This is to certify that the

thesis entitled

THE EFFECTS OF AN INDUSTRIAL EFFLUENT
ON THE QUALITY OF A MICHIGAN
WARMWATER STREAM

presented by

Ethan Jay Nedeau

has been accepted towards fulfillment
of the requirements for

M.S. degree in Entomology

Major professor

Date 16 December, 1999

THE EFFECTS OF AN INDUSTRIAL EFFLUENT ON THE QUALITY OF A MICHIGAN WARMWATER STREAM

By

Ethan Jay Nedeau

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Entomology

1999

ABSTRACT

THE EFFECTS OF AN INDUSTRIAL EFFLUENT ON THE QUALITY OF A MICHIGAN WARMWATER STREAM

By

Ethan Jay Nedeau

I studied the effect of an industrial effluent on the quality of a second-order, warmwater stream. The effluent increased the total stream discharge by 50-150%; this significantly improved downstream physical habitat quality. The effluent carried high levels of iron precipitate, and caused a considerable increase in water temperatures. Artificial substrates were used to compare macroinvertebrate colonization upstream and downstream of the effluent, with no effort to standardize habitat quality. There were few significant trends in colonization. Macroinvertebrate community composition and colonization was then compared among sites with comparable habitat quality (riffles with gravel/cobble substrata). There were clear, significant differences in macroinvertebrate diversity and colonization among these sites, with riffles immediately downstream of the effluent supporting the lowest diversity and highest proportion of pollution-tolerant taxa. Growth and mortality bioassays were conducted with the mayfly *Stenacron interpunctatum*. Though not statistically significant, results from the two bioassays suggest that food quality, not water quality per se, likely explains the scarcity of mayflies immediately downstream of the effluent. This study illustrates the importance of considering both habitat quality and water quality when assessing the effects of point-source pollutants on stream ecosystems.

DEDICATION

Well, dad, I've earned a lot if titles in the last 24 years – CHAMPION Runner, EXPERT Fisherman (no laughing), BACHELOR of Science and Woman, ROYAL Pain in the Neck.....Sorry you left before seeing me become a MASTER of Science. I dedicate this to you.

ACKNOWLEDGEMENTS

Through the drivers-side mirror of my 1984 Dodge station wagon I watched the sun rise over the hills of western Massachusetts. I was heading west. A passenger seat full of junk food wrappers, one new alternator, and 900 miles later I arrived at Michigan State University. Three days later I arrived at Site 001 - the outfall of Pharmacia & Upjohn's industrial wastewater into Portage Creek. My leg sank deeply into black mud when I stepped into the creek for the first time, and my heart sank with it. Did I really come all this way to solve a riddle of how an industry can possibly make a severely degraded stream even worse? My impressions from that first afternoon at Portage Creek were only reinforced through two years of intensive investigation. As the sun rose over the broad fields of eastern Michigan, and the engine of the U-Haul seemed to be roaring in eager anticipation of a late-August sea-breeze on the coast of Maine, I was left with the question of what I had gained from my midwest experience. I thought of all my friends – sharing a beer with Loren at the boathouse as the sun set over Gull Lake, stumbling home from the bar with Jeremy, waking in my tent to the sound of Nate yelling, "ETHAN, GET UP. YOU HAVE TO WRITE YOUR THESIS!" You all know who you are, and I can't believe you are reading my thesis - get a life! Some of you helped me above and beyond the day-to-day maintenance of sanity, and deserve special recognition – Jeremy, Kelly Nate, Eric, John, Keri, Bill (the finest bottom picker around), and Julie (not so bad herself). I also thought of the lessons I had learned, such as how to jimmy a car lock with a landscaping flag, the value of patching neoprene waders before spending a January afternoon standing around in a stream, and how to make a graph in MSExcel without having the tail end of the y-axis label get cut off (a lesson I still struggle with). I also

thought of the guidance and training I received from my committee members – especially Mike Kaufman for being practical, anal-retentive, and tolerant. Thanks also to my primary advisor Rich Merritt for granting me a chance to represent Michigan State at the NABS 5K, and making the lab a more festive place to work in. I also learned to deal with loss during my time at Michigan State (beyond data, equipment, and sanity), because my father was killed on the same morning that I was in a quagmire of raw data and SAS© printouts. I thought about canning graduate school and returning east to be nearer my family, but ultimately it was the friendship and support of my friends in Michigan that allowed me to weather the ordeal. I thank you all.

Finally, I must give thanks to the Department of Entomology for research and travel funds, the Pharmacia & Upjohn Company for funding my research assistantship, Kellogg Biological Station for summer housing, lab space, and support, and the Michigan Department of Environmental Quality for project oversight.

TABLE OF CONTENTS

LIST OF TABLES vii

LIST OF FIGURES viii

CHAPTER ONE
POSITIVE AND NEGATIVE EFFECTS OF AN INDUSTRIAL EFFLUENT ON THE BENTHIC MACROINVERTEBRATES OF A MICHIGAN WARMWATER STREAM

INTRODUCTION 1
STUDY SITE 6
METHODS 8
RESULTS 12
DISCUSSION 18
SUMMARY 24
LITERATURE CITED 27

CHAPTER TWO
GROWTH AND SURVIVAL OF *STENACRON INTERPUNCTATUM* (EPHEMEROPTERA: HEPTAGENIIDAE) EXPOSED TO AN INDUSTRIAL EFFLUENT

INTRODUCTION 52
METHODS 54
RESULTS 58
DISCUSSION 59
LITERATURE CITED 65

APPENDIX 1
QUALITATIVE LIST OF MACROINVERTEBRATE TAXA COLLECTED BY THE DIFFERENT SAMPLING TECHNIQUES, JUNE 1996 – JUNE 1998 74

LIST OF TABLES

CHAPTER ONE

TABLE 1. Water quality and habitat quality variables, adapted from Karr (1991).....32

TABLE 2. Explanation of metrics and indices used to compare macroinvertebrate communities collected by the three sampling methods (HD = Hester-Dendy artificial substrates, Hess = Hess samples, Tile = ceramic tiles).....33

TABLE 3. List of aquatic macrophyte species found within the study area. Relative proportions of these species are indicated for upstream, downstream, and within the industrial effluent (0 = Absent, 1 = < 20%, 2 = 20-50%, 3 = 50-80%, 4 = >80%).....34

CHAPTER TWO

TABLE 1. Temperature and flow data for *in situ* bioassay with the mayfly *Stenacron interpunctatum*. Experiment 1: May 16 – May 22 (6 days), 1997. Experiment 2: May 18-May 23 (5 days), 1998. Degree Day = Σ daily mean temperatures > 0° for duration of experiment. Velocity not measured in Experiment 2.....67

TABLE 2. Summary data for the *in situ* bioassay with the mayfly *Stenacron interpunctatum*. Experiment 1: May 16 – May 22 (6 days), 1997. Experiment 2: May 18-May 23 (5 days), 1998. Mean Growth: Δ Dry Weight. The p-values for the overall ANOVA are indicated; tests are considered significant of the p-value < 0.05. See Figures 4-6 for graphical summary, and Table 1 for temperature/flow data.....67

TABLE 3. Summary data for the *in situ* bioassay with the mayfly *Stenacron interpunctatum*: Experiment 2. Dates: May 18 – May 23 (5 days), 1998. The p-values for the overall ANOVA are indicated; tests are considered significant if the p-value < 0.05.....68

LIST OF FIGURES

CHAPTER ONE

FIGURE 1. Map of Portage Creek. A) Broader view of Portage Creek (Scale: 1 inch = 1000 meters), showing the three riffle "sites" used for Hess samples and ceramic tile colonization studies. The dashed lines perpendicular to the stream channel are locations for habitat and flow analyses. B) Narrow view of Portage Creek (Scale: 1 inch = ~200 meters), showing 5 locations where Hester-Dendy artificial substrates were deployed, including the discharge channel itself. The circle in Figure 1.B indicates the location where mussel shells were found following scouring from the storm.....35

FIGURE 2. A) Pharmacia & Upjohn's discharge into Portage Creek. B) Confluence of the discharge channel with Portage Creek.....36

FIGURE 3. A) Trout habitat restoration site, and the upstream site for Hess sampling and ceramic tile colonization. B) Short riffle section created by railroad construction, and upstream site for Hess sampling, collection site for mayflies, and test location for mayfly growth experiment 1.....37

FIGURE 4. A) Riffle section located just upstream of the Kilgore Road bridge, and downstream site for Hess sampling and ceramic tile colonization. B) Portage Creek in the lower reaches of its watershed, about 5 miles downstream from the study area....38

FIGURE 5. Mean depth (m), and mean velocity (m/s) upstream and downstream of the industrial effluent (+ Standard Errors). Values are taken from microhabitat measurements of the Hester-Dendy artificial substrates (See Figure 1 for sampling locations)..... 39

FIGURE 6. Two representative depth profiles from upstream and downstream of the industrial effluent. The X-axis is inverted to illustrate the profile as a cross-section of the stream at each transect. See Figure 1 for transect locations.... 39

FIGURE 7. Percentage of substrate types upstream and downstream of the industrial effluent. Values are taken from microhabitat measurements of Hester-Dendy artificial substrates. See Figure 1 for sampling locations..... 40

FIGURE 8. Percentage of instream-cover upstream and downstream of the industrial effluent during the growing season (June-September) and non-growing season (October-May). Values are taken from microhabitat measurements of Hester-Dendy artificial substrates. See Figure 1 for sampling locations. A list of aquatic macrophytes is presented in Table 3..... 40

LIST OF FIGURES (CONTINUED)

FIGURE 9. Log (# Individuals) colonizing Hester-Dendy artificial substrates at each sampling unit over the three colonization periods (+ Standard Errors). N = 11-14 (Upstream), 4-7 (Discharge Channel), 10-12 (Downstream). Overall ANOVA p-values: October 0.0429, January 0.0001, April 0.0029. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.....41

FIGURE 10. Mean # Taxa colonizing Hester-Dendy artificial substrates at each sampling unit over the three colonization periods (+ Standard Errors). N = 11-14 (Upstream), 4-7 (Discharge Channel), 10-12 (Downstream). Overall ANOVA p-values: October 0.0019, January 0.0001, April 0.0001. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.....41

FIGURE 11. Mean # EPT Taxa colonizing Hester-Dendy artificial substrates at each sampling unit over the three colonization periods (+ Standard Errors). N = 11-14 (Upstream), 4-7 (Discharge Channel), 10-12 (Downstream). Overall ANOVA p-values: October 0.1070, January 0.0011, April 0.0002. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.....42

FIGURE 12. Mean # Ephemeroptera individuals colonizing Hester-Dendy artificial substrates at each sampling unit over the three colonization periods (+ Standard Errors). N = 11-14 (Upstream), 4-7 (Discharge Channel), 10-12 (Downstream). Overall ANOVA p-values: October 0.1791, January 0.0372, April 0.0333. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.....42

FIGURE 13. Mean # Trichoptera individuals colonizing Hester-Dendy artificial substrates at each sampling unit over the three colonization periods (+ Standard Errors). N = 11-14 (Upstream), 4-7 (Discharge Channel), 10-12 (Downstream). Overall ANOVA p-values: October 0.4311, January 0.1487, April 0.0429. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations..... 43

FIGURE 14. H' diversity of invertebrates colonizing Hester-Dendy artificial substrates at each sampling unit over the three colonization periods (+ Standard Errors). Oligochaetes and chironomids were excluded when calculating H'. N = 11-14 (Upstream), 4-7 (Discharge Channel), 10-12 (Downstream). Overall ANOVA p-values: October 0.0001, January 0.0001, April 0.0001. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.....43

LIST OF FIGURES (CONTINUED)

FIGURE 15. Biotic index of invertebrates colonizing Hester-Dendy artificial substrates at each sampling unit over the three colonization periods (+ Standard Errors). Oligochaetes and chironomids were excluded when calculating the biotic index. N = 11-14 (Upstream), 4-7 (Discharge Channel), 10-12 (Downstream). Overall ANOVA p-values: October 0.3632, January 0.0001, April 0.0054. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.... 44

FIGURE 16. Log (# Individuals) collected with the modified-Hess sampler at riffle sites upstream and downstream of the industrial effluent (+ Standard Errors), exclusive of the chironomids and oligochaetes. N = 5 per site. Overall ANOVA p-values: June 0.0079, November 0.0010. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.....45

FIGURE 17. Mean # Taxa collected with the modified-Hess sampler at riffle sites upstream and downstream of the industrial effluent (+ Standard Errors). N = 5. Overall ANOVA p-values: June 0.0006, November 0.0029. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.....45

FIGURE 18. Mean # EPT Taxa collected with the modified-Hess sampler at riffle sites upstream and downstream of the industrial effluent (+ Standard Errors). N = 5. Overall ANOVA p-values: June 0.0027, November 0.0004. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.....46

FIGURE 19. Mean # Ephemeroptera individuals collected with the modified-Hess sampler at riffle sites upstream and downstream of the industrial effluent (+ Standard Errors). N = 5. Overall ANOVA p-values: June 0.0077, November 0.0093. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.....46

FIGURE 20. Mean # Trichoptera individuals collected with the modified-Hess sampler at riffle sites upstream and downstream of the industrial effluent (+ Standard Errors). N = 5. Overall ANOVA p-values: June 0.2907, November 0.374. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.....47

LIST OF FIGURES (CONTINUED)

FIGURE 21. H' Diversity of samples collected with the modified-Hess sampler at riffle sites upstream and downstream of the industrial effluent (+ Standard Errors), exclusive of chironomidae and oligochaeta. N = 5. Overall ANOVA p-values: June 0.0332, November 0.0012. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.....47

FIGURE 22. Biotic index calculated for samples collected with the modified-Hess sampler at riffle sites upstream and downstream of the industrial effluent (+ Standard Errors), exclusive of oligochaeta and chironomidae. N = 5. Overall ANOVA p-values: June 0.0539, November 0.1494. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.....48

FIGURE 23. Functional feeding group composition of samples collected with the modified Hess sampler at riffle sites upstream and downstream of the industrial effluent. Figures are based on presence/absence of taxa. See Figure 1 for sampling locations.....48

FIGURE 24. Log (# Individuals) colonizing ceramic tiles at the three riffle sites over the four colonization periods (± Standard Errors). N = 4–5. Overall ANOVA p-values: July 0.0717, August 0.0229, September 0.0332, November 0.1765. See Figure 1 for sampling locations.....49

FIGURE 25. Mean # Taxa colonizing ceramic tiles at the three riffle sites over the four colonization periods (± Standard Errors). N = 4–5. Overall ANOVA p-values: July 0.1060, August 0.1392, September 0.2238, November 0.0618. See Figure 1 for sampling locations.....49

FIGURE 26. % Midges and Worms (Oligochaeta)) colonizing ceramic tiles at the three riffle sites over the four colonization periods (± Standard Errors). N = 4–5. Overall ANOVA p-values: July 0.0004, August 0.0009, September 0.0001, November 0.1502. See Figure 1 for sampling locations.....50

FIGURE 27. Mean # EPT Taxa colonizing ceramic tiles at the three riffle sites over the four colonization periods (± Standard Errors). N = 4–5. Overall ANOVA p-values: July 0.0688, August 0.2242, September 0.1445, November 0.0127. See Figure 1 for sampling locations.....50

FIGURE 28. Mean # Ephemeroptera colonizing ceramic tiles at the three riffle sites over the four colonization periods (± Standard Errors). N = 4–5. Overall ANOVA p-values: July 0.0223, August 0.0066, September 0.0838, November 0.0657. Figure 1 for sampling locations.....51

FIGURE 29. Mean # Trichoptera colonizing ceramic tiles at the three riffle sites over the four colonization periods (± Standard Errors). N = 4–5. Overall ANOVA p-values: July 0.2862, August 0.0581, September 0.2743, November 0.1666. Figure 1 for sampling locations.....51

CHAPTER TWO

FIGURE 1. Map of Portage Creek from Portage Police Station (Upstream Riffle) to Milham Park (Downstream Riffle), showing locations used during the course of the mayfly growth experiments. Scale: 1 inch = ~1000 meters.....69

FIGURE 2. Elements of the mayfly growth experiment. A) Ceramic tiles placed in the discharge channel. B) Ceramic tiles after 1 month in the discharge channel, displaying a heavy accumulation of ferric hydroxide and bacteria. C) A pair of growth chambers used in Experiment 1; 1 is open to show the arrangement of food tiles. D) A growth chamber used in Experiment 2, showing the divider used to separate the chamber into 2 compartments. E) A pair of growth chambers suspended within the cage and immersed in the stream. F) All growth chambers (6 chambers, 3 cages) at the industrial outfall during Experiment 2.....70

FIGURE 3. Experimental design for the mayfly growth experiments. See Figure 1 for a map of test locations, and Figure 2 for photographs of ceramic tiles, growth chambers, cages, and the experiment in progress.....71

FIGURE 4. Mean growth (g) of caged *Stenacron interpunctatum* for the *in situ* growth/mortality experiment conducted May 16-May 22, 1997 (+ Standard Errors). N (cages) = 4 (upstream), 4 (discharge), and 3 (downstream). Overall ANOVA not significant (p-value 0.5918). See Table 2 for summary data, and Figure 1 for site locations.....72

FIGURE 5. Mean growth (g) of caged *Stenacron interpunctatum*, corrected for total degree-day accumulation at each location (+ Standard Deviation). *In situ* growth/mortality experiment conducted May 16-May 22, 1997. N (cages) = 4 (upstream), 4 (discharge), and 3 (downstream). Overall ANOVA not significant (p-value 0.8677). Degree days = Σ daily mean temperatures >0°C. See Table 2 for summary data, and Figure 1 for site locations.....72

FIGURE 6. Percent survival (+ Standard Deviation) of caged *Stenacron interpunctatum* for the *in situ* growth/mortality experiment conducted May 16-May 22 1997. N (cages) = 4 (upstream), 4 (discharge), and 3 (downstream). Overall ANOVA not significant (p-value 0.6019). See Table 2 for summary data, and Figure 1 for site locations.....73

LIST OF FIGURES (CONTINUED)

FIGURE 7. Mean growth (g) of caged *Stenacron interpunctatum* exposed to different test locations and food sources (upstream versus discharge channel) (+ Standard Errors). N (chambers) = 6 for each combination. Dates: May 18 – May 23 1998. Two-factor ANOVA not significant (p-values: Location 0.1978, Food 0.1332, Location x Food 0.9284). See Table 2 for summary data and Figure 1 for site locations.....75

CHAPTER ONE
THE EFFECTS OF AN INDUSTRIAL EFFLUENT ON THE BENTHIC MACROINVERTEBRATES OF A MICHIGAN WARMWATER STREAM.

INTRODUCTION

There are five primary classes of water resource variables that determine the structure and function of lotic ecosystems: water quality, habitat structure, flow regime, energy source, and biotic interactions (Karr 1991, Allan 1995). From a conservation or management standpoint, it would be valuable to know the relative importance of these classes of variables. This would aid in establishing protection criteria, or allow focused restoration or remediation efforts to achieve more efficient and beneficial use of public resources (Karr 1990, 1991, Allan and Flecker 1993, Scrimgeour and Wicklum 1996). Unfortunately, these variables operate at different spatial and temporal scales within and among lotic ecosystems, and most are confounded, such that it becomes difficult to tease apart the relative importance of each to overall ecosystem structure and function (Power et al. 1988, Allan 1995).

