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# LINKING EMBEDDEDNESS AND MACROINVERTEBRATE HEALTH IN TWO SOUTHWEST OHIO STREAMS

THESIS

Jonathan P. Kochersberger, Captain, USMC

AFIT/GES/ENV/08-M03

**DEPARTMENT OF THE AIR FORCE**
**AIR UNIVERSITY**

## AIR FORCE INSTITUTE OF TECHNOLOGY

**Wright-Patterson Air Force Base, Ohio**

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THESIS

Presented to the Faculty

Department of Systems and Engineering Management

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In Partial Fulfillment of the Requirements for the

Degree of Master of Science in Environmental Engineering and Science

Jonathan P. Kochersberger

Captain, USMC

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# LINKING EMBEDDEDNESS AND MACROINVERTEBRATE HEALTH IN TWO SOUTHWEST OHIO STREAMS

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## Abstract

Environmental managers utilize a variety of tools when assessing lotic systems for stressors attributed to anthropogenic sources. Stream deposited sediment has been recognized as one of the major stressors affecting streams in the U.S. The detrimental effect on aquatic biota of sediment depositing within the interstitial spaces of stream substrate (embeddedness) has been established, yet lacking is an effective *in situ* method of quantifying embeddedness over short time periods. The goal of this research was to develop a short-term embeddedness (EMB) quantification method that can be linked to benthic macroinvertebrate health. Such a method would be a valuable tool when conducting biological and physical habitat assessments of wadeable streams and rivers. An *in situ* embeddedness chamber was developed to capture sediment deposited within the interstitial spaces of a uniformly sized substrate. Using sediment accumulation and macroinvertebrate colonization as endpoints, three exposure periods were evaluated (4, 7, and 14 days) on a small order stream (Honey Creek, New Carlisle, Ohio, USA) and a medium order stream (Stillwater River, Covington, Ohio, USA). The experiment was conducted during low flow conditions with little variation in flow, turbidity, and total suspended solids. Three treatment areas located downstream of the EMB chambers also were established to assess benthic macroinvertebrate colonization rates. Different levels of substrate disturbance (disturbed, slightly disturbed, and undisturbed) were mimicked by removing the embedded fine sediments. Embeddedness chamber results show correlations between newly deposited fine sediment and insect colonization rates.

Measured percent embeddedness (USGS method) results at both sites were close to a natural stream condition (~33-35% embedded). Increases in both sediment and insect colonization within the EMB chambers during the three sampling periods show that the chambers had not reached the embeddedness equilibrium for the stream conditions at that time. Regression analyses run between chamber abiotic and biotic parameters reveal interesting correlations showing possible influence of fine sediment fractions on the biotic responses. Treatment area invertebrate results showed higher densities with the undisturbed areas indicating the need for a longer study period to assess true colonization potential. Further exploration, calibration, and validation of an effective *in situ* embeddedness quantification method for lotic systems is needed for accurate stream assessments.

## Dedication

To my wife and children. Without your love, support, and patience throughout this whole process none of this would have been possible. To my son, your curiosity and excitement that "daddy takes care of water and bugs" gives me hope for the future. To my sweet little girl, may you grow to love nature for all of its beauty and wonder as I have. To my lovely wife, your quiet strength and belief in me is the fuel that makes this man run.

**Acknowledgments**

I would like to thank Lt. Col David A. Smith for allowing me the flexibility to pursue research in the stream ecology and aquatic toxicology fields rather than being bound to strictly environmental engineering research. I also owe many thanks to Dr. Allen Burton for taking me on as a graduate student and providing me with the opportunity to gain valuable field and laboratory experience while working under his guidance. Your guidance and advice throughout this research, while allowing me the freedom to "experiment" and learn, is the formula for making self-motivated and self-disciplined scientists. I am deeply grateful. I would also like to thank Dr. Charles Bleckmann for agreeing to be a committee member. None of this research would have been possible without the frequent guidance and help from my friend Kevin Custer. Kevin, your enthusiasm for the outdoors, macroinvertebrates, and stream ecology kept an air of excitement throughout many long hours both in the field and in the lab. Whenever I needed a question answered or a thought sanity-checked you were there to help. I owe you big time! To Keith Taulbee, thank you so much for helping me understand the statistics involved with this research and some deeper thoughts on the inner workings of a stream ecosystem. Thanks are also due for the rest of the "Burton Lab" for donating some of your time to help me along the way. Padrick especially, you were always willing to help.

Jonathan P. Kochersberger

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# I. Introduction

## Background

It is widely accepted that anthropogenic activities have altered aquatic and terrestrial ecosystems. Challenges such as change in global climate, loss of habitat and biodiversity, and inputs of anthropogenic chemicals must be considered in the environmental risk assessment process in order to fully characterize each environmental issue (Hope, 2006). The United States Environmental Protection Agency (USEPA) defines part of the analysis phase of an ecological risk assessment as the creation of summary profiles describing exposure to a stressor(s) and the relationship between the stressor and the response to that stressor. Terrestrial and aquatic organisms typically are the receptors that are affected by single or multiple stressors. Therefore, evaluating the habitat to which they restrict the bulk of their activities is an important step in establishing a stressor-response relationship. Both suspended and deposited sediments have been identified as the major pollutant of US waters; the effect on aquatic organisms is well established (Lemly, 1982; Newcombe and MacDonald, 1991; Waters, 1995; Wood and Armitage, 1997; USEPA, 2002). Large amounts of sediment moving through lotic systems tend to have effects, both direct and indirect, on aquatic organisms. Impacts of increased sediment input on stream communities, such as reduced light penetration, smothering, habitat reduction, and the introduction of absorbed pollutants (pesticides, nutrients, and metals), have been clearly documented (Oschwald, 1972; Newcomb and MacDonald, 1991; Hynes, 1970). A vital physical characteristic of aquatic habitats, particularly lotic systems, is the degree of substrate embeddedness that occurs as a result of fine sediments filling the voids of interstitial spaces in the streambed. Although

alteration of streambed habitat is recognized as one of the most important stressors of benthic organisms, the reliability of the findings from the existing embeddedness methodologies have been questioned (Sylte and Fischenich, 2002).

**Research Objectives**

1. Design a chamber for *in situ* embeddedness assessment for lotic systems that contains a uniformly sized clean substrate and that allows sediment accumulation and subsequent embedding of the test substrate.

Hypothesis: Using sediment weight, porosity, and benthic macroinvertebrate colonization as endpoints, an effective and efficient chamber and method can be designed to quantify the relationship between embeddedness and colonization.

2. Compare embeddedness chamber endpoints of sediment dry weight and benthic macroinvertebrate colonization to determine the relationship between each endpoint within the chambers.

Hypothesis: There will be an inverse relationship between the amount of sediment in the chambers and the number and composition of colonizing macroinvertebrates.

3. Compare benthic macroinvertebrate colonization of stream substrate between three areas which exhibit varying degrees of substrate disturbance.

Hypothesis: There will be an observable difference among colonization of benthic macroinvertebrates in the three substrate treatment areas with the disturbed area (less embedded) exhibiting a more diverse assemblage than the undisturbed area (more embedded).

## Methodology

This research utilized established methods for assessing physical and biological parameters within lotic systems in addition to an experimental method that attempts to draw relevant correlations. Previous studies have used various means of assessing macroinvertebrate colonization of introduced and natural substrates as well as the effect that fine sediment has on macroinvertebrate colonization. This research employed an *in situ* chamber with an introduced substrate to assess macroinvertebrate colonization and sediment accumulation over periods of 4, 7, and 14 days. Four sediment fractions (representing gravel, sand, silt, and clay) were analyzed from sediment that accumulated within the interstitial spaces of the chamber substrate. Furthermore, the porosity of each chamber was estimated using the bulk density of the chamber sediments (determined through a sequential loss on ignition process). Three treatment areas within a section of the sampling site were modified by removing variable levels of fine sediment and were analyzed for macroinvertebrate colonization at 4-Day and 14-Day points. A 10 day sediment toxicity test was conducted, using sediment from each site, to determine if any background factors were present that could alter macroinvertebrate colonization (i.e., sediment toxicity). A thorough characterization of the substrate at each site, including percent embedded substrate size fraction, was conducted to better understand the site characteristics.

## Assumptions/Limitations

Due to the brief window for field studies and subsequent sample processing, relatively short exposures were used to evaluate the *in situ* embeddedness chamber. Desired exposure times for benthic macroinvertebrate colonization tend to span at least 5

weeks (Roby *et al.,* 1978; Shaw and Minshall, 1980; Lamberti and Resh, 1985). Even so, one colonization study with an introduced substrate showed stabilization of colonizing macroinvertebrates between 9 and 14 days (Wise and Molles, 1979). The rapid accumulation of sediment in the embeddedness chambers during the pilot study provided confidence for evaluating the *in situ* embeddedness chambers and treatment areas after short exposures. A major assumption was that stream conditions would be relatively stable (i.e., low flow) and during such short exposure periods, thus minimizing confounding by any major environmental variables.

**Implications**

The development of an *in situ* embeddedness assessment technique would be a valuable stream assessment tool when conducting biological and physical habitat assessments of wadeable streams and rivers. Accurately assessing the *in situ* conditions that lead to embeddedness and the degradation of benthic macroinvertebrate habitat would be an improvement to the current methods of merely quantifying the percent embeddedness of a particular reach at one point in time. An *in situ* embeddedness assessment technique could provide an improved means of quantifying embeddedness that is quicker and yields less variance than the current subjective methods. Such an *in situ* technique would also provide a means of linking exposure effects more effectively.

# II. Literature Review

## Habitat Assessments and the Ecological Risk Assessment Process

The use of habitat assessments in the ecological risk assessment process provides a means of differentiating habitat changes resulting from physical, chemical, and biological factors (Rand and Newman, 1998). Habitat assessment methods are a structured, logical, and systematic approach for determining habitat alterations because they consider important life requirements and environmental variables crucial to organisms (Rand and Newman, 1998). There are a wide variety of habitat assessments that serve as important tools during all phases of an ecological risk assessment. Common habitat assessments include the USEPA Rapid Bioassessment Protocol (RBP) and the Ohio Environmental Protection Agency (OEPA) Qualitative Habitat Evaluation Index (QHEI). Rand and Newman (1998) conducted a review of the aforementioned assessments that discussed their applicability to ecological risk assessments: in the problem formulation phase, habitat assessments can be used to identify potential habitats that are at risk and subsequently used to determine assessment endpoints; in the analysis phase, they can be used to define exposure in habitats and to identify dose-response relationships. Moreover, in the risk characterization phase, habitat assessments can be used to emphasize the importance of habitats at risk. Finally, in the risk management phase, they can be used to define habitat management actions (Rand and Newman, 1998).

Habitat assessments are not without criticism. The qualitative nature of most methods has been questioned due to deficient study design and to the poor reproducibility of surveys due to variation among observers (Bauer and Ralph, 2001; Roper and Scarnecchia, 1995).

Conversely, Wang *et al.* (1996) reported that little variation existed among trained observers for most physical habitat variables. Wang *et al.* (1996) noted that if adequate initial training was conducted the experience of observers had little effect on the accuracy and precision of habitat estimates. However, the Wang *et al.* study (1996) cautioned that, aside from most other observed habitat variables, there was a high degree of variation among trained observers when estimating gravel embeddedness. Further, Bauer and Ralph (2001) emphasized the need for developing systematic procedures that meet rigorous data quality objectives. Bauer and Ralph pointed out that identical habitat indicators can be both quantitatively and qualitatively assessed and that information derived from these methods are not readily comparable.

Although arguments on both sides of the issue are valid, performing a habitat assessment is still a fundamental part of establishing the stressor-response relationship for use in an ecological risk assessment.

**Sediment in Streams**

In a recent USEPA (2006) report, approximately 25% of the nations' streams (167,092 miles) are in poor condition in terms of streambed sediment compared to a regional reference condition. Excessive sediments are ranked as the fourth highest stressor out of eight major stressors identified nationwide. Sediment becomes a stressor when excess inputs occur within a system as well as when normal levels of sediment become vectors of exposure to pollutant contamination. Along with geology, geomorphology, and vegetation, sediment input is a vital factor to consider when

assessing a watershed or aquatic system (Allan *et al.*, 1997). Sediment inputs are an important factor to consider when assessing a watershed or aquatic system along with geology, geomorphology, and vegetation (Allan *et al.*, 1997; Polf and Huryn, 1998; Church, 2002). Actions such as land disturbances and removal of native vegetation increase sediment delivery rates in contrast to the natural, dynamic processes in watersheds (Waters, 1995; Jones *et al.*, 2001). Impacts on stream ecosystems from large-scale agricultural operations have been well documented, and, in most cases, have been linked to declining water quality and increasing sediment loading (Waters, 1995; Johnson *et al*., 1997; Walser and Bart, 1999).

Certain land use practices, such as converting land to pasture, greatly increase the input of sediment directly onto certain substrate forms and the filling of interstitial spaces. One such substrate form is natural lateral gravel bars which tend to be inhabited by invertebrates whose patterns of distribution correlate strongly with water chemistry and surface/subsurface exchange (Boulton and Stanley, 1995). Important reactions between epi-benthic habitat and chemical and microbial processes occur within the hyporheos and can have substantial ramifications for surface biota (Boulton, 1993; Stanford and Ward, 1993; Findlay, 1995). As with any assessment of stressors within a system, a more holistic approach is required for a reliable interpretation. Elevated fine sediment loadings have different effects on hyporheic exchange and associated ecological processes depending on local hydrologic and geomorphic conditions (Brunke and Gonser, 1997; Rehg *et al*., 2005). Excess sediment within a lotic system has the potential to have detrimental effects on large-scale ecosystem functions that may be strongly influenced by hyporheic (subsurface) exchange processes. Hyporheic exchange strongly

influences the larger-scale transport of solutes and fine particulate matter to include nutrients, contaminants, dissolved organic carbon, and particulate organic carbon (Findlay, 1995; Brunke & Gonser, 1997; Mulholland *et al.,* 1997; Winter *et al.*, 1998; Jones & Mulholland, 2000; Minshall *et al.*, 2000).

Studies have shown that hyporheic exchange is an important component of both good quality habitat for aquatic invertebrates and for fish spawning (Ward *et al.*,, 1998; Baxter & Hauer, 2000) and that deposition of fine sediment into streambeds can greatly reduce hyporheic exchange (Packman & Mackay, 2003). Changes occurring within the hyporheic zone as a result of increased fine particulate deposition may include altered hydraulic conductivity and bulk porosity (Packman & Mackay, 2003; Rehg *et al.*, 2005). The clogging of the interstitial spaces with depositing fine particulates not only effects physical properties such as decreasing available habitat for biota, it also prevents the down-welling of oxygen-rich surface water and promotes the development of large hypoxic or anoxic zones that are further undesirable to hyporheic invertebrates (Boulton *et al.*, 1997). Studies have identified the relationship between large amounts of fine interstitial sediments and low hyporheic dissolved oxygen (Poole & Stewart, 1976; Strommer and Smock, 1989; Bretschko, 1994) and particles <1 mm in diameter are known to reduce the availability of dissolved in stream gravels (Tagart, 1984).

One of the major issues with sediments and fine particulates that have embedded within the interstices is the persistence of such conditions. Particles becoming trapped within the interstices of a streambed is an irreversible condition under steady streamflow conditions (Packman *et al.*, 2000), yet clay particles are readily remobilized due to increased shear stress when bed sediment transport occurs (Packman and Brooks, 2001).

Fine sediment accumulates primarily below the region of active bed sediment transport and this depth varies depending on the degree of mobilization of the bed (Rehn *et al.*, 2005). Streams with either continuous bed sediment transport or frequent episodic transport events relative to the input of fine sediments exhibit little fine sediment accumulation in the uppermost layers of the streambed, but there will still be accumulation at some depth in the bed (Rehn *et al.*, 2005). In streams experiencing episodic sediment transport events, seepage of fines into the deeper interstitial spaces occurring during inter-flood periods can further reduce permeability and dissolved oxygen (Boulton *et al.*, 1997). The periods of dynamic equilibrium with regard to discharge and flow may see reduced sediment loads, although previous deposition of fines can continue to be a stressor. The reduction of flushing flows to remove interstitial silt and clay has been shown to lead to low hyporheic dissolved oxygen concentrations and reduced colonization by surface benthos (Boulton *et al.*, 1997; Brunke and Gonser, 1997).

A recirculating flume study conducted by Rehg *et al.* (2005) showed clay concentrations within the water column reaching nearly zero after only 55 hours due to stream-subsurface exchange, settling, and filtration within the interstitial spaces of a homogenous sand bed. Their results, showing a lack of significant subsurface exchange, indicated that clay deposition in the uppermost layer of the substrate was quite effective in preventing water exchange through the substrate (Rehg *et al.*, 2005). Another study using an *in situ* flume evaluated the critical threshold required for entraining fine material. Results from this study indicated that loss of fine material within the substrate at a critical flow threshold caused larger substrate particles to shake and this caused an

increase in macroinvertebrate drift (Gibbons *et al.*, 2007). Though this study was not evaluating the direct effect of fine sediment on the biota, it did reinforce the idea that the relationship between fine sediment and biota within a system can be complex.

Physicochemical attributes associated with stream sediment pose multidimensional avenues of stress to aquatic environments. In streams experiencing contamination, fine colloidal particles have the potential to carry a large pollutant load (Axtmann and Luoma, 1991; Kimball *et al.*, 1995; Harvey and Fuller, 1998; Ren and Packman, 2002). Both suspended and deposited sediments have the potential to impair a system and have both direct and indirect negative effects on aquatic organisms. Some of these negative effects will be covered in the section devoted to biotic implications of excess sediment in the aquatic environment.

**Previous Studies on Fine Sediment and Biota**

There have been numerous studies (Lenat *et al.*, 1981; Richards and Bacon, 1994; Angradi, 1999; Runde and Hellenthal:, 2000; Rabeni *et al.*, 2005; Gibbons *et al.*, 2007; Bo *et al.*, 2007) employing a variety of methods that have evaluated the effects of fine sediment on aquatic biota. Research has shown the usefulness of recirculating flumes in understanding the variation of stream-subsurface exchange rates and obtaining results applicable to natural streams (Elliott and Brooks, 1997; Packman *et al.*, 2000; Ren and Packman, 2002; Wörman *et al.*, 2002). Packman *et al.* (2000) found that clay particles can be almost completely removed from suspension within a flume because particle trapping in the substrate becomes essentially irreversible under steady flow conditions.

Several field studies have been conducted to assess the relationship between fine sediment inputs and aquatic macroinvertebrates (i.e., Bjorn *et al.*, 1977; Wesch *et al.*, 1989; Richards and Bacon, 1994; Runde and Hellenthal, 2000; Kaller and Hartman, 2004; Bo *et al.*, 2007). A number of approaches have had similar results showing a negative correlation between fine sediment and macroinvertebrate densities. Richards and Bacon (1994) used small basket samplers called Whitlock Vibert (WV) boxes to assess the effect of varying levels of fine sediment substrate on macroinvertebrate colonization within uniformly size gravel. Their design used two WV box positions within the stream substrate (flush with the surrounding substrate surface and buried ~30 cm below the substrate surface). After a 10 wk exposure, their results showed a larger amount of fine sediment accumulation in the below-surface boxes than in the surface boxes (Richards and Bacon, 1994). Their experiments with fine sediment accumulation and macroinvertebrate colonization indicated that fine sediment abundance did have distinct effects on macroinvertebrate colonization within the hyporheos (Richards and Bacon, 1994).

These results reinforce the theory that deposited fine sediments within a streambed will infiltrate fairly deep within a substrate in the absence of flushing stream flows. A recent study (Bo *et al*., 2007) in northwest Italy used an approach to that of Richards and Bacon (1994) using sediment accumulation traps filled with varying mixtures of gravel and sand substrate. The intent of this study was to assess how fine sediment accumulation can influence the colonization process and community composition of macroinvertebrates (Bo et al, 2007). The traps were randomly placed in a

riffle habitat with the apex of the traps flush with the surrounding substrate. Forty eight traps were used during two sampling periods (20 & 40 days) to evaluate the macroinvertebrate colonization of selected traps. Results showed significant differences in number of taxa and decreases in abundance with increased substrate clogging.

**Biotic Implications of Increased Sediment Input**

Changes in macroinvertebrate assemblages have been routinely used in assessing habitat and other facets of lotic systems in part because they exhibit consistent long-term changes to watershed activities that influence substrate characteristics (Richards and Minshall, 1992). Aquatic macroinvertebrates and vertebrates are exposed to a multitude of stressors that can have synergistic effects and cause considerable impairment. Contaminant exposure, excess nutrients, and excess sediment plague the biota of many aquatic systems throughout the U. S. As covered in a previous section, excess stream sediment has been recognized as a major stream stressor with both physical and chemical impacts. Distribution of benthic macroinvertebrates is influenced in part by the amount of fine sediment within the substrate. Effects of excess fine sediment on stream macroinvertebrates can range from small changes in abundance or assemblage structure to the complete replacement of a cobble/gravel habitat-adapted assemblage by a sand habitat-adapted assemblage (Lenat *et al.*, 1979, Lenat *et al.*, 1981). The results of numerous studies suggest that substrate size and composition determine the distribution of benthic taxa (Minshall, 1984) and those substrates composed of smaller particles exhibit lower numbers of taxa and lower productivity (Nuttall, 1972; Cederholn and Lestelle, 1974; Allan, 1975; Ward, 1975; Alexander and Hansen, 1986).

Decreases in substrate heterogeneity also tend to decrease richness of benthic invertebrates (Vinson and Hawkins, 1998). An early study by Bjorn *et al.* (1977) found macroinvertebrate densities to be greater in riffles with low amounts of fine sediment than riffles with higher proportions of fines. Exposure to sustained suspended sediment loads tend to clog feeding structures and reduce feeding efficiency of filter feeding macroinvertebrates (Hynes, 1970). Increases in suspended sediment loads have also been shown to decrease algal productivity which can have a detrimental effect on secondary productivity (Newcomb and MacDonald, 1991). Kaufmann and Hughes (2006) found that streambed instability and an increase in fine particle inputs associated with riparian disturbance and road construction were highly correlated with low abundance and richness of salmonids, tailed frogs, and other coldwater and sediment intolerant taxa.

Uneven distribution of benthic macroinvertebrates within the substrate results from a variety of physical and chemical factors. Maridet *et al.* (1992) suggested that effective porosity of stream substrate is the primary factor determining the vertical distribution of invertebrates. Substrate size can influence the velocity within the substrate and also provides interstitial space for a variety of benthic dwelling organisms to inhabit (Maridet and Philippe, 1995; Lazorchak, 1998).

**Embeddedness**

A vital physical characteristic of aquatic habitats, particularly lotic systems, is the degree of substrate embeddedness that occurs as a result of fine sediments filling in the interstitial spaces in the streambed. Although alteration of streambed habitat is recognized as one of the most important stressors for benthic organisms, the reliability of

the outcomes from the varying methodologies for measuring embeddedness has been questioned (Sylte and Fischenich, 2002). Misconceptions exist about the term *embeddedness* which has led to inaccurate portrayals of embeddedness and the physical factors it describes. Two of these common misconceptions are embeddedness as a direct measure of the volume of fine sediment and as a measure of substrate mobility (Sylte and Fischenich, 2002).

Researchers have formulated a multitude of definitions for embeddedness. Sylte and Fischenich (2002) listed a number of these definitions in their review of techniques for measuring embeddedness. Their summary of the definition of embeddedness seems adequate: the degree to which larger substrate particles are covered with finer particles. This definition uses a length term which represents a volume of fines surrounding coarser substrates, and is placed in relative proportion to rock height in the plane of embeddedness (Figure 1) (Sylte & Fischenich, 2002).

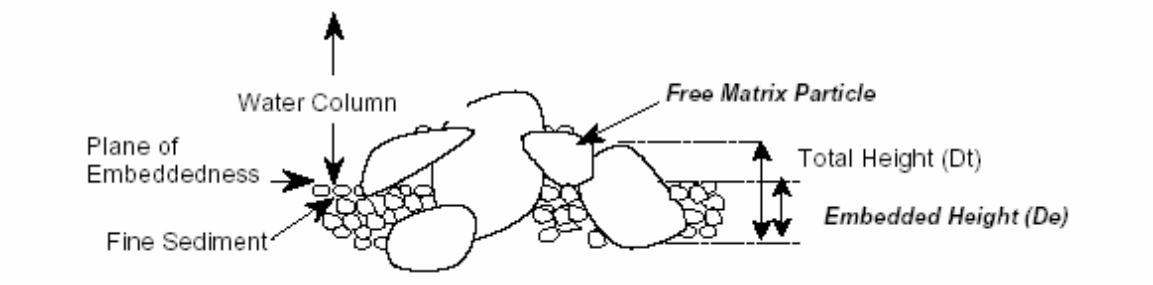



**Figure 1. Schematic Representation of Embeddedness (Sylte and Fischenich, 2002)**

Similarly, Whitman *et al.* (2003) defined stream embeddedness as a description of the extent to which fines the size of sand or smaller (≤2mm) fill the interstices between larger streambed particles.

A theme in many of the embeddedness definitions is the description of the filling of voids or spaces around larger substrate particles (commonly referred to as interstitial spaces) by smaller particles (referred to as fines). This interstitial space is a vital characteristic in the suitability of stream substrates for supporting aquatic organisms. Benthic invertebrates are often used in monitoring sediment conditions in streams because substrate (and interstitial space) is believed to be the most important factor in benthic invertebrate distribution and abundance (Cummins and Lauff, 1968; Minshall, 1984; Quinn and Hickey, 1990). Rabeni *et al.* (2005) appropriately identified benthic invertebrates as potential receptors in a watercourse that is experiencing sedimentation due to the influence of deposited sediment on stream substrate conditions. The results of many studies (Lemly, 1982; Zweig and Rabeni, 2001; Rabeni et al., 2005) have shown that increasing levels of deposited sediment have resulted in a decrease in invertebrate density and tax richness, both of which are used as metrics to deposited sediment. Waters (1995) presents a dramatic example of the effect of deposited sediment on organisms: A gravel washing operation in the Truckee River in California experienced a 90 % reduction in bottom fauna densities and biomass after up to a foot of fine sediment was deposited on the river bottom.

The United States Geological Survey (USGS) official definition of sedimentation is the act or process of forming or accumulating sediment in layers; the process of deposition of sediment (Fitzpatrick, 1998). This definition describes the process of

sediments accumulating but does not imply that the process includes the filling of interstitial spaces. Waters (1995) concedes that measuring the degree of streambed sedimentation is difficult in terms that have biological meaning. Waters (1995) also suggests possible terms that break sedimentation into aspects, such as area of streambed covered, depth of coverage, percent of defined fine, and percent saturation of interstitial space or embeddedness.

Measures of embeddedness need to be clearly separated from other sediment related factors in order for specific physical properties to be identified in stressor-response relationships. The USEPA's RBP differentiates between sediment deposition and embeddedness: embeddedness being the extent to which rocks and snags are covered or sunken into the silt, sand, or mud of a stream bottom; and sediment deposition, being the amount of sediment that has accumulated in pools as a result of large-scale movement of sediment (Barbour, et al.,1999). The results of studies by Bjorn *et al.* (1974, 1977) concluded that a 33 % or less level of embeddedness is probably the normal operating range in proper functioning streams. Waters (1995) pointed out that Bjorn *et al.*'s (1977) conclusions showed that, at embeddedness levels greater than 33 %, insect abundance decreased by about 50 % and mayflies and stonefly numbers increased dramatically once the study section of stream was cleared of fine sediment.

**Methods for Quantifying Embeddedness**

Increases in deposited sediment and subsequent embeddedness of substrate have a negative effect on most benthic invertebrates. However, there is much contention about

actual methods of measuring substrate embeddedness. This section will summarize some common methods of quantifying substrate embeddedness and will comment on published limitations and requirements for further study.

There are a few methods of measuring embeddedness (Platts *et al*., 1983; Burns, 1984; Plafkin *et al*., 1989; Skille and King, 1989; Fitzpatrick, 1998; Osmundson and Scheer, 1998; Bain and Stevenson, 1999), but there is no comprehensive standard that actually describes embeddedness and how to quantify it. Sylte and Fischenich (2002) reviewed the most common methods of embeddedness measurement techniques compiled from journal articles, agency reports, and personal files of those involved in the development of the techniques. Some of the methods are simply refinements of earlier methods. The majority of the published works are for use in wadeable streams or rivers. In addition, Edsall *et al.* (1997) describes some alternative techniques for surveying the physical habitat of large rivers.

***Platts/Bain Method.***

The Platts/Bain method was developed from two studies, one by Platts *et al*. (1983) and the other by Bain and Stevenson (1999). According to the Platts/Bain method, embeddedness measures in terms of surface area the degree to which larger particles are surrounded or covered by fine sediment. Descriptions such as negligible, low, moderate, high, and very high are designated by researchers as ratings in five or more representative habitats at midstream locations. This method is a very subjective substrate embeddedness technique that has the potential to show a wide range of results among different observers.

***Burns Method.***

The Burns method (unpublished data) was described in a 1984 study examining of embeddedness of salmonid habitat in Idaho. The method used embeddedness levels to refer to the proportion of an individual particle surrounded by fine sediment. Substrate particles considered were 4.5 cm to 30 cm and fines were defined as particles less than 6.3 mm in diameter. The proportion of particle surrounded by fine sediment was calculated by dividing the embedded depth by the total depth of rock that lies perpendicular to the plane of embeddedness (Figure 1) (Burns and Edward, 1985). This method treated an embeddedness measurement made for one rock as one observation. Moreover, it employed a 60 cm steel hoop to delineate the area of the substrate to be examined (making at least 100 observations). Then, particles within the sampling hoop were measured for dimensions using a 30 cm transparent ruler. Requirements for particular stream velocities (float time across the hoop) and stream depth were sought to determine suitable winter cover for over-wintering salmonids (Sylte and Fischenich, 2002).

***BSK Method.***

Developed by Skille and King (1989), the method is a modified version of the Burns method. It is essentially the same procedure as the Burns method except that the BSK method does not focus hoop placement on specific substrates. The BSK method allows for a randomized sampling technique that provides data that representing the entire stream reach.

### *United States Fish & Wildlife Service-Upper Colorado River Measurement Method.*

This Upper Colorado River Measurement Method of the United States Fish & Wildlife Service (USFW) quantifies embeddedness by measuring the amount of particle exposed or depth to embeddedness (DTE) rather than the percent embedded measurements utilized by the other methods (Sylte and Fischenich, 2002). Twenty measurements that include one run and one riffle are taken at 15 sites. This technique consists of laying one hand on the substrate particle surface layer while holding the other hand perpendicular to the first, then extending the fingers down between the thumb and forefinger of the first hand until the tip of the index finger reaches the top of the layer of embeddedness (Osmundson and Scheer, 1998). These embeddedness measurements are then averaged to represent the site. An interesting aspect of this method is that it takes into account the number of rocks above the embeddedness line. Sylte and Fischenich (2002) note that this method is better for assessing a specific site over time rather than drawing conclusions between sites because of the dependence of depth to embeddedness on substrate particle distribution.

### **USEPA Environmental Monitoring and Assessment Program (EMAP) Method.**

The USEPA RBP (1999) states that embeddedness observations should be taken upstream and within central portions of riffles and cobble substrate areas in order to avoid confusion with sediment deposition. The EPA's EMAP method for determining substrate embeddedness is derived from a combination of methods adapted from Wolman (1954),

Bain *et al.* (1985), Platts *et al.* (1983), and Plafkin *et al.* (1989). A cross-section is defined by laying a surveyors rod or tape to span the wetted channel. For this method, five substrate particles (those larger than sand) from each of 11 transects are evaluated for surface stains, markings, and algae. The average percent embeddedness of the particles within a 10 cm diameter of the measuring rod is recorded. As a result of this method, embeddedness therefore is classified as the fraction of the particle's surface that is surrounded by (embedded in) fine sediments on the stream bottom. Substrate particle sizes are visually estimated and classified into categories according to the Wentworth Scale. As a reference, the method defines sands and fines being embedded 100 % and bedrock and hardpan being embedded 0 %. Since it is strictly a visual method, this approach is very subjective

***USGS National Water Quality Assessment Program Method.***

As described in the USGS (1998) substrate particle size is measured and percent embeddedness is estimated. This method characterizes stream substrate utilizing the Wolman (1954) pebble count method for courser particles and a laboratory analysis for sand or finer material. These quantitative measurements are particularly useful for analyzing fish and invertebrate habitats (Fitzpatrick *et al.*, 1998). Substrate embeddedness is determined much like the USEPA (1998) EMAP method, estimating to the nearest 10 %, the percentage of the surface area of gravel or larger particles that is covered by sand or fines. To minimize subjectivity, this method recommends the use of a graded ruler or calipers to measure the height of the embedding mark (Figure 2) on the substrate particle as a percentage of the total height of the particle.

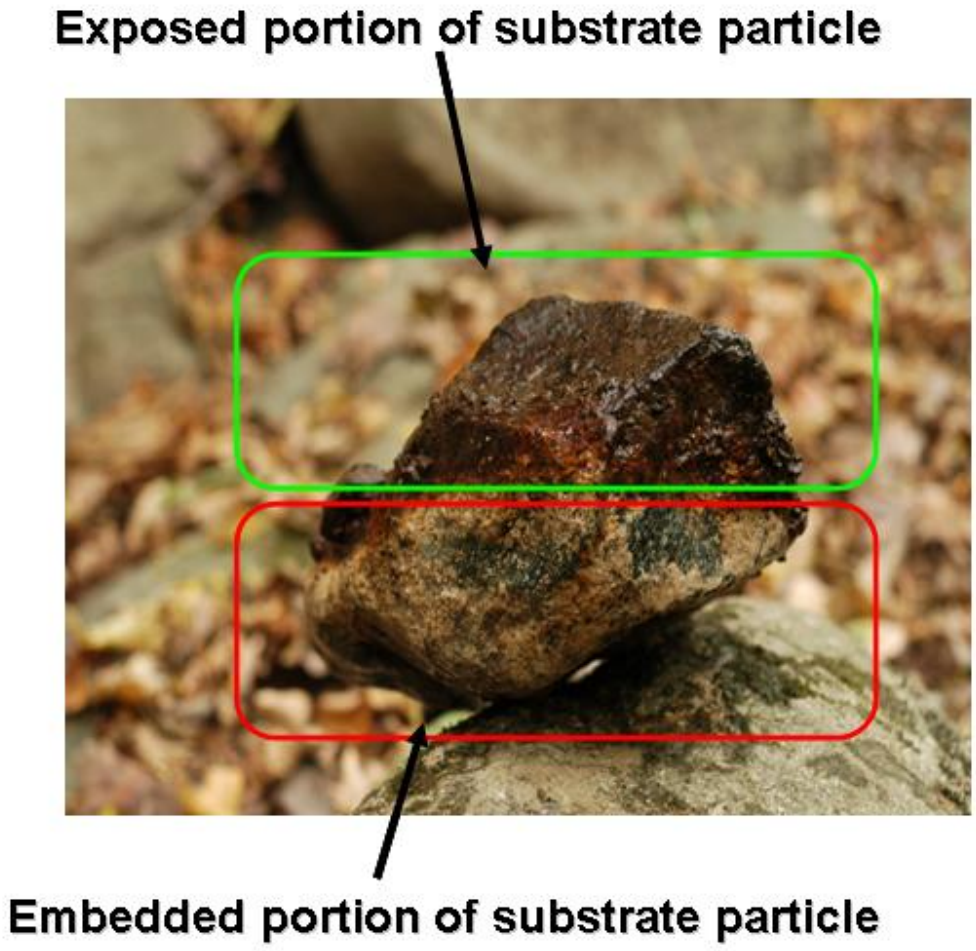



**Figure 2. Embedded Substrate Particle from Honey Creek.**

***Additional Embeddedness Measurement Techniques.***

Whitman *et al.* (2003) developed photographic techniques to characterize coarse (>2 mm) and fine (<2 mm) substrate particle sizes and compared their results with other sampling techniques such as Platts *et al.* (1983) and the Wolman (1954) pebble count. The method provides a quantitative result of embeddedness through digitizing surface

fines and evaluating the photographs. The photographic techniques developed yielded a greater number of particles measured compared to the Wolman pebble count. Interestingly, the results of embeddedness indices from field and photographic observations had significant variation in five out of the twelve sampling reaches (Whitman *et al.*, 2003). This method does have limitations such as camera cost and film processing logistics. Wang *et al.* (1996) reported that photographic techniques for analyzing substrates is limited to clear shallow areas and takes considerably longer to perform overall than visual techniques. If time is not a factor, digital photography seems like an obvious choice when experimenting with photographic techniques for determining embeddedness.

Freeze core sampling is a technique that has been used to obtain streambed samples for analysis. Rood and Church (1994) used a McNeil-freeze core apparatus to collect samples of alluvial gravel in Alaska. This method involved two individuals working a core barrel 30 cm into the substrate. Subsequently, a freeze core probe using liquid nitrogen was utilized to deliver extremely cold temperatures to the substrate sample. Once the core was frozen, it was removed for substrate analysis. Some drawbacks of this technique include the material costs involved and the inadequacy of the core samples to characterize the complete grain size in cobble/gravel bed rivers (Rood and Church, 1994).

Although the existing methodologies for determining substrate embeddedness have some similarities, the different qualitative and quantitative results make it difficult to compare data among different studies using various methods. Recently, the USEPA

(2006) finalized a report of a two year wadeable streams assessment that was conducted across the entire U.S. This research was initiated, in part, from a 2000 report from the General Accounting Office (GAO) that noted the EPA and the states were unable to make statistically valid inferences about water quality. Moreover, this GAO report claimed that insufficient data existed to support management decisions (USEPA, 2006). A portion of this study included comparisons of the different sampling protocols applied by the EMAP, USGS, and state agency methods; a current analysis is underway to explore how new data can be used (USEPA, 2006). A nationally standardized methodology for determining embeddedness would serve as a valuable tool in quantifying such a critical physicochemical stressor on aquatic organisms.

Habitat assessments have been primarily used as management tools to evaluate the impacts of development because they allow comparisons of different habitats and habitat characteristics of various areas at the same point in time and the same area at future points in time (Rand and Newman, 1998). Refining habitat evaluations such as stream substrate embeddedness techniques are essential in all phases of the ecological risk assessment process. Even so, further exploration and enhancement of standard techniques are imperative. Existing methods for determining substrate embeddedness were developed to capture a point in time estimate. What has not been addressed in the literature is the development of a method to determine embeddedness on an event basis. Developing a method to determine embeddedness on an event basis would be useful in ascertaining the magnitude of a stressor per event. Additional research adapting embeddedness quantification techniques to *in situ* toxicity testing techniques may provide valuable insights in linking physical stressors to effects on receptors.

