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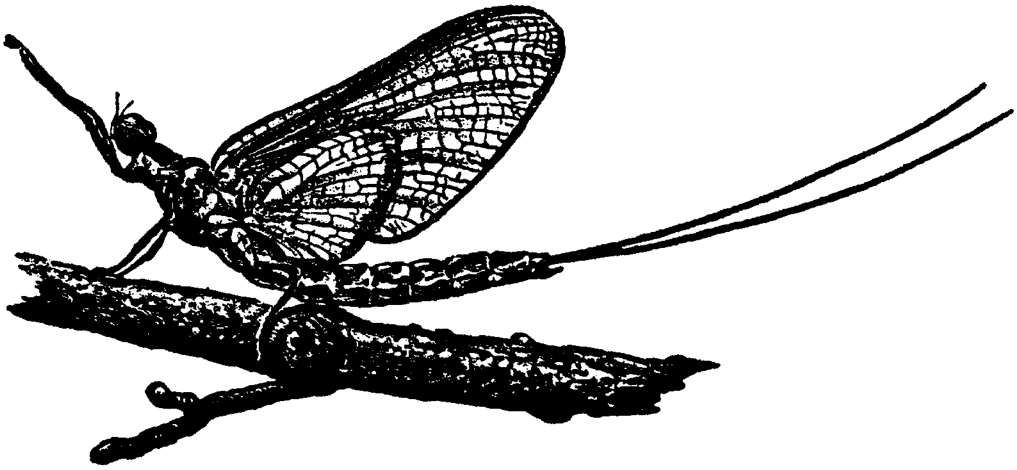

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COMPARISON OF PREDICTED HABITAT CHANGE AND BENTHIC MACROINVERTEBRATE RESPONSE TO A SIMULATED IRRIGATION WITHDRAWAL IN HUNT CREEK, MICHIGAN

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ABSTRACT

We diverted approximately 50% of the summer stream flow from a 0.7 km section of Hunt Creek from June 1-August 31, 1992-94 to simulate the impacts of flow withdrawal for irrigation on the benthic macroinvertebrate assemblage. We also simulated the impacts of the withdrawal on the benthic macroinvertebrate habitat in the treatment section by use of the Physical Habitat Simulation System (PHABSIM), and compared the changes in habitat with observed densities of benthic macroinvertebrates in the treated section of Hunt Creek. We developed habitat suitability criteria (HSC) from benthic macroinvertebrate samples collected in the treated section of Hunt Creek. The withdrawal of 50% did not decrease the benthic macroinvertebrate habitat of most of the taxa examined, but did reduce habitat of riffle dwelling taxa (e.g. Heptageniidae) by up to 38%. The total density of benthic macroinvertebrates in the treatment section of Hunt Creek did not change as a result of the reduced flow in relation to the total density of benthic macroinvertebrates in a control section. However, the densities of Heptageniidae in a riffle sampled in 1994 did decrease in relation to a control riffle ($p=0.05$), indicating that reduced flow may have resulted in a reduction of Heptageniidae density.

Introduction

The Physical Habitat Simulation System (PHABSIM) is the computer based habitat modeling component of the Instream Flow Incremental Methodology (IFIM) developed by the U.S. Fish and Wildlife Service that models stream physical habitat as a function of discharge (Milhous et al. 1989). PHABSIM is widely used to evaluate the impact of altered flow regimes on stream fish habitat (Reiser et al. 1989) with the assumption that stream physical habitat is directly related to stream fish population abundance or biomass (Figure 1) (Bovee 1978, Orth and Maughan 1982, Mathur et al. 1985). PHABSIM is widely used in the western United States for fish habitat evaluations (Reiser et al. 1989), but has not been as widely used for modeling stream benthic macroinvertebrate habitat. Also, the PHABSIM system has not been widely applied in the midwestern United States where the geology, hydrology, and faunal composition are different from western streams.

In addition to evaluating effects of reduced streamflows on fish populations, it is necessary to determine impacts on benthic macroinvertebrates because benthic macroinvertebrates are the primary source of food for fishes, particularly game species such as the trouts and charrs (Elliott 1973, Alexander and Gowing 1976, Allan 1981, Bechara, Morceau and Planas 1992, Nielsen 1992). In many trout streams the density of benthic macroinvertebrates and the occurrence of macroinvertebrates in the drift may limit the growth rate of individual fish or may limit the population size (Chapman 1966). Orth (1987) also argued for consideration of ecological factors other than space occupied by fish alone when evaluating the impacts of altered streamflows, yet most PHABSIM analyses of altered streamflows are centered on the changes in game fish habitat. We are only aware of one published study which applied the PHABSIM system to benthic macroinvertebrates. Bovee (1985) studied the impacts of a peaking hydropower operation on the benthic macroinvertebrate habitat in a Colorado stream, but no comparison was made between the output of the PHABSIM modeling and observed benthic macroinvertebrate abundance. Gowan (1984) also modeled the habitat of two genera of macroinvertebrates in a marginal trout stream in Michigan and determined that withdrawals for irrigation reduced habitat by up to 11%, but that fish habitat losses were more severe. However, he did not compare PHABSIM model output with observed benthic macroinvertebrate densities.

Several studies have documented benthic macroinvertebrate habitat use patterns and have published habitat suitability criteria (HSC) which could be used in a PHABSIM analysis (Gore and Judy 1981, Orth and Maughan 1983, Gore 1989). However, the availability of these data have not led to an increase in the use of benthic macroinvertebrates in predicting impacts of flow regulation in streams.

Very little is known about the relationships between natural streamflow patterns and benthic macroinvertebrate communities and abundance. Ward (1976) reviewed the impacts of regulated streamflows on the benthos below large dams and noted that streamflow regulation can result in changes in community composition and enhancement or reduction of standing crop, depending on flow regime. Others (Poff and Ward 1991, Minshall and Winger 1968, Corrarino and Brusven 1983) have documented the impacts of altered streamflows on invertebrate drift, noting that altered streamflows can result in increased drift rates. Current velocity (Rabeni and Minshall 1977, Orth and Maughan 1983, Degani et al. 1993), depth (Degani et al. 1993) and substrate composition (Minshall 1984) are important factors influencing the distribution and abundance of benthic macroinvertebrates. Of these three factors, depth and velocity will change as streamflow is altered. Also, substrates may become embedded with fines if velocity is sufficiently reduced.

Therefore, altered streamflows can be expected to have an influence on benthic macroinvertebrate communities.

Here we present the results of a PHABSIM analysis of benthic macroinvertebrate habitat in a Michigan stream during a simulated irrigation withdrawal. The objectives were to determine the impacts of a simulated irrigation withdrawal on the benthic macroinvertebrates in Hunt Creek, and to evaluate the PHABSIM model as a quantitative predictor of the changes in the benthic macroinvertebrate assemblage resulting from the altered streamflow.

Methods

We examined macroinvertebrate habitat and populations in two sections of Hunt Creek: one nontreatment section (section C) and a treatment section (section B; Figure 1). Prior to sampling macroinvertebrates and macroinvertebrate habitat we measured and marked sections B and C (Figure 1) into approximately 50 m contiguous reaches, and omitted the small area of impounded water at the downstream end of sections B and C and the disturbed habitat at the upstream end of section B immediately below the bulkhead. We were left with four 50 m reaches in section C and seven reaches in section B.

We sampled benthic macroinvertebrates from randomly selected locations in sections B and C during May-September, 1992 and April-September, 1993 to construct habitat suitability criteria (HSC). We moved upstream through the sections and collected 20 samples each month in each section from randomly selected locations. We sampled macroinvertebrates using a modified Hess sampler or a petite Ponar grab in water that was either too deep or too shallow to sample with the Hess sampler. The net on the Hess sampler was constructed of 500 mm mesh and the area sampled by the Hess sampler was 0.023 m^2. The Ponar grab sampled 0.026 m^2. We preserved benthic samples in the field in 95% ethyl alcohol. At each sample location, we also measured depth to the nearest cm, water velocity to the nearest $cm \cdot s^{-1}$, and visually estimated dominant substrate composition using the codes in Table 1. We measured water velocity with either a mechanical pygmy Gurley or an electronic Marsh-McBirney current meter. We compared velocity measurements between the two meters in Hunt Creek on several occasions by measuring velocity at specific points in the stream with both meters. We found no consistent differences in measurements of velocity between the two meters and velocity measurements were always in close agreement.

We separated macroinvertebrates from inorganic material in the samples by floating the sample contents in a saturated sugar solution (Anderson 1959). We also thoroughly sorted a second time through 20 samples from a variety of habitats (depositional, erosional) after they were sorted once to evaluate the effectiveness of the initial sorting.

We identified macroinvertebrates to family using the keys in Merritt and Cummins (1984) and Pennak (1989). We counted the number of organisms by family in the samples and converted these to density estimates by dividing by the sampled area.

We constructed HSC from the combined data collected in 1992-93 in section B using the nonparametric tolerance limits method (Bovee 1986). HSC were constructed from habitat use data by use of the formula:

$$NSI=2(1-P), \qquad (1)$$

where NSI is the normalized suitability index and P is the central proportion of the data frequency distribution (Bovee 1986). We constructed the HSC for depth and mean column velocity with this approach by defining P as the central 50, 75, 90, and 95% portions of the distribution by use of the nonparametric tolerance limits table found in Somerville (1958) and a confidence level of 95%. We constructed the HSC for substrate by normalizing the frequency of use data for each of the substrate categories. We normalized the substrate data by dividing the frequency of use data in each category by the frequency for the most commonly used substrate (Bovee 1986). We combined the substrate data by largest particle size and ignored the percent embeddeded classification because sample sizes were small for some of the substrate categories. Because We were concerned about statistical independence of the observations, We did not weight the value of the habitat measured at a sample location by the number of organisms in the sample. This resulted in HSC constructed from presence-absence data only. We also did not correct the HSC based on habitat availability because we randomly selected sample sites and therefore sampled all habitat types in approximate proportion to their availability.