Water quality has traditionally been the variable of interest in water resource management and biological assessment (Prati et al. 1971, Karr 1991). Water quality includes an array of variables of both natural and anthropogenic origin that collectively make up the physico-chemical nature of the water, including dissolved and suspended materials (Table 1). These variables provide a means of quantitatively comparing water quality within and among water bodies. Early legislation emphasized the importance of water quality because it was thought that clean water would ensure the biological

integrity of aquatic systems (Karr 1991). Much of the focus was on regulating point-sources of pollution, and developing water quality standards based on laboratory toxicity tests (Cairns and Pratt 1989, Maltby and Calow 1989). Little or no emphasis was placed on examining non-point source pollution, or human activities that altered other classes of water resource variables such as habitat quality and flow regime. The combined effects of non-point source pollutants and other human activities on aquatic systems precluded the ability to associate water quality standards with biological integrity (Karr 1991). In the 1980's, there was a strong push for water resource assessment and protection programs that emphasized direct measurement of biological integrity, rather than relying on water quality standards as surrogate measures (Cairns and Pratt 1989, Karr 1991).

Direct measures of biological integrity have focused on a variety of organisms, most commonly invertebrates (Plafkin et al. 1989, Rosenberg and Resh 1993), and fish (Karr 1981, 1991). Macroinvertebrates are commonly used as surrogates for biological integrity because of their importance in the structure and function of lotic ecosystems (Merritt et al. 1984, Rosenberg and Resh 1993, Wallace and Webster 1996). The growing understanding of the biotic and abiotic variables that determine macroinvertebrate community structure (Power et al. 1988) has increased the ability to detect biological impairment in aquatic systems (Rosenberg and Resh 1993). Yet, identifying the specific cause(s) of biological impairment has remained elusive due to the confounded nature of environmental variables, and the bias towards water quality as the variable of interest in pollution assessments.

Among other water resource variables, habitat quality is of critical importance to the biological integrity of aquatic systems. Habitat quality is defined by a number of

variables that describe the physical/structural nature of the system (both within the aquatic system and the adjacent riparian corridor), and collectively determine the degree of niche heterogeneity and ultimately species diversity (Table 1). Niemi et al. (1990) reviewed the literature for studies that documented resilience of aquatic systems to disturbance. They found that recovery from most types of disturbance were generally less than three years, unless the physical habitat of the system was altered. Nearly all cases in which recovery times exceeded five years involved some sort of habitat degradation or simplification. This suggests that habitat quality is at least as important as water quality in determining biological integrity, and should perhaps be given equal consideration in water resource management and biological assessment.

This is not to suggest that habitat quality is ignored in current bioassessment procedures, or considered unimportant in water resource management (Rankin 1995). A description of habitat quality is a component of nearly all bioassessment procedures (Platts et al. 1983, Plafkin et al.1989, Rankin 1995). However, the mere description of habitat quality variables does little more to predict biological integrity of aquatic systems than did the mere description of water quality variables called for in early pollution assessment programs. The question remains the same: how well do descriptions of variables reflect the actual biological integrity of the system? Water resource assessments have taken an important step to directly measure biological integrity rather than rely on water quality measures. The same philosophy should be applied to habitat quality measures. Both water quality and habitat quality should be expressed in terms of their contribution to the overall productivity and diversity that the stream ecosystem can support (Karr 1995, Rankin 1995).

A more holistic understanding of the factors contributing to the biological integrity of aquatic systems becomes especially important in watersheds that have been severely impacted by human activity, and receive both point and non-point sources of pollution that degrade both water quality and habitat quality (Karr and Schlosser 1978, Karr et al. 1985, Karr 1991, Roth et al. 1995). The distinction between water quality and habitat quality is critical from a restoration or remediation standpoint. For example, habitat restoration programs that seek to improve biological integrity of aquatic systems by increasing habitat heterogeneity would not produce the desired effects if water quality were the limiting factor (NRC 1992). Likewise, targeting point-sources of pollution in order to improve water quality will not improve the biological integrity of the system if habitat quality were the limiting factor.

Portage Creek is typical of streams throughout the midwestern United States in that it's watershed has been subjected to intensive agricultural, industrial, and urban development over the last 150 years (Karr et al. 1985, Lamberti and Berg 1995). In addition to a myriad of other point and non-point sources of pollution that continually threaten both water quality and habitat quality, a pharmaceutical company releases its cooling water into Portage Creek. The non-contact cooling water is drawn from a single large aquifer, distributed within the plant for cooling purposes, and is then discharged into Portage Creek at a rate of 5-8 million gallons per day. According to the Pharmacia & Upjohn Company, Portage, MI, the only alterations to the water other than thermal are chlorination processes to minimize bacterial growth within the plant, and then dechlorination (sulfonation) processes prior to its discharge into the environment. The

effluent also contains input from the storm water drainage system at the manufacturing facility.

Potential negative effects of this effluent on Portage Creek are ferric hydroxide precipitation and deposition, and elevated water temperatures. Groundwater in this region of southwestern Michigan is very high in ferrous iron (Fe^{2+}), and when this is brought to the surface and exposed to oxygen, it is oxidized to ferric iron (Fe^{3+}). In neutral water most of this ferric iron is hydrolyzed to ferric hydroxide, which forms a yellow-orange precipitate ("yellowboy") and settles out on the substratum. It is known to inhibit the growth of benthic algae (Sheldon and Skelly 1990, Wellnitz and Sheldon 1995), depress macroinvertebrate diversity by interfering with feeding and respiration (Koryak et al. 1972, Rasmussen and Lindegaard 1988, Wellnitz et al. 1994), and has consequences for higher trophic levels (Letterman and Mitsch 1978). The effluent is also thermally constant, ranging from 17-24 °C throughout the year. In winter it ranges from 15-20 °C above ambient stream temperature, and once mixed with the stream water increases the stream temperature 8-12 °C. Temperature is a critical factor influencing the life cycle of most aquatic invertebrates (Ward and Stanford 1992, Sweeney 1984). It has direct effects on hatching time and survivorship of eggs, larval growth period, timing of emergence, and adult size at emergence. It can also affect the population and community ecology of aquatic invertebrates (Ward 1976), and ecosystem processes (Cairns 1976, Paul et al. 1978).

Although the industry consistently meets water quality standards for discharges into local surface waters, the impact of the effluent on Portage Creek is not fully understood. Previous biological assessments carried out by the Michigan Department of

Natural Resources (MDNR *unpublished reports*), and independent consulting firms (Fishbeck, Thompson, Carr & Huber, Inc. 1991) were unable to conclusively determine the overall effect of the effluent on Portage Creek. Among the shortcomings of each of the studies was a failure to adequately characterize in-stream habitat quality, provide a quantitative analysis of the macroinvertebrate communities, and a failure to recognize the importance of habitat-specific sampling and confounding variables. The challenge was to make a fair assessment of the impact of this point-source of pollution on the quality of Portage Creek, while considering other historical and contemporary influences on ecosystem quality. The objectives of this study were to:

(1) assess the immediate impact of the industrial effluent on the quality of Portage Creek, considering both habitat quality and water quality,

(2) incorporate this assessment with the larger story of historical and contemporary influences on the quality of Portage Creek, and

(3) offer insight into the problems associated with conducting upstream-downstream pollution assessments in an urban/agricultural watershed.

STUDY SITE

Portage Creek is a third-order tributary of the Kalamazoo River in Kalamazoo County, in southwestern Michigan. Its 52-square kilometer drainage area is a glacial outwash plain, with up to 350 feet of sand and gravel outwash overlying a shale bedrock valley. It originates from groundwater seeps and wetlands, and travels north for about 15 kilometers to its confluence with the Kalamazoo River in the city of Kalamazoo. It receives substantial recharge from both the groundwater and adjacent wetlands.

Originally, the watershed was classified as an oak savannah, and probably included patches of dry prairie. The dominant tree species included oaks (*Quercus spp.*), and hickories (*Carya spp.*). Marshes lined the creek along most of its length. The watershed was converted to an agricultural landscape in the 19th and early 20th centuries. In the upper reaches of the watershed, most of the agricultural activity has been abandoned, and much of the area is now undergoing rapid urbanization. Land use in the lower reaches of the watershed is primarily industrial and urban. Several large industries are clustered along the creek in the city of Kalamazoo, including two large paper industries that are now closed down. Much of this historical industrial complex is now condemned, and listed as an EPA Superfund Site due to terrestrial and groundwater contamination (Figure 4B). There are many documented cases of chemical spills, contaminated groundwater venting to the creek, and other toxic discharges into Portage Creek over the past few decades. The industrial effluent which is the focus of this study is located well upstream of the urban/industrial complex in the city of Kalamazoo, but still downstream of a myriad of point and non-point sources of pollution in the city of Portage, and outlying areas.

Though not accessible to the general public, people can rent canoes upstream of the discharge channel and paddle past it. There was concern over the yellow-brown precipitate that covered the substratum within and downstream of the discharge channel, and a more general concern that such a conspicuous pollution source detracted from the aesthetic beauty of the creek. This prompted the Michigan Department of Environmental Quality to initiate an investigation into the effects of this effluent on Portage Creek.

METHODS

I. Habitat Analysis

I focused on the following variables to describe the habitat quality upstream and downstream of the industrial effluent: water depth, water velocity, substrate type, in-stream cover (i.e. macrophytes, woody material). Water velocity was measured with a Marsh-McBirney portable flow meter. Substrate was examined visually and placed into one of two categories: fine material (silt and sand), or coarse material (gravel and cobble). Macrophytes were identified to species using keys of Voss (1985). The habitat assessment was conducted within 5 12-meter stream sections upstream and downstream of the discharge channel (2 upstream, 3 downstream) (Figure 1). Habitat variables were measured at six randomly selected locations within each of the stream sections. In addition, four sites were selected in order to determine flow heterogeneity and total discharge (Figure 1). At each of these sites I randomly established two cross-stream transects, and measured depth and flow velocity at 30 centimeter increments along each transect. From these data I computed total discharge (m^3/s), and depth profiles.

The following large-scale habitat descriptors were assessed visually using field surveys and aerial photographs: channel alteration, channel sinuosity, run-bend ratio, nature of the riparian zone, land use, and potential sources of pollution. These descriptors reflect historical and contemporary landscape-level disturbances that could confound our assessment of the impact of a single source of pollution. The large-scale habitat descriptors, and other historical and contemporary influences on Portage Creek, are addressed in the discussion section.

II. BENTHIC MACROINVERTEBRATE ASSESSMENT

The initial approach to assess the benthic macroinvertebrate community focused on a 600-meter section of Portage Creek, from 100 meters upstream of the effluent to 500 meters downstream of the effluent (Figure 1). Artificial substrates were deployed into 6 stream sections (Figure 1), which included the five sites used for the habitat assessment and an additional site within the discharge channel itself. Each stream section was 12 meters long, and divided into 24 quadrats. Artificial substrates were placed into six randomly selected quadrats within each site, and habitat variables were measured at the exact location at which each artificial substrate was placed (See also: **HABITAT ANALYSIS**). The samplers were deployed for five one-month periods from October 1996 to September 1997. I used Hester-Dendy (HD) artificial substrates; HD substrates consist of seven circular masonite plates stacked onto a large eyebolt with spacers in between each (Hester and Dendy 1962). They were attached to a heavy basal plate, and placed on the stream bottom (Mathers and Martin 1967). This sampler provides a uniform surface area for invertebrate colonization, and is accessible to both drifting and crawling individuals. Samples were collected by placing a pint canning jar over the sampler so that the jar fit tightly against the basal plate, and inverting it so that the multi-plate portion was contained within the jar. The sample was eliminated from further analysis if sediment or leaves buried the sampler.

It became apparent that Site 3 actually encompassed two distinct environments: the west side (3W) was outside of the effluent plume and more similar to upstream conditions, and the east side (3E) was within the effluent plume and more similar to the discharge channel itself. For the analysis, sites 1,2 and 3W were pooled into a single

"Upstream" site, site 3E and the discharge channel were pooled into a single "Discharge Channel" site, and sites 4 and 5 were pooled into a single "Downstream" site.

Since sampling sites and habitat quality were confounded in the previous approach, I tried a second approach where I sampled similar habitats upstream and downstream of the discharge channel. I sampled in five riffles located within a 6-kilometer reach of Portage Creek (Figure 1). The first upstream riffle is a 75-meter reach that was restored for trout by the Michigan Department of Natural Resources. The second upstream riffle is where a railroad bed and old service road cross the creek. These two sites comprise the "Upstream" site for the analysis. Samples were taken within a 400-meter stretch of riffle habitat immediately downstream of the discharge channel ("Impact Zone"). To test for downstream recovery, I chose riffles at least 3-kilometers downstream of the effluent ("Recovery"). All samples were taken within 200 meters of the bridge at Kilgore Road (Figure 1). All of the riffle sections chosen were similar in terms of depth, flow velocity, and substrate type.

I sampled these riffles with a modified Hess-sampler (Merritt and Cummins 1996). The sampler is planted firmly in the substrate, with a 250 μm net pointing downstream. Sediments are stirred within the enclosed area, and all fine particles and invertebrates are washed into the net. Larger stones were examined for attached invertebrates (such as the limpet *Ferrissia rivularis*, or the caddisfly *Psychomyia flavida*). Placement of the Hess sampler on the stream bottom was non-random - I was careful to sample locations with similar depth, flow velocity, and substrate. I used this non-random approach because I was only interested in assessing differences in water quality between sites, without having to take a large number of samples. If I were interested in assessing

the spatial distribution or abundance of macroinvertebrates, then a random approach requiring large sample sizes would be necessary. Five samples were taken at each of the "sites" (Upstream, Impact Zone, Recovery), on two sampling dates (June 1997, November 1997), for a total of 30 samples.

Ceramic tiles were used to assess colonization processes in the same riffles used for the Hess sampling (Figure 1). Six-inch square ceramic tiles were placed within a narrow range of depth, flow velocity, and substrate type, with the corrugated surface pointing down. The tiles were intended to sample clinging/sprawling invertebrates that graze the surface biofilm. These types of invertebrates would be more sensitive to ferric hydroxide deposition. The tiles also allowed for a more quantitative assessment of the differences in chironomid midge colonization between the three riffle sites. The colonization period was 28-32 days, and they were placed in the riffles for four consecutive months.

III. Sample Processing, Sorting, and Analysis

All samples were either preserved in 90% ethanol in the field, or placed on ice and transported back to the lab for processing. Hester-Dendy and tile samples were rinsed through a 250 μm sieve, and Hess samples were rinsed through a 500 μm sieve. Samples were stored permanently in 70% ethanol. Rose-bengal stain was added to the Hess samples to facilitate sorting. Taxa were identified to genus or species, except for the following: Class Hydracarina (water mites), Class Oligochaeta (worms), Family Chironomidae (midges), Family Ceratopogonidae (biting midges), and some early instar

insects. Oligochaetes and chironomids were ennumerated for the HD and tile samples, but not for the Hess samples.

A number of metrics and indices were used to characterize the macroinvertebrate communities collected by the two different sampling methods. An annotated list of these metrics and indices is provided in Table 2. The metrics and indices are displayed graphically, and analyzed statistically. Analysis of variance was performed on each of the metrics and indices using the ANOVA (equal sample sizes) or GLM (General Linear Methods for unequal sample sizes) procedures in the SAS® statistical software program. Comparisons among treatment means were performed with Tukey's LSD test in the same software package. The significance level was $p \leq 0.05$ unless otherwise indicated.

RESULTS

I. Habitat Analysis

The industrial effluent increases the total discharge of Portage Creek by 50 to 150%, based on my flow measurements and historical data provided by USGS gauging stations upstream and downstream of the industrial effluent. This has immediate and pronounced effects on in-stream habitat quality. The added volume of water did not have an immediate effect on mean stream width, or mean velocity (Figure 5). However, maximum velocities range from 25-100% higher downstream of the discharge channel, indicating a faster and more sharply defined thalweg. Mean depth is 0.2 meters greater downstream of the discharge channel (Figure 5). Perhaps more striking than the mean depths are the depth profiles illustrated in Figure 6. The depth profiles and flow analyses illustrate a greater heterogeneity of depth and flow conditions downstream of the effluent

due to the substantial increase in total discharge. Substrate was 100% sand and silt upstream of the discharge channel (Figure 7). Downstream of the discharge channel, there was a larger proportion of gravel and cobble substrate.

Macrophytes represented a large proportion of total in-stream cover in Portage Creek. During the summer growing season (June-September), there was nearly 85% in-stream cover upstream of the discharge channel, and only 60% downstream (Figure 8). However, most of the in-stream cover upstream is the macrophyte *Potamogeton filiformis*, which is an important but transient cover type. It's root system is weak, and since it is rooted in a fairly unstable sand substratum, it is quickly uprooted and washed downstream once it senesces in the fall. In the fall and winter (post-senescence), the upstream reaches are nearly denuded of any in-stream cover, and what little exists was woody debris that is confined to the margins of the channel. There was not such a strong seasonal difference for in-stream cover downstream of the discharge channel (Figure 8). Downstream, there was a larger percentage of the macrophytes *Sparganium sp.* and *Elodea canadensis*, both of which are somewhat more resistant to senescence and degradation than *Potamogeton filiformis*. Downstream, the macrophytes were rooted in a more stable substratum, and exist in a more stable thermal environment. Each of these factors may enhance their ability to remain active during the winter months. There was also a larger amount of large woody debris at the surface of the streambed downstream of the discharge channel; this is in part due to the effluent because the added discharge allowed the stream to scour away much of the fine sediments, thereby preventing the material from getting buried. I observed much woody debris upstream of the discharge

channel that was buried under fine sediment and thus not available for colonization by invertebrates.

Overall, aquatic macrophyte diversity was considerably higher downstream of the discharge channel (Table 3). Four species were found immediately upstream, though *Potamogeton filiformis* comprises >80% of the biomass. Eight species were found immediately downstream, and the two most abundant species were *Sparganium sp.* and *Elodea canadensis*. *Ludwigia palustris* was dominant within the discharge channel itself. This species was never found upstream of the effluent, nor was it found further than 75 meters downstream of the effluent.

II. Benthic Macroinvertebrate Assessment

The HD samplers were colonized by a total of 46 taxa (Appendix 1); though worms (oligochaetes) and midges (Chironomidae) comprised from 65-95% of the individuals in nearly all samples. The worms and midges were so abundant that they essentially swamped out all other taxa when computing community indices (H'Diversity, Biotic Index), so these indices were calculated after excluding these two groups. The data are shown graphically in Figures 9-15.