## III. Methodology

### Test Sites

Test sites were selected after reviewing several previous biological and water quality assessments (OEPA, 1996 and 2001). Sites that exhibited a fairly high degree of water quality and a diverse macroinvertebrate assemblage were selected. This research intended to assess two different stream orders to evaluate the magnitude of physical and biological differences that have been described in the River Continuum Concept (Vannote *et al.*, 1980).

#### *Honey Creek.*

Honey Creek, Clark County, Ohio (latitude and longitude N 39° 58' 17.8"/W 084° 08' 07.5") (Figure 3) was chosen as a small order stream site for this research because of its high water quality, and diverse macroinvertebrate community. With headwaters located in Champaign County, Honey Creek flows south through Clark County and eventually into Miami County, where it ends at its confluence with the Great Miami River. Honey Creek drains an area approximately 91.6 square miles and is 18.6 miles long with an average gradient of 19.6 ft/mile (OEPA, 1996). Honey Creek exhibits many positive physical habitat attributes such as an abundance of functional in-stream cover, coarse glacial substrates, pooled areas greater than 40 centimeters in depth, and a persistent wooded riparian corridor (OEPA, 1996). The upper reaches of the creek have been designated as Exceptional Warmwater Habitat (EWH) by the Ohio Environmental Protection Agency (OAC). A biological and water quality study conducted of the Upper Great Miami River in 1995, which included Honey Creek, inferred that mild organic enrichment from the New Carlisle Wastewater Treatment Plant (WWTP) was likely a

contributing factor to the middle and lower reaches of Honey Creek not meeting the EWH criteria. A Qualitative Habitat Evaluation Index conducted as part of the 1995 OEPA study found Honey Creek having a mean reach QHEI value of 74.3, which suggested that the near in-stream habitats were sufficient to support a community of aquatic organisms consistent with EWH biological criteria (Rankin, 1989).

The section of the creek where the embeddedness study was conducted was located approximately 370 meters upstream of the location where State Route 202 crosses over Honey Creek. During the 1995 biological and water quality study, the macroinvertebrate community in this section of Honey Creek (downstream of RM 3.2) was similar to that of the upper section of the creek (upstream of the New Carlisle WWTP RM 10), with Invertebrate Community Index (ICI) scores of 44 and 40, respectively. In addition, the section of Honey Creek where the embeddedness study was conducted exhibited an EPT taxa richness of 25 (OEPA, 1996). For the EMB study, the aquatic macroinvertebrate community was sampled (using a D-Frame dip net) once on September 28, 2007, according to the USEPA RBP multi-habitat approach (Barbour *et al.*, 1999). The sampling sites for the EMB chambers and the treatment areas were located in a run approximately 0.5 meters downstream of a small riffle area. The riparian area along the test site was stable with many hardwood trees and low lying shrubs along the banks of the river. The river substrate consisted mainly of cobbles and boulders with sand and pebbles mixed throughout. Stream depth at the sampling site averaged approximately 0.25 meters.

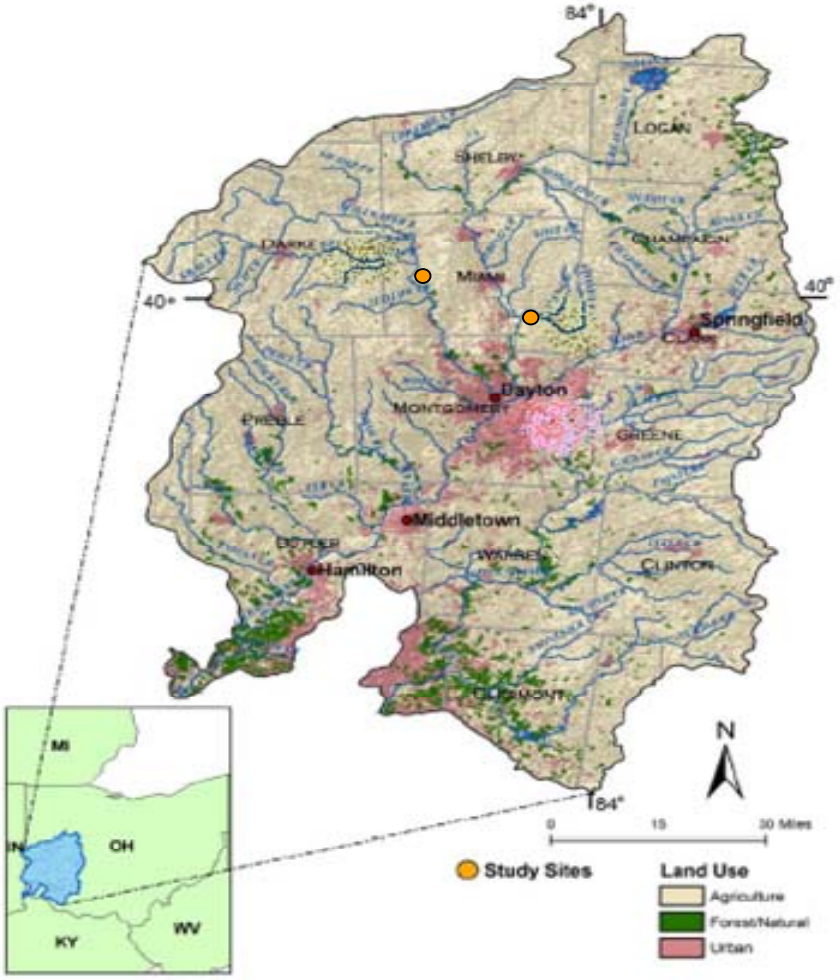



**Figure 3. Map of both study sites and land use in the Great and Little Miami River Basins, Southwest Ohio. Honey Creek (Clark County) was the small order stream site and the Stillwater River (Miami County) was the medium order stream site.**

***Stillwater River.***

The Stillwater River, Miami County, Ohio (latitude and longitude N 40° 05' 54.1"/W 084° 21' 16.0") (Figure 3) was chosen as a site for this research because it also exhibits high water quality and a diverse macroinvertebrate community. Relative to the Honey Creek test site, the Stillwater River serves as a larger stream order site. The Stillwater River is designated as EWH from RM 57 to its confluence with the Great Miami River just north of Dayton (OEPA, 2001). The Stillwater's headwaters are located in eastern Indiana and the northern part of Darke County; it flows east through Darke County into Miami County. The river then flows south through Miami County into Montgomery County until it intersects the Great Miami River. The Stillwater River is 67.2 miles in length and its watershed covers approximately 673 square miles with an average gradient of 4.2 feet per mile (OEPA, 2001). The primary land uses within the watershed are agriculture (80%), pasture, woodland, and urban (totaling ~11%), with light manufacturing in some areas. There are roughly 25 point source facilities contributing pollution within the watershed; the larger sources are the WWTPs spread throughout the watershed (OEPA, 2001). Use designations for the Stillwater River are Industrial Water Supply, Agricultural Water Supply, Primary Contact for Recreation, and Public Water Supply at RM 18 (OEPA, 2001). No sediment bioassay toxicity was detected downstream of the Covington WWTP; its effluent was identified as having no receiving water impairment (OEPA, 2001).

The sampling site for this EMB study was located at ~RM 30, approximately 200 m upstream from the Faulknor Road Bridge. This section of the river exhibited the highest Composited Index Scores during a 2001 study and relatively high scores during

previous studies conducted in 1990 and 1982 (OEPA, 2001:). For the EMB study, the aquatic macroinvertebrate community was sampled using a D-Frame dip net once on 28-September-07, according to the USEPA RBP multi-habitat approach (Barbour *et al.*, 1999). The sampling sites for the EMB chambers and the treatment areas were located in a run approximately 2-3 meters downstream of a riffle area. Comparable to the Honey Creek zone, the riparian area along the test site was stable with many hardwood trees and low lying shrubs along the banks of the river. The river substrate consisted mainly of cobbles and boulders with sand and pebbles mixed throughout. Stream depth at the sampling site averaged approximately 0.5 meters.

**Experimental Design**

Embeddedness chambers were placed at the head of a run habitat that exhibited adequate flow. Immediately downstream of the chambers, three substrate treatment areas were established in a run habitat. These treatment areas were established to assess benthic macroinvertebrate colonization rates by mimicking different levels of substrate disturbance (disturbed, slightly disturbed, and undisturbed) by removing the embedded fine sediments. A random sampling scheme was developed to sample both the embeddedness chambers (for macroinvertebrates and sediment) and the treatment areas (for macroinvertebrates only) at three different time periods. The random sampling design accounted for non-uniform stream conditions across the width of the sampling area and for the natural patchy distribution of benthic macroinvertebrates (Pringle *et al.*, 1988).

Percent embeddedness measurements were taken at each site only once during the course of the study. A substrate sample was collected at each site and analyzed for possible sediment toxicity. Diffusive Gradient in Thin Films (DGTs) were exposed for 24 hours in each of the treatment areas at both sites and analyzed for any metal (Zn, Cd, Ni, Cu, and Pb) toxicity that may have influenced macroinvertebrate colonization. DGTs measure the concentration of chemical species or fluxes from solid phase to solution in bulk sediment, such as in the pore water of interstitial spaces (Davison *et al.*, 2000). Water quality parameters; temperature, pH, conductivity, dissolved oxygen, and turbidity were monitored in addition to stream flow. Water samples for the determination of total suspended solids (TSS) were taken at different intervals throughout the embeddedness chamber exposures. The majority of the field work for this study occurred from 1-October-07 to 22-October-07.

## Embeddedness Chamber Development

### *Chamber Design.*

The embeddedness chamber was developed using a previously designed *in situ* toxicity chamber (Burton *et al.*, 2005), with some substantial modifications to meet the requirements for a completely flow-through system. Unlike the *in situ* toxicity chambers, the embeddedness chamber was designed to allow organisms and larger sediment access into the interior of the chamber. Initially, the embeddedness chambers were created with smaller mesh sizes (1 & 2 mm), but were revised with a larger 4 mm opening mesh to allow for more infiltration of organisms and sediment. Furthermore, the embeddedness

chambers originally were designed with no holes in the end caps, but were then modified with 4 mm holes drilled into each end cap. These holes were added to allow the chamber design to accommodate hyporheic (subsurface) flow and provide an environment more closely exhibiting in-stream conditions.

The *in situ* embeddedness chamber was constructed of cylindrical cellulose acetate butyrate tubing with a 6.67 cm inner diameter (ID), 6.98 cm outer diameter (OD), 0.16 cm wall thickness, and a length of 12.7 cm. Two rectangular sections (8.5 cm x 4 cm) were removed on each core tube leaving an 8.5 cm x 1.0 cm section of the tube remaining intact on the top portion of the chamber for support (Figure 4). Each end of the cylindrical tubing was capped with a polyethylene closure that had approximately 1/3 of the end portion removed (Figure 5). Each end cap had nine evenly spaced 4 mm holes drilled into the flat end portion of the cap (Figure 5). The constructed bare chamber with caps was then enclosed with mesh with 4 cm diameter openings (soft nylon laundry bag mesh) using DAP® clear silicone sealant (Figure 6). The nylon mesh was affixed only to one end cap, allowing for easy removal of the other end cap. When complete, the chamber resembled a slightly reinforced, lengthwise half-cylinder with a flat mesh top (Figure 7). Next, the chamber was filled with precisely 110 clear glass spherical marbles, all with a uniform 14 mm diameter. The non-secured end cap was placed on the chamber and the mesh flap was pulled tight over the cap. A plastic zip tie was tightened around the circumference of the cap securing the mesh flap in place and the marbles in the chamber.

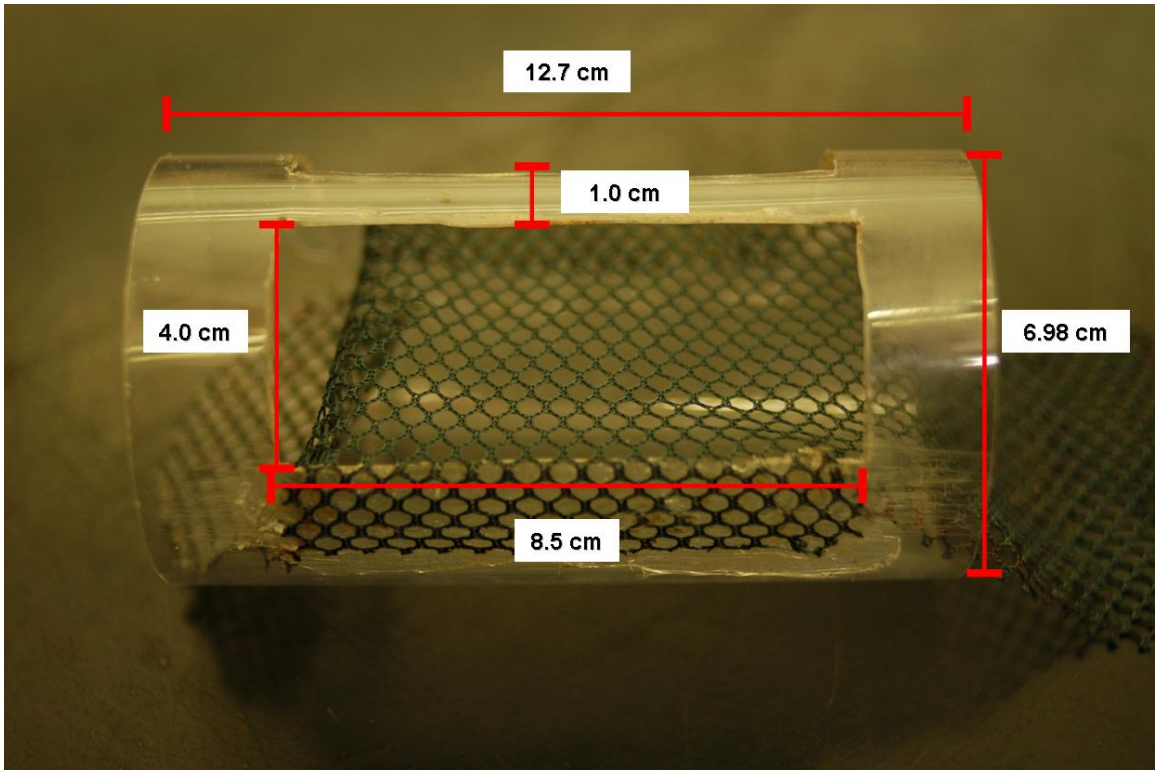



**Figure 4. Unassembled Embeddedness Chamber and Dimensions with Nylon Mesh Attached.**

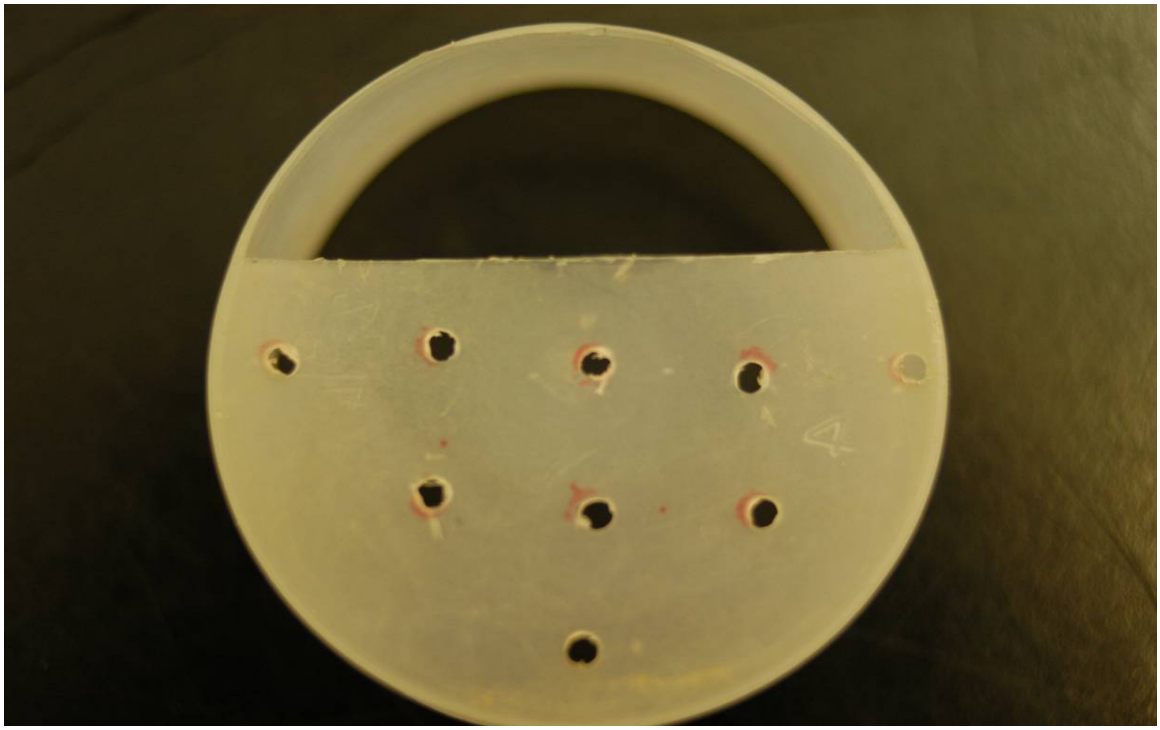


**Figure 5. Unassembled Polyethylene End Cap with 4 mm Holes to Allow for Subsurface Flow Through the Chambers.**

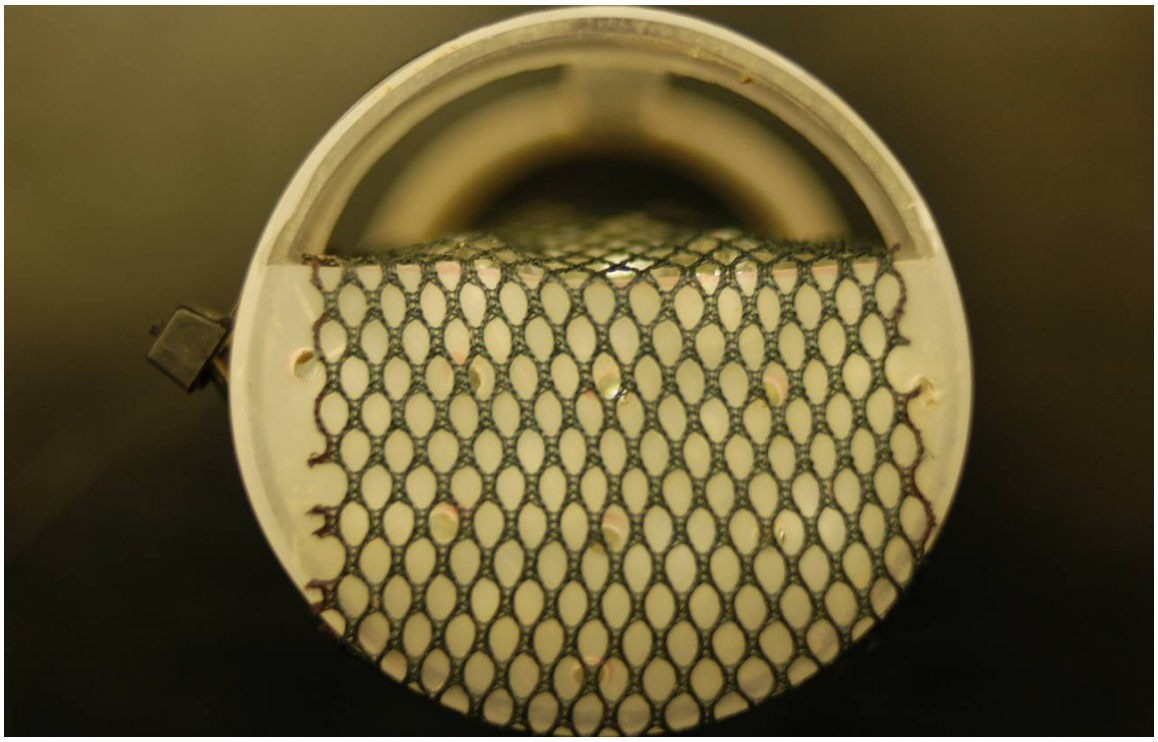


**Figure 6. Assembled Polyethylene End Cap and Flow-through View of Embeddedness Chamber.**

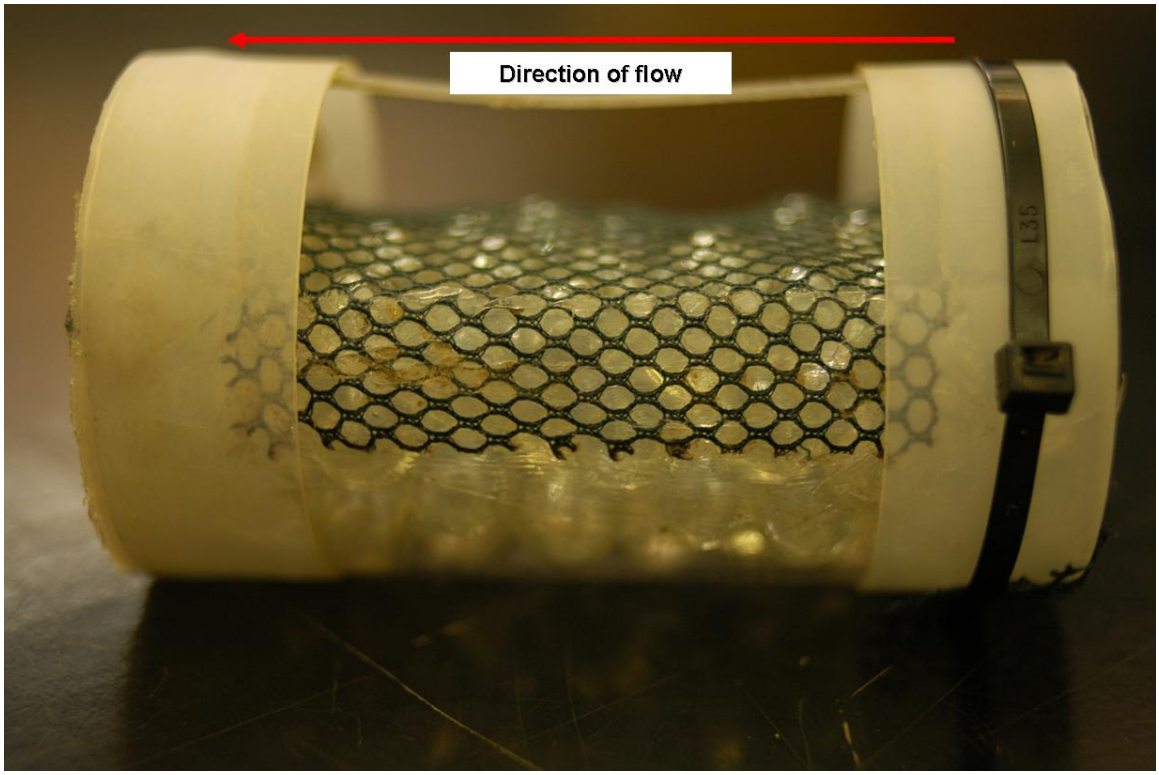



**Figure 7. Fully Assembled Embeddedness Chamber Ready for Deployment.**

***Embeddedness Chamber Cover.***

Covers were designed to minimize any unwanted sediment depositing in the chambers during deployment and to minimize any sediment loss from the chamber upon retrieval. A 12.7 cm length of cylindrical cellulose acetate butyrate tubing (the same used to construct the chambers) was cut lengthwise in half and then a polyethylene cap was hot-glued to each end of the section of tubing. The polyethylene caps were then trimmed so a circular flap would cover the holes in the chamber caps when the cover was installed on the chamber (Figure 8).

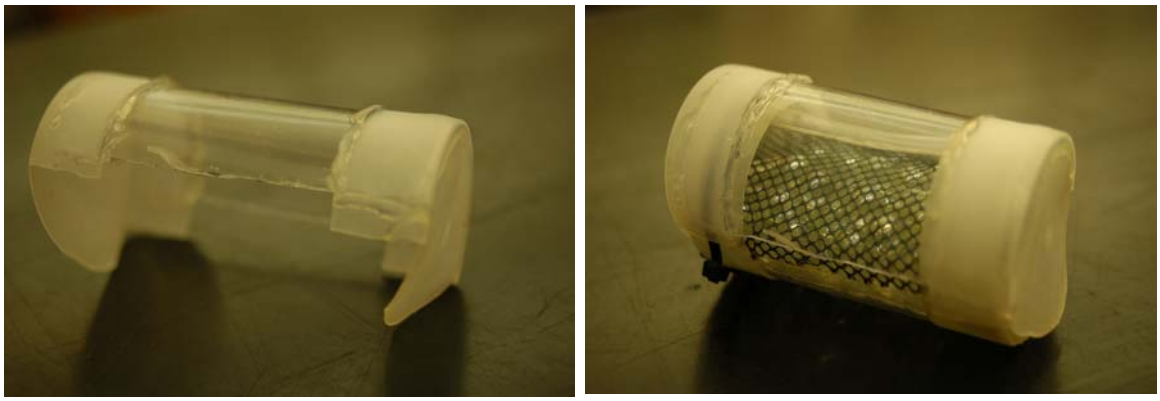


**Figure 8. Embeddedness Chamber Deployment and Retrieval Cover.**

**Pilot Study**

A pilot study was conducted with the preliminary design of the embeddedness chamber (1 mm mesh, no end cap holes) to gather data that was analyzed to determine the number of replicates needed to determine a significant difference (at the desired confidence interval) between sites. Accumulated sediment weight was the only endpoint for this study. The original experimental design planned for conducting the study on one

river and analyzing the differences between sites. Four sites were used on the Mad River within the city limits of Dayton, Ohio. A specific location was chosen at each site that allowed for adequate flow across the chambers. Each tray location exhibited a run habitat that was approximately 1/4 of a meter deep.

***Chamber Deployment.***

Four chambers were secured to each metal *in situ* basket and two baskets were placed at each site, equaling a total of eight embeddedness chambers per site. At each site both baskets were placed adjacent to each other in similar conditions. *In situ* baskets were partially buried in a cleared out section of the streambed so that when the chambers were installed the flat mesh portion would be relatively flush with the surrounding undisturbed substrate. The baskets were placed perpendicular to the flow at each site. Each *in situ* basket was secured by driving a metal rod into the streambed and then securing the basket to the rod with a plastic zip tie. Each chamber was carefully placed into the *in situ* basket (with the end cap with the affixed mesh facing upstream) and secured by elastic straps.

Once the chambers were secured, substrate that was removed to bury the basket was then carefully arranged around the upstream and downstream sections of the chambers so that the flat upper portions of the chambers were flush with the surrounding undisturbed sediment. Care was taken to not disturb any fine sediment while ensuring the substrate around the chambers was flush. Care also taken to not walk upstream of the chambers once they were secured in the baskets.

### *Chamber Retrieval.*

The embeddedness chambers were retrieved after seven days. Each chamber was individually removed from the *in situ* basket and sealed in a plastic bag. Care when removing the chambers from the river prevented any significant loss of collected sediment.

### *Sample Processing.*

In the laboratory, each chamber was removed from its bag and emptied into a 1 mm sieve and into a collection tray. The retained marbles were rinsed with water and the remaining sediment and water mixture was poured into a 500 mL pre-weighed glass beaker. Then the beakers were dried in an oven at 104 °C for seven days. Subsequently, they were reweighed to obtain a dry sediment weight.

## Primary Embeddedness Chamber Experiment

### *Embeddedness Chamber Deployment.*

Improvements derived from the pilot study were incorporated into the final chamber deployment. Changes from the pilot study included a larger mesh size (4 mm) on embeddedness chambers, a total of 24 deployed chambers per site, utilization of a different *in situ* tray, the use of macroinvertebrate colonization as an endpoint, and the securing of chambers to *in situ* baskets with zip ties instead of elastic straps.

In preparation for the final deployment, a total of 24 chambers were prepped in the lab, with each chamber receiving precisely 110 clear glass marbles. Loose caps were installed on each chamber and mesh flaps were secured with zip ties. Prior to installing

the *in situ* trays in the stream, three embeddedness chambers were secured to one metal *in situ* tray using two zip ties per chamber, one around each rigid end of the chamber (Figure 9). The three chambers were placed adjacent to each other so there was no space between the sides of the chambers (Figure 9). Once the chamber covers were installed on each chamber, the *in situ* tray was installed in the stream. The placement area for the *in situ* tray was carefully prepared by removing enough substrate so that the flat portion of the chamber would be flush with the surrounding undisturbed substrate when the trays were in place (Figure 10).

A total of eight *in situ* trays were installed at each site, with each tray being positioned so that the end cap with the adhered mesh was facing perpendicular to (into) the stream flow. *In situ* trays were all placed adjacent to each other, each tray end flush to the next, across the width of the sampling area (Figure 11). Each tray was secured with plastic zip ties to a steel rod driven into the substrate on either side of the tray. Once the trays were secured in place, the substrate that was removed to allow tray placement was then carefully spread around the chambers and trays so that the substrate was level with the undisturbed substrate in the area. After the site was allowed to settle for ~2 minutes the chamber covers were removed. When deployed, only the upper halves of the embeddedness chambers were visible (Figure 10). As with the pilot study, care was taken to avoid walking upstream of the chambers and disturbing any substrate that would cause fine sediment to flow across the deployed chambers.

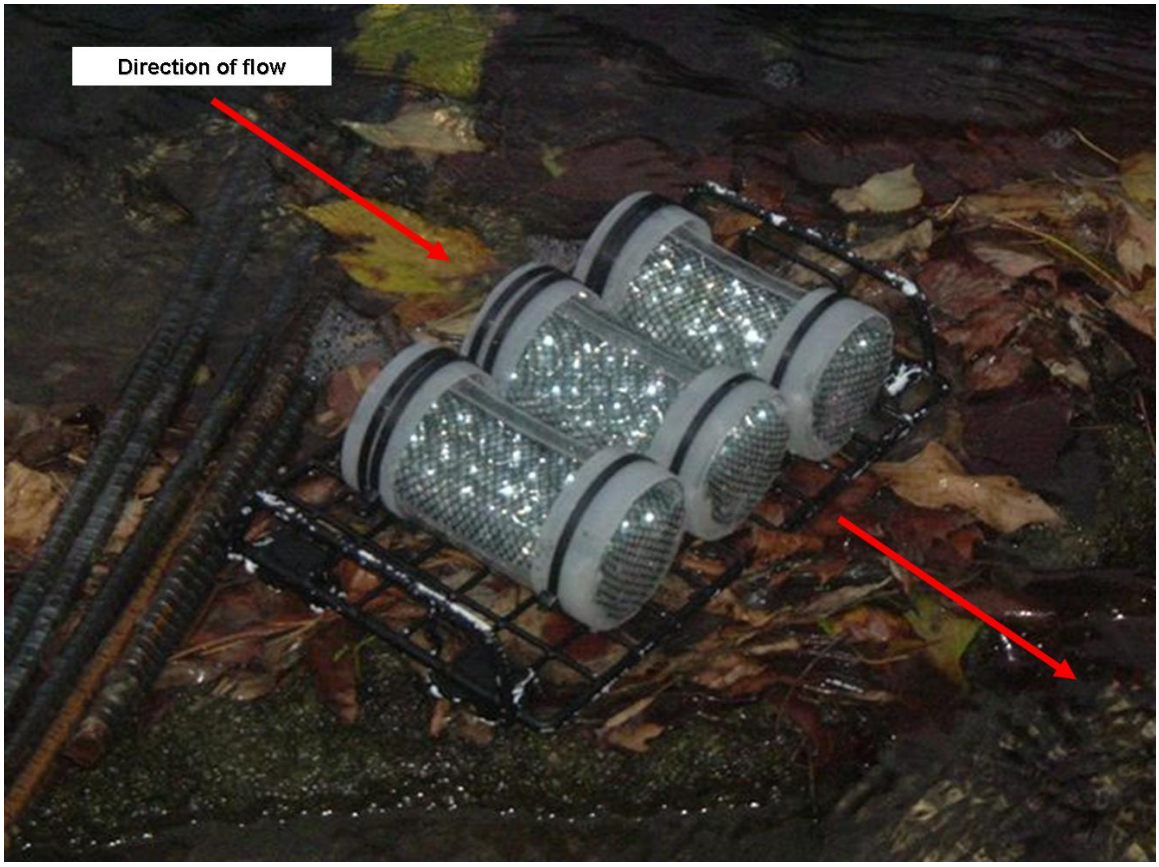



**Figure 9. Embeddedness Chambers Attached to an In Situ Tray and Ready for Deployment.**

**Figure 10. Embeddedness Chambers Deployed on In Situ Tray (Honey Creek).**

**Figure 11. Fully Deployed Series of Embeddedness Chambers at the Honey Creek Site.**

***Embeddedness Chamber Retrieval.***

The primary concerns when retrieving embeddedness chambers were to minimize any loss of sediment that had collected in the chambers and to not introduce any unnatural sediment flow across the chambers. When removing chambers, care was taken not to disturb any of the substrate upstream of the chamber area as well as immediately downstream of the chamber area. Any unnecessary disturbance of fine sediment would affect the treatment areas downstream of the chamber areas. Chamber covers were installed on chambers that were to be recovered for the particular sampling period, according to a randomized sampling scheme. Care was taken, when installing the

chamber covers, to gently push the cover flaps over the bottom portion of the chambers in order to block the end cap holes and prevent any unnecessary disturbance of the substrate surrounding the chamber. Once the chamber covers were in place, wire cutters were used to remove the zip ties on both the front and back of the chamber to be removed. The individual removing the chamber maintained pressure on it (flow conditions being strong enough to move the unsecured chamber) until the chamber was removed from stream and placed in the plastic bag. The chamber was removed gently yet quickly and immediately placed in the plastic bag which was quickly sealed. In turn, this bag was sealed inside another plastic bag as an added precaution against any sediment loss. Once the chamber was removed, a small amount of substrate was placed in the space where the chamber was so that the adjacent chamber's flat mesh portion was flush with the surrounding undisturbed substrate.

**Treatment Areas**

Each sampling site had three treatment areas that were located approximately one meter downstream of and parallel to the embeddedness chambers. Each treatment area consisted of a 3 m wide x 1m long rectangular area marked at each corner by a 20 cm metal stake with an orange tip. The three treatment areas were designated as such: Undisturbed-- (reference) with no disturbance of the in-stream substrate conditions; Slightly disturbed--all substrate particles within this treatment area that were not fully embedded or buried were wiped clean (by hand) of all loose particles and organisms; Disturbed--all surface substrate particles as well as all substrate particles down to approximately 10 centimeters deep were loosened and agitated with shovels and the fine

substrate particulates and organisms were allowed to flow out of the treatment area. The three treatment areas were arranged in order of undisturbed, slightly disturbed, and disturbed downstream of the embeddedness chambers (Figure 12).

## Random Sampling Design

### *Embeddedness Chambers.*

A total of 24 chambers designated for both benthic invertebrate colonization sampling and total sediment sampling were deployed and labeled from 1 to 12 starting from the left bank looking downstream (chambers were arranged across the channel). The embeddedness chambers for each site were split into two series consisting of 12 chambers per series, numbered 1 through 12. When situated in the stream, facing downstream, the two series were numbered as such: 1, 1; 2, 2; 3, 3; …12, 12 (Figure 12). A fair coin was tossed to determine which series of embeddedness chambers were dedicated to macroinvertebrate colonization (benthos) and which series were dedicated to sediment accumulation and porosity. As a result of the coin toss the chambers in the first series were dedicated to macroinvertebrate colonization and the second to total sediment accumulation and porosity.

One random number set was generated (1:12) and the results were divided into thirds. Another random number set was generated (1:3) to determine which chambers in their respective third of the random number set from 1 to 12 were sampled during the three sampling time points (4 d, 7 d, & 14 d) (Figure 12). The results of the random number generation from 1 to 3 were each assigned a sampling time point (Figure 12).

It neither can be assumed that sediment deposition/embedding occurs evenly across the river width due to variable velocity and flow and that the patchy distribution of benthic macroinvertebrates holds true (Pringle *et al.*, 1988). To account for this phenomenon, the random number set (1-12) was used to give a randomized selection of the embeddedness chambers across the width of the sampling area.

***Treatment Areas.***

Each 3 m x 1 m treatment area was divided into six 1 m wide x ½ m long subareas. The treatment subareas were numbered from 1 to 6, starting at the upstream left bank block of the treatment area (Figure 12). One random number set (1-6) was generated and the results divided into thirds. Another random number set was generated (1-3) to determine which subareas in their respective third of the random number set from 1 to 6 were sampled during the selected sampling times (Figure 12).

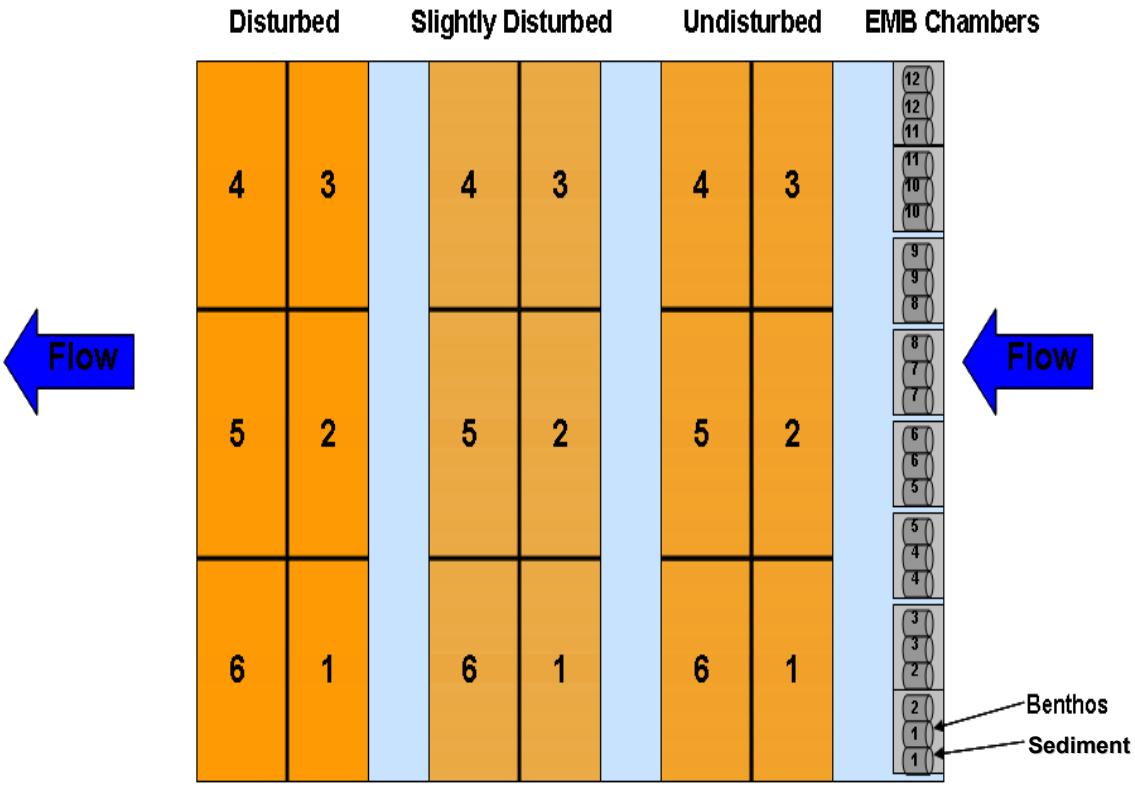



**Figure 12. Schematic of the Experimental Design of Both the Embeddedness Chambers and the Treatment Areas. Two Numbers from Each Treatment Area Were Randomly Selected For Each Sampling Event. Four Embeddedness Chamber Numbers (both for benthos and sediment) Were Randomly Selected for Each of the Three Sampling Events.**

## Sediment Quality Testing

A 10-day sediment toxicity test using *Hyalella azteca* was conducted according to USEPA methods to determine if there was any background toxicity within the sediments at the sites that may affect macroinvertebrate colonization (USEPA, 2000). As an additional precaution, DGTs were exposed for 24 h in each of the treatment areas at both sites to detect sediment metal toxicity in particular. DGTs have been found to provide a time-averaged effect of the separation of chemical species and quickly reach a near

steady state situation, thus allowing for the direct interpretation of the measurement as a mean flux or a concentration (Davison *et al*., 2000). One DGT was installed in each of the three treatment areas at both sites. Each DGT was inserted with care into the sediment as far as possible (length-wise) without physically forcing it too much. DGTs were placed with the narrow portion facing the flow to minimize surface area exposed to floating debris.

