related blooms of the native diatom *Didymosphenia geminata*. Our project has some study elements similar to the work of Hauer and Stanford (1997) but since they were primarily interested in the effects of hydrological regime, we had to deviate from some aspects of their design. For example, we used the same equipment to sample benthos, but we did not sample stranded or drifting invertebrates. We used several analytical methods to summarize and explain differences among the sites, methods not used by earlier investigators. In the interest of comparison, we tried to express our results using units and measures similar to those of Hauer and Stanford (1997). However, given the differences in goals, this was not always practical.

2.1. Study Sites

The most important similarity to the Hauer and Stanford (1997) report was our use of the same study sites--we sampled the same five sites on the Kootenai River in northwestern Montana and northeastern Idaho (Fig.2.1). The region has not changed significantly since the description by Hauer and Stanford (1997). That is, the river still drains about 30,420 km^2 of coniferous forest land in Montana and Canada.

The five study sites were used to describe the longitudinal gradient of changes in the macroinvertebrate assemblages below Libby Dam. The upstream study site was located about 3km below Libby Dam, near the Dunn Creek access site, and was designated "1-DUN." The next site downriver was near the Elkhorn RV Campground and Resort, about 18 km downstream from Libby Dam, and was designated "2-ELK." The middle site was just upstream from the mouth of Pipe Creek, about 35 km from Libby Dam, and was designated "3-PIP." The fourth study site designated "4-KOA," was near the KOA campground downstream from the town of Troy, MT (5km), upstream from the confluence with the Yaak River, and about 65 km downstream from Libby Dam. The downstream site was about 5 km upstream from the town of Bonners Ferry, ID, at a river access known locally as "Crossport." The downstream site was about 110 km below Libby Dam and was designated "5-CPT."

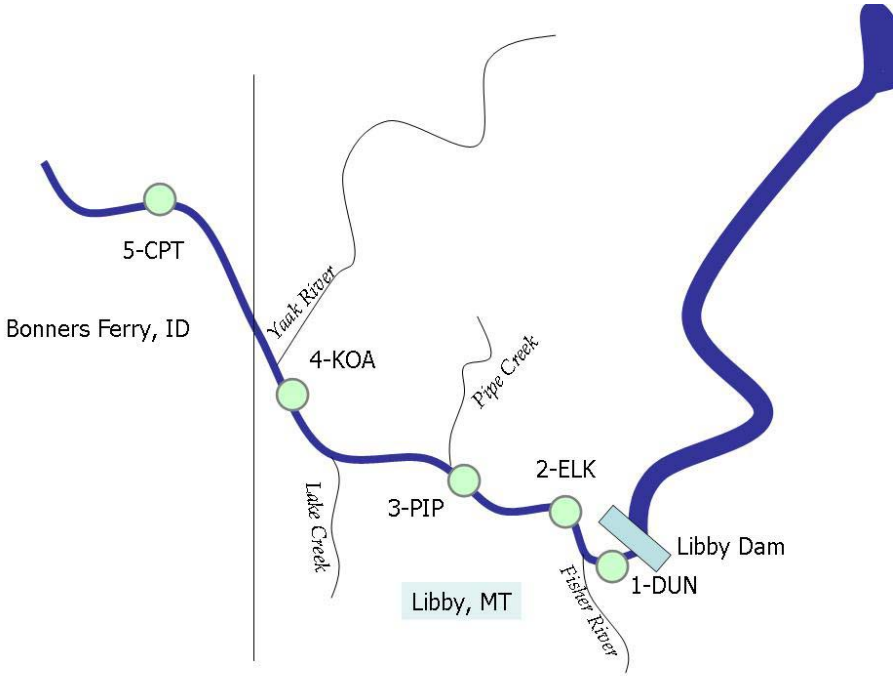


Figure 2. Simplified schematic of study area. The study sites were all located on the Kootenai River between Libby Dam (Montana) and the town of Bonners Ferry (Idaho). Flow is from east to west. The vertical line delineates the boarder between Idaho and Montana.

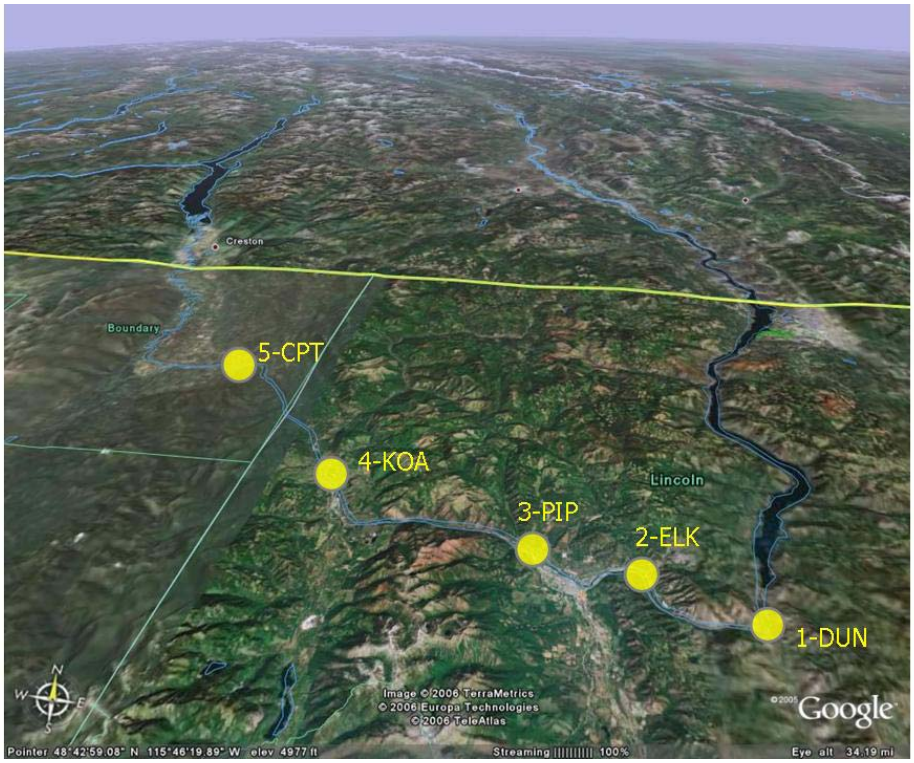


Figure 2.2. Satellite images of study area. This figure shows the general topography and land use of the study area. These are the same study sites examined by Hauer and Stanford (1997). (Image provided courtesy of Google Earth)

2.2 Sample Design and Collection

We sampled each of the five sites three times during 2005. Ideally, we would have sampled on the exact same dates (degree-dates) that Hauer and Stanford (1997) sampled to minimize the effects of life histories (especially emergence) on aquatic invertebrate abundances. Unfortunately, this was not possible because extremely high river discharge persisted the entire summer and prevented safe sample collection (Fig.2.4). We sampled when river levels had been stable for several days and provided access to habitat that had been submerged for several months. Thus, September and October were ideal sampling times and we were able to sample conditions similar to those occurring when Hauer and Stanford conducted their survey. None of our sampling periods were similar to the July sampling period during which Hauer and Stanford (1997) were able to collect in 1994.

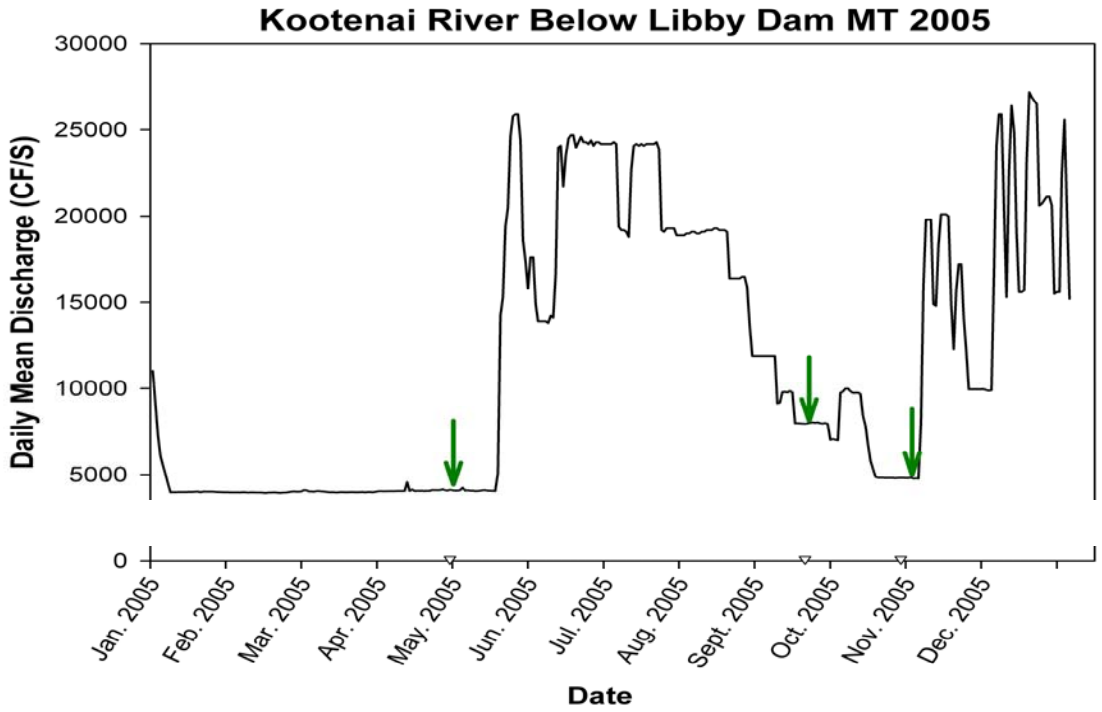


Figure 2.4. 2005 Kootenai River Hydrograph. The sample dates are marked with arrows.

Five samples were collected from each site on each date. The budget included funds for analysis of three samples, but we collected two extra samples in case three had insufficient statistical power to detect ecologically relevant trends. The samples remain in storage at our laboratory in Moscow, ID, and are available if any additional laboratory analysis is required.

The placement of the sampler was determined by the near-substrate flow readings. A Marsh-McBirney digital flow meter was used to measure the flow as close as possible to the river's bottom. The goal was to record the flow's influence on each sample's macroinvertebrate community composition so that the flow data could be correlated with the macroinvertebrate data. The flows used to select individual sample units fell across an entire range of high (~0.6m/s) and low (~0.1m/s). Thus, the flow data collected for this project should not be used to compare flow rates at individual sites; all sites will appear to have the same flow because of the methods we used in the field. However, this method allowed us to partially control for the influence of flow on community structure and to address the effects of *Didymosphenia geminata* with fewer replicates than would otherwise be necessary.

We also qualitatively described the amount of different sized substrata occurring in each sample. These data were assembled into a particle size index based on the intermediate disturbance hypothesis, the relation of surface area, and stability and colonization rates of invertebrates. The stability and colonization rates method weights the particles according to their colonization potential into an Invertebrate Particle Colonization Index (IPCI; Formula 2.1). The idea is that larger stones roll less frequently and therefore accumulate more of the less mobile taxa. Boulders are weighted less because they provide less surface area and are less effectively sampled by area-delimiting sample methods. The IPCI ranges from 400 (100% large cobble) to zero (100% fines), as developed by Marshall (1997) for the Academy of Natural Sciences. The goal is to have a covariate that can be used to describe the overall composition of substrata particles at a scale relating to invertebrate community structure. IPCI provides an advantage over using individual particle sizes in that zero-values are less frequent and more meaningful.

Formula 2.1

IPCI = 0*Fines + 1*Fine Gravel + 2*Coarse Gravel + 3*Small Cobble + 4*Large Cobble + 1*Boulder

Where: variables are the % composition of various inorganic substrate sizes.

We also quantified the amount of algae in each sample several ways. First, we qualitatively estimated the relative amount of the substrata surface covered by periphyton. This measurement included any periphyton growth thick enough to obscure the color of benthic substrata. We also measured the depth of algae growth on substrata within each sample to describe the depth of algae growth to the nearest 1.0mm. We also collected three quantitative algae samples from the area delimited by each benthic sample. These were used to estimate the dry mass and ash-free-dry-mass (AFDM) of periphyton. Since the amount of algae can vary greatly within a small area, the average of the three algae grabs was used to represent the average biomass of periphyton in each sample. That is, the individual algae grabs were not used as true replicates in any statistical tests.

After the supporting data were collected for an individual sample, all substrata in the 0.25m^2 sample area were scrubbed with a stiff brush and collected in net downstream. Each rock within the sample area was inspected for clinging and attached invertebrates which were added to the sample. The samples were preserved in the field using 95% ethanol and labeled to match the supporting data.

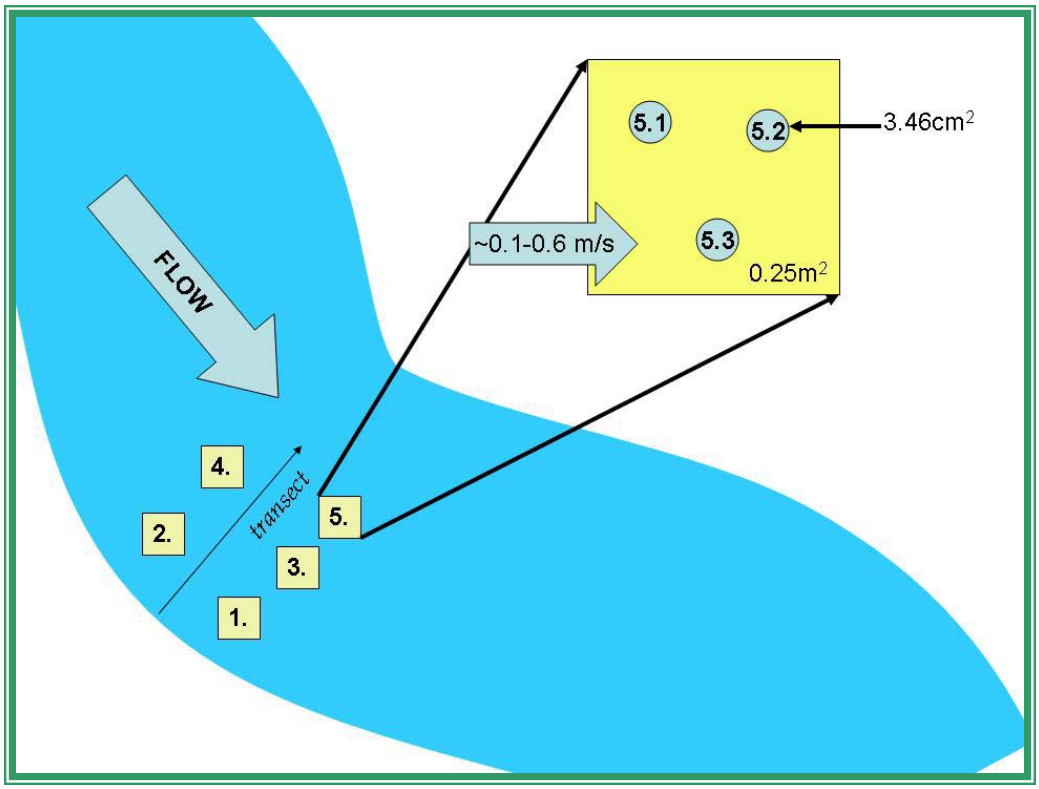


Figure 2.4. Sampling plan. The blue area represents the Kootenai River and the squares represent benthic samples. Samples were collected from flow ranges of about 0.1-0.6 m/s, with greater flows usually farther from shore. For each individual sample, the substrate composition, near-substrate water velocity, algae cover, algae depth, and algae biomass were described. Algae biomass was averaged to describe the mean biomass per cm^2 in each sample. The detailed enlargement of sample 5 illustrates the invertebrate sample area ($0.25m^2$) and the three quantitative $3.46cm^2$ algae samples. These data were collected for all samples, not just sample 5, however only samples 1, 3, and 5 were analyzed for this study.

2.3 Laboratory Analysis

After collection, all benthic samples were logged into the Moscow, ID, laboratory and inspected for damage. There were no leaking or desiccated samples associated with this project; all the samples were in good condition.

Samples were subsampled using a fixed-count, known-area subsampling technique and a modified Caton 1991 subsampling device. The target number of organisms was 500. The invertebrates were removed from detritus using dissecting microscopes and a minimum of 6x

magnification. We identified all specimens in the samples that contained fewer than 500 organisms, but most samples contained many more than 500 specimens.

We identified macroinvertebrate specimens using our modern taxonomic library (Appendix 1). Some of the principle references include Merrit and Cummins (1996), Stewart and Stark (2003), Wiggins (1997), and Thorp and Covich (2002). Although there have been extensive taxonomic revisions of many invertebrate families and genera since the work of Hauer and Stanford (1997), the systematic status of the taxa upon which they based their discussion have not changed.

In keeping with the work of Hauer and Stanford (1997), we only identified the chironomid midges (Chironomidae) and black flies (Simuliidae) to the Family level. Oligochaetes were identified to Class. In hindsight, more rigorous taxonomy may have proven useful because midges are one of the dominant taxonomic groups occurring among the mucilaginous mass associated with *D. geminata* blooms.

Quality assurance of laboratory work included a re-sorting of every sample by additional technicians to calculate sorting efficiency. Sorters for this project exceeded 95% efficiency, which exceeds the industry standard of 90%.

2.4. Data Analysis-Supporting data and covariates

Differences in supporting data were described using two-way Analysis of Variance (ANOVA) with SITE and MONTH as treatment effects. These analyses were followed by Tukey's LSD test, when appropriate, to describe significant differences among levels of the treatment SITE.

Occasionally, we observed significant interaction effects that limited our ability to make generalized conclusions about the treatment effects. The interaction effects were generally due to our sampling design and due to the way the algae data responded seasonally. Supporting data (habitat, flow, algae-etc.) are not the focus of our analysis and their real value is as covariates in the analysis of trends among the benthos. In this capacity, the significant interaction of treatments is not an impediment. These data should not be used to discuss spatial or temporal changes in flow or habitat because they were specifically collected to be similar among sites—therefore, these interactions can be largely ignored.

2.5. Data Analysis-Qualitative Macroinvertebrate Abundances

Macroinvertebrate taxonomic abundances were entered into our proprietary database system. The raw taxonomic data are presented in an appendix to allow independent validation of our calculations (Appendix 2).

The taxonomic data was the basis of the Hauer and Stanford (1997) analysis. Their analysis consisted primarily of a discussion of the dominant taxa occurring in the samples and how the taxa varied among sites, seasons and flow regimes, but Hauer and Stanford (1997) had very little quantitative analysis. To facilitate our comparisons with the results of Hauer and Stanford (1997), we also considered the taxa discussed by Hauer and Stanford (1997). However, a direct comparison with their findings is of limited use because their analytical methods were qualitative. This part of the report is lengthy and verbose—it is similar to methods used by Hauer and Stanford.

2.6. Data Analysis-Quantitative Macroinvertebrate Abundances

Considering changes in the abundance of every single taxon can be mind boggling. On one hand it provides some insights regarding the causes of changes in community structure. On the other hand, there are so many species, each with species-specific habitat preferences and tolerances, that considering each individually invites spurious correlations and confusion. If we were to take each of our taxa and compare 1-to-1 changes in percent abundances for each season at each station, the result would be a very large hodge-podge of contradictory information that would ultimately, be explained by subjective discussion. We wanted to take a more quantitative approach to comparing the abundance of taxa along a gradient below the dam and to compare these findings with Hauer and Stanford (1997).

We calculated community similarity for all the samples we collected and all the samples collected by Hauer and Stanford (1997). We used the Bray-Curtis Dissimilarity index (BCD) because of its familiarity to ecologists; it is the measure often used by cluster analysis to generate classification dendrograms. The BCD scores can range from zero (completely identical communities) to one (communities with no taxa in common). This index is sometimes expressed as the Bray-Curtis Similarity (BCS) index; to convert our results to BCS, subtract BCD from one. Thus, 0.25 BCD = 0.75 BCS.