The discharge channel consistently supported fewer total individuals than upstream or downstream sites; this was statistically significant in January and April (Figure 9). Upstream and downstream sites were never statistically different in terms of total individuals, though there were consistently more upstream. The discharge channel supported significantly fewer taxa for all three sampling periods (Figure 10). Upstream and downstream sites were not significantly different, though in January there were more

taxa collected downstream of the industrial effluent (Figure 10). The discharge channel supported consistently fewer EPT taxa (Figure 11), mayfly individuals (Figure 12) and caddisfly individuals (Figure 13) than other sites; in fact, no mayflies or caddisflies were collected from the discharge channel in January or April. There was a high variability of mayfly and caddisfly individuals collected at upstream and downstream sites, and thus it was difficult to demonstrate a significant difference between the sites. Upstream and downstream were never significantly different for any of the EPT metrics, though there were more mayflies collected downstream of the industrial effluent in January and April (Figure 12), and more caddisflies collected downstream of the industrial effluent for all three sampling dates (Figure 13). The discharge channel consistently supported a lower diversity than upstream or downstream sites, and this was significant in October and April (Figure 14). Upstream diversity was not significantly different than discharge channel diversity in January, and downstream diversity was significantly greater than all other sites in January. Upstream and downstream sites were not significantly different in October or April (Figure 14).

The biotic index provided less consistent results than the metrics or the diversity index (Figure 15). In October, there was virtually no difference between each of the sites in terms of the biotic index. The discharge channel did have a significantly greater (more pollution tolerance) biotic index score in January and April. Upstream and downstream sites were virtually identical for all three sampling dates.

A total of 53 taxa were collected with the modified-Hess sampler at the riffle sites (Appendix 1). Forty taxa (39 in June, 23 in November) were collected at the upstream sites, twenty-two taxa (22 in June, 15 in November) were collected within the impact

zone, and thirty taxa (30 in June, 25 in November) were collected at the downstream recovery sites. The riffles in the impact zone supported significantly fewer total individuals than upstream or recovery sites for both sampling dates (Figure 16). Upstream and recovery sites did not differ in June, but in November the upstream sites had significantly greater number of individuals (Figure 16). The impact zone also supported a significantly fewer number of taxa than upstream or recovery sites for both sampling dates (Figure 17), though the other two sites did not differ significantly. The same is true for the mean # EPT taxa, though it is interesting to note that the recovery zone had a greater number of EPT taxa for both sampling periods, although this was not significant (Figure 18). The recovery sites had a greater number (though not significant) of mayfly (Figure 19) and caddisfly (Figure 20) individuals in June, though in November the upstream sites had greater numbers of both (significant only for caddisflies). The impact zone had significantly fewer mayflies than upstream or recovery sites for both sampling dates (Figure 19). However, the impact zone was not significantly different than upstream or recovery sites in terms of caddisfly individuals in June, and was not different than recovery sites in November (Figure 20).

In June, there was no significant difference in diversity between the upstream and recovery sites, though the recovery sites did have a slightly higher diversity (Figure 21). The impact zone was significantly less diverse than the upstream or recovery sites. In November, the recovery sites were significantly more diverse than upstream or impact zone sites, and the impact zone and upstream sites were not significantly different. There was no significant difference in the biotic index between the sites for either sampling date (Figure 22). Identification of worms and chironomids to more meaningful taxonomic

levels would probably help elucidate trends in the biotic index. Since sample sizes were small (n=5 for each "site"/sampling date), the fact that most of the tests showed significant differences suggests that biological differences between the riffles are quite robust. Overall, the data indicate that benthic invertebrate abundance and diversity had recovered to at least upstream levels by 3 kilometers downstream of the discharge channel.

Figure 23 shows the trend in the relative proportion of functional feeding groups at the three sites. Scrapers comprised only 10% of the taxa downstream of the industrial discharge, compared to 28% upstream and 27% at Kilgore Road. Shredders comprised 26% of the taxa downstream of the effluent, compared to 15% upstream and only 5% at Kilgore Road. The number of collector-gatherer taxa gradually increased in a downstream direction, and there were slightly more predators upstream (Figure 23).

Macroinvertebrate abundance and diversity data collected with the ceramic tiles are generally consistent with that from the Hess samples. There were few statistically significant trends for the measured metrics, largely because of small sample sizes and high degree of variability in macroinvertebrate colonization. Ceramic tiles in the impact zone had a larger number of individuals than other sites in July and September, yet the Hess samples indicated a statistically significant reduction in numbers of organisms from this same area. The contrasting results are due to the fact that midges (Chironomidae) and oligochaetes were ennumerated for the tile samples but not for the Hess samples; the impact zone had a comparatively higher percentage of these organisms than the upstream or recovery sites (Figure 26). The impact zone consistently supported fewer number of macroinvertebrate taxa (Figure 25) and EPT Taxa (Figure 27) than upstream or recovery

sites. The recovery sites supported the greatest number of taxa for 3 of the 4 sampling dates, and had a greater number of EPT Taxa than upstream sites in July and August. These effects were not statistically significant. The upstream sites usually supported the greatest numbers of mayfly individuals (Figure 28) and caddisfly individuals (Figure 29), despite the fact that the recovery sites had a greater number of taxa. Although few of the observations were statistically significant, the consistent trends in colonization at the three "sites" indicate a classic example of impact and subsequent downstream recovery.

DISCUSSION

I. Habitat Quality

Over the last 150 years, the entire Portage Creek watershed has been subjected to intensive agricultural and urban development. I obtained aerial photographs for the Portage Creek watershed dating back to 1935, and they showed that much of the watershed was under intensive agriculture at some point in the last century, but the amount of land under intensive agriculture has decreased in the last two to three decades. Much of the land that was cleared for agricultural purposes has reverted back to early-successional forest, and this natural regeneration has obscured the level to which the watershed was disturbed in the late 1800's and early 1900's. Despite the positive changes that have taken place within the terrestrial landscape, the habitat quality of Portage Creek remains poor. The upper reaches of Portage Creek are almost entirely channelized, with homogeneous depth and flow, 100% sand and silt substrates, and low structural diversity.

Pharmacia & Upjohn Co. pumps 5-8 million gallons of heated water into Portage Creek every day, increasing the total discharge by 50-150%. This has immediate effects on habitat quality because the added volume of water gives the stream enough energy to keep fine sediment in suspension and transport it downstream. This increases depth and flow heterogeneity, and exposes the gravel and cobble substrates that comprised the original streambed. The shift in substrate particle size has positive effects on stream invertebrates (Cummins and Lauff 1969). The increase in habitat heterogeneity also allows for a greater diversity of aquatic macrophytes, which are an important source of cover and food for aquatic animals (Iversen et al. 1985, Carpenter and Lodge 1986, Humphries 1996).

An alternative explanation for this shift in habitat quality is that the industry built its discharge channel at a natural break in the stream continuum, where it made a transition from a low gradient reach to a higher gradient reach. However, stream gradient below the discharge channel is not different from upstream. Stronger evidence was attained using shells of freshwater bivalves in the Family Unionidae. Hundreds of broken and heavily eroded bivalve shells were found in the riffles downstream of the industrial effluent, yet none were found in the silt/sand substratum upstream. A flash flood in June of 1997 nearly doubled the total discharge of Portage Creek for a brief period, and flushed the sediment out from a 15 meter section of the creek about 100 meters upstream of the industrial effluent. The flash flood exposed a gravel/cobble streambed by deepening the channel by 0.2 to 0.6 meters. Unionid shells were numerous in this short section, representing all of the species that had been found downstream. Within two weeks, this short section of stream was filled in with sand, and the shells were once again

buried. This chance event is of particular interest for this study. It suggests that many sections of Portage Creek that are now 100% sand and silt substrate once had a substrate type suitable for freshwater mussels, and presumably other riffle fauna. Over time, agricultural and urban development in the watershed caused excessive sedimentation of the creek. This buried the existing streambed, including the assemblage of freshwater mussels that inhabited the stream. The industry discharged enough water into the creek to allow it to regain some of its initial habitat quality. It took a point-source pollutant to reveal what a history of non-point source pollution had buried. Unfortunately, no live unionid mussels were found in Portage Creek after two years of study.

II. Benthic Macroinvertebrate Assessment

The results from the Hess samples and ceramic tile samples indicate that the industrial effluent has a negative impact on the quality of Portage Creek. The riffles immediately downstream of the effluent ("the impact zone") consistently supported fewer taxa, a lower community diversity, and have fewer numbers of pollution-intolerant taxa such as mayflies (Ephemeroptera) and caddisflies (Trichoptera) than riffles upstream or much farther downstream. Despite the fact that the industrial effluent elevates total stream temperature by as much as 8-12 °C in the winter, temperature is unlikely to be the cause for the reduction in diversity we see immediately downstream of the industrial effluent. Although the stream temperature at the recovery sites was slightly cooler than sites immediately downstream of the discharge channel, it remained 6-10°C above upstream (natural) temperatures. Despite only a minor recovery of stream temperature at the recovery sites, there was a very substantial recovery of the macroinvertebrate

community. The recovery zone consistently supported a greater number of pollution-intolerant EPT taxa than the upstream site, indicating that conditions were as good or better than upstream conditions. This suggests that the factor that limits the macroinvertebrate community in the impact zone must be localized. This is not meant to imply that temperature has no effect on the benthic invertebrates. Some taxa were found at Kilgore Road that were not found at the upstream sites, including the mayflies *Tricorythodes sp., Ephemerella lata, Ephemera simulans,* the caddisfly *Psychomyia flavida*, and the snail *Goniobasis livescens*. Perhaps thermal constancy and warmer temperatures is the factor that allows these taxa to exist at these sites. The introduced bivalve, *Corbicula fluminea*, is also found at the Kilgore Road riffles, and its presence is almost certainly attributed to the warmer temperatures provided by the effluent. This clam is widely distributed throughout the upper midwest in streams that are either warmed by industrial effluents (such as nuclear power plants), or whose temperature does not drop below this species lower lethal limit of 4-5 °C (Mattice and Dye 1978, Graney et al. 1980). Although the Kilgore Road communities are comprised of slightly different taxa, the overall community composition reflects favorable water quality and habitat quality. So although temperature may have an effect on the invertebrates, it is unlikely that the effect of temperature alone is negative.

Iron deposition is more likely to have altered the macroinvertebrate community structure in the riffles within the impact zone. Ferric hydroxide is evident within the discharge channel and for about 1000 meters downstream, where the sediments and aquatic macrophytes are covered with a fine layer of rusty-colored material. Ferric hydroxide deposition has been shown to have both direct and indirect effects on stream

biota. It inhibits the colonization and growth of diatoms and green algae, which comprises an important primary food source for aquatic food webs (Cummins and Klug 1979, Sode 1983, Sheldon and Skelly 1990, Wellnitz and Sheldon 1994). In a separate study in Portage Creek, Kaufman (*unpublished data*) found that tiles placed in the discharge channel had significantly fewer diatoms, and lower chlorophyll-a than tiles placed upstream or further downstream. In a small mountain stream in Vermont, Sheldon and Skelly (1990) found that over a distance of 17 meters, the epilithic community of diatoms and green algae was almost entirely replaced by an iron-depositing bacterium *Leptothrix ochracea.* The percent cover of *L. ochracea* increased from 0.1 % to 99.8 % over that short distance, and this was due to a 24-fold increase in iron concentration and a 20-fold increase in manganese concentration. Within ~300 meters, the percentage of *L ochracea* dropped to less than 10%, and the diatoms and filamentous algae regained dominance. At the same study site, Wellnitz et al. (1994) found that macroinvertebrate diversity and abundance was greatly reduced within the *L. ochracea* bloom, though further downstream the diversity and abundance approached that of upstream. These patterns were also evident in other streams in northern Vermont that also had blooms of iron-depositing bacteria (Wellnitz et al. 1994). Many other studies have documented similar drops in the diversity of the macroinvertebrate and fish communities due to iron deposition, and the subsequent downstream recovery (Koryak et al.1972, Letterman and Mitsch 1978, Rasmussen and Lindegaard 1988).

It is possible that ferric hydroxide and temperature act in synergy immediately downstream of the discharge channel. The combined effects of poor food quality and high temperature (increased metabolism) could have a large effect on some animals.

Clogging of gills with ferric hydroxide coupled with high temperature (increased respiratory demands) could also have a large effect on some animals, especially those with external gills (such as heptageniid mayflies).

Results from the Hester-Dendy artificial substrates indicate that the industrial effluent has no effect on the biological integrity of Portage Creek. The discharge channel itself supports a sparse community of pollution-tolerant invertebrates, but whatever was inhibiting the development of a healthy macroinvertebrate community within the discharge channel was apparently not operating in the creek itself. Important riffle insects such as hydropsychid caddisflies and heptageniid mayflies were more abundant downstream of the discharge channel than upstream. This indicates that the industrial effluent may actually improve conditions for some important pollution-intolerant taxa. Given the difference in habitat conditions between upstream and downstream sites, it is not surprising that we should find a more diverse macroinvertebrate community downstream of the industrial effluent. *All else being equal*, an artificial substrate placed in a good microhabitat (gravel or cobble) should be colonized by a greater diversity of macroinvertebrates than one placed in a poor microhabitat (sand or silt) because there is a greater local source pool of potential colonizers (Osman 1982). If anything, it was surprising that we didn't see an even greater number and diversity of macroinvertebrates downstream of the industrial effluent compared to upstream.

However, in addition to improving habitat quality (as measured by depth, flow velocity, and substrate particle size), the industrial effluent also reduces water quality due to thermal pollution and ferric hydroxide precipitation. The latter ultimately degrades habitat quality by blanketing the stream substratum. This reduction in water quality is

thought to account for the discrepancy between invertebrate community that could potentially colonize the favorable habitat downstream of the effluent versus the invertebrate community that actually inhabits such sites.

This result is partially dependent on the sampling method chosen. If one were interested in expressing the shift in habitat quality in terms of what it means for the macroinvertebrate community, then it would not be valid to use a sampling method that attempts to overcome habitat differences between sites (such as artificial substrates). A more active sampling method (such as a Hess sampler or a Surber sampler) would be more effective at showing differences in macroinvertebrate communities due to habitat quality. If an active sampling method had been used, a much larger positive effect of the industrial effluent on the stream biota would have been demonstrated. This is because the sand habitat is inhabited by only a few taxa in Portage Creek: nematodes (Nematoda), flatworms (Turbellaria), worms (Oligochaeta), water mites (Hydracarina), clams (Sphaeriidae), midges (Chironomidae) a few beetles (Coleoptera), and dragonfly and damselfly larvae (Odonata). Many more taxa are found in riffles downstream of the discharge channel.

SUMMARY

The variable of interest in water resource management and biological assessment has always been water quality; this is especially true for assessing the impacts of point-source pollutants on aquatic systems (Karr 1991). Other water resource variables, such as habitat quality and flow regime, are often relegated to the status of confounding variables. Standard procedures emphasize the importance of sampling identical habitats

upstream and downstream of a source of pollution, so that differences in macroinvertebrate communities can be attributed to water quality. Artificial substrates are tremendously popular in aquatic research, and much of their appeal is that they provide a uniform colonization area so that the biological community can be assessed independent of variation in the natural substratum (Rosenberg and Resh 1982, 1993).

What if a point-source of pollution alters habitat quality? In this case, habitat quality should not be considered merely a confounding factor that serves only to obscure water quality differences between sites. Instead, it should become as important as water quality in the overall assessment of the pollutant's impact on the stream (Rankin 1995). Equal consideration of water quality and habitat quality causes the complexity of the assessment to increase, but also provides a more comprehensive and realistic assessment of the overall impact of the pollutant on biological integrity. The success of restoration or remediation programs is dependent on an accurate assessment of the causes of observed effects (Karr 1991, NRC 1992, Davis and Simon 1995).

In this study, if only the results from the Hess samples were presented, then it would be clear that the industry was having a negative impact on the quality of Portage Creek. This assumes that the control sites are representative of their respective reaches. We know this is not the case, because the two upstream riffle sites were the *only* sites upstream of the discharge channel that had a riffle habitat suitable for the establishment of a macroinvertebrate community that we typically consider indicative of a healthy, unpolluted stream. Using the same sampling method, riffles immediately downstream of the discharge channel would compare favorably to ~ 95% of the locations upstream of the discharge channel.

If only the results from the artificial substrates were presented, then we would probably conclude that the industry was having no effect on the biological integrity of Portage Creek, or even a slightly positive effect. This is due to the fact that the effluent increases total stream discharge and improves in-stream habitat quality. The habitat analysis and the two sampling approaches provide comprehensive insight into the overall effect of the industrial effluent on Portage Creek: by improving habitat it has a slightly positive effect on biological integrity at one scale, but by reducing water quality it has a negative effect on biological integrity at another scale.

In this situation, the water resource manager faces a bit of a dilemma: is it better to have good water quality at the expense of habitat quality, or good habitat quality at the expense of water quality? Shutting down the effluent would cause a rapid degradation of habitat quality, and lower the productivity and diversity of Portage Creek. We would be sacrificing habitat quality for water quality. Alternatively, allowing the industry to continue business as usual would maintain current biological integrity. We would be sacrificing localized reductions in water quality for habitat quality. Perhaps it is unwise to set a precedent of trading water quality for habitat quality. However, restoring biological integrity to aquatic systems that have been virtually destroyed by decades or centuries of human activities is no easy task. It requires an understanding of which factors most strongly limit biological integrity within each system, and an evaluation of the costs and benefits of different restoration or remediation strategies. Taking the quickest and easiest approach to try to improve biological integrity, such as targeting point sources of pollution rather than addressing non-point sources, may yield no net improvement in the health of our aquatic systems (Karr 1991). This study demonstrates

that targeting a point-source of pollution may actually be detrimental to overall biological integrity.

LITERATURE CITED

Allan, J.D. 1995. Stream ecology: Structure and Function of Running Waters. Chapman & Hall. 388 pages.

Allan, J.D., and A.S. Flecker. 1993. Biodiversity conservation in running waters: identifying the major factors that threaten the destruction of riverine species and ecosystems. Bioscience 43:32-43.

Cairns, J. Jr., and J.R. Pratt. 1989. The scientific basis of bioassays. Hydrobiologia 188/189:5-20.

Cairns, J.Jr. 1976. Heated waste-water effects on aquatic ecosystems. Pages 32-38 In: *Thermal Ecology II* (G.W. Esch and R.W. McFarlane, editors) ERDA Symposium Series (CONF-750425), pp. 302-307.

Carpenter, S.R., and D.M. Lodge. 1986. Effects of submersed macrophytes on ecosystem processes. Aquatic Botany 26:341-370

Cover, E.C., and R.C. Harrel. 1978. Sequences of colonization, diversity, biomass, and productivity of macroinvertebrates on artificial substrates in a freshwater canal. Hydrobiologia 59:81-95.

Cummins, K.W., and G.H. Lauff. 1969. The influence of substrate particle size on the microdistribution of stream macrobenthos. Hydrobiologia 34:145-181.

Cummins, K.W., and M.J. Klug. 1979. Feeding ecology of stream invertebrates. Annual Review of Ecology and Systematics. 10:147-172.

Davis, W.S., and T.P. Simon. 1995. *Biological Assessment and Criteria: Tools for Water Resource Planning and Decision Making.* Lewis Publishers.

Gagnier, D.L., and R.C. Bailey. 1994. Balancing loss of information and gains in efficiency in characterizing stream sediment samples. Journal of the North American Benthological Society 13:170-180.