We used a representative reach approach for modeling the habitat in section B of Hunt Creek with PHABSIM. We randomly selected two of the 50 m reaches in section B to model by use of PHABSIM (reaches B2 and B4 in Figure 1). We established transect locations in each of the reaches, and used changes in meso habitat (riffle, run, pool) within the reach to guide transect placement. We classified substrate along each transect by use of the same codes used for the benthic macroinvertabrate habitat use observations (Table 1). We collected flow data in the two reaches in section B at three discharges; 0.46, 0.23, and 0.11 $m^3 \cdot s^{-1}$. We measured depths to the nearest cm with a wading rod and velocities to the nearest

$cm \cdot s^{-1}$ with either a mechanical Pygmy-Gurley or an electronic March-McBirney current meter. We calibrated the PHABSIM model and simulated benthic macroinvertebrate habitat for selected families over a range of flows from summer baseflow to 0.01 $m^3 \cdot s^{-1}$ (2% of baseflow) for the reaches in sections B. We selected families based on their frequency of occurrence in the 1992-93 samples and based on habitat use characteristics. We modeled habitat for macroinvertebrate families that occurred in 20-80% of the samples in 1992-93 and selected additional families to provide data for habitat types that were not sufficiently represented in the initial selection procedure.

Because early results of the PHABSIM modeling indicated that riffle dwelling macroinvertebrates (e.g. Hydropsychidae) were more likely to be impacted by the reduction in flow than macroinvertebrates found more commonly in pool or depositional habitats, We altered the macroinvertebrate sampling design during the final year of the study. Prior to the withdrawal period in 1994 we selected two riffles, one in section C and one in section B, that had similar microhabitat characteristics under baseflow conditions. The riffle selected in section B was approximately 25 m upstream of the upper end of modeled reach B2. The depths, mean column velocities, and substrate characteristics of the selected riffle were very similar to those found in the riffle habitats in reach B2. We measured the width and length of each of the selected riffles and established a two dimensional grid of cells using permanent markers in the stream bank. The cells were approximately the same size as the area sampled by the Hess sampler. We then collected benthic samples in seven randomly selected cells in each riffle at three week intervals from May 12-August 23. We used the same sampling protocol as for the 1992-93 samples.

We formulated hypotheses concerning the impacts of the reduced streamflow on the density of benthic macroinvertebrate families in section B of Hunt Creek in 1992 and 1993 based on the relation between WUA and discharge curves and the assumption that WUA is positively and linearly related to macroinvertebrate abundance (Bovee 1978, Orth and Maughan 1982, Mathur et al. 1985). We used profile analysis (Morrison 1990) to compare the total benthic macroinvertebrate density trends between sections B and C during the summer for the 1992 data and used Before After Control Impact (BACI) analysis (Stewart-Oaten et al. 1986, 1992) to evaluate the predictions of the PHABSIM model for the 1993 data. For the BACI analysis of the 1993 and 1994 macroinvertebrate data, we determined the mean pretreatment difference between sections B and C from the benthic samples collected prior to 1 June and compared that mean difference to the mean difference between the sections from the samples collected after 1 June. Because the riffle sampled in 1994 in section B was close to reach B2 and was similar to the riffles in reach B2, we evaluated the relation between WUA and discharge for riffle transects in reach B2 to formulate hypotheses concerning the impacts of the withdrawal on the macroinvertebrate assemblage in the sampled riffle. We evaluated hypotheses of withdrawal impacts on the total macroinvertebrate density and on the densities of the most abundant families in 1994 by use of BACI statistics by again comparing the pretreatment mean difference to the treatment mean difference.

We also evaluated the impacts of reduced flow on the macroinvertebrate drift density in Hunt Creek. We collected macroinvertebrate drift data across a fixed transect in the treatment and the control sections of Hunt Creek. We sampled the drift by use of rectangular drift nets with a mouth opening 75x15.5 cm and a net constructed from 64 mm mesh nylon netting. Three nets were set across the transect which bisected a riffle, and the nets sampled the entire water column. We sampled the invertebrate drift for 20 minutes at four-hour intervals over 24 hours approximately every four weeks during summer, 1993 in section C and sampled the drift in section B on approximately the same dates but for up to 48 hours, depending on the withdrawal schedule. We measured depth and velocity at each net location immediately prior to sampling and used these data to estimate the volume sampled in the 20 minute period. We sorted the drift samples in a saturated sugar solution immediately after collection and preserved macroinvertebrates in 95% ethyl alcohol as they were retrieved from the samples. We thoroughly sorted through 15 samples a second time to determine the effectiveness of the procedure. We calculated total number of invertebrates in the drift per m^3 for each sample and determined the mean number of invertebrates per m^3 for each sample date and time. We used BACI analysis to evaluate the impacts of the withdrawal on the macroinvertebrate drift densities by comparing pretreatment mean difference in drift density between section B and C for the 24 hour period prior to the withdrawal on June 2 with the treatment period mean differences for the remaining sample dates.

Results

Benthic Macroinvertebrate Assemblage

We collected 199 benthic samples in 1992 and 237 benthic samples during 1993. We sorted 26 benthic samples a second time after sugar floating and determined We were retrieving 88% (range 69-100%) of the macroinvertebrates from the samples with the sugar floating procedure. Because we retrieved nearly all invertebrates from the samples we did not adjust the sample data by the efficiency of the initial sorting procedure. The benthic samples contained macroinvertebrates from 45 families representing 13 orders (Table 2). We collected 83 benthic samples in 1994, 42 from the riffle in section B and 41 from the riffle in section C. The 1994 samples contained macroinvertebrates from 29 families representing 11 orders (Table 2). Oligochaetes, baetids, gammarids, elmid larvae and chironomids were the most frequently occurring taxa in 1992-93, when samples were collected at random locations throughout sections B and C. Oligochaetes were the most frequently collected taxa in the 1994 samples in sections B and C. Other frequently occurring taxa in 1994 were baetids, heptageniids, glossosomatids, chironomids, elmid larvae, and gammarids (Table 2).

We selected 13 families of macroinvertebrates for habitat modeling using PHABSIM (Table 2). In addition to the families selected based on frequency of occurrence, we selected the Tipulidae (present in 16.3% of the 1992-93 samples) to provide an additional taxon that occurs primarily in pool or depositional habitats. We also excluded the Limnephilidae because the genera of limnephilids found in Hunt Creek included both scrapers (*Goera* sp.) and shredders-herbivores (*Limnephilus* sp.) which occupy different habitats (Merritt and Cummins 1984). The majority of the taxa selected were found primarily in riffles (Table 2) and the remaining taxa were habitat generalists found primarily in runs and depositional habitats but also in riffles.

Habitat suitability criteria (HSC) for depth were very similar for all the taxa irrespective of the habitat type selected most frequently (Table 3). The narrowest optimal (suitability=1.0) depth range was from 16-31 cm for the Hydropsychidae and the widest optimal depth range was from 13-33 cm for the Ceratopogonidae. The range of usable (0.0<suitability<1.0) depths was also very similar between taxa and was also similar to the range of depths sampled in 1992-93. The minimum usable depth was 2 cm (Ceratopogonidae) and the maximum usable depth was 61 cm (6 taxa, Table 3). The depth HSC did not differ between taxa that primarily occupied riffles in comparison to taxa that primarily occupied pool or depositional habitats (Figure 2). For example, the optimal depth range for Hydropsychidae (riffles) was from 16-31 cm and was from 15-35 cm for Tipulidae and 13-33 cm for Ceratopogonidae, both found primarily in depositional areas (Table 3).

Although there was considerable overlap in usable mean column velocities between the taxa, mean column velocity HSC were less similar between taxa than depth HSC and were better indicators of the habitat types selected by the taxa (Table 3). For example, the optimal velocity range for Hydropsychidae was 34-61 $cm \cdot s^{-1}$ and 24-58 $cm \cdot s^{-1}$ for Tipulidae (Figure 3). Usable velocity ranges also overlapped considerably for the taxa selected for habitat modeling. The maximum usable velocity was between 71-81 $cm \cdot s^{-1}$. The minimum usable velocity was 0 $cm \cdot s^{-1}$ for most taxa but was as high as 4 $cm \cdot s^{-1}$ for the Simuliidae and the Hydropsychidae, both filter feeding taxa (Merritt and Cummins 1984).

Substrate HSC also reflected the habitat selectivity of the taxa (Table 4, Figure 4). The optimal substrate size (medium gravel) was the same for all the taxa selected for modeling. However, this undoubtedly reflects the fact that medium gravel was the most frequently sampled substrate during 1992-93. Large gravel was the most suitable substrate after medium gravel for all taxa except Ceratopogonidae (Table 4). Sand and silt in combination and sand alone were the second most suitable substrates for the ceratopogonids.

PHABSIM Model Results

The 50% reduction in summer stream flow in the treatment section resulted in a very minor loss of stream surface area in both modeled reaches (Figure 5). In reach B2 total surface area decreased from 206 to 195 $m^2 \cdot 100\ m^{-1}$, a reduction of only 5.6%. In reach B4, total surface area decreased from 292 to 275 $m^2 \cdot 100\ m^{-1}$, a reduction of only 5.7%. Model results indicated that reducing flow in section B to a discharge of 0.01 $m^3 \cdot s^{-1}$ would reduce total surface area to 132 $m^2 \cdot 100\ m^{-1}$ in reach B2 (35.9% loss in surface area) and 176 $m^2 \cdot 100\ m^{-1}$ in reach B4 (39.7% loss of surface area). This result suggests that considerable pool habitat would remain in section B if discharge was reduced to 0.01 $m^3 \cdot s^{-1}$. The difference in the total surface area estimates between the two modeled reaches is due to differences in channel width.

The reduction of summer stream flow by 50% increased WUA for all but three of the taxa in reach B2 (Heptageniidae, Rhyacophilidae, and Elmidae adults, Table 5). The increases in WUA ranged from 1% to 22% (Perlodidae and Tipulidae, respectively). In general, the taxa that were most commonly found in pool and depositional habitats had the largest increases in WUA. The Heptageniidae, Rhyacophilidae, and Elmidae adults all had reduced WUA as a result of the 50% reduction in summer stream flow, although the losses were minor (4-15%). In contrast, WUA decreased in reach B4 for all taxa except Ceratopogonidae when summer stream flow was reduced 50%. The WUA estimate at 50% reduced flow for ceratopogonids remained virtually unchanged (from 279.8 $m^2 \cdot 100\ m^{-1}$ to 280.3 $m^2 \cdot 100\ m^{-1}$) (Table 5). For the remaining taxa, WUA decreased between 9% and 38% (Nemouridae and Heptageniidae, respectively). The WUA estimates for taxa most commonly found in pool and depositional habitats decreased less than for riffle dwelling taxa (e.g. Figure 6).