**Physicochemical Water Quality Parameters**

To evaluate whether the embeddedness chambers would be effective instruments to quantify embeddedness during steady conditions, it was necessary to monitor certain water quality parameters. Stream flow was monitored to determine if major fluctuations in flow, due most likely to storm events, would cause increased bed flow movement of sand and gravel particles across the surface of the embeddedness chambers. Flow rates were recorded using a Swoffer 3000 flow meter (Swoffer Instruments, Seattle, WA) on six occasions over the course of the final embeddedness chamber exposure periods. Steel rods were hammered into each bank of the stream during the first flow rate measurement occasion so that all flow rate measurements could be conducted at the same location on each occasion.

Water samples were taken and analyzed for TSS on five occasions to clarify whether any major fluctuations occurred during the chamber exposure period. TSS was determined according to Standard Methods (1998). The water quality parameters turbidity, dissolved oxygen, specific conductance, temperature, and pH were monitored

using a YSI 6920 Sonde (Yellow Springs Instruments, Yellow Springs, OH). In particular, turbidity was scrutinized as an indicator of possible fine sediment transport, especially when accompanied by higher flow events.

## Substrate Characterization

### *Grain Size Fractional Analysis of EMB Chamber Sediment.*

The basic method for analyzing the material within the embeddedness chambers was wet sieving to separate the desired size fractions. Subsequently, drying was performed to obtain the dry weight of the sediment as well as loss on ignition weight. The method used to determine grain size fractional analysis of the embeddedness chambers was adapted from the USEPA's Experimental Stream Facility standard operating procedure (unpublished data) for the determination of sediment size fractions accumulated in cobble trays. The desired endpoint of sediment weight for this part of the study made wet sieving feasible. However, if the sediments were to be chemically analyzed, an alternate method would have been required. Wet sieving has been shown to substantially change the physicochemical characteristics of a sediment sample (i.e., decreased percent total organic carbon and decreased total PCBs) (Day *et al.*, 1995). Sequential Loss on Ignition (LOI) is an accepted and widely used method to estimate both the organic and carbonate content of sediments (Dean, 1974). Dry weight of the sediment was determined after drying in an oven, organic content was determined after firing the sediment at 550 °C, and carbonate content was determined after firing at 1000 °C. The theory behind the last step is that at 1000 °C carbon dioxide is evolved from

carbonate (calcium carbonate), leaving only oxides behind (Dean, 1974; Heiri *et al.*, 2001). The determination of the organic and carbonate fractions of the embeddedness chamber sediments facilitated the calculation of chamber porosity using the bulk density and the wet sediment volume obtained from this process.

Prior to processing the embeddedness chambers, several items were prepared. All weights were recorded on a lab sheet to the nearest 0.01 g. A 5.7 L plastic bucket for capturing the <63 µm fraction slurry was rinsed with water, dried, and then weighed. Three 100 mL capacity ceramic crucibles (one each for 2mm, 250 µm, and 63 µm sediment fractions) per embeddedness chamber were pre-ashed in a muffle furnace at 550 °C for 1 h and then weighed. One capped 53 mL plastic bottle (used to take an aliquot from the <63 µm slurry) was weighed. One crucible per embeddedness chamber, to hold a 47 mm diameter, 1.2 µm pore size quartz filter (SKC, Eighty Four, PA), was pre-ashed at 550 °C in a muffle furnace for 1 h then weighed. Finally, two 4.25 cm diameter, 1.2 µm quartz filters per embeddedness chamber were pre-ashed in a muffle furnace at 550 °C for 1 h and then weighed.

Each embeddedness chamber that had been designated for sediment analysis was processed to obtain four different grain size fractions (>2 mm, 2 mm - 250 µm, 250 µm - 63 µm, and 63 µm – 1.2 µm). Each individual chamber and zip lock bag in which it was stored was weighed prior to emptying. Individual embeddedness chambers were emptied into a sieve stack consisting of the following 25.4 mm diameter sieves, from top to bottom, respectively: 5.6 mm (retained the marbles), 2 mm, 250 µm, and 63 µm. The 5.7 L plastic bucket was placed under the sieve stack and the entire arrangement was placed in a deep sink.

Next, a spray nozzle attached to a hose (deionized source) was used to rinse all of the material out of the zip lock bag and the embeddedness chamber through the top sieve (5.6 mm). The marbles retained on the 5.6 mm sieve were thoroughly rinsed to remove any noticeable material and then the 5.6 mm sieve was removed from the stack. The rinsed zip lock bag, marbles, chamber, and zip tie were set aside for determination of chamber pore volume. The material remaining on the 2 mm sieve was thoroughly rinsed, using the spray nozzle, washing any material smaller than 2 mm through the sieve and into the 250 µm sieve below. Then the 2 mm sieve was removed from the stack and the material retained on the 2 mm sieve was placed into a pre-weighed 100 mL crucible and dried in an oven at 104 °C for 24 h. Once cool, the crucible was reweighed and then placed in a muffle furnace and fired for 1 h at 550 °C. The crucibles were again weighed after cooling and then fired one last time in a muffle furnace at 1000 °C for 1 h. The crucible was weighed a final time to determine the Loss on Ignition (LOI) weight due to carbonate burn off. The above procedure of rinsing, drying (104 °C), ashing (550 °C and 1000 °C), and weighing was repeated for the material retained on the 250 µm and 63 µm sieves, as well.

After cooling on a brick tray, all crucibles were placed in a desiccator for thorough drying (for at least 30 min) before weighing. Lastly, the plastic bucket (with no remaining sieves) containing the rinse water and material smaller than 63 µm was weighed. A small mixer attached to an electric drill was used to stir the contents of the bucket as quickly as possible without allowing a vortex to form. When the contents of the bucket appeared to be uniformly mixed, the 53 mL plastic bottle was submerged (inverted) halfway down into the sediment solution and then turned upright to fill with

sediment and capped. The exterior of the bottle was then dried off and the bottle was weighed.

A vacuum filtration apparatus with a three port filter manifold was used to filter the entire contents of the 53 mL bottle through a 1.2 µm pore size, 47 mm diameter quartz filter (SKC, Eighty Four, PA). Any material retained inside the bottle and on the side of the filter holder was rinsed onto the filter. The filter used for one sample (one embeddedness chamber) was placed in a pre-weighed crucible and dried in an oven at 104 °C for 24 h. The crucible and filter were then weighed and processed in the same manner as the other size fractions (muffle furnace at 550 °C & 1000 °C for 1 h each).

***Sediment Dry Weight Calculations.***

The formula for the weight of the dry fraction (DF) of sediments retained on the 2 mm, 250 µm, and 63 µm sieves is:

$$DF_f = SW_f - TW_f \quad (1)$$

Where $DF_f$ = Dry fraction of sediments retained on the particular sieve
$SW_f$ = Weight of crucible and dried sample
$TW_f$ = Weight of crucible
f=fraction being analyzed (2 mm, 250 µm, and 63 µm)

The formula for the estimated weight of the dry fraction of sediments that retained on the 1.2 µm filter is:

$$DF_{1.2} = \frac{\left(SW_{1.2} - TW_{1.2} - TW_{(f1-f2)}\right) * \left(SW_{Sedsoln}\right)}{SW_{Sedsoln}} \quad (2)$$

Where $DF_{1.2}$ = Dry fraction of sediments retained on 1.2 µm filter
$SW_{1.2}$ = Weight of crucible, filters, and dried sample
$TW_{1.2}$ = Weight of crucible
$TW_{(f1-f2)}$ = Weight of filters (as needed)
$SW_{Sedsoln}$ = Weight of sediment solution
$SW_{ft}$ = Weight of falcon tube filled with sediment solution
$TW_{ft}$ = Weight of empty falcon tube

The formula for the Total Solids Accumulated is:

$$Tot\,DF = DF_2 + DF_{250} + DF_{63} + DF_{1.2} \tag{3}$$

***Sediment LOI Calculations.***

The LOI for the organic fraction of the sediment is:

$$LOI_{550} = \frac{\left(DF_{104} - DF_{550}\right)}{DF_{104}} * 100 \tag{4}$$

The LOI for the carbonate fraction of the sediment is:

$$LOI_{1000} = \frac{\left(DF_{550} - DF_{1000}\right)}{DF_{104}} * 100 \tag{5}$$

***Embeddedness Chamber Porosity.***

The rinsed zip lock chamber storage bag and chamber (marbles installed, end cap secured, and zip tie taut over the mesh flap) were placed in an oven at (50 °C) to dry for 24 h. Once dry and cool, the zip lock bag and chamber were weighed and the weight was recorded.

***Chamber Porosity Calculations.***

The formula for the density of the sediment sample particles is:

$$\rho_{particle} = \frac{\left(SW_{wet\,sed} - TW_{chamber}\right) - \left(SW_{wet\,sed} - SW_{df}\right)}{\left[Vol - \left(\frac{SW_{wet\,sed} - SW_{df}}{\rho_{water}}\right)\right]} = g/cm^3 \tag{6}$$

Where $SW_{wet\ sed}$ = Weight of EMB chamber sediment/water (chamber wet weight – clean chamber dry weight) (g)

$TW_{chamber}$ = Tare weight of embeddedness chamber (test substrate and storage bag included) (g)

$SW_{df}$ = Weight of dried sediment fraction (2 mm, 250 µm, 63 µm, or 1.2 µm) (g)

Vol = Volume of wet sediment from chambers see equation 5 ($cm^3$)

$\rho_{water}$ = Density of water (1 $g/cm^3$)

The volume of wet sediment within the chambers is:

$$Vol = \frac{\left(SW_{df} - SW_{df\,550}\right)}{1.4} + \frac{\left(SW_{df\,550} - SW_{df\,1000}\right) * 2.274}{2.7} +$$

$$\frac{\left[2.74 * \left(SW_{df\,1000} - 1.274\right) * \left(SW_{df\,550} - TW_{df}\right)\right]}{2.6} + \qquad (7)$$

$$\left(SW_{df} - TW_{df}\right) * \frac{\%\ water\ content}{100} * \frac{100}{\left(1 - \%\ water\ content\right)} = g = mL = cm^3$$

The water content of the sediment is:

$$Water\ Content\ (\%) = \frac{\left(SW_{wet\ sed} - Tot\ DF_{104}\right)}{SW_{wet\ sed}} * 100 \qquad (8)$$

Where Tot $DF_{104}$ = Total dry fraction of the dried chamber sediments (24 h at 104°C)

The percent of organic matter in the sediment is:

$$\%\ Organic = \frac{\left(Tot\ DF_{104} - Tot\ DF_{550}\right)}{Tot\ DF_{104}} * 100 \qquad (9)$$

Where Tot $DF_{550}$ = Total dry fraction of sediment after firing in a muffle furnace at 550°C for 1h

The percent $CaCO_3$ in the sediment sample is:

$$\%\,CaCO_3 = \frac{\left(Tot\,DF_{550} - Tot\,DF_{1000}\right)}{Tot\,DF_{104}} * 2.274 * 100 \qquad (10)$$

Where Tot DF $_{1000}$ = Total dry fraction of sediment after calcification in a muffle furnace at 1000°C for 1h.

The percent inorganic content of the sediment is:

$$\%\,Inorganic = 100 - \left(\%\,CaCO_3 + \%\,Organic\right) \qquad (11)$$

**Benthic Macroinvertebrate Characterization**

***RBP.***

Following the methods described in Chapter 7 of the USEPA's Rapid Bioassessment Protocol (Barbour *et al.*, 1999), the aquatic macroinvertebrate community was sampled once at each site on (Honey Creek and Stillwater River) 28-September-07. Preserved macroinvertebrate samples were emptied into a 500 µm sieve and rinsed with deionized water to remove any fine particulates. Any large organic matter, such as leaves and twigs as well as any large substrate particles were rinsed on the sieve and then discarded. Each sample was then spread evenly in a 30 cm x 36 cm standardized subsampling tray with 30 grids (6 cm x 6 cm). A random number set from 1 to 30 was generated to determine which previously numbered grids were to be sampled. The contents of each grid were sorted under a dissecting microscope (10x magnification) using a modified Bogorov counting chamber. The subsamples were sorted into insects,

non-insects, and debris. Subsequently, organisms were preserved with a 70% ethanol (ETOH) solution and sorted debris with a 95% ETOH solution. Only insects were used in analysis due to time constraints. All insects were identified to family level or lowest practical taxa using Peckarsky *et al*. (1990) and Merritt *et al.* (2008).

***EMB Chamber Macroinvertebrate Colonization.***

The embeddedness chambers designated for macroinvertebrate colonization were processed in the lab immediately upon returning from retrieving chambers in the field. Chambers were emptied into a sieve stack containing a 5.6 mm sieve for retaining the marbles on top of a 500 µm sieve for retaining the invertebrates. The marbles and chamber were thoroughly rinsed and inspected for any clinging invertebrates and then the 5.6 mm sieve was removed from the stack. The sample retained on the 500 µm sieve was rinsed with deionized water to remove any fine particulate matter; the unsorted sample was then preserved in a 70% ETOH solution in a glass vial. Samples were sorted and enumerated under a dissecting microscope (10x magnification) using a modified Bogorov counting chamber. Samples were separated into insects, non-insects, and debris and then preserved with either a 70% ethanol (ETOH) solution (organisms) and a 95% ETOH solution (sorted debris). All insects were identified to family level or lowest practical taxa using Peckarsky *et al*. (1990) and Merritt *et al.* (2008).

***EMB Chamber Macroinvertebrate Metrics.***

A variety of macroinvertebrate metrics were chosen to assess each sampling site. The following richness measures (predicted to show a decrease with increasing perturbation) were assessed: Total number of taxa, Number of EPT taxa, Number of Ephemeroptera taxa, Number of Plecoptera taxa, and Number of Trichoptera taxa, and Number of Diptera taxa. Richness measures such as those listed above reflect the diversity of an aquatic macroinvertebrate assemblage (Resh *et al.*, 1995). An increase in diversity correlates with the increasing health of an assemblage and suggests that ecological conditions are adequate to support many species (Barbour *et al.*, 1999). The following composition measures (predicted to show a decrease with increasing perturbation) were assessed: % EPT, % Ephemeroptera, % Plecoptera, and % Trichoptera. The following additional composition measures that are predicted to show an increase with increasing perturbation were assessed: % Diptera and % Chironomidae. Measures of composition provide information on the make-up of the macroinvertebrate assemblage and on the relative contribution of particular populations to the total ecosystem fauna (Barbour *et al.*, 1999).

Tolerance and intolerance measures are used to assess the relative sensitivity to perturbation and are normally non-specific to the type of stressor (Barbour *et al*., 1999). However, some metrics such as the Family Biotic Index (FBI) developed by Hilsenhoff (1998) provide a means of evaluating a particular stressor, such as organic pollution. Two tolerance/intolerance measures (% Dominant taxa, Family Biotic Index, and % Hydropsychidae to Trichoptera) were assessed, both of which are predicted to show an increase with increasing perturbation. A feeding measure such as functional feeding

groups provides information on a system's feeding strategy balance within the benthic macroinvertebrate assemblage and imbalances can reflect stressed conditions (Barbour *et al*., 1999). Two feeding measures that were assessed, which are predicted to decrease in response to increasing perturbation, were %Shredders and % Grazers & Scrapers. Two additional feeding measures were assessed (% Filterers & % Predators), both of which have a variable predicted response to increasing perturbation. Additional measures of interest that were evaluated were total number of Chironomidae and number of Hydropsychidae.

The intent of assessing these metrics, in both the embeddedness chambers and treatment areas, was to determine any correlations in noticeable responses and changes in fine sediment deposition into areas inhabited by benthic macroinvertebrates (insects only).

***Treatment Area Sampling.***

Each treatment area was sampled during the three sampling periods according to a random sampling scheme that selected two sub-areas. A modified Hess sampler (Figure 13) with 250 µm mesh was used to sample the treatment areas. The modified Hess sampler had an inside diameter of 21.9 cm. The area used for insect densities (# insects/$m^2$) was calculated from the following equations:

$$Area\ of\ a\ circle = \pi r^2 \qquad (12)$$

Where: r = 10.95 cm (radius of the modified Hess sampler)

$$Ratio\ of\ \ Hess\ Sampler\ Area\ to\ 1m^2 = Area\,(cm^2) * \frac{1m^2}{10{,}000\,cm^2} \qquad (13)$$

$$Density\,(\#\,in\,\mathrm{sec}\,ts / m^2) = \#\,in\,\mathrm{sec}\,ts / Hess\ sample * \ factor\,(m^2) \qquad (14)$$

All large substrate particles within the sampler were brushed clean by hand. A garden trowel was used to disturb the substrate within the sampler down to a depth of approximately 10 cm. Care was taken to minimize the amount of debris that was collected in the 250 μm mesh. Once the sub-area was sampled, the modified Hess sampler was placed with the meshed end in the current so all organisms could be washed into the cod end of the mesh and collected. All debris and organisms were funneled into a 1 L plastic bottle and preserved with an 80% ETOH solution. All insects were identified to family level or lowest practical taxa using Peckarsky *et al*. (1990) and Merritt *et al.* (2008).

**Figure 13. Modified Hess Sampler Used to Sample Treatment Areas.**

***Macroinvertebrate Sorting and Identification.***

Preserved samples were emptied into a 500 µm sieve and rinsed with deionized water to remove any fine particulates. Any large organic matter, such as leaves and twigs as well as any large substrate particles were rinsed over the sieve and then discarded. Each sample was then placed in a 25 cm x 20 cm white plastic counting tray. The contents of each sample were sorted under a dissecting microscope (10x magnification) using a modified Bogorov counting chamber. All insects were identified to family level or lowest practical taxa using Peckarsky *et al*. (1990) and Merritt *et al.* (2008).

## IV. Results and Analysis

### Pilot Study

The first EMB chamber prototype was tested at four locations on the Mad River and a statistical power analysis (Minitab V15) was performed on the sediment dry weight data. The results from this analysis helped determine how many replicate chambers were needed at each site to determine differences. Power analysis revealed that with an α-level of 0.05 and an assumed standard deviation of 3.0, only four chambers per site would be sufficient in order to detect differences. This initial deployment was conducted over a seven day period from 18-June-07 to 25-June-07. There was a moderate flow event during this initial exposure of the embeddedness chambers which may have led to the chambers rapidly accumulating sediment (Figure 15).

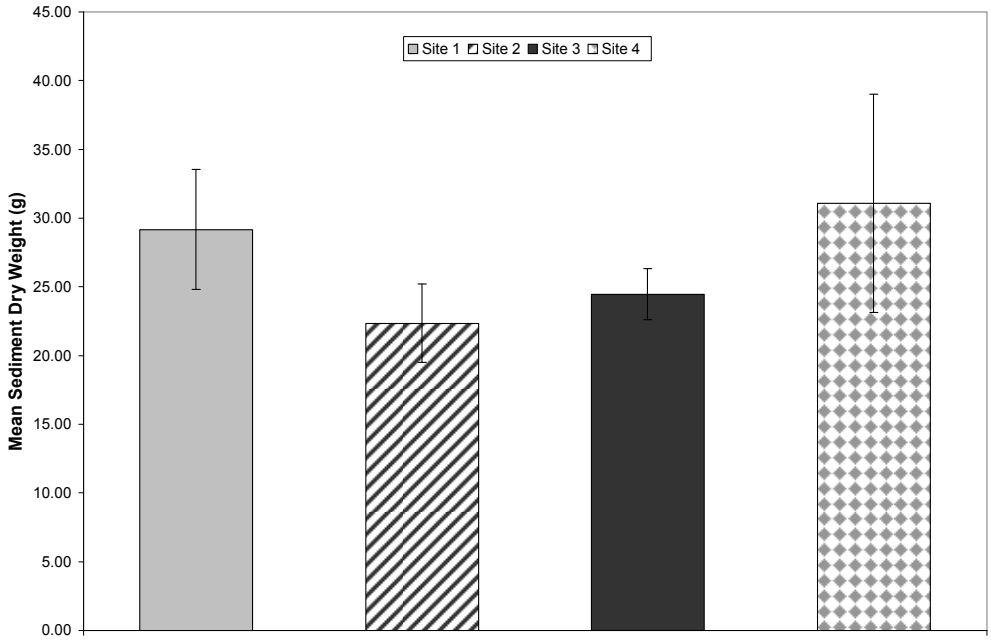



**Figure 14. Pilot Study Embeddedness Chamber Dry Weight.**

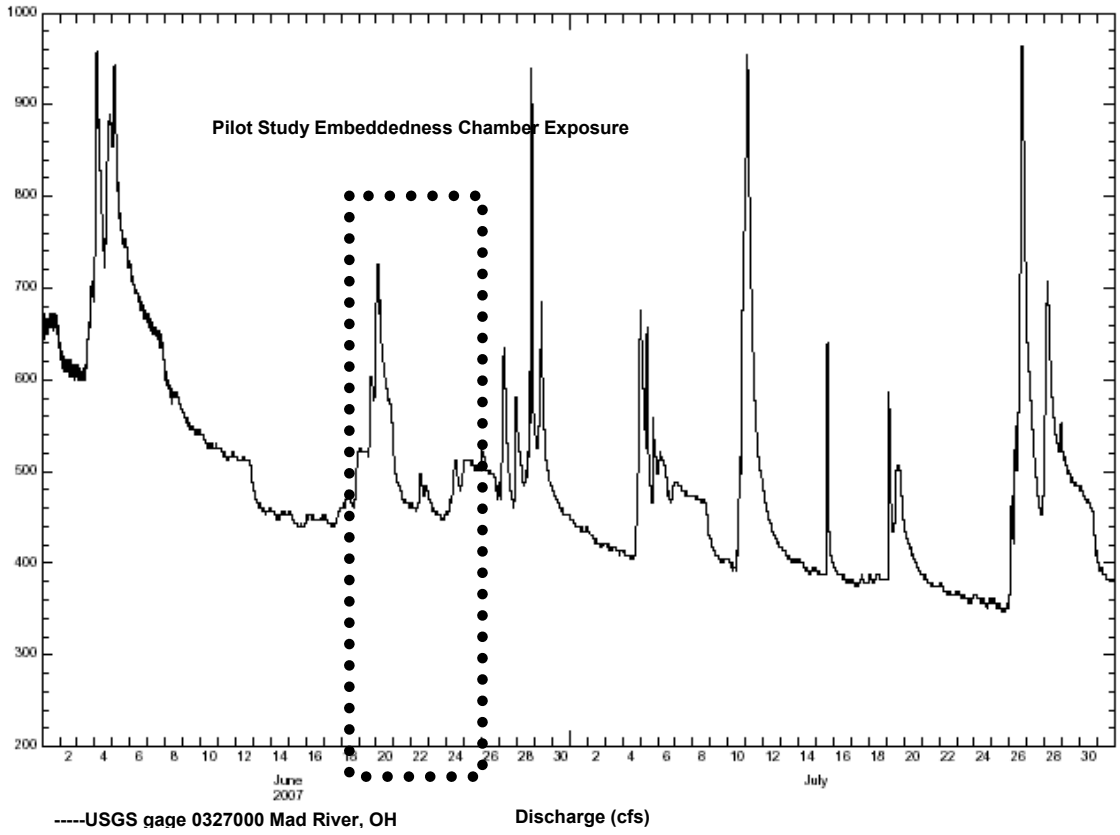



**Figure 15. Hydrograph June/July 2007 from USGS Gage Station Located Near Huffman Dam on the Mad River near Dayton, OH.**

## EMB Chamber Optimization

A noticeable amount of sediment accumulated within the chambers starting at day 4 of the exposure period and appeared to increase steadily through the 7-Day and 14-Day exposures. There were no lost chambers throughout the three exposure periods and all chambers appeared to be in the same position from deployment, indicating that the zip ties were sufficient to hold the chambers in position on the *in situ* trays. Flow remained relatively steady and low during the entirety of the exposure periods (Figures 19 and 20)

thus, the deployed chamber arrangement has yet to be tested under relatively high flow conditions. Results from the chamber colonization indicate that the 4 mm mesh openings were large enough to allow a wide variety and variable sizes of organisms to enter the chambers. All chambers remaining after the 4-day exposure had developed a fine layer of algae on the surface. Some chambers had collected leaf litter on the upstream side of the chamber, an inevitable consequence of the early October exposure periods. Leaf litter was carefully removed from all affected chambers on their respective sampling day.

**Sediment Quality Testing**

As noted in a previous section, a sediment toxicity test was conducted to determine if preexisting background toxicity impacted macroinvertebrate colonization. The test was conducted at approximately 23 °C with a 16 h light/8 h dark photoperiod. Test containers were 300 mL high form glass beakers filled with 100 mL of site sediment (100 mL of Ottawa sand for the lab controls) and 175 mL of overlying culture water. Four replicates and four controls were tested for the each of the two study site sediments. Ten 7-14 day old amphipods were added to each beaker at the start of the test. Overlying water changes were conducted twice a day using an apparatus that accurately delivered a volume of water to the test beakers (Zumwalt *et al.*, 1994). After an overlying water change, amphipods in all beakers were fed approximately 0.15 g of crushed rabbit food pellets (Nutriphase) once a day. The following water quality parameters were monitored at specified times throughout the 10 day test: pH, dissolved oxygen, conductivity, temperature, alkalinity, and hardness. Results from a two-sample t-test show no significant differences in mean survival between Honey Creek sediment (87.5% survival,

p=0.138) and the control (97.5%) and Stillwater River sediment (90%, p=0.391) and the control (Figure 16).

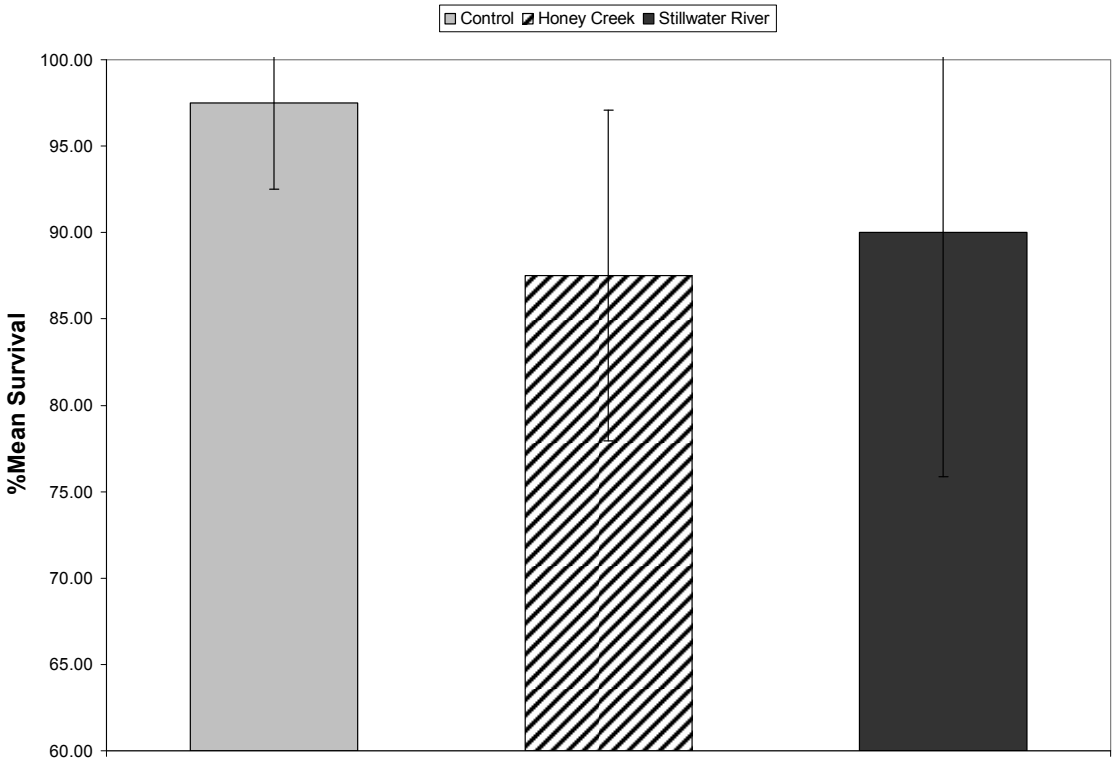



**Figure 16. Ten-Day USEPA Sediment Toxicity Test Results for the Two Site Sediments.**

Following a 24 h exposure in the treatment area sediment DGTs were analyzed for the following metals: cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni), and zinc (Ni). Results indicate that Zn and Cu had the highest flux from both sites with the more disturbed treatment areas at Honey Creek (slightly disturbed and disturbed) showing higher flux than the undisturbed treatment area (Figure 17).

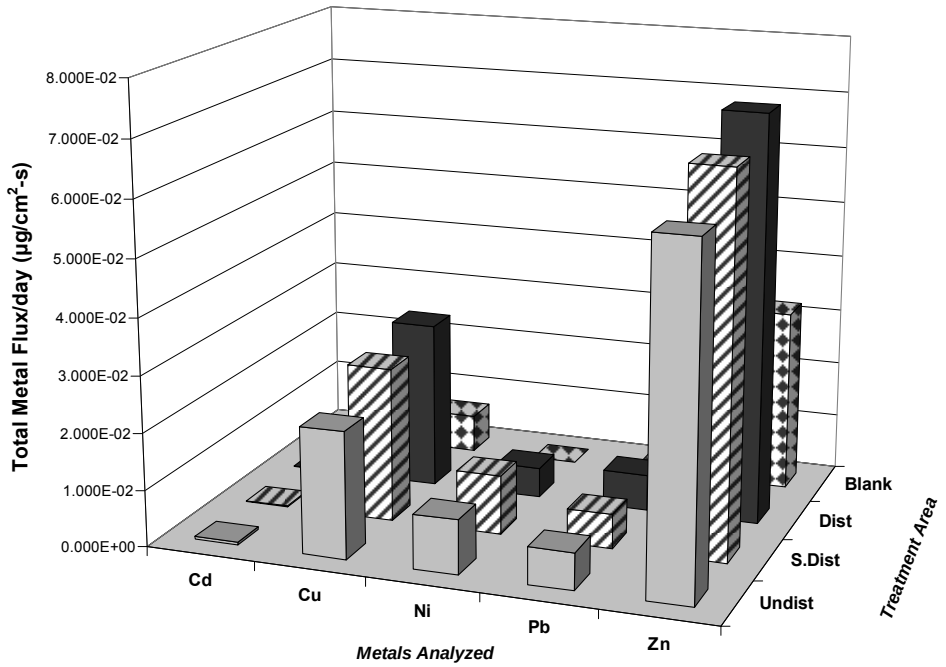



**Figure 17. Total Metal Flux/Day from Sediment to DGTs (Honey Creek).**

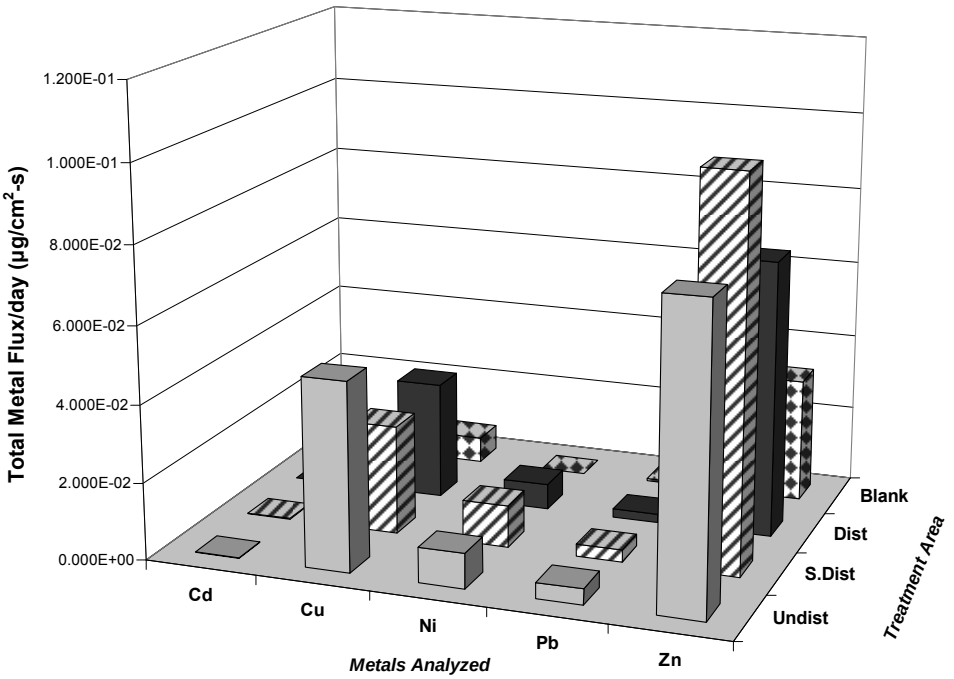



**Figure 18. Total Metal Flux/Day from Sediment to DGTs (Stillwater River).**

The Stillwater River site however, showed higher flux in CU and Zn in the less disturbed areas (undisturbed and slightly disturbed) (Figure 18). The blank DGTs show fairly high concentrations of Cu and Zinc suggesting possible cross-contamination during the analytical laboratory analysis (Figures 17 and 18).

## Physicochemical Water Quality Parameters

### *Discharge.*

Stream flow (discharge) was monitored on six occasions over the 4-14 day embeddedness chamber exposure period in order to determine whether the embeddedness chambers would be effective in representing embeddedness occurring during steady stream flow conditions. No major fluctuations in flow at both sites were recorded and discharge remained relatively stable over the 14 day embeddedness exposure period (Figures 19 & 20, Table 1). No major rainfall (0.04 in on 1-October-07 and 12-October-07) was recorded and the greater Dayton area experienced extremely dry conditions during the exposure period (Figure 21). The Honey Creek site maintained a mean flow of 0.311 $m^3$/sec with flow increasing slowly from 0.293 $m^3$/sec on 3-October-07 to 0.319 $m^3$/sec on 15-October-07. The Stillwater River site experienced small fluctuations in flow and did not increase steadily like Honey Creek. The Stillwater River site had a mean flow of 0.852 $m^3$/ sec over the exposure period and experienced the lowest flow on 15-October-07 and the highest flow on 5-October-07 (Table 1).