Graney, R.L., D.S. Cherry, J.H. Rodgers Jr., and J.Cairns Jr. 1980. The influence of thermal discharges and substrate composition on the population structure and distribution of the Asiatic clam, *Corbicula fluminea*, in the New River, Virginia. The Nautilus 94:130-135.

Hester, F.E., and J.S. Dendy. 1962. A multiple-plate sampler for aquatic macroinvertebrates. Transactions of the American Fisheries Society 91:420-421.

Hilsenhoff, W.H. 1987. An improved biotic index of organic stream pollution. The Great Lakes Entomologist 20:31-39.

Humphries, P. 1996. Aquatic macrophytes, macroinvertebrate associations and water levels in a lowland Tasmanian river. Hydrobiologia 321:219-233.

Iversen, T.M., J. Thorup, T. Hansen, J. Lodal, and J. Olsen. 1985. Quantitative estimates and community structure of invertebrates in a macrophyte rich stream. Archiv fur Hydrobiologie 102:291-301.

Karr, J.R. 1981. Assessment of biotic integrity using fish communities. Fisheries 6:21-27.

Karr, J.R. 1990. Biological integrity and the goal of environmental legislation: lessons for conservation biology. Conservation Biology 4:244-250.

Karr, J.R. 1991. Biological integrity: a long-neglected aspect of water resource management. Ecological Applications 1:66-84.

Karr, J.R., and I.J. Schlosser. 1978. Water resources and the land-water interface. Science 201:229-234.

Karr, J.R., L.A. Toth, and D.R. Dudley. 1985. Fish communities of midwestern rivers: a history of degradation. Bioscience 35:90-95.

Koryak, M., M.A. Shapiro, and J.L. Sykora. 1972. Riffle zoobenthos in streams receiving acid mine drainage. Water Research 6:1239-1247.

Lamberti, G.A., and M.B. Berg. 1995. Invertebrates and other benthic features as indicators of environmental change in Juday Creek, Indiana. Natural Areas Journal 15:249-258.

Lamberti, G.A., and V.H. Resh. 1979. Substrate relationships, spatial distribution patterns, and sampling variability in a stream caddisfly population. Environmental Entomology 8:561-567.

Lenat, D.R. 1993. A biotic index for the southeastern United States: derivation and list of tolerance values, with criteria for assigning water-quality ratings. Journal of the North American Benthological Society 12:279-290.

Letterman, R.D., and W.J. Mitsch. 1978. Impact of acid mine drainage on a mountain stream in Pennsylvania. Environmental Pollution 17:53-73.

Maltby, L., and P. Calow. 1989. The application of bioassays in the resolution of environmental problems: past, present and future. Hydrobiologia 188/189:65-76.

Mason, W.T., and P.P. Yevich. 1967. The use of phloxine B and rose bengal stains to facilitate sorting benthic samples. Transactions of the American Microscopical Society 86:221-223.

Mathers, C.K., and T.R. Martin. 1969. A new multiplate sampler for collecting macroinvertebrates of the stream biocies. Transactions of the Illinois Academy of Science 62:331-333.

Mattice, J.S., and L.L. Dye. 1976. Thermal tolerance of the adult Asiatic clam. Pages 130-135 in: *Thermal Ecology II* (G.W. Esch and R.W. McFarlane, editors) ERDA Symposium Series (CONF-750425), pp. 302-307.

Meier, P.G., D.L. Penrose, and L. Polak. 1979. The rate of colonization by macro-invertebrates on artificial substrate samplers. Freshwater Biology 9:381-392.

Merritt, R.W., and K.W. Cummins. 1996. Trophic relations of macroinvertebrates. Pages 453-474 in: Methods in Stream Ecology (F.R. Hauer and G.A. Lamberti, eds.) Academic Press.

Merritt, R.W., K.W. Cummins, and T.M. Burton. 1984. The role of aquatic insects in the processing and cycling of nutrients. Pages 134-163 In: *The Ecology of Aquatic Insects* (V.H. Resh and D.M. Rosenberg, eds.) Praeger Scientific, New York 625 p.

Michigan Department of Natural Resource (MDNR). 1991. Qualitative biological and habitat survey protocols for wadeable streams and rivers. GLEAS Procedure No. 51. Michigan DNR, Surface Water Quality Division, Great Lakes Environmental Assessment Section.

Niemi, G.J., P. DeVore, N. Detenbeck, D. Taylor, A. Lima, J. Pastor, J.D. Yount, and R.J. Naiman. 1990. Overview of case studies on recovery of aquatic systems from disturbance. Environmental Management 14:571-587.

NRC (National Research Council). 1992. *Restoration of Aquatic Ecosystems: Science, Technology, and Public Policy.* National Academy Press, Washington, D.C.

Osman, R.W. 1982. Artificial substrates as ecological islands. Pages 71-130 in: Artifical Substrates (J. Cairns Jr., editor). Ann Arbor Science.

Paul, R.W.Jr., E.F. Benfield, and J. Cairns Jr. 1978. Effects of thermal discharge on leaf decomposition in a river ecosystem. International Journal of Applied and Theoretical Limnology 20:1759-1766.

Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross, and R.M. Hughes. 1989. *Rapid bioassessment protocols for use in streams and rivers. Benthic macroinvertebrates and fish.* EPA 440-4-89-001. Office of Water Regulations and Standards, U.S. Environmental Protection Agency, Washington, D.C.

Platts, W.S., W.F. Megahan, and G.W. Minshall. 1983. *Methods for evaluating stream, riparian, and biotic conditions*. General Technical Report No. INT-138, U.S. Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station, Ogden, Utah.

Power, M.E., R.J. Stout, C.E. Cushing, P.P. Harper, F.R. Hauer, W.J. Matthews, P.B. Moyle, B. Statzner, and I.R. Wais De Badgen. 1988. Biotic and abiotic controls in river and stream communities. Journal of the North American Benthological Society 7:456-479.

Prati, L., R. Pavanello, and F. Pesarin. 1971. Assessment of surface water quality by a single index of pollution. Water Research 5:741-751.

Rankin, E.T. 1995. Habitat indices in water resource quality assessments. Pages 181-208 in: *Biological Assessment and Criteria: Tools for Water Resource Planning and Decision Making* (W.S. Davis and T.P. Simon, editors). Lewis Publishers 415 pages.

Rasmussen, K., and C. Lindegaard. 1988. Effects of iron compounds on macroinvertebrate communities in a Danish lowland river system. Water Research 22:1101-1108.

Richards, C., G.E Host, and J.W. Arthur. 1993. Identification of predominant environmental factors structuring stream macroinvertebrate communities within a large agricultural catchment. Freshwater Biology 29:285-294.

Rosenberg, D.M., and V.H. Resh. 1982. The use of artificial substrates in the study of freshwater benthic macroinvertebrates. Pages 175-235 in: *Artificial Substrates* (J. Cairns Jr., editor). Ann Arbor Science, Ann Arbor, Michigan.

Rosenberg, D.M., and V.H. Resh. 1993. *Freshwater biomonitoring and benthic macroinvertebrates*. Chapman & Hall. 488 pages.

Roth, N.E., J.D. Allan, and D.L. Erickson. 1995. Landscape influences on stream biotic integrity assessed at multiple spatial scales. Landscape Ecology xxxxxxx.

Scrimgeour, G.J., and D. Wicklum. 1996. Aquatic ecosystem health and integrity: problems and potential solutions. Journal of the North American Benthological Society 15:254-261.

Sheldon, S.P., and D.K. Skelly. 1990. Differential colonization and growth of algae and ferromanganese-depositing bacteria in a mountain stream. Journal of Freshwater Ecology 5:475-485.

Sode, A. 1983. Effect of ferric hydroxide on algae and oxygen consumption by sediment in a Danish stream. Archives fur Hydrobiologie 1:134-162.

Voss, E.G. 1972. *Michigan Flora: A guide to the identification and occurrence of the native and naturalized seed-plants of the state: Part II Dicots (Saururaceae-Cornaceae).* Cranbook Institute of Science Bulletin 59.

Wallace, J.B., and J.R. Webster. 1996. The role of macroinvertebrates in stream ecosystem function. Annual Review of Entomology 41:115-139.

Ward, J.V. 1976. Effects of thermal constancy and seasonal temperature displacement on community structure of stream macroinvertebrates. In: *Thermal Ecology II* (G.W. Esch and R.W. McFarlane, editors), ERDA Symposium Series (CONF-750425), pp. 302-307.

Ward, J.V., and J.A. Stanford. 1982. Thermal responses in the evolutionary ecology of aquatic insects. Annual Review of Entomology 27:97-117.

Wellnitz, T.A., and S.P. Sheldon. 1995. The effects of iron and manganese on diatom colonization in a Vermont stream. Freshwater Biology 34:465-470.

Wellnitz, T.A., K.A. Grief, and S.P Sheldon. 1994. Response of macroinvertebrates to blooms of iron-depositing bacteria. Hydrobiologia 281:1-17.

Williams, D.D., and M.R. Smith. 1996. Colonization dynamics of river benthos in response to local changes in bed characteristics. Freshwater Biology 36:237-248.

TABLE 1. Water quality and habitat quality variables, as defined by Karr (1991).

CLASS	COMPONENTS
Water Quality	Temperature, dissolved oxygen, pH, turbidity, nutrients (primarily nitrogen and phosphorus), organic and inorganic chemicals, heavy metals, other toxic substances
Habitat Quality	Substrate type and distribution, water depth, current velocity, habitat diversity (pools, riffles), in-stream cover (woody debris, undercut banks), spawning and nursery areas, basin size and shape

TABLE 2. Explanation of metrics and indices used to compare macroinvertebrate communities collected by the three sampling methods (HD = Hester-Dendy artificial substrates, Hess = Hess samples, Tile = ceramic tiles).

METRIC/INDEX	METHOD	DESCRIPTION
Log (# Individuals)	HD, Hess, Tile	Log-transformed number of macroinvertebrates in a sample or collection of samples.
Mean # Taxa	HD, Hess, Tile	Average number of macroinvertebrate taxa in a sample or collection of samples.
Mean # EPT Taxa	HD, Hess, Tile	Average number of taxa in a sample or collection of samples which belong to the insect families Ephemeroptera, Plecoptera, or Trichoptera.
Ephemeroptera Individuals	HD, Hess, Tile	Average number of individuals in a sample or collection of samples that belong to the Family Ephemeroptera.
Trichoptera Individuals	HD, Hess, Tile	Average number of individuals in a sample or collection of samples that belong to the Family Trichoptera.
% Midges and Worms	Tile	Percentage of total individuals in a sample belonging to the family Chironomidae or the Class Oligochaeta.
H' Diversity	HD, Hess	Shannon-Weiner diversity. This diversity measure is calculated as: $H' = -\sum(N_i/N) \log_{10} (N_i/N)$ Where N_i = number of individuals of species i, and N = total number of individuals in a sample. The higher the value of H', the more diverse our sample (Hayek and Buzas 1997).
Biotic Index	HD, Hess	Combines two commonly used biotic indices for eastern North America (Hilsenhoff 1987, Lenat 1993). Hilsenhoff's values were used preferentially, but Lenat's were used when Hilsenhoff failed to provide a value (particularly for non-insect taxa). Scores range from 0 to 10, with low scores indicating little pollution tolerance.
Functional Feeding Groups	Hess	Percentage of each of the major functional feeding groups (Scrapers, Filtering-Collectors, Gathering-Collectors, Shredders, and Predators), based on presence/absence of taxa (Merritt and Cummins 1995).

TABLE 3. List of aquatic macrophyte species found within the study area. Relative proportions of these species are indicated for upstream, downstream, and within the industrial effluent (0 = Absent, 1 = < 20%, 2 = 20-50%, 3 = 50-80%, 4 = >80%).

SPECIES	UPSTREAM	EFFLUENT	DOWNSTREAM
Elodea canadensis	1	0	2
Ludwigia palustris	0	4	1
Potamogeton crispus	1	0	1
Potamogeton filiformis	4	0	1
Potamogeton zosteriformis	1	0	1
Sparganium spp.	0	0	3
Zannichellia palustris	0	0	1
Unidentified Poaceae	0	1	1

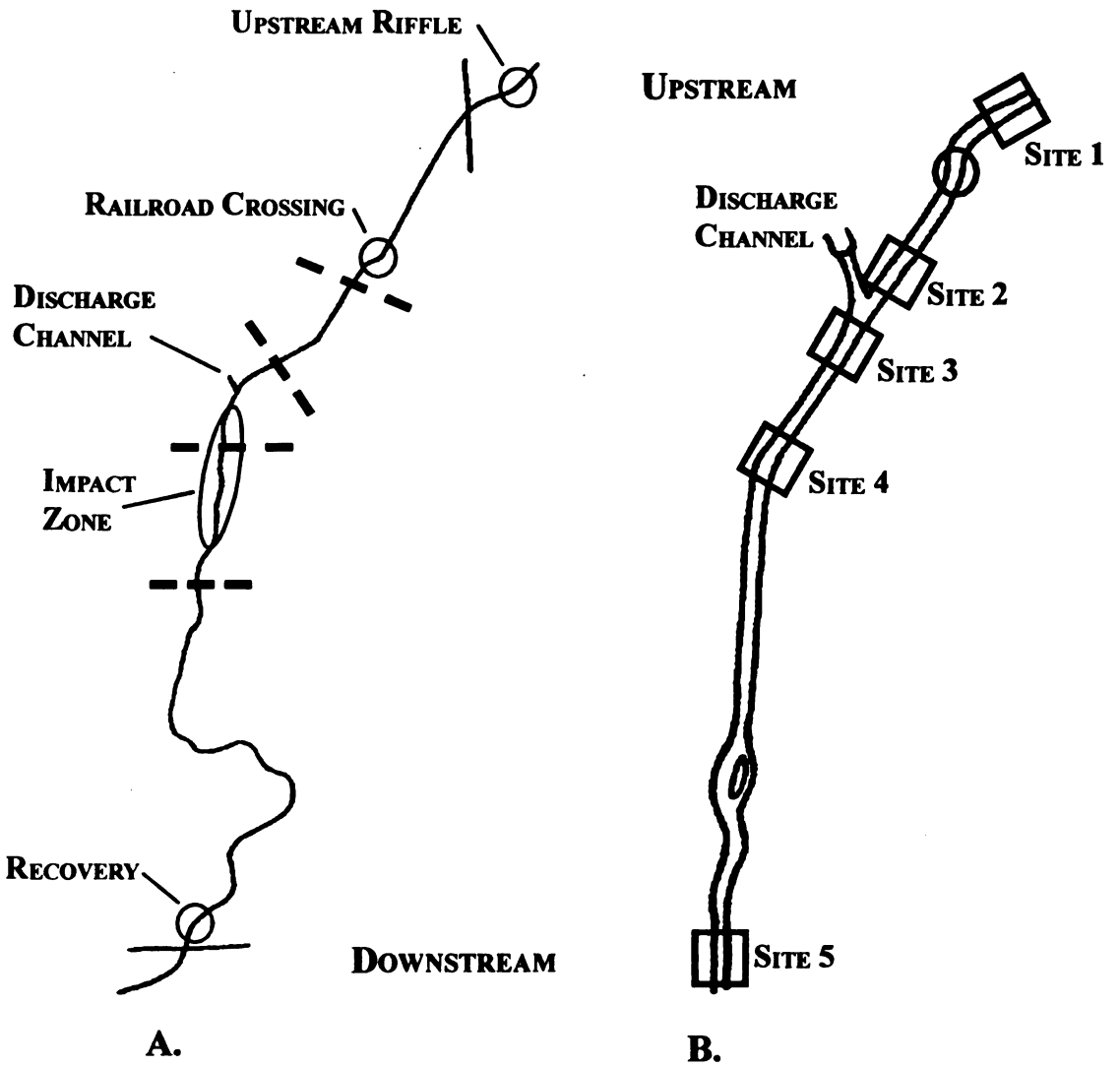


FIGURE 1. Map of Portage Creek. A) Broader view of Portage Creek (Scale: 1 inch = ~ 1000 meters), showing the three riffle "sites" used for Hess samples and ceramic tile colonization. The dashed lines perpendicular to the stream channel are locations for habitat and flow analyses. B). Narrower view of Portage Creek (Scale: 1 inch = ~200 meters), showing the five stream sections where Hester-Dendy artificial substrates were deployed, including the discharge channel itself. The circle in Figure 1.B indicates the location where mussel shells were found following excavation by the storm.

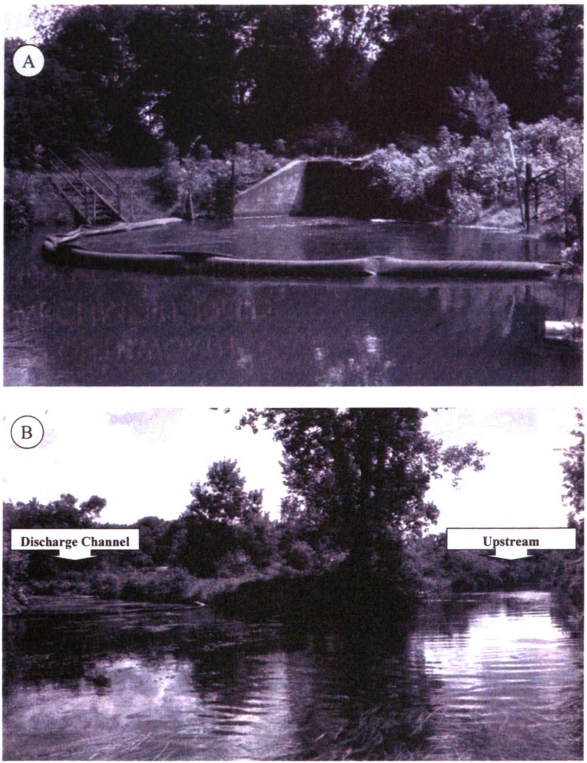


Figure 2. A) Pharmacia & Upjohn's industrial outfall. B) Confluence of the discharge channel with Portage Creek.

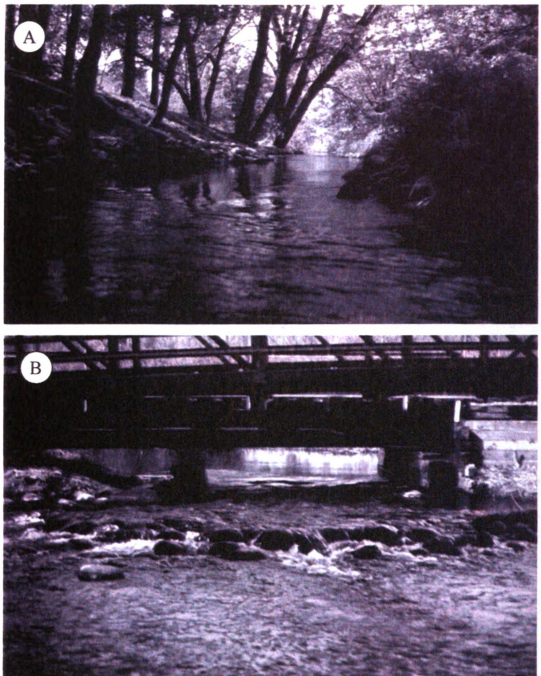


Figure 3. A) Trout habitat restoration site; one upstream site for Hess sampling and ceramic tile colonization. B) Short riffle section created by the railroad bridge construction; upstream site for Hess sampling, collection site for mayflies, and test location for mayfly growth experiment.