Reducing flow to less than 50% of baseflow during the summer months would result in reduced WUA for all taxa modeled (Table 5). Also, the model predicted that reducing flow to 2% of baseflow (0.01 $m^3 \cdot s^{-1}$) would reduce WUA between 51-96% in reach B2 (Ceratopogonidae larvae and Elmidae adults respectively) and 61-99% in reach B4 Ceratopogonidae and Simuliidae larvae/Elmidae adults respectively).

Based on the WUA curves for reaches B2 and B4 we hypothesized that the 50% reduction in flow should not have resulted in a decrease in total benthic macroinvertebrate density in section B in 1992 or 1993. The profile analysis of the 1992 density data support this hypothesis because the test indicated the trends in total macroinvertebrate densities (Table 6, Figure 7) were not different between the sections ($F=0.30$, $df=4,194$, $p<0.001$). Similarly, the results of the BACI analysis of the 1993 data (Table 6, Figure 8) support the conclusion that the withdrawal did not reduce total macroinvertebrate density in section B (Student's t test, $p=0.84$).

The relation between WUA and discharge for the riffle transects in reach B2 were virtually unchanged by excluding the run and pool habitats, although the magnitude of the WUA estimates at any one discharge did decrease. Therefore, we hypothesized that the 50% reduction in summer stream flow would have no impact on the abundance of any of the benthic macroinvertebrate taxa in the sampled riffle in section B during summer, 1994. We tested these hypotheses on the taxa for which we modeled habitat and also on the Gammaridae, Chironomidae, and Elmidae larvae. The 50% reduction in summer stream flow did not impact the abundance of the families tested nor did it impact the total macroinvertebrate abundance estimates in section B (Table 7). Total macroinvertebrate density in section B did not decline as a result of the withdrawal and the trend in total density through the summer closely matched the trend in section C (Table 7). The only instance in which the trends in total macroinvertebrate numbers departed was between the samples collected on July 12 when the total numbers in section C declined slightly from the previous sample whereas the total number in section B on July 12 increased over the previous sample period (Figure 9).

The only taxa-specific BACI test that produced a significant result was for the Heptageniidae (Table 8). However, it is not clear whether the difference between pretreatment and treatment mean differences was due to the reduced flow because the densities of Heptageniidae in both sections B and C were declining prior to the withdrawal period and continued to decline through the summer. However, the rate of decline in Heptageniidae density was faster in section B before the withdrawal was initiated and continued at a faster rate through most of the summer, although the densities of Heptageniidae in sections B and C were virtually identical at the time the final sample was collected (Table 7). This difference in rates of decline between the sections resulted in the sign and magnitude of the treatment mean difference changing after the withdrawal was initiated. We also was unable to detect a mean differences for the three most abundant taxa (Gammaridae, Chironomidae, and Elmidae larvae, Table 8).

Invertebrate Drift

Invertebrate drift density (individuals$\cdot$m^{-3}) was elevated in section B immediately following the initiation of each water withdrawal event (June 2 and August 22) but returned to prewithdrawal levels within 24 hours (Figures 10-12). The elevated drift densities on June 2 were not significantly different from pretreatment levels ($t=-1.39$, $df=10$, $p=0.19$) but the elevated densities on August 22 were significantly different ($t=-2.98$, $df=10$, $p=0.02$). Also, total drift densities were similar between sections B and C during the July and early August sampling.

Discussion

Total benthic macroinvertebrate density in Hunt Creek did not decline as a result of the 50% reduction in summer stream flow in the treatment section. Further, there is no evidence for an impact on any of the individual families examined except, perhaps, the Heptageniidae (Table 7). The significance of the test result for Heptageniidae density may be due to chance alone because we did not adjust p values for multiple comparisons as suggested by Smith et al. (1993). The families we selected for habitat modeling and the additional three families selected for BACI analysis represented 93% of the benthic macroinvertebrate fauna in Hunt Creek. It is unlikely that any of the remaining taxa were impacted by the reduced flow. This result is consistent with the observation that the withdrawal reduced the total surface area of the stream less than 6%. We recognize that we probably did not adequately sample the first and second instars of many of the taxa because of the mesh size used on the Hess sampler. However, it is unlikely that sampling the early instars would have influenced the results of the study because the model predictions matched the observed trends in density of the macroinvertebrates.

The PHABSIM model indicated that reducing stream flow more than 50% would reduce WUA in both reaches for all taxa and that WUA losses would be substantial. A PHABSIM analysis of brook trout habitat in section B of Hunt Creek during the same period indicated that brook trout WUA would not be substantially reduced by reducing the streamflow until the discharge was equal to approximately 20% of baseflow, or to 0.09 $m^3 \cdot s^{-1}$ (Baker and Coon 1995). These results indicated that brook trout food resources may be impacted by a lesser reduction in flow, one that would not be sufficient to reduce brook trout foraging or resting habitat. The benthic macroinvertebrates in Hunt Creek comprise over 85% of the diet of the brook trout, both by volume and caloric value (Alexander and Gowing 1976). A flow reduction in Hunt Creek greater than 50% could adversely affect the brook trout population even if other measures of brook trout habitat did not decline.

There were no obvious differences in the relation between WUA and discharge among the various taxa. The relation between WUA and discharge for taxa that were most common in riffle habitats (e.g. Heptageniidae and Hydropsychidae) had a similar shape when compared to the relations for taxa that were more common in depositional or pool habitats (e.g. Tipulidae and Empididae) (Figure 6). The only clear difference in the relation between WUA and discharge was between reaches

B2 and B4. In general, the WUA relations for reach B4 indicated a reduction in WUA for benthic macroinvertebrates in Hunt Creek when summer stream flow was reduced 50%, although the reductions were minor. In contrast, WUA curves from reach B2 indicated a modest increase in WUA when summer stream flow was reduced 50%. The differences in the WUA curves between reaches B2 and B4 are undoubtedly due to the differences in velocity and depth between the two reaches.

The similarity between the HSC and the WUA-discharge relations for the taxa modeled is probably due to the methods used to construct the HSC. We did not weight the value of the habitat parameters for the individual samples because the habitat parameters measured and modeled with PHABSIM (depth and mean column velocity) may not be the most important parameters influencing the abundance of macroinvertebrates at the sample location. For example, Peckarsky (1984) noted that predation can be an important factor influencing the distribution and abundance of both predator and prey species of macroinvertebrates in localized areas. Further, it is unlikely that mean column velocity and depth are as important as the shear stress and thickness of the boundary layer in determining the abundance of macroinvertebrate taxa at specific locations in a stream (Osborne et al. 1985). The substrate composition at a location is undoubtedly important in determining the abundance of specific taxa of macroinvertebrates at locations in a stream (Minshall 1984). However, the PHABSIM system assumes substrate composition is constant and therefore, is not as important in determining the shape of WUA-discharge relation as depth and mean column velocity.

We did not conduct the PHABSIM analysis on the habitat of the macroinvertebrate families that occurred in less than 20% of the samples because these families represented less than 7% of the benthic organisms encountered during sampling and habitat suitability criteria constructed for extremely small sample sizes are generally not representative of the actual habitat suitability requirements of the taxa (Bovee 1986). If a habitat analysis for the infrequently occurring taxa was necessary it would require altering the sampling protocol to more adequately sample those taxa. In addition, we did not analyze the habitat for the most frequently encountered taxa (found in more than 80% of the samples) because nearly all microhabitats sampled were suitable for those taxa. As a result, the HSC for those taxa would have been too broad to be useful as a predictor or the change in habitat for those taxa and the WUA-discharge relation would have looked similar to the Total Area-discharge relation (Figure 5).

In addition to macroinvertebrate habitat, invertebrate drift can be affected by changes in discharge (Anderson and Lehmkuhl 1968, Minshall and Winger 1968, Hooper and Ottey 1988, Poff and Ward 1991). Poff and Ward (1991) demonstrated that drift increased for most taxa during simultaneous experimental streamflow reductions and elevations. We documented an increase in invertebrate drift densities (number$\cdot$m^{-3}) in the 24 hour period immediately following flow reduction but drift densities returned to prewithdrawal levels quickly, indicating the response of the invertebrates in section B to the reduced discharge was a short term, catastrophic drift response (Waters 1972). The only aspect of the total macroinvertebrate drift that might have been altered by the reduction of flow was the rate of downstream movement of the invertebrates due to the reduced velocity in the treatment section. This reduced rate of downstream movement may alter macroinvertebrate colonization dynamics in dewatered streams, even if other measures of habitat quality and quantity are not reduced by reduced flows.

Although We were not able to document a short term response to the reduced flow in Hunt Creek, it is possible that reduced flows could have a long term impact, particularly if stream edge habitats important for oviposition are subjected to drying over successive years. If stream edge habitats were dewatered it could lead to reduced reproductive success for taxa that use overhanging vegetation and stream edge substrates for egg laying. This in turn could lead to reduced production of macroinvertebrates in the stream.

The results presented here also point out that habitat components normally not considered in a PHABSIM analysis may be important in determining the response of fish to reduced flows. Orth (1987) argued for including broader ecological analyses in impact assessment, yet most of the PHABSIM studies conducted since 1987 have focused on fish microhabitat alone (Conder and Annear 1987, Scott and Shirvell 1987, Bovee et al. 1994). In this study we found that brook trout food-producing habitat may be decreased by a reduction in flow that would not produce a reduction in brook trout WUA (Baker and Coon 1995). For example, the PHABSIM model indicated benthic macroinvertebrate WUA in section B would be reduced approximately 52% and 66% in reaches B2 and B4, respectively if summer flow was reduced 85% but brook trout WUA would only be reduced 10%.

Finally, we want to stress that the results presented here are unique to Hunt Creek and should not be applied to streams throughout Michigan or the midwest. It is likely that a withdrawal of 50% of summer baseflow would have a greater impact on both fish and benthic macroinvertebrate habitat in marginal trout streams or streams that do not have high quality habitat under summer baseflow conditions. For example, a withdrawal of 50% of summer baseflow in Fish Creek, a marginal brown trout stream in Ingham County, Michigan would result in a 30% reduction of WUA for Hydropsyche spp. and a 25% reduction of WUA for Ephemerella spp. (esimated from figures in Gowan 1984). Clearly, the potential impacts of proposed water withdrawals from streams must be evaluated stream by stream.

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Table 1. Codes used to classify substrate use and availability in Hunt Creek.