The BCD, like other similarity measures, compares the abundance of every taxon in one sample to the corresponding abundance in another, resulting in a single number that describes the relative difference of each sample's taxonomic composition. Our data matrix allowed for 1035 pair-wise similarity measurements in 2006 and a greater number from Hauer and Stanford's data (1997) because they used more replicate samples. These BCD values can be used in number of ways. For our purposes, it is especially important to use them to describe shifts in community composition down stream from Libby Dam. Thus we selected a subset of similarities comparing the composition of samples to the upstream site. Using this method, all samples from the site (DUN-1) were compared to each other to attain an estimate of the within-site variation in community structure (3 BCD values). We also selected the BCD values that compared each other site with the upstream site (9 BCD values each).

Selecting this subset of BCD values from the complete dissimilarity matrix allowed us to quantify a gradient of change in the overall taxonomic composition. We fit both a linear and non-linear model to describe how BCD changed downstream. If all the values were zero, it would indicate that each sample had exactly the same taxa in the same abundance. If all the values were equal to the within site variation at DUN-1, but the slope was not significant, it would indicate that all the sites had some heterogeneity in taxa and abundance but that this was relatively uniform at all sites.

2.7. Data Analysis-Quantitative Macroinvertebrate Community Function

This project could not support a detailed study of secondary production and energy flow of benthic food webs; it would simply be too expensive and time consuming. However, the most important changes in benthic food-webs are related to community function. Therefore, we also performed statistical analyses on some ecologically relevant summary measures known as "metrics." Metrics are often used, in combination with regional reference criteria, for biological assessment of ecological health. However, this is not the capacity in which we used them. We used metrics to summarize the taxonomic composition of samples in terms of ecological function. This is not the most comprehensive method to describe ecosystem function because biomass, biomass turnover, survival and growth can have significant effects on ecosystem function (e.g, Allen Paradox (Allen 1949)). Yet, metrics provide a cost effective method of comparing taxonomic-abundance data in terms of community function. The most important questions

regarding the effects of Libby Dam (or *D. geminata*) on benthic assemblages are related to changes in ecological function.

For the sake of discussion, we divided metrics into three groups: Ecological Community Metrics (ECM), Community Stress Metrics (CSM), and Community Function Metrics (CFM). The ECM included measures like Taxa richness, Total abundance, Diversity, Evenness, and Taxa: Abundance Ratio. These are general metrics that are used in a variety of biological sciences. Our specific hypotheses for these metrics are:

- Total Abundance should be greatest at DUN-1, which is the site closest to Libby Dam;
- Taxa Richness should be lowest at DUN-1, increasing downstream;
- Diversity and Evenness should be greatest downstream;
- Richness: Abundance Ratio should be very low at DUN-1 and increase at downstream sites.

The Community Stress metrics are measures that are based on the hypothesized response of lotic benthic assemblages to disturbance, stress, or ecological perturbation. These included the Ephemeroptera, Plecoptera, Trichoptera: (EPT) Richness metric, %EPT abundance, %Chironomidae, %Oligocheata %Non-Insect abundance. Specific Hypotheses included:

- EPT metrics should be lowest upstream, and greatest downstream;
- %Chironomidae should be greatest upstream because these sites have in previous years exhibited the greatest amount of *D. geminata* mucilage;
- Percent Non-Insects (worms, snails, crustaceans, mussels etc.) are usually a minor constituent of North American lotic communities and their abundance should decrease with increasing distance from Libby Dam.

Community Function Metrics are based on the roles of invertebrate taxa in processing organic materials. They five primary Functional Feeding Groups: Shredders, Scrapers, Collector-gatherers, Collector-filterers, and Predators. If disturbances are sufficient in magnitude to influence large numbers of taxa (or many individuals of few taxa) they may alter the trophic basis of the ecosystem's production. Although these measures are no substitute for a secondary production study they can provide insights into the general nature of trophic changes of sufficient magnitude. Karr and Chu (1999) criticized these metrics because they required a large amount of

change before they responded to disturbance. However, Marshall (2001) found that even though they required large amounts of change to describe significant differences, they did change dramatically (and statistically significantly) when disturbances were sufficient in magnitude to change trophic structure. Disturbances usually result in increased abundances of generalist taxa (Collectors) and a corresponding decrease in the abundance more specialized groups (e.g., Shredders, Scrapers).

- Typically, Collector-filterers increase below impoundments, and we expect DUN-1 to exhibit the greatest relative abundance of Collector-filterers.
- Collector-gatherers and Collector-filterers naturally partition resources by their collection method. Both feed on small (<1mm) particles, but filterers are usually more abundant where flow suspends particles in the water column and gatherers tend to feed where flow caused particles to deposit among substrata.
- Shredders and Scrapers receive most their nutrition from biofilms. Scrapers remove epilithic biofilms from substrata, and Shredders consume organic substrata to attain biofilms. These taxa may show low abundances where biofilm growth is sparse and increased abundance where it is thick—until it hinders their ability to effectively forage.

Quantitative analysis of metrics

Another advantage of biological metrics is they allow easier use of statistical hypothesis testing tools. To describe the patterns of functional changes in the macroinvertebrate assemblage, we used a two-way Analysis of Variance (ANOVA) with the Treatments assigned by the variables SITE and MONTH. These analyses were followed by Tukey's LSD test, when appropriate, to describe significant differences among SITES. Analyzing the metrics this way allowed us to discern differences among sites, given natural seasonal variation occurring throughout the study period.

We followed up these analyses of the metrics with a model that is very similar to the two-way ANOVA model, but added the habitat variables (excluding algae AFDM) as covariates to the model. We then tested for differences among sites after controlling for the influence of habitat. This Analysis of Covariance (ACOVA) allowed us to examine the correlation of metrics with habitat variables and to determine which sites were different from each other after the variance related to habitat was accounted for. We started this model with all habitat variables in the model

and used a stepwise iterative variable selection tool to remove variables from the model. Thus, we began the analysis with all covariates (except algae AFDM), and systematically removed the variable that explained the least variation in the model. This process was repeated until only terms that were significantly related to the metrics were maintained in the model. The rationale for "backwards stepwise" variable selection is that we specifically selected variables that are known to affect the composition of lotic benthos.

We re-ran the ACOVA to include algae AFDM as an independent variable along with the other habitat measures. The stepwise variable selection procedure discussed above was repeated for each metric. These analyses, when compared to the ACOVA models excluding AFDM, helped explain the effects of *Didymosphenia geminata* on community function.

The results of the ANOVA and ACOVA models were summarized in tabular format and differences discussed as relevant to the relative importance of Libby Dam, physical habitat, and biofilm depth.

For all statistical tests in this report we assumed that the effects were statistically significant if the probability of a type-1 statistical error was <0.05. To reduce the chance of type-2 statistical error, we allowed effects to be considered "marginally significant" if the probability of type-1 statistical error was <0.10.

2.8. Data Analysis-Quantitative Response to D. geminata

Both shredders and scrapers feed upon biofilm-colonized substrata. Therefore we expected that changes in the quantity or quality of biofilms could affect the success (and thereby abundance) of these two functional feeding groups. In areas of very low epilithic growth, these taxa should be less common than in areas with moderate growth. In the case of the Kootenai River and the spread of *D. geminata*, biofilms are likely to become sufficiently thick as to interfere with feeding and mobility of these taxa, many of which are clingers. Therefore we performed a nonlinear regression to fit a dose-response curve for the abundance of scrapers and shredders relative to the thickness of epilithic diatoms. This allowed us to identify threshold levels of epilithic biofilms that are beneficial and detrimental to natural ecosystem function.

We plotted the density of biofilms downstream of Libby Dam and overlayed the thresholds estimated by the dose-response curve (preceding paragraph). This should show the greatest concentrations of D. geminata relative to other sites studied. However, recall that the samples were stratified to select a range of flows. These data are not habitat weighted and may not describe the relative amount area covered by *D. geminata*.

While the first draft was in review, we were asked to correlate the physical thickness of epilithic biofilms with algae AFDM. The correlations are provided at the end of the results section.

3. RESULTS

3.1 Supporting field data and Covariates

Since we used individual thermometer temperature measures when benthic samples were collected, there was no replication within sites and daily temperature fluctuations confounded differences in site temperature. The only meaningful comparison of temperature for this study is seasonal variation which steadily increased throughout the study. Specifically, April was significantly cooler than September and October (ANOVA, $P<0.001$). Figures for these data are provided in Appendix-2.

Similarly, our two-way ANOVA did not detect significant spatial or seasonal differences in the variables DEPTH or FLOW (Table 3.1). This makes sense because we used near-substrate flow velocities to stratify the precise sample locations at each site and the depth sampled was limited to depths effectively sampled by the sample gear.

Our two-way ANOVA detected both seasonal and spatial differences in the qualitative measure of EMBEDDEDNESS. This is a measure of how tightly fine particles pack around larger particles of the substratum. Embeddedness was greatest at the middle three sites and lowest at the upstream (1-DUN) and downstream (5-CPT) sites. Seasonal variation in embeddedness indicated that October had the lowest amount fines packed among coarse substrata (6%) and September had the highest amount (17%). Thus, there were differences among sites and between seasons.

The overall composition of the substratum was significantly different among sites but not between seasons (Table 3.1). The interpretation of these results was complicated by a statistically significant interaction among the spatial and temporal aspects of the ANOVA test which was mainly caused by a dramatic decrease in the size of particles sampled in September and October (Fig. 3.1). This is the result of using flow-rate stratify sampling during a period of changing river levels—not due to actual changes in the substrata of the river. Seasonal differences at other sites did not result in sampling different sized particles—this caused a statistically significant interaction effect between the Treatments SITE and PERIOD.

The only algae measurement that did not describe a significant difference among the sites or seasons was the ALGAE DEPTH variable; all other physical descriptors of algae showed some significant differences. PERCENT ALGAE measured the degree to which the substrata appeared to be covered by a distinct algae film. There were significant differences among the sites and seasons as well a significant interaction effect. Generally, the greatest percent cover was observed in October and the lowest was observed in September. The significant interaction effect was largely due to changes in percent cover at the 3-PIP site, which had a very high portion of substrata covered in thick algae in April and a very low portion covered in September.

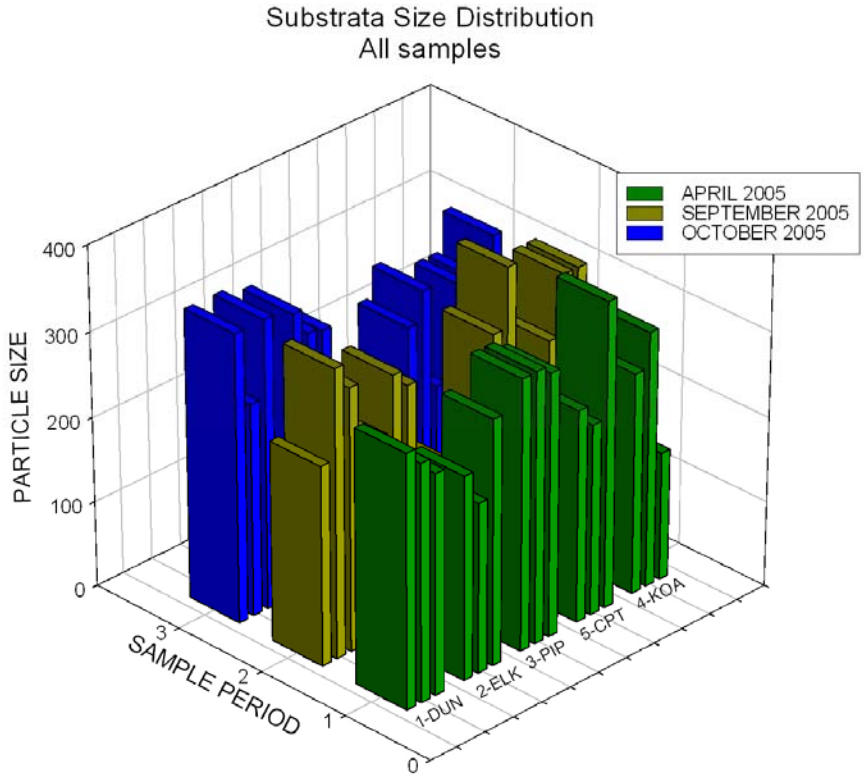


Figure 3.1. Substrata size distribution for all samples. Our two-way ANOVA resulted in significant interaction between the treatment effects (PERIOD, SITE) for the substrata index; the cause of this can be seen here. The graph shows the particle size index for every sample in the study. However, the September and October sampling period contained many smaller particles than the April sampling at the 3-PIP site. Most other sites did not change among seasons. Hence, the interaction was an artifact of the accessible habitat.

The laboratory measurements of periphyton dry mass (DM) and ash-free dry mass (AFDM) both indicated significant differences among sites and seasons--and a significant interaction between these two treatment effects. The interaction effect was strongly influenced by three samples that had exceptionally high amounts of inorganic material in them. Most samples had less than 100mg/cm^2 DM periphyton. However, three samples had dramatically elevated periphyton biomass. Specifically, in September, two samples from the 3-PIP site (~550mg/cm^2, 870mg/cm^2 DM) and one sample from the 4-KOA site (~500mg/cm^2 DM) were much higher than the other samples.

The algae measures were correlated with each other, and we used the most reliable measure. Here after, when the variable ALGAE is mentioned, it refers to the ash-free dry mass of periphyton. This measure was quantitative, standardized for inorganic content, and the values used to correlate with benthos represent the mean condition of the sample (n=3). To avoid problems with colinearity/autocorrelation, we ultimately used the most reliable variable: ALGAE = Periphyton_Ash_Free_Dry_Mass.

3.2. Benthic Macroinvertebrate Sampling Overview

We collected approximately 115,300 invertebrates throughout the study and the number of specimens collected did not vary seasonally (Fig 3.2). Although the sampling effort was uniform among the sites, there were many more specimens collected from immediately below Libby Dam (1-DUN) than from the other sites (Fig. 3.3). Patterns such as this are expected below dams of oligotrophic rivers because many species benefit from seston enrichment from lentic outfalls (e.g., Parker and Voshell 1983).

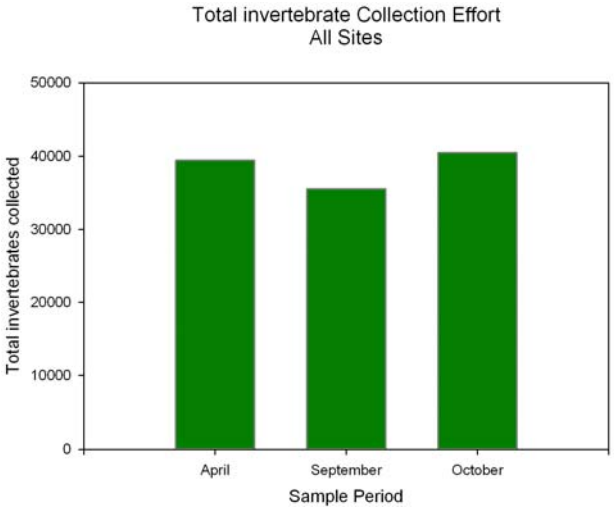


Figure 3.2. Total invertebrates collected by season. These data are the sum estimated total abundance from all sites for each season.

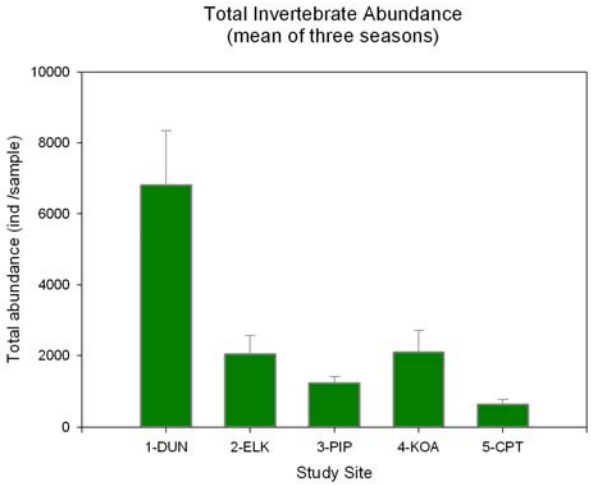


Figure 3.3. Mean invertebrate abundance by site. These data are the mean total abundance of each site, over all three months ± 1 SEM. The data show that total abundance was much higher at 1-DUN than at the other sites.

3.3. Results-Qualitative Macroinvertebrate Abundances

Hauer and Stanford considered specific dominant taxa as the primary endpoint of their study (1997). Since their work is the foundation upon which this study builds, we also considered how these specific taxa change seasonally and spatially below the dam. One of the weaknesses of these types of analyses is high natural variation in the abundance of individual species—even within a site. An individual taxon might not occur in some samples and may occur in very high abundance in others. This limits the kinds of statistical hypothesis testing that may be used as descriptive tools. Thus, Hauer and Stanford were unable to statistically test differences in these taxa. Their analyses consisted of a large number of bar-graphs for a few members of some important orders of aquatic insects.

Ephemeroptera

Hauer and Stanford found that generally the Baetid mayfly, *Baetis tricaudatus,* had an abundance < ~ 200 individuals per m^2—with two exceptions. In April, they found exceptionally high abundances of *B. tricaudatus* at the 1-DUN site, immediately below Libby Dam. They also found very low abundances (< 10) of this cosmopolitan species during all sampling seasons at site 5-CPT. Our results for this taxon were very similar to theirs, but we did not observe nearly as much seasonal variation as they did. We observed a mean maximum abundance of about 3,100 ind / m^2 at the 1-DUN site. The abundance only dropped as low as 1,680. Seasonal differences lacked statistical significance in 2005, but we also observed a clear reduction in the abundance of *B. tricaudatus* at sites farther downstream from Libby Dam (Fig. 3.4). Beatid mayflies are commonly called "small minnow mayflies" because of their swimming behavior and ability to move freely, in a fish-like swimming motion, in swift currents. They are multivoltine and excellent colonizers of frequently disturbed habitat.

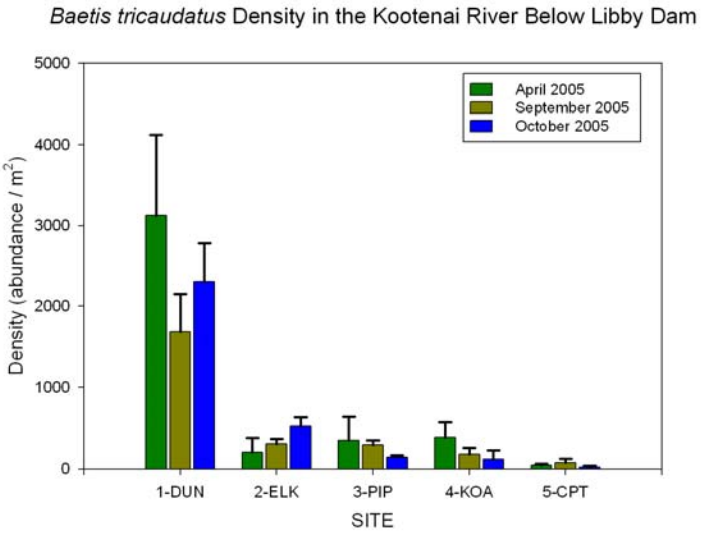


Figure 3.4. *Baetis tricaudatus* density. The mean density of *B. tricaudatus* is presented as the number of individuals per m2. The error associated with the estimate of each mean is ± 1 SEM.