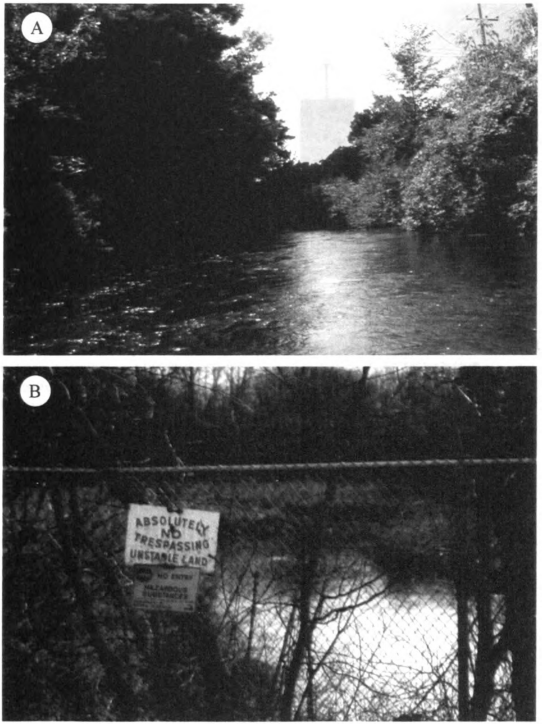


Figure 4. A) Riffle located just upstream of the Kilgore Road bridge; downstream site for Hess sampling and ceramic tile colonization. B) Portage Creek in the lower reaches of its watershed, about 5 miles downstream of the study area. Two crumbling paper mills in this area have left the soils and water contaminated.

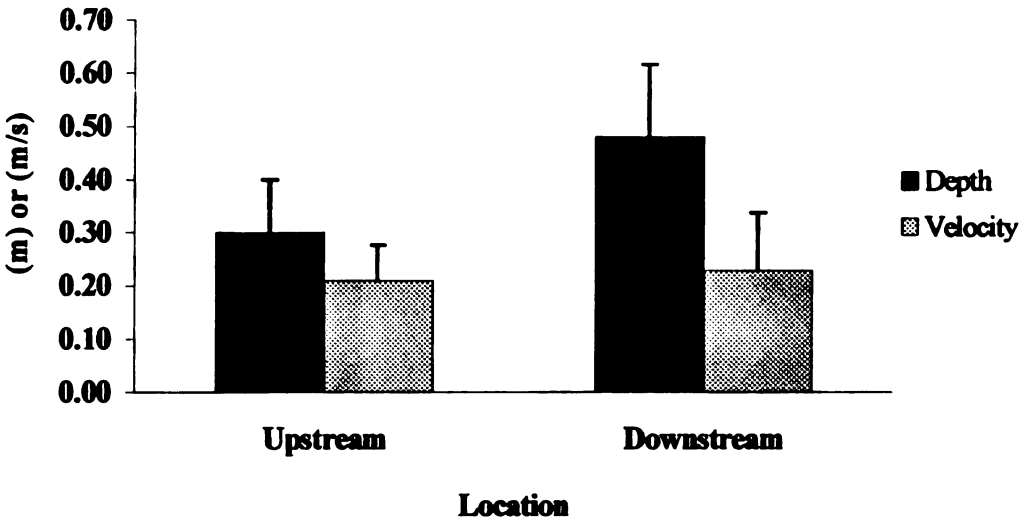


FIGURE 5. Mean depth (m), and mean velocity (m/s) upstream and downstream of the industrial effluent (+ Standard Errors). Values are taken from microhabitat measurements of the Hester-Dendy artificial substrates (See Figure 1 for sampling locations).

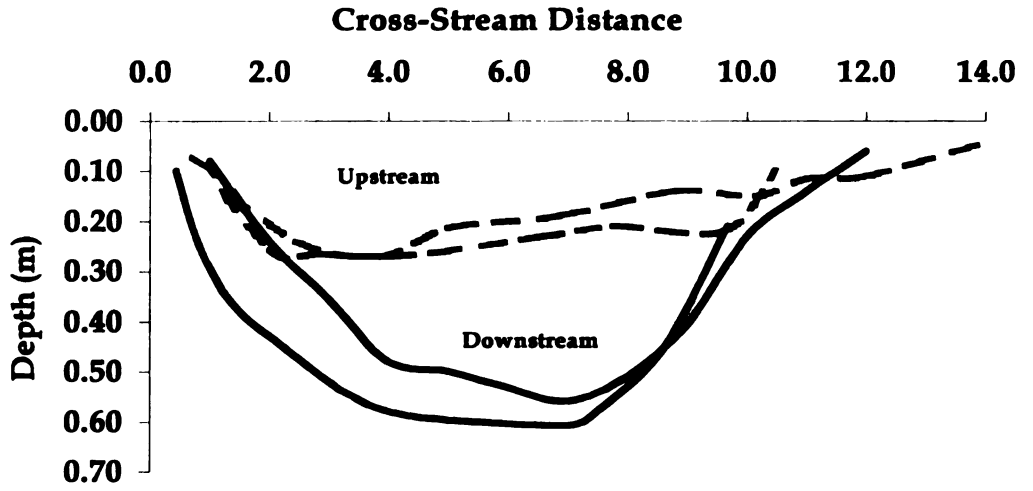


FIGURE 6. Two representative depth profiles from upstream and downstream of the industrial effluent. The X-axis is inverted to illustrate the profile as a cross-section of the stream at each transect. See Figure 1 for transect locations.

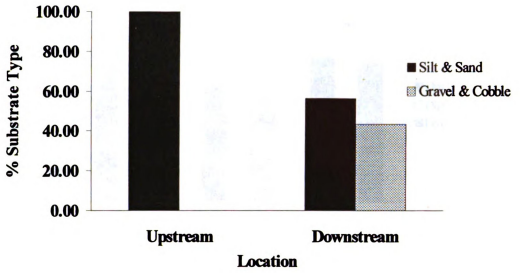


FIGURE 7. Percentage of substrate types upstream and downstream of the industrial effluent. Values are taken from microhabitat measurements of Hester-Dendy artificial substrates. See Figure 1 for sampling locations.

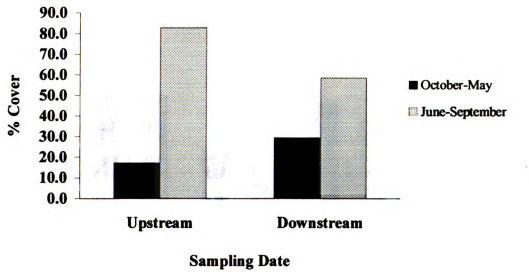


FIGURE 8. Percentage of instream-cover upstream and downstream of the industrial effluent during the growing season (June-September) and non-growing season (October-May). Values are taken from microhabitat measurements of Hester-Dendy artificial substrates. See Figure 1 for sampling locations. A list of aquatic macrophytes is presented in Table 3.

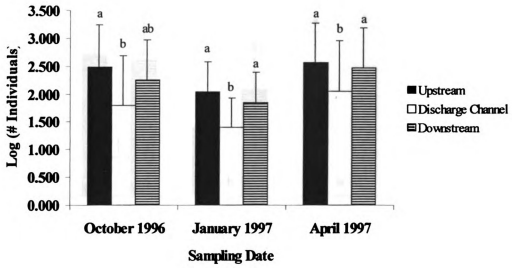


FIGURE 9. Log (# Individuals) colonizing Hester-Dendy artificial substrates at each sampling unit over the three colonization periods (+ Standard Errors). N = 11-14 (Upstream), 4-7 (Discharge Channel), 10-12 (Downstream). Overall ANOVA p-values: October 0.0429, January 0.0001, April 0.0029. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.

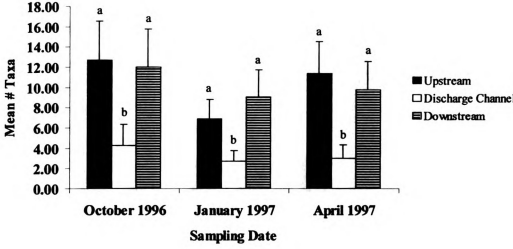


FIGURE 10. Mean # Taxa colonizing Hester-Dendy artificial substrates at each sampling unit over the three colonization periods (+ Standard Errors). N = 11-14 (Upstream), 4-7 (Discharge Channel), 10-12 (Downstream). Overall ANOVA p-values: October 0.0019, January 0.0001, April 0.0001. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.

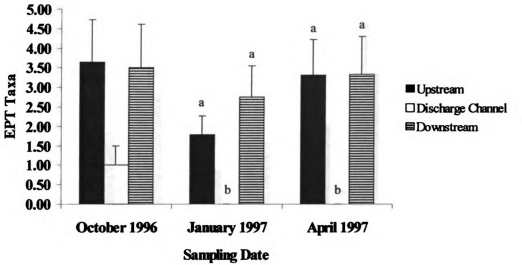


FIGURE 11. Mean # EPT Taxa colonizing Hester-Dendy artificial substrates at each sampling unit over the three colonization periods (+ Standard Errors). N = 11-14 (Upstream), 4-7 (Discharge Channel), 10-12 (Downstream). Overall ANOVA p-values: October 0.1070, January 0.0011, April 0.0002. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.

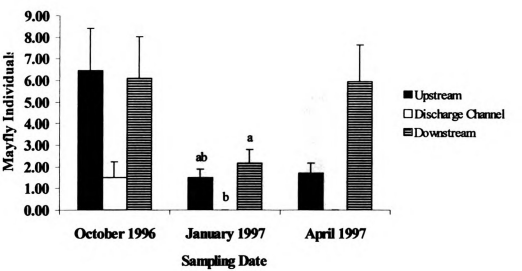


FIGURE 12. Mean # Ephemeroptera individuals colonizing Hester-Dendy artificial substrates at each sampling unit over the three colonization periods (+ Standard Errors). N = 11-14 (Upstream), 4-7 (Discharge Channel), 10-12 (Downstream). Overall ANOVA p-values: October 0.1791, January 0.0372, April 0.0333. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.

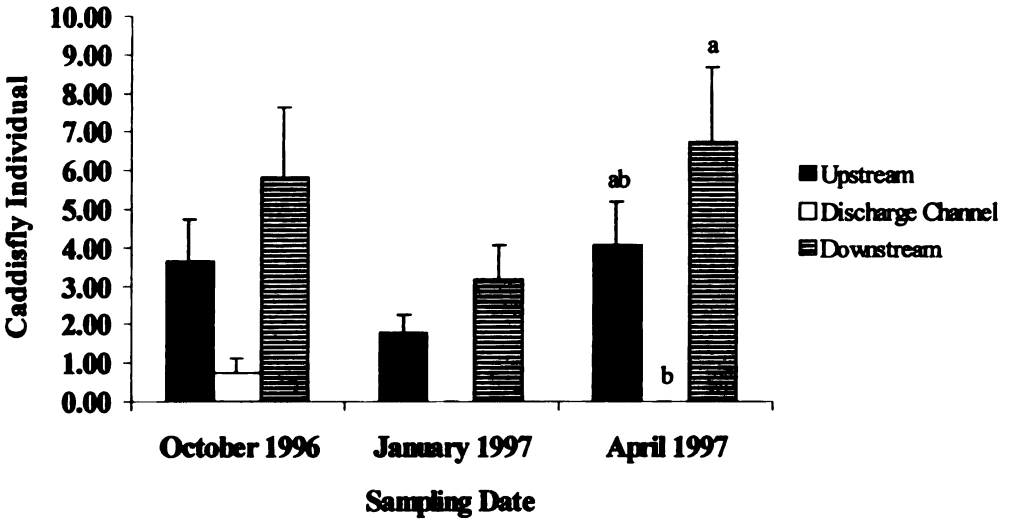


FIGURE 13. Mean # Trichoptera individuals colonizing Hester-Dendy artificial substrates at each sampling unit over the three colonization periods (+ Standard Errors). N = 11-14 (Upstream), 4-7 (Discharge Channel), 10-12 (Downstream). Overall ANOVA p-values: October 0.4311, January 0.1487, April 0.0429. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.

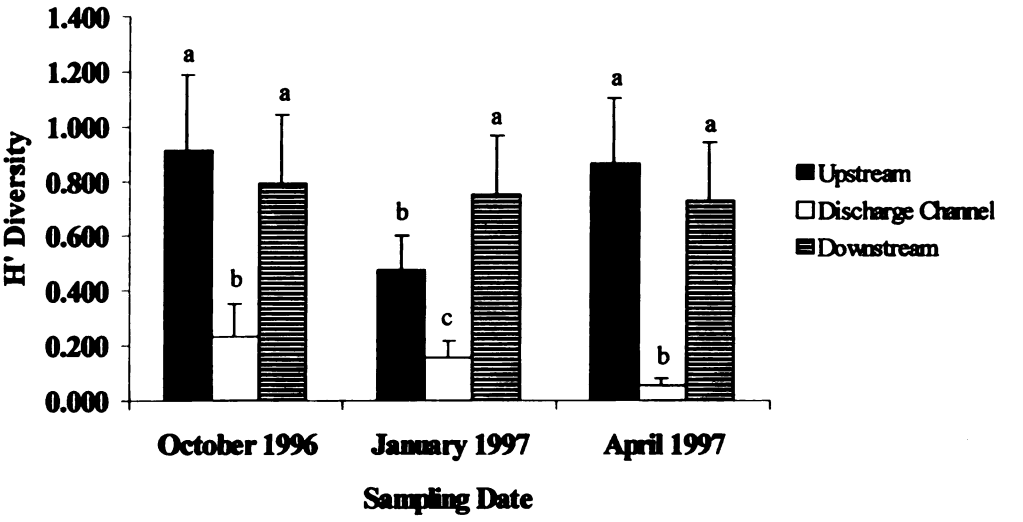


FIGURE 14. H' diversity of invertebrates colonizing Hester-Dendy artificial substrates at each sampling unit over the three colonization periods (+ Standard Errors). Oligochaetes and chironomids were excluded when calculating H'. N = 11-14 (Upstream), 4-7 (Discharge Channel), 10-12 (Downstream). Overall ANOVA p-values: October 0.0001, January 0.0001, April 0.0001. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.

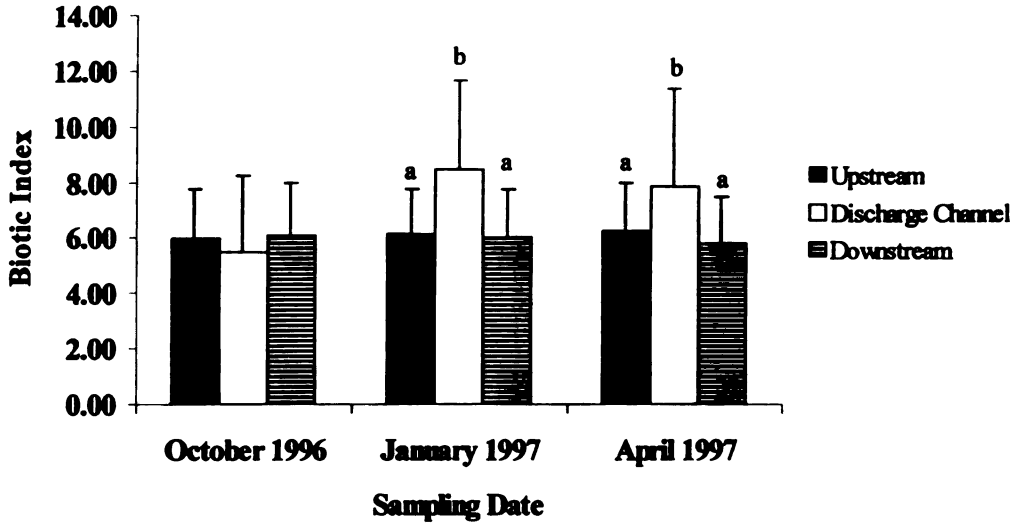


FIGURE 15. Biotic index of invertebrates colonizing Hester-Dendy artificial substrates at each sampling unit over the three colonization periods (+ Standard Errors). Oligochaetes and chironomids were excluded when calculating the biotic index. N = 11-14 (Upstream), 4-7 (Discharge Channel), 10-12 (Downstream). Overall ANOVA p-values: October 0.3632, January 0.0001, April 0.0054. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.

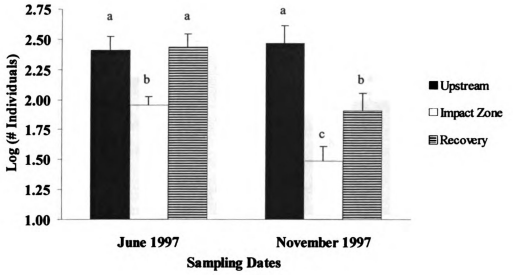


FIGURE 16. Log (# Individuals) collected with the modified-Hess sampler at riffle sites upstream and downstream of the industrial effluent (+ Standard Errors), exclusive of the chironomids and oligochaetes. N = 5 per site. Overall ANOVA p-values: June 0.0079, November 0.0010. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.

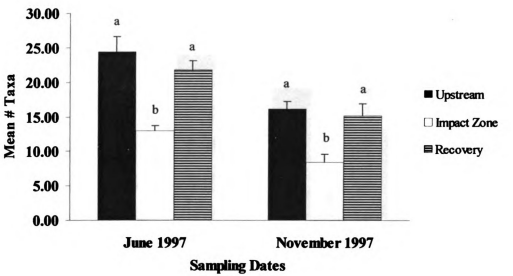


FIGURE 17. Mean # Taxa collected with the modified-Hess sampler at riffle sites upstream and downstream of the industrial effluent (+ Standard Errors) N = 5. Overall ANOVA p-values: June 0.0006, November 0.0029. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.

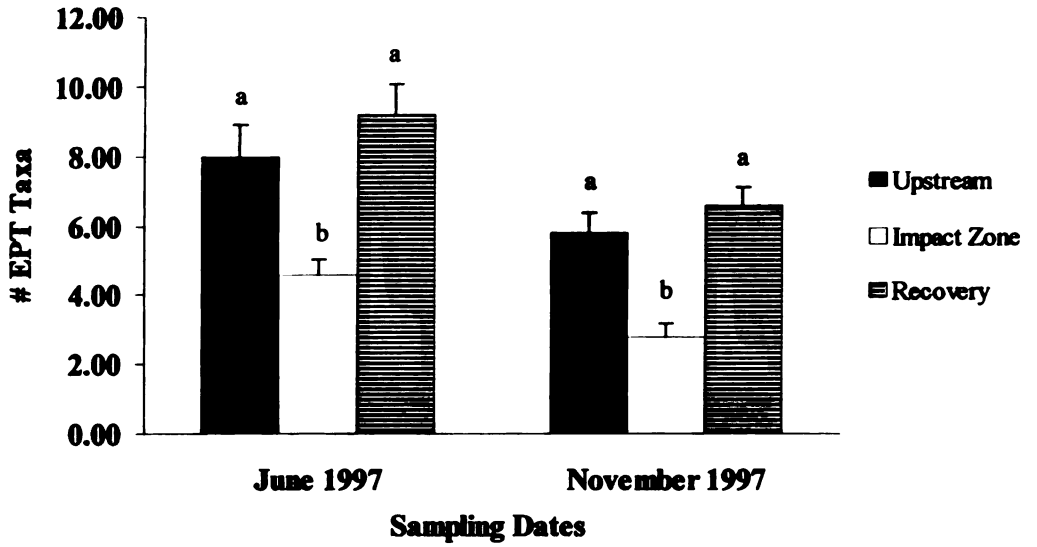


FIGURE 18. Mean # EPT Taxa collected with the modified-Hess sampler at riffle sites upstream and downstream of the industrial effluent (+ Standard Errors). N = 5. Overall ANOVA p-values: June 0.0027, November 0.0004. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.