Substrate Code[*]	**Substrate Description**
1	Fines composed of sand and silt
2	Sand
3.X	Small gravel, Diameter < 0.6 cm
4.X	Medium gravel, diameter ≥ 0.6 cm and less than 2.5 cm
5.X	Large gravel, diameter > 2.5 cm

[*] Substrate classifications for gravels included an estimate of the embeddedness of the gravel, X=1,2,3, and 4 where 1=up to 25%, 2=26-50%, 3=51-75%, and 4=76-100% embedded. For example, a substrate classification of 4.2 denotes medium gravel embedded between 26-50%.

Table 2. Summary of benthic macroinvertebrate taxa collected and percent frequency of occurrence in benthic samples in sections B and C of Hunt Creek, 1992-94. Taxa selected for habitat modeling are in bold face type.

Class	**Order**	**Family**	**% Occurrence in 1992-93 (n=436)**	**% Occurrence in 1994 (n=83)**
Insecta	Ephemeroptera	**Baetidae**	72.5	97.6
		Ephemerellidae	37.6	53.0
		Ephemeridae	5.1	
		Heptageniidae	38.8	83.1
		Leptophlebiidae	1.6	2.4
		Tricorythidae	0.2	
	Trichoptera	**Hydropsychidae**	31.9	47.0
		Glossosomatidae	67.7	90.4
		Limnephilidae	23.6	33.7
		Rhyacophilidae	36.2	53.0
		Philopotamidae	12.6	20.5
		Brachycentridae	15.8	14.5
		Lepidostomatidae	3.2	
		Hydroptilidae	1.6	4.8
		Psychomyiidae	0.2	
	Plecoptera	**Perlodidae**	23.2	32.5
		Nemouridae	30.3	69.9
		Taenyopterygidae	0.2	

		Leuctridae	6.0	26.5
		Perlidae	0.2	
	Odonata	Cordulegasteridae	9.2	1.2
		Gomphidae	1.4	
		Aeshnidae	0.2	
		Calopterygidae	0.7	
	Coleoptera	Elmidae larvae	81.9	98.8
		Elmidae adults	33.5	84.3
		Dytiscidae (larvae)	0.2	
	Diptera	Chironomidae	90.6	86.7
		Tipulidae	16.3	3.6
		Simuliidae	34.9	51.8
		Empididae	50.7	68.7
		Ceratopogonidae	25.0	6.0
		Tabanidae	9.2	2.4
		Athericidae	3.7	10.8
		Ptychopteridae	0.7	
		Muscidae	0.2	1.2
		Dixidae	0.9	
		Stratomyiidae	0.2	
	Megaloptera	Corydalidae	7.1	10.8
		Sialidae	1.8	
	Lepidoptera	Pyralidae	0.2	
Arachnida	Acari	Hydracarina	1.4	15.7
Bivalvia	Pelecypoda	Sphaeriidae	0.9	
Malacostraca	Amphipoda	Gammaridae	91.7	97.6

	Isopoda	Asselidae	6.7	14.5
Oligochaeta	Undetermined	Undetermined	100.0	100.0
Hirudinea	Undetermined	Undetermined		1.2

Table 3. Summary of mean column velocity and depth habitat suitability ranges for the 13 families of benthic macroinvertebrates selected for habitat modeling in Hunt Creek. Column headings (1, 0.5, 0.2, 0.1, and usable) are suitability values.

Mean Column Velocity ($\mathrm{cm \cdot s^{-1}}$)

Taxa	1	0.5	0.2	0.1	**Usable**
Baetidae	21-56	9-64	0-73	0-78	0-80
Ephemerellidae	21-55	12-63	1-70	0-74	0-79
Heptageniidae	42-65	27-73	6-78	4-79	0-80
Hydropsychidae	34-61	23-69	15-77	7-78	4-79
Glossosomatidae	30-58	16-65	10-74	2-79	0-80
Rhyacophilidae	34-63	27-70	12-78	0-79	0-80
Perlodidae	32-63	22-73	15-79	4-80	3-81
Nemouridae	27-58	10-66	2-73	0-79	0-80
Elmidae Adult	34-60	27-70	15-78	11-79	0-80
Tipulidae	24-58	5-64	0-69	0-70	0-71
Simuliidae	28-59	21-73	14-78	14-79	4-80
Empididae	27-57	14-65	8-77	0-79	0-81
Ceratopogonidae	4-43	0-54	0-73	0-77	0-78
			Depth (cm)		
Baetidae	17-36	12-46	8-58	5-61	4-63
Ephemerellidae	17-37	11-47	5-58	4-58	2-63
Heptageniidae	20-38	13-49	11-58	6-61	4-63
Hydropsychidae	16-31	11-42	6-49	4-55	4-58

Glossosomatidae	17-37	12-47	6-55	4-61	4-63
Rhyacophilidae	17-36	13-44	11-50	7-56	6-61
Perlodidae	13-38	7-43	5-47	4-61	3-62
Nemouridae	13-31	8-40	5-47	4-58	3-59
Elmidae Adult	17-37	13-46	7-58	5-61	3-64
Tipulidae	15-35	11-43	9-49	6-56	5-57
Simuliidae	13-30	11-46	6-58	4-58	3-59
Empididae	16-37	12-48	8-56	4-61	2-64
Ceratopogonidae	13-33	10-48	3-52	2-56	1-57

Table 4. Summary of substrate suitability values for the 13 families of benthic macroinvertebrates selected for habitat modeling in section B of Hunt Creek. Percent embeddedness of the substrate was not included in the substrate suitability calculations.

	Substrate Code				
Taxa	**1 (sand and silt)**	**2 (sand)**	**3 (small gravel)**	**4 (medium gravel)**	**5 (large gravel)**
Baetidae	0.13	0.13	0.17	1	0.70
Ephemerellidae	0.11	0.11	0.2	1	0.65
Heptageniidae	0.02	0	0.02	1	0.83
Hydropsychidae	0	0.11	0.11	1	0.80
Glossosomatidae	0	0.04	0.13	1	0.55
Rhyacophilidae	0.02	0.08	0.06	1	0.67
Perlodidae	0	0.04	0.04	1	0.88
Nemouridae	0.08	0.24	0.20	1	0.76
Elmidae Adult	0	0	0.07	1	0.65
Tipulidae	0.23	0.14	0.09	1	0.82
Simuliidae	0.04	0.12	0.27	1	0.96
Empididae	0.08	0.12	0.17	1	0.61
Ceratopogonidae	0.69	0.41	0.14	1	0.38

Table 5. Relation between Weighted Usable Area (WUA, $m^2 \cdot 100\ m^{-1}$) and discharge for the 13 benthic macroinvertebrate families selected for habitat modeling in section B of Hunt Creek.

	Discharge ($m^3 \cdot s^{-1}$)						
Reach B2	**0.01**	**0.04**	**0.07**	**0.11**	**0.17**	**0.23**	**0.46**
Baetidae	24.8	60.0	91.3	123.1	160.7	180.5	160.0
Ephemerellidae	26.2	64.5	96.9	127.8	162.8	180.6	155.8
Heptageniidae	6.7	20.8	35.9	59.1	99.2	130.7	153.4
Hydropsychidae	5.2	24.7	50.6	85.7	130.9	155.1	147.8
Glossosomatidae	11.0	37.4	66.2	97.8	138.1	161.2	148.0
Rhyacophilidae	7.4	17.8	33.7	62.3	109.1	140.0	146.5
Perlodidae	9.1	36.2	68.6	104.8	145.3	166.6	165.4
Nemouridae	29.8	77.0	115.1	148.4	180.2	193.1	164.7
Elmidae Adult	3.4	16.6	35.5	65.0	111.0	141.3	147.3
Tipulidae	30.8	66.0	99.7	135.8	173.8	192.2	157.4
Simuliidae	4.7	29.6	65.7	108.7	153.0	175.8	169.2
Empididae	13.9	42.1	72.8	107.4	148.7	171.0	156.6
Ceratopogonidae	88.1	132.8	163.1	188.9	212.2	217.3	180.3
Reach B4							
Baetidae	28.5	67.5	108.1	156.7	197.2	216.7	249.4
Ephemerellidae	30.6	67.9	108.2	157.5	201.1	219.8	247.0
Heptageniidae	6.5	22.4	40.5	63.8	99.5	131.9	212.8
Hydropsychidae	3.9	21.5	43.9	75.5	131.6	166.6	225.7
Glossosomatidae	11.8	36.4	66.9	108.8	162.1	190.8	234.1
Rhyacophilidae	8.4	19.4	35.7	62.0	108.8	142.6	220.3

Perlodidae	8.5	34.2	58.8	91.1	153.9	189.2	242.3
Nemouridae	34.5	86.0	125.2	163.7	208.6	230.0	252.2
Elmidae Adult	3.2	14.6	30.6	57.1	109.9	145.3	220.2
Tipulidae	33.0	80.2	123.6	166.1	201.3	220.8	254.1
Simuliidae	3.2	23.5	54.3	93.8	156.6	194.6	241.7
Empididae	15.0	42.8	79.1	125.3	174.7	202.3	244.4
Ceratopogonidae	109.1	171.8	217.1	249.0	265.6	280.3	279.8

Table 6. Benthic macroinvertebrate density estimates (number·m^{-2}, standard error estimates in parentheses) from random sample locations in sections B and C for 1992-93.