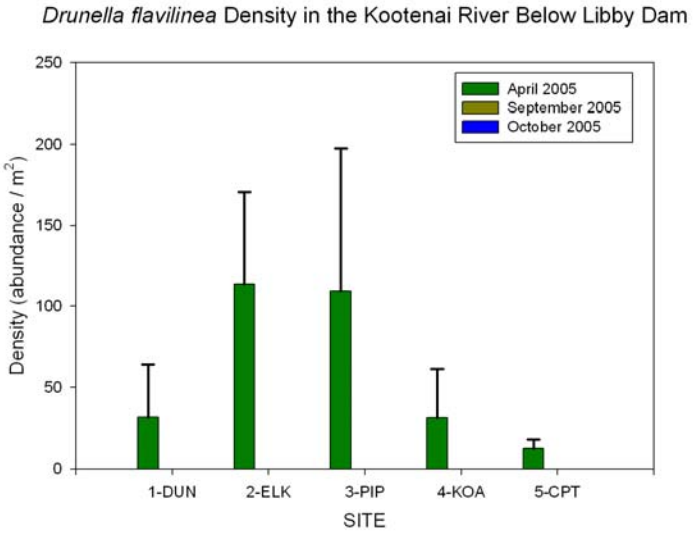


Figure 3.5. *Drunella flavilinea* density. The mean density of *D. flavilinea* is presented as the number of individuals per m2. The error associated with the estimate of each mean is ± 1 SEM.

Hauer and Stanford (1997) also reported relatively high densities of several Ephemerellid mayflies—commonly called "spiny crawlers" because of their morphology and locomotive methods. One of the important taxa they found was *Drunella flavilinea*—small stout ephemerellid often associated with moss, algae or interstitial spaces in clean water. They are usually collector-gatherers but may incidentally ingest algae or other materials. Hauer and Stanford only collected this species in April and July with the greatest abundance at the 3-PIP site in April (~380 ind. / m^2). We were unable to collect in July 2005 because high water prevented river access. Our findings for April and October were very similar to Hauer and Stanford's (1997) results. Our mean densities for this taxon were lower than theirs (~110 ind. / m^2) and highly variable (Fig. 3.5).

Another spiny crawler mayfly was abundant at all sites in 2005 and in 1994 (Hauer and Stanford 1997). *Ephemerella inermis* was collected at all sites sampled in 1993/1994, but was most abundant in April. They also observed much lower abundance at 5-CPT (~100 ind. / m^2) than the other sites (~1,000-1,500 ind. / m^2). Our results were similar to theirs, with a few exceptions. The earlier study reported the highest abundance at 1-DUN in April (1,500 ind. / m^2). We found a similar density at 2-ELK in April (1,600 ind. / m^2), but also found high abundances at 4-KOA (~1,280 ind. / m^2) and 5-CPT (690 ind. / m^2) in October 2005. The spatial-temporal abundance pattern shows the greatest abundance was in April at 2-ELK, tapering off farther down stream (Fig. 3.6).

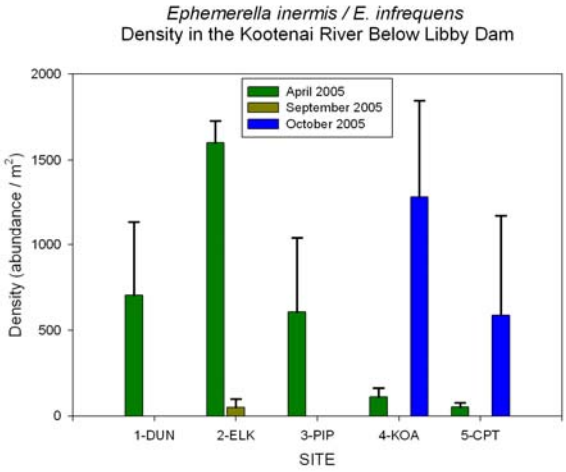


Figure 3.6. Abundance of *Ephemerella inermis* 2005. Hauer and Stanford reported this taxon as *E. inermis*, but it is not possible to separate larvae of this taxon from *E. infrequens*—and there is significant range overlap. We present a little more conservative taxonomic classification of the group and allow that there may be a few *E. infrequens* among the prevalent *E. inermis*.

Hauer and Stanford presented data for another spiny crawler mayfly, *Serratella tibialis*, that was abundant at all sites except 1-DUN in July (and only collected in July). They generally reported abundances between 500 -1,000 ind. / m^2 but found as many as 1,500 ind. / m^2 at the 4-KOA site. We only collected *S. tibialis* in September (Fig. 3.7) and at much lower abundances (~10-30 ind. / m^2). This is probably an artifact of the life history of *S. tibialis* rather than due to a fundamental change in the river system. The early instars are usually abundant in Montana around July, and emerge in September. Thus, our sampling efforts may have missed many of the *S. tibialis* because they were in flight at the time. This also explains why no *S. tibialis* were collected in October (1994 and 2005).

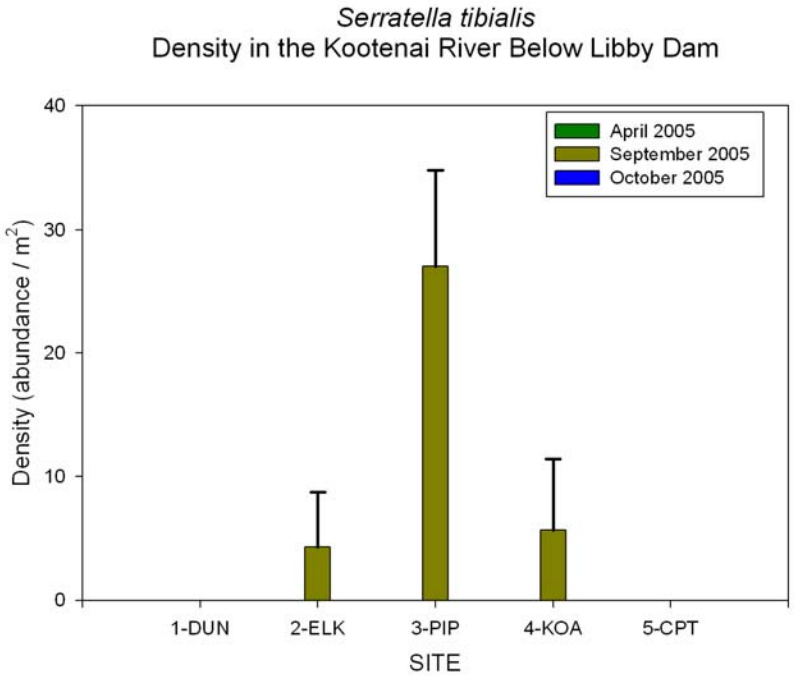


Figure 3.7. Abundance of *Serratella tibialis* 2005. Hauer and Stanford (1997) collected high abundances of this taxon in July 1994, but none in October 2004. This is probably due to the life-history of *S. tibialis*, which has early instars in July and emerges in September (in Montana). This difference does not indicate large fundamental changes in the structure or function of benthic assemblages of the Kootenai River.

Plecoptera

Hauer and Stanford reported four abundant stonefly taxa among their samples: *Pteronarcys californica*, *Taenionema pacificum*, *Claassenia subsalusa* and *Hesperoperla pacifica*. We report more conservative taxonomy for two taxa, *Pteronarcys* and *Taenionema*, because some larvae were not able to be definitively identified to the species level. Some *Pteronarycs* larvae could only be identified to genus. Although these are probably *P. californica* as well, we followed the taxonomic convention to use the most conservative taxonomic unit. The Terniopterygidae in the samples were probably *Taenionema*, but all were very immature and could not be definitively identified even to genus. We left them all at the family level. Since Hauer and Stanford did not have any immature taeniopteryigids, it is likely that they lumped all of them into the *T. pacificum* taxon based on distributional studies or some of their unpublished records for the region. Regardless, our taxon Taeniopterygidae is probably synonymous with their taxon *T. pacificum*.

Hauer and Stanford (1997) noted that Perry (1984) also collected *Sweltsa* from many samples, whereas they did not. The greatest density they collected was about 8/m^2 but Perry (1984) collected about 20/m^2 from most sites. Our results were much more similar to Hauer and Stanford (1997). In April we collected a few *Sweltsa* from 3-PIP (μ~2/m^2) and 4-KOA (μ~3.5/m^2). Most of these small, predatory stoneflies were collected in September, and only from 3-PIP (μ~13.3/m^2) and 4-KOA (μ~14.6/m^2). In October, abundances again reduced to average at 2-5/m^2. The taxon has a patchy distribution and includes many zero values, and our sample with the greatest abundance contained about 40/m^2.

In July, October, and September, Hauer and Stanford (1997) collected *Pteronarcys*, a large, semivoltine, shredder-detritivore. They did not collect any *Pteronarcys* from 1-DUN, but collected some from all other sites. All of their observations were fewer than 30/m^2 and most were less than 10/m^2. Low abundances like these are typical of large, long-lived invertebrate taxa. Our results were very similar (fig. 3.8). We collected no *Pteronarcys* from 1-DUN and found a maximum mean abundance of 40/m^2.

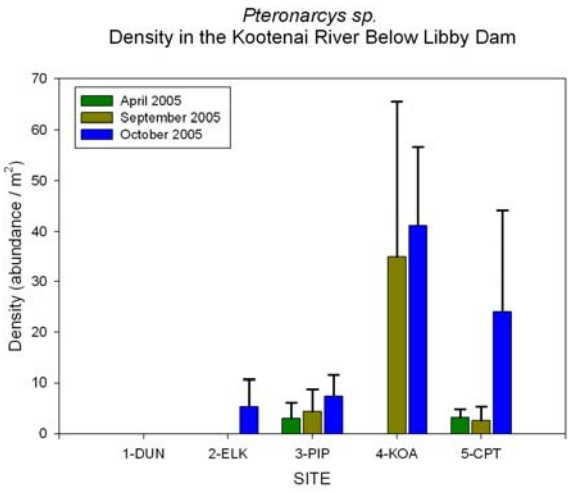


Figure 3.8. Abundance of *Pteronarcys* sp. 2005. The bars represent the mean abundance ± 1 SEM.

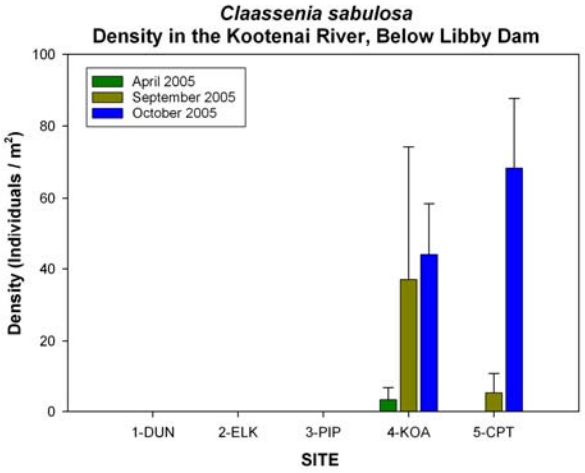


Figure 3.9. Abundance of *Claassenia sabulosa* 2005. The bars represent the mean abundance ± 1 SEM.

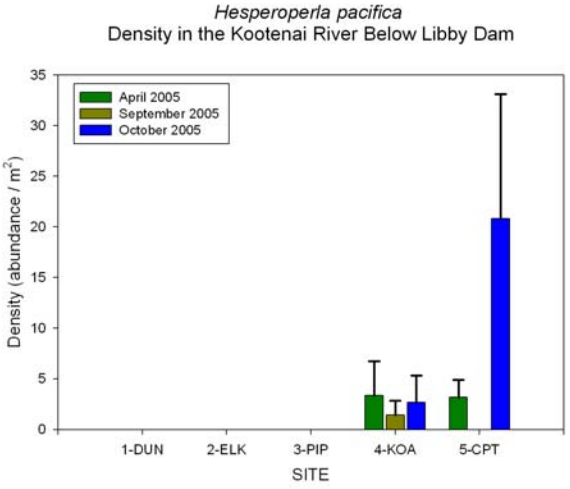


Figure 3.10. Abundance of *Hesperoperla pacifica* 2005. The bars represent the mean abundance ± 1 SEM.

Pteronarcys larvae (occasionally called "nymphs") are important food for salmonids in the Kootenai River, so it is encouraging that the abundances appear to have changed little over the last decade.

The two perlid stoneflies collected were *Hesperoperla pacifica* (Fig. 3.10) and *Claassenia sabulosa* (Fig. 3.9). Like the rest of this family, they are predators. These specific taxa are large semivoltine and probably important forage for fish. We found much higher densities of *C. sabulosa* than Hauer and Stanford (1997) who only found 20-25/m^2 at 3-PIP and 5-CPT in October—all other site dates had fewer. We collected as many 70 /m^2 from 5-Crossport, and about 40/m^2 from 4-KOA, but we did not collect them from any other site in the survey. These findings are a little disconcerting because they suggest that *C. sabulosa* no longer occurs at the other sites. However, Hauer and Stanford (1997) found very few individuals (<1/m^2 at 1-DUN and 2-ELK). Taxa occurring in low densities are often under-represented by randomized sampling. Nonetheless, it would be reassuring to verify the occurrence of these taxa at other sites—especially since the sites from which they appear to be lacking are the sites with the greatest amount of coverage by *Didymosphenia geminata*.

Our findings were very similar for *H. pacifica*. Where Hauer and Stanford (1997) found low abundances of *H. pacifica* (<1/m^2 at 1-DUN, <2/m2 at 2-ELK and 3-PIP), we collected none. They collected about 3/m^2 at 4-KOA and less than 4/m^2 at 5-CPT. We found similar abundances to those reported by Hauer and Stanford at 4-KOA, but we collected many more at 5-CPT (~20/m^2) in October. We suspect the omission of these taxa from 1-DUN, 2-ELK and 3-PIP, may be due to under-sampling low-density taxa. However, since these are probably important forage for fishes, it would be reassuring to verify the occurrence of these taxa at other sites—especially since the sites from which they appear to be lacking are the sites with the greatest amount of coverage by *Didymosphenia geminata*.

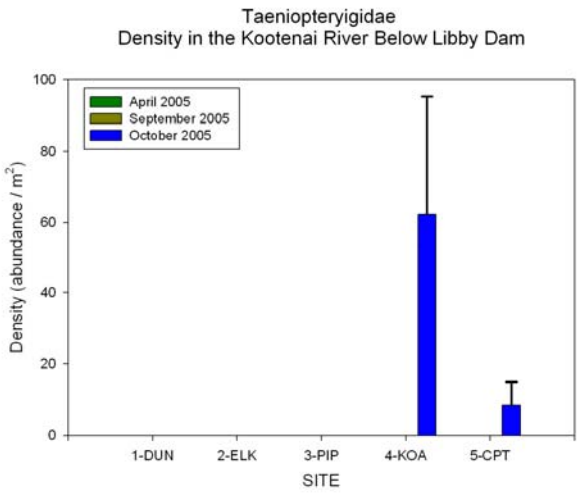


Figure 3.11. Abundance of Taenipterygidae 2005. The bars represent the mean abundance ± 1 SEM.

Hauer and Stanford reported rather high abundances for the taeniopterygid stoneflies at 4-KOA (~80/m²) and 5-CPT (~40/m²) in October. This corresponds to the beginning of their larval life-cycle which consists of winter growth and spring emergence as adults, resulting in the common name "winter stoneflies." Our results were similar to theirs, but we found fewer taeniopterygids at 5-CPT than they did (Fig. 3.11).

Trichoptera.

Hauer and Stanford reported several *Hydropsyche* species that we did not find. This includes *H. occidentalis*, which is indistinguishable from several other common local species for the majority of its aquatic life, and *H. orris*, which is most commonly collected from woody debris in fine-sediment coastal-streams of the Gulf Coast. To avoid confusion, we lumped all of our *Hydropsyche* together for this discussion (although for metrics and other calculations our species determinations were maintained; see appendices).

We observed a distinct pattern of decreasing *Hydropsyche* abundance downstream from Libby Dam (Fig. 3.12) with the greatest average abundance occurring at 1-DUN in October (2,300 /m²). Gradients such as these are common below impoundments because reservoirs, like lakes, develop planktonic food webs that are not usually found in oligotrophic rivers. Plankton contribute a much higher than average concentration of

fine particulate organic material to the outfall of most reservoirs. As a result, it is very common to find extremely high densities of filter-feeders below dams and to have those densities taper-off farther downstream. *Hydropsyche* and *Cheumatopsyche* are two very common genera of filter-feeding caddisfly and thus the distributional pattern and abundance we observed was not unexpected. The total abundance was much higher than that observed by Hauer and Stanford (1997) who collected less than 600 *Hydropsyche* / m^2 at the upstream site (1-DUN) and about 800 *Hydropsyche*/m^2 at 2-ELK. It is interesting that the typical pattern of very high abundance of *Hydropsyche* tapering to lower abundance was not more apparent in 1994/1995. We were able to rule out eutrophication of Lake Koocanusa (up stream of Libby Dam) because the lake remains highly oligotrophic and plankton monitoring has indicated that there should be no increase in seston export below the dam (Dunnigan et al. 2007).

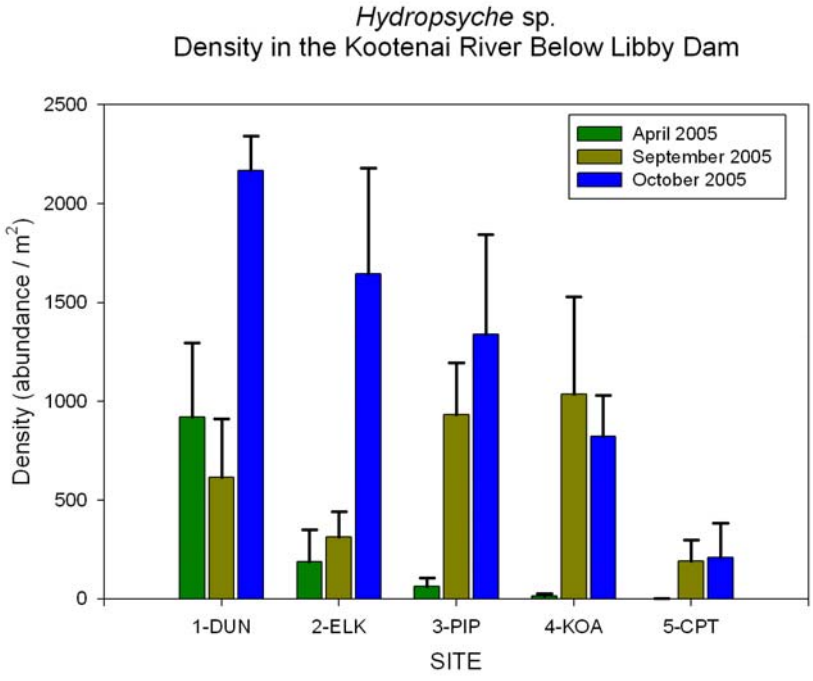


Figure 3.12. Abundance of *Hydropsyche* sp. 2005. The bars represent the mean abundance ± 1 SEM.

3.4. Results-Quantitative Macroinvertebrate Abundances

Our data produced a similarity matrix of 1,035 comparisons of Bray-Curtis Dissimilarity (BCD). Linear regression of these values produced highly significant regressions for September ($p<0.0001$, $r^2=0.64$) and October ($p<0.0001$, $r^2=0.478$), but not April ($p=0.84$, $r^2=0.001$). This indicates that in September and October, the sites became increasingly dissimilar from 1-DUN along a down-stream gradient. However, there was not a significant relationship between dissimilarity and down-stream distance in April. The data were better fit by a non-linear model, which is provided for descriptive purposes (eq. 3.1-3.4; April did not exhibit a significant trend).