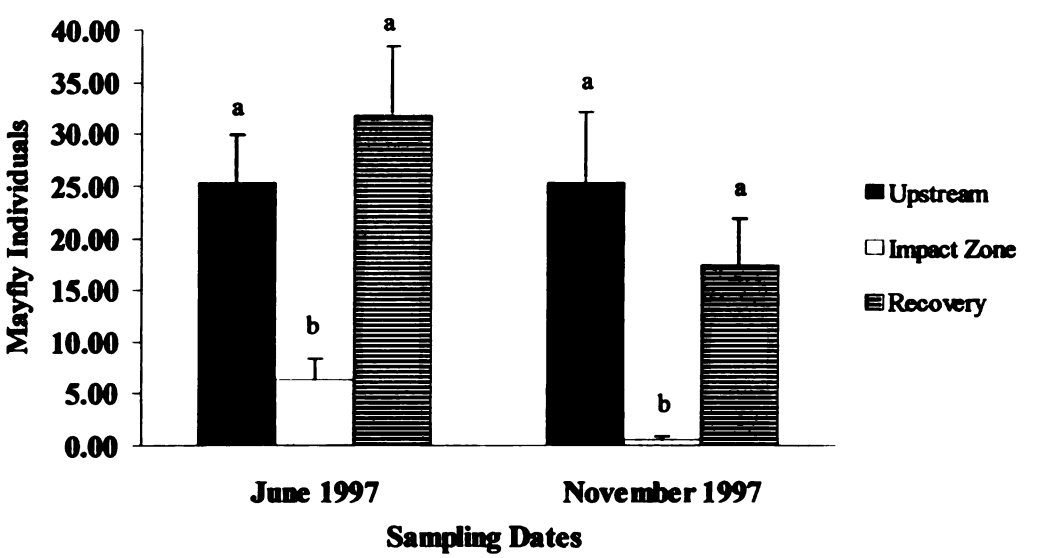


FIGURE 19. Mean # Ephemeroptera individuals collected with the modified-Hess sampler at riffle sites upstream and downstream of the industrial effluent (+ Standard Errors). N = 5. Overall ANOVA p-values: June 0.0077, November 0.0093. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.

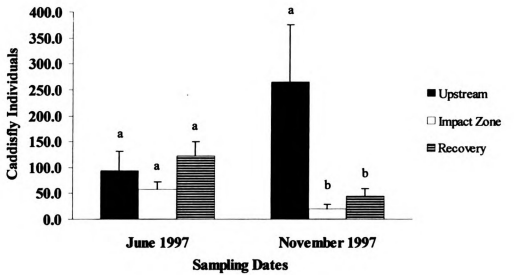


FIGURE 20. Mean # Trichoptera individuals collected with the modified-Hess sampler at riffle sites upstream and downstream of the industrial effluent (+ Standard Errors). N = 5. Overall ANOVA p-values: June 0.2907, November 0.374. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.

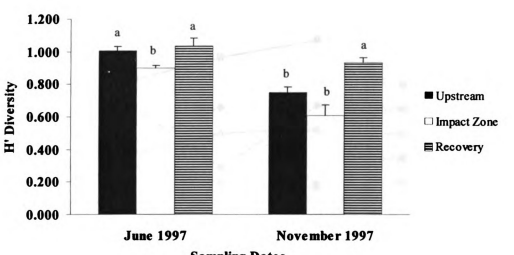


FIGURE 21. H' Diversity of samples collected with the modified-Hess sampler at riffle sites upstream and downstream of the industrial effluent (+ Standard Errors), exclusive of chironomidae and oligochaeta. N = 5. Overall ANOVA p-values: June 0.0332, November 0.0012. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.

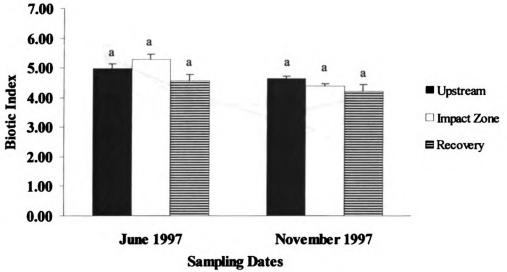


FIGURE 22. Biotic index calculated for samples collected with the modified-Hess sampler at riffle sites upstream and downstream of the industrial effluent (+ Standard Errors), exclusive of oligochaeta and chironomidae. N = 5. Overall ANOVA p-values: June 0.0539, November 0.1494. Sites marked with the same letter are not significantly different (Tukeys LSD Method, $p > 0.05$). See Figure 1 for sampling locations.

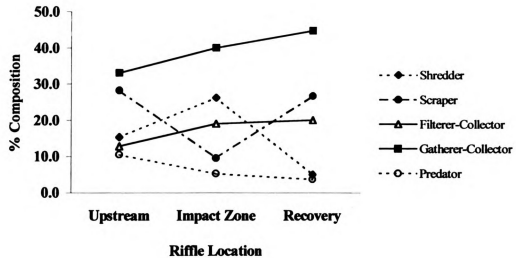


FIGURE 23. Functional feeding group composition of samples collected with the modified Hess sampler at riffle sites upstream and downstream of the industrial effluent. Figures are based on presence/absence of taxa. See Figure 1 for sampling locations.

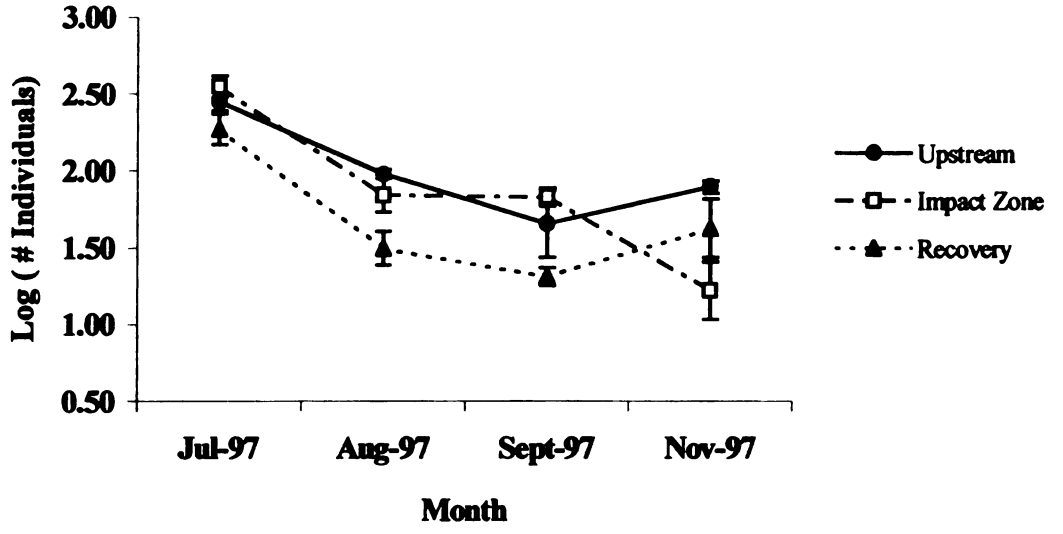


FIGURE 24. Log (# Individuals) colonizing ceramic tiles at the three riffle sites over the four colonization periods (± Standard Errors). N = 4–5. Overall ANOVA p-values: July 0.0717, August 0.0229, September 0.0332, November 0.1765. See Figure 1 for sampling locations.

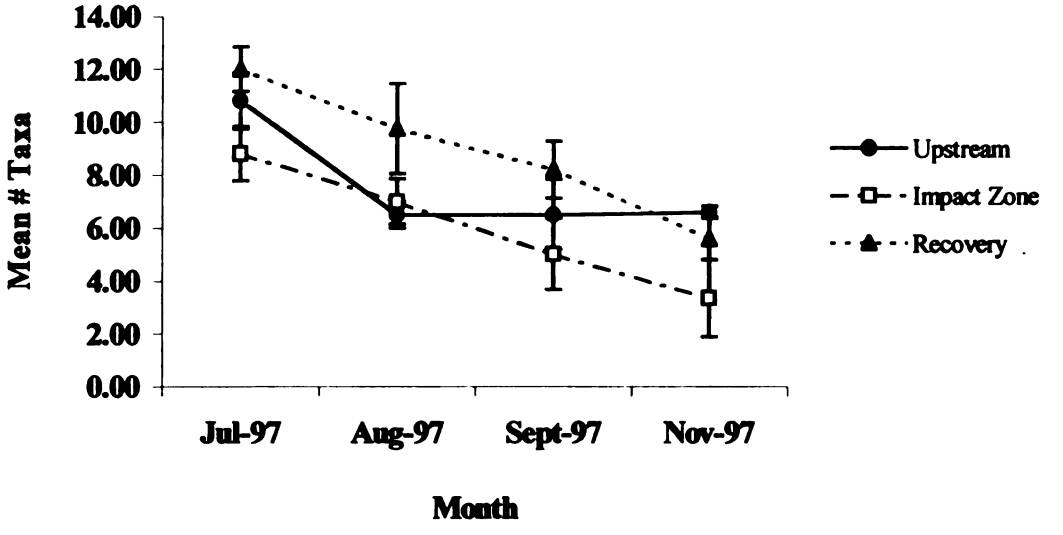


FIGURE 25. Mean # Taxa colonizing ceramic tiles at the three riffle sites over the four colonization periods (± Standard Errors). N = 4–5. Overall ANOVA p-values: July 0.1060, August 0.1392, September 0.2238, November 0.0618. See Figure 1 for sampling locations.

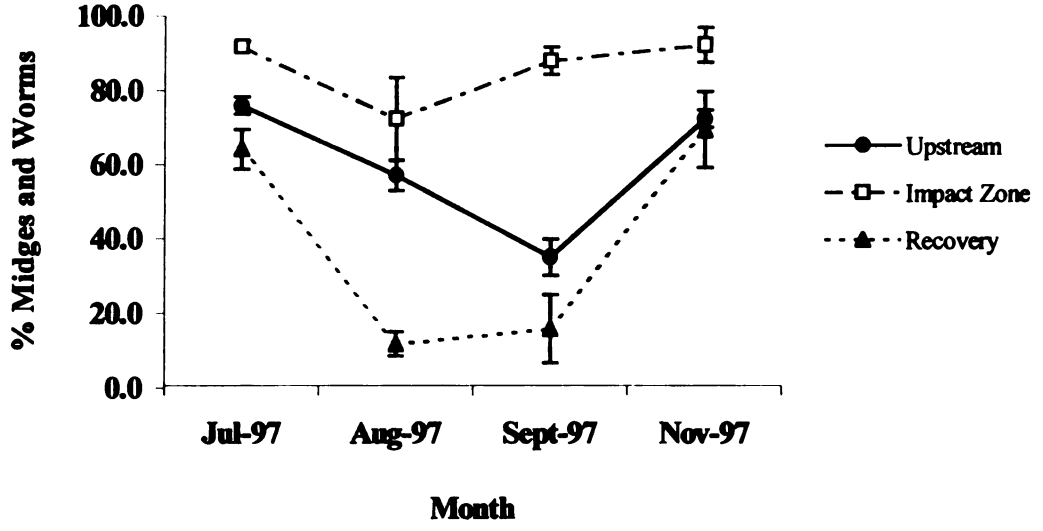


FIGURE 26. % Midges and Worms (Oligochaeta)) colonizing ceramic tiles at the three riffle sites over the four colonization periods (± Standard Errors). N = 4–5. Overall ANOVA p-values: July 0.0004, August 0.0009, September 0.0001, November 0.1502. See Figure 1 for sampling locations.

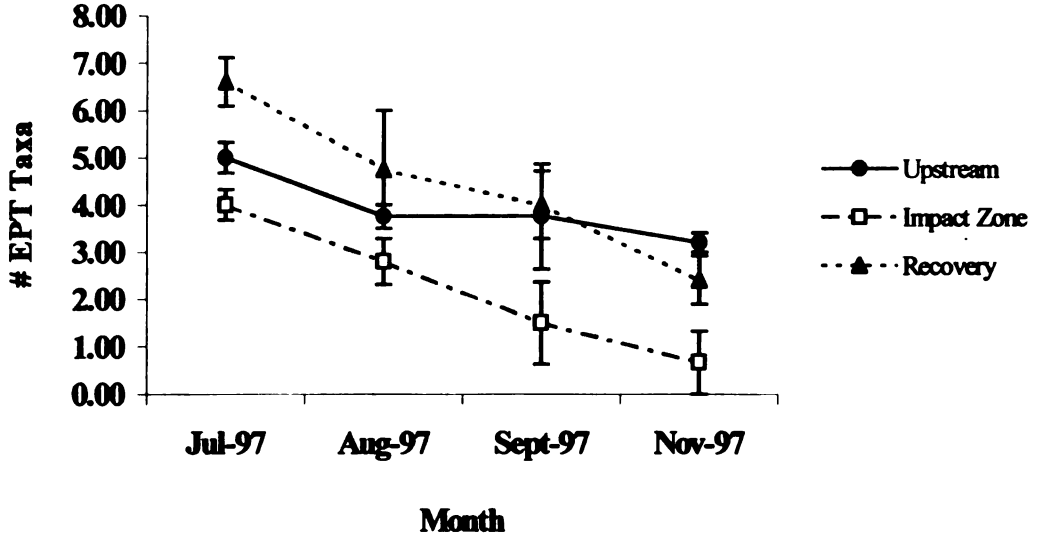


FIGURE 27. Mean # EPT Taxa colonizing ceramic tiles at the three riffle sites over the four colonization periods (± Standard Errors). N = 4–5. Overall ANOVA p-values: July 0.0688, August 0.2242, September 0.1445, November 0.0127. See Figure 1 for sampling locations.

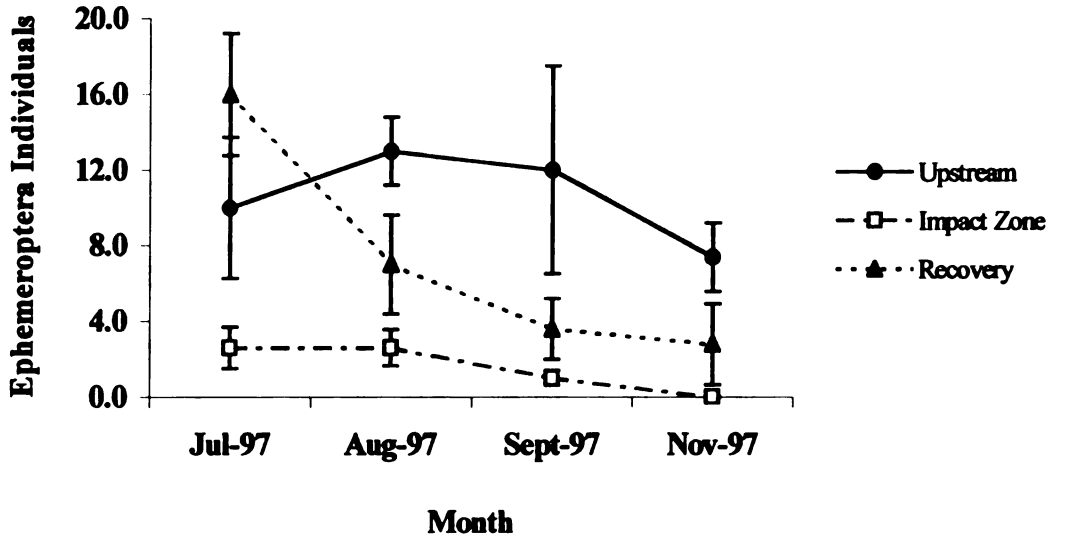


FIGURE 28. Mean # Ephemeroptera colonizing ceramic tiles at the three riffle sites over the four colonization periods (± Standard Errors). N = 4–5. Overall ANOVA p-values: July 0.0223, August 0.0066, September 0.0838, November 0.0657. Figure 1 for sampling locations.

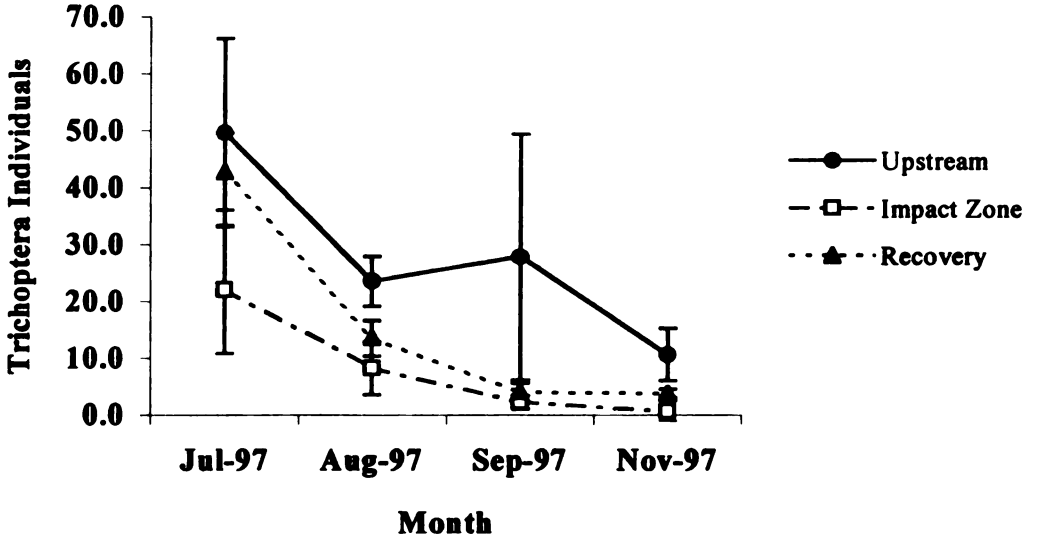


FIGURE 29. Mean # Trichoptera colonizing ceramic tiles at the three riffle sites over the four colonization periods (± Standard Errors). N = 4–5. Overall ANOVA p-values: July 0.2862, August 0.0581, September 0.2743, November 0.1666. Figure 1 for sampling locations.