Family/Year	**Section**	**April**	**May**	**June**	**July**	**August**	**September**
1992							
Baetidae	B		75(21)	110(30)	273(87)	321(106)	18(6)
	C		136(41)	641(106)	427(85)	318(112)	185(68)
Ephemerellidae	B		138(38)	112(27)	104(24)	9(6)	2(2)
	C		91(37)	40(17)	47(13)	5(3)	11(5)
Heptageniidae	B		236(56)	11(8)	4(3)	9(4)	90(24)
	C		460(109)	65(23)	14(8)	20(20)	91(37)
Hydropsychidae	B		61(33)	24(11)	258(93)	446(222)	256(145)
	C		11(5)	9(5)	12(7)	241(106)	510(307)
Glossosomatidae	B		1042(253)	661(190)	927(334)	410(141)	845(156)
	C		173(57)	234(63)	366(140)	310(90)	1108(265)
Rhyacophilidae	B		37(12)	2(2)	27(9)	111(35)	198(55)
	C		33(10)	31(21)	23(10)	31(14)	107(30)
Perlodidae	B		4(3)	11(5)	18(12)	38(14)	62(18)
	C		5(3)	29(10)	42(15)	36(15)	111(28)
Nemouridae	B		44(23)	78(59)	62(30)	13(7)	0(0)
	C		20(8)	698(317)	206(104)	20(9)	2(2)
Elmidae adults	B		94(41)	35(12)	36(20)	58(20)	76(29)
	C		0(0)	0(0)	0(0)	0(0)	0(0)
Tipulidae	B		18(6)	13(6)	11(4)	36(10)	13(5)
	C		7(5)	2(2)	14(7)	2(2)	13(6)
Simuliidae	B		13(7)	13(8)	322(202)	125(58)	4(3)
	C		57(29)	546(298)	330(157)	138(106)	120(82)
Empididae	B		24(8)	52(18)	79(21)	20(9)	22(7)

	C		2(2)	80(25)	66(24)	38(10)	18(8)
Ceratopogonidae	B		20(14)	26(10)	37(14)	49(16)	44(11)
	C		13(9)	22(10)	19(9)	29(17)	29(16)
Total	B		4031(647)	5569(771)	8301(1256	6109(940)	4148(470)
	C		2309(315)	6078(1050	8865(1195	6451(1001	5443(963)
<u>1993</u>							
Baetidae	B	277(58)	114(25)	110(29)	244(33)	392(71)	130(26)
	C	251(82)	78(19)	180(72)	592(125)	435(117)	300(70)
Ephemerellidae	B	317(81)	168(38)	191(39)	175(36)	21(12)	13(9)
	C	49(16)	26(13)	26(15)	0(0)	2(2)	0(0)
Heptageniidae	B	239(134)	275(93)	38(18)	7(5)	12(8)	141(51)
	C	378(93)	321(86)	236(81)	81(28)	40(18)	18(9)
Hydropsychidae	B	146(81)	61(26)	38(18)	404(120)	442(202)	280(168)
	C	45(24)	5(3)	0(0)	14(8)	25(16)	58(24)
Glossosomatidae	B	306(72)	248(71)	249(75)	245(79)	222(56)	630(182)
	C	448(159)	109(36)	28(13)	236(77)	305(102)	117(48)
Rhyacophilidae	B	131(38)	11(6)	9(7)	20(10)	59(18)	150(32)
	C	36(12)	12(7)	21(9)	14(7)	29(12)	36(13)
Perlodidae	B	11(6)	5(3)	5(5)	7(4)	7(4)	49(16)
	C	7(4)	2(2)	5(5)	2(2)	11(4)	16(8)
Nemouridae	B	70(40)	90(39)	40(23)	54(25)	5(5)	0(0)
	C	61(26)	31(14)	99(57)	36(13)	16(5)	2(2)
Elmidae adults	B	33(9)	90(27)	74(25)	52(24)	116(39)	146(42)
	C	40(20)	59(21)	45(19)	31(12)	29(9)	119(54)
Tipulidae	B	18(7)	14(7)	7(4)	9(5)	7(5)	0(0)
	C	11(7)	0(0)	2(2)	5(3)	25(22)	0(0)
Simuliidae	B	16(10)	491(427)	5(3)	168(60)	113(52)	11(4)
	C	20(11)	83(47)	5(3)	139(58)	94(39)	38(12)
Empididae	B	47(11)	105(30)	186(55)	197(57)	90(30)	61(21)
	C	85(22)	59(21)	66(29)	96(29)	88(33)	108(29)

Ceratopogonidae	B	45(29)	38(18)	29(11)	49(23)	38(13)	52(20)
	C	2(2)	52(50)	2(2)	5(3)	9(9)	7(5)
Total	B	6236(1043	4509(655)	5308(713)	9053(1131	6312(751)	4480(727)
	C	4241(783)	2403(346)	2984(737)	4036(690)	4646(751)	4030(918)

Table 7. Benthic macroinvertebrate density estimates (number·m^{-2}, standard error estimates in parentheses) from one riffle each in sections B and C, 1994.

		Date					
Taxa	**Section**	**May 12**	**June 1**	**June 20**	**July 12**	**August 3**	**August 23**
Baetidae	B	448(83)	91(33)	149(42)	1143(218)	2331(576)	1052(210)
	C	759(142)	442(92)	545(74)	992(187)	2182(389)	935(215)
Ephemerellidae	B	643(55)	350(78)	253(33)	117(41)	39(21)	19(14)
	C	58(16)	26(13)	13(8)	0(0)	13(13)	0(0)
Heptageniidae	B	1149(136)	851(106)	273(49)	110(31)	39(15)	26(13)
	C	649(106)	539(83)	487(62)	326(84)	130(30)	19(13)
Hydropsychidae	B	195(55)	84(50)	208(67)	32(13)	636(148)	740(180)
	C	6(6)	6(6)	0(0)	0(0)	13(13)	26(13)
Glossosomatidae	B	1818(483)	1117(269)	1442(263)	234(73)	312(38)	130(47)
	C	571(141)	253(31)	221(53)	76(22)	65(17)	84(40)
Rhyacophilidae	B	52(25)	13(8)	52(38)	13(13)	136(37)	52(15)
	C	39(15)	39(6)	52(15)	38(18)	19(9)	19(9)
Perlodidae	B	6(6)	0(0)	26(9)	0(0)	97(48)	39(12)
	C	6(6)	6(6)	19(9)	23(16)	45(24)	6(6)
Nemouridae	B	162(47)	104(32)	435(90)	45(24)	97(44)	26(13)
	C	117(61)	162(78)	539(166)	159(66)	104(45)	19(14)
Elmidae adults	B	377(80)	214(56)	273(44)	149(52)	299(62)	390(66)
	C	78(32)	162(65)	182(65)	106(46)	84(27)	84(51)
Tipulidae	B	0(0)	0(0)	0(0)	0(0)	19(14)	6(6)
	C	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Simuliidae	B	52(27)	32(22)	6(6)	104(38)	156(119)	32(26)
	C	468(250)	156(80)	26(26)	60(19)	149(72)	65(40)
Empididae	B	136(49)	130(55)	208(70)	117(28)	65(34)	13(8)
	C	149(43)	136(43)	227(95)	98(36)	97(34)	32(19)

Ceratopogonidae	B	13(13)	0(0)	6(6)	0(0)	13(8)	6(6)
	C	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Total	B	7188(485)	4188(713)	6195(892)	6948(727)	7591(1031)	5409(726)
	C	4844(441)	2981(366)	4500(805)	3614(524)	6325(805)	4149(1262)

Table 8. Results of BACI analysis of the 1994 benthic macroinvertebrate abundance data in sections B and C of Hunt Creek (df=4 for all tests).

Taxa	**Pretreatment Mean Difference (#·m^{-2})**	**Treatment Mean Difference (#·m^{-2})**	**t statistic**	**p value**
Baetidae	-331.0	5.3	-2.5	0.09
Ephemerellidae	454.5	100.5	2.5	0.19
Heptageniidae	406.0	-128.5	4.9	0.05
Hydropsychidae	133.5	394.3	-1.5	0.21
Glossosomatidae	1055.5	418.0	1.9	0.13
Rhyacophilidae	-6.5	31.3	-1.0	0.36
Perlodidae	-3.0	17.3	-1.2	0.30
Nemouridae	-6.5	-54.5	0.8	0.52
Elmidae adult	175.5	163.8	0.1	0.94
Elmidae larvae	571.5	1198.8	-2.7	0.06
Simuliidae	-270.0	-0.5	-1.8	0.31
Empididae	-9.5	-12.8	0.3	0.79
Ceratopogonidae	6.5	6.3	0.04	0.98
Chironomidae	-90.9	29.5	-0.7	0.51
Gammeridae	-419.0	-736.7	0.5	0.63
Total Density	1775.5	1888.8	-0.15	0.89

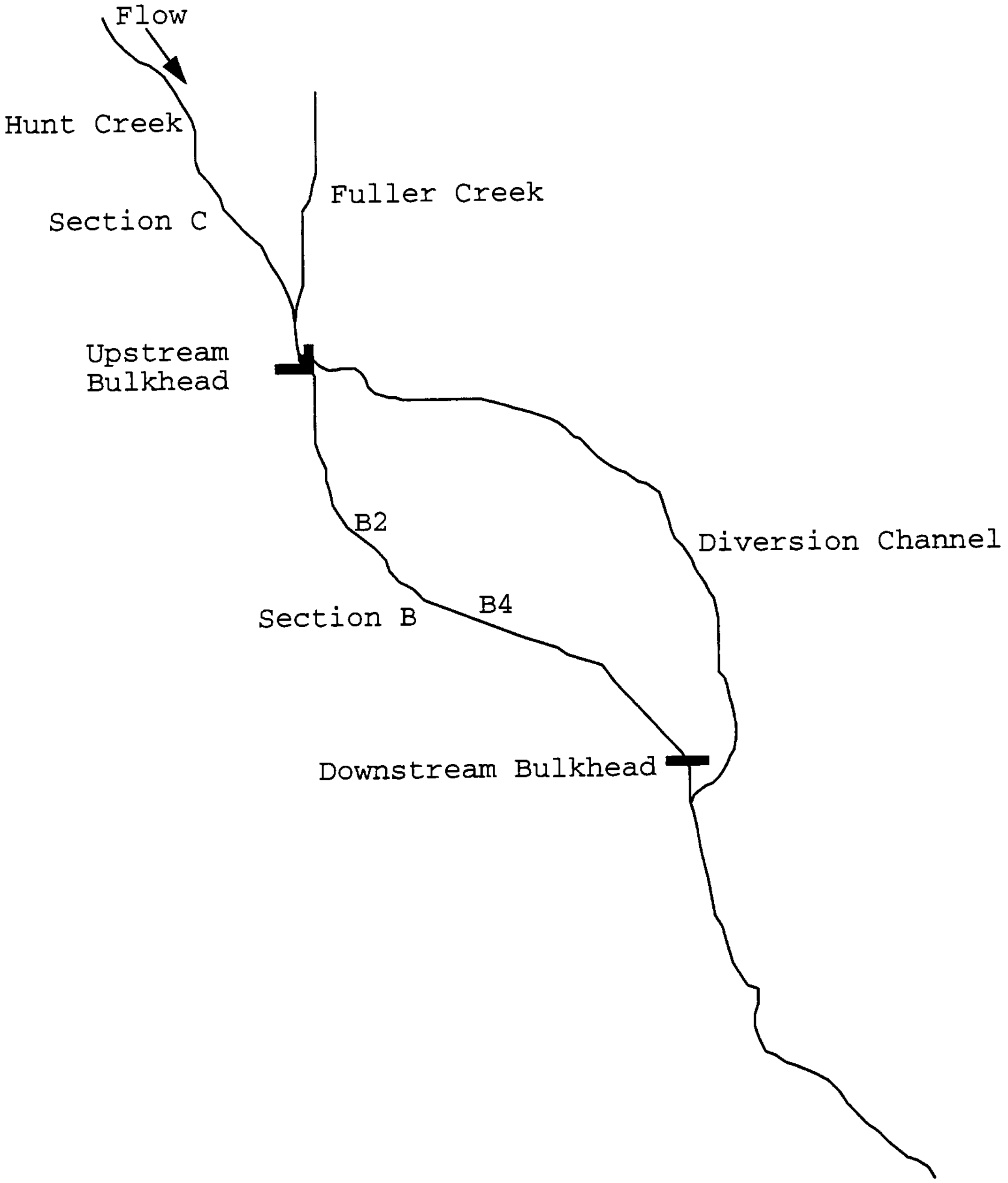

Figure 1. Map of Hunt Creek study area. The upstream bulkhead is the boundary between sections C and B.