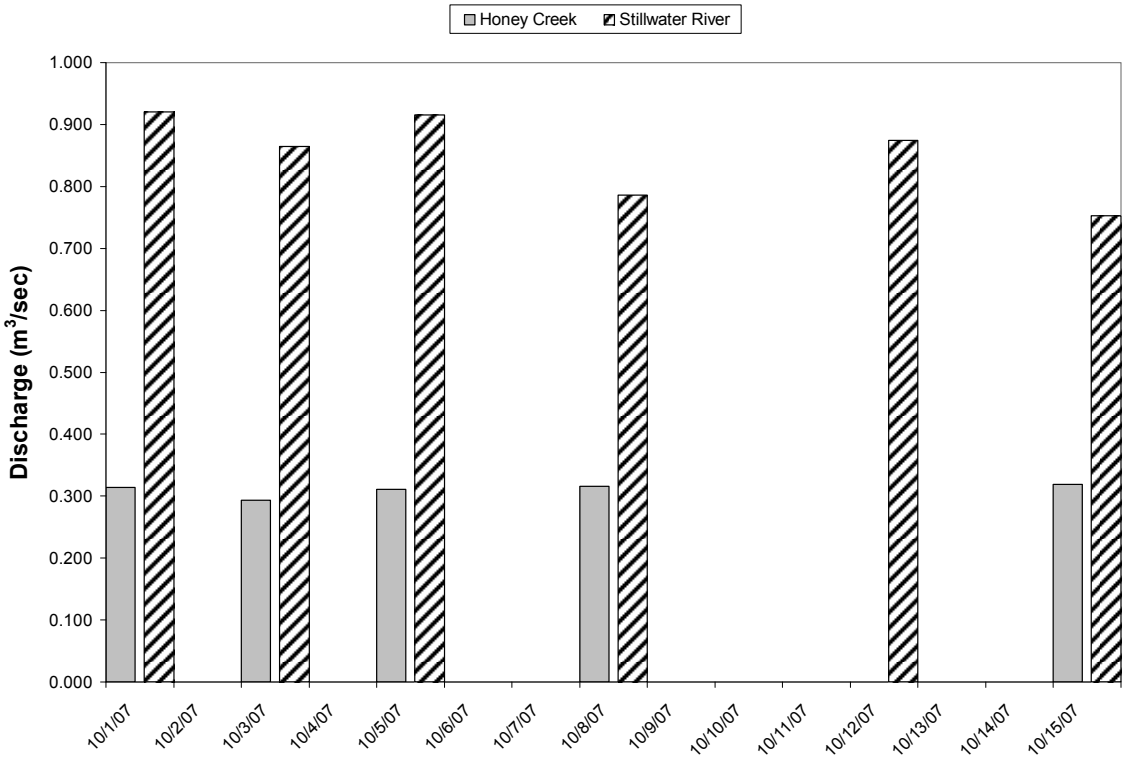



**Figure 19. Discharge for the Two Sampling Sites over Embeddedness Chamber Exposure Periods.**

**Table 1. Discharge for the Two Sampling Sites over the Embeddedness Chamber Exposure Periods.**

| Honey Creek | | Stillwater River | |
|---|---|---|---|
| **Date** | **Flow** | **Date** | **Flow** |
| 10/1/2007 | 0.314 | 10/1/2007 | 0.920 |
| 10/3/2007 | 0.293 | 10/3/2007 | 0.864 |
| 10/5/2007 | 0.312 | 10/5/2007 | 0.916 |
| 10/8/2007 | 0.316 | 10/8/2007 | 0.786 |
| 10/12/2007 | * | 10/12/2007 | 0.874 |
| 10/15/2007 | 0.319 | 10/15/2007 | 0.753 |
| Mean Flow | 0.311 | Mean Flow | 0.852 |

***Readings not taken on this date**

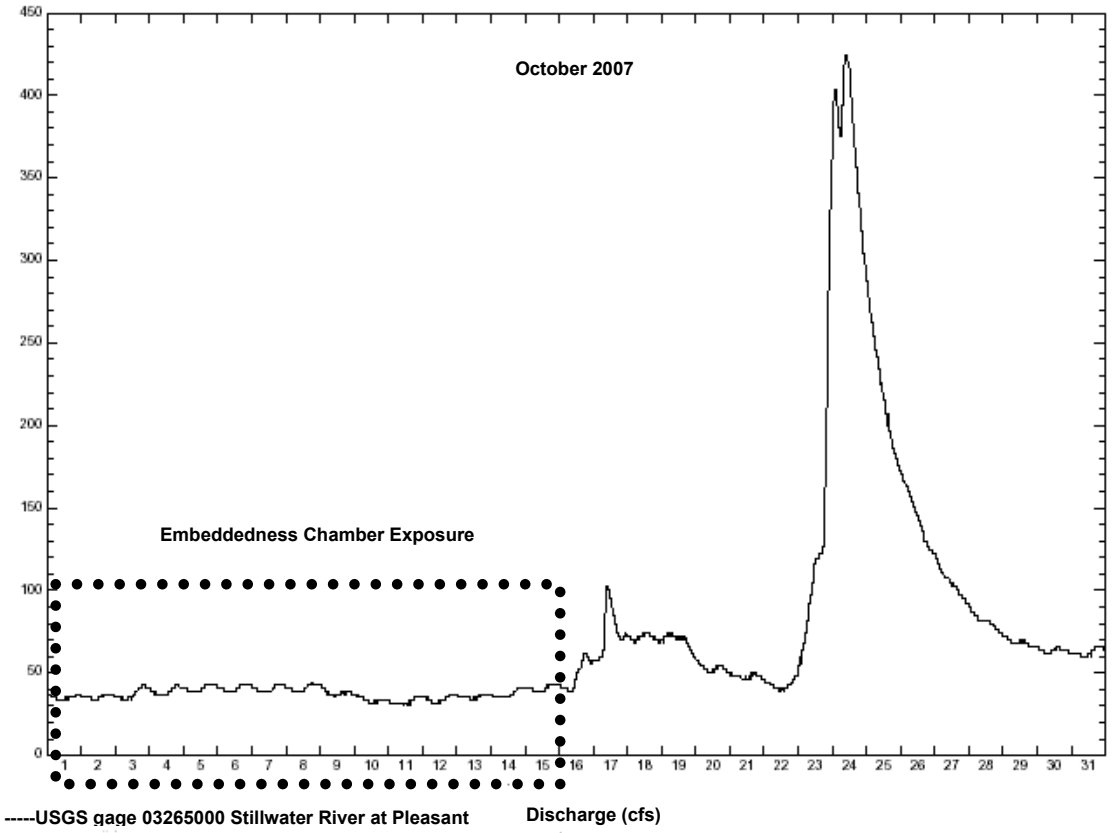



**Figure 20. Hydrograph for October 2007 from USGS Gage Station Located Downstream of the Stillwater River Site.**

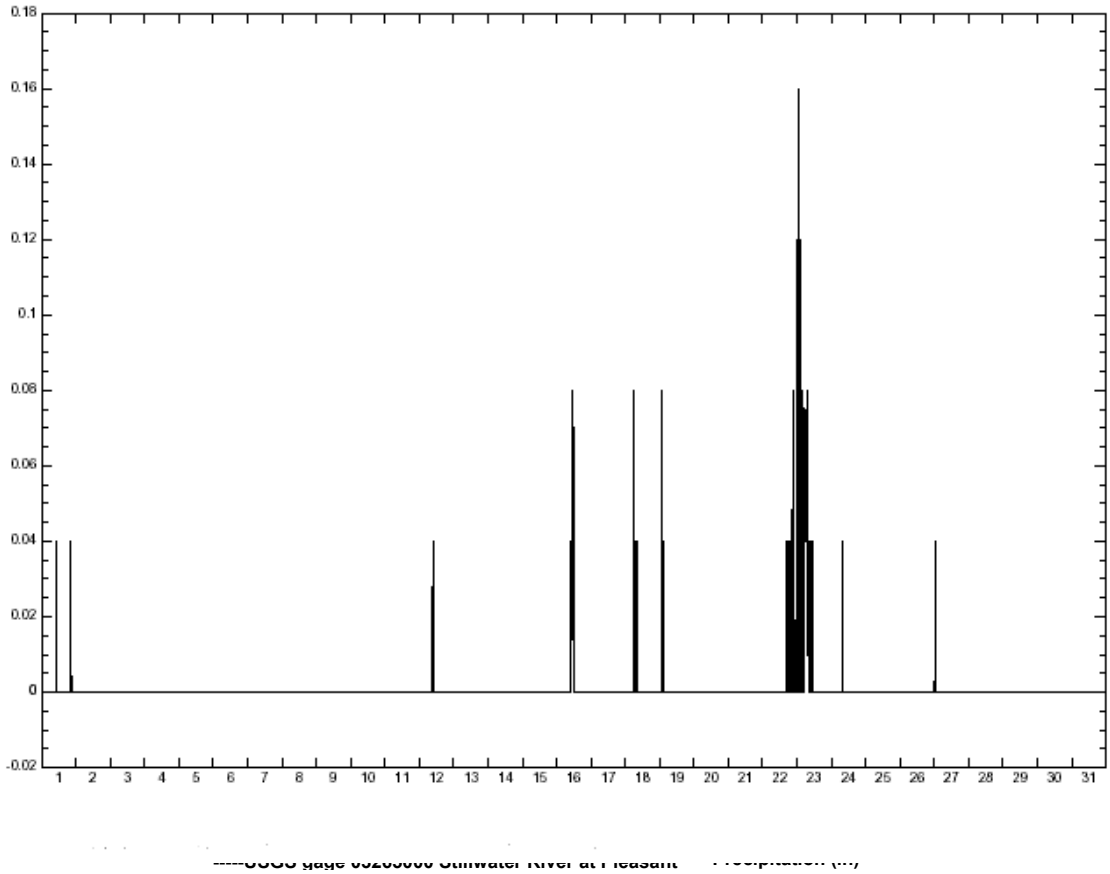



**Figure 21. Precipitation for October 2007 from USGS Gage Station Located Downstream of the Stillwater River Site.**

***Total Suspended Solids.***

Water samples were taken at both study sites on three occasions over the embeddedness chamber exposure periods and analyzed for total suspended solids. Concentrations obtained from analyzing the Honey Creek water samples were recorded with the lowest TSS concentration being 3.8 mg/L and the high concentration of 9.0 mg/L (Table 2). Stillwater River TSS concentrations exhibited a low of 1.3 mg/L and a

high of 6.0 mg/L. No concentrations of over 10.0 mg/L were recorded over the sampling period (Figure 22).

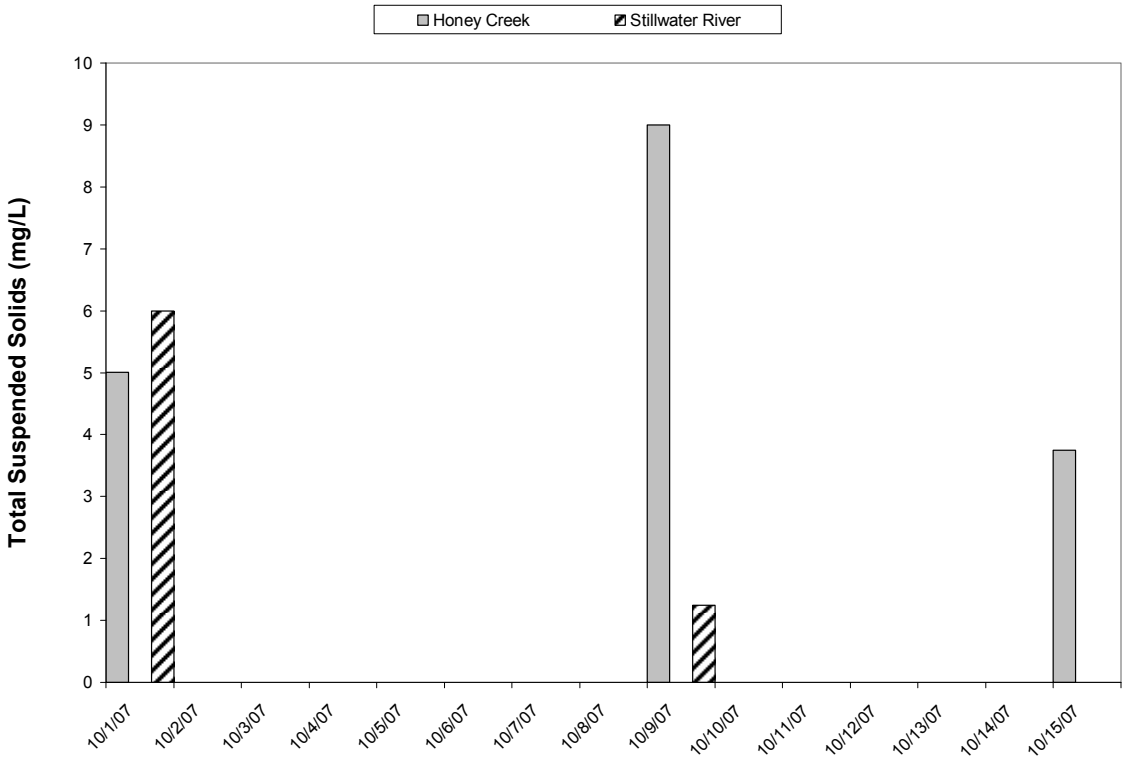



**Figure 22. Total Suspended Solids Concentrations Sampled During the Embeddedness Chamber Exposure Period.**

**Table 2. TSS Concentrations from the Primary Embeddedness Chamber Exposure Periods.**

| Date | Site | TSS (mg/L) |
|---|---|---|
| 10/1/2007 | HY | 5.00 |
| 10/9/2007 | HY | 9.00 |
| 10/15/2007 | HY | 3.75 |
| 10/8/2007 | SR | 6.00 |
| 10/15/2007 | SR | 1.25 |

***Turbidity.***

Turbidity measurements taken during the embeddedness chamber exposure periods indicate that there was very little fluctuation at both Honey Creek and the Stillwater River (Figure 23). Turbidity at Honey Creek varied from near zero to only 1.5 NTUs (Table 3). Turbidity readings from the Stillwater River varied from 0.8 NTUs to a maximum of 2.8 NTUs (Table 3).

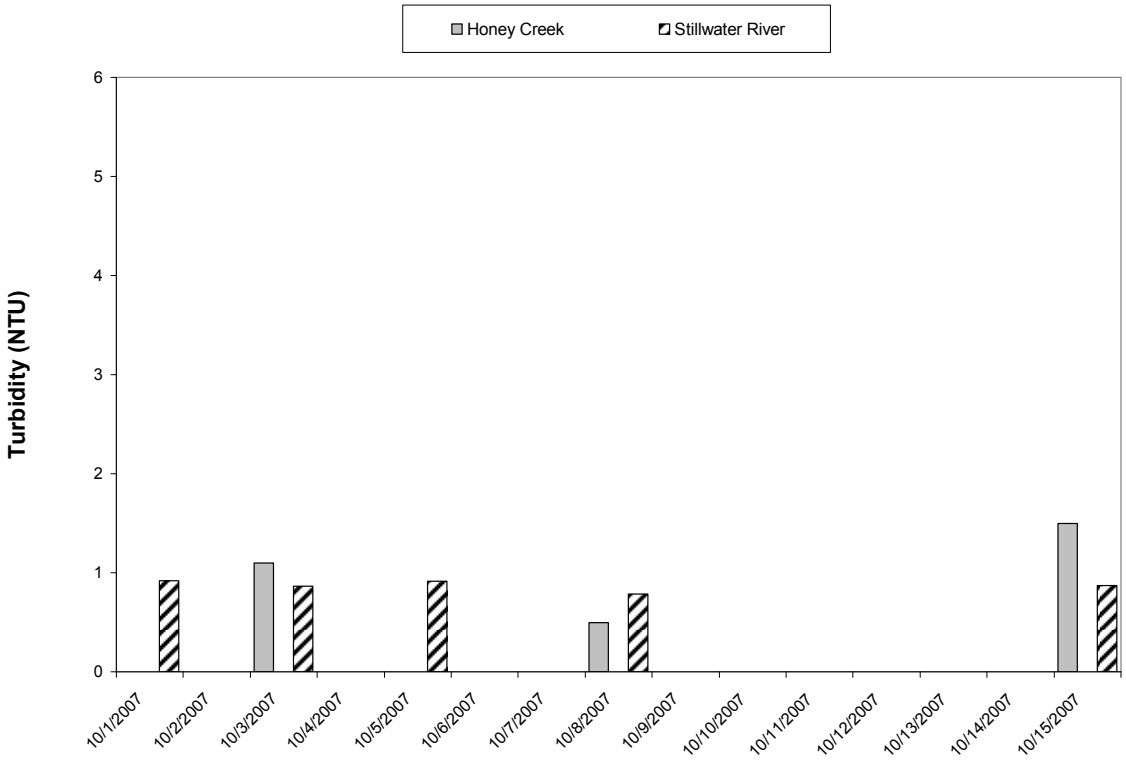



**Figure 23. Turbidity Measurements from the Study Sites during Embeddedness Chamber Exposures.**

**Table 3. Turbidity Readings (NTU) from the Primary Embeddedness Chamber Exposure Periods.**

| Date | Site | Turbidity (NTU) |
|---|---|---|
| 10/1/2007 | HY Cr | 0 |
| 10/3/2007 | HY Cr | 1.1 |
| 10/5/2007 | HY Cr | 0 |
| 10/8/2007 | HY Cr | 0.5 |
| 10/15/2007 | HY Cr | 1.5 |
| 10/1/2007 | SR | * |
| 10/3/2007 | SR | 1.8 |
| 10/5/2007 | SR | 1.8 |
| 10/8/2007 | SR | 2.8 |
| 10/12/2007 | SR | 1 |
| 10/15/2007 | SR | 0.8 |

*****Equipment Failure

***Conductivity.***

Conductivity readings taken at each site show very little fluctuation with the exception of a noticeable drop on 8-October-07 (Figure 24). Conductivity readings at Honey Creek varied from 492 µS/cm °C to 816 µS/cm °C (Table 4). Readings from the Stillwater River remained quite steady only varying from 686 µS/cm °C to 785 µS/cm °C (Table 4).

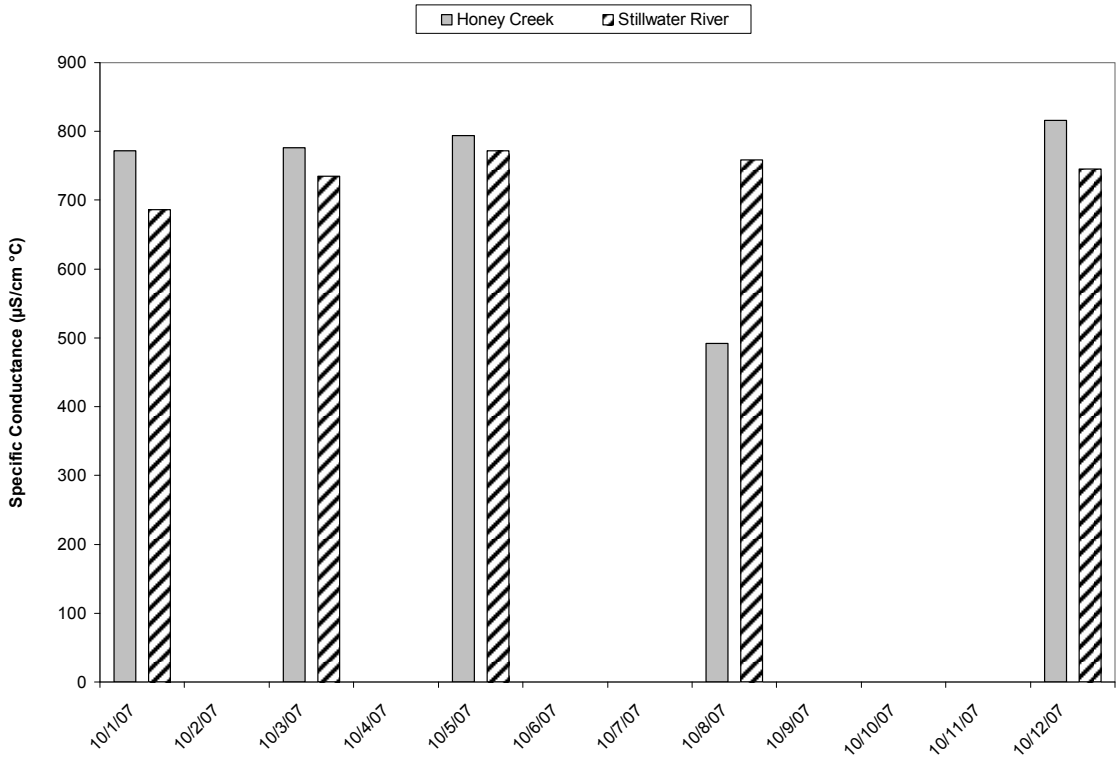



**Figure 24. Conductivity Readings from the Study Sites during Embeddedness Chamber Exposures.**

**Table 4. Conductivity Readings from the Primary Embeddedness Chamber Exposure Periods.**

| Date | Site | Cond (µS/cm °C) |
|---|---|---|
| 10/1/2007 | HY Cr | 772 |
| 10/3/2007 | HY Cr | 776 |
| 10/5/2007 | HY Cr | 794 |
| 10/8/2007 | HY Cr | 492 |
| 10/15/2007 | HY Cr | 816 |
| 10/1/2007 | SR | 686 |
| 10/3/2007 | SR | 735 |
| 10/5/2007 | SR | 772 |
| 10/8/2007 | SR | 759 |
| 10/12/2007 | SR | 745 |
| 10/15/2007 | SR | 785 |

## Substrate Characterization

### *Embeddedness.*

The results from conducting percent embeddedness using the method described in a USGS publication (1998) indicate that both sites were experiencing relatively low embeddedness (31.5 % at Honey Creek; 27.2 % at Stillwater River) (Figure 25). Percent embeddedness values for both sites fall in the range of what has been considered to be the normal operating range (<33-35 %) of a properly functioning stream (Bjorn *et al.*, 1974, 1977, Waters, 1995).

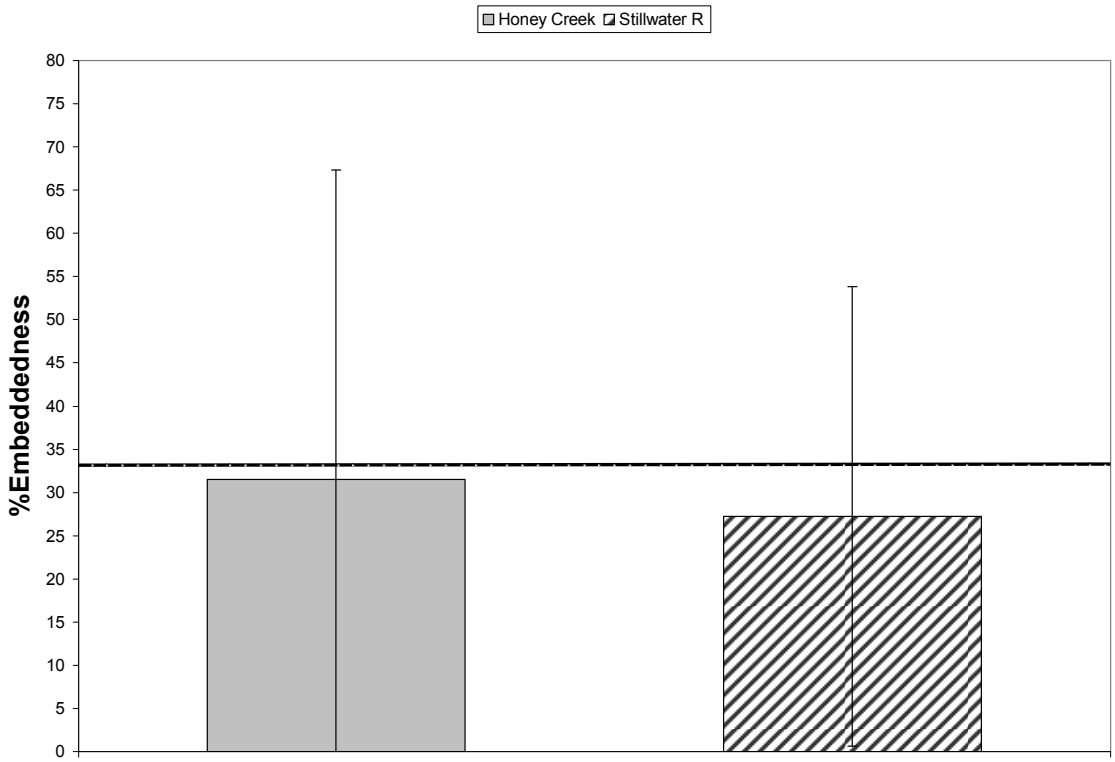



**Figure 25. Percent Embeddedness (USGS method) for Honey Creek and Stillwater River (31.5 & 27.2 respectively). Honey Creek Measurements Taken on 22-October-07. Stillwater River Measurements Taken on 12-October-07.**

***Grain Size Fractional Analysis of Chamber Sediment.***

Results from the embeddedness chamber processing indicate that the majority of the sediment that accumulated was in the size range of < 2 mm to > 63 µm (Figures 30 and 31). At day four the 63 µm – 250 µm fraction accounted for the majority (57%) of the sediment accumulating in the embeddedness chambers deployed at Honey Creek (Figure 26). However, after day four there was a clear increasing trend over time in the 250 µm – 2 mm size fraction (53% and 62% respectively) (Figure 27). The Stillwater River results show the 250 µm – 2 mm size fraction clearly being the majority of the sediment accumulating over the 4, 7, and 14-day sampling points (87%, 82%, and 78% respectively) (Figures 28 and 29). Results did indicate a decrease in dominance of the 250 µm – 2 mm size fraction over time along with an increase in the 63 µm – 250 µm size fraction (Figure 31). The > 2 mm size fraction was a relatively small percentage of the overall sediment accumulating at both sites and at all time points (Figures 30 and 31). The 1.2 µm - 63 µm size fraction remained relatively stable throughout the entire chamber exposure period (Figures 30 and 31). Mean bulk porosity within the embeddedness chambers showed an overall trend of decreasing porosity from day 4 to day 14 at both sites (Figure 32).

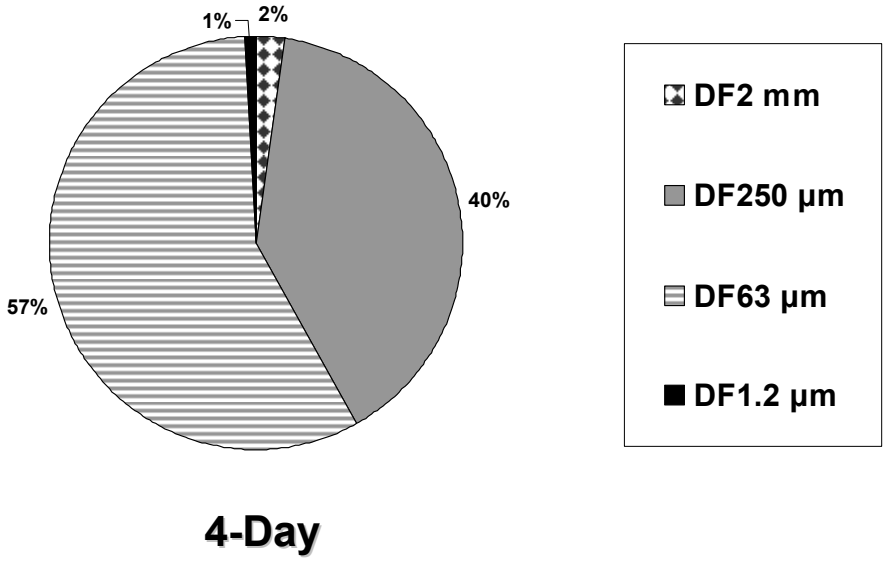



**Figure 26. Percent of Total Dry Fraction of Chamber Sediment Accumulated for Honey Creek (4-Day).**

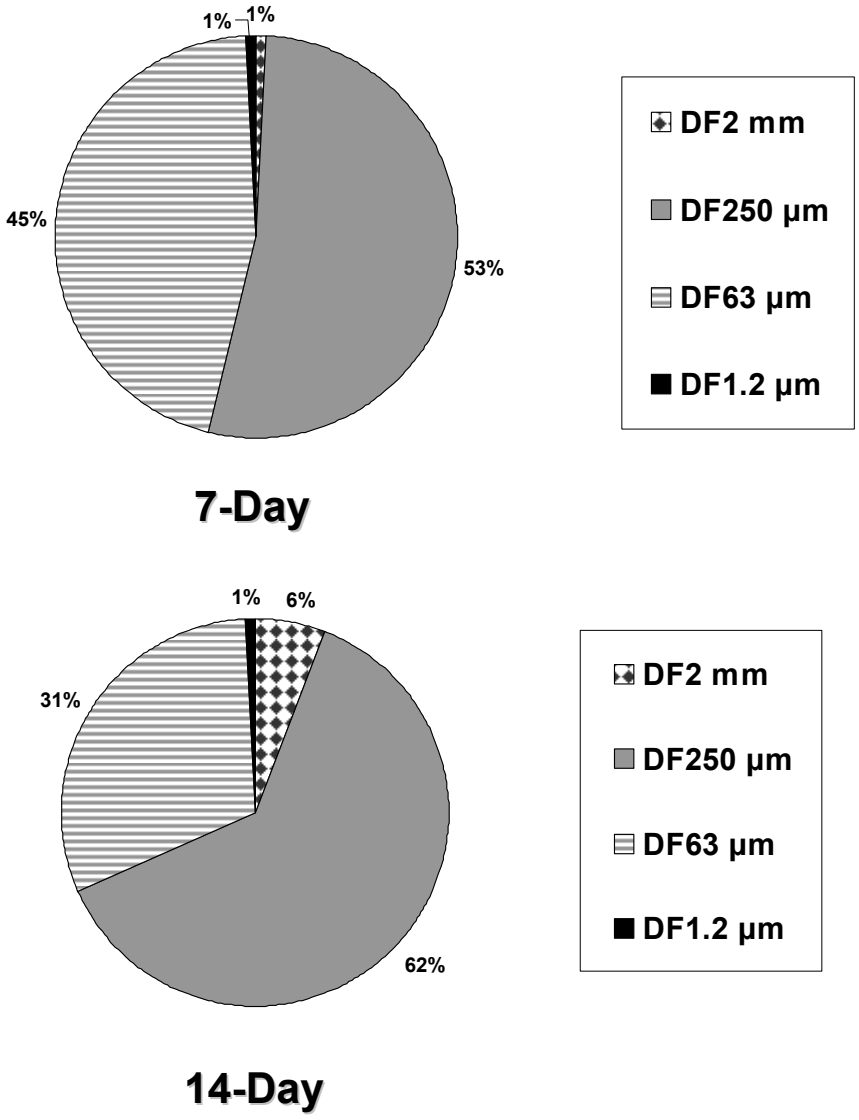
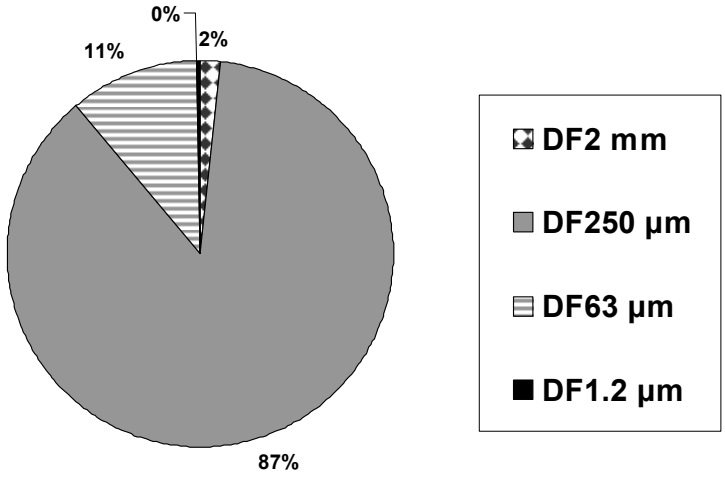



**Figure 27. Percent of Total Dry Fraction of Chamber Sediment Accumulated for Honey Creek (7&14-Day).**



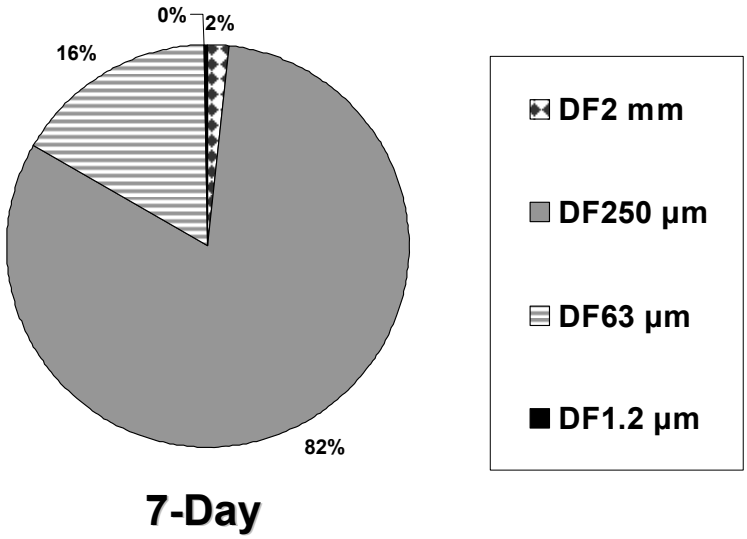




**Figure 28. Percent of Total Dry Fraction of Chamber Sediment Accumulated for Stillwater River (4&7-Day).**

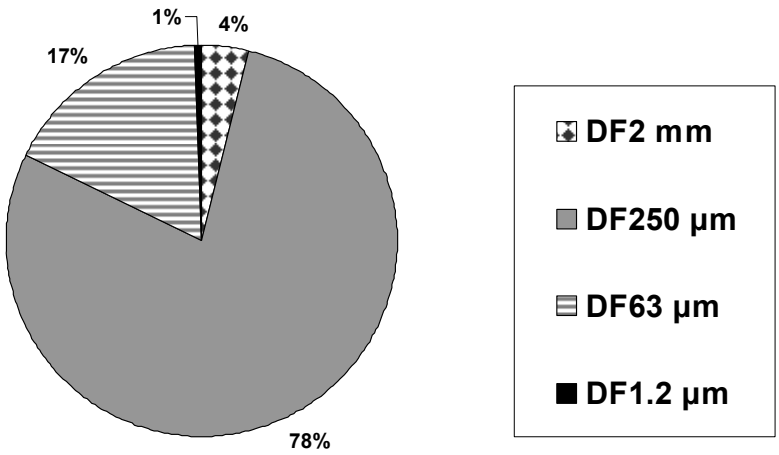



**Figure 29. Percent of Total Dry Fraction of Chamber Sediment Accumulated for Stillwater River (14-Day).**

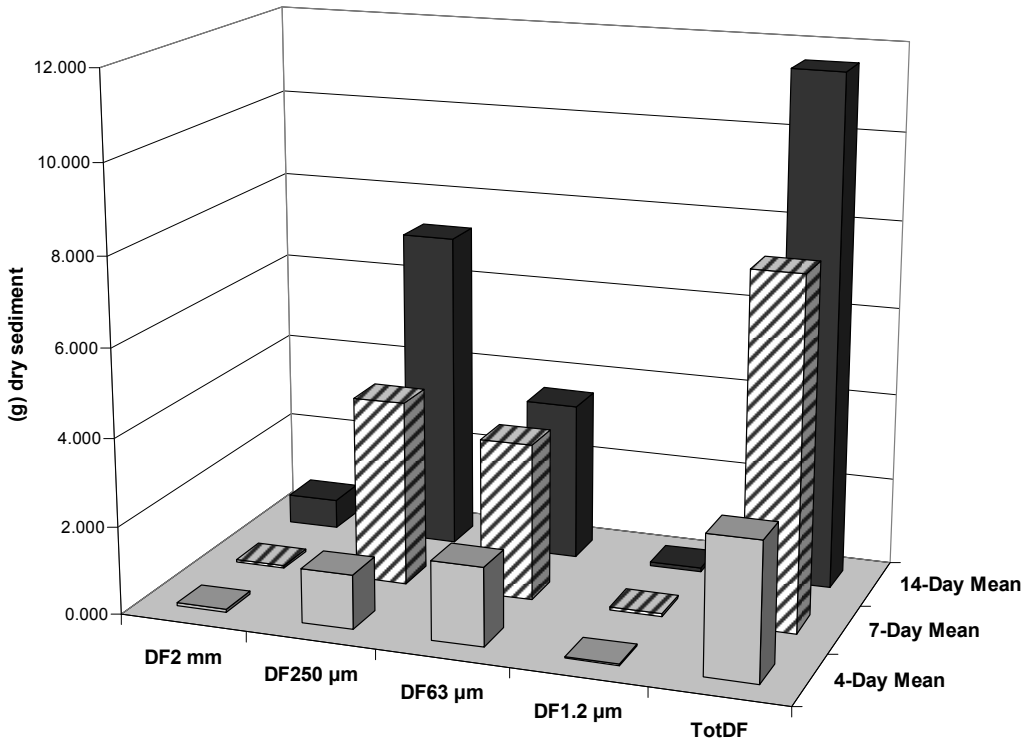



**Figure 30. Temporal Representation of Percent of Dry Fractions of Chamber Sediment Accumulated for Honey Creek.**

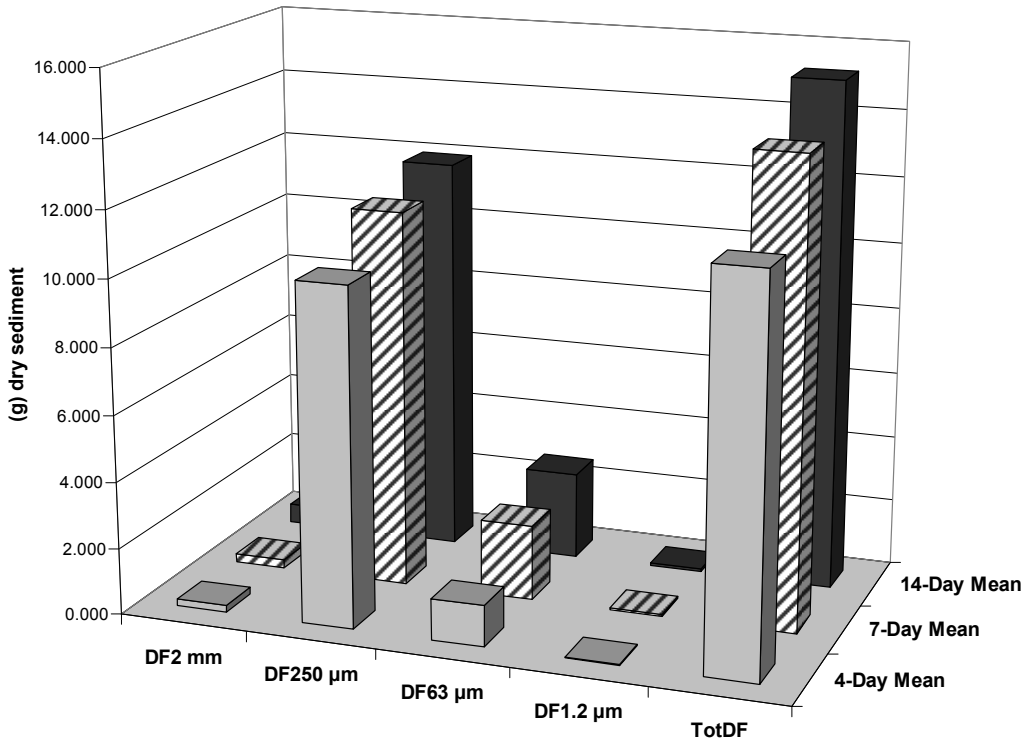



**Figure 31. Temporal Representation of Percent of Dry Fractions of Chamber Sediment Accumulated for Stillwater River.**

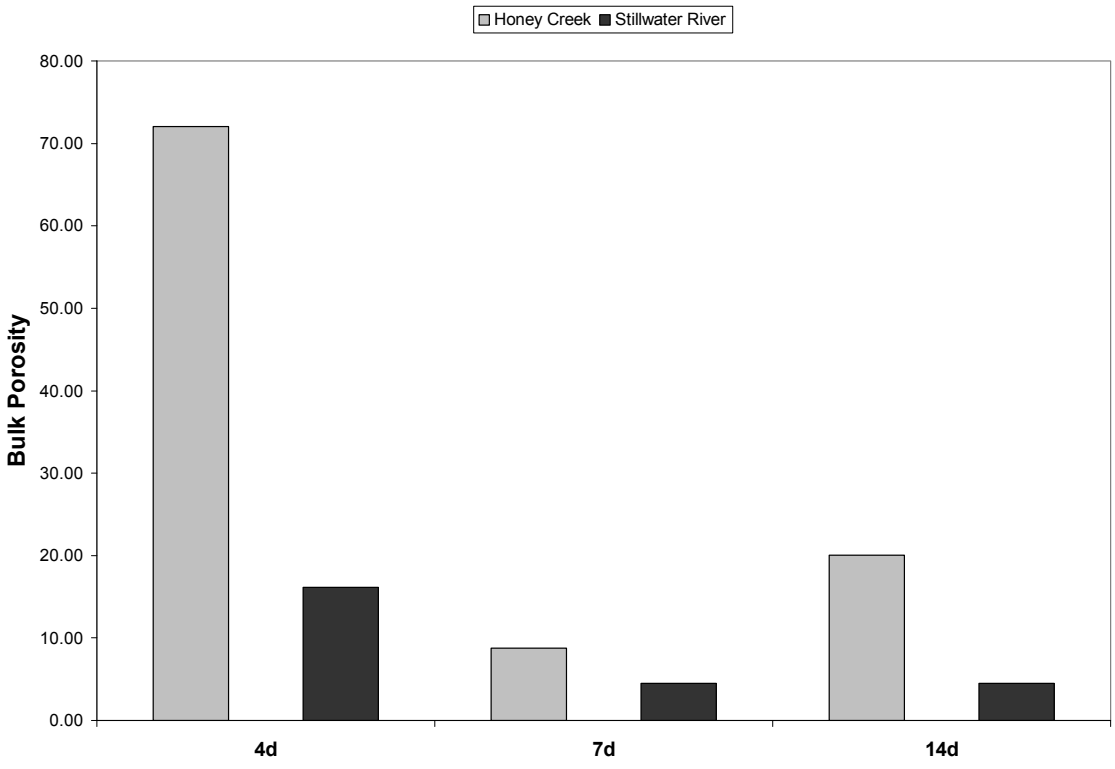



**Figure 32. Embeddedness Chamber Sediment Bulk Porosity over the Three Time Points.**

## Benthic Macroinvertebrate Characterization

### *RBP Metrics.*

The benthic macroinvertebrate metrics derived from the RBP samples and the taxa information provide background information on each particular site for reference. Though this information was not used in the traditional reference data sense, it merely provided information on what benthic macroinvertebrates occupied that particular stretch of stream (Figure 33). The intent of this research was not to assess the changes occurring within the reach compared to historical or reference data, but rather to assess the embeddedness chamber effectiveness in representing natural processes that are occurring within the stream.