CHAPTER TWO
GROWTH AND SURVIVAL OF *STENACRON INTERPUNCTATUM* (EPHEMEROPTERA: HEPTAGENIIDAE) EXPOSED TO AN INDUSTRIAL EFFLUENT

INTRODUCTION

A critical component of water resource management is the prediction or detection of adverse effects of stressors on aquatic ecosystems (Rosenberg and Resh 1993, Davis and Simon 1995). Historically, much of the focus of water resource assessment and management programs was on establishing water quality standards for physico-chemical variables, and controlling point-sources of pollution (Karr 1991, Yoder 1995). Considerable emphasis was placed on the development of acute and chronic laboratory bioassays for a suite of pollutants (Malins 1989, Maltby and Calow 1989). The ultimate goal of these laboratory bioassays was to predict the impacts of pollutants on entire ecosystems. Despite strict regulation of point-source pollution by state and federal regulatory agencies in the past few decades, there is still potential for these to compromise the biological integrity of aquatic ecosystems. This is due to the fact that laboratory bioassays will rarely provide an accurate and realistic prediction or assessment of the effects of a point-source of pollution on ecosystem quality (Cairns 1983, Cairns and Pratt 1989, Maltby and Calow 1989). This is because they often fail to address potential synergistic interactions of the toxicant with physical, chemical, or biological modifying factors in the environment (Sprague 1995). Bioassays conducted within natural systems (*in situ*) have become popular in the last 15 years (Winger et al. 1984, Munawar and Munawar 1987, Chappie and Burton 1997). Test conditions are not necessarily meant to emulate the natural system, but are meant to enclose the organisms

within a defined space where they can be exposed to some of the dynamic environmental variables that would often go unaccounted for in laboratory studies. *In situ* bioassays are particularly well suited for determining the impact of point-source pollutants on representative aquatic species (Muliss et al. 1996), and ideally community structure (Cairns and Pratt 1989).

An example of a point-source pollutant that may have negative impacts on aquatic ecosystems yet is largely unregulated is cooling water effluents. We studied the effect of an industrial cooling-water effluent on benthic macroinvertebrate community structure. The effluent is thermally constant, ranging from 17 to 25 °C throughout the year. In the winter months the effluent raises the stream temperature 8 to 12°C above ambient. The effluent contains high levels of ferrous iron, which is oxidized to ferric iron and then hydrolyzed to ferric hydroxide, which forms a yellow-brown precipitate ("yellowboy") that settles out on the substratum. It is known to inhibit the growth of benthic algae (especially diatoms and green algae) (Sheldon and Skelly 1990, Wellnitz and Sheldon 1995), depress macroinvertebrate diversity by interfering with feeding and respiration (Koryak et al. 1972, Rasmussen and Lindegaard 1988, Wellnitz et al. 1994), and has consequences for higher trophic levels such as fish (Letterman and Mitsch 1978).

Neither the thermal nature of the effluent nor the levels of iron exceed water quality standards. However, there could be an additive effect of temperature and ferric hydroxide in the effluent, or there may be some other factor that acts in synergy with these factors to influence the biota. Mayflies (Ephemeroptera) were conspicuously scarce or absent in riffles immediately downstream of the industrial effluent (Chapter One), though were abundant at sites further upstream and much farther downstream. Their

scarcity caused particular concern about the effect that the industrial effluent was having on the biological integrity of Portage Creek. We conducted *in situ* bioassays to try to assess the specific effect of the effluent on the mayfly *Stenacron interpunctatum* (Family Heptageniidae). Our specific objectives were:

(1) Compare mayfly growth and mortality within and downstream of the industrial effluent versus and upstream control (Experiment 1),

(2) Try to separate the effects of temperature and food quality on mayfly growth and mortality (Experiment 2).

METHODS

STUDY SITE: Portage Creek is a third-order tributary of the Kalamazoo River in Kalamazoo County, Michigan (Figure 1). Its 52-square kilometer drainage area is a glacial outwash plain, with up to 350 feet of sand and gravel overlying a shale bedrock valley. It originates from seeps and wetlands, and travels north for about 15 kilometers to its confluence with the Kalamazoo River in the city of Kalamazoo. Land use in the watershed in primarily agricultural, urban, and industrial. The industrial effluent that is the focus of this study is located in the city of Portage (Figure 3A, 3B). The effluent is non-contact cooling water utilized by a large pharmaceutical company. Though not accessible to the general public, people can rent canoes upstream of the industrial effluent and canoe past it. There was concern over the yellow-brown precipitate that covered the substratum within and downstream of the discharge channel, and a more general concern that such a conspicuous source of pollution was detracting from the aesthetic beauty of

the creek. This prompted the Michigan Department of Environmental Quality to initiate an investigation into the effects of this effluent on Portage Creek.

CHOICE OF TEST ORGANISM: Results from comparative field sampling (Chapter One) show that mayflies are scarce or absent within and immediately downstream (~1000 meters) of the industrial effluent. The scarcity of the mayfly *Stenacron interpunctatum* is particularly noticeable because it is abundant at riffles upstream and well downstream (>1500 meters) of the industrial effluent. Late-instars are readily distinguished from similar mayflies in the genus *Stenonema* by their pointed gills and gray/black coloration pattern. Behavioral studies indicate that it is an opportunistic collector-gatherer (McShaffrey and McCafferty 1986). It obtains most of its nutrition by brushing loosely adhered material from the surfaces of stones. *S. interpunctatum* was chosen because of its abundance in Portage Creek, its apparent sensitivity to the physico-chemical environment immediately downstream of the industrial effluent (based on its scarcity/absence), its nutritional requirements, and ease of handling and identification.

EXPERIMENT ONE: The first experiment was designed to determine if *S. interpunctatum* was capable of surviving and growing within and downstream of the discharge channel, compared to an upstream control. The experimental design is illustrated in Figure 3. Twelve growth chambers were partially filled with gravel (1-3 cm diameter) from the mayfly collection site, along with eight 4 cm^2 ceramic tiles previously colonized with periphyton in Augusta Creek (Kalamazoo County, Michigan). Organisms were collected at the Livery Canoe Launch located about 1500 meters upstream of the discharge

channel, where a short riffle section supports abundant mayflies (Figure 1). Ten mayflies were placed into each of the growth chambers; twenty individuals were set aside to determine initial size and weight.

Four growth chambers were placed into each of three acclimation chambers (50-gallon buckets); two were transported to the downstream test sites, and the third remained at the collection (control) site (Figure 2). The acclimation chambers were placed at their respective test sites until the water temperature equilibrated with the stream temperature, which took about 3-4 hours. Two growth chambers were then placed into each of two cages suspended in the stream, for a total of 4 growth chambers (40 individual mayflies) per site. The growth chambers were left in the stream for six days (May 16 – May 22 1997). Daily measurements of temperature (min/max thermometer) and flow velocity (Marsh-McBirney flowmeter) were taken. At the conclusion of the experiment, all mayflies were killed, measured (total length, head capsule width), and weighed (dry weight).

EXPERIMENT TWO: The second experiment was designed to examine the growth and survival of *S. interpunctatum* provided with different food types at different site locations. This was a 2-way factorial, with two levels of the location treatment (upstream, discharge channel), and two levels of the food treatment (good quality food, poor quality food). The experimental design is illustrated in Figure 3. The good quality food consisted of gravel (1 to 3 cm diameter) and 2.5 cm^2 ceramic tiles that had been placed in a riffle upstream of the industrial effluent, and was coated with a biofilm high in diatoms, filamentous green algae, and FPOM. The poor quality food consisted of

gravel (1 to 3 cm. Diameter) and 2.5 cm^2 ceramic tiles that had been placed in the discharge channel, and was coated with a thick gelatinous matrix of ferric hydroxide, a blue-green bacterium, and perhaps other inorganic substances.

Each of the 12 growth chambers used in Experiment One were divided into two chambers, and each half was partially filled with either good quality food or poor quality food. Due to an unusually warm spring, the first cohort of *S. interpunctatum* had already emerged from upstream sections of Portage Creek. Thus, test animals were collected at a riffle section about 3 kilometers downstream of the industrial effluent (Kilgore Road) (Figure 1). These individuals probably represented the second cohort of the summer, since this site is strongly influenced by the thermal nature of the industrial effluent, and growth/development of mayflies is accelerated at these sites. Eight animals were placed into each half of the growth chamber (food treatment), for a total of 16 animals per chamber. No measures were taken to acclimate the animals to the temperature at their respective test sites because the temperature differences were small (~2-4 °C). Two growth chambers were placed into each of three cages suspended in the stream, for a total of 6 growth chambers, and 6 replicates per food treatment. The growth chambers were left in the stream for five days (May 18 – May 23 1998). Daily measurements of temperature (min/max thermometer) were taken. At the conclusion of the experiment, all mayflies were killed, and weighed (dry weight).

RESPONSE VARIABLES: In the first experiment, total length (mm), head capsule width (mm), and dry weight (grams) were determined for each of the test animals. All three were very well correlated with each other, so in the second experiment only dry weights

(grams) were determined. For uniformity, only dry weights were used in the statistical analysis to determine a growth response. Growth was defined as:

Growth = Final Dry Weight – Initial Dry Weight

For survival determination, all missing animals were presumed dead. Individuals that had emerged within the growth chambers were considered survivors, but were not included in the growth analysis.

For Experiment 1, I used a one-factor ANOVA to test the effect of site on mayfly growth and survival. For Experiment 2, I used a two-factor ANOVA to test the effect of site, food quality, and the interaction on mayfly growth and survival. I used the SAS© statistical software program for the analyses. A test was considered significant of the p-value ≤ 0.05. Further statistical analysis, with a more accurate portrayal of randomization, blocking, and treatment effects were only considered if the overall ANOVA was significant.

RESULTS

EXPERIMENT ONE: The temperature and flow data are presented in Table 1. Water temperatures were significantly warmer in the discharge channel and the downstream test site, as shown by degree-day accumulations. Therefore, growth was corrected for degree- day accumulations (Mean Growth / ° Day). Summary data for the growth/survival response is presented in Table 2, and presented graphically in Figures 4-6.

There were no statistically significant results from Experiment 1 (where significance is judged as a p-value less than 0.10). *S. interpunctatum* exhibited the

greatest absolute growth in the discharge channel (0.00219 grams), and the least growth at the control site (0.00113 grams) (Figure 4), though the overall ANOVA for mean growth was not significant (p-value 0.5918). *S. interpunctatum* exhibited the greatest growth at the downstream site when corrected for degree-day accumulation, and grew the least at the upstream control site (Figure 5), though the overall ANOVA for mean growth corrected for temperature is not significant (p-value 0.8677). There was no trend in survival between the three sites, and the overall ANOVA p-value was 0.6019 (Figure 6).

EXPERIMENT TWO: The temperature data is presented in Table 1. Flow velocity was not measured in Experiment 2, but was similar between the two sites. Summary data for the growth/survival response is presented in Table 3. Figure 7 shows the interaction of food and location on mayfly growth. Results from the two-factor ANOVA indicate few statistically significant results from Experiment 2 (Table 3) (where significance is judged as a p-value less than 0.10). Food quality appears to have the most obvious effect on growth (p-value 0.1332), and growth normalized for degree days (p-value 0.0863). Location, and the location x food interaction appear to have little effect on growth. There was no difference in survival between the different treatments.

DISCUSSION

Stenacron interpunctatum grew faster within and downstream of the discharge channel than upstream, and exhibited no survival response to the effluent. The higher temperatures are thought to have caused the slightly positive growth response. Temperature is perhaps the most critical factor in the life cycle of most aquatic insects

(Vannote and Sweeney 1980, Ward and Stanford 1982, Sweeney 1984). Many studies have shown that warmer temperatures generally accelerate the life cycles of aquatic insects by decreasing egg hatching time (Newell and Minshall 1978, Humpesch and Elliot 1980), and accelerating larval growth and emergence (Humpesch 1981, Sweeney and Vannote 1984, 1986). Development continues to increase with temperature until a thermal threshold is reached (Sweeney 1984), and though this is species dependent, aquatic insects can generally tolerate temperatures of up to 30-35 °C. The maximum recorded temperature during the growth/survival experiments was 25 °C, which is likely still within the optimal growth range for *Stenacron interpunctatum*. Flowers and Hilsenhoff (1978) report that this species is often abundant in slower currents of small eutrophic streams of southern Wisconsin, and has an unusually high tolerance for silty environments. McCafferty and Huff (1978) describe a complex life cycle for this species in Indiana, with emergence periods in the late spring, summer, and early fall. These life history studies suggest that *S. interpunctatum* has a fairly high tolerance for warmer temperatures.

Food quantity and quality are also critical factors in the life cycle of aquatic invertebrates (Cummins and Klug 1979, Anderson and Cummins 1979, Sweeney 1984). Food quantity and quality have been shown to affect larval development time, size at maturity, and fecundity of aquatic insects (Colbo and Porter 1979, Collins 1980, Webb and Merritt 1987). In this study, *S. interpunctatum* grew poorly when provided with a low quality food source (a gelatinous ferric hydroxide/bacterial biofilm that developed within the discharge channel), suggesting that food quality rather than temperature or water quality are inhibiting some pollution-intolerant taxa from inhabiting riffles within

and immediately downstream of the effluent. Other studies have demonstrated a general reduction in macroinvertebrate community composition in areas with high dissolved iron and ferric hydroxide (Koryak et al. 1972, Greenfield and Ireland 1978, Letterman and Mitsch 1978, Rasmussen and Lindegaard 1988, Wellnitz et al. 1994), and have often attributed this to a reduction in the quantity and quality of food, destabilization of substrate by flocculent iron, or direct toxic effects of Fe ions and/or the closely associated iron-depositing sheathed bacteria *Leptothrix spp.*. Wellnitz et al. (1994) looked at substrate choice, weight gain, and survival of selected aquatic insects on ferric hydroxide/*Leptothrix ochracea* substrates. Two of the three heptageniid mayflies (*Epeorus, Heptagenia)* preferred substrates free of the iron, and *Stenonema* showed no preference. All three genera showed a greater mortality within the bloom of iron-depositing bacteria, and though all three ingested the iron bacteria, only *Heptagenia* gained weight after 10 days. They found that *Leptothrix ochracea* encrusted the tracheal gills of these mayflies, and appeared to hamper gill motion.

The quality and quantity of food resources is temperature-dependent. For instance, temperature can influence the quantity of periphytic algae (McIntire and Phinney 1965), or the microbiota that colonize detrital material and enhance its nutritional quality (Webster and Benfield 1986). So determining the relative importance of temperature and food resources on aquatic invertebrate population and community ecology is problematic because temperature affects both the quantity and quality of food, *and* the ingestion and assimilation of the food by consumers (Sweeney 1984). In this study there are several possible combined effects of temperature and ferric hydroxide precipitation on macroinvertebrates. Elevated temperature will increase respiratory

demand, and combined with gill clogging or abrasion by particulate iron could have a more detrimental effect than either one alone. Elevated temperature will increase metabolic demand, and combined with poor food quality due to particulate iron in suspension or on the stream substrate could also have a greater effect than either one alone. In addition, temperature and iron could act in synergy with other environmental variables. Thermal stress can be additive with toxic stress, especially when it is accompanied by low oxygen (Sprague 1995). Thermal stress accompanied by changes in pH or dissolved oxygen can also have additive effects. Oxygen and pH can also act in synergy with the toxic stress of dissolved iron, suspended iron (gill abrasion), and iron-coated substrates (poor nutrition, gill abrasion). The physiological, biochemical, or behavioral traits of a species will determine its relative sensitivity to a particular stressor (Sparague 1995). Life stage or size of the organism is a particularly important in determining sensitivity. In this experiment, late-instar individuals were used, and these larger, older individuals will generally be less sensitive to the adverse effects of temperature or food quality than early-instar individuals.

These experiments were not designed to address all of the possible synergistic effects of iron and temperature with other environmental variables. The experiments were primarily designed to try to understand the why mayflies, especially *Stenacron interpunctatum*, were scarce or absent in suitable habitats immediately downstream of the industrial effluent. The results are not conclusive. If the only effect of temperature were to accelerate larval development, then *S. interpunctatum* might be expected to be found downstream of the effluent, but perhaps display an asynchronous emergence pattern. However, benthic samples were taken during all times of the year, and mayflies

were never more than scarce. One might expect early instars to be more sensitive to the warmer thermal regime, but this is unlikely given the multivoltine life cycle of *S. interpunctatum,* and the results of previous studies on similar species. Based on Experiment Two, ferric hydroxide precipitation and deposition perhaps better explains the scarcity of mayflies in the discharge channel and riffles immediately downstream. This result is supported by the studies that have examined macroinvertebrate community structure in streams impacted by iron compounds.

Clearly, water quality standards are not protecting aquatic life in Portage Creek. Comparative field sampling of the benthic macroinvertebrate communities show that the riffles immediately downstream of the effluent consistently support fewer number of taxa, and a larger proportion of pollution-tolerant taxa. Yet the industry consistently meets effluent quality standards for all physico-chemical criteria. Further studies in this system with a broader range of taxa, a broader range of life stages, and exposure to a broader range of environmental conditions (seasonal effects) might help to better understand the scarcity of mayflies in riffles downstream of the industrial effluent. This would greatly enhance our understanding of the overall effect of the industrial effluent on the biological integrity of Portage Creek, and allow water resource managers to hone their restoration or remediation efforts.

LITERATURE CITED

Anderson, N.H., and K.W. Cummins. 1979. Influences of diet on the life histories of aquatic insects. Journal of the Fisheries Research Board of Canada 36:335-342.

Cairns, J. Jr., and J.R. Pratt. 1989. The scientific basis of bioassays. Hydrobiologia 188/189:5-20.

Cairns, J.Jr. 1983. Are single species toxicity tests alone adequate for estimating environmental hazard? Hydrobiologia 100:47-57.

Chappie, D.J., and A. Burton Jr. 1997. Optimization of in situ bioassays with *Hyalella azteca* and *Chironomus tentans*. Environmental Toxicology and Chemistry 16:559-564.

Colbo, M.H., and G.N. Porter. 1979. Effects of the food supply on the life history of Simuliidae (Diptera). Canadian Journal of Zoology 57:301-306.

Collins, N.C. 1980. Developmental responses to food limitation as indicators of environmental conditions for *Ephydra cinerea* Jones (Diptera). Ecology 61:650-661.

Cummins, K.W., and M.J. Klug. 1979. Feeding ecology of stream invertebrates. Annual Review of Ecology and Systematics. 10:147-172.

Davis, W.S., and T.P. Simon. 1995. *Biological Assessment and Criteria: Tools for Water Resource Planning and Decision Making*. Lewis Publishers.

Flowers, R.W., and W.L. Hilsenhoff. 1978. Life cycles and habitats of Wisconsin Heptageniidae (Ephemeroptera). Hydrobiologia 60:159-171.

Greenfield, J.P., and M.P. Ireland. 1978. A survey of the macrofauna of a coal-waste polluted Lancashire fluvial system. Environmental Pollution 16:105-122.

Humpesch, U.H. 1981. Effect of temperature on larval growth of *Ecdyonurus dispar* (Ephemeroptera: Heptageniidae) from two English lakes. Freshwater Biology 11:441-457.

Humpesch, U.H., and J.M. Elliot. 1980. Effect of temperature on the hatching time of eggs of three *Rhithrogena* spp. (Ephemeroptera) from Austrian streams and an English stream and river. Journal of Animal Ecology 49:643-661.