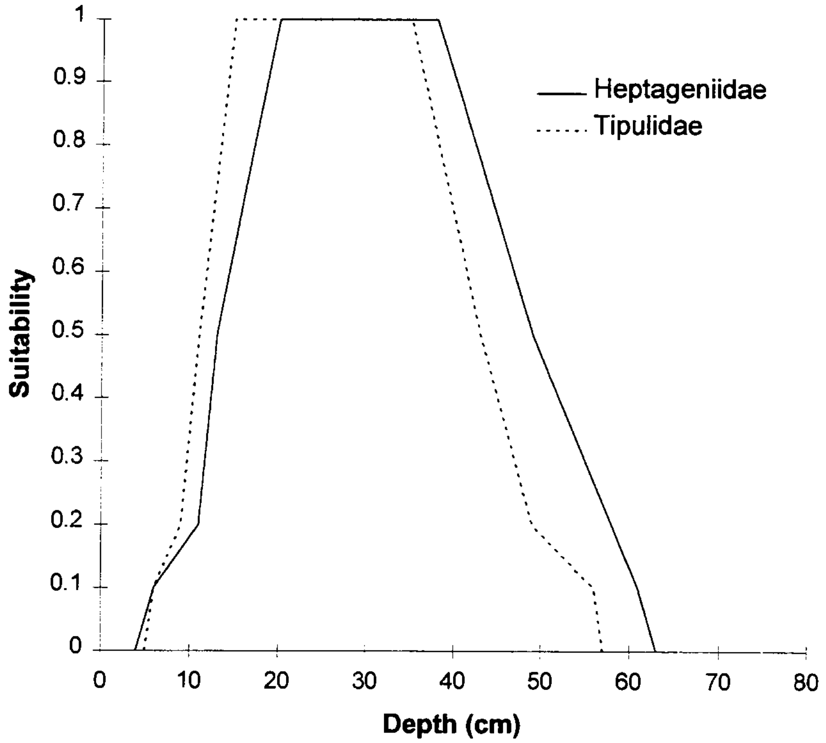

Figure 2. Depth habitat suitability criteria for Heptageniidae and Tipulidae calculated from habitat use data collected in section B of Hunt Creek, summer 1992-93.

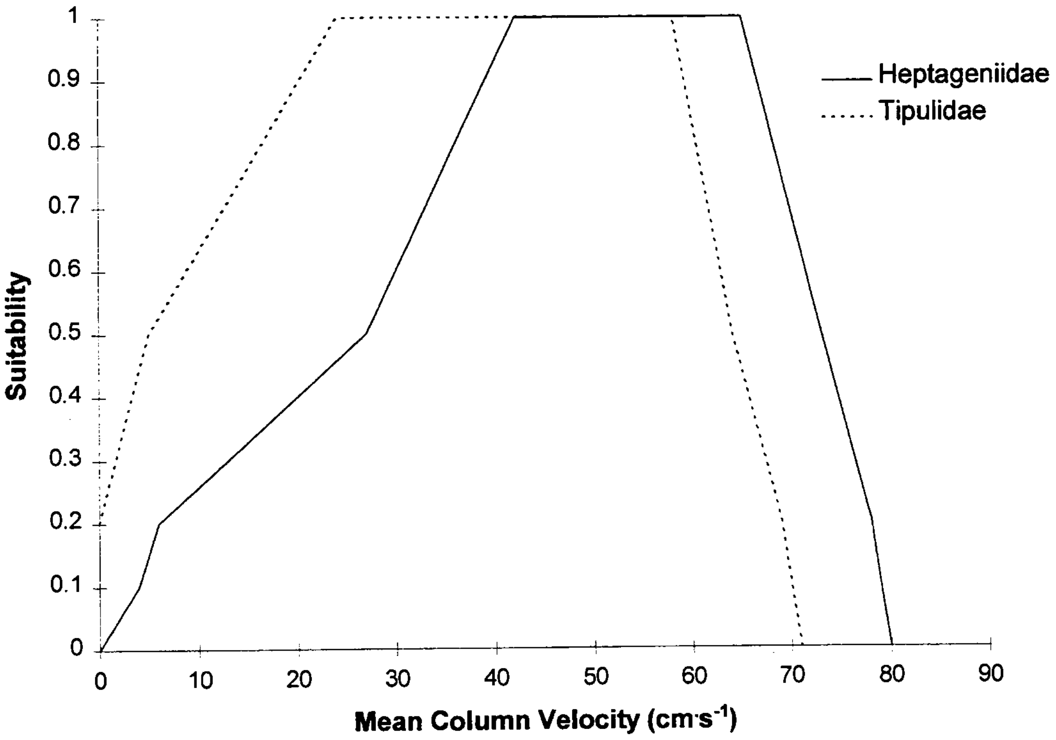

Figure 3. Mean column velocity habitat suitability criteria for Heptageniidae and Tipulidae calculated from habitat use data collected in section B of Hunt Creek, summer, 1992-93.

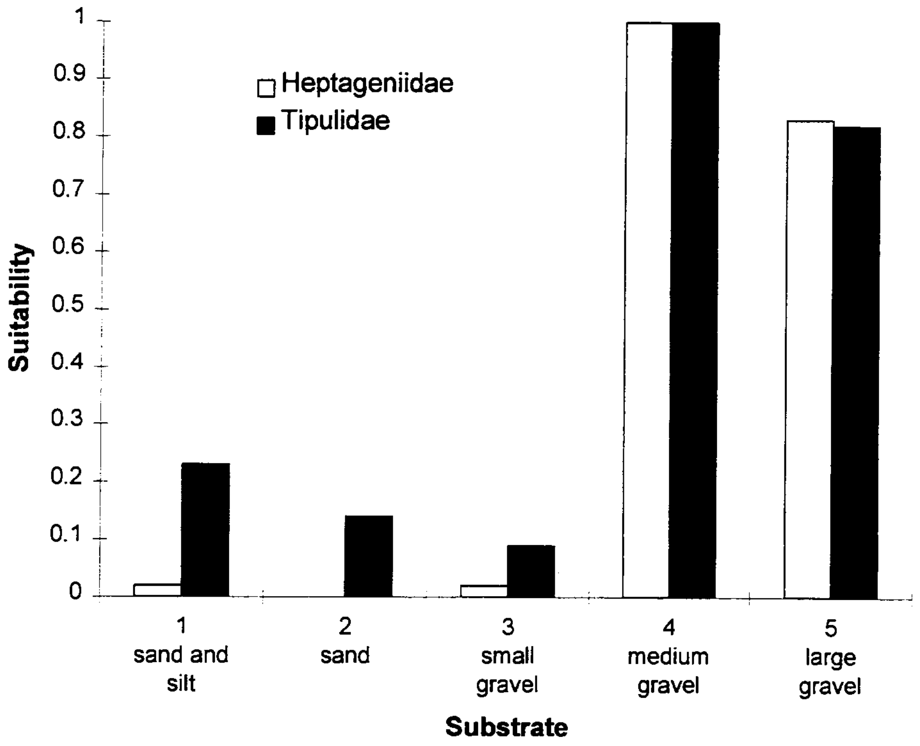

Figure 4. Substrate suitability criteria for Heptageniidae and Tipulidae calculated from habitat use data collected in section B of Hunt Creek, summer, 1992-93.

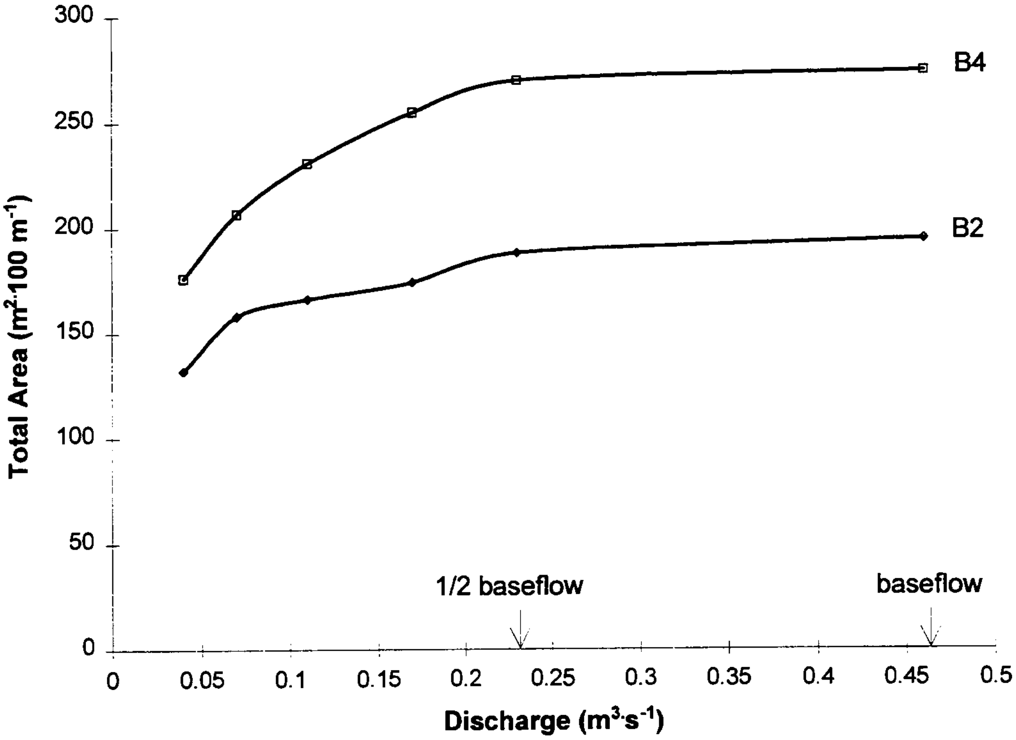

Figure 5. Total area ($m^2 \cdot 100\ m^{-1}$) as a function of discharge ($m^3 \cdot s^{-1}$) for reaches B2 and B4 in Hunt Creek.