Equation 3.1 (Linear, September 2005,)

$$BCD = 2.98 + 0.004*(DISTANCE(km)$$

Equation 3.2 (Linear, October 2005)

$$BCD = 2.98 + 0.004*(DISTANCE(km)$$

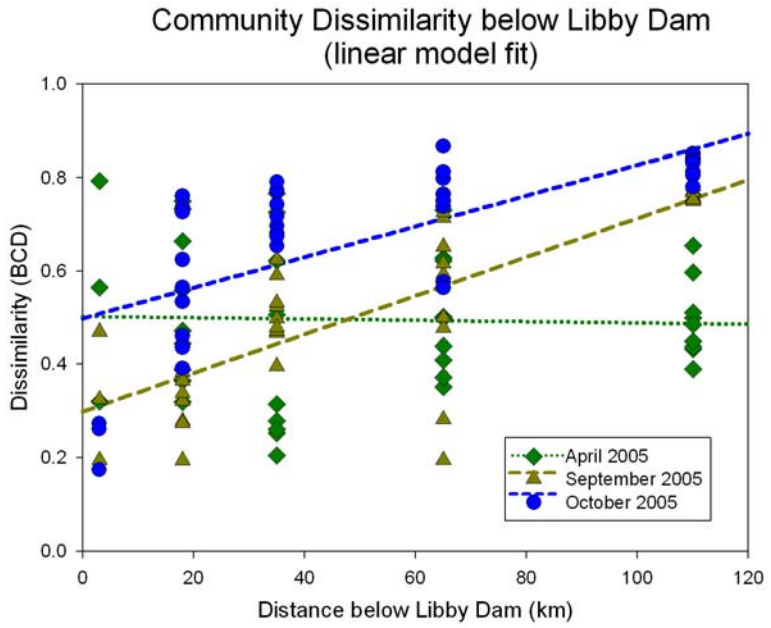
Equation 3.3. (non-linear, September 2005)

$$BCD = -0.947 + DISTANCE(km)^{0.105}$$

Equation 3.4. (non-linear, October 2005)

$$BCD = -0.797 + DISTANCE(km)^{0.105}$$

A.



B.

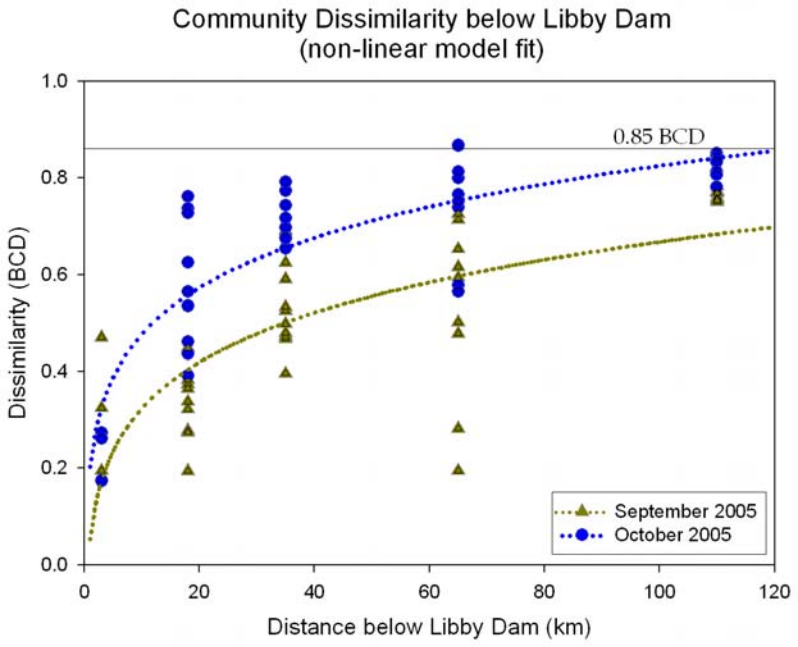
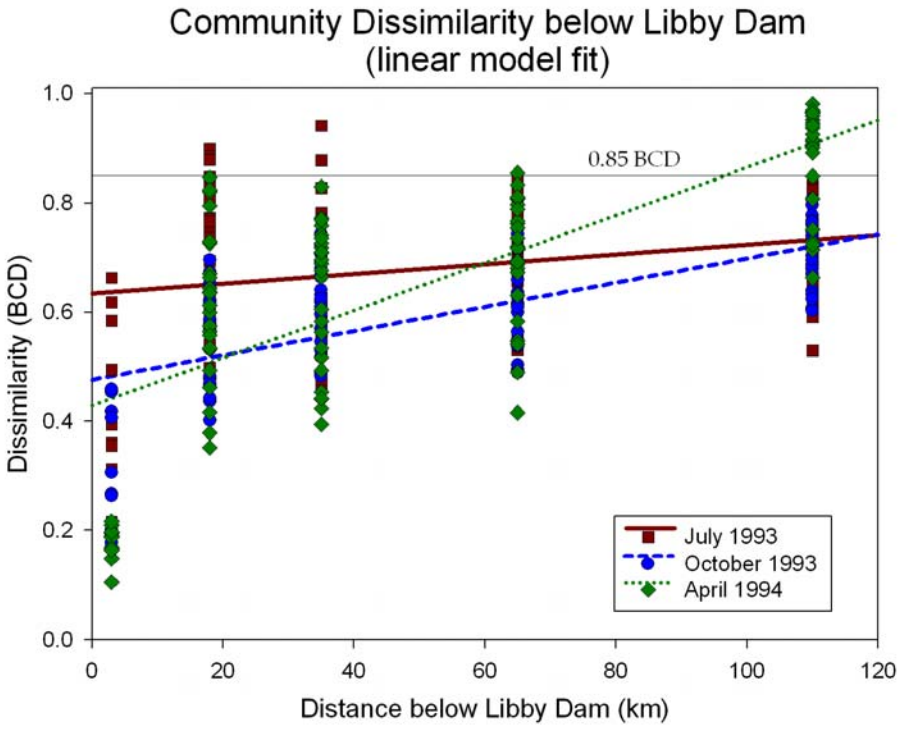


Figure 3.13. Bray-Curtis Dissimilarity Downstream of Libby Dam. Low BCD values indicate samples with very similar taxonomic composition—both in the taxa present and their abundance. Values of 1.0 are attained when the samples share no taxa. There was no significant trend among the data collected in April 2005. The graphs show the fit of the linear (A) and non-linear models (B) applied to the data.

The dissimilarity analysis suggests that in September and October (2005), the communities became more dissimilar from the upstream site as distance below Libby Dam increased. This is consistent with the expectations of benthic community structure below impounded oligotrophic rivers because reservoirs enrich riverine seston with plankton from the lentic reservoir food webs (e.g., Parker and Voshell 1983). However, the key question for this survey is: *Are these results different than they had been in earlier investigations – before the increase in D. geminata production*? Thus, we revisited the original data from Hauer and Stanford (1997) and recalculated the Bray-Curtis Dissimilarity index to determine if the downstream change in assemblage structure was similar to those exhibited historically.

We found there was a significant change in the dissimilarity of the benthic community structure along a downstream gradient in all months of Hauer and Stanford's data (Eq. 3.5-3.10; Fig 3.12). However, it differed from the 2005 gradients observed in several ways. First, all months showed a statistically significant trend, even if the slope was slight (e.g., July 1993). Second, April (1994) was the month during which the community dissimilarity gradient was most pronounced, whereas in 2005, the data produced a slope not significantly different from zero. Third, the upstream site was more dissimilar from the downstream sites in 1993/1994 than it was in 2005—these differences were especially pronounced in April 1994. Only one of our 2005 samples exceeded a BCD value of 0.85 (BCD=0.86, 4-KOA, Fig 3.11), whereas many of the earlier samples exceeded this value (Fig 3.12A).

A.



B.

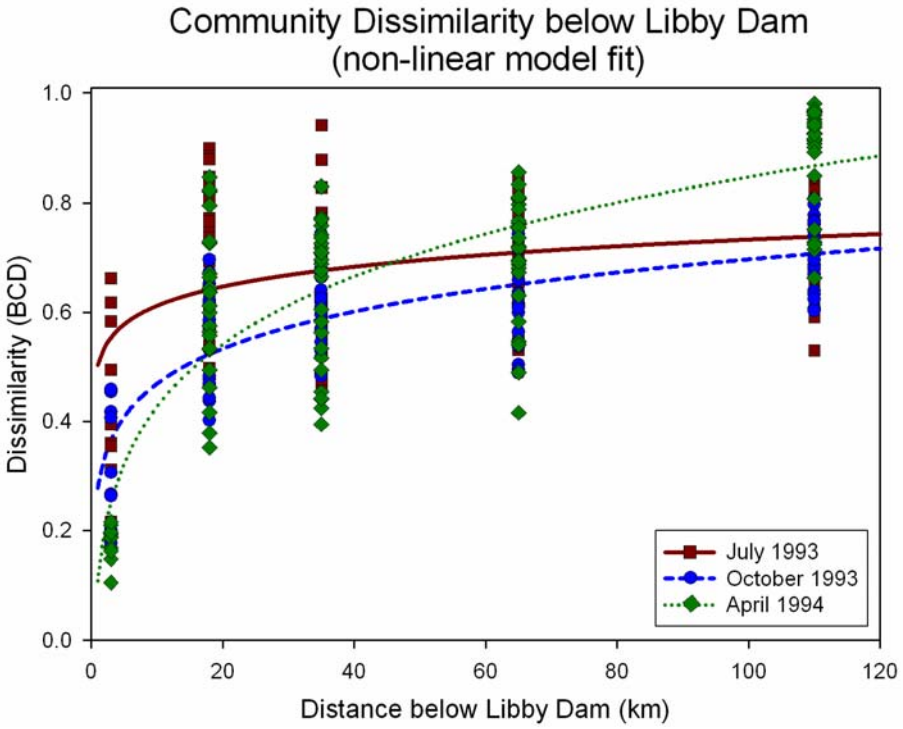


Figure 3.14. Bray-Curtis Dissimilarity Downstream of Libby Dam. Low BCD values indicate samples with very similar taxonomic composition—both in the taxa present and their abundance. Values of 1.0 are attained when the samples share no taxa. There was no significant trend among the data collected in April 2005. The graphs show the fit of the linear (A) and non-linear models (B) applied to the data.

Equations 3.5-3.10. These formulae are linear and non-linear model fits describing the relationship between distances downstream from the dam and community dissimilarity (BCD) from samples collected at the upstream site (1-DUN). Values at 1-DUN were generated by comparing all samples collected from that site with each other (whereas values at downstream sites compare each of their samples to each of the samples collected at 1-DUN).

Equation 3.5 (Linear, July 1993, P=0.009, r^2=0.062)

BCD = 0.634 + 0.001*(DISTANCE(km)

Equation 3.6 (Linear, October 1993, P <0.001, r^2=0.412)

BCD = 0.732 + 0.002*(DISTANCE(km)

Equation 3.7 (Linear, April 1994, P <0.001, r^2=0.540)

BCD = 0.429 + 0.004*(DISTANCE(km)

Equation 3.8. (non-linear, July 1993, r^2=0.009)

$BCD = -0.497 + DISTANCE(km)^{0.045}$

Equation 3.9. (non-linear, October 1993, r^2=0.540)

$BCD = -0.722 + DISTANCE(km)^{0.076}$

Equation 3.10. (non-linear, April 1994, r^2=0.0692)

$BCD = -0.891 + DISTANCE(km)^{0.120}$

We expect that overall community structure should change with distance below an impoundment because the reservoir's influence on food web structure declines with downstream distance (e.g., Parker and Voshell 1983). Although our results suggest that in 2005, community structure was influenced by Libby Dam, they also suggest that the direct influences of Libby Dam on benthic fauna of the Kootenai River were more pronounced in Hauer and Stanford's (1997) study than in 2005. These results could occur when another factor begins having significant influence on the structure and function of benthic food webs such as *D. geminata* blooms, or watershed-scale disturbances. *D. geminata* out-breaks are likely related to other large scale influences, and the combined effects cannot be resolved using this study design. However, our results are consistent with the hypothesized influence of *D. geminata* on benthic community structure. Although we conclude that *D. geminata* could have caused changes in community structure, we also acknowledge that other unmeasured disturbances may have similar direct or indirect effects on the biota of the Kootenai River.

3.5. Results-Quantitative Macroinvertebrate Community Function

All of the ECM and CSM, as well as 5 of the 6 CFM measured for this study indicated there was a statistically significant difference among the sites after correction for seasonal differences with the treatment MONTH (Table 3.1). Not surprisingly, many of the metrics showed a significant interaction among the treatments SITE and MONTH, and need to be considered conservatively. Nonetheless, the results are presented here for descriptive purposes, with the caveat that the p-values may be confounded by interactions among the treatments.

Ecological Community Metrics (ECM)

Total abundance was greatest at the upstream site and lowest at the downstream site. The intermediate sites were not significantly different from each other (Table 3.1, Fig 3.3); however, a reverse trend was observed in taxa richness, where the higher values were generally observed farther downstream. After correcting for seasonal variation, the statistical groupings were similar to those of total abundance (Table 3.1) but opposite in terms of magnitude. That is, the upstream site had the lowest taxa richness, the downstream site had the greatest taxa richness, and the middle sites were not significantly different from each other. The Shannon Wienner Diversity index (H'; $\log_2$) did not show such a clear trend. The upstream site had the lowest mean, but was not significantly different from 2-ELK or 4-KOA (Table 3.1). The T:N ratio indicated that the 5-CPT site had significantly greater richness per unit abundance than all the other sites, which were not significantly different from each other (Table 3.1). Moreover, the data appear to show a gradient of increasing T:N downstream (Fig. 3.15).

The ECM results support the *a priori* hypotheses we defined in the methods section of this document. That is, richness was greatest downstream, abundance was greatest upstream, diversity and T:N were greatest downstream. The trends for Taxa and Abundance are summarized in figure of T:N (Fig.3.16) and Diversity (Fig. 3.17) is presented to illustrate the subtle response gradient of this metric.

Table 3.1. Differences among the treatments SITE and MONTH. The results of the two-way ANOVA indicate that there were significant differences among the sites for all metrics except the abundance of predators. There were also significant differences among the months sampled for many metrics. Tukey's HSD test was used to describe differences among sites. The Groupings formed are defined by letters. Sites sharing letters were not significantly different from each other. For example Taxa richness at 1-DUN (A) was significantly different from all other sites, but 2-ELK (B) was only significantly different from 1-DUN(A) and 5-CPT(C), but not the other sites. The ANOVA indicated a significant interaction effect between the treatments for several of the metrics (Denoted by an asterisk (*)); these groupings should be interpreted conservatively because different sites responded different seasonally when interactions are significant.

Metrics	TWO-WAY ANOVA						TUKEY'S HSD GROUPING				
	SITE		MONTH		INTERACTION						
	F – Start	P – Value	F – Start	P – Value	F – Start	P – Value	1-DUN	2-ELK	3-PIP	4-KOA	5-CPT
ECOLOGICAL COMMUNITY METRICS											
Taxa	14.50	<0.001	12.07	<0.001	2.255	0.051	A	B	BC	BC	C
Total Abundance	11.3	<0.001	0.888	0.422	0.974	0.474	A	B	BC	BC	C
Diversity	4.2	0.008	12.68	<0.001	1.87	0.104	A	AB	B	AB	B
T:N	6.79	<0.001	0.75	0.48	0.523	0.829	A	A	A	A	B
COMMUNITY STRESS METRICS											
EPT	27.97	<0.001	8.95	<0.001	2.73	0.022*	A	B	C	C	C
% EPT	10.03	<0.001	25.95	<0.001	4.991	0.001*	A	BC	BC	AB	C
%Chironomidae	4.113	0.009	10.71	<0.001	3.000	0.013*	A	AB	AB	AB	B
% Oligochaeta	2.901	0.038	0.998	0.380	0.507	0.842	A	AB	AB	B	B
% Non-insects	3.069	0.031	5.194	0.012	0.744	0.653	A	AB	AB	AB	B
COMMUNITY FUNCTION METRICS											
Gatherers	5.701	0.002	2.31	0.117	3.923	0.003*	A	AB	BC	C	C
Filterers	3.003	0.034	0.433	0.652	2.927	0.015*	AB	A	AB	B	AB
Predators	0.486	0.746	5.007	0.013	0.851	0.567	--	--	--	--	--
Shredders	5.36	0.002	15.1	<0.001	10.381	<0.001*	A	B	AC	BC	ABC
Scrapers	39.56	<0.001	33.64	<0.001	10.338	<0.001*	A	A	B	B	C
Collectors	4.198	0.008	2.198	0.132	6.459	<0.001*	A	AB	AB	B	B

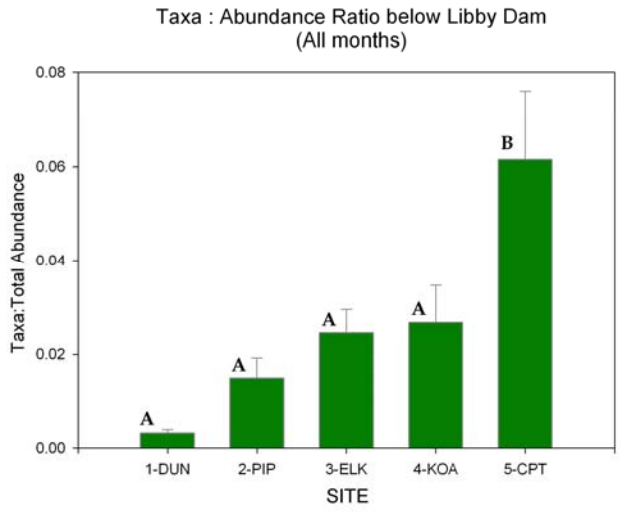


Figure 3.16. Taxa: Abundance ratio (T:N). This relationship was used to summarize the relationship between taxa richness and total abundance. Total abundance was high below Libby dam and richness was low. Conversely taxa richness was high at 5-CPT, but total abundance was lower. Error-bars denote ±1SEM. Letters denote the Tukey's HSD grouping after variation is considered fro both SITE and MONTH (see Table 3.1)

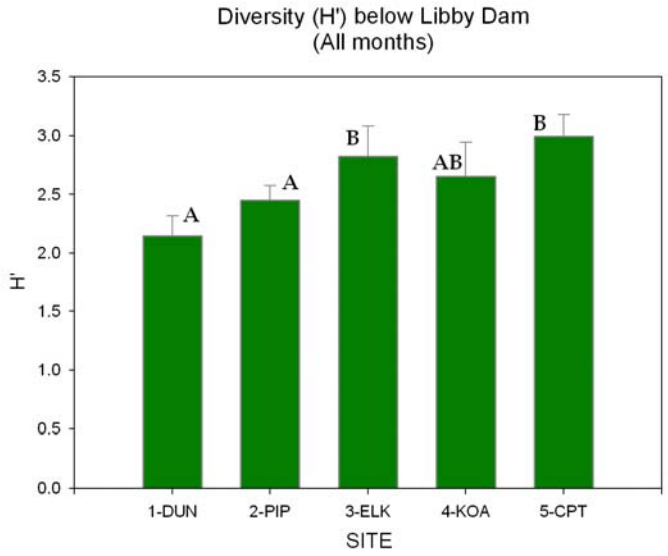


Figure 3.17. Shannon Diversity (H'). Shannon Diversity was calculated as it was originally published using base-2 logarithms. Error-bars denote ±1SEM. Letters denote the Tukey's HSD grouping after variation is considered fro both SITE and MONTH (see Table 3.1)

Community Stress Metrics (CSM)

All five of the CSM indicated that there was a significant difference among the sites, and all but %Oligochaeta indicated that there was a significant difference among seasons. The metrics dealing with non-insects (%Oligochaeta and %non-insect abundance) were the only CSM to not exhibit a significant interaction among the spatial and temporal treatments (Table 3.1).

Although there were many overlapping groups formed by the two-way ANOVA and Tukey's HSD, the overall patterns displayed by these metrics are consistent with our hypotheses defined earlier (methods section 2.4). Specifically, they define a response gradient consistent with upstream perturbation and downstream recovery of the Kootenai River (Figs 3.18-3.22). The most noteworthy is the low abundance of EPT taxa at the upstream site, because the Hydropsychidae are often very abundant below dams. This suggests that the community composition is perturbed by something other than Libby Dam, as is consistent with our hypothesized effect of *D. geminata* on the biota of the Kootenai River.

The relative Oligochaeta abundance and non-insect abundance were much higher than expected at 1-DUN. Typically, "clean" streams in Montana have < 5% oligochaetes represented in the community, and have < 8-10% of the community represented by non-insect taxa (e.g., Marshall and Kerans 2003). This deviation from the norm may be related to the mucilaginous retaining fine particulate organic matter or to the flow refugia for non-insects and midges.