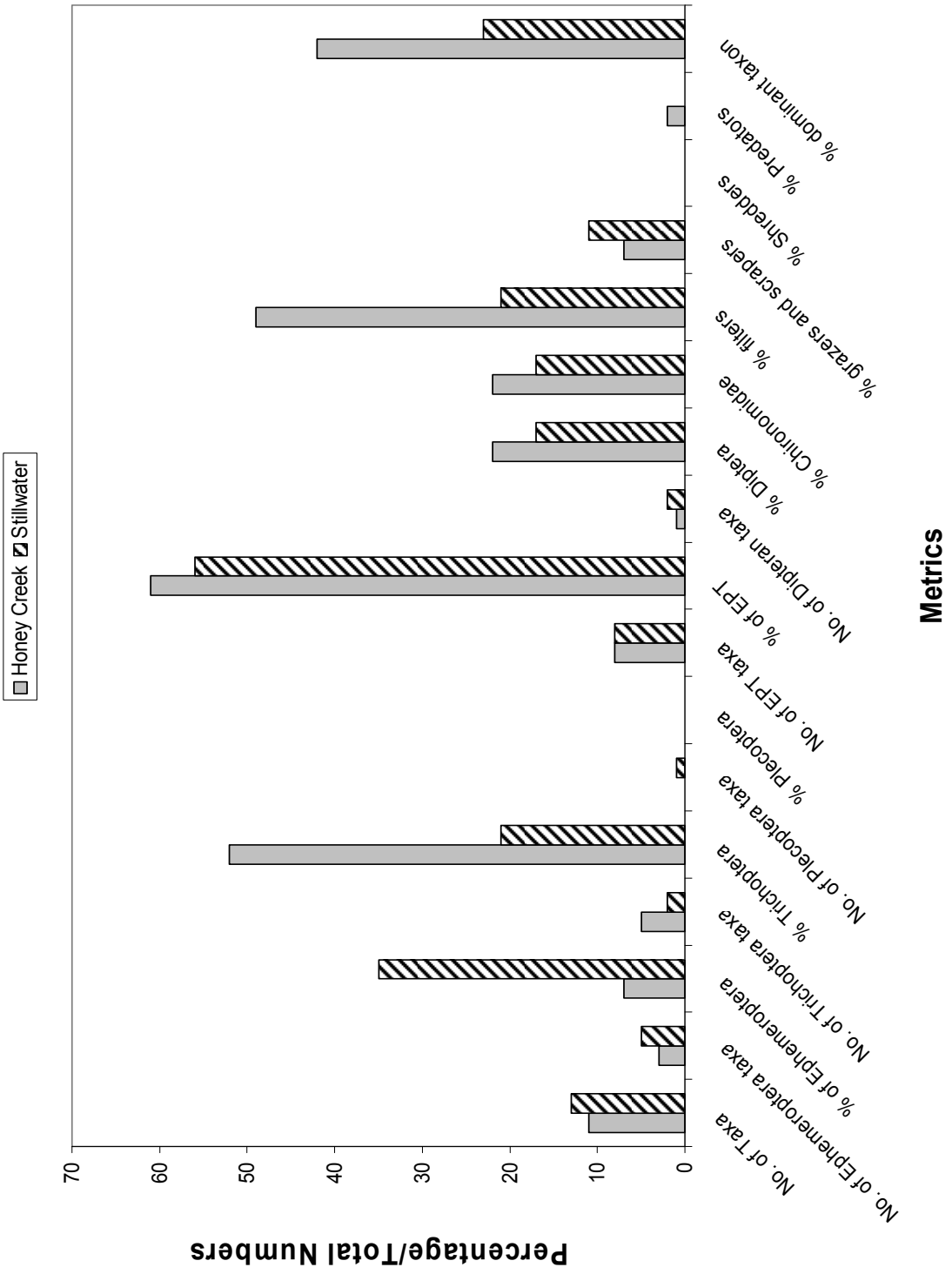



**Figure 33. Honey Creek and Stillwater River Macroinvertebrate RBP Results Sampled on 28-September-07.**

**Table 5. Taxa List for the Macroinvertebrate RBP at Honey Creek and Stillwater River Sites for 28-September-07.**

| Class | Honey Creek 28-September-07 Order | Family | Total | Functional Feeding Group | Tolerance Value | Family Biotic Index Values | % Abund. |
|---|---|---|---|---|---|---|---|
| Insecta | Ephemeroptera | Baetidae | 1 | GC | 4 | 4 | 1 |
| | | Heptageniidae | 8 | SC | 4 | 32 | 5 |
| | | Caenidae | 2 | GC | 7 | 14 | 1 |
| | Ephemeroptera | | 3 | | | 0 | 2 |
| Insecta | Odonata | Coenagrionidae | 3 | PR | 6 | 18 | 2 |
| Insecta | Trichoptera | Philopotamidae | 10 | FC | 3 | 30 | 6 |
| | | Polycentropodidae | 2 | FC | 6 | 12 | 1 |
| | | Hydropsychidae | 70 | FC | 4 | 280 | 42 |
| | | Hydroptilidae | 1 | SC | 4 | 4 | 1 |
| | | Glossosomatidae | 2 | SC | 0 | 0 | 1 |
| | | Trichoptera | 1 | | | 0 | 1 |
| Insecta | Coleoptera | Elmidae | 28 | GC | 4 | 112 | 17 |
| Insecta | Diptera | Chironomidae | 37 | GC | 6 | 222 | 22 |
| | | **Total Insecta #** | 168 | | | | |

| Class | Stillwater River 28-September-07 Order | Family | Total | Functional Feeding Group | Tolerance Value | Family Biotic Index Value | % Abund. |
|---|---|---|---|---|---|---|---|
| Insecta | Ephemeroptera | Baetidae | 10 | GC | 4 | 40 | 5 |
| | | Heptageniidae | 22 | SC | 4 | 88 | 10 |
| | | Leptohyphidae | 1 | GC | 2 | 2 | 0 |
| | | Caenidae | 5 | GC | 7 | 35 | 2 |
| | | Tricorythidae | 40 | GC | 4 | 160 | 18 |
| | Ephemeroptera | | 1 | | | 0 | 0 |
| Insecta | Odonata | Coenagrionidae | 1 | PR | 6 | 6 | 0 |
| Insecta | Pleoptera | Perlodidae | 1 | PR | 2 | 2 | 0 |
| Insecta | Trichoptera | Polycentropodidae | 2 | FC | 6 | 12 | 1 |
| | | Hydropsychidae | 44 | FC | 4 | 176 | 20 |
| Insecta | Coleoptera | Psephenidae | 3 | SC | 4 | 12 | 1 |
| | | Elmidae | 51 | GC | 4 | 204 | 23 |
| Insecta | Diptera | Chironomidae | 38 | GC | 6 | 228 | 17 |
| | | Tipulidae | 1 | SH | 3 | 3 | 0 |
| | | **Total Insecta #** | 220 | | | | |

PR=predator
GC=gather/collector
FC=filterer/collector
SC=scraper

***Embeddedness Chamber Metrics.***

Metrics data from the embeddedness chamber samples was compared between the three sampling times (4, 7, & 14 days) and significance was determined by running a one-way ANOVA with Tukey's Post Hoc test to determine if there were significant differences between the two sampling dates. All total numbers data was LN transformed prior to running statistical analysis in order to help the data fit the assumption of a normal distribution. All proportional data (% abundances) were transformed using an Arcsine Square Root transformation to meet the assumption of normal distribution. Both sites exhibited a steady increasing trend over time in the total number of insects colonizing the chambers (Figure 37). There was a significant difference in Number of Taxa ($p = 0.012$, increasing trend) between the 4-Day and 14-Day sampling events at Honey Creek (Figure 35). There was also a significant difference between the 4-Day and 7-Day samples in the number of Trichoptera taxa ($p = 0.048$, increasing trend) at Honey Creek (Figure 35). Significant differences between 4-Day and 14-Day sampling events were found in the following Stillwater River chamber metrics: % Trichoptera ($p = 0.046$) and % EPT taxa ($p = 0.027$) increased over time, and % Diptera ($p = 0.024$) decreased over time (Figure 36).

Regression analyses were run on the wide array of data from the embeddedness chambers. The biotic metrics were run against abiotic factors such as total dry fraction of sediment, porosity, fractions of the dry sediment weight (2 mm, 250 μm, 63 μm, and 1.2 μm total dry weights), % organic content of chamber sediments (LOI), and time. Regressions run on the data from both Honey Creek and the Stillwater River resulted in

numerous significant p-values and $r^2$-values showing moderate correlations for most significant tests (Tables 6 and 7). There were hypothesized correlations that were validated by the analyses such as a correlation between porosity and total dry fraction of chamber sediments (Stillwater R, Tot DF, $p = 0.008$, $r^2 = 46.9$ %) showing a strong negative correlation (Table 7). Correlations between sediment and porosity observed at the Stillwater River suggest that the smaller fractions may have the most influence on porosity ($DF_{250}$, $p = 0.014$, $r^2 = 46.9$ %; $DF_{63}$, $p = 0.042$, $r^2 = 35.1$ %) (Table 7). Similar correlations between porosity and sediment with the Honey Creek chamber data also suggested the smaller fractions were more related to changes in porosity (Tot $DF_{250}$, $p = 0.037$, $r^2 = 43.7$; $DF_{1.2}$, $p = 0.006$, $r^2 = 30.5$ %, negative correlation) (Table 6).

The benthic metric data for Honey Creek showed a variety of significant p-values when run against the chamber sediment data (Table 6) yet most $r^2$ values were relatively weak. Significant correlations to note from the regression data are between the 1.2 μm dry fraction and the number of Chironomidae ($p = 0.005$, $r^2 = 32.0\%$) and the number of Hydropsychidae ($p = 0.003$, $r^2 = 34.7\%$) both exhibiting positive correlations (Table 6). The Stillwater River data showed a noticeable trend in significant correlations between benthic metrics and the 63 μm dry sediment fraction with 14 out of the 20 metrics exhibiting a significant correlation (Table 7).

**Table 6. Significant Observations from Regression Analysis between Embeddedness Chamber Biotic and Abiotic Parameters (Honey Creek).**

| | Porosity | | | Time | | | DF 1.2 µm | | | %Organic | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | p | $r^2$ (%) | *s | p | $r^2$ (%) | s | p | $r^2$ (%) | s | p | $r^2$ (%) | s |
| % of Ephemeroptera | | | | | | | | | | 0.009 | 28.5% | - |
| No. of Plecoptera taxa | | | | | | | | | | 0.006 | 30.5% | - |
| Total insects | | | | 0.000 | 54.1% | + | | | | | | |
| No. of EPT taxa | | | | 0.005 | 31.4% | + | | | | | | |
| No. Chironomidae | | | | 0.002 | 36.0% | + | 0.005 | 32.0% | + | | | |
| No. Hydropsychidae | | | | 0.001 | 41.3% | + | 0.003 | 34.7% | + | | | |
| DF 250 µm | 0.037 | 43.7% | - | | | | | | | | | |
| DF 1.2 µm | 0.006 | 30.5% | - | | | | | | | | | |

*s = slope

**Table 7. Significant Observations from Regression Analysis between Chamber Biotic and Abiotic parameters (Stillwater River).**

| | Porosity | | | DF 63 µm | | |
|---|---|---|---|---|---|---|
| | p | $r^2$ (%) | *s | p | $r^2$ (%) | s |
| Total insects | | | | 0.016 | 45.6 | + |
| No. of Taxa | | | | 0.001 | 65.6 | + |
| No. of Ephemeroptera taxa | | | | 0.010 | 50.4 | + |
| % of Ephemeroptera | | | | 0.017 | 45.2 | + |
| No. of Trichoptera taxa | | | | 0.023 | 41.8 | + |
| % Trichoptera | | | | 0.004 | 57.5 | + |
| No. of EPT taxa | | | | 0.001 | 68.2 | + |
| % of EPT | | | | 0.001 | 69.9 | + |
| % Chironomidae | | | | 0.001 | 65.6 | - |
| % grazers & scrapers | | | | 0.031 | 38.6 | + |
| % Shredders | | | | 0.011 | 49.0 | + |
| % dominant taxon | | | | 0.002 | 63.8 | - |
| Family Biotic Index | | | | 0.002 | 37.4 | - |
| No. Hydropsychidae | | | | 0.020 | 43.3 | + |
| | | | | | | |
| Total DF | 0.008 | 51.7 | - | | | |
| DF 250 µm | 0.014 | 46.9 | - | | | |
| DF 63 µm | 0.008 | 52.1 | - | | | |
| %Organic | 0.042 | 35.1 | + | | | |

*s = slope

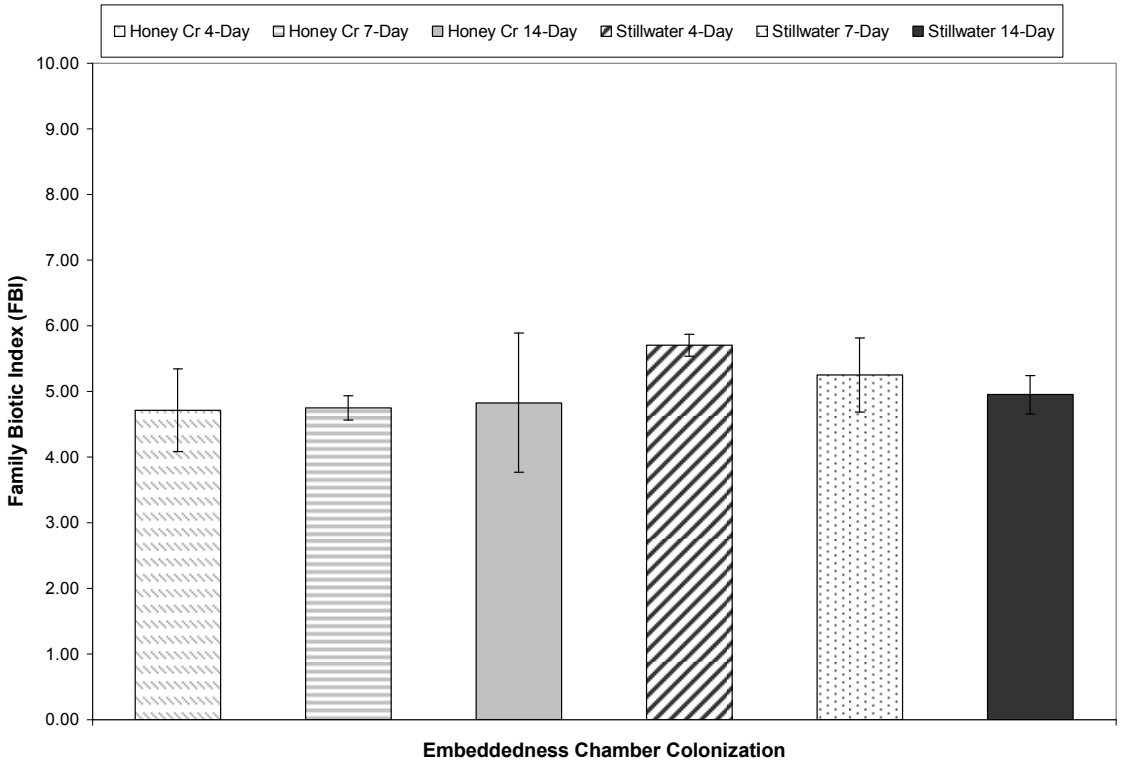



**Figure 34. Honey Creek and Stillwater River Macroinvertebrate Family Biotic Index from Embeddedness Chamber Exposure Periods (4,7, and 14-Day).**

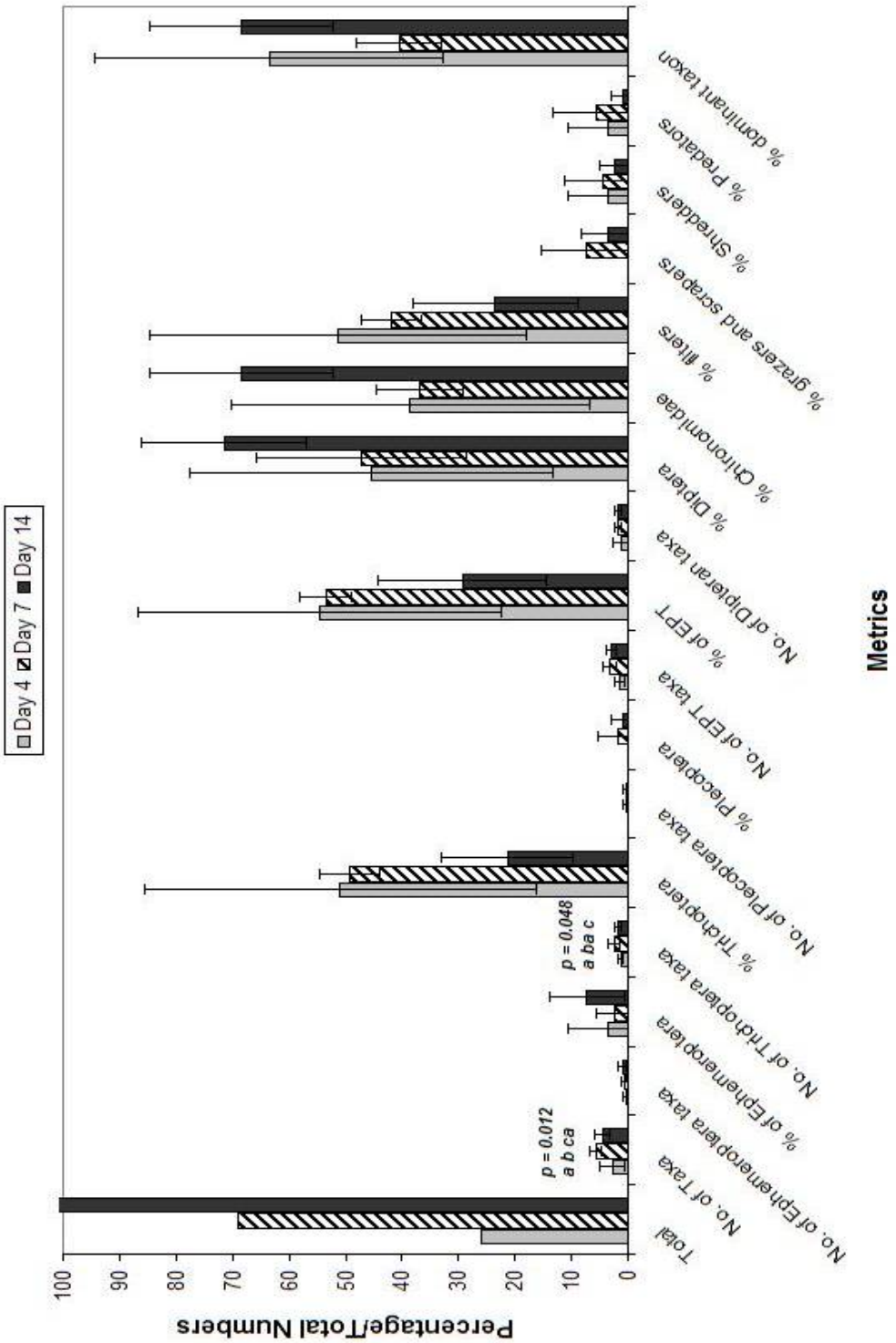



**Figure 35. Honey Creek Embeddedness Chamber Macroinvertebrate Metrics (4, 7, & 14-Day). Single Letters (a, b, c) Indicate No Significant Difference; Double Letters (ba, cb, ca) Indicate Significant Difference.**

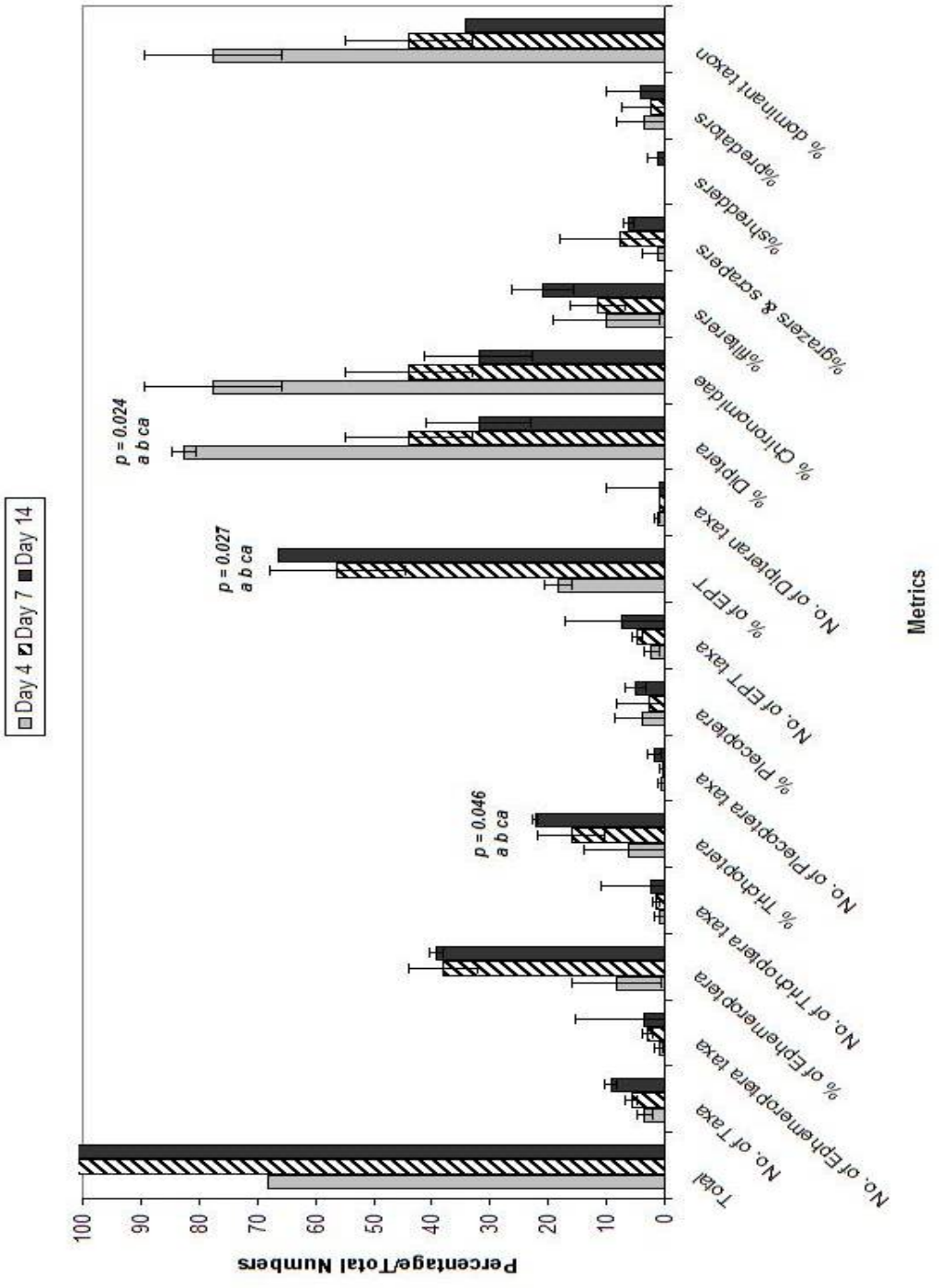



**Figure 36. Stillwater River Embeddedness Chamber Macroinvertebrate Metrics (4, 7, & 14-Day). Single Letters (a, b, c) Indicate No Significant Difference; Double Letters (ba, cb, ca) Indicate Significant Difference.**

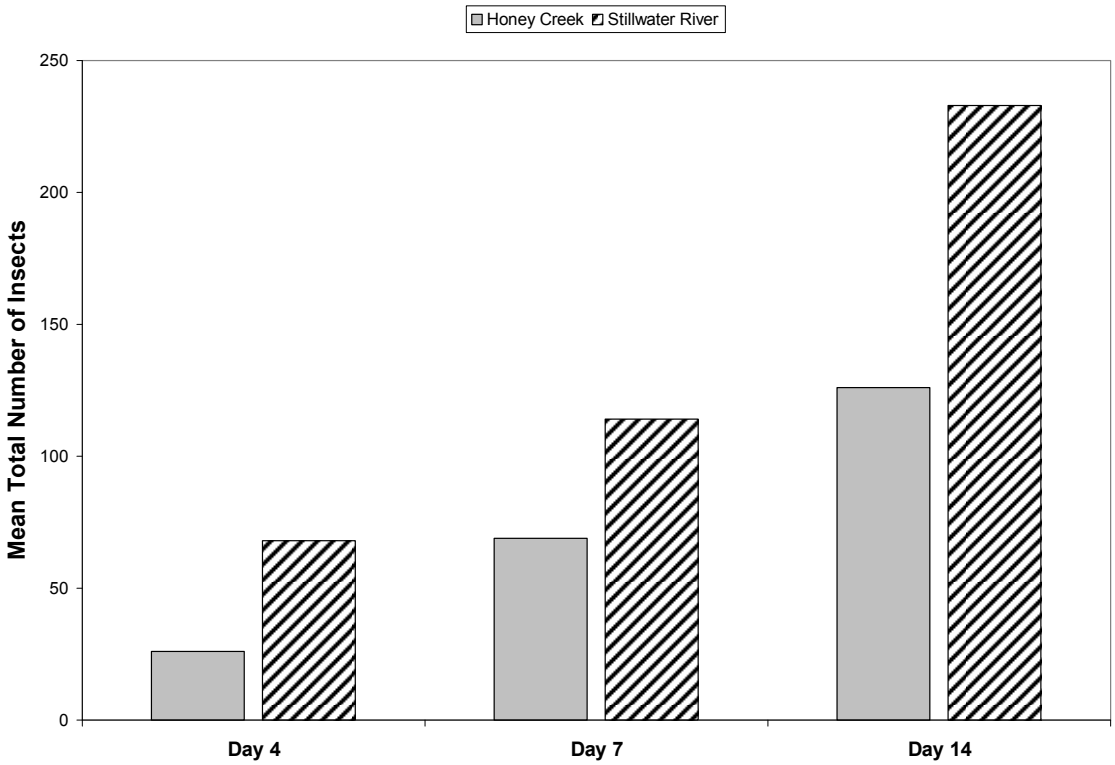



**Figure 37. Mean Total Numbers of Embeddedness Chamber Colonizing Insects.**

**Table 8. Taxa List for Honey Creek Embeddedness Chambers.**

| Order/Family | Functional Feeding Group | Tolerance Value | 4d Total | 7d Total | 14d Total |
|---|---|---|---|---|---|
| **Ephemeroptera** | | | | | |
| Isonychiidae | FC | 2 | 1 | 1 | 2 |
| Heptageniidae | SC | 4 | | 1 | 3 |
| Caenidae | GC | 7 | | | 1 |
| **Odonata** | | | | | |
| Coenagrionidae | PR | 6 | | 1 | |
| Calopterygidae | PR | 5 | | 1 | |
| **Pleoptera** | | | | | |
| Taeniopterygidae | SH | 2 | | 1 | |
| **Trichoptera** | | | | | |
| Philopotamidae | FC | 3 | | 1 | 1 |
| Polycentropodidae | FC | 6 | | 2 | 2 |
| Hydropsychidae | FC | 4 | 10 | 25 | 21 |
| Limnephilidae | SH | 4 | 1 | | |
| Hydroptilidae | SC | 4 | | 4 | |
| Leptoceridae | GC | 4 | | 1 | |
| **Coleoptera** | | | | | |
| Elmidae | GC | 4 | | 1 | |
| **Diptera** | | | | | |
| Simulidae | FC | 6 | 1 | | 1 |
| Chironomidae | GC | 6 | 12 | 27 | 93 |
| Tipulidae | SH | 3 | | 2 | 2 |
| Emphididae | PR | 6 | 1 | 1 | |
| **Total Insecta #** | | | 26 | 69 | 126 |

**PR=predator**
**GC=gather/collector**
**FC=filterer/collector**
**SC=scraper**

**Table 9. Taxa List for Stillwater River Embeddedness Chambers.**

| Order/Family | Functional Feeding Group | Tolerance Value | 4day | 7day | 14day |
|---|---|---|---|---|---|
| **Ephemeroptera** | | | | | |
| Isonychiidae | FC | 2 | 0 | 1 | 0 |
| Baetidae | GC | 4 | 2 | 0 | 1 |
| Heptageniidae | SC | 4 | 0 | 3 | 10 |
| Caenidae | GC | 7 | 4 | 20 | 36 |
| Potamanthidae | GC | 4 | 0 | 0 | 7 |
| Tricorythidae | GC | 4 | 0 | 17 | 32 |
| Siphlonuridae | GC | 7 | 0 | 0 | 1 |
| **Odonata** | | | | | |
| Coenagrionidae | PR | 6 | 0 | 0 | 1 |
| **Pleoptera** | | | | | |
| Perlodidae | PR | 2 | 2 | 2 | 8 |
| Taeniopterygidae | SH | 2 | 0 | 0 | 3 |
| **Trichoptera** | | | | | |
| Philopotamidae | FC | 3 | 0 | 0 | 1 |
| Polycentropodidae | FC | 6 | 0 | 1 | 2 |
| Hydropsychidae | FC | 4 | 3 | 10 | 49 |
| Hydroptilidae | SC | 4 | 0 | 6 | 1 |
| Glossosomatidae | SC | 0 | 1 | 0 | 2 |
| **Lepidoptera** | | | | | |
| Crambidae | | | 0 | 0 | 1 |
| **Coleoptera** | | | | | |
| Elmidae | GC | 4 | 0 | 0 | 1 |
| **Diptera** | | | | | |
| Simulidae | FC | 6 | 2 | 0 | 0 |
| Chironomidae | GC | 6 | 54 | 54 | 77 |
| **Total Insecta #** | | | 68 | 114 | 233 |

**PR=predator**
**GC=gather/collector**
**FC=filterer/collector**
**SC=scraper**

***Treatment Area Metrics.***

Treatment area sampling design facilitated two replicates per treatment area (Undisturbed, Slightly Disturbed, and Disturbed) per sampling time. Metric data was compared between the two sampling times (4 & 14 days) and significance was determined by running a one-way ANOVA with Tukey's Post Hoc test to determine if there were significant differences between the two sampling dates. All total numbers data was LN transformed prior to running statistical analysis in order to help the data fit the assumption of a normal distribution. All proportional data (% abundances) were transformed using an Arcsine Square Root transformation to meet the normal distribution assumption.

It was hypothesized that there would be significant differences between treatment areas during each sampling period and significant differences between treatment areas and each sampling period. The macroinvertebrate metrics from the Honey Creek site treatment area samples showed some significant differences but no significant differences were seen in the metrics from the Stillwater River site. Significant differences in the number of EPT taxa were found between all three of the Honey Creek treatment areas for Day 14 ($p = 0.021$) with the Disturbed site exhibiting the larger number of EPT taxa (Figures 38, 41, and 42). There was a significant difference ($p = 0.029$) in % Chironomidae between the Disturbed treatment area and the Undisturbed treatment area at the Honey Creek site for Day 14 with the Disturbed treatment area exhibiting a higher percentage (Figures 38 and 42). The number of Ephemeroptera taxa at Honey Creek showed a significant difference ($p = 0.047$) (Figure 38 and 42) and though Tukey's pair-wise comparison did not indicate significance between the Undisturbed and Disturbed

treatment areas at Day 14, review the statistical program output of the confidence intervals suggest so. Though not significant ($p = 0.098$) there was a possible trend noticed for % Predators at the Honey Creek site.

Two-sample t-tests were run to determine any significant differences between a particular treatment area and the two sampling dates (i.e. Undisturbed Day 4 vs. Undisturbed Day 14). The only significant differences (increasing trends) between the two sampling dates were in the Slightly Disturbed treatment area at Honey Creek for % Diptera ($p = 0.047$) (Figure 41) and the Family Biotic Index ($p = 0.05$) (Figure 40). There were several metrics that exhibited possible trends at the Honey Creek site even though the p-values were >0.05. In the slightly disturbed treatment area at Honey Creek possible differences existed in % Chironomidae ($p = 0.092$, increasing trend), % Plecoptera ($p = 0.066$, decreasing trend), % Shredders ($p = 0.066$, decreasing trend), and % Dominant Taxon ($p = 0.088$, increasing trend) (Figure 41). Possible differences in the Slightly Disturbed area at the Stillwater River site were also noted in % Ephemeroptera ($p = 0.095$, increasing trend) and % Dominant Taxon ($p = 0.055$, decreasing trend) (Figure 44). Two other metrics at the Honey Creek site also showed possible differences, % Diptera in the Undisturbed area ($p = 0.076$, decreasing trend) (Figure 38) and % Ephemeroptera in the Disturbed area ($p = 0.072$, increasing trend) (Figure 42).