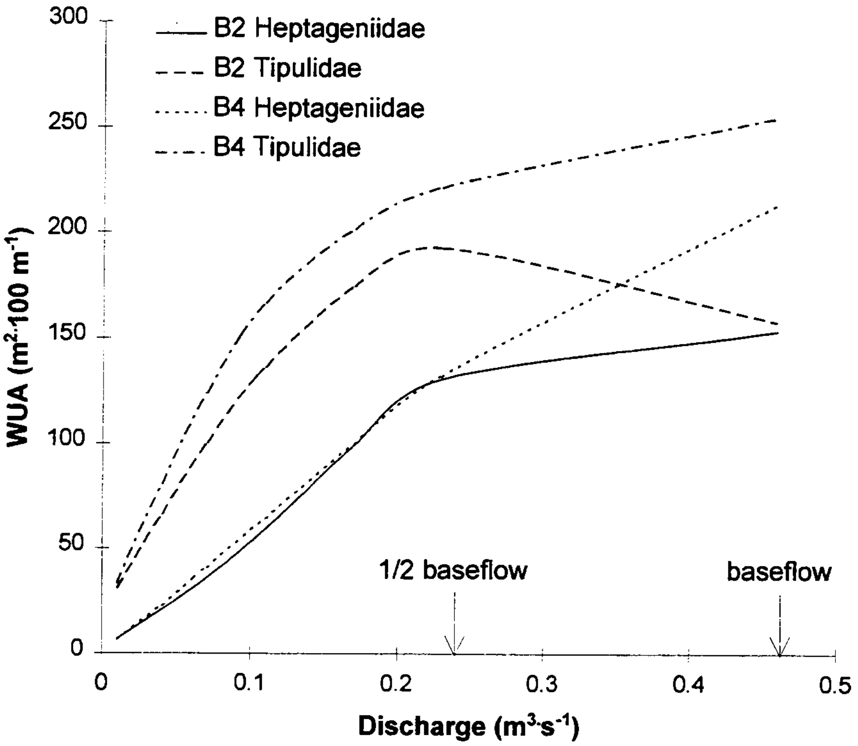

Figure 6. Weighted usable area ($m^2 \cdot 100\ m^{-1}$) at discharge ($m^3 \cdot s^{-1}$) for Heptageniidae and Tipulidae in section B of Hunt Creek.

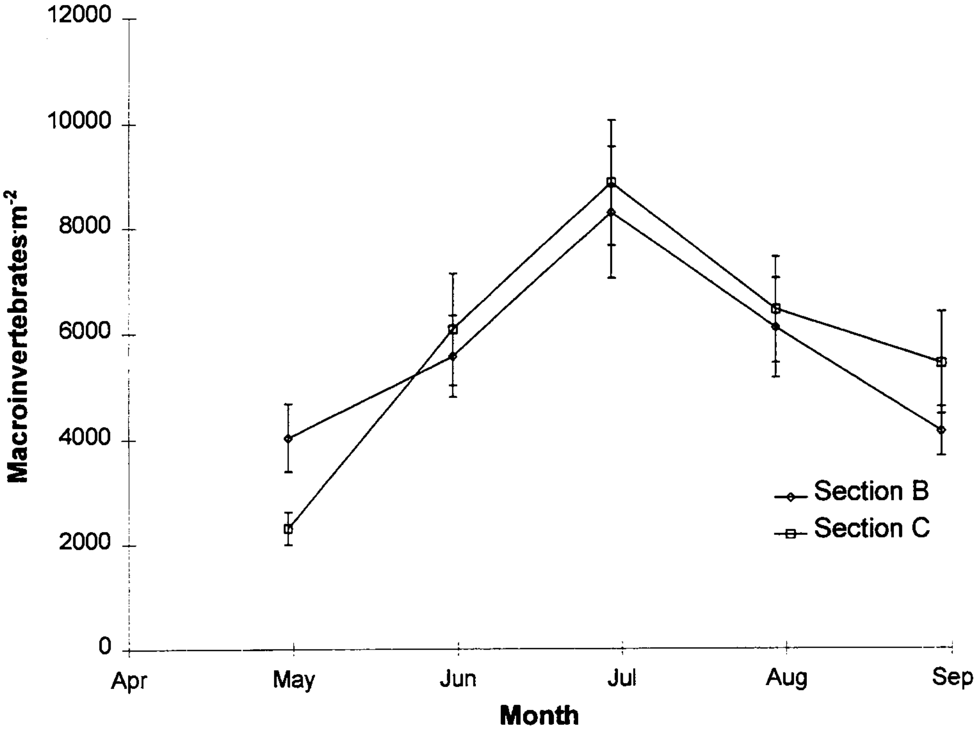

Figure 7. Total macroinvertebrate density (number·m^{-2}, with standard error bars) in sections B and C of Hunt Creek, summer, 1992.

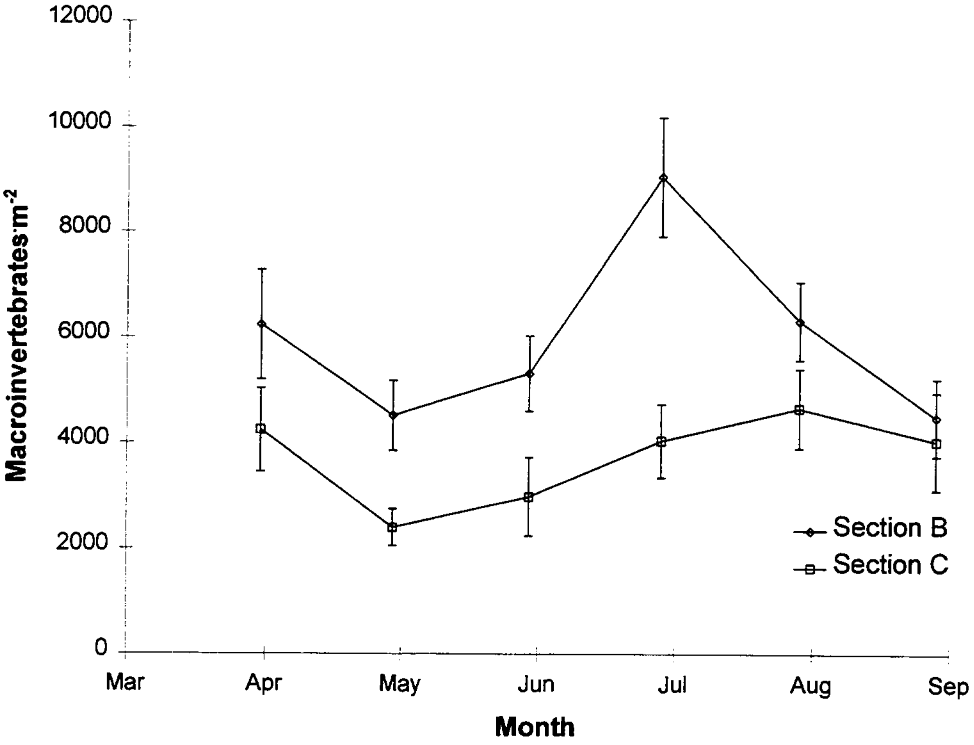

Figure 8. Total macroinvertebrate density (number·m^{-2}, with standard error bars) in sections B and C of Hunt Creek, summer, 1993.

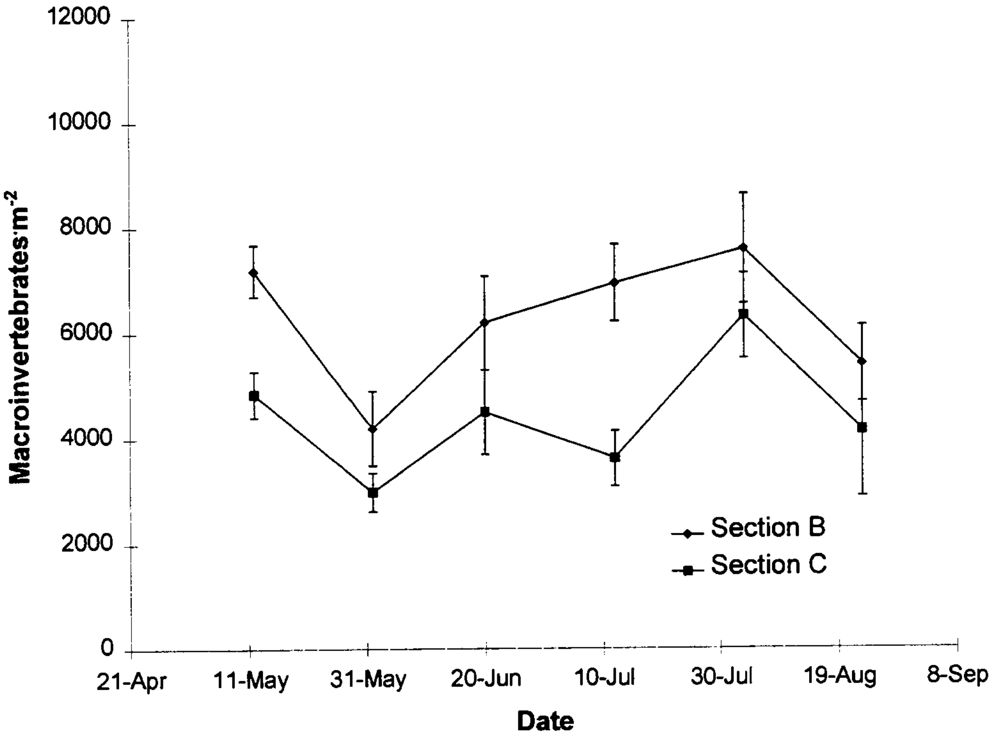

Figure 9. Total macroinvertebrate density (number·m^{-2}, with standard error bars) in sampled riffles in sections B and C of Hunt Creek, summer, 1994. June 1 samples were collected immediately prior to the start of the withdrawal period.