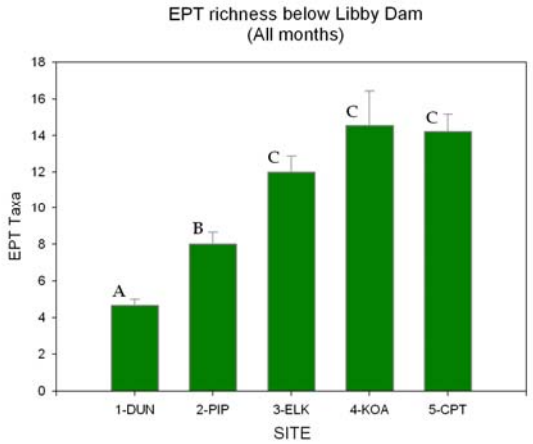


Figure 3.18. EPT richness. The mean richness of EPT taxa is shown (±1SEM). The letters denote the results of a two-way ANOVA and a Tukey's HSD test to determine statistically significant groups. All sites sharing a letter are not significantly different. Note that the test considers seasonal variation as well, so that groups may not correspond as expected based on visual examination of these graphs.

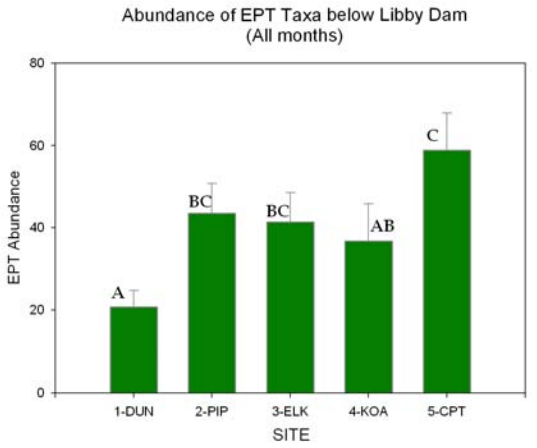


Figure 3.19. EPT Abundance. The mean Abundance of EPT taxa is shown (±1SEM). The letters denote the results of a two-way ANOVA and a Tukey's HSD test to determine statistically significant groups. All sites sharing a letter are not significantly different. Note that the test considers seasonal variation as well, so that groups may not correspond as expected based on visual examination of these graphs.

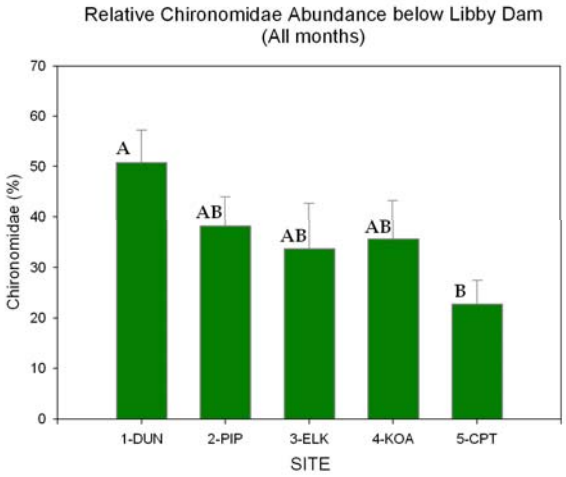


Figure 3.20. Chironomid Abundance. The mean chironomid abundance is shown (±1SEM). The letters denote the results of a two-way ANOVA and a Tukey's HSD test to determine statistically significant groups. All sites sharing a letter are not significantly different. Note that the test considers seasonal variation as well. groups may not correspond as expected based on visual examination.

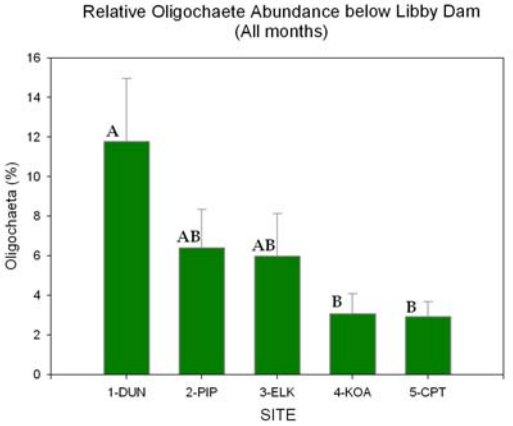


Figure 3.21. Oligochaeta Abundance. The mean Oligochaeta abundance is shown (±1SEM). The letters denote the results of a two-way ANOVA and a Tukey's HSD test to determine statistically significant groups. All sites sharing a letter are not significantly different. Note that the test considers seasonal variation as well, so that groups may not correspond as expected based on visual examination of these graphs.

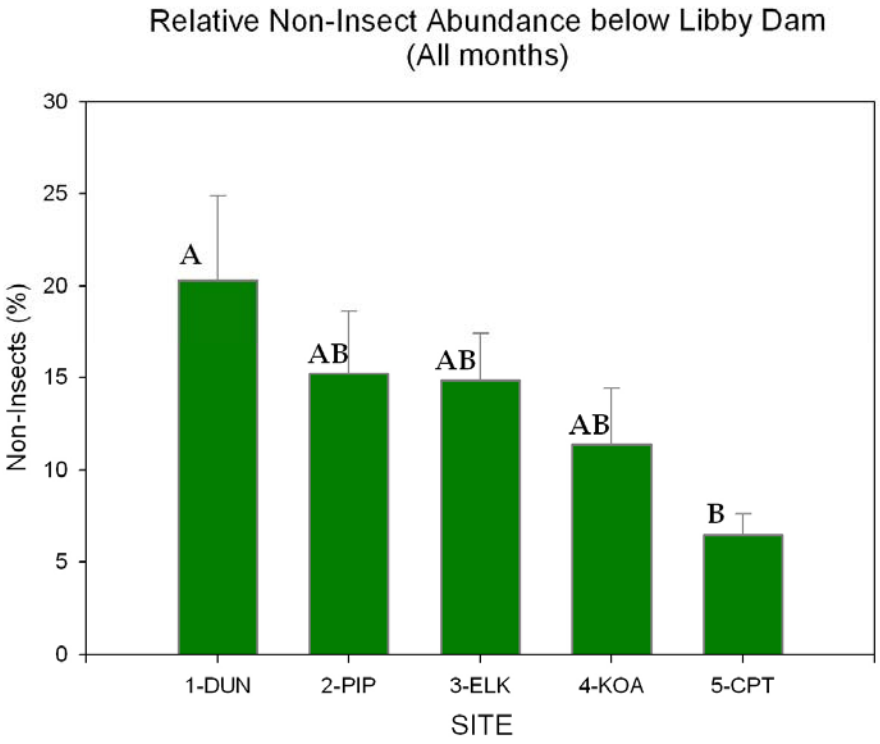


Figure 3.21. Non-Insect Abundance. The mean non-insect abundance is shown (±1SEM). The letters denote the results of a two-way ANOVA and a Tukey's HSD test to determine statistically significant groups. All sites sharing a letter are not significantly different. Note that the test considers seasonal variation as well, so that groups may not correspond as expected based on visual examination of these graphs.

Community Function Metrics (CFM)

Five of the six CFM measured indicated there was a significant difference among the sites after correction for seasonal variation (Table 3.1). The exception was the relative abundance of predators, which remained fairly constant throughout the study.

Collector-gatherers were much more abundant at the upstream site than at downstream sites and, although collector-filterers did not show as a clear trend, the combined abundance of gatherers and filterers (i.e., collectors) resembled the trend for gatherers (with greater amplitude and less variation among sites). Both the shredders and the scrapers did not show a significant trend, but this is often the case when communities become dominated by collectors. Thus, the differences in ecosystem function appear to be related to the dominance of collectors, and collector-gatherers in particular.

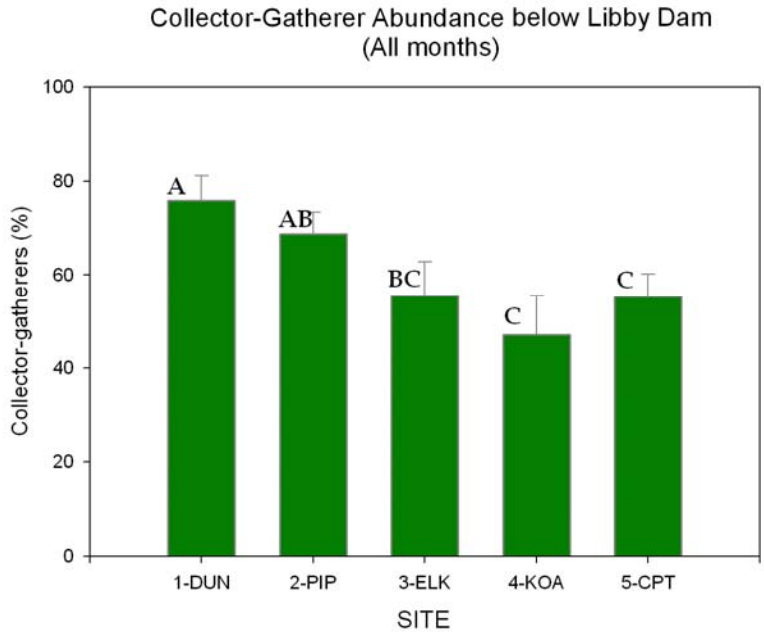


Figure 3.22. Collector-Gatherer Abundance. The mean collector-gatherer abundance is shown (±1SEM). The letters denote the results of a two-way ANOVA and a Tukey's HSD test to determine statistically significant groups. All sites sharing a letter are not significantly different. Note that the test considers seasonal variation as well, so that groups may not correspond as expected based on visual examination of these graphs.

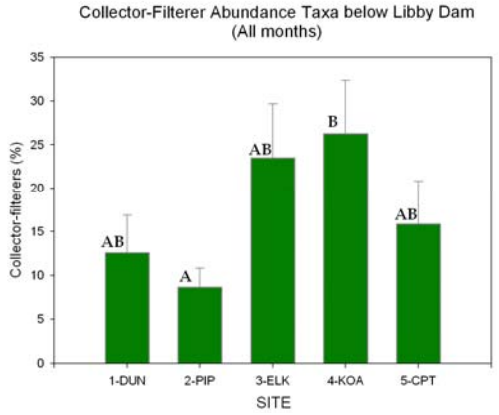


Figure 3.23. Collector-filterer Abundance. The mean Collector-filterer abundance is shown (±1SEM). The letters denote the results of a two-way ANOVA and a Tukey's HSD test to determine statistically significant groups. All sites sharing a letter are not significantly different. Note that the test considers seasonal variation as well, so that groups may not correspond as expected based on visual examination of these graphs.

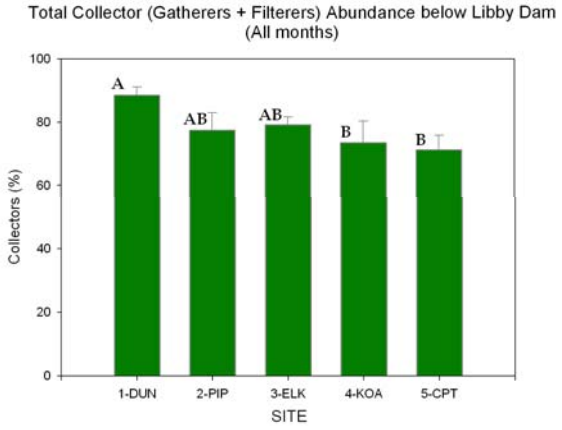


Figure 3.24. Collector Abundance. The mean Collector abundance is shown (±1SEM). The letters denote the results of a two-way ANOVA and a Tukey's HSD test to determine statistically significant groups. All sites sharing a letter are not significantly different. Note that the test considers seasonal variation as well, so that groups may not correspond as expected based on visual examination of these graphs.

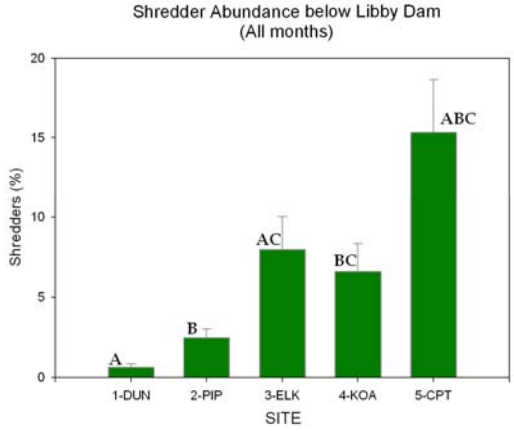


Figure 3.25. Shredder Abundance. The mean Shredder abundance is shown (±1SEM). The letters denote the results of a two-way ANOVA and a Tukey's HSD test to determine statistically significant groups. All sites sharing a letter are not significantly different. Note that the test considers seasonal variation as well, so that groups may not correspond as expected based on visual examination of these graphs.

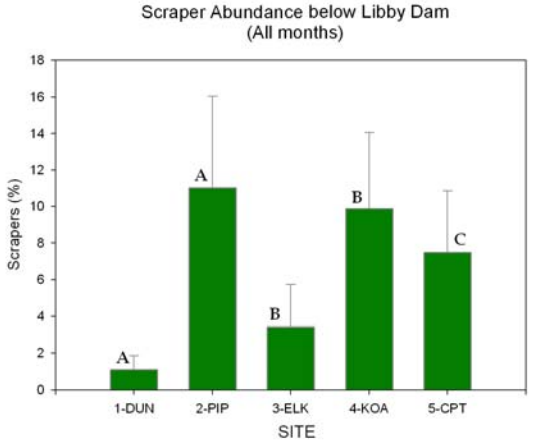


Figure 3.26. Scraper Abundance. The mean scraper abundance is shown (±1SEM). The letters denote the results of a two-way ANOVA and a Tukey's HSD test to determine statistically significant groups. All sites sharing a letter are not significantly different. Note that the test considers seasonal variation as well, so that groups may not correspond as expected based on visual examination of these graphs.

3.6. The Role of Habitat and D. geminata

When we replaced the two-way ANOVA design (discussed above to describe differences among sites), with a GLM procedure with a forward stepwise variable selection algorithm, site-related differences became obscured. This is because once habitat and temporal (i.e., MONTH) variables were considered, SITE no longer explained a significant portion in the variance of biological metrics. That is, the differences among sites appeared to be able to be explained by habitat, but seasonal factors continued to be important for some metrics (TABLE 3.2). This suggests that differences among sites might be due to habitat differences among them. The only variables that were significantly different among the sites were Embeddedness, and the particle size index (see Section 3.1). Thus, it appears that at least some of the variation in biological metric values can be explained by these variables. However, some of the variables for which ANOVA indicated a site-difference could not be statistically fit to any habitat variables using the forward step-wise algorithm.

Recall that we collected three periphyton samples from each macroinvertebrate sample. These data are used as a measure of the density of *D. geminata*, because *D. geminata* was by far the dominant taxon represented in the samples. When we added the average periphyton biomass (AFDM, mg/cm^2) as a variable (ALGAE) and repeated the procedure above, something very interesting happened (Table 3.3). The variable ALGAE contributed significantly to every model. Metrics for which no models could be derived using the step-wise selection algorithm with habitat covariates, showed significant relationships. Additionally, terms in the original models that were significant were frequently replaced by algae. Generally, periphyton AFDM was the strongest single predictor of benthic community structure. This is exciting because flow is usually the single strongest predictor of community structure (e.g., Hart and Fonseca 1991).

Table 3.2. Habitat factors significantly correlated with metrics. These models were developed using a forward, step-wise algorithm. Treatment effects for sites and months were coded for inclusion and interaction effects were also included. Algae variables were not included, these are considered elsewhere (table 3.3). The threshold for inclusion to the model was set at p=0.15. This liberal p-value was selected to avoid type-2 statistical error.

			P-value	R^2
ECM				
	Total Abundance	= 6.17 + Particle*(0.004)	0.075	0.072
	Taxa Richness	= 15.1 + Embed*0.137 + Month*(4.73)	0.031	0.192
	Diversity (H')	=1.89 + Month*(0.75)+Particle*Month(0.001)	<0.001	0.333
CSM				
	EPT Richness	*No terms met tolerance criteria*	N.S.	n/a
	% EPT (abund)	= 6.49 + Month*(16.9)	<0.001	0.314
	% Chironomidae	= 60.2 -21.5*(Month) + 0.037(Particle*Month)	0.001	0.299
	% Oligochaete	*No terms met tolerance criteria*	N.S.	n/a
	% Non-Insect	= 17.9 -11.5*(Flow)	0.103	0.061
CFM				
	Gatherers	= 40.4 +0.103(Particle)-9.24(Flow*Month)	0.004	0.229
	Filterers	= 2-.4 + 32.5 (Flow) -0.059(Particle)	<0.001	0.307
	Collectors	*No terms met tolerance criteria*	N.S.	n/a
	Shredders	*No terms met tolerance criteria*	N.S.	n/a
	Scrapers	= (-1.52) + 4.06 (Month)	0.002	0.198

Table 3.3. Habitat and algae factors significantly correlated with metrics. These models were developed using a forward, step-wise algorithm. Treatment effects for sites and months were coded for inclusion and interaction effects were also included. Algae variables were included; models excluding algae are considered elsewhere (table 3.2). The threshold for inclusion to the model was set at p=0.15. This liberal p-value was selected to avoid type-2 statistical error.

			P-value	Algae P	R^2
ECM					
	Total Abundance	= 6.50 + 0.154 (Algae)	<0.001	<0.001	0.288
	Taxa Richness	= 24.1 – 0.241 (Algae)	0.034	0.034	0.317
	Diversity (H')	= 2.01 + Month*(0.733) + Particle*Month(0.001) + Algae(0.066)	<0.001	0.008	0.439
CSM					
	EPT Richness	= 11.5 + 4.84 (Flow) – 0.51 (Algae)	0.011	0.008	0.198
	% EPT (abund)	= 48.9 -1.68 (Algae)	0.093	0.093	0.064
	% Chironomidae	= 48.7 + 0.355(Embed) -16.8(Month) + 3.28(Algae)	<0.001	<0.001	0.549
	% Oligochaeta	= 1.57 + 0.863 (Algae)	0.001	0.001	0.241
	% Non-Insect	= 24.8 -14.6(Flow) + 1.12(Algae) -0.044(Particle)	0.007	0.005	0.253
CFM					
	Gatherers	= 37.7 -24.5(Flow) + 2.05(Algae)+ 0.081 (Particle)	<0.001	0.007	0.348
	Filterers	= 9.98 +36.4(Flow) – 1.17(Algae)	<0.001	0.033	0.325
	Collectors	= 80.8 - 5.76 (Month) +1.68(Algae)	0.013	0.007	0.187
	Shredders	= 10.5 – 7.58(Algae)	0.075	0.075	0.072
	Scrapers	= 11.7 – 0.164 (Embed) – 0.614(Algae)	0.039	0.040	0.143

There are some limitations to these models that need to be considered before the implications are discussed. Generally, these models should not be used for prediction of benthic assemblage structure. We reduced the level of replication required by using near-substrate water velocity as a factor to select sites. Thus the variable flow was tightly constrained. Similarly the measures of algae biomass used to develop these models were excessive, nuisance levels of biofilms. Obviously, the predicative value of these models should not be extrapolated beyond the range represented by the data—and particularly not when biofilm density is < 3mg/cm^2 (q.v., Section 3.6.3.). Additionally, the fit of these models is relatively poor and the use as a predictor is likely to result in some error.

Despite these limitations, the directional correlations (positive or negative) of metrics with the variable ALGAE provide a means to discuss our results relative to our original hypotheses. That is, by using the variable ALGAE as a surrogate for *D. geminata* thickness, we can specifically discuss the effects of *D. geminata* on the biota of the Kootenai River below Libby dam.