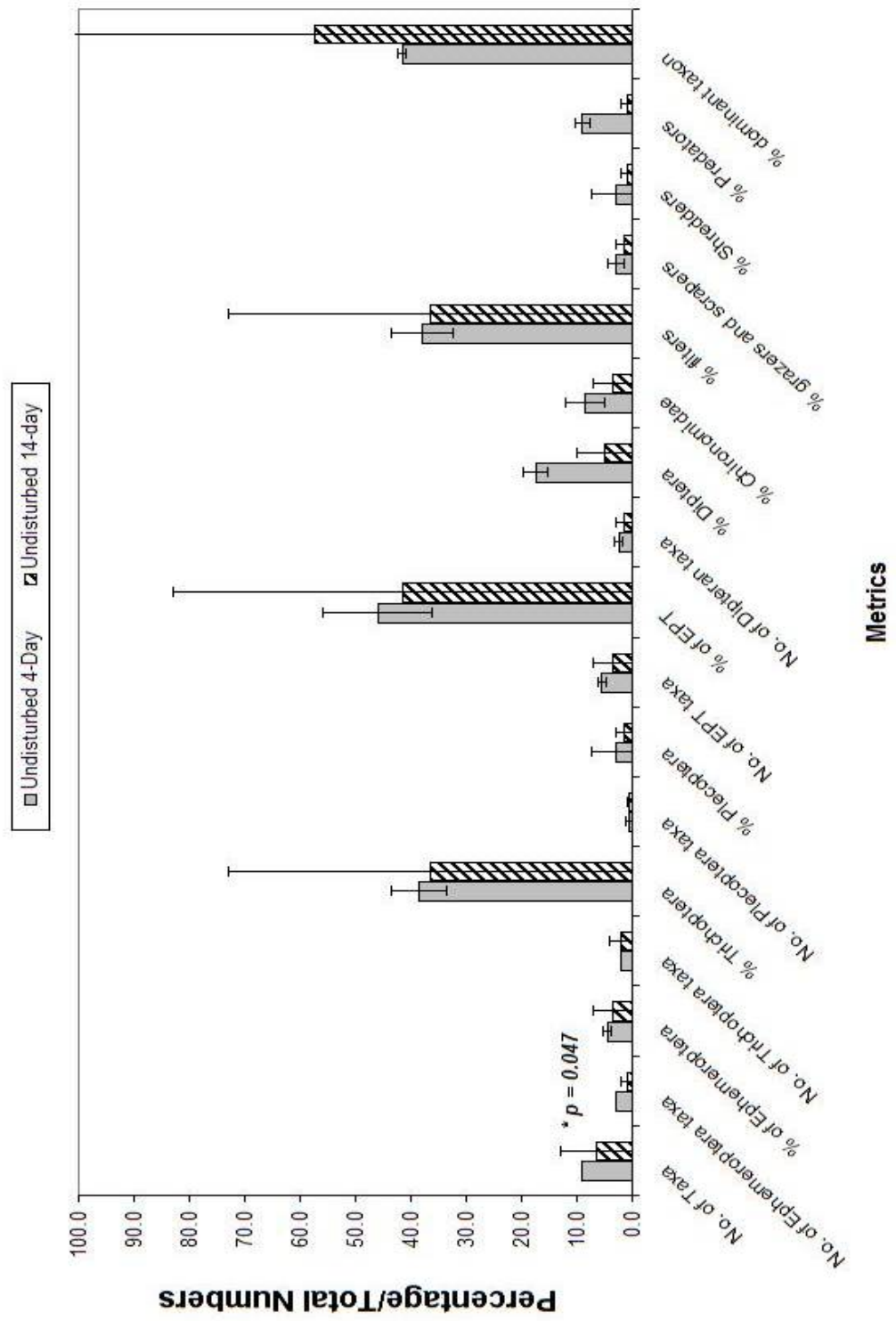



**Figure 38. Honey Creek Undisturbed Treatment Area Metrics (4 & 14-Day).**

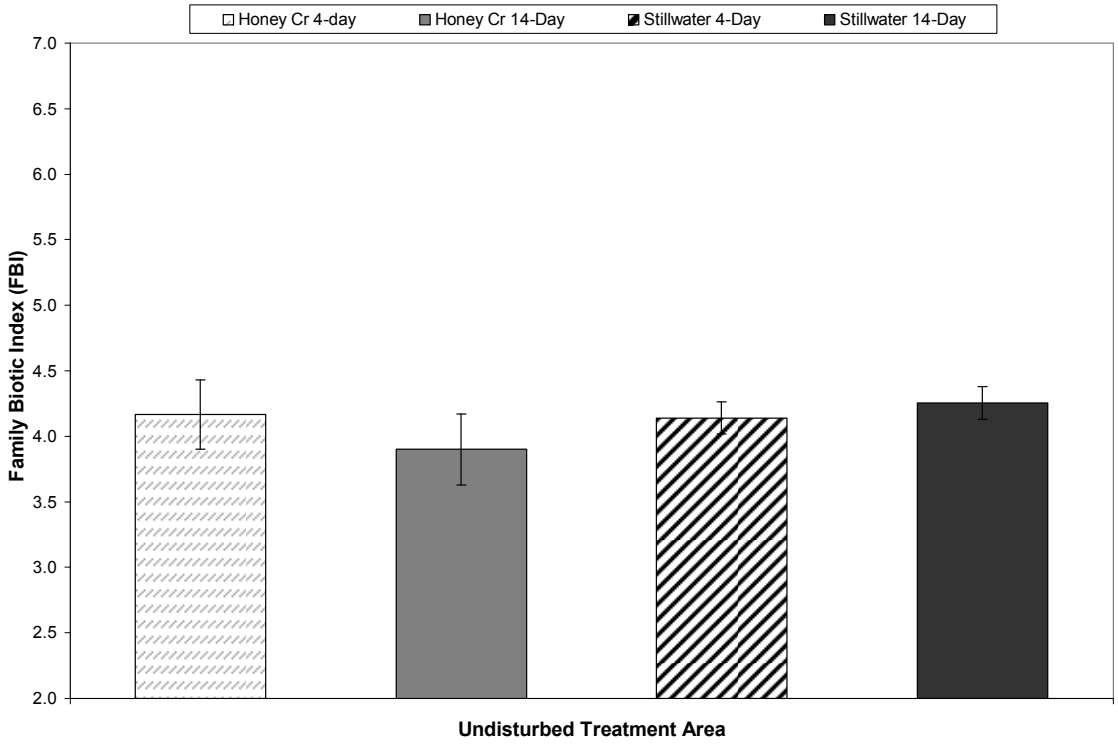



**Figure 39. Undisturbed Treatment Area Family Biotic Index Metric (4 & 14-Day) for both Sampling Sites.**

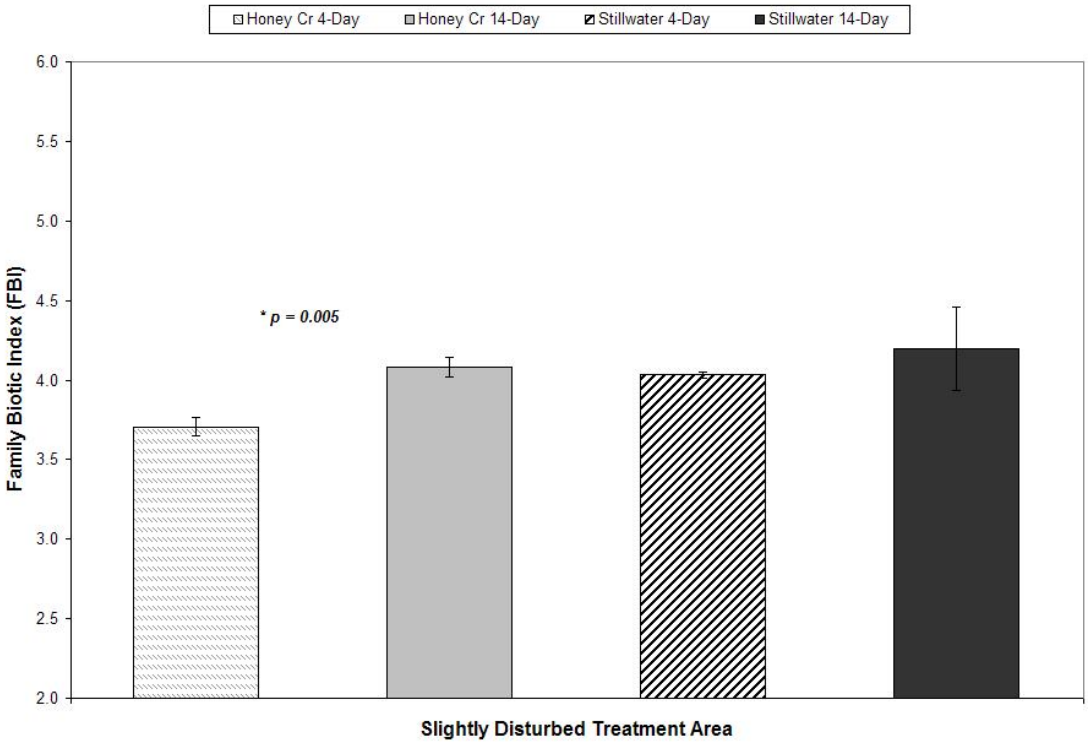



**Figure 40. Slightly Disturbed Treatment Area Family Biotic Index Metric (4 & 14-Day) for both Sampling Sites.**

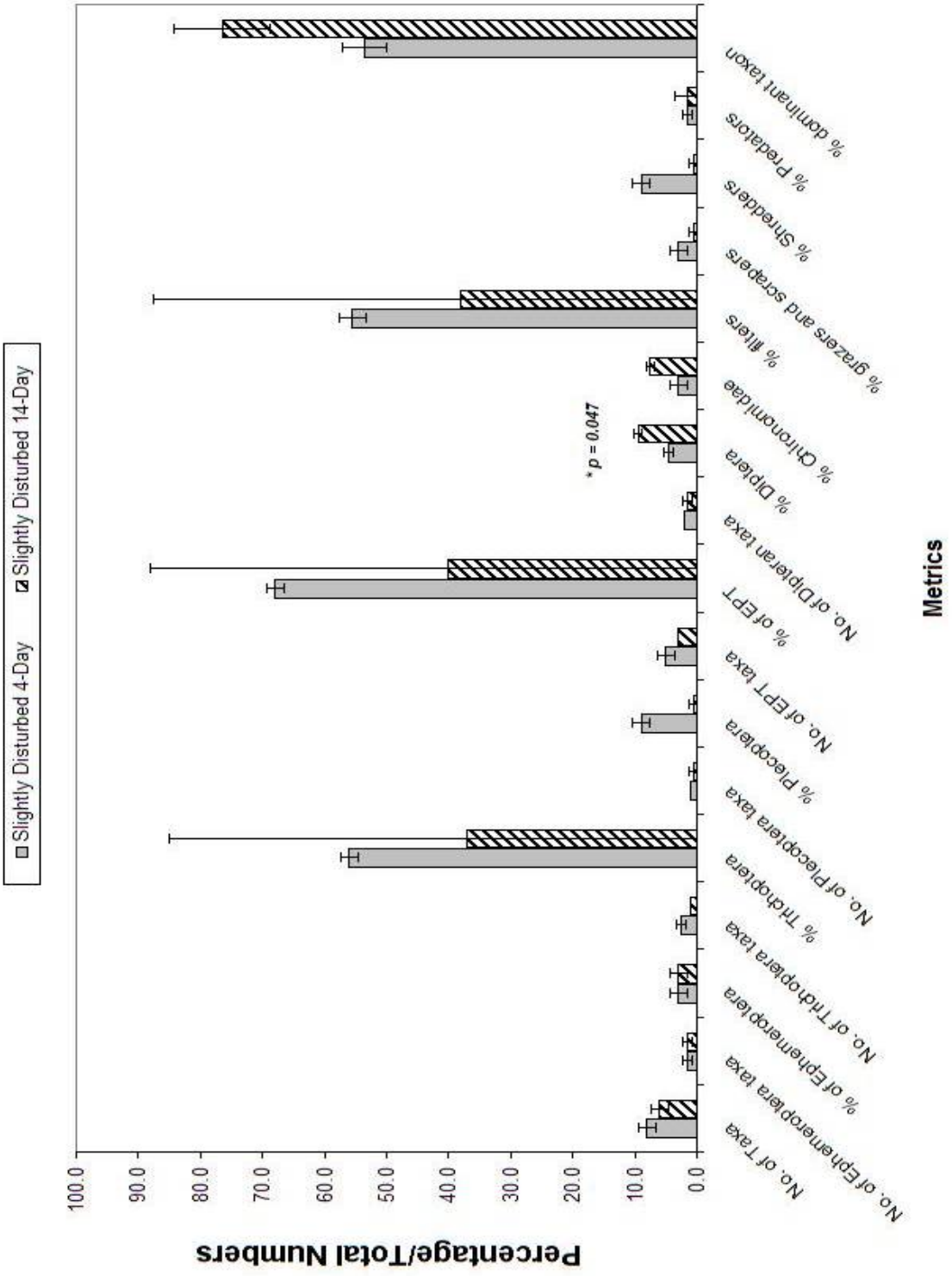



**Figure 41. Honey Creek Slightly Disturbed Treatment Area Metrics (4 & 14-Day).**

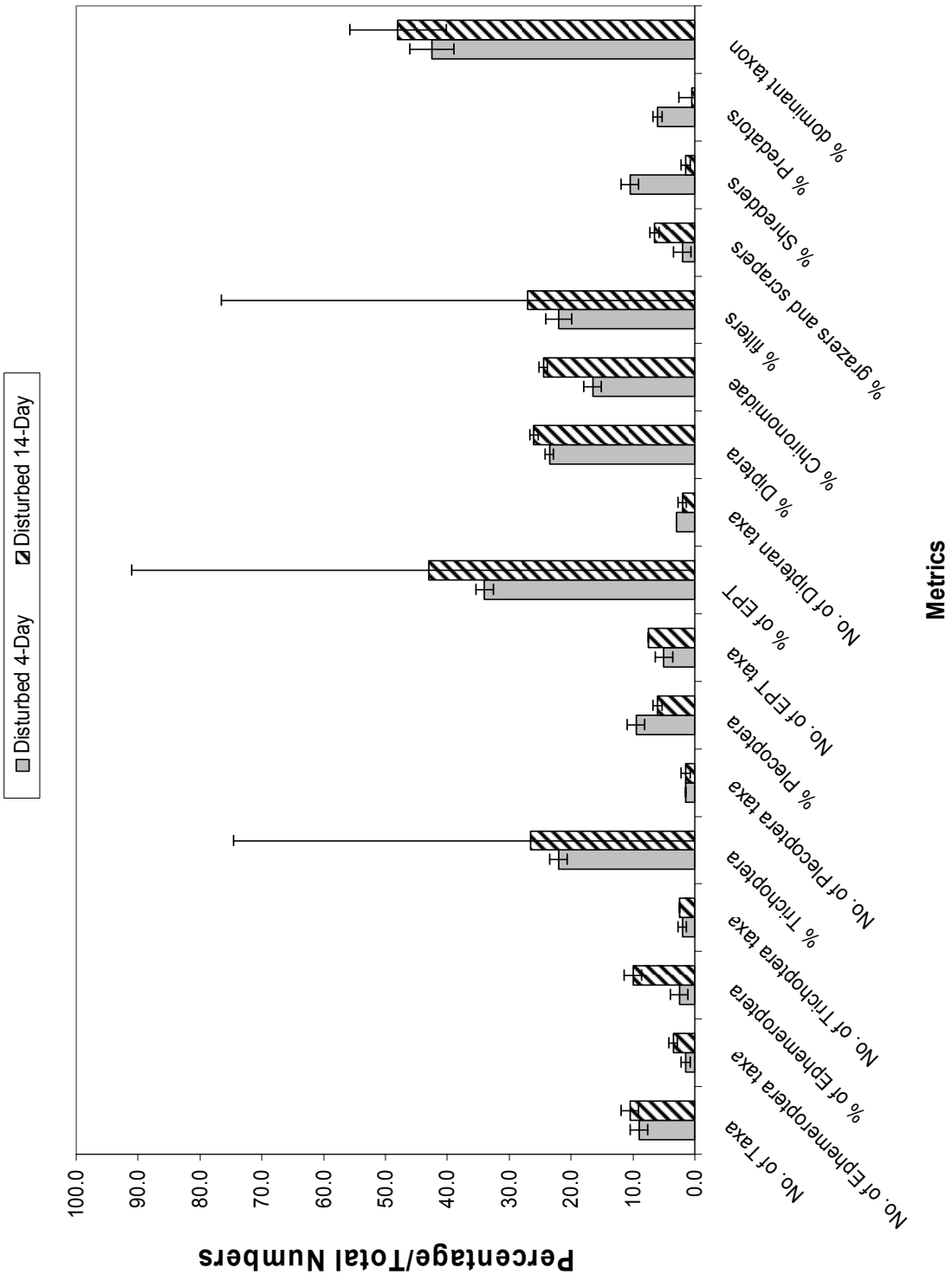



**Figure 42. Honey Creek Disturbed Treatment Area Metrics (4 & 14-Day).**

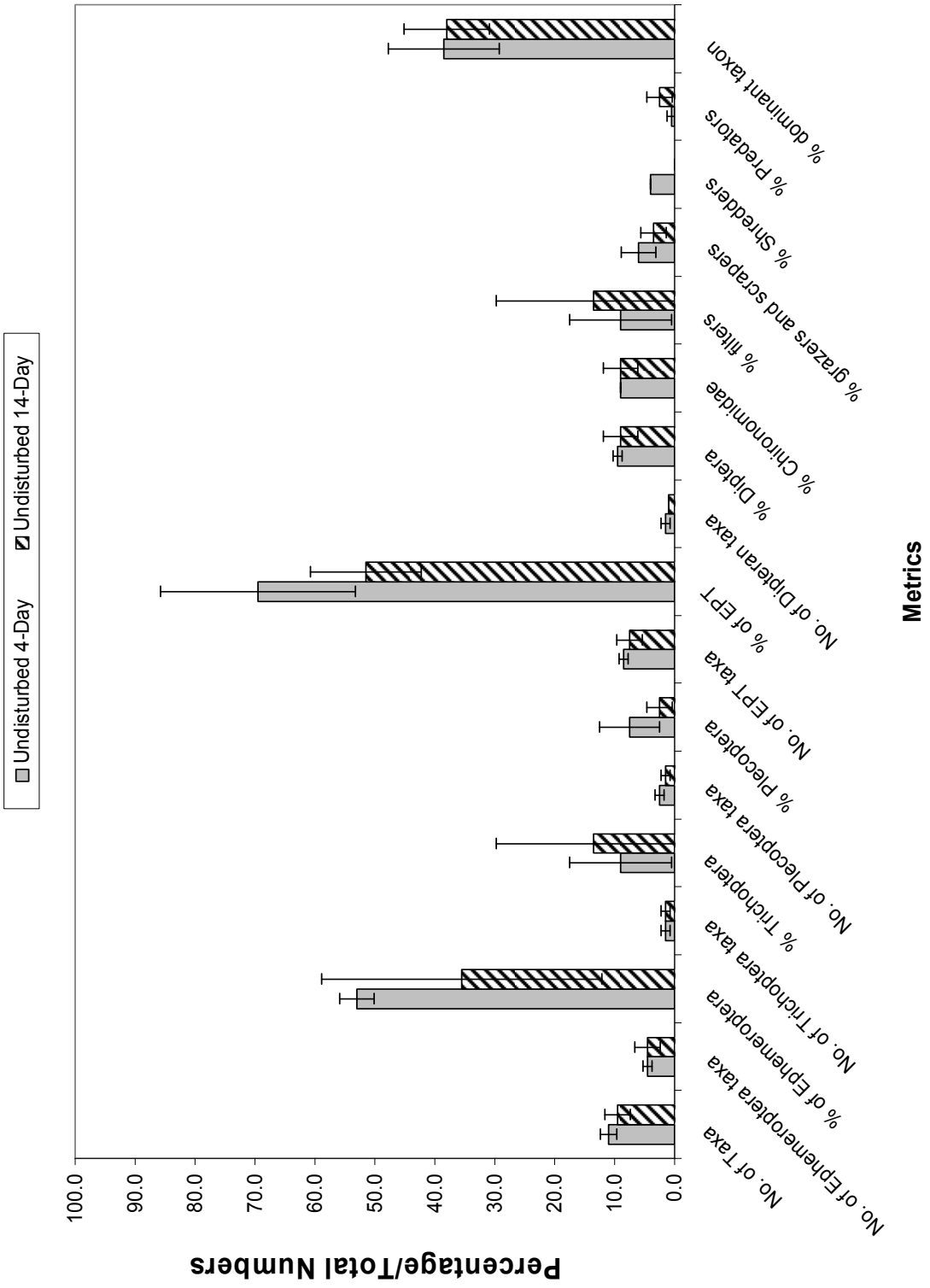



**Figure 43. Stillwater River Undisturbed Treatment Area Metrics (4 & 14-Day).**

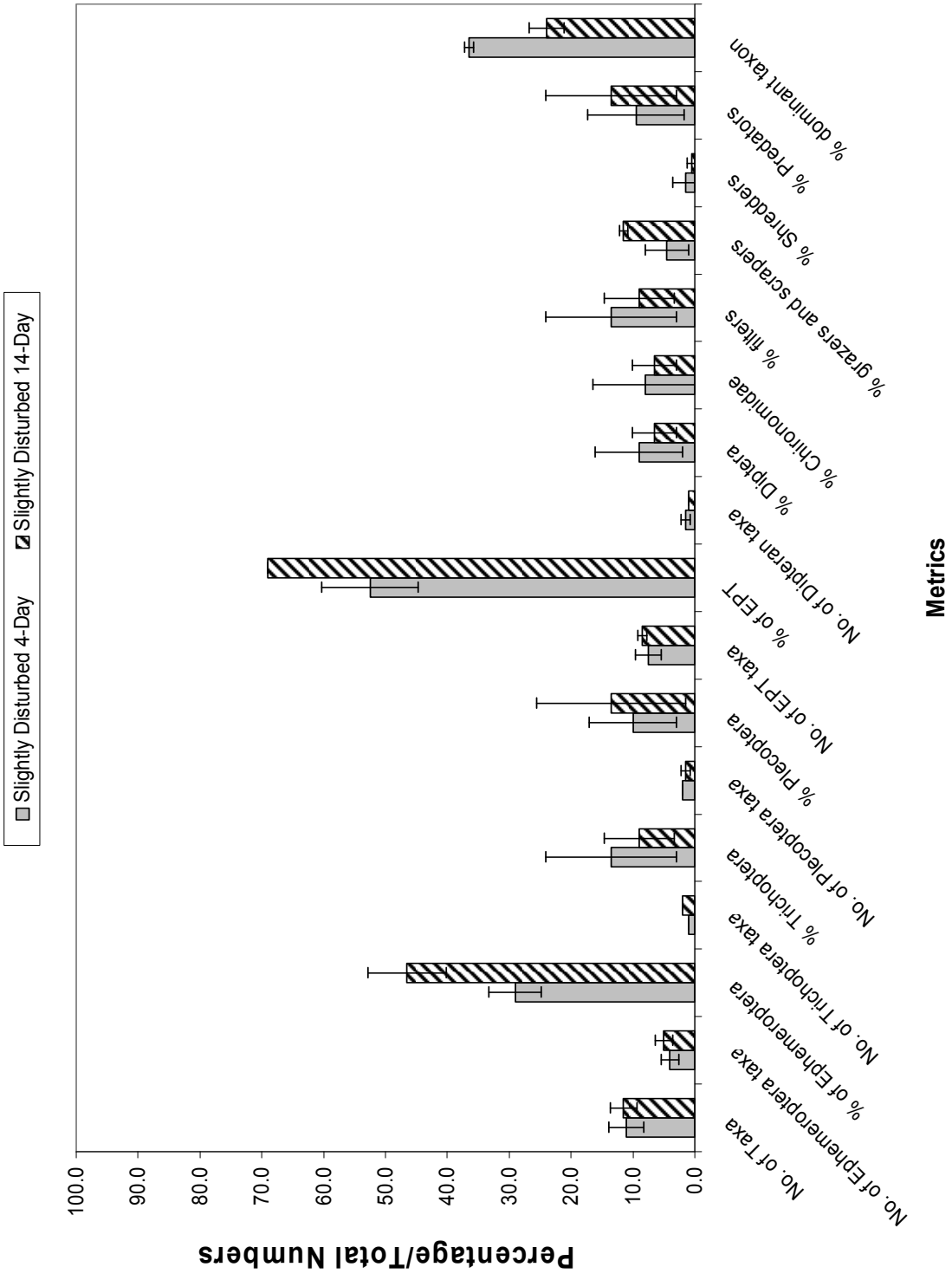



**Figure 44. Stillwater River Slightly Disturbed Treatment Area Metrics (4 & 14-Day).**

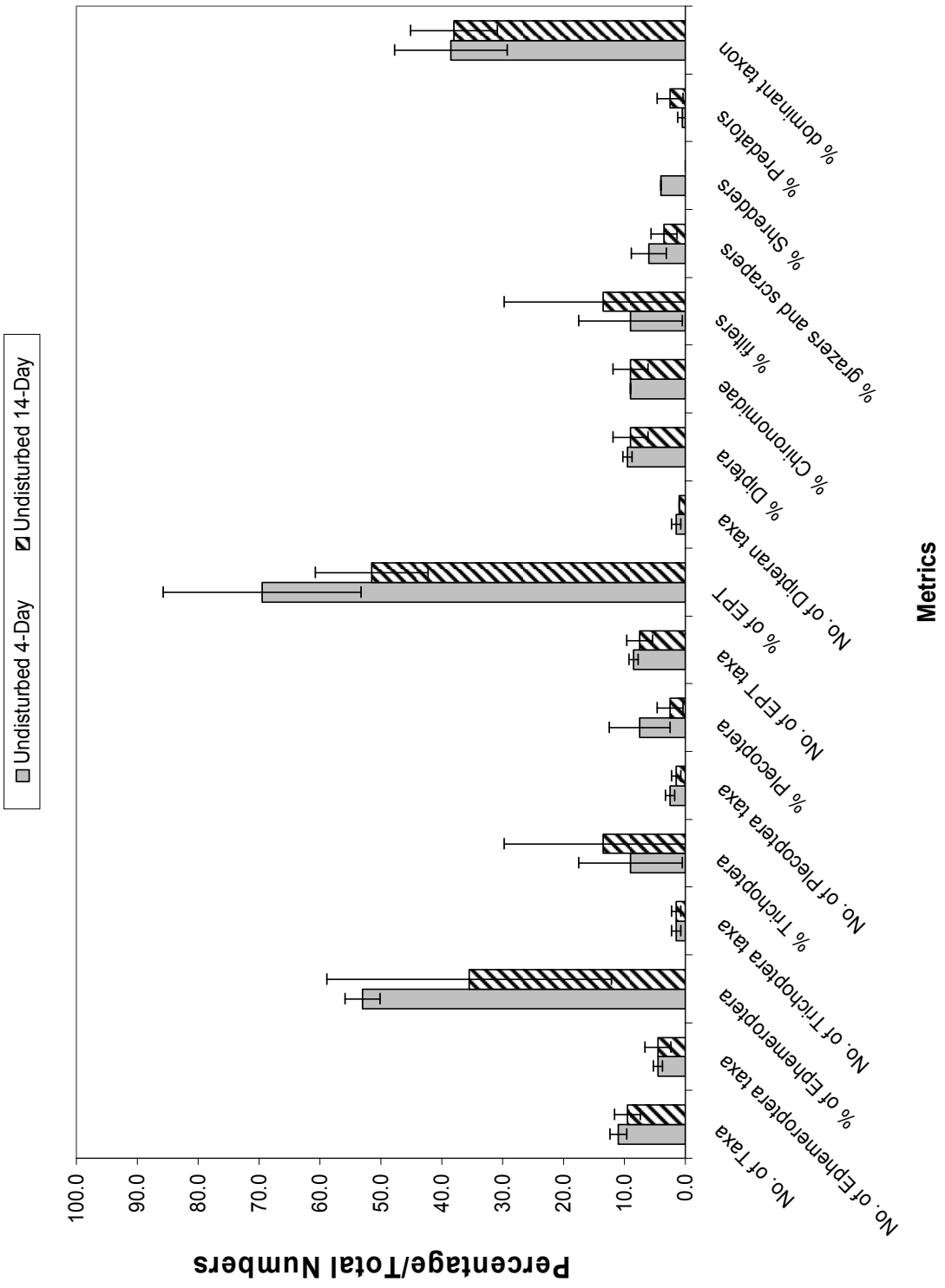



**Figure 45. Stillwater River Disturbed Treatment Area Metrics (4 & 14-Day).**

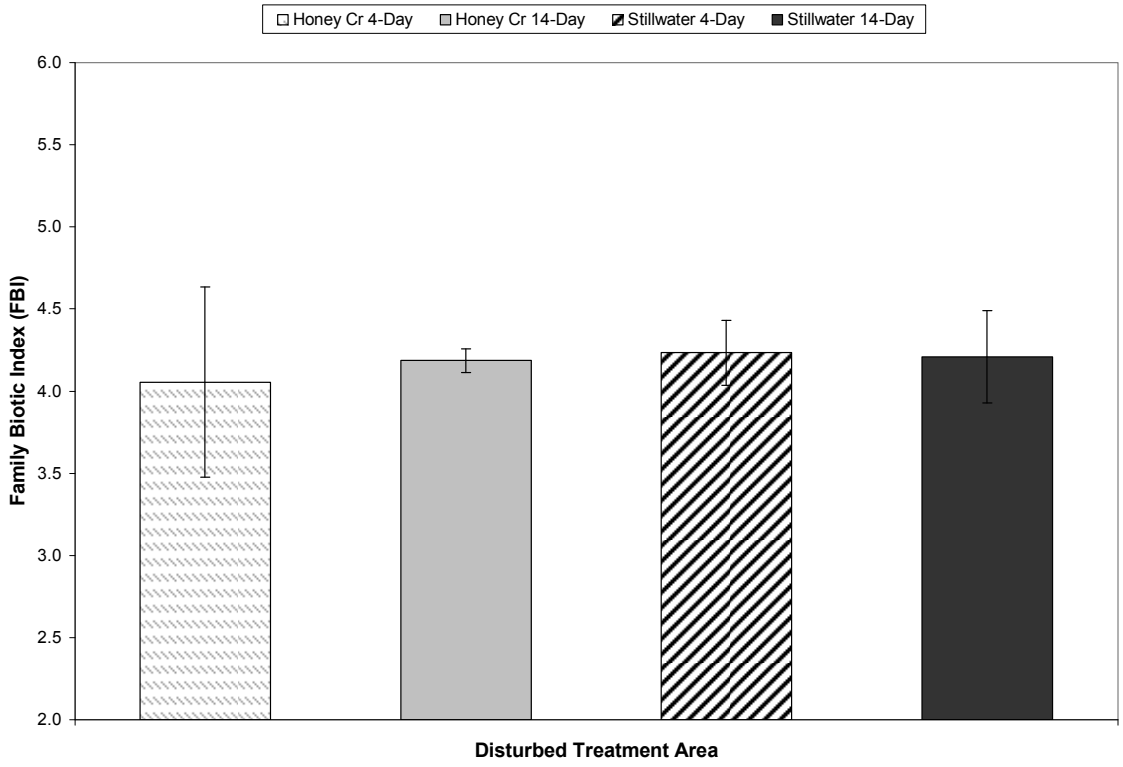



**Figure 46. Disturbed Treatment Area Family Biotic Index Metric (4 & 14-Day) for both sampling sites.**

# V. Discussion

## Pilot Study

The initial embeddedness chamber exposure on the Mad River during the pilot study validated the design of the embeddedness chamber showing that it was capable of accumulating sediment bound for the interstices of the stream substrate. Though not originally intended for use as a benthic macroinvertebrate colonization tool, observations from the processed chambers indicated that the resident fauna were rapidly taking advantage of the clean substrate (marbles). This size of the mesh covering the chambers was changed from 1 mm openings to 4 mm openings in large part due to this observation. Though a detailed identification of the colonizing macroinvertebrates was not undertaken cursory observations indicated that the major taxa where from the orders Trichoptera and Diptera (Chironomidae), both of which have taxa that are relatively tolerant to perturbation. The rapid accumulation of sediment within the embeddedness chambers during this pilot study also influenced the decision to run the primary chamber study at relatively short exposures to capture what is occurring during the initial stages of embeddedness. Sediment dry weight data from the pilot study was initially intended to determine the how many replicates would be needed to detect differences in accumulating sediment between sites. Revision of the overall study design included a shift in focus from between sites within a stream to differences over time at one site. The power analysis results were still valid in determining how many replicates would be needed to detect differences between different sampling times at the desired α-level.

**Embeddedness Chamber Optimization**

The embeddedness chamber design that was modified from the pilot study demonstrated that it was capable of capturing both accumulating sediment within the substrate and colonizing macroinvertebrates. Improvements in design such as the larger mesh size and end cap holes provided attributes that were more representative of the natural substrate. Observations of several net spinning caddis fly larvae cases inside the chambers and adjacent to the end cap holes during processing indicates that the chamber design seemed to provide adequate subsurface water flow. The chambers held up quite well during the 14-Day exposure but the design is yet to be tested over longer durations and during high flow conditions. The *in situ* tray design worked as intended; keeping the embeddedness chambers securely in place during the exposure. The system used to secure the chambers to the *in situ* tray was also effective in facilitating removal of single chambers at selected times without disturbing adjacent chambers. Like the embeddedness chambers, the in situ tray design for this experiment has yet to be tested over long exposures and during high flow events. Embeddedness chamber deployment and retrieval covers appeared to work in minimizing sediment loss but further validation through a small scale test in the field would confirm their effectiveness. The glass marbles serving as artificial substrate were easily cleaned of any visible organic and inorganic matter during chamber processes yet longer field exposures may see a noticeable biofilm develop on the surface of the marbles which would affect the dynamics of the imposed habitat (i.e. food availability, adsorption of colloidal material).

## Sediment Quality Testing

Results from the ten day sediment toxicity test indicate no apparent toxicity within the site sediments. The diversity of the macroinvertebrate community; as seen in the RBP samples, chamber colonization, and treatment area samples is indicative of the quality of the substrate at the study sites (Honey Creek and Stillwater River). Further studies at these sites would require no additional chemical toxicity endpoints to infer that site substrate toxicity was influencing macroinvertebrate colonization of the embeddedness chambers. DGT results for the 24 h exposure periods at each site were inconclusive indicate flux of Cu and Zn at levels higher than Cd, Ni, and Pb. Cu fluxes appear to have been greater in the more disturbed treatment areas at Honey Creek (Figure 17) and greater in the less disturbed treatment areas at the Stillwater River site (Figure 18). Zinc fluxes at both sites were higher than all of the other metals analyzed (Figures 17 and 18) but show a varied response to the treatment areas at each site. Unfortunately the blanks indicate high levels of Cu and Zn which point towards possible cross-contamination having occurred during the laboratory analysis. With the contamination possibility in mind, results from the areas must weighted carefully.

## Physicochemical Water Quality Parameters

All of the parameters sampled were intended to provide a better picture of some of the major variables involved with sediment movement and macroinvertebrate colonization. When viewed with a weight of evidence approach each piece can help to confirm conclusions made from both the sediment and macroinvertebrate data. Results

from the stream flow measurements taken on selected dates as well as the USGS gage data suggest that stream flow fluctuations were minimal during the primary chamber exposures (Figure 20). This is important in order to determine what is occurring at base flow conditions with depositing sediment (and subsequent embedding sediment). Large variations in flow would only serve to confound observations made during this initial analysis of the *in situ* embeddedness method. This fact holds true for both TSS and turbidity as well, both of which can be used as an indicator of possible sediment and nutrient load within a system. The TSS results indicate small fluctuations in suspended solids concentration (Figure 22) but it would be useful in further studies to include more frequent samples over an exposure period. Turbidity readings as well as Conductivity readings from the selected sampling times indicate that there was minimal fluctuation over the embeddedness chamber exposure periods. However, continuous readings provided by a data Sonde deployed during the duration of an exposure would be more useful in assessing stream conditions.

## Substrate Characterization

### *Embeddedness.*

Percent embeddedness measurements from each site indicate that these stretches of streams were not experiencing excessive stress due to substrate embeddedness. Results for both sites are below what has been considered to be the normal range for low to moderate gradient systems such Honey Creek and the Stillwater River (~33-35%)

(Figure 25). The presence of sediment in lotic systems in a natural phenomenon and some level of substrate embeddedness is inevitable in a lower gradient system that is not experience excessive sediment inputs. Bed load movement of substrate is also a natural phenomenon and its magnitude is determined largely in part to discharge. When stream discharge reaches a critical value of stability for a given systems, the relatively stable layer of embedded substrate (armour layer) will begin to break up and the embedded fine particulates will be flushed downstream (Schälchli, 1992). The flushing of embedded fine sediment is dependent on higher flow events that in most natural systems occur as a result of precipitation or snow melt events. In the absence of frequent higher discharges, a system experiencing excessive sediment inputs will likely exhibit a higher percent embeddedness of the substrate.

***Grain Size Fractional Analysis of Chamber Sediment.***

Embeddedness chamber sediment results indicate that the sand and silt (250 µm - 2 mm and 63 µm - 250 µm respectively) fractions were the dominant particles that were embedding within the chambers (Figures 30 and 31). The term embedding is used loosely here because the sediment within the chambers continued to increase steadily over the 14 days of exposures. The steady increase in sediment (Total Dry Fraction) at both sites indicates that the chambers had yet to reach a dynamic equilibrium with the surrounding substrate (Figures 30 and 31). These sediment results compared to the colonization data suggest that the significant differences (both in sediment and macroinvertebrate data) over time may be the result of a clean substrate moving towards conditions existing in the surrounding substrate. Longer embeddedness chamber

exposure times are necessary to better examine the physical processes that are occurring with the sediment within the chambers. It is expected that the embeddedness chambers would continue to accumulate sediment over longer exposure times and would eventual exhibit an asymptotical response as the chambers reached equilibrium for the particular system.

Porosity data indicated a clear trend in decreasing porosity from Day-4 to Day-14 at both Honey Creek and the Stillwater River (Figure 32). Regression analysis revealed negative correlations between porosity and the total dry fraction, the 1.2 µm - 63 µm dry fraction and the 250 µm - 2 mm dry fraction of chamber sediments at the Honey Creek site (Table 6). Regression analysis revealed correlations between porosity and the total dry fraction, 63 µm - 250 µm dry fraction, the 250 µm - 2 mm dry fraction, and % Organic content of chamber sediments at the Stillwater River site (Table 7). All of these data suggest that the embeddedness chamber design and subsequent sample and data processing is able to capture the well supported phenomenon of decreasing substrate porosity with increasing fine sediment.

## Benthic Macroinvertebrate Characterization

### *RBP Metrics.*

The macroinvertebrate RBP results show both sites experiencing high values of the following metrics: % Trichoptera, % EPT taxa, % Filterers, and % Dominant taxa (Figure 33). These results indicate that the bulk of the macroinvertebrate assemblage (insects only) is comprised of mainly taxa that have a moderate pollution tolerance. RBP

results from Honey Creek indicate that Trichoptera taxa are the primary contributors to the % EPT taxa metric (the majority being Hydropsychidae) (Table 5). Contrary to Honey Creek, the Stillwater River metric results indicate that Ephemeroptera taxa were the primary contributors to % EPT taxa (the majority being Heptageniidae and Tricorythidae) (Table 5). Both dominant families of Ephemeroptera taxa (Heptageniidae and Tricorythidae) have moderate pollution tolerance values (4). RBP results from both sites provided a background list of macroinvertebrates that could be expected to colonize the embeddedness chambers during an exposure.

***Embeddedness Chamber Metrics.***

Macroinvertebrate metric analyses results indicate significant differences in the number of total taxa, the number of Trichoptera taxa, and the number of EPT taxa between day 4 and day 14. Analyses revealed that the significant differences and correlations between particular metrics and time at Honey Creek (No. of Trichoptera, No. of EPT taxa) (Table 6) suggest that the chambers are representing actions of taxa that are known early colonizers and opportunists. This information validates that the embeddedness chambers are effective in representing what naturally occurs in many systems.

Results from the statistical analysis of embeddedness chamber macroinvertebrate colonization data from the Stillwater River site indicate significant correlations between the majority of metrics and the 63 μm – 250 μm fraction of the chamber sediments. This is interesting because the 250 μm – 2 mm size fraction is the dominant proportion of the

total sediment for all three sampling points at this site (Figures 31). Had the dominant fraction been the 63 μm – 250 μm size, then it would have been easy to suggest that the correlations between the metrics and the particular sediment size were occurring mainly due to proportionality. Further investigations are warranted to identify these interesting correlations. Analyses revealed that metrics responding to the 63 μm – 250 μm fraction (% Trichoptera, No. of EPT taxa, % EPT, % Chironomidae, and % Dominant Taxon) (Table 7) consisted of taxa that are known early colonizers and opportunists. This information also validates that the embeddedness chambers are effective in representing what naturally occurs in many systems, as was seen with the Honey Creek data.

***Treatment Area Metrics.***

Results from analyses of the treatment area metrics indicate very few significant differences compared to the embeddedness chamber results. ANOVA results from the Honey Creek treatment areas reveal significant differences between the number of EPT taxa on Day-14 between all of the treatment areas (Figures 38, 41 and 42). Also noted were significant differences in % Chironomidae between the disturbed and undisturbed treatment areas on day 14 (Figures 38 and 42). T-test analysis between treatments and sampling times revealed significant differences between the slightly disturbed treatment areas and % Diptera and the FBI values for both 4-Day and 14-Day samples (Figures 42 and 40). No significant relationships were discovered for the Stillwater River data. All of the results for the treatment areas suggest that there needs to be more time elapsing between disturbances in order to detect noticeable differences in macroinvertebrate response to various levels of disturbance.

## Conclusion

The acceptance of excessive sediment inputs in lotic systems as a major stressor has been well documented and numerous papers have been devoted to the effect of sediment on aquatic macroinvertebrates as well as the effect of embeddedness and colmation on hyporheic exchange processes. Physical and ecological assessment of aquatic systems requires an assortment of tools and techniques for use in a weight of evidence approach. *In situ* methods are becoming more established as viable means of assessing what is actually occurring in the system of concern because the methods allow for assessment of the effects of multiple exposures. Embeddedness quantification techniques have undergone much scrutiny and the subjective nature of many techniques has led to the decrease in the perceived importance of percent embeddedness as an endpoint in habitat assessments. It can be assumed that the creation of a more quantitative means of expressing embeddedness and linking its effect on biota would be well accepted in the environmental assessment field.

Results from this research suggest that the in situ embeddedness chamber as a means of linking embeddedness and macroinvertebrate health holds much promise. Further research into how well the embeddedness chamber reflects base-line *in situ* conditions may provide more and stronger evidence of significant correlations. Examination of chamber dynamics under various base-flow conditions including different habitats (i.e. riffles) and during different seasons will provide insight into significant relationships between embeddedness and biotic responses. Evaluating chamber dynamics during different flow regimes may also provide useful information in the calibration and validation of the embeddedness chamber as an effective tool in

assessing embeddedness of a system. Inclusion of non-insect taxa into the analysis may provide additional significance needed to validate assumptions. Though this study evaluated relatively short colonization times, results suggest that there are some significant correlations present. Additional research evaluating longer colonization and embedding times could reveal an expected asymptotical response of colonization and accumulated sediment within the artificial substrate over time. Strengthening the linkage between the current percent embeddedness quantification and this experimental approach is needed to further determine important relationships. A possible benefit of this *in situ* embeddedness methodology is a means of assessing the abiotic and biotic effects of a multi sediment-related stressor exposure. Additional chambers during an exposure could be devoted to assessing pollutant and nutrient loads associated with depositing sediment within the chambers. The ultimate goal of developing an *in situ* embeddedness quantification method is a sound, well-tested system that can be standardized for use in many situations. The path to standardization of such a method would require testing and calibration in many different eco-regions under a variety of situations examining a multitude of variables.