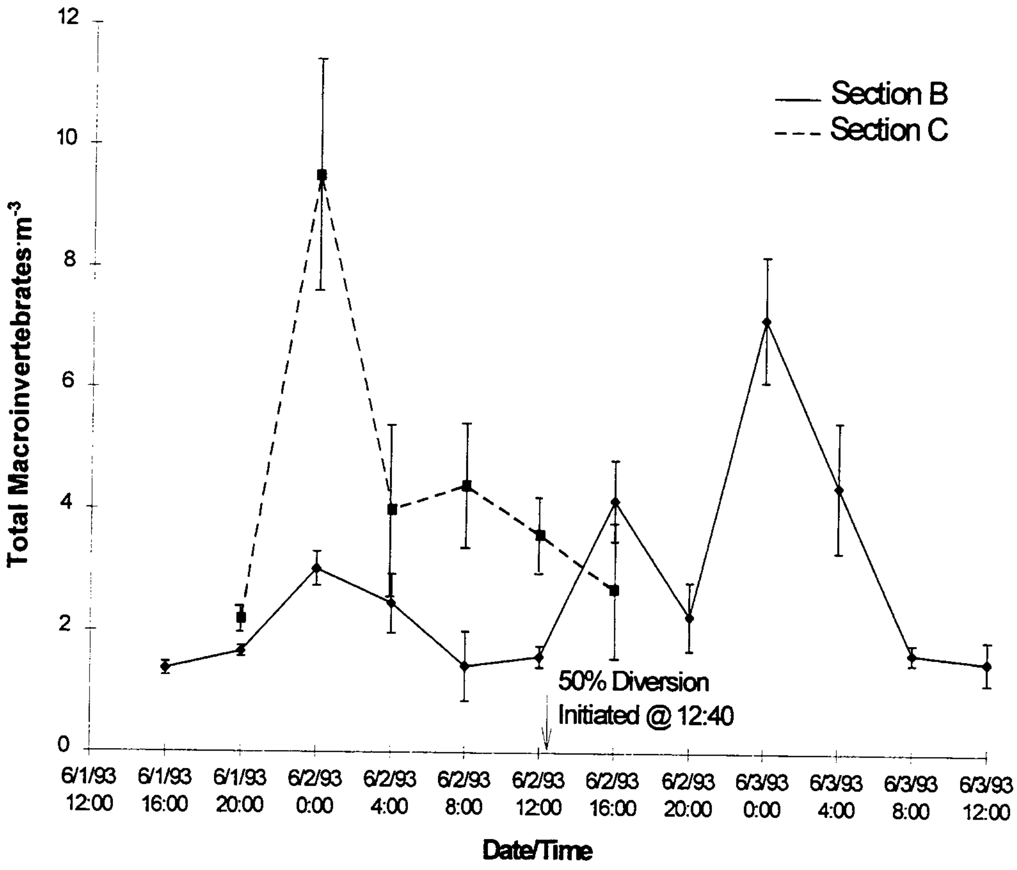

Figure 10. Macroinvertebrate drift density (number·m^{-3}, with standard error bars) in section B immediately before and after the initiation of the withdrawal period in 1993 and in section C. Withdrawal period began at approximately 12:40 on June 2, after the 12:00 sampling was completed. Section C drift samples were collected on June 6 and 7.

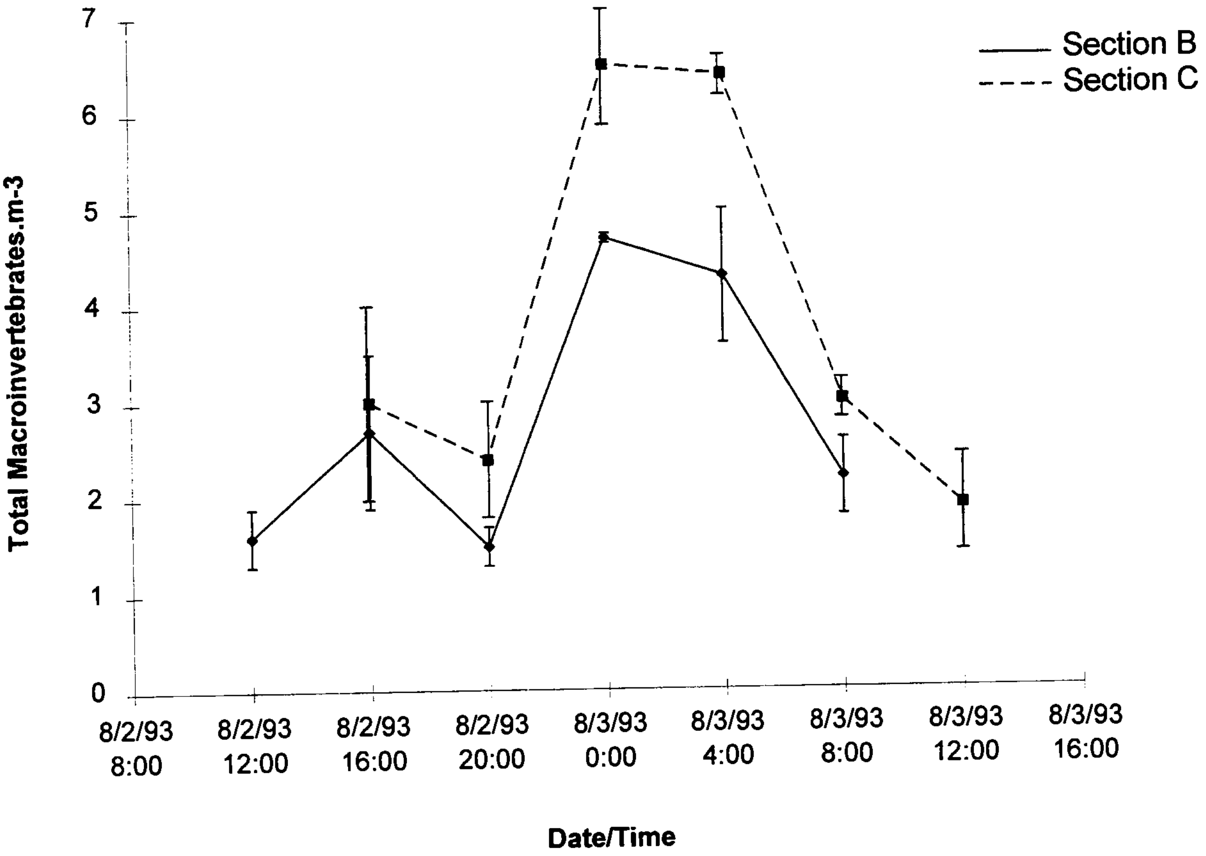

Figure 11. Macroinvertebrate drift density (number$\cdot$m^{-3}, with standard error bars) in section B on August 1-3, 1993. Section B samples were collected on August 1 and 2 and section C samples were collected on August 2-3.

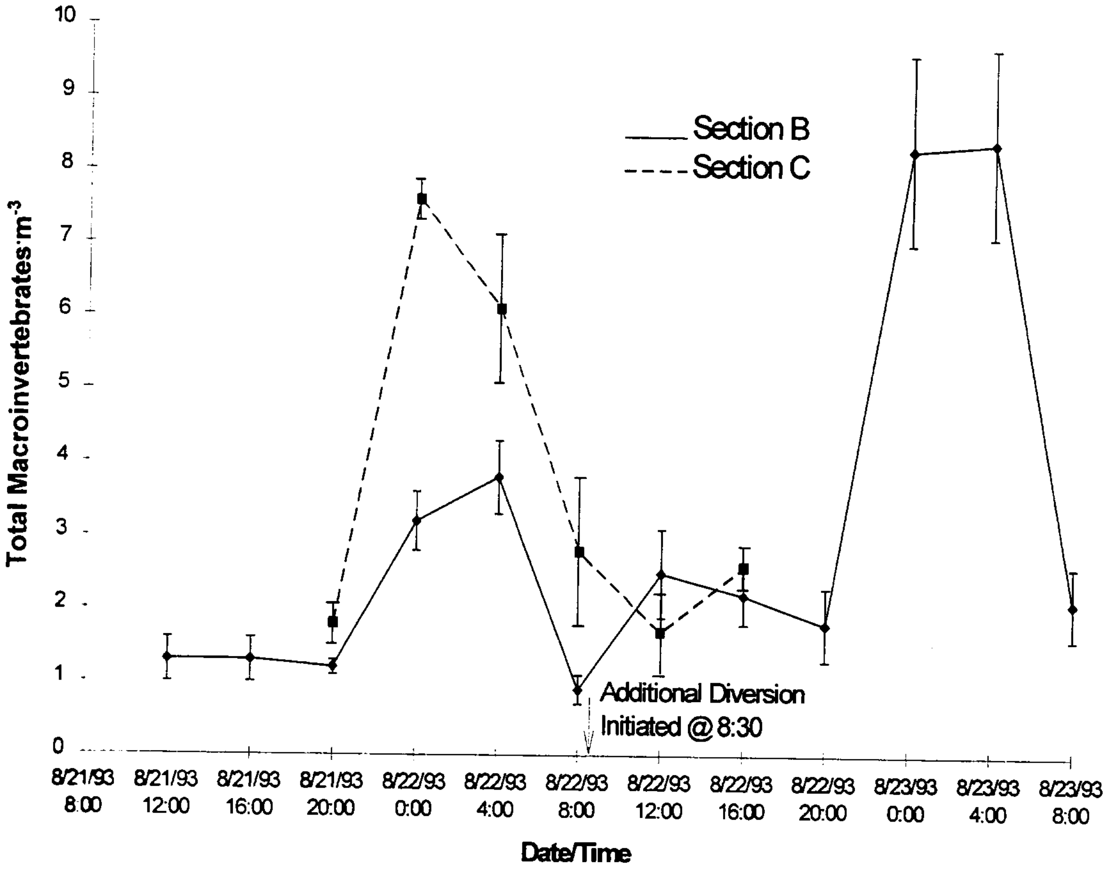

Figure 12. Macroinvertebrate drift density (number·m^{-3}, with standard error bars) in section B immediately before and after the initiation of the additional withdrawal period in August, 1993. Withdrawal period began at approximately 8:30 on August 22. Section C samples were collected on August 25 and 26.