3.6.1. Effects of D. geminata on ECM

Total abundance of macroinvertebrates was positively correlated with periphyton AFDM, whereas the Taxa Richness and Shannon Diversity (H') of benthic assemblages were negatively correlated with periphyton AFDM. This appeared to occur because thick mats of *D. geminata* provided habitat for midges and oligochaetes, but many non-burrowing invertebrates were excluded from thick mucilaginous material.

3.6.2. Effects of D. geminata on CSM

The same phenomenon affecting the ECM also affected the CSM. Specifically, the richness and abundance of EPT taxa—most of which are clingers/crawlers—were negatively correlated with AFDM of *D. geminata*. Midges, worms and the percent abundance of non-insects were significantly positively correlated with periphyton AFDM. The association of burrowing midges and oligochaete worms with thick mats of *D. geminata* suggests that they may benefit from *D. geminata* in some way. Certainly, the biofilms provide shelter from predators and from scouring flows. The mucilaginous material may trap fine organic particles that they require for sustenance. We believe it is unlikely that these groups derive any significant nutrition from *D. geminata* itself

because we analyzed the gut contents of invertebrates from a high AFDM sample and found no *D. geminata* remains. There may be some occasional grazing occurring because one of our taxonomists was able to identify a *D. geminata* frustule in the gut of a slide-mounted midge specimen.

3.7. Data Analysis-Quantitative Response to D. geminata

The high abundance of worms and midges among samples containing high periphyton biomass translated to a positive correlation with collector gatherers, collector-filterers, and collectors (gatherers + filterers). Along with Algae, gatherers were negatively correlated with flow, whereas filterers were positively correlated with flow. Shredders and scrapers were negatively correlated with periphyton biomass. We usually attribute such correlations to the reciprocal relationship of these measures with changes in the collectors. That is, a large increase in the percent abundance of one functional feeding group, requires a corresponding decrease in the percent abundance of one or more other functional feeding groups. However, we observed some very important patterns in the abundance of both shredders and scrapers.

No shredders were collected at periphyton densities > 8.0mg/cm^2 (Fig. 3.27). Similarly, the scrapers did not comprise more than 2.5 % of the community when the biofilms were >8.0 mg/cm^2 (Fig 3.27). Both shredders and scrapers derive their sustenance from the composition of biofilms. Scrapers directly feed on biofilms. Shedders usually consume a coarser substrate of lower nutritive quality (e.g., leaves) but derive their nutrition from the biofilms growing on it (Cummins 1974). Thus, some level of epilithic biofilms is "good" and indicative of healthy biofilms. The model that we fit to our observations suggests that the optimum density of epilithic film is around 3 mg/ cm2. Below this threshold, both shredders and scrapers exhibit a positive correlation with ALGAE, thereafter, their contribution to the community begins to decline. Although our models (Eq. 3.11, 3.12) reached optima at ~3.0mg / cm^2, high abundances of shredders and scrapers were observed through a biofilm density of ~5mg/cm^2.

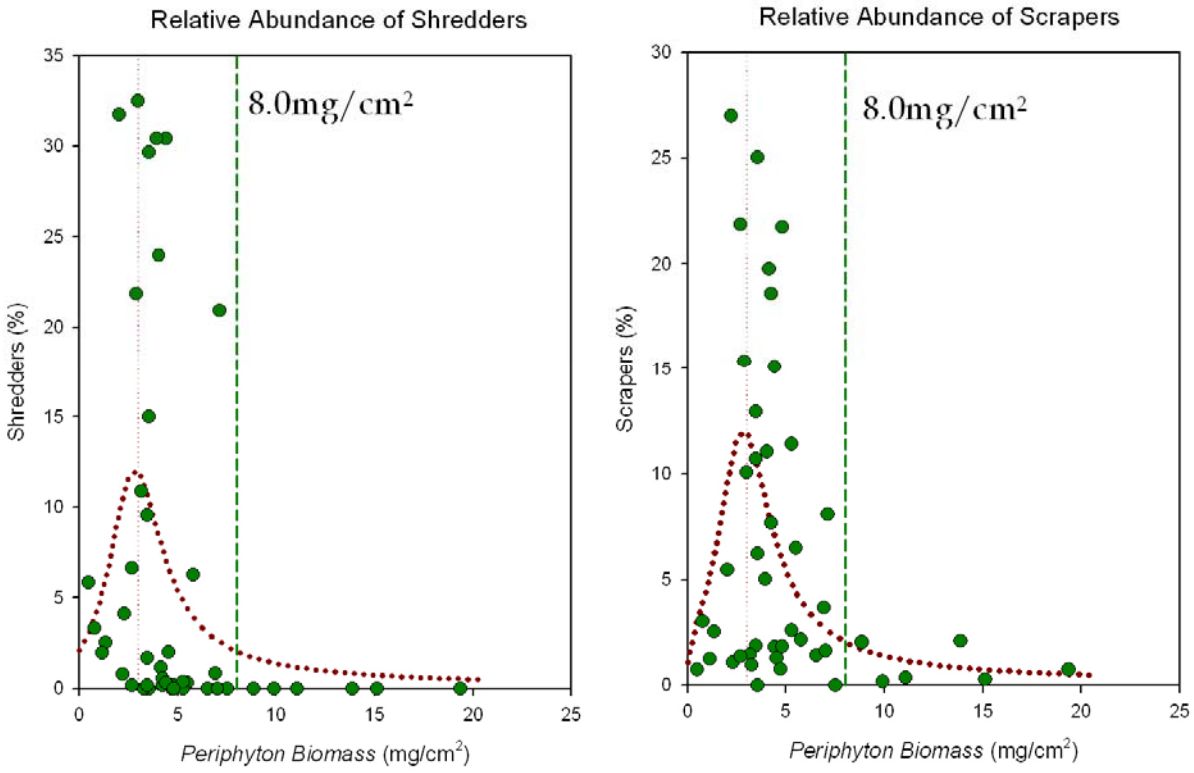


Figure 3.27. Abundance of shredders and scrapers compared to biomass of periphyton. The abundance of scrapers and shredders were greatly reduced beyond 8mg/cm² (dashed-line threshold) and the models (Eq. 3.11, 3.12) peaked at about 3mg/cm² (fine dotted line).

Equation 3.11.Relationship among shredders and density of biofilms (r^2=0.151)

$$\%Shredders = \frac{1}{(-0.525 + 0.618^{ALGAE} + 0.124(ALGAE)}$$

Equation 3.12.Relationship among scrapers and density of biofilms (r^2=0.204)

$$\%Scrapers = \frac{1}{(-0.075 + 3.19^{ALGAE} + 0.045(ALGAE)}$$

The effect of excluding production by scrapers and shredders is cause for concern because these organisms link higher trophic levels to biofilm production in the Kootenai River. So it is reassuring to know that we observed relatively few (six) data points in excess of the 8 mg/cm2 threshold. These occurred only at the two upstream sites, but occurred in all three months sampled (Fig. 3.28). However, all the sites had values that exceeded the 3mg/cm^2 optimum, after which the relative abundances of scrapers and shredders begin to decline.

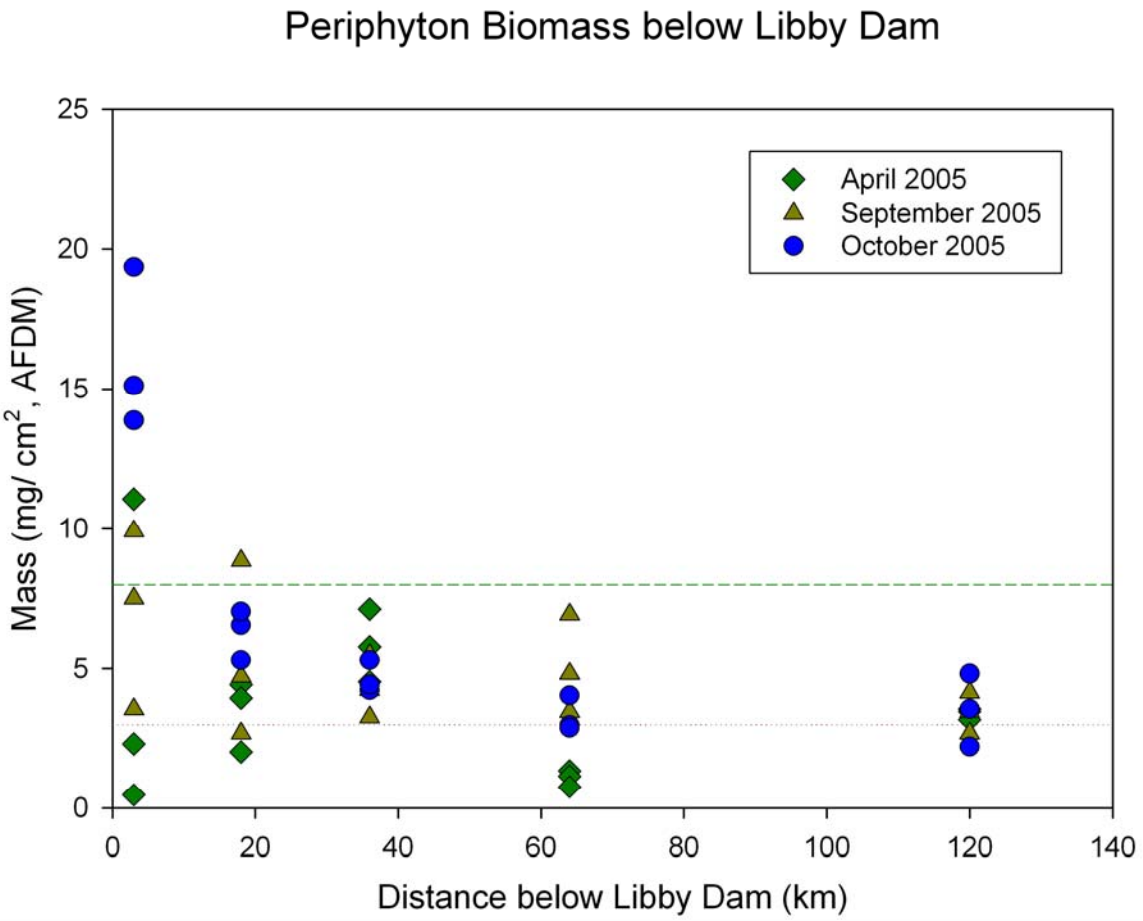


Figure 3.28. Downstream trends in periphyton biomass. The red (dotted) and green (dashed) lines represent the 3mg/cm^2 and 8mg/cm^2 thresholds from Fig 3.27. the low-biomass values occurred in the spring. High biomass biofims occurred mainly at the site immediately downstream from Libby Dam.

Ideally, we should be able to use our data to recommend a thickness of biofilms that corresponds to the 8 mg/cm2 threshold and the 3 mg/cm2 optimum level. However, our measures of bioflim thickness were independent of biomass estimates. Only one measure was collected per invertebrate sample, whereas, the biomass measures are the mean of three algae samples collected from within each invertebrate sample. Thus the thickness data do not correlate with biomass data (Fig. 3.29) and the resulting recommendations would not be meaningful.

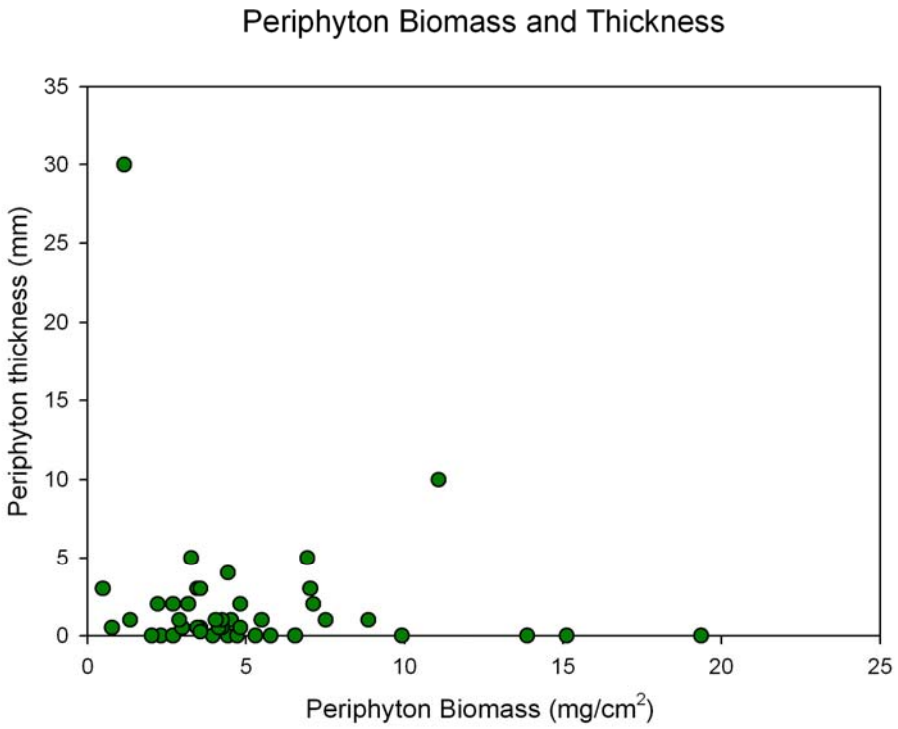


Figure 3.29. Correlation of epilithic biomass to thickness measures. It would be useful to estimate the biomass of epilithic biofilms using a ruler in the field. Unfortunately, our thickness measures were not recorded directly from the biomass sample, and the correlation between the variables too poor to allow the data to be used this way.

3.7. Results summary

When we reviewed the taxonomic composition data using the methods of Hauer and Stanford, we observed many of same taxa and phenomena they described (Hauer and Stanford 1997). Some of the abundance patterns we observed (e.g., Hydropsyche abundance; Fig. 3.12) exhibited patterns commonly associated with impounded rivers. However, examining each of the 98 taxa we identified individually makes it difficult to test any specific hypothesis. Therefore, we examined the taxonomic composition using a community dissimilarly matrix which compared the relative abundance of each of these taxa and condensed the comparisons into one single measure of dissimilarity—the Bray-Curtis Dissimilarity index (BCD).

We found that the BCD of communities compared to the upstream site increased with distance—which is expected below dams. However, when we repeated the analysis using Hauer and Stanford's (1997) data, we found that the trend was more pronounced in the 1990's than it was in 2005. We considered this an indication that the structure of the benthos is less directly influenced by Libby dam than it was when Hauer and Stanford (1997) studied it.

Next we addressed how these structural changes relate to ecosystem function. Since we did not conduct a secondary production study (they are very expensive), we used metrics to summarize community structure in terms that are functionally relevant. We found that sites had statistically significant differences in 14 of 15 metrics tested (Table 3.1). Moreover, the results were usually reflective of a down stream gradient of change.

Then we included sample-specific habitat measures, along with SITE and MONTH (coded as effects) and applied GLM with a forward stepwise algorithm to select the variables most strongly correlated with the metrics. The results indicated that differences among sites may actually be related to habitat, but not all metrics resulted in significant models (Table 3.2).

We repeated the GLM-variable selection algorithm with the addition of periphyton biomass (ALGAE) as a "habitat variable." The variable ALGAE was a significant predictor for every metric tested (Table 3.3). In fact, it replaced most other habitat measures and improved the fit of the models significantly. We then discussed, in general terms, the implications of these findings.

Below, we specifically address the directional response of metrics compared using ANOVA, ACOVA and a variety of covariates.

Specific ECM hypotheses:

1. *Total abundance is elevated at upstream sites due to seston enrichment.*

   Total abundance was elevated as expected, but we believe *D. geminata* may have also contributed to high abundance by providing refuge to burrowing midges and oligochaetes.

2. *Taxa Richness is depressed upstream because of high densities of filter feeders, and/or because of habitat exclusion related to D. geminata coverage.*

   Taxa richness was depressed at upstream sites, probably due to a combination of seston enrichment and *D. geminata* coverage.

3. *Diversity should be greatest downstream.*

   Diversity was greatest downstream, but some of the sites were (2-PIP, 4-KOA) were not significantly different from the upstream site.

4. *T:N ratio will be greater downstream, where the influence of D. geminata and seston enrichment are reduced.*

   The T:N ratio was tiny upstream and much greater downstream. The middle sites were not significantly different from the upstream site, but were intermediate to the up- and downstream extremes.

Specific CSM hypotheses :

1. *EPT richness will be greatest downstream, where the influence of D. geminata and seston enrichment are reduced*.

   This hypothesis was correct.

2. *Percent EPT abundance will be greatest down stream, if D. geminata excludes colonization of filter feeding Trichoptera from upstream substrata.*

   EPT abundance was greatest downstream, but filter feeders were not excluded from upstream substrata.

3. *Percent abundance of chironomid midges will be elevated upstream if they are able to benefit (food, refuge, cover etc.) from D. geminata coverage at upstream sites.*

   Chironomids were most abundant at the upstream site. We collected many more midges than Hauer and Stanford at the upstream site. Midge abundance was positively correlated with *D. geminata* thickness.

4. *Percent Non-insects will increase under the same conditions as chironomid midges.*

   This hypothesis was supported by our findings, but the effects were much more pronounced when oligochaete worms were considered separately from other non-insects (e.g., Amphipoda, Gastropoda).

Our specific CFM hypotheses are that:

1. *Collector-gatherers will be elevated at upstream sites if D. geminata improves their success by providing sustenance (direct, or indirect) or protection (from invertebrate predators, fish or scour).*

   Our results support this hypothesis. The abundance of Collector-gatherers was strongly correlated with the mass of *D. geminata* mats and negatively correlated with water velocity.

2. *Collector filterers will be elevated at the upstream sites due to seston enriched outfall if D. geminata does not exclude colonization below the dam.*

   Our results support this hypothesis. The abundance of Collector-filterers was correlated with the mass of *D. geminata* mats and water velocity.

3. *Total collectors (gatherers + filterers) will be elevated at upstream sites as a result of elevated Collector-gatherers, or Collector-filterers, or both.*

   Our results support this hypothesis. The abundance of Collector-filterers was correlated with the mass of *D. geminata* mats and the effects-coded variable MONTH –indicating significant seasonal variation.

4. *Predators will remain unchanged, unless D. geminata reduces their mobility sufficiently to reduce their success.*

   Predators were not significantly different among sites. A post hoc analysis of predators indicated that they were most strongly influenced by particle size and flow—both negatively.

5. *Shredders will be depressed at upstream sites for several reasons. Increases in relative abundance of Collectors will necessitate a reduction in other groups. Reduced CPOM crop at upstream sites also likely—due to both the Dam (retention, scour) and D. geminata (covering CPOM deposits).*

   Shredders were in very low abundance below Libby dam and much higher in abundance at the down stream site. Moreover they were negatively associated with periphyton biomass (Fig. 3.27)

6. *Scrappers will be depressed at upstream sites for the same reasons as shredders. Most Scrappers are also clingers, and may be excluded from feeding areas by mucilaginous coating of substrata.*

   Scrapers did not show a significant gradient effect like shredders. They were least abundant at the upstream site, but highly variable. However, they were negatively associated with periphyton biomass (Fig. 3.27)

Other findings

We also identified an optimum amount of periphyton that should occur in the Kootenai River (~3mg/cm^2) and a threshold that should not be exceeded (8mg/cm^2). The threshold (8mg/cm^2) was only exceeded at the two upstream sites (1-DUN and 2-ELK), but many of the sites exceeded our estimated optimum biofilms biomass (~3mg/cm^2).

4. IMPLICATIONS OF *D. GEMINATA* FOR THE KOOTENAI RIVER

Didymosphenia geminata is the only native North American species in the genus *Didymosphenia.* It is a stalk-growing diatom from the family Cybellacea (Spalding 1997), that has been historically collected in low abundances from cool, clear, oligotrophic waters (Patrick and Reimer 1975). The algae grows on simple stalks formed of complex polymers they secrete as they grow. The stalks split when the cells reproduce, with each daughter cell growing on the end of a newly bifurcated strand of polymer. The result is the growth of tufts and, in some cases, mats of *D. geminata*.