# Appendix

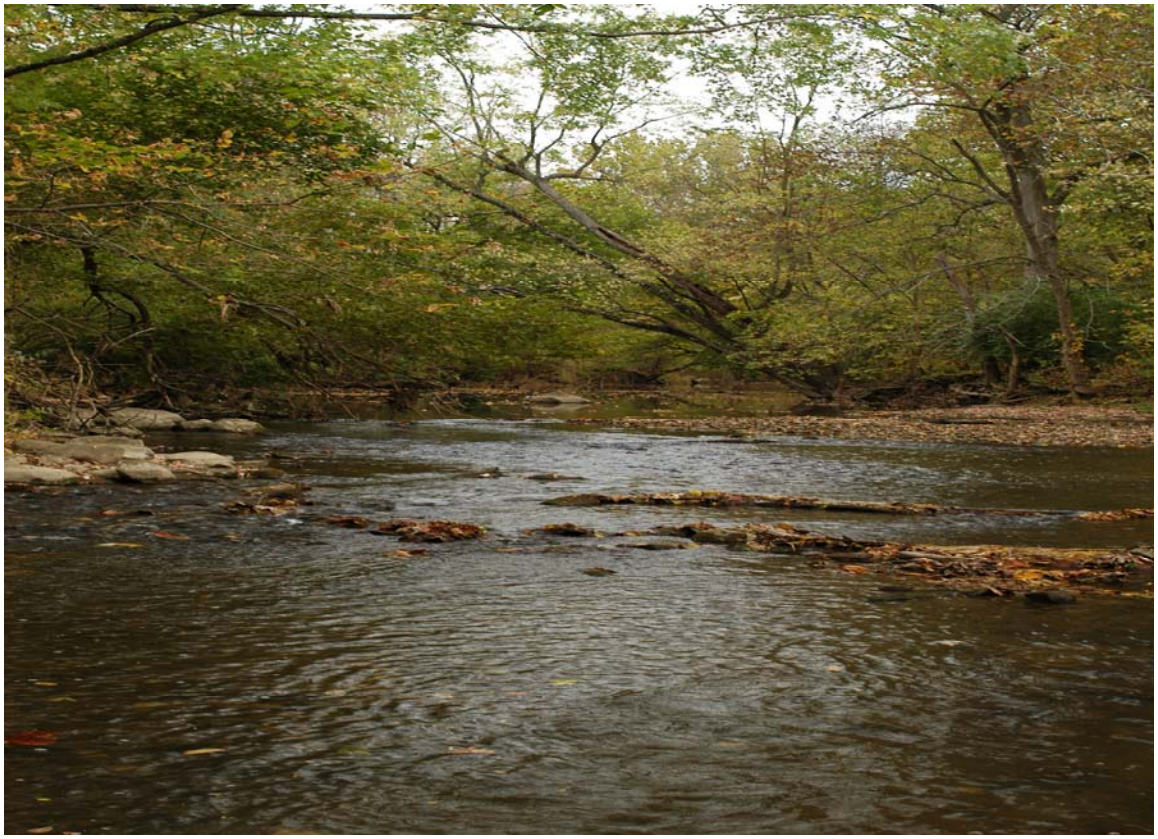


**Figure 47. Photograph of the Honey Creek Study Site**

**Figure 48. Photograph of the Stillwater River Study.**

**Table 10. Pilot Study Embeddedness Chamber Raw Sediment Data**

| Embeddedness (EMB) Pilot Study | | | | | | | |
|---|---|---|---|---|---|---|---|
| Sample | Beaker (Empty) Wt grams | Beaker (w/ Dry Sediment) Wt grams | Dry Sediment Wt grams | | Site | Mean Dry Sediment Wt | Std Dev |
| Site 1-1 | 219.24 | 245.17 | 25.93 | | 1 | 29.18 | 4.38 |
| Site 1-2 | 222.16 | 252.43 | 30.27 | | 2 | 22.35 | 2.85 |
| Site 1-3 | 222.64 | 250.37 | 27.73 | | 3 | 24.47 | 1.85 |
| Site 1-4 | 224.54 | 251.06 | 26.52 | | 4 | 31.07 | 7.94 |
| Site 1-5 | 220.17 | 254.32 | 34.15 | | | | |
| Site 1-6 | 222.89 | 257.35 | 34.46 | | | | |
| Site 1-7 | 223.12 | 255.46 | 32.34 | | | | |
| Site 1-8 | 227.12 | 249.17 | 22.05 | | | | |
| Site 2-1 | 224.09 | 245.15 | 21.06 | | | | |
| Site 2-2 | 221.07 | 237.04 | 15.97 | | | | |
| Site 2-3 | 226.82 | 250.77 | 23.95 | | | | |
| Site 2-4 | 223.86 | 246.46 | 22.6 | | | | |
| Site 2-5 | 219.61 | 244.08 | 24.47 | | | | |
| Site 2-6 | 221.22 | 243.54 | 22.32 | | | | |
| Site 2-7 | 222.56 | 247.24 | 24.68 | | | | |
| Site 2-8 | 223.52 | 247.25 | 23.73 | | | | |
| Site 3-1 | 221.59 | 246.73 | 25.14 | | | | |
| Site 3-2 | 223.98 | 250.17 | 26.19 | | | | |
| Site 3-3 | 222.34 | 246.82 | 24.48 | | | | |
| Site 3-4 | 221.64 | 245.38 | 23.74 | | | | |
| Site 3-5 | 222.88 | 247.39 | 24.51 | | | | |
| Site 3-6 | 226.6 | 253.76 | 27.16 | | | | |
| Site 3-7 | 220.10 | 243.56 | 23.46 | | | | |
| Site 3-8 | 221.97 | 243.02 | 21.05 | | | | |
| Site 4-1 | 224.38 | 259.84 | 35.46 | | | | |
| Site 4-2 | 222.98 | 259.61 | 36.63 | | | | |
| Site 4-3 | 223.57 | 238.51 | 14.94 | | | | |
| Site 4-4 | 220.15 | 246.71 | 26.56 | | | | |
| Site 4-5 | 223.14 | 251.92 | 28.78 | | | | |
| Site 4-6 | 219.10 | 248.95 | 29.85 | | | | |
| Site 4-7 | 220.13 | 259.61 | 39.48 | | | | |
| Site 4-8 | 219.42 | 256.27 | 36.85 | | | | |
| | | | | | | | |
| Oven Temp | 100 °C | NOTES: | | | | | |
| Start Date | 27-Jun-07 | Pulled from oven, let stand for 5 min to cool then weighed | | | | | |
| Start Time | 1800 | Beakers cooled on bench paper and cart. | | | | | |
| End Date | 3-Jul-07 | Temp range (80-104°C) | | | | | |
| End Time | 1215 | | | | | | |

## Table 11. Sediment Toxicity Test Raw Data

| Treatment | Replicate | Org Survival | %Survival | Control | |
|---|---|---|---|---|---|
| Control | 1 | 9 | 90.00 | Mean | 97.50 |
| Control | 2 | 10 | 100.00 | Std Dev | 5.00 |
| Control | 3 | 10 | 100.00 | | |
| Control | 4 | 10 | 100.00 | **Honey Creek** | |
| Honey Cr. | 1 | 9 | 90.00 | Mean | 87.50 |
| Honey Cr. | 2 | 8 | 80.00 | Std Dev | 9.57 |
| Honey Cr. | 3 | 8 | 80.00 | | |
| Honey Cr. | 4 | 10 | 100.00 | **Stillwater R.** | |
| Stillwater R. | 1 | 7 | 70.00 | Mean | 90.00 |
| Stillwater R. | 2 | 10 | 100.00 | Std Dev | 14.14 |
| Stillwater R. | 3 | 10 | 100.00 | | |
| Stillwater R. | 4 | 9 | 90.00 | | |

**2-Sample t-test**

| | |
|---|---|
| HY & Control | p=0.138 |
| SR & Control | p=0.391 |

**Kolomogrov-Smirnov Normality**

| | |
|---|---|
| HY & Control | p>0.15 |
| SR & Control | p>0.15 |

**Lenene's Equal Variance Test**

| | |
|---|---|
| HY & Control | p=0.207 |
| SR & Control | p=0.228 |

**Table 12. Sediment Toxicity Test Water Quality Data**

| Date | Day 1 (9/29/07) | Day 2 (9/30/07) | Day 3 (10/01/07) | Day 4 (10/02/07) | Day 5 (10/03/07) | Day 6 (10/04/07) | Day 7 (10/05/07) | Day 8 (10/06/07) | Day 9 (10/07/07) | Day 10 (10/08/07) |
|---|---|---|---|---|---|---|---|---|---|---|
| **CONTROL** | | | | | | | | | | |
| pH | 7.88 | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | 7.35 | XXXXXXX |
| DO | 6.07 | 6.18 | 6.22 | 6.46 | 6.87 | 6.8 | 7.13 | 6.53 | 6.92 | 6.19 |
| Cond | 644 | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | 644 | XXXXXXX |
| Hardness | 122 | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | 131 | XXXXXXX |
| Alk | | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | X | XXXXXXX |
| Temp | 21.3 | 21.3 | 21 | 21.7 | 21.3 | 21.4 | 21.2 | 21.7 | 21.6 | 20.9 |
| | | | | | | | | | | |
| **HONEY CREEK** | | | | | | | | | | |
| pH | 7.63 | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | | XXXXXXX |
| DO | 6.96 | 6.79 | 6.92 | 6.84 | 6.44 | 6.57 | 7.09 | 7.21 | 7.26 | 6.97 |
| Cond | 739 | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | 617 | XXXXXXX |
| Hardness | 166 | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | 158 | XXXXXXX |
| Alk | | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | 152 | XXXXXXX |
| Temp | 21.7 | 21.3 | 21 | 21.3 | 20.9 | 21.1 | 21.2 | 21.7 | 21.5 | 20.6 |
| | | | | | | | | | | |
| **STILLWATER RIVER** | | | | | | | | | | |
| pH | 6.79 | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | 7.5 | XXXXXXX |
| DO | 7.26 | 6.17 | 6.78 | 5.92 | 6.05 | 6.23 | 6.64 | 5.03 | 6.51 | 4.88 |
| Cond | 652 | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | 623 | XXXXXXX |
| Hardness | 165 | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | 155 | XXXXXXX |
| Alk | | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | XXXXXXX | 152 | XXXXXXX |
| Temp | 21.5 | 20.8 | 20.6 | 21.3 | 21.2 | 20.9 | 21.2 | 21.9 | 21.4 | 20.6 |

## Table 13. DGT Flux Calculations Raw Data

| Date | Sample ID | Position | Units | t (hrs) | Gel slice size (cm) | Cd (µg/L=Ce | Cu (µg/L=Ce | Ni (µg/L=Ce | Pb (µg/L=Ce | Zn (µg/L=Ce | $V_{HNO3}$ (mL) | $V_{gel}$ (mL) | $f_e$ | MCd(µg) | MCu(µg) | MNi(µg) | MPb(µg) | MZn(µg) | Δg(cm) | Temp. (°C) | t/s) | A (cm2) | F(Cd) [µg/cm2-s-1] | F(Cu) [µg/cm2-s-1] | F(Ni) [µg/cm2-s-1] | F(Pb) [µg/cm2-s-1] | F(Zn) [µg/cm2-s-1] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10/8/2007 | HY-U-1 | 1 | µg/L | 24 | 2.0 | 0.34 | 16.00 | 9.30 | 1.90 | 44.00 | 1 | 0.288 | 0.8 | 0.0009547 | 0.0225760 | 0.0149733 | 0.0003969 | 0.0278940 | 0.093 | 20 | 86400 | 3.6 | 1.76E-09 | 8.28E-08 | 4.81E-08 | 9.83E-09 | 2.27E-07 |
| 10/8/2007 | HY-U-2 | 2 | µg/L | 24 | 2.0 | 0.37 | 19.00 | 7.00 | 9.80 | 50.00 | 1 | 0.288 | 0.8 | 0.0009396 | 0.0305940 | 0.0112700 | 0.0145778 | 0.0895500 | 0.093 | 20 | 86400 | 3.6 | 1.91E-09 | 9.83E-08 | 3.62E-08 | 5.07E-08 | 2.63E-07 |
| 10/8/2007 | HY-U-3 | 3 | µg/L | 24 | 2.0 | 0.35 | 15.00 | 5.10 | 2.90 | 41.00 | 1 | 0.288 | 0.8 | 0.0009504 | 0.0224150 | 0.0008211 | 0.0044969 | 0.0660100 | 0.093 | 20 | 86400 | 3.6 | 1.81E-09 | 7.76E-08 | 2.64E-08 | 1.50E-08 | 2.12E-07 |
| 10/8/2007 | HY-S-1 | 1 | µg/L | 24 | 2.0 | 0.00 | 18.00 | 9.60 | 3.30 | 36.00 | 1 | 0.288 | 0.8 | 0.0000000 | 0.0289680 | 0.0116835 | 0.0053133 | 0.0479600 | 0.093 | 20 | 86400 | 3.6 | 0.00E+00 | 9.31E-08 | 3.41E-08 | 1.70E-08 | 1.86E-07 |
| 10/8/2007 | HY-S-2 | 2 | µg/L | 24 | 2.0 | 0.27 | 22.00 | 8.30 | 4.30 | 46.00 | 1 | 0.288 | 0.8 | 0.0009435 | 0.0354420 | 0.0133653 | 0.0066923 | 0.0744900 | 0.093 | 20 | 86400 | 3.6 | 1.39E-09 | 1.13E-07 | 4.29E-08 | 2.22E-08 | 2.38E-07 |
| 10/8/2007 | HY-S-3 | 3 | µg/L | 24 | 2.0 | 0.00 | 21.00 | 8.50 | 9.00 | 68.00 | 1 | 0.288 | 0.8 | 0.0000000 | 0.0338310 | 0.0139895 | 0.0099600 | 0.1094400 | 0.093 | 20 | 86400 | 3.6 | 0.00E+00 | 1.08E-07 | 4.40E-08 | 3.10E-08 | 3.52E-07 |
| 10/8/2007 | HY-D-1 | 1 | µg/L | 24 | 2.0 | 0.00 | 22.00 | 4.70 | 1.90 | 69.00 | 1 | 0.288 | 0.8 | 0.0000000 | 0.0354420 | 0.0076547 | 0.0039269 | 0.0969600 | 0.093 | 20 | 86400 | 3.6 | 0.00E+00 | 1.13E-07 | 2.43E-08 | 9.83E-09 | 3.10E-07 |
| 10/8/2007 | HY-D-2 | 2 | µg/L | 24 | 2.0 | 0.00 | 24.00 | 4.00 | 1.40 | 51.00 | 1 | 0.288 | 0.8 | 0.0000000 | 0.0378840 | 0.0065140 | 0.0029284 | 0.0728110 | 0.093 | 20 | 86400 | 3.6 | 0.00E+00 | 1.24E-07 | 2.07E-08 | 7.24E-09 | 2.64E-07 |
| 10/8/2007 | HY-D-3 | 3 | µg/L | 24 | 2.0 | 0.27 | 20.00 | 3.20 | 11.00 | 50.00 | 1 | 0.288 | 0.8 | 0.0009435 | 0.0322000 | 0.0031629 | 0.0117710 | 0.0885500 | 0.093 | 20 | 86400 | 3.6 | 1.39E-09 | 1.03E-07 | 1.65E-08 | 5.69E-08 | 2.58E-07 |
| 10/8/2007 | SR-U-1 | 1 | µg/L | 24 | 2.0 | 0.00 | 36.00 | 7.00 | 5.10 | 84.00 | 1 | 0.288 | 0.8 | 0.0000000 | 0.0576960 | 0.0112700 | 0.0092911 | 0.1352400 | 0.093 | 20 | 86400 | 3.6 | 0.00E+00 | 1.86E-07 | 3.62E-08 | 2.64E-08 | 4.34E-07 |
| 10/8/2007 | SR-U-2 | 2 | µg/L | 24 | 2.0 | 0.30 | 37.00 | 7.20 | 2.70 | 50.00 | 1 | 0.288 | 0.8 | 0.0009483 | 0.0595720 | 0.0114592 | 0.0043547 | 0.0885500 | 0.093 | 20 | 86400 | 3.6 | 1.55E-09 | 1.91E-07 | 3.72E-08 | 1.39E-08 | 2.58E-07 |
| 10/8/2007 | SR-U-3 | 3 | µg/L | 24 | 2.0 | 0.00 | 36.00 | 5.10 | 1.50 | 40.00 | 1 | 0.288 | 0.8 | 0.0000000 | 0.0576960 | 0.0080821 | 0.0026145 | 0.0644400 | 0.093 | 20 | 86400 | 3.6 | 0.00E+00 | 1.86E-07 | 3.15E-08 | 7.78E-09 | 2.07E-07 |
| 10/8/2007 | SR-S-1 | 1 | µg/L | 24 | 2.0 | 0.00 | 24.00 | 7.80 | 2.60 | 77.00 | 1 | 0.288 | 0.8 | 0.0000000 | 0.0389440 | 0.0125283 | 0.0041986 | 0.1223670 | 0.093 | 20 | 86400 | 3.6 | 0.00E+00 | 1.24E-07 | 4.03E-08 | 1.34E-08 | 3.98E-07 |
| 10/8/2007 | SR-S-2 | 2 | µg/L | 24 | 2.0 | 0.00 | 21.00 | 10.00 | 2.10 | 86.00 | 1 | 0.288 | 0.8 | 0.0000000 | 0.0338310 | 0.0161400 | 0.0033281 | 0.1384760 | 0.093 | 20 | 86400 | 3.6 | 0.00E+00 | 1.08E-07 | 5.17E-08 | 1.08E-08 | 4.43E-07 |
| 10/8/2007 | SR-S-3 | 3 | µg/L | 24 | 2.0 | 0.36 | 18.00 | 9.70 | 2.30 | 62.00 | 1 | 0.288 | 0.8 | 0.0009380 | 0.0289680 | 0.0161787 | 0.0037293 | 0.0998200 | 0.093 | 20 | 86400 | 3.6 | 1.86E-09 | 9.31E-08 | 3.46E-08 | 1.19E-08 | 3.20E-07 |
| 10/8/2007 | SR-D-1 | 1 | µg/L | 24 | 2.0 | 0.00 | 24.00 | 5.60 | 1.30 | 56.00 | 1 | 0.288 | 0.8 | 0.0000000 | 0.0389440 | 0.0089616 | 0.0018083 | 0.0901600 | 0.093 | 20 | 86400 | 3.6 | 0.00E+00 | 1.24E-07 | 2.89E-08 | 6.72E-09 | 2.89E-07 |
| 10/8/2007 | SR-D-2 | 2 | µg/L | 24 | 2.0 | 0.00 | 23.00 | 3.90 | 1.70 | 49.00 | 1 | 0.288 | 0.8 | 0.0000000 | 0.0370120 | 0.0062769 | 0.0027227 | 0.0788900 | 0.093 | 20 | 86400 | 3.6 | 0.00E+00 | 1.19E-07 | 2.01E-08 | 8.80E-09 | 2.53E-07 |
| 10/8/2007 | SR-D-3 | 3 | µg/L | 24 | 2.0 | 0.00 | 21.00 | 5.40 | 1.90 | 55.00 | 1 | 0.288 | 0.8 | 0.0000000 | 0.0338310 | 0.0089694 | 0.0033169 | 0.0885510 | 0.093 | 20 | 86400 | 3.6 | 0.00E+00 | 1.08E-07 | 2.79E-08 | 9.83E-09 | 2.84E-07 |
| 10/8/2007 | Blank | | µg/L | 24 | 2.0 | 0.00 | 15.00 | 0.00 | 1.40 | 72.00 | 1 | 0.288 | 0.8 | 0.0000000 | 0.0281453 | 0.0000000 | 0.0002624 | 0.1152920 | 0.093 | 20 | 86400 | 3.6 | 0.00E+00 | 7.76E-08 | 0.00E+00 | 7.24E-09 | 3.72E-07 |

Positions:

1 Sediment/water interface

2 Top Sediment layer

3 Bottom Sediment Layer

## Table 14. DGT Mean Flux Data

| Honey Creek | Undist | S.Dist | Dist | Blank |
|---|---|---|---|---|
| Cd | 4.741E-04 | 1.208E-04 | 1.208E-04 | 0.000E+00 |
| Cu | 2.236E-02 | 2.728E-02 | 2.952E-02 | 6.708E-03 |
| Ni | 9.571E-03 | 1.047E-02 | 5.322E-03 | 0.000E+00 |
| Pb | 6.529E-03 | 6.082E-03 | 6.395E-03 | 6.261E-04 |
| Zn | 6.038E-02 | 6.708E-02 | 7.200E-02 | 3.220E-02 |

| Stillwater R. | Undist | S.Dist | Dist | Blank |
|---|---|---|---|---|
| Cd | 1.342E-04 | 1.610E-04 | 0.000E+00 | 0.000E+00 |
| Cu | 4.875E-02 | 2.818E-02 | 3.041E-02 | 6.708E-03 |
| Ni | 9.079E-03 | 1.096E-02 | 6.664E-03 | 0.000E+00 |
| Pb | 4.159E-03 | 3.131E-03 | 2.191E-03 | 6.261E-04 |
| Zn | 7.782E-02 | 1.006E-01 | 7.156E-02 | 3.220E-02 |

*** Metal Flux to DGTs per day (µg/cm2-s)**

## Table 15. Site Water Quality Raw Data

| Date | Site | Cond (µS/cm °C) | River Temp (°C) | pH | River DO (mg/L) | Turbidity (NTU) | Flow |
|---|---|---|---|---|---|---|---|
| 10/1/2007 | HY Cr | 772 | 15.6 | xx | 7.88 | 0 | 0.314 |
| 10/3/2007 | HY Cr | 776 | 17.56 | 8.1 | 9.79 | 1.1 | 0.293 |
| 10/5/2007 | HY Cr | 794 | 18.16 | 7.95 | 7.83 | 0 | 0.312 |
| 10/8/2007 | HY Cr | 492 | 19.53 | 7.93 | 8.09 | 0.5 | 0.316 |
| 10/15/2007 | HY Cr | 816 | 13.14 | 7.97 | 8.12 | 1.5 | 0.319 |
| 10/1/2007 | SR | 686 | 18.5 | xx | 9.49 | xx | 0.920 |
| 10/3/2007 | SR | 735 | 19.17 | 8.38 | 10.35 | 1.8 | 0.864 |
| 10/5/2007 | SR | 772 | 22.01 | 8.37 | 10.08 | 1.8 | 0.916 |
| 10/8/2007 | SR | 759 | 23.19 | 8.31 | 9.89 | 2.8 | 0.786 |
| 10/12/2007 | SR | 745 | 14.24 | 8.47 | 11.21 | 1 | 0.874 |
| 10/15/2007 | SR | 785 | 15.89 | 8.51 | 13.44 | 0.8 | 0.753 |

**Table 16. Honey Creek Embeddedness Raw Data.**

| USGS Embeddedness Measurements | | | | | | | |
|---|---|---|---|---|---|---|---|
| Site: | Honey Creek | Site EMB | 34.16 | | | | |
| Date: | 10/22/2007 | Std Dev | 35.22 | | | | |
| Sampler: | jpk | | | | | | |
| | | | | | | | |
| # | Particle Height (mm) | Height of EMB (mm) | % EMB | # | Particle Height (mm) | Height of EMB (mm) | % EMB |
| 1 | 19 | 12 | 63.16 | 51 | 21 | 11 | 52.38 |
| 2 | 14 | 0 | 0.00 | 52 | 68 | 45 | 66.18 |
| 3 | 8 | 8 | 100.00 | 53 | 19 | 0 | 0.00 |
| 4 | 25 | 0 | 0.00 | 54 | 9 | 0 | 0.00 |
| 5 | 12 | 4 | 33.33 | 55 | 22 | 0 | 0.00 |
| 6 | 6 | 6 | 100.00 | 56 | 10 | 0 | 0.00 |
| 7 | 12 | 2 | 16.67 | 57 | 19 | 0 | 0.00 |
| 8 | 11 | 6 | 54.55 | 58 | 76 | 0 | 0.00 |
| 9 | 26 | 10 | 38.46 | 59 | 17 | 0 | 0.00 |
| 10 | 21 | 16 | 76.19 | 60 | 19 | 19 | 100.00 |
| 11 | 31 | 0 | 0.00 | 61 | 81 | 23 | 28.40 |
| 12 | 54 | 0 | 0.00 | 62 | 13 | 0 | 0.00 |
| 13 | 17 | 0 | 0.00 | 63 | 37 | 0 | 0.00 |
| 14 | 22 | 0 | 0.00 | 64 | 28 | 0 | 0.00 |
| 15 | 46 | 0 | 0.00 | 65 | 38 | 0 | 0.00 |
| 16 | 47 | 27 | 57.45 | 66 | 42 | 22 | 52.38 |
| 17 | 32 | 0 | 0.00 | 67 | 32 | 0 | 0.00 |
| 18 | 34 | 0 | 0.00 | 68 | 15 | 0 | 0.00 |
| 19 | 18 | 0 | 0.00 | 69 | 26 | 20 | 76.92 |
| 20 | 18 | 0 | 0.00 | 70 | 60 | 0 | 0.00 |
| 21 | 43 | 16 | 37.21 | 71 | 25 | 18 | 72.00 |
| 22 | 30 | 30 | 100.00 | 72 | 30 | 23 | 76.67 |
| 23 | 59 | 25 | 42.37 | 73 | 26 | 0 | 0.00 |
| 24 | 45 | 29 | 64.44 | 74 | 25 | 0 | 0.00 |
| 25 | 42 | 25 | 59.52 | 75 | 50 | 26 | 52.00 |
| 26 | 23 | 10 | 43.48 | 76 | 8 | 0 | 0.00 |
| 27 | 19 | 12 | 63.16 | 77 | 24 | 0 | 0.00 |
| 28 | 15 | 0 | 0.00 | 78 | 40 | 0 | 0.00 |
| 29 | 37 | 14 | 37.84 | 79 | 49 | 19 | 38.78 |
| 30 | 60 | 0 | 0.00 | 80 | 8 | 0 | 0.00 |
| 31 | 41 | 24 | 58.54 | 81 | 100 | 75 | 75.00 |
| 32 | 40 | 0 | 0.00 | 82 | 15 | 0 | 0.00 |
| 33 | 27 | 0 | 0.00 | 83 | 47 | 20 | 42.55 |
| 34 | 19 | 0 | 0.00 | 84 | 8 | 8 | 100.00 |
| 35 | 13 | 0 | 0.00 | 85 | 16 | 16 | 100.00 |
| 36 | 41 | 19 | 46.34 | 86 | 30 | 14 | 46.67 |
| 37 | 38 | 34 | 89.47 | 87 | 17 | 0 | 0.00 |
| 38 | 18 | 0 | 0.00 | 88 | 14 | 11 | 78.57 |
| 39 | 11 | 11 | 100.00 | 89 | 21 | 0 | 0.00 |
| 40 | 23 | 12 | 52.17 | 90 | 37 | 0 | 0.00 |
| 41 | 22 | 0 | 0.00 | 91 | 10 | 10 | 100.00 |
| 42 | 72 | 26 | 36.11 | 92 | 41 | 14 | 34.15 |
| 43 | 17 | 12 | 70.59 | 93 | 16 | 0 | 0.00 |
| 44 | 79 | 23 | 29.11 | 94 | 19 | 5 | 26.32 |
| 45 | 14 | 14 | 100.00 | 95 | 5 | 0 | 0.00 |
| 46 | 192 | 153 | 79.69 | 96 | 36 | 20 | 55.56 |
| 47 | 125 | 16 | 12.80 | 97 | 21 | 14 | 66.67 |
| 48 | 22 | 0 | 0.00 | 98 | 24 | 0 | 0.00 |
| 49 | 153 | 69 | 45.10 | 99 | 11 | 0 | 0.00 |
| 50 | 30 | 0 | 0.00 | 100 | 14 | 14 | 100.00 |

**Table 17. Stillwater River Embeddedness Raw Data.**

| USGS Embeddedness Measurements | | | | | | | |
|---|---|---|---|---|---|---|---|
| Site: | Stillwater River | **Site EMB:** | **27.20** | | | | |
| Date: | 10/12/2007 | Std Dev | 26.60 | | | | |
| Sampler: | jpk | | | | | | |
| | | | | | | | |
| # | Particle Height (mm) | Height of EMB (mm) | % EMB | # | Particle Height (mm) | Height of EMB (mm) | % EMB |
| 1 | 45 | 24 | 53.33 | 51 | 44 | 16 | 36.36 |
| 2 | 27 | 15 | 55.56 | 52 | 25 | 0 | 0.00 |
| 3 | 71 | 10 | 14.08 | 53 | 28 | 10 | 35.71 |
| 4 | 45 | 16 | 35.56 | 54 | 43 | 0 | 0.00 |
| 5 | 22 | 0 | 0.00 | 55 | 117 | 33 | 28.21 |
| 6 | 16 | 10 | 62.50 | 56 | 66 | 17 | 25.76 |
| 7 | 46 | 25 | 54.35 | 57 | 50 | 22 | 44.00 |
| 8 | 22 | 0 | 0.00 | 58 | 42 | 0 | 0.00 |
| 9 | 21 | 0 | 0.00 | 59 | 26 | 15 | 57.69 |
| 10 | 42 | 0 | 0.00 | 60 | 30 | 10 | 33.33 |
| 11 | 32 | 14 | 43.75 | 61 | 44 | 40 | 90.91 |
| 12 | 36 | 7 | 19.44 | 62 | 37 | 0 | 0.00 |
| 13 | 54 | 6 | 11.11 | 63 | 4 | 0 | 0.00 |
| 14 | 122 | 22 | 18.03 | 64 | 48 | 0 | 0.00 |
| 15 | 98 | 26 | 26.53 | 65 | 200 | 86 | 43.00 |
| 16 | 21 | 9 | 42.86 | 66 | 75 | 65 | 86.67 |
| 17 | 96 | 36 | 37.50 | 67 | 7 | 0 | 0.00 |
| 18 | 46 | 12 | 26.09 | 68 | 82 | 0 | 0.00 |
| 19 | 16 | 11 | 68.75 | 69 | 420 | 150 | 35.71 |
| 20 | 28 | 7 | 25.00 | 70 | 58 | 18 | 31.03 |
| 21 | 17 | 8 | 47.06 | 71 | 35 | 0 | 0.00 |
| 22 | 9 | 9 | 100.00 | 72 | 44 | 13 | 29.55 |
| 23 | 200 | 66 | 33.00 | 73 | 70 | 30 | 42.86 |
| 24 | 7 | 0 | 0.00 | 74 | 72 | 37 | 51.39 |
| 25 | 180 | 83 | 46.11 | 75 | 102 | 60 | 58.82 |
| 26 | 34 | 0 | 0.00 | 76 | 55 | 30 | 54.55 |
| 27 | 31 | 21 | 67.74 | 77 | 35 | 0 | 0.00 |
| 28 | 13 | 7 | 53.85 | 78 | 16 | 7 | 43.75 |
| 29 | 12 | 0 | 0.00 | 79 | 85 | 0 | 0.00 |
| 30 | 99 | 30 | 30.30 | 80 | 5 | 0 | 0.00 |
| 31 | 180 | 111 | 61.67 | 81 | 88 | 50 | 56.82 |
| 32 | 48 | 48 | 100.00 | 82 | 55 | 36 | 65.45 |
| 33 | 11 | 0 | 0.00 | 83 | 10 | 0 | 0.00 |
| 34 | 64 | 20 | 31.25 | 84 | 111 | 48 | 43.24 |
| 35 | 135 | 10 | 7.41 | 85 | 72 | 24 | 33.33 |
| 36 | 9 | 0 | 0.00 | 86 | 43 | 17 | 39.53 |
| 37 | 67 | 17 | 25.37 | 87 | 115 | 26 | 22.61 |
| 38 | 66 | 0 | 0.00 | 88 | 117 | 80 | 68.38 |
| 39 | 85 | 56 | 65.88 | 89 | 10 | 0 | 0.00 |
| 40 | 40 | 0 | 0.00 | 90 | 10 | 0 | 0.00 |
| 41 | 52 | 30 | 57.69 | 91 | 54 | 26 | 48.15 |
| 42 | 7 | 0 | 0.00 | 92 | 100 | 12 | 12.00 |
| 43 | 100 | 4 | 4.00 | 93 | 50 | 10 | 20.00 |
| 44 | 65 | 37 | 56.92 | 94 | 42 | 15 | 35.71 |
| 45 | 20 | 0 | 0.00 | 95 | 15 | 0 | 0.00 |
| 46 | 50 | 14 | 28.00 | 96 | 51 | 7 | 13.73 |
| 47 | 70 | 0 | 0.00 | 97 | 48 | 10 | 20.83 |
| 48 | 103 | 0 | 0.00 | 98 | 39 | 0 | 0.00 |
| 49 | 16 | 0 | 0.00 | 99 | 56 | 0 | 0.00 |
| 50 | 28 | 0 | 0.00 | 100 | 32 | 0 | 0.00 |

## Table 18. Embeddedness Chamber Sediment Raw Data

| | DF2 (104°C) | DF2 (550) | DF2 (1000°C) | DF250 (104°C) | DF250 (550°C) | DF250 (1000°C) | DF63 (104°C) | DF63 (550°C) | DF63 (1000°C) | DF1.2 (104°C) | DF1.2 (550°C) | DF1.2 (1000°C) | Total Dry Fraction 1.2 (104°C) | TotDF | %Organic (104°C) | % CaCO3 | % Inorganic | Porosity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| EMB-HY-B-3 4d | 0.125 | 0.122 | 0.100 | 0.918 | 0.889 | 0.683 | 0.77 | 0.695 | 0.631 | 0.029 | 0.03 | 0.03 | 0.029 | 1.842 | 5.8 | 36.0 | 58.2 | 142.73 |
| EMB-HY-B-6 4d | 0.011 | 0.002 | 0.002 | 1.341 | 1.279 | 1.075 | 2.047 | 1.859 | 1.663 | 0.026 | 0.026 | 0.026 | 0.026 | 3.425 | 7.6 | 26.6 | 65.9 | 42.22 |
| EMB-HY-B-10 4d | 0.072 | 0.060 | 0.045 | 1.440 | 1.375 | 1.147 | 2.512 | 2.325 | 2.055 | 0.034 | 0.035 | 0.035 | 0.034 | 4.058 | 6.5 | 28.7 | 64.8 | 31.07 |
| EMB-HY-B-5 7d | 0.018 | 0.004 | 0.004 | 3.058 | 2.941 | 2.398 | 4.508 | 4.169 | 3.692 | 0.045 | 0.045 | 0.041 | 0.045 | 7.629 | 6.2 | 30.4 | 63.4 | 7.33 |
| EMB-HY-B-8 7d | 0.050 | 0.042 | 0.034 | 6.485 | 6.296 | 5.167 | 3.344 | 3.135 | 2.773 | 0.068 | 0.061 | 0.061 | 0.068 | 9.947 | 4.2 | 34.3 | 61.6 | 3.25 |
| EMB-HY-B-9 7d | 0.061 | 0.050 | 0.050 | 5.220 | 4.996 | 4.204 | 3.918 | 3.68 | 3.25 | 0.091 | 0.091 | 0.091 | 0.091 | 9.29 | 5.1 | 29.9 | 65.0 | 3.61 |
| EMB-HY-B-11 7d | 0.093 | 0.085 | 0.063 | 2.081 | 1.999 | 1.642 | 2.497 | 2.334 | 2.069 | 0.085 | 0.073 | 0.073 | 0.085 | 4.756 | 5.6 | 30.8 | 63.6 | 20.96 |
| EMB-HY-B-1 14d | 0.049 | 0.046 | 0.046 | 4.236 | 4.115 | 3.316 | 3.735 | 3.446 | 3.039 | 0.094 | 0.081 | 0.081 | 0.094 | 8.114 | 5.3 | 33.8 | 61.0 | 4.90 |
| EMB-HY-B-2 14d | 0.170 | 0.161 | 0.094 | 1.364 | 1.311 | 1.044 | 1.212 | 1.119 | 0.999 | 0.06 | 0.052 | 0.052 | 0.060 | 2.806 | 5.8 | 36.8 | 57.4 | 73.12 |
| EMB-HY-B-7 14d | 0.128 | 0.098 | 0.063 | 7.952 | 7.664 | 6.469 | 5.512 | 5.235 | 4.634 | 0.118 | 0.103 | 0.103 | 0.118 | 13.71 | 4.4 | 30.4 | 65.2 | 1.60 |
| EMB-HY-B-12 14d | 2.347 | 2.303 | 1.586 | 15.484 | 15.255 | 11.921 | 3.943 | 3.774 | 3.353 | 0.106 | 0.094 | 0.092 | 0.106 | 21.88 | 2.1 | 46.5 | 51.4 | 0.69 |
| EMB-SR-B-3 4d | 0.633 | 0.623 | 0.408 | 15.904 | 15.714 | 13.007 | 1.418 | 1.309 | 1.134 | 0.044 | 0.037 | 0.037 | 0.044 | 17.999 | 1.8 | 39.1 | 59.1 | 1.13 |
| EMB-SR-B-4 4d | 0.049 | 0.048 | 0.042 | 3.164 | 3.119 | 2.636 | 0.75 | 0.698 | 0.615 | 0.025 | 0.021 | 0.021 | 0.025 | 3.988 | 2.6 | 32.6 | 64.8 | 26.76 |
| EMB-SR-B-6 4d | 0.141 | 0.136 | 0.089 | 18.863 | 18.635 | 15.882 | 1.363 | 1.271 | 1.118 | 0.03 | 0.025 | 0.025 | 0.030 | 20.397 | 1.6 | 32.9 | 65.5 | 0.69 |
| EMB-SR-B-10 4d | 0.002 | 0.002 | 0.002 | 2.599 | 2.493 | 2.207 | 1.444 | 1.319 | 1.149 | 0.042 | 0.036 | 0.036 | 0.042 | 4.087 | 5.8 | 25.4 | 68.8 | 35.96 |
| EMB-SR-B-5 7d | 0.745 | 0.738 | 0.561 | 26.093 | 25.819 | 21.97 | 2.301 | 2.161 | 1.875 | 0.043 | 0.038 | 0.038 | 0.043 | 29.182 | 1.5 | 33.6 | 64.9 | 0.30 |
| EMB-SR-B-8 7d | 0.005 | 0.005 | 0.005 | 5.420 | 5.331 | 4.624 | 1.868 | 1.734 | 1.496 | 0.054 | 0.048 | 0.048 | 0.054 | 7.347 | 3.1 | 29.2 | 67.6 | 7.21 |
| EMB-SR-B-9 7d | 0.020 | 0.020 | 0.016 | 10.625 | 10.455 | 9.091 | 2.594 | 2.398 | 2.09 | 0.049 | 0.043 | 0.043 | 0.049 | 13.288 | 2.8 | 28.7 | 68.5 | 1.69 |
| EMB-SR-B-11 7d | 0.213 | 0.205 | 0.135 | 3.215 | 3.141 | 2.693 | 2.337 | 2.169 | 1.868 | 0.071 | 0.063 | 0.063 | 0.071 | 5.836 | 4.4 | 31.9 | 63.7 | 8.89 |
| EMB-SR-B-1 14d | 0.196 | 0.190 | 0.127 | 14.705 | 14.511 | 12.458 | 3.006 | 2.815 | 2.397 | 0.099 | 0.085 | 0.085 | 0.099 | 18.006 | 2.2 | 32.0 | 65.7 | 0.70 |
| EMB-SR-B-2 14d | 1.031 | 1.000 | 0.668 | 22.658 | 22.382 | 18.972 | 2.631 | 2.462 | 2.117 | 0.087 | 0.074 | 0.072 | 0.087 | 26.407 | 1.9 | 35.2 | 62.9 | 0.28 |
| EMB-SR-B-7 14d | 0.706 | 0.696 | 0.451 | 7.219 | 7.083 | 6.175 | 2.931 | 2.722 | 2.361 | 0.085 | 0.073 | 0.07 | 0.085 | 10.941 | 3.4 | 31.5 | 65.1 | 2.28 |
| EMB-SR-B-12 14d | 0.394 | 0.389 | 0.359 | 3.227 | 3.156 | 2.592 | 1.974 | 1.829 | 1.579 | 0.063 | 0.055 | 0.051 | 0.063 | 5.658 | 4.0 | 34.1 | 61.9 | 14.71 |