Through the mid-1980's and 1990's, researchers began noticing nuisance out-breaks (or blooms) of *D. geminata* through out the northern hemisphere. In 2004, the first verified occurrence of *D. geminata* was reported for the southern hemisphere, when it was encountered during routine monitoring in New Zealand. Dense out-breaks continue to spread throughout New Zealand (Biggs et al. 2006). Thus, the scale of *D. geminata* invasions has reached global proportions.

Out-breaks in the United States are causes of public concern as well. Although it is a native species here, it has historically only occurred in low abundances. Thus when people encounter large mats of slimy material resembling raw sewage—a perception that is not aided by toilet-paper-like dried mats along the shore—they become concerned. Furthermore, mats of algae may detach with elevated flows and interfere with sport fishing and with river aesthetics.

In 2002, the Kootenai Tribe of Idaho found that *D. geminata* began dominating their routine monitoring samples collected on the Kootenai River (Holderman and Handy 2004). Unattached mats are common in the Kootenai River after power generation by Libby Dam. However, at the time of this report the biological effects of *D. geminata* out-breaks were unknown.

The purpose of this portion of the report is to combine our results with what is known about *D. geminata* and to decide if it poses an ecological risk.

Our results indicate that levels of *D. geminata* exceeding 8 mg /cm^2 AFDM significantly reduce the abundance of both shredders and scrapers. Furthermore, we found that it significantly increased the abundance of midges and oligochaete worms, while generally suppressing EPT taxa. We acid-digested all the invertebrates from one high-*D. geminata* sample and found no frustules, indicating that the invertebrates probably receive only limited benefits from the coverage of this algae. However, we also found at periphyton biomasses up to 3mg/cm^2 shredders and scrapers appeared to show increased success; invertebrates may feed on *D. geminata* at lower levels when the mucilage is not thick enough to impede their movement.

Thus, coverage of substrata by *D. geminata* has potential to seriously alter the structure and function of the Kootenai River's Food webs. Shifting secondary production from large stoneflies to small midges with high biomass turnover rates could have implications for fish populations because individuals will need to expend more energy to consume the same amount of food. Furthermore, if invertebrate production is forced to the hyporheos, less will be available for fish consumption. Thus, the impacts of *D. geminata* on benthic food webs are of general interest, not simply a matter of academic curiosity.

One implication that we have not seen discussed in the literature, or even heard at meetings is the potential interrelation of *D. geminata* and whirling disease. We did not identify the oligochaetes for this study below the level of Class, but one of the most common oligochaetes in Montana is *Tubifex tubifex*, a vector of whirling disease. Since *D. geminata* has potential to turn much of the river bottom into *T. tubifex* habitat, the whirling-disease processes that were previously relegated to side-channels and depositional areas could occur across nearly the entire rivers substrata for much of the year—greatly increasing exposure of fish to the parasite *Myxobolus cerebralis*. If fish are more stressed from increased foraging effort (above), the effects could be synergistic.

We believe the *D. geminata* poses significant risk to healthy structure and function of the Kootenai River. These changes could have economic implications for fisheries in Kootenai River as well as other western rivers with power generating reservoirs. Therefore, it is important to determine the causes of recent out-breaks.

Most of the early work on *D. geminata* was descriptive (e.g. Patrick and Reimer 1975, Antoine and Benson 1984). Specimens were infrequently collected and since they never presented a problem there was little need to model the response of a highly variable diatom. Although there is now a flurry of research on this species, this activity is a result of recent problem blooms.

Kawecka and Sanecki (2003) reviewed the distribution of *D. geminata* in southern Poland. They report that in the 1960's, *D. geminata* occurred only sporadically from oligotrophic rivers. However, in the 1990's, they reported *D. geminata* from larger rivers across southern Poland and found large mats of the algae below dams on the River San. They also reported a large suite of chemical parameters that did not seem to explain the large populations. They concluded that either the original description of *D. geminata* was incorrect, or that a unique strain of *D. geminata* had developed in Poland. Several other investigators (Biggs et al 2006, Bothwell 2006) have casually mentioned that there may be a new and especially invasive variety of *D. geminata* that forms nuisance blooms.

At the same time that the entire northern hemisphere began noticing these blooms, there was something else going on. Stratospheric ozone concentrations had plummeted to incredibly low levels (Weatherhead and Andersen 2006), increasing the amount of Ultra-Violet Radiation (UVR) reaching the earth—and the surface of clear, low nutrient streams. Bothwell et al (2006) have dismissed the contributing role of UVR in the response of *D. geminata*—presumably because UVR can actually hinder the growth and production of many algae (Bothwell et al. 1993, Bothwell et al. 1994, Kelly et al. 2003).

However, Falkowski and LaRoche (1991) explain how algae physiologically respond to increased light intensity and changes in spectral quality of light. In the case of increased intensity, or increased photosynthetic wave lengths (Like UVR), the cells shift the allocation of carbon from manufacture of photosynthetic pigments, to the formation of carbohydrates and lipids. This response could easily explain why the growth of *D. geminata* has appeared to increase recently, even though local environmental factors appear to have changed little. Increased secretion of a carbohydrate polymer stalk could be a response to UVR and the production of excess carbohydrates.

Furthermore, algae species differ in their ability to photo-acclimate (Falkowski and LaRoche 1991). Species that normally co-occur with *D. geminata* and are unable to adapt to increase irradiance would become disadvantaged—giving *D. geminata* a competitive advantage for the sparse resources of oligotrophic waters. If *D. geminata* competitively displaces some algae species the net primary production could remain unchanged, or even decrease. Thus this mode of action does not necessarily contradict findings of UVR-related production declines (Bothwell et al. 1993, Bothwell et al. 1994).

This mode of action also makes sense because *D. geminata* has a tradition of being collected only from cool, clear waters. If there is a sudden circumpolar development of a new genetic strain, there should be a sudden circumpolar selective pressure favoring that strain—otherwise, similar out breaks should have been observed since the species description in the 1800's.

There may or may not be a new strain of *D. geminata* . What is important is the ecology of the *D. geminata* and the global factors that cause it to form nuisance mats, and interfere with natural ecosystem function. There is insufficient information on the conditions that cause problem blooms and it is impossible to recommend a comprehensive management plan for *D. geminata* in the Kootenai River.

Recommendations:

1. If possible, Libby Dam should generate occasional scour flows to keep the biomass of *D. geminata* below 5-8 mg/cm^2.
2. A combination of laboratory and controlled field studies could determine the specific conditions leading to *D. geminata* blooms. These should focus on the roles of UVR, nutrients, and competition among algae species.
3. A small study of *D. geminata* thickness and its relationship to biomass should be conducted to allow mangers to conduct expedient field surveys to identify potential algae problems.
4. Genetic studies to determine the validity of the "new variety hypothesis" should be lower priority, because this is not relevant to management's immediate needs.

5.0 LITERATURE CITED

5.0 LITERATURE CITED

Biggs B.J.F, C. Kilroy, and C. C. Vieglais. 2006. A New Zealand science response to help manage *Didymosphenia geminata* - an unwanted diatom invader of freshwaters. Current knowledge of *Didymosphenia geminata* and developing a research and management response. Bozeman, MT: American Division of Fisheries Society Annual Meeting, Western Division.

Bothwell M., D. Sherbot, J. Deniseger, H. Wright, D. Lynch, and D. Kelly. 2006. Blooms of *Didymosphenia geminata* in rivers on Vancouver Island 1990 to present: A sign of environmental change or a new invasive species? Current knowledge of *Didymosphenia geminata* and developing a research and management response. Bozeman, MT: American Division of Fisheries Society Annual Meeting, Western Division.

Bothwell M., D. Sherbot, A. Roberge, and R. Daley. 1993. Influence of natural ultraviolet-radiation on lotic periphytic diatom community growth, biomass accrual, and species composition - short-term versus long-term effects. Journal of Phycology 29(1):24-35.

Bothwell M., D. Sherbot, and C. Pollock. 1994. Ecosystem response to solar ultraviolet-b radiation - influence of trophic-level interactions. Science 265(5168):97-100.

Cummins K.W. 1974. Structure and function of stream ecosystems. BioScience 24(11):631-641.

Dunnigan, J. 2004. Investigation of the macrozoobenthos ecology of the Kootenai River. RFP05-1009P. 1-33p.

Falkowski P.G., and J. LaRoche. 1991. Acclimation to spectral irradiance in algae. Journal of Phycology 27:8-14.

Fonseca D.M., and D. D. Hart. 1996. Density-dependent dispersal of black fly neonates is mediated by flow. OIKOS 75(1):49-58.

Hart D.D., and D. M. Fonseca. 1996. An important confluence for stream ecology. Trends in Ecology & Evolution 11(7):272-2.

Hauer F.R., and J. A. Stanford. 1997. Long-Term Influence Of Libby Dam Operation On The Ecology of Macrozoobenthos Of The Kootenai River, Montana and Idaho. Polson, MT: Montana Department of Fish, Wildlife & Parks. 1-65 p.

Holderman C.E., and R. S. Hardy. 2004. Kootenai River Fisheries Investigations Ecosystem Rehabilitation Project: Nutrient Restoration Work Plan.

Kawecka B., and J. Sanecki. 2003. *Didymosphenia geminata* in running waters of southern Poland- symptoms of change in water quality? Hydrobiologia (495):193-201.

Kelly D., M. Bothwell, and D. Schindler. 2003. Effects of solar ultraviolet radiation on stream benthic communities: An intersite comparison. Ecology 84(10):2724-2740.

Kilroy C. 2004. A new alien diatom, *Didymosphenia geminata* (Lyngbye) Schmidt: its biology, distribution, effects and potential risks for New Zealand fresh waters. Christchurch, New Zealand: National Institute of Water & Atmospheric Research, Ltd. 34 p.

Kociolek J.P., and S. A. Spaulding. 2003. Symmetrical naviculoid diatoms. In: Wehr JD, Sheath RG, editors. Freshwater Algae of North America Ecology and Classification. San Diego, CA: Academic Press.

Kociolek J.P., and S. A. Spaulding. 2003. Eunotioid and asymmetrical naviculoid diatoms. In: Wehr JD, Sheath RG, editors. Freshwater Algae of North America Ecology and Classification. San Diego, CA: Academic Press.

Marshall, B.D. 2001. An Evaluation of the Sensitivity of a Macroinvertebrate Biomonitoring Study in Headwater Streams of New River Gorge National River. Journal of Freshwater Ecology 16(3): 415-428.

Marshall B.D., and B. L. Kerans. 2003. A critical appraisal of Montana's Rapid Bioassessment Protocols (MT RBP) for evaluating the ecological condition of steams and rivers using benthic macroinvertebrates: Part 2, current reference criteria and metrics. Bozeman, MT: Montana State University, Department of Ecology. 1-51 p.

Merritt R.W., and K. W. Cummins. 1996. An introduction to the aquatic insects of North America. Merritt RW, Cummins KW, edn. Dubuque. IA: Kendall/Hunt Publishing Co. 862 p.

Parker C., and J. Voshell. 1983. Production of filter-feeding trichoptera in an impounded and a free-flowing river. Canadian Journal of Zoology 61(1):70-87.

Patrick R., and C. W. Reimer. 1975. The diatoms of the United States, exclusive of Alaska and Hawaii. Vol. 2, part 1. Monographs of the Academy of Natural Sciences of Philadelphia. 13. Acad. Nat. Sci. Phila..

Perry SA. 1984. Comparative ecology of benthic communities in natural and regulated areas of Flathead and Kootenai Rivers, Montana. Denton, TX: North Texas State University.

Perry SA, and J. E. Huston. 1983. Aquatic insect study: October, 1979 through June, 1982. US Army Corps of Engineers.

Perry S.A., and W. Perry. 1986. Effects of experimental flow regulation on invertebrate drift and stranding in the Flathead and Kootenai Rivers, Montana, USA. Hydrobiologia 134:171-182.

Perry S.A., W. B. Perry, and J. A. Stanford. 1986. Effects of stream regulation on density, growth, and emergence of two mayflies (Ephemeroptera: ephemerellidae) and a caddisfly (Trichoptera: hydropsychidae) in two rocky mountain rivers (U.S.A.). Canadian Journal of Zoology 64(3):656-666.

Southwood, T.R.E. 1991. Ecological methods with particular reference to the study of insect populations, 2nd edn. Chapman and Hall, New York, NY.

Stewart K. W., and B. P. Stark. 2002. Nymphs of North American stonefly genera (Plecoptera). 2nd Ed. The Caddis Press. Columbus, Ohio. xii + 510p.

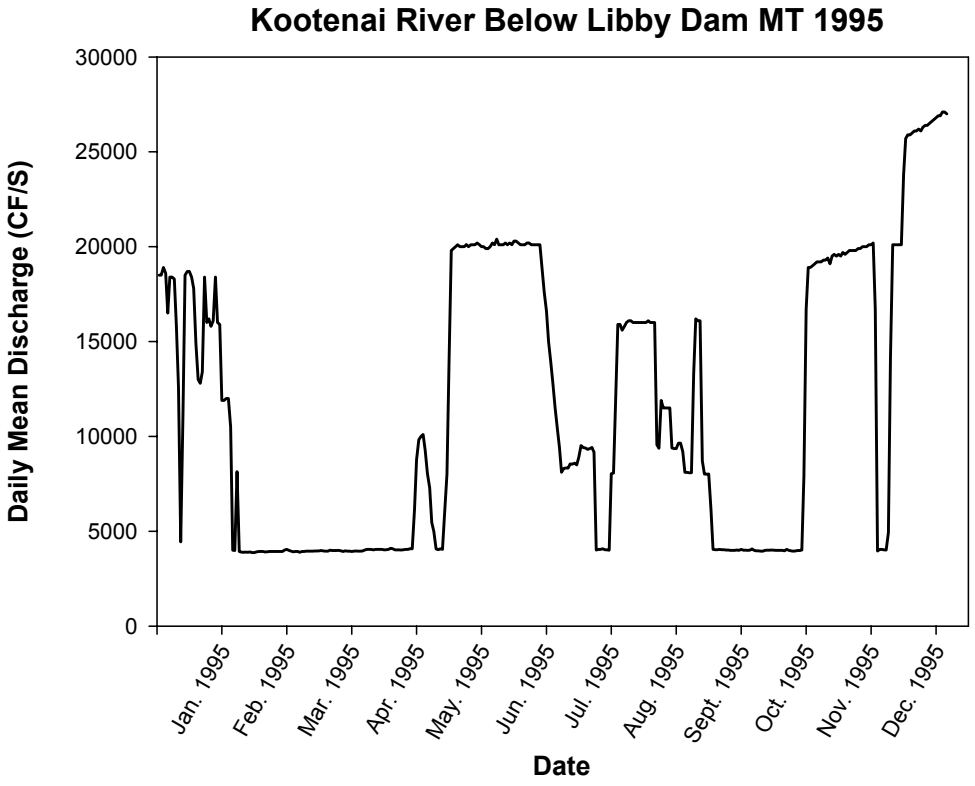
Snyder E., and G. Minshall. 1996. Ecosystem metabolism and nutrient dynamics in the Kootenai River in relation to impoundment and flow enhancement for fisheries management. Final Report. Stream Ecology Center, Idaho State University, Pocatello, Idaho.

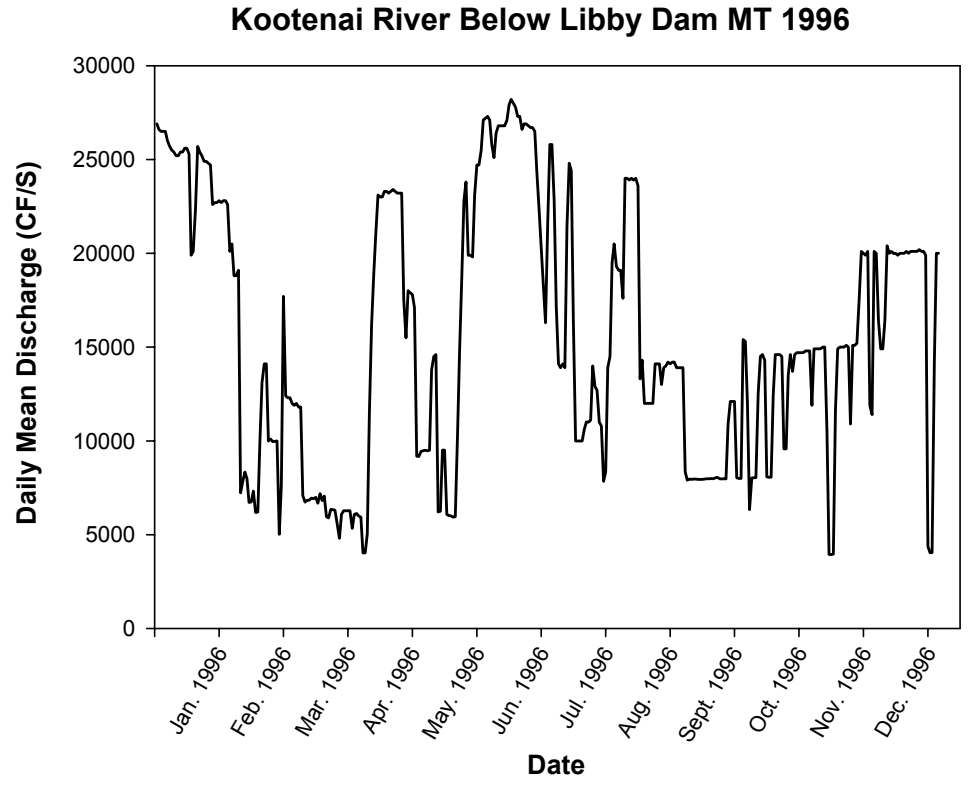
Thorp J.H., and A. P. Covich. 2001. Ecology and Classification of North American Freshwater Invertebrates. Thorp JH, Covich AP, editors: Academic Press. 1-992 p.

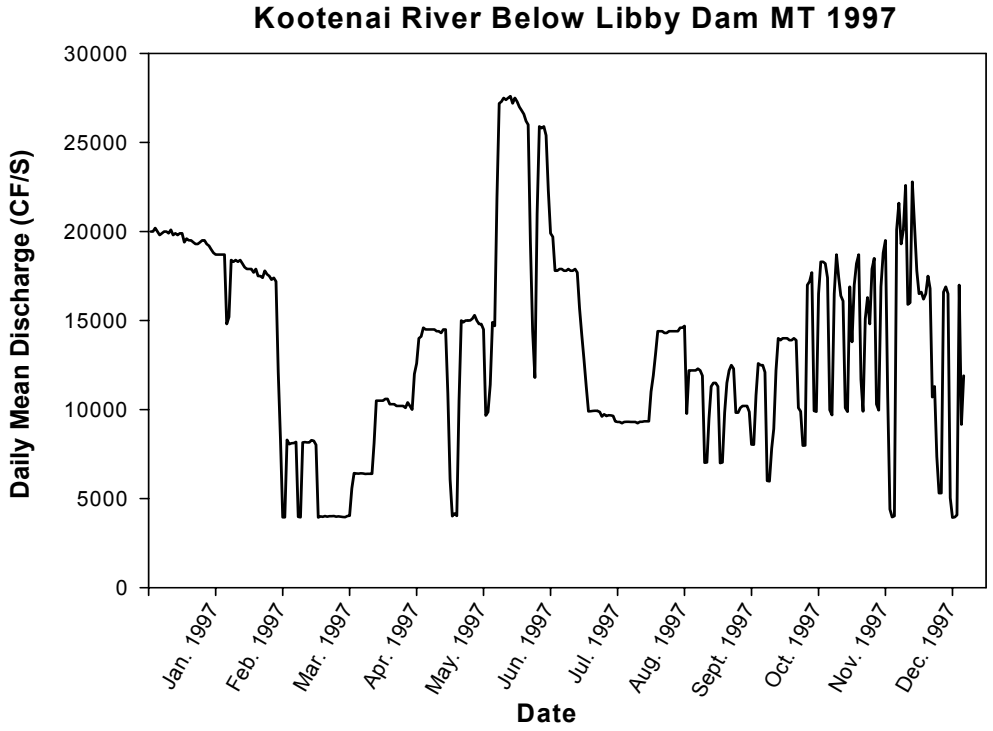
Weatherhead E.C., and S. B. Andersen. 2006. The search for signs of recovery of the ozone layer. Nature 441(4):39-45.

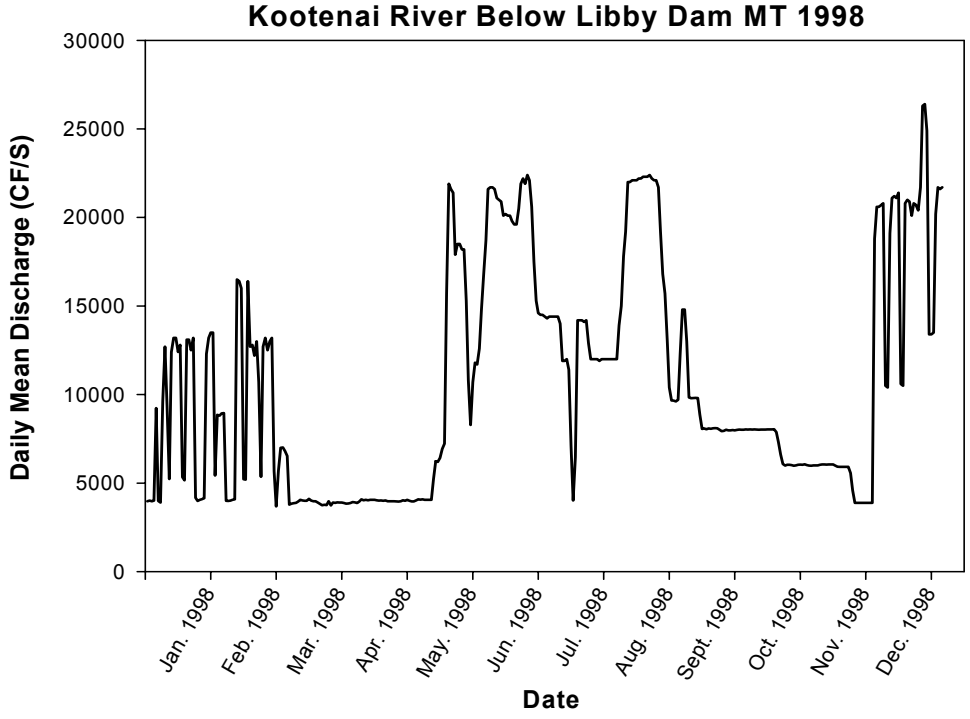
Wiggins, G.B. 1998. Larvae of the North American caddisfly genera (trichoptera), 2nd edn. University of Toronto Press, Inc., Toronto, Canada.

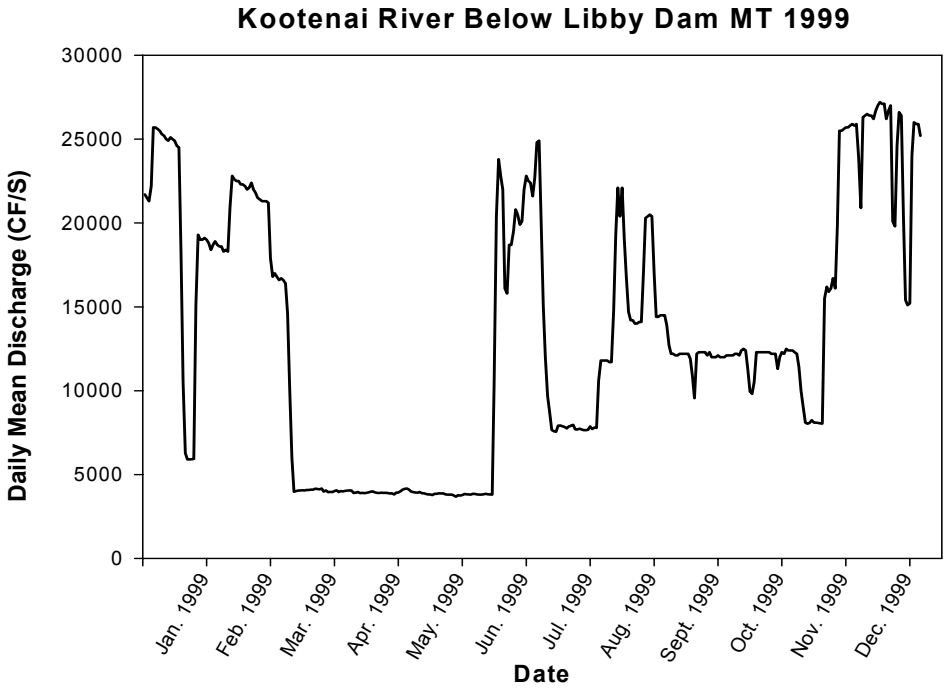
Appendix 1. Libby Dam Hydrographs since Hauer and Stanford (1997).

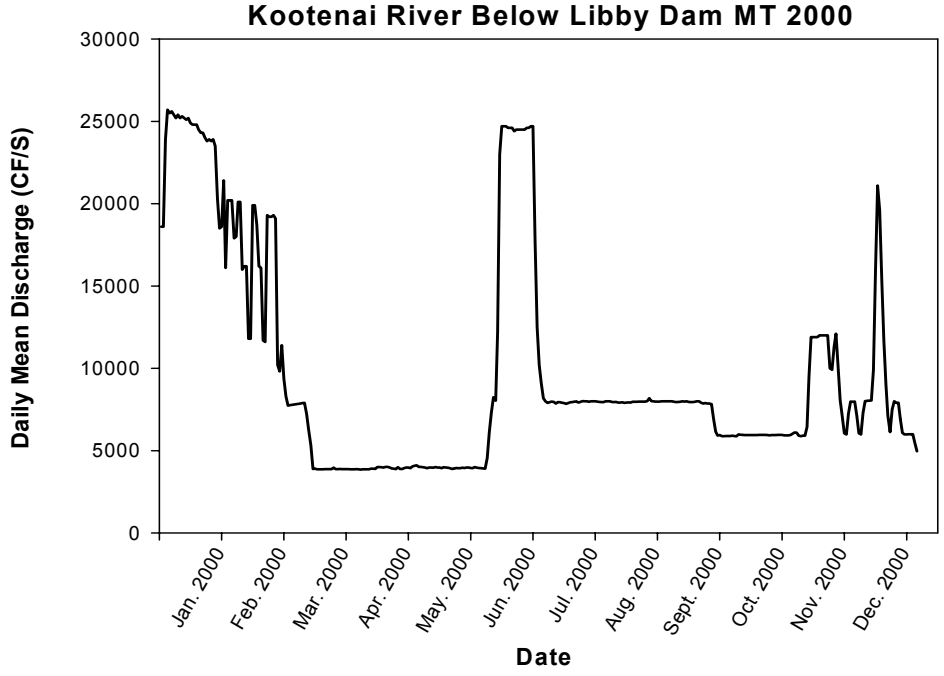


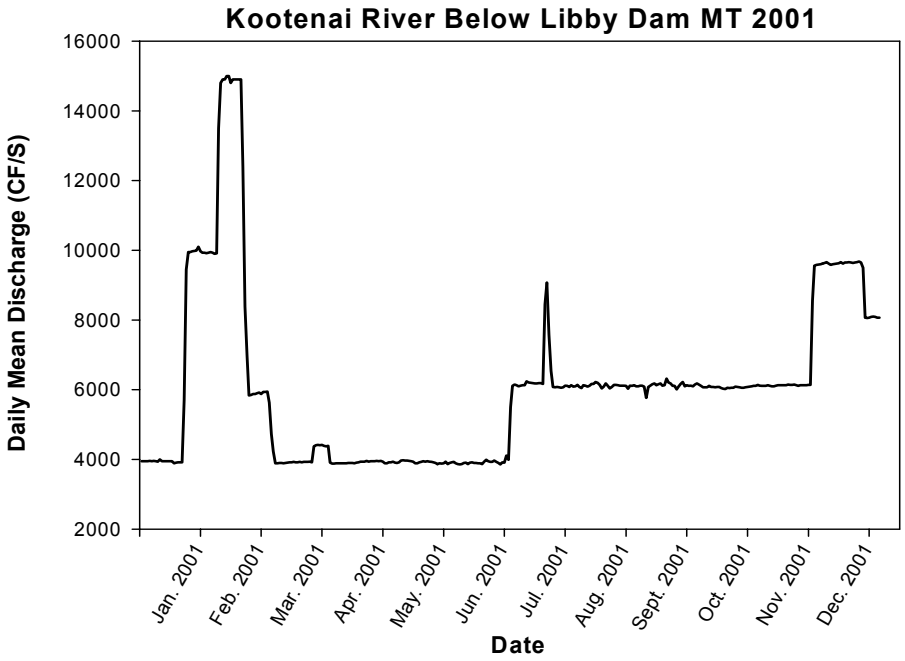


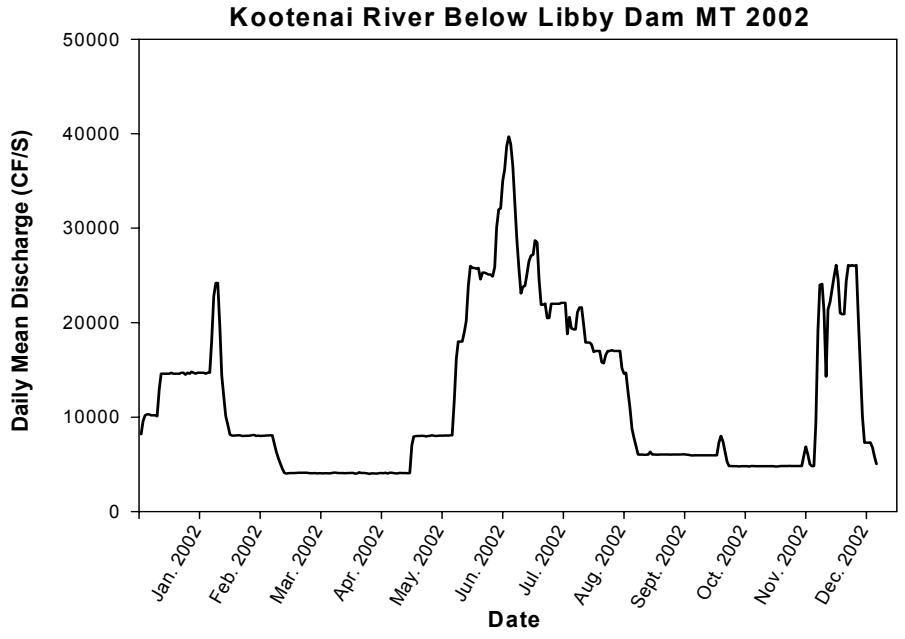

Kootenai River Below Libby Dam MT 1997
Daily Mean Discharge (CF/S)
30000
25000
20000
15000
10000
5000
0
Jan. 1997
Feb. 1997
Mar. 1997
Apr. 1997
May. 1997
Jun. 1997
Jul. 1997
Aug. 1997
Sept. 1997
Oct. 1997
Nov. 1997
Dec. 1997
Date

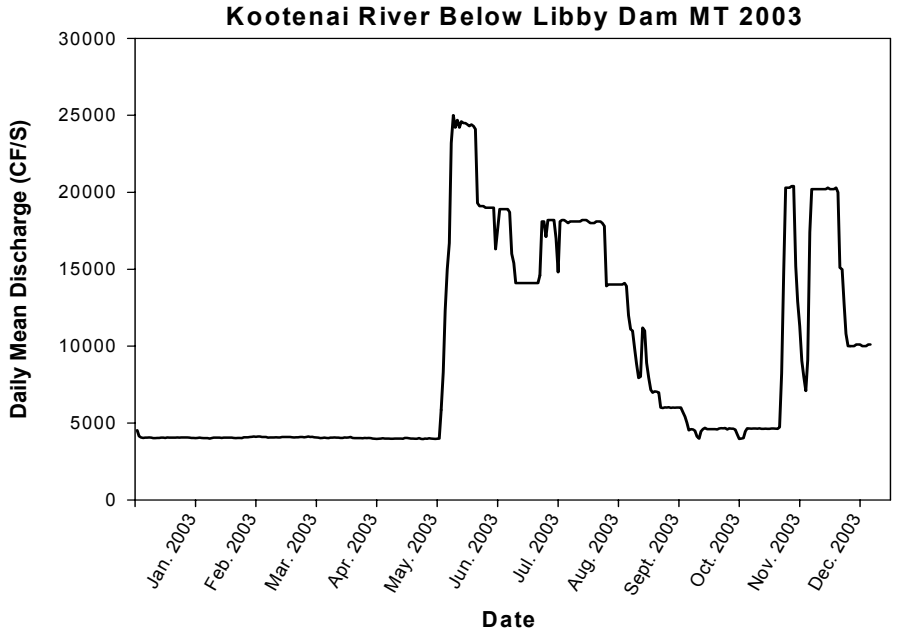

Kootenai River Below Libby Dam MT 1998
Daily Mean Discharge (CF/S)
30000
25000
20000
15000
10000
5000
0
Jan. 1998
Feb. 1998
Mar. 1998
Apr. 1998
May. 1998
Jun. 1998
Jul. 1998
Aug. 1998
Sept. 1998
Oct. 1998
Nov. 1998
Dec. 1998
Date

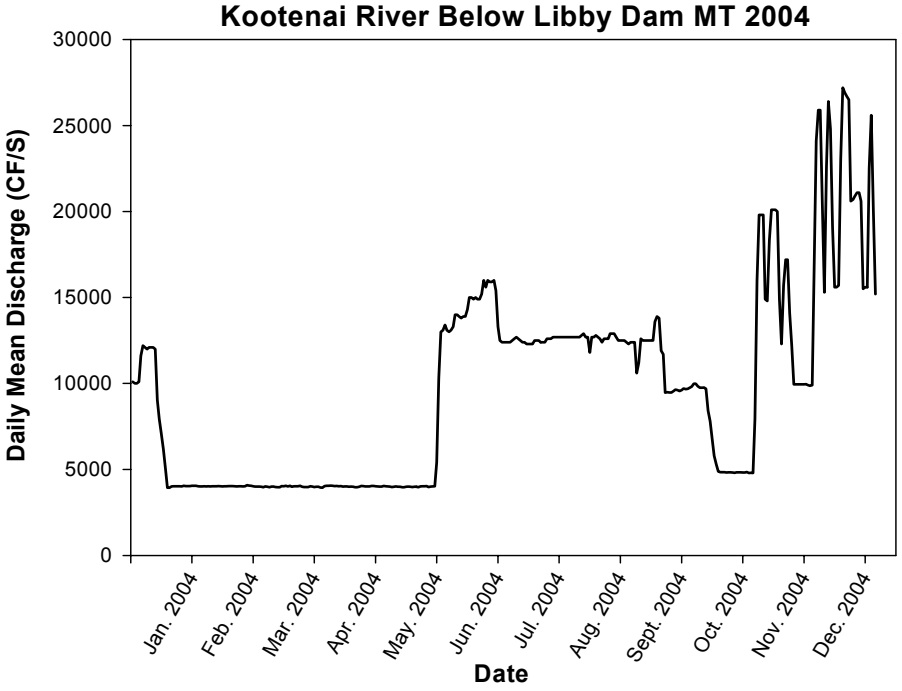

Kootenai River Below Libby Dam MT 1999
Daily Mean Discharge (CF/S)
30000
25000
20000
15000
10000
5000
0
Jan. 1999
Feb. 1999
Mar. 1999
Apr. 1999
May. 1999
Jun. 1999
Jul. 1999
Aug. 1999
Sept. 1999
Oct. 1999
Nov. 1999
Dec. 1999
Date

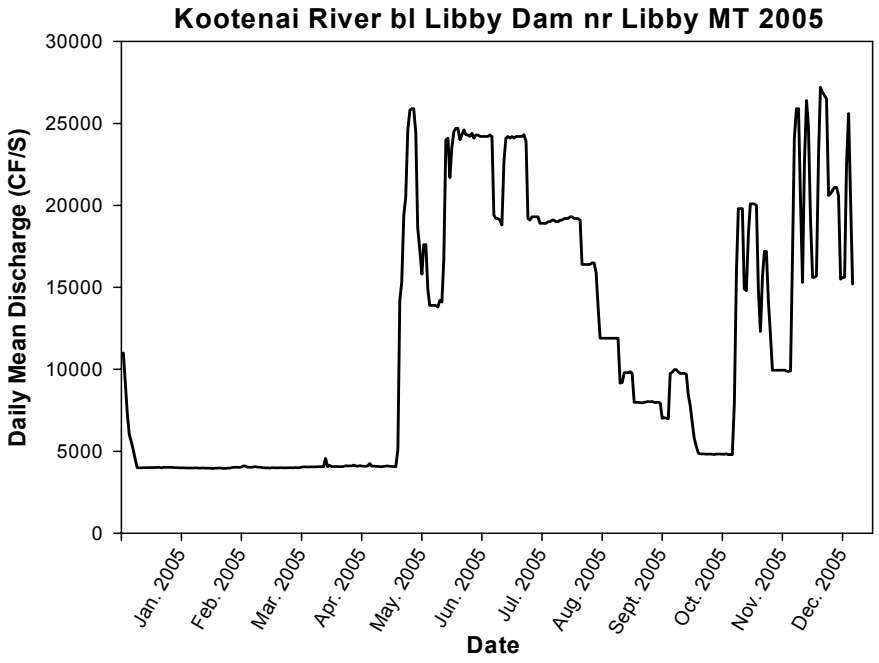

Kootenai River Below Libby Dam MT 2000
Daily Mean Discharge (CF/S)
30000
25000
20000
15000
10000
5000
0
Jan. 2000
Feb. 2000
Mar. 2000
Apr. 2000
May. 2000
Jun. 2000
Jul. 2000
Aug. 2000
Sept. 2000
Oct. 2000
Nov. 2000
Dec. 2000
Date


Kootenai River Below Libby Dam MT 2001
Daily Mean Discharge (CF/S)
16000
14000
12000
10000
8000
6000
4000
2000
Jan. 2001
Feb. 2001
Mar. 2001
Apr. 2001
May. 2001
Jun. 2001
Jul. 2001
Aug. 2001
Sept. 2001
Oct. 2001
Nov. 2001
Dec. 2001
Date


Kootenai River Below Libby Dam MT 2002
Daily Mean Discharge (CF/S)
50000
40000
30000
20000
10000
0
Jan. 2002
Feb. 2002
Mar. 2002
Apr. 2002
May. 2002
Jun. 2002
Jul. 2002
Aug. 2002
Sept. 2002
Oct. 2002
Nov. 2002
Dec. 2002
Date


Kootenai River Below Libby Dam MT 2003
Daily Mean Discharge (CF/S)
30000
25000
20000
15000
10000
5000
0
Jan. 2003
Feb. 2003
Mar. 2003
Apr. 2003
May. 2003
Jun. 2003
Jul. 2003
Aug. 2003
Sept. 2003
Oct. 2003
Nov. 2003
Dec. 2003
Date


Kootenai River Below Libby Dam MT 2004
Daily Mean Discharge (CF/S)
30000
25000
20000
15000
10000
5000
0
Jan. 2004
Feb. 2004
Mar. 2004
Apr. 2004
May. 2004
Jun. 2004
Jul. 2004
Aug. 2004
Sept. 2004
Oct. 2004
Nov. 2004
Dec. 2004
Date


Kootenai River bl Libby Dam nr Libby MT 2005
Daily Mean Discharge (CF/S)
30000
25000
20000
15000
10000
5000
0
Jan. 2005
Feb. 2005
Mar. 2005
Apr. 2005
May. 2005
Jun. 2005
Jul. 2005
Aug. 2005
Sept. 2005
Oct. 2005
Nov. 2005
Dec. 2005
Date