**Table 19. Embeddedness Chamber Benthos Raw Data (untransformed).**

| Sample | Total | No.of Taxa | No.of Taxa (Mean Stddev) | No.of Ephemeroptera taxa (Mean Stddev) | % of Ephemeroptera (Mean Stddev) | No.of Trichoptera taxa (Mean Stddev) | % Trichoptera (Mean Stddev) | No.of Plecoptera taxa (Mean Stddev) | % Plecoptera (Mean Stddev) | No.of EPT taxa (Mean Stddev) | % of EPT (Mean Stddev) | No.of Diptera taxa (Mean Stddev) | % Diptera (Mean Stddev) | % Chironomidae (Mean Stddev) | % Chironomidae (Mean Stddev dev) | % Filterers (Mean Stddev) | % grazers and scrapers (Mean Stddev) | % Shredders (Mean Stddev) | % Predators (Mean Stddev) | % dominant taxon (Mean Stddev) | Family Biotic Index (FBI) (Mean Stddev) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| EMEHY-B-3-140 | 26 | 2 | 2.0 | 0 | 0.3 | 0 | 3.5 | 1 | 1.3 | 51 | 51.0 | 0 | 0.0 | 1 | 1.5 | 51 | 94.5 | 1 | 1.3 | 50 | 45.5 | 50 | 38.5 | 39 | 31.3 | 0 | 3.5 | 0 | 3.5 | 50 | 63.5 | 4.50 | 4.71 |
| EMEHY-B-4-140 | 1 | 2.2 | 0 | 0.5 | 0 | 7.0 | 1 | 0.5 | 100 | 34.7 | 0 | 0.0 | 0 | 0.0 | 1 | 1.0 | 100 | 32.2 | 0 | 1.3 | 0 | 32.1 | 0 | 31.8 | 100 | 33.3 | 0 | 0.0 | 0 | 7.0 | 100 | 30.8 | 4.00 | 0.63 |
| EMEHY-B-6-140 | 6 | | 1 | | 14 | 2 | | 0 | | 0 | | 3 | | 3 | | 42 | | 0 | | 29 | | 42 | | 14 | | 14 | | 29 | | 4.95 | | |
| EMEHY-B-10-140 | 2 | 0 | | 0 | | 1 | 25 | 0 | 0 | | 1 | | 25 | 1 | | 75 | | 75 | | 25 | | 0 | | 0 | | 75 | | 5.50 | | |
| EMEHY-B-3-70 | 69 | 5 | 5.0 | 1 | 0.5 | 6 | 2.5 | 2 | 2.5 | 53 | 49.3 | 0 | 0.3 | 0 | 1.8 | 59 | 53.5 | 1 | 1.8 | 35 | 47.3 | 35 | 36.8 | 35 | 41.8 | 0 | 4.5 | 0 | 5.8 | 35 | 40.5 | 4.47 | 4.75 |
| EMEHY-B-9-70 | 5 | 1.0 | 0 | 0.6 | 0 | 3.0 | 2 | 1.0 | 54 | 5.2 | 0 | 0.5 | 0 | 3.5 | 2 | 1.3 | 54 | 4.5 | 2 | 0.5 | 39 | 10.6 | 31 | 7.7 | 45 | 5.3 | 0 | 6.6 | 16 | 7.6 | 46 | 7.6 | 4.65 | 0.19 |
| EMEHY-B-8-70 | 7 | | 1 | | 4 | 4 | | 0 | | 0 | | 5 | | 4.8 | | 75 | | 4.8 | | 40 | | 4 | | 0 | | 0 | | 4.8 | | | | | 4.80 | |
| EMEHY-B-11-70 | 6 | | 0 | | 0 | 2 | | 1 | | 7 | | 3 | | 5.3 | | 40 | | 2 | | 33 | | 45 | | 7 | | 14 | | 33 | | | | | 4.97 | |
| EMEHY-B-1-140 | 425 | 5 | 4.5 | 1 | 1.0 | 15 | 7.3 | 2 | 1.8 | 15 | 21.3 | 0 | 0.3 | 0 | 1.0 | 30 | 29.3 | 2 | 1.8 | 72 | 71.5 | 67 | 69.5 | 15 | 23.5 | 10 | 2.3 | 0 | 1.0 | 67 | 69.5 | 5.19 | 4.83 |
| EMEHY-B-2-140 | 69 | 6 | 1.3 | 2 | 0.8 | 10 | 6.6 | 2 | 0.5 | 30 | 11.6 | 0 | 0.5 | 0 | 2.0 | 40 | 44.9 | 2 | 0.5 | 60 | 14.6 | 57 | 16.3 | 40 | 14.6 | 0 | 2.6 | 0 | 2.0 | 57 | 16.3 | 3.33 | 1.06 |
| EMEHY-B-7-140 | 69 | 3 | | 0 | | 0 | | 2 | | 8 | | 2 | | 8 | | 92 | | 1 | | 92 | | 8 | | 0 | | 0 | | 0 | | 92 | | | 5.82 | |
| EMEHY-B-4-140 | 69 | 4 | | 1 | | 4 | | 1 | | 32 | | 1 | | 4 | | 39 | | 3 | | 62 | | 39 | | 31 | | 4 | | 4 | | 59 | | | 4.96 | |
| EMEHY-B-3-140 | 69 | 6 | 3.5 | 1 | 1.0 | 5 | 8.3 | 2 | 0.8 | 10 | 8.3 | 1 | 0.5 | 5 | 3.8 | 4 | 2.3 | 2 | 1.3 | 82 | 82.5 | 82 | 77.5 | 5 | 10.0 | 5 | 1.3 | 0 | 0.0 | 4 | 3.5 | 82 | 77.5 | 5.50 | 5.70 |
| EMEHY-B-4-140 | 69 | 3 | 1.3 | 2 | 0.8 | 18 | 7.7 | 0 | 1.0 | 0 | 7.5 | 0 | 0.6 | 0 | 4.8 | 2 | 1.3 | 18 | 2.4 | 1 | 0.5 | 83 | 11.7 | 0 | 9.1 | 0 | 2.5 | 0 | 0.0 | 0 | 4.7 | 83 | 11.7 | 5.91 | 0.17 |
| EMEHY-B-8-140 | 69 | 4 | | 1 | | 10 | | 0 | | 0 | | 1 | | 10 | | 2 | | 21 | | 2 | | 80 | | 20 | | 0 | | 0 | | 10 | | | 5.70 | |
| EMEHY-B-11-140 | 69 | 2 | | 0 | | 0 | | 1 | | 15 | | 0 | | 0 | | 1 | | 15 | | 1 | | 85 | | 15 | | 0 | | 0 | | 0 | | 85 | | | 5.69 | |
| EMEHY-B-3-70 | 114 | 5 | 5.8 | 3 | 3.0 | 44 | 38.0 | 1 | 1.5 | 16 | 16.0 | 0 | 0.3 | 0 | 2.8 | 4 | 4.8 | 4 | 1.0 | 40 | 44.0 | 40 | 44.0 | 16 | 11.5 | 4 | 7.8 | 0 | 0.0 | 0 | 2.5 | 40 | 44.0 | 5.28 | 5.25 |
| EMEHY-B-9-70 | 223 | 5 | 1.0 | 2 | 0.8 | 40 | 5.9 | 1 | 0.6 | 16 | 5.7 | 1 | 0.5 | 11 | 5.5 | 4 | 1.0 | 65 | 11.7 | 1 | 0.0 | 35 | 11.0 | 35 | 4.7 | 0 | 10.3 | 0 | 0.0 | 10 | 5.0 | 35 | 11.0 | 4.60 | 0.57 |
| EMEHY-B-8-70 | 114 | 7 | | 4 | | 38 | | 2 | | 23 | | 0 | | 0 | | 6 | | 61 | | 1 | | 41 | | 8 | | 23 | | 0 | | 0 | | 41 | | | 5.15 | |
| EMEHY-B-11-70 | 223 | 6 | | 3 | | 30 | | 2 | | 9 | | 0 | | 0 | | 5 | | 39 | | 1 | | 60 | | 7 | | 4 | | 0 | | 0 | | 60 | | | 5.98 | |
| EMEHY-B-1-140 | 223 | 10 | 9.3 | 5 | 3.5 | 49 | 39.3 | 2 | 2.3 | 13 | 22.3 | 2 | 1.0 | 6 | 5.0 | 9 | 7.5 | 6 | 1.0 | 31 | 32.0 | 31 | 32.0 | 11 | 21.0 | 13 | 6.3 | 2 | 1.3 | 4 | 4.0 | 31 | 34.3 | 4.53 | 4.95 |
| EMEHY-B-2-140 | 111 | 5 | 1.5 | 3 | 1.0 | 22 | 11.9 | 4 | 1.3 | 29 | 8.5 | 2 | 0.5 | 4 | 1.2 | 9 | 1.7 | 5 | 0.5 | 1 | 0.0 | 43 | 9.5 | 28 | 9.4 | 1 | 5.4 | 1 | 1.0 | 4 | 1.6 | 43 | 6.0 | 5.21 | 0.28 |
| EMEHY-B-7-140 | 8 | | | 3 | | 44 | | 1 | | 2 | | 2 | | 6 | | 78 | | 1 | | 21 | | 21 | | 30 | | 3 | | 2 | | 2 | | 30 | | | 4.99 | |
| EMEHY-B-4-140 | 8 | | | 3 | | 42 | | 2 | | 17 | | 1 | | 6 | | 65 | | 1 | | 33 | | 33 | | 15 | | 8 | | 0 | | 6 | | 33 | | | 5.08 | |

**Table 20. Embeddedness Chamber Benthos Raw Data (transformed).**

| x-formed data | Sample | Cham # | Site | Time | Total insects | No. of Taxa | No. of Ephemeroptera taxa | % of Ephemeroptera | No. of Trichoptera taxa | % Trichoptera | No. of Plecoptera taxa | % Plecoptera | No. of EPT taxa | % of EPT | No. of Dipteran taxa | % Diptera | % Chironomidae | % filters | % grazers and scrapers | % Shredders | % Predators | % dominant taxon | Family Biotic Index (FBI) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| EMB-HY-B-3 4d | HY-4d | 3 | HY | 4 | 7 | 1.10 | 0.000 | 0.00 | 0.69 | 0.80 | 0.00 | 0.00 | 0.69 | 0.80 | 0.69 | 0.79 | 0.79 | 0.66 | 0.00 | 0.00 | 0.00 | 0.79 | 1.70 |
| EMB-HY-B-6 4d | HY- 4d | 6 | HY | 4 | 7 | 1.79 | 0.693 | 0.38 | 1.10 | 0.56 | 0.00 | 0.00 | 1.39 | 0.71 | 1.39 | 0.86 | 0.57 | 0.71 | 0.00 | 0.38 | 0.38 | 0.57 | 1.77 |
| EMB-HY-B-10 4d | HY-4d | 10 | HY | 4 | 8 | 0.69 | 0.000 | 0.00 | 0.69 | 0.52 | 0.00 | 0.00 | 0.69 | 0.52 | 0.69 | 1.05 | 1.05 | 0.52 | 0.00 | 0.00 | 0.00 | 1.05 | 1.87 |
| EMB-HY-B-5 7d | HY-7d | 5 | HY | 7 | 16 | 1.61 | 0.693 | 0.25 | 1.10 | 0.82 | 0.00 | 0.00 | 1.39 | 0.88 | 0.69 | 0.63 | 0.63 | 0.63 | 0.44 | 0.00 | 0.00 | 0.63 | 1.70 |
| EMB-HY-B-8 7d | HY-7d | 8 | HY | 7 | 13 | 1.61 | 0.000 | 0.00 | 1.10 | 0.83 | 0.00 | 0.00 | 1.10 | 0.83 | 1.10 | 0.67 | 0.59 | 0.75 | 0.29 | 0.00 | 0.41 | 0.75 | 1.77 |
| EMB-HY-B-9 7d | HY-7d | 9 | HY | 7 | 25 | 1.95 | 0.693 | 0.20 | 1.61 | 0.72 | 0.00 | 0.00 | 1.79 | 0.77 | 1.10 | 1.05 | 0.77 | 0.68 | 0.20 | 0.20 | 0.00 | 0.77 | 1.76 |
| EMB-HY-B-11 7d | HY-7d | 11 | HY | 7 | 15 | 1.79 | 0.000 | 0.00 | 1.10 | 0.76 | 0.69 | 0.27 | 1.39 | 0.82 | 1.10 | 0.68 | 0.61 | 0.75 | 0.00 | 0.38 | 0.27 | 0.61 | 1.77 |
| EMB-HY-B-1 14d | HY-14d | 1 | HY | 14 | 20 | 1.61 | 0.693 | 0.40 | 1.10 | 0.40 | 0.00 | 0.00 | 1.39 | 0.58 | 1.10 | 1.01 | 0.96 | 0.40 | 0.32 | 0.23 | 0.00 | 0.96 | 1.82 |
| EMB-HY-B-2 14d | HY-14d | 2 | HY | 14 | 30 | 1.79 | 1.099 | 0.32 | 1.10 | 0.58 | 0.00 | 0.00 | 1.61 | 0.68 | 1.10 | 0.89 | 0.86 | 0.68 | 0.00 | 0.00 | 0.00 | 0.86 | 1.47 |
| EMB-HY-B-7 14d | HY-14d | 7 | HY | 14 | 51 | 1.10 | 0.000 | 0.00 | 1.10 | 0.29 | 0.00 | 0.00 | 1.10 | 0.29 | 0.69 | 1.28 | 1.28 | 0.29 | 0.00 | 0.00 | 0.00 | 1.28 | 1.92 |
| EMB-HY-B-12 14d | HY-14d | 12 | HY | 14 | 25 | 1.39 | 0.693 | 0.20 | 0.69 | 0.60 | 0.69 | 0.20 | 1.39 | 0.67 | 1.10 | 0.91 | 0.87 | 0.59 | 0.20 | 0.20 | 0.20 | 0.87 | 1.79 |
| EMB-SR-B-3 4d | SR-4d | 3 | SR | 4 | 22 | 1.61 | 0.693 | 0.23 | 1.10 | 0.32 | 0.69 | 0.23 | 1.61 | 0.46 | 0.69 | 1.13 | 1.13 | 0.23 | 0.23 | 0.00 | 0.20 | 1.13 | 1.87 |
| EMB-SR-B-4 4d | SR-4d | 4 | SR | 4 | 23 | 1.10 | 1.099 | 0.44 | 0.00 | 0.00 | 0.00 | 0.00 | 1.10 | 0.44 | 0.69 | 1.15 | 1.15 | 0.00 | 0.00 | 0.00 | 0.00 | 1.15 | 1.93 |
| EMB-SR-B-6 4d | SR-4d | 6 | SR | 4 | 10 | 1.39 | 0.693 | 0.32 | 0.00 | 0.00 | 0.69 | 0.32 | 1.10 | 0.46 | 1.10 | 1.11 | 0.89 | 0.46 | 0.00 | 0.00 | 0.32 | 0.89 | 1.90 |
| EMB-SR-B-10 4d | SR-4d | 10 | SR | 4 | 13 | 0.69 | 0.000 | 0.00 | 0.69 | 0.40 | 0.00 | 0.00 | 0.69 | 0.40 | 0.69 | 1.17 | 1.17 | 0.40 | 0.00 | 0.00 | 0.00 | 1.17 | 1.90 |
| EMB-SR-B-5 7d | SR-7d | 5 | SR | 7 | 25 | 1.61 | 1.386 | 0.73 | 0.69 | 0.41 | 0.00 | 0.00 | 1.61 | 0.89 | 0.69 | 0.68 | 0.68 | 0.41 | 0.20 | 0.00 | 0.00 | 0.68 | 1.84 |
| EMB-SR-B-8 7d | SR-7d | 8 | SR | 7 | 19 | 1.61 | 1.099 | 0.68 | 0.69 | 0.41 | 0.69 | 0.34 | 1.61 | 0.94 | 0.69 | 0.63 | 0.63 | 0.40 | 0.00 | 0.00 | 0.32 | 0.63 | 1.72 |
| EMB-SR-B-9 7d | SR-7d | 9 | SR | 7 | 27 | 1.95 | 1.609 | 0.66 | 1.10 | 0.50 | 0.00 | 0.00 | 1.95 | 0.90 | 0.69 | 0.69 | 0.69 | 0.29 | 0.50 | 0.00 | 0.00 | 0.69 | 1.82 |
| EMB-SR-B-11 7d | SR-7d | 11 | SR | 7 | 43 | 1.79 | 1.386 | 0.58 | 1.10 | 0.30 | 0.00 | 0.00 | 1.79 | 0.67 | 0.69 | 0.89 | 0.89 | 0.27 | 0.20 | 0.00 | 0.00 | 0.89 | 1.94 |
| EMB-SR-B-1 14d | SR-4d | 1 | SR | 14 | 45 | 2.30 | 1.792 | 0.78 | 1.10 | 0.37 | 1.10 | 0.25 | 2.30 | 0.97 | 0.69 | 0.59 | 0.59 | 0.34 | 0.37 | 0.14 | 0.20 | 0.59 | 1.71 |
| EMB-SR-B-2 14d | SR-14d | 2 | SR | 14 | 76 | 2.40 | 1.386 | 0.49 | 1.61 | 0.57 | 1.10 | 0.20 | 2.30 | 0.84 | 0.69 | 0.72 | 0.72 | 0.56 | 0.10 | 0.10 | 0.20 | 0.72 | 1.83 |
| EMB-SR-B-7 14d | SR-14d | 7 | SR | 14 | 60 | 2.08 | 1.386 | 0.73 | 0.69 | 0.58 | 1.10 | 0.20 | 1.95 | 1.08 | 0.69 | 0.48 | 0.48 | 0.58 | 0.17 | 0.14 | 0.14 | 0.58 | 1.79 |
| EMB-SR-B-12 14d | SR-14d | 12 | SR | 14 | 52 | 2.08 | 1.386 | 0.71 | 1.10 | 0.42 | 0.69 | 0.25 | 1.95 | 0.94 | 0.69 | 0.61 | 0.61 | 0.40 | 0.29 | 0.00 | 0.25 | 0.61 | 1.80 |

## Table 21. Treatment Area Metric Raw Data (untransformed).

| Site/Treatment Area | No. of Taxa | No. of Ephemeroptera taxa | % of Ephemeroptera | No. of Trichoptera taxa | % Trichoptera | No. of Plecoptera taxa | % Plecoptera | No. of EPT taxa | % of EPT | No. of Dipteran taxa | % Diptera | % Chironomidae | % filters | % grazers and scrapers | % Shredders | % Predators | % dominant taxon | Family Biotic Index (FBI) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Means | | | | | | | | | | | | | | | | | | |
| EMB-HY-Undist-4d | 9.0 | 3.0 | 4.5 | 2.0 | 38.5 | 0.5 | 3.0 | 5.5 | 46.0 | 2.5 | 17.5 | 8.5 | 38.0 | 3.0 | 3.0 | 9.0 | 41.5 | 4.2 |
| EMB-HY-Undist-14d | 6.5 | 1.0 | 3.5 | 2.0 | 36.5 | 0.5 | 1.5 | 3.5 | 41.5 | 1.5 | 5.0 | 3.5 | 36.5 | 1.5 | 1.0 | 1.0 | 57.5 | 3.9 |
| Std Devs | | | | | | | | | | | | | | | | | | |
| EMB-HY-Undist-4d | 0.0 | 0.0 | 0.7 | 0.0 | 4.9 | 0.7 | 4.2 | 0.7 | 9.9 | 0.7 | 2.1 | 3.5 | 5.7 | 1.4 | 4.2 | 1.4 | 0.7 | 0.3 |
| EMB-HY-Undist-14d | 0.7 | 0.0 | 3.5 | 1.4 | 21.9 | 0.7 | 2.1 | 0.7 | 16.3 | 0.7 | 2.8 | 4.9 | 21.9 | 2.1 | 1.4 | 1.4 | 12.0 | 0.3 |
| Means | | | | | | | | | | | | | | | | | | |
| EMB-HY-S.Dist-4d | 8.0 | 1.5 | 3.0 | 2.5 | 56.0 | 1.0 | 9.0 | 5.0 | 68.0 | 2.0 | 4.5 | 3.0 | 55.5 | 3.0 | 9.0 | 1.5 | 53.5 | 3.7 |
| EMB-HY-S.Dist-14d | 6.0 | 1.5 | 3.0 | 1.0 | 37.0 | 0.5 | 0.5 | 3.0 | 40.0 | 1.5 | 9.5 | 7.5 | 38.0 | 0.5 | 0.5 | 1.5 | 76.5 | 4.1 |
| Std Devs | | | | | | | | | | | | | | | | | | |
| EMB-HY-S.Dist-4d | 1.4 | 0.7 | 1.4 | 0.7 | 1.4 | 0.0 | 1.4 | 1.4 | 1.4 | 0.0 | 0.7 | 1.4 | 2.1 | 1.4 | 1.4 | 0.7 | 3.5 | 0.1 |
| EMB-HY-S.Dist-14d | 1.4 | 0.7 | 1.4 | 0.0 | 48.1 | 0.7 | 0.7 | 0.0 | 48.1 | 0.7 | 0.7 | 0.7 | 49.5 | 0.7 | 0.7 | 2.1 | 7.8 | 0.1 |
| Means | | | | | | | | | | | | | | | | | | |
| EMB-HY-Dist-4d | 9.0 | 1.5 | 2.5 | 2.0 | 22.0 | 1.5 | 9.5 | 5.0 | 34.0 | 3.0 | 23.5 | 16.5 | 22.0 | 2.0 | 10.5 | 6.0 | 42.5 | 4.1 |
| EMB-HY-Dist-14d | 10.5 | 3.5 | 10.0 | 2.5 | 26.5 | 1.5 | 6.0 | 7.5 | 43.0 | 2.0 | 26.0 | 24.5 | 27.0 | 6.5 | 1.5 | 0.5 | 48.0 | 4.2 |
| Std Devs | | | | | | | | | | | | | | | | | | |
| EMB-HY-Dist-4d | 2.8 | 0.7 | 0.7 | 1.4 | 1.4 | 0.7 | 12.0 | 1.4 | 12.7 | 1.4 | 2.1 | 0.7 | 0.0 | 0.0 | 14.8 | 4.2 | 9.2 | 0.6 |
| EMB-HY-Dist-14d | 0.7 | 0.7 | 2.8 | 0.7 | 23.3 | 0.7 | 0.0 | 0.7 | 26.9 | 0.0 | 5.7 | 4.9 | 25.5 | 2.1 | 2.1 | 0.7 | 8.5 | 0.1 |
| Means | | | | | | | | | | | | | | | | | | |
| EMB-SR-Undist-4d | 11.0 | 4.5 | 53.0 | 1.5 | 9.0 | 2.5 | 7.5 | 8.5 | 69.5 | 1.5 | 9.5 | 9.0 | 9.0 | 6.0 | 4.0 | 0.5 | 38.5 | 4.1 |
| EMB-SR-Undist-14d | 9.5 | 4.5 | 35.5 | 1.5 | 13.5 | 1.5 | 2.5 | 7.5 | 51.5 | 1.0 | 9.0 | 9.0 | 13.5 | 3.5 | 0.0 | 2.5 | 38.0 | 4.3 |
| Std Devs | | | | | | | | | | | | | | | | | | |
| EMB-SR-Undist-4d | 1.4 | 0.7 | 2.8 | 0.7 | 8.5 | 0.7 | 4.9 | 0.7 | 16.3 | 0.7 | 0.7 | 0.0 | 8.5 | 2.8 | 0.0 | 0.7 | 9.2 | 0.1 |
| EMB-SR-Undist-14d | 2.1 | 2.1 | 23.3 | 0.7 | 16.3 | 0.7 | 2.1 | 2.1 | 9.2 | 0.0 | 2.8 | 2.8 | 16.3 | 2.1 | 0.0 | 2.1 | 7.1 | 0.1 |
| Means | | | | | | | | | | | | | | | | | | |
| EMB-SR-S.Dist-4d | 11.0 | 4.0 | 29.0 | 1.0 | 13.5 | 2.0 | 10.0 | 7.5 | 52.5 | 1.5 | 9.0 | 8.0 | 13.5 | 4.5 | 1.5 | 9.5 | 36.5 | 4.0 |
| EMB-SR-S.Dist-14d | 11.5 | 5.0 | 46.5 | 2.0 | 9.0 | 1.5 | 13.5 | 8.5 | 69.0 | 1.0 | 6.5 | 6.5 | 9.0 | 11.5 | 0.5 | 13.5 | 24.0 | 4.2 |
| Std Devs | | | | | | | | | | | | | | | | | | |
| EMB-SR-S.Dist-4d | 2.8 | 1.4 | 4.2 | 0.0 | 10.6 | 0.0 | 7.1 | 2.1 | 7.8 | 0.7 | 7.1 | 8.5 | 10.6 | 3.5 | 2.1 | 7.8 | 0.7 | 0.0 |
| EMB-SR-S.Dist-14d | 2.1 | 1.4 | 6.4 | 0.0 | 5.7 | 0.7 | 12.0 | 0.7 | 0.0 | 0.0 | 3.5 | 3.5 | 5.7 | 0.7 | 0.7 | 10.6 | 2.8 | 0.3 |
| Means | | | | | | | | | | | | | | | | | | |
| EMB-SR-Dist-4d | 9.5 | 4.5 | 34.0 | 2.0 | 19.0 | 0.5 | 1.5 | 7.0 | 54.5 | 1.5 | 7.5 | 7.0 | 20.0 | 3.5 | 0.0 | 2.0 | 38.0 | 4.2 |
| EMB-SR-Dist-14d | 10.5 | 4.0 | 33.5 | 1.0 | 9.5 | 2.0 | 6.5 | 7.0 | 49.5 | 1.5 | 13.5 | 12.0 | 9.5 | 9.5 | 3.0 | 5.0 | 34.5 | 4.2 |
| Std Devs | | | | | | | | | | | | | | | | | | |
| EMB-SR-Dist-4d | 2.1 | 0.7 | 2.8 | 0.0 | 17.0 | 0.7 | 2.1 | 1.4 | 17.7 | 0.7 | 4.9 | 4.2 | 18.4 | 2.1 | 0.0 | 2.8 | 11.3 | 0.2 |
| EMB-SR-Dist-14d | 0.7 | 1.4 | 12.0 | 0.0 | 4.9 | 0.0 | 0.7 | 1.4 | 17.7 | 0.7 | 4.9 | 7.1 | 4.9 | 7.8 | 2.8 | 1.4 | 20.5 | 0.3 |

**Table 22. Treatment Area Metric Raw Data (transformed).**

| Sample | Site | Day | No. of Taxa | No. of Ephemeroptera taxa | % of Ephemeroptera | No. of Trichoptera taxa | % Trichoptera | No. of Plecoptera taxa | % Plecoptera | No. of EPT taxa | % of EPT | No. of Dipteran taxa | % Diptera | % Chironomidae | %filterers | %grazers & scrapers | %shredders | %predators | % dominant taxon | Family Biotic Index |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| EMB-HY-Undist-4 4d | HY | 4 | 2.30 | 1.39 | 0.20 | 1.10 | 0.63 | 0.0 | 0.00 | 1.79 | 0.67 | 1.39 | 0.45 | 0.34 | 0.62 | 0.14 | 0.00 | 0.29 | 0.71 | 1.47 |
| EMB-HY-Undist-5 4d | HY | 4 | 2.30 | 1.39 | 0.23 | 1.10 | 0.71 | 0.7 | 0.25 | 1.79 | 0.82 | 1.10 | 0.41 | 0.25 | 0.71 | 0.20 | 0.25 | 0.32 | 0.69 | 1.38 |
| EMB-HY-S-Dist-4 4d | HY | 4 | 2.08 | 0.69 | 0.14 | 1.10 | 0.86 | 0.7 | 0.32 | 1.39 | 0.98 | 1.10 | 0.20 | 0.14 | 0.86 | 0.14 | 0.32 | 0.14 | 0.85 | 1.30 |
| EMB-HY-S-Dist-5 4d | HY | 4 | 2.30 | 1.10 | 0.20 | 1.39 | 0.84 | 0.7 | 0.29 | 1.79 | 0.96 | 1.10 | 0.23 | 0.20 | 0.83 | 0.20 | 0.29 | 0.10 | 0.80 | 1.32 |
| EMB-HY-Dist-4 4d | HY | 4 | 2.48 | 0.69 | 0.14 | 1.39 | 0.50 | 1.1 | 0.44 | 1.79 | 0.72 | 1.61 | 0.49 | 0.41 | 0.49 | 0.14 | 0.48 | 0.17 | 0.64 | 1.29 |
| EMB-HY-Dist-5 4d | HY | 4 | 2.08 | 1.10 | 0.17 | 0.69 | 0.48 | 0.7 | 0.10 | 1.39 | 0.52 | 1.10 | 0.52 | 0.42 | 0.49 | 0.14 | 0.00 | 0.30 | 0.78 | 1.50 |
| EMB-HY-Undist-3 14d | HY | 14 | 1.95 | 0.69 | 0.10 | 1.39 | 0.81 | 0.0 | 0.00 | 1.39 | 0.82 | 0.69 | 0.27 | 0.27 | 0.81 | 0.00 | 0.00 | 0.00 | 0.78 | 1.41 |
| EMB-HY-Undist-6 14d | HY | 14 | 2.08 | 0.69 | 0.25 | 0.69 | 0.48 | 0.7 | 0.17 | 1.10 | 0.58 | 1.10 | 0.17 | 0.00 | 0.48 | 0.17 | 0.14 | 0.14 | 0.95 | 1.31 |
| EMB-HY-Dist-3 14d | HY | 14 | 2.48 | 1.61 | 0.35 | 1.10 | 0.72 | 1.1 | 0.25 | 2.08 | 0.91 | 1.10 | 0.58 | 0.56 | 0.74 | 0.29 | 0.17 | 0.00 | 0.71 | 1.44 |
| EMB-HY-Dist-6 14d | HY | 14 | 2.40 | 1.39 | 0.29 | 1.39 | 0.32 | 0.7 | 0.25 | 1.95 | 0.51 | 1.10 | 0.49 | 0.48 | 0.30 | 0.23 | 0.00 | 0.10 | 0.83 | 1.42 |
| EMB-HY-S-Dist-3 14d | HY | 14 | 1.79 | 1.10 | 0.20 | 0.69 | 1.00 | 0.0 | 0.00 | 1.10 | 1.04 | 0.69 | 0.30 | 0.29 | 1.02 | 0.10 | 0.00 | 0.00 | 1.00 | 1.40 |
| EMB-HY-S-Dist-6 14d | HY | 14 | 2.08 | 0.69 | 0.14 | 0.69 | 0.17 | 0.7 | 0.10 | 1.10 | 0.25 | 1.10 | 0.32 | 0.27 | 0.17 | 0.00 | 0.10 | 0.17 | 1.13 | 1.42 |
| EMB-SR-Undist-4 4d | SR | 4 | 2.56 | 1.79 | 0.80 | 1.10 | 0.17 | 1.1 | 0.20 | 2.20 | 0.87 | 1.10 | 0.32 | 0.30 | 0.17 | 0.29 | 0.20 | 0.10 | 0.60 | 1.44 |
| EMB-SR-Undist-5 4d | SR | 4 | 2.40 | 1.61 | 0.84 | 0.69 | 0.40 | 1.4 | 0.34 | 2.08 | 1.12 | 0.69 | 0.30 | 0.30 | 0.40 | 0.20 | 0.20 | 0.00 | 0.74 | 1.40 |
| EMB-SR-S-Dist-4 4d | SR | 4 | 2.30 | 1.39 | 0.60 | 0.69 | 0.48 | 1.1 | 0.23 | 1.79 | 0.87 | 1.10 | 0.20 | 0.14 | 0.48 | 0.14 | 0.17 | 0.20 | 0.64 | 1.40 |
| EMB-SR-S-Dist-5 4d | SR | 4 | 2.64 | 1.79 | 0.54 | 0.69 | 0.25 | 1.1 | 0.40 | 2.20 | 0.76 | 0.69 | 0.38 | 0.38 | 0.25 | 0.27 | 0.00 | 0.40 | 0.65 | 1.39 |
| EMB-SR-Dist-4 4d | SR | 4 | 2.48 | 1.79 | 0.60 | 1.10 | 0.27 | 0.7 | 0.17 | 2.08 | 0.71 | 1.10 | 0.34 | 0.32 | 0.27 | 0.14 | 0.00 | 0.20 | 0.75 | 1.48 |
| EMB-SR-Dist-5 4d | SR | 4 | 2.20 | 1.61 | 0.64 | 1.10 | 0.59 | 0.0 | 0.00 | 1.79 | 0.96 | 0.69 | 0.20 | 0.20 | 0.61 | 0.23 | 0.00 | 0.00 | 0.58 | 1.41 |
| EMB-SR-Undist-3 14d | SR | 14 | 2.48 | 1.95 | 0.81 | 0.69 | 0.14 | 1.1 | 0.20 | 2.20 | 0.87 | 0.69 | 0.27 | 0.27 | 0.14 | 0.14 | 0.00 | 0.20 | 0.61 | 1.47 |
| EMB-SR-Undist-6 14d | SR | 14 | 2.20 | 1.39 | 0.45 | 1.10 | 0.52 | 0.7 | 0.10 | 1.79 | 0.74 | 0.69 | 0.34 | 0.34 | 0.52 | 0.23 | 0.00 | 0.10 | 0.72 | 1.43 |
| EMB-SR-S-Dist-3 14d | SR | 14 | 2.64 | 1.95 | 0.80 | 1.10 | 0.37 | 0.7 | 0.23 | 2.20 | 0.98 | 0.69 | 0.20 | 0.20 | 0.37 | 0.34 | 0.00 | 0.25 | 0.54 | 1.48 |
| EMB-SR-S-Dist-6 14d | SR | 14 | 2.40 | 1.61 | 0.71 | 1.10 | 0.23 | 1.1 | 0.49 | 2.08 | 0.98 | 0.69 | 0.30 | 0.30 | 0.23 | 0.35 | 0.10 | 0.48 | 0.49 | 1.39 |
| EMB-SR-Dist-3 14d | SR | 14 | 2.40 | 1.79 | 0.71 | 0.69 | 0.37 | 1.1 | 0.27 | 2.08 | 0.91 | 0.69 | 0.42 | 0.42 | 0.37 | 0.40 | 0.10 | 0.25 | 0.46 | 1.48 |
| EMB-SR-Dist-6 14d | SR | 14 | 2.48 | 1.39 | 0.52 | 0.69 | 0.25 | 1.1 | 0.25 | 1.79 | 0.65 | 1.10 | 0.32 | 0.27 | 0.25 | 0.20 | 0.23 | 0.20 | 0.78 | 1.39 |

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| **13. SUPPLEMENTARY NOTES** | | | |

**14. ABSTRACT**

Environmental managers utilize a variety of tools when assessing lotic systems for stressors attributed to anthropogenic sources. Stream deposited sediment has been recognized as one of the major stressors affecting streams in the U.S. The detrimental effect on aquatic biota of sediment depositing within the interstitial spaces of stream substrate (embeddedness) has been established, yet lacking is an effective *in situ* method of quantifying embeddedness over short time periods. The goal of this research was to develop a short-term embeddedness (EMB) quantification method that can be linked to benthic macroinvertebrate health. Such a method would be a valuable tool when conducting biological and physical habitat assessments of wadeable streams and rivers. An *in situ* embeddedness chamber was developed to capture sediment deposited within the interstitial spaces of a uniformly sized substrate. Using sediment accumulation and macroinvertebrate colonization as endpoints, three exposure periods were evaluated (4, 7, and 14 days) on a small order stream (Honey Creek, New Carlisle, Ohio, USA) and a medium order stream (Stillwater River, Covington, Ohio, USA). The experiment was conducted during low flow conditions with little variation in flow, turbidity, and total suspended solids. Three treatment areas located downstream of the EMB chambers also were established to assess benthic macroinvertebrate colonization rates. Different levels of substrate disturbance (disturbed, slightly disturbed, and undisturbed) were mimicked by removing the embedded fine sediments. Embeddedness chamber results show correlations between newly deposited fine sediment and insect colonization rates. Measured percent embeddedness (USGS method) results at both sites were close to a natural stream condition (~33-35% embedded). Increases in both sediment and insect colonization within the EMB chambers during the three sampling periods show that the chambers had not reached the embeddedness equilibrium for the stream conditions at that time. Regression analyses run between chamber abiotic and biotic parameters reveal interesting correlations showing possible influence of fine sediment fractions on the biotic responses. Treatment area invertebrate results showed higher densities with the undisturbed areas indicating the need for a longer study period to assess true colonization potential. Further exploration, calibration, and validation of an effective *in situ* embeddedness quantification method for lotic systems is needed for accurate stream assessments.

**15. SUBJECT TERMS**

Emeddedness, *in situ*, macroinvertebrate colonization, sediment

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