Karr, J.R. 1991. Biological integrity: a long-neglected aspect of water resource management. Ecological Applications 1:66-84.

Koryak, M., M.A. Shapiro, and J.L. Sykora. 1972. Riffle zoobenthos in streams receiving acid mine drainage. Water Research 6:1239-1247.

Letterman, R.D., and W.J. Mitsch. 1978. Impact of acid mine drainage on a mountain stream in Pennsylvania. Environmental Pollution 17:53-73.

Malins, D.C. 1989. The use of environmental assays for impact assessment. Hydrobiologia 188/189:87-91.

Maltby, L., and P. Calow. 1989. The application of bioassays in the resolution of environmental problems: past, present and future. Hydrobiologia 188/189:65-76.

McCafferty, W.P., and B.L. Huff Jr. 1978. The life cycle of the mayfly *Stenacron interpunctatum* (Ephemeroptera: Heptageniidae). Great Lakes Entomologist 11:209-216.

McIntire, J.W., and H.K. Phinney. 1965. Laboratory studies of periphyton production and community metabolism in lotic environments. Ecological Monographs 35:237-258.

McShaffrey, D., and W.P. McCafferty. 1986. Feeding behavior of *Stenacron interpunctatum* (Ephemeroptera: Heptageniidae). Journal of the North American Benthological Society 5:200-210.

Muliss, R.M., D.M. Revitt, and R.B.E. Schutes. 1996. A statistical approach for the assessment of the toxic influences on *Gannarus pulex* (Amphipoda) and *Asellus aquaticus* (Isopoda) exposed to urban aquatic discharges. Water Research 5:1237-1243.

Munawar, M., and I.F. Munawar. 1987. Phytoplankton bioassays for evaluating toxicity of in situ sediment contaminants. Hydrobiologia 149:87-105.

Newell, R.L., and G.W. Minshall. 1978. Effect of temperature on the hatching time of *Tricorythodes minutus* (Ephemeroptera: Tricorythidae). Journal of the Kansas Entomological Society 51:504-506.

Rasmussen, K., and C. Lindegaard. 1988. Effects of iron compounds on macroinvertebrate communities in a Danish lowland river system. Water Research 22:1101-1108.

Rosenberg, D.M., and V.H. Resh. 1993. *Freshwater biomonitoring and benthic macroinvertebrates*. Chapman & Hall. 488 pages.

Sheldon, S.P., and D.K. Skelly. 1990. Differential colonization and growth of algae and ferromanganese-depositing bacteria in a mountain stream. Journal of Freshwater Ecology 5:475-485.

Sprague, J.B. 1995. Factors that modify toxicity. Pages 1012-1051 In: *Fundamentals of Aquatic Toxicology* (G.M. Rand, editor). Taylor & Francis.

Sweeney, B.W. 1984. Factors influencing life history patterns of aquatic insects. Pages 56-100 In: *The Ecology of Aquatic Insects* (V.H. Resh and D.M. Rosenberg, editors). Praeger Publishers.

Sweeney, B.W., and R.L. Vannote. 1984. Influence of food quality and temperature on the life history characteristics of the parthenogenetic mayfly, *Cloeon triangulifer*. Freshwater Biology 14:621-630.

Sweeney, B.W., and R.L. Vannote. 1986. Growth and production of a stream stonefly: influences of diet and temperature. Ecology 67:1396-1410.

Vannote, R.L., and B.W. Sweeney. 1980. Geographic analysis of thermal equilibria: a conceptual model for evaluating the effect of natural and modified thermal regimes on aquatic insect communities. The American Naturalist 115:667-695.

Ward, J.V., and J.A. Stanford. 1982. Thermal responses in the evolutionary ecology of aquatic insects. Annual Review of Entomology 27:97-117.

Webb, K.M., and R.W. Merritt. 1987. The influence of diet on the growth of *Stenonema vicarium* (Walker) (Ephemeroptera: Heptageniidae). Hydrobiologia 153:253-259.

Webster, J.R., and E.F. Benfield. 1986. Vascular plant breakdown in freshwater ecosystems. Annual Review of Ecology and Systematics 17:567-594.

Wellnitz, T.A., and S.P. Sheldon. 1995. The effects of iron and manganese on diatom colonization in a Vermont stream. Freshwater Biology 34:465-470.

Wellnitz, T.A., K.A. Grief, and S.P Sheldon. 1994. Response of macroinvertebrates to blooms of iron-depositing bacteria. Hydrobiologia 281:1-17.

Winger, P.V., M.J. Imlay, W.E. McMillan, T.W. Martin, J Takekawa, and W.W. Johnson. 1984. Field and laboratory evaluation of the influence of copper-diquat on apple snails in southern Florida. Environmental Toxicology and Chemistry 3:409-424.

Yoder, C.O. 1995. Policy issues and management applications of biological criteria. Pages 327-343 In: *Biological Assessment and Criteria: Tools for Water Resource Planning and Decision Making* (W.S. Davis and T.P. Simon, editors). Lewis Publishers.

TABLE 1. Temperature and flow data for *in situ* bioassay with the mayfly *Stenacron interpunctatum*. Experiment 1: May 16 – May 22 (6 days), 1997, Experiment 2: May 18-May 23 (5 days), 1998. Degree Days = Σ daily mean temperatures > 0° for duration of experiment. Velocity not measured in Experiment 2.

PARAMETER	UPSTREAM	DISCHARGE	DOWNSTREAM
EXPERIMENT 1			
Flow Velocity (m/s)	0.22	0.22	0.25
Mean Temperature (° C)	12.2	21.3	16.1
Min / Max (° C)	10 / 15	14 / 24	13 / 19
Degree Days (° D)	73.2	127.8	96.6
EXPERIMENT 2			
Mean Temperature (° C)	16.5	19.3	
Min / Max (° C)	(12 / 20)	(15 / 24)	
Degree Days (° D)	82.5	96.5	

TABLE 2. Summary data for the *in situ* bioassay with the mayfly *Stenacron interpunctatum*: Experiment 1. Dates: May 16-May 22 (6 days), 1997. Mean Growth: Δ Dry Weight. The p-values for the overall ANOVA are indicated; tests are considered significant if the p-value < 0.05. See Figures 4-6 for graphical summary, and Table 1 for temperature/flow data.

	STATISTIC		
	MEAN	SD	P-VALUE
MEAN GROWTH (BY SITE)			0.5918
Upstream	1.13 E - 03	8.32 E - 04	
Discharge Channel	2.19 E - 03	1.29 E - 03	
Downstream	2.10 E - 03	2.42 E - 03	
MEAN GROWTH/DEGREE DAY (BY SITE)			0.8677
Upstream	1.55 E - 05	1.14 E - 05	
Discharge Channel	1.71 E - 05	1.00 E - 05	
Downstream	2.18 E - 05	2.50 E - 05	
MEAN SURVIVAL (BY SITE)			0.6019
Upstream	62.50	20.62	
Discharge Channel	65.00	5.77	
Downstream	53.33	15.28	

TABLE 3. Summary data for the *in situ* bioassay with the mayfly *Stenacron interpunctatum*: Experiment 2. Dates: May 18-May 23 1998 (5 days). The p-values for the overall ANOVA are indicated; tests are considered significant if the p-value < 0.05.

TEST	MEAN	SD
GROWTH (LOCATION)		
Upstream	3.62 E – 05	9.67 E – 04
Discharge	6.99 E – 04	9.87 E – 04
GROWTH (FOOD)		
Upstream Food	6.92 E – 04	9.19 E – 04
Discharge Food	4.35 E – 05	1.04 E – 03
GROWTH (LOCATION X FOOD)		
Upstream x Upstream Food	2.91 E – 04	9.20 E – 04
Upstream x Discharge Food	-2.18 E – 04	1.03 E – 03
Discharge x Upstream Food	1.09 E – 03	7.91 E – 04
Discharge x Discharge Food	3.05 E – 04	1.07 E – 03
PERCENT SURVIVAL (LOCATION)		
Upstream	62.5	10.7
Discharge	58.3	16.3
PERCENT SURVIVAL (FOOD)		
Upstream Food	62.5	13.1
Discharge Food	58.3	14.4

DEPENDENT VARIABLE	SOURCE	P-VALUE
GROWTH	Location	0.1978
	Food	0.1332
	Location x Food	0.9284
SURVIVAL	Location	0.8296
	Food	0.8096
	Location x Food	0.8385

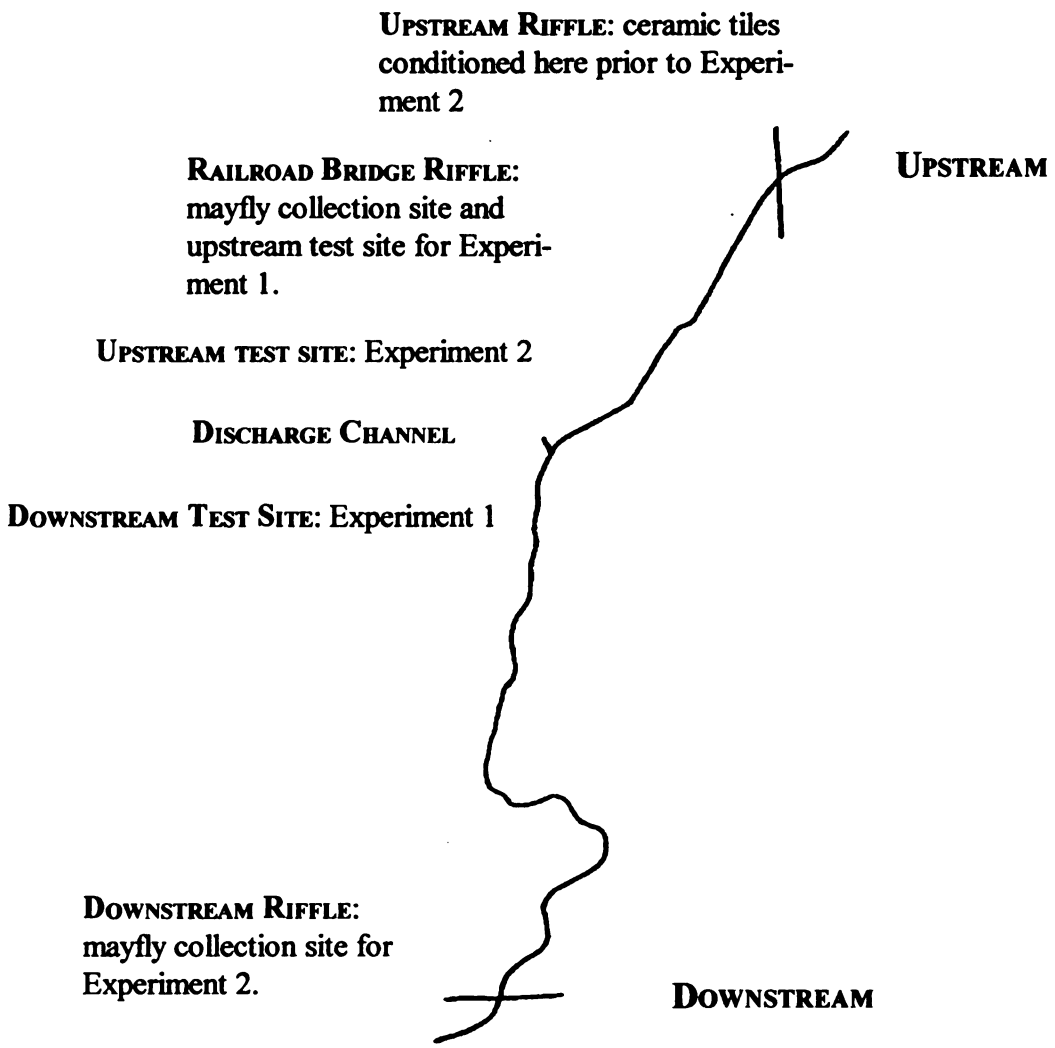


FIGURE 1. Map of Portage Creek. from Portage Police Station (Upstream Riffle) to Milham Park (Downstream Riffle), showing locations used during the course of the mayfly growth experiments. Scale: 1 inch = ~ 1000 meters. See text for details.

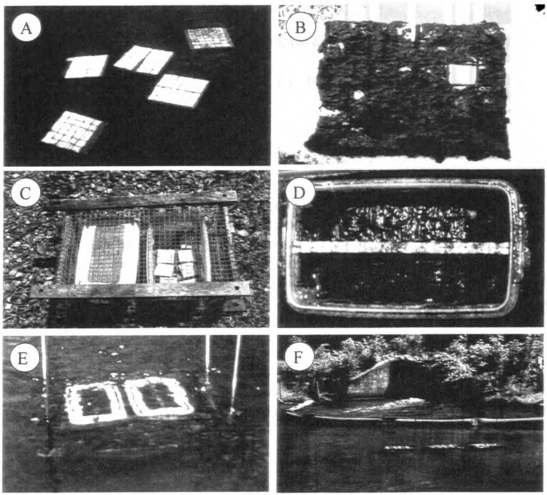
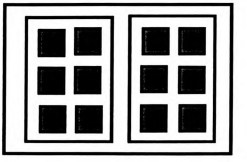


Figure 2. Elements of the mayfly growth experiment. A) Ceramic tiles placed in the discharge channel. B) Ceramic tiles after ~1 month in the discharge channel, displaying a heavy accumulation of ferric hydroxide and bacteria. C) A pair of growth chambers used in Experiment One; one is open to show the arrangement of food tiles. D) A growth chamber used in Experiment Two, showing the divider used to separate the chamber into two compartments. E) A pair of growth chambers suspended within the cage and immersed in the stream. F) All growth chambers (6 chambers, 3 cages) at the industrial effluent outfall during Experiment Two.

Experiment 1

Food Type: High Quality
Locations: Upstream, Discharge Channel, Downstream
Replications: 4 chambers (2 cages) per site, 10 mayflies per chamber.



Experiment 2

Food Types: Upstream (diatoms and green algae), and Discharge (ferric hydroxide and bacteria).
Locations: Upstream, Discharge Channel
Replications: 6 chambers (3 cages) per site, 2 compartments (food types) per chamber, 8 mayflies per compartment.

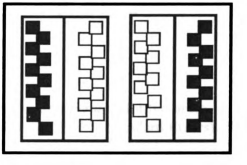


Figure 3. Experimental design for the mayfly growth experiments. See Figure 1 for a map of test locations, and Figure 2 for phtographs of ceramic tiles, growth chambers, cages, and the experiment in progress.

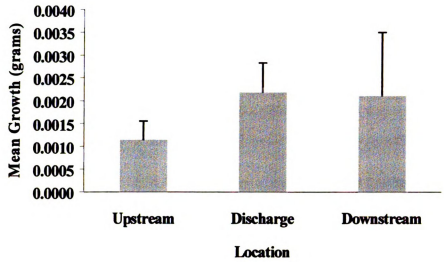


FIGURE 4. Mean Growth (grams) of caged *Stenacron interpunctatum* for the *in situ* growth/mortality experiment conducted May 16 – May 22, 1997 (+ Standard Errors). N (cages) = 4 (upstream), 4 (discharge), 3 (downstream). Overall ANOVA not significant (p-value 0.5918). See Table 2 for summary data, and Figure 1 for site locations.

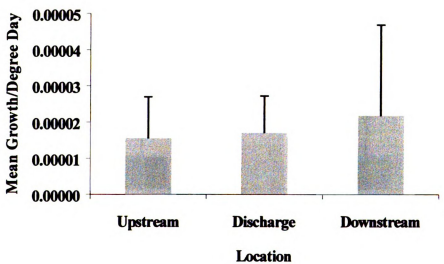


FIGURE 5. Mean growth (g) of caged *Stenacron interpunctatum*, corrected for total degree-day accumulation at each location (+ Standard Deviation). *In situ* growth/mortality experiment conducted May 16 - May 22, 1997. N (cages) = 4 (upstream), 4 (discharge), 3 (downstream). Overall ANOVA not significant (p-value 0.8677). Degree Days = Σ daily mean temperatures > 0° for 6-day experiment. See Table 2 for summary data, and Figure 1 for site locations.

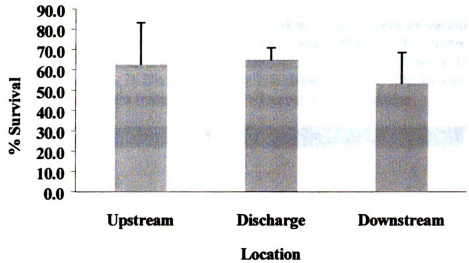


FIGURE 6. Percent survival of caged *Stenacron interpunctatum* for the *in situ* growth/mortality experiment conducted May 16-May 22 1997 (+ Standard Deviation). N (cages) = 4 (upstream), 4 (discharge), 3 (downstream). Overall ANOVA not significant (p-value 0.6019). See Table 2 for summary data, and Figure 1 for site locations.

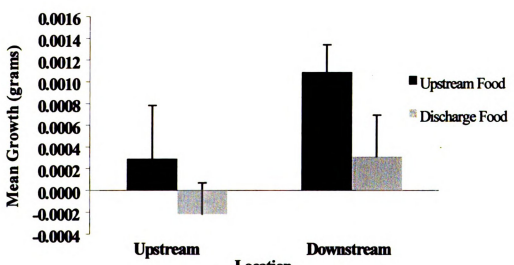


FIGURE 7. Mean growth (grams) of caged *Stenacron interpunctatum* exposed to different test locations and food sources (+ Standard Errors). N (chambers) = 6 for each combination. Dates: May 18 – May 23 1998. Two-ANOVA not significant (p-values: Location 0.1978, Food 0.1332, Location x Food 0.9284). See Table 2 for summary data, and Figure 1 for site locations.

APPENDIX ONE

Qualitative list of macroinvertebrate taxa collected in Portage Creek by various sampling methods over the course of the study (June 1996 – June 1998). 1: HD Upstream, 2: HD Discharge, 3: HD Downstream, 4: Hess Upstream, 5: Hess Impact Zone, 6: Hess Downstream Recovery, 7: Tile Upstream, 8: Tile Impact Zone, 9: Tile Downstream Recovery, 10: Qualitative (entire stream). * Denotes iron deposition.

TAXA	1	2*	3*	4	5*	6	7	8*	9	10
PORIFERA										•
CNIDARIA										
Hydra sp.			•	•						•
ECTOPROCTA										
Cristatella mucedo										•
TURBELLARIA										
Dugesia tigrina	•	•	•	•	•	•	•			•
NEMATODA	•	•	•	•	•	•				•
OLIGOCHAETA										
Unident. Oligochaeta	•	•	•	•	•	•	•	•	•	•
Bothrioneurum sp.										•
Rhyacodrilus sp.										•
Tubifex sp.		•								•
Potamothrix sp.										•
Tubificidae w/o caps		•								•
Ophidonais sp.										•
Nais sp.										•
Dero sp.										•
Pristina sp.										•
Sparganophilus tamesis	•		•							•
Lumbriculus variegatus										•
Aelosoma sp.		•								•
HIRUDINEA										
Erpobdellidae										•
Helobdella stagnalis	•	•	•							•
Helobdella triserialis	•	•	•							•
Batrachobdella phalera										•
Placobdella papillifera			•							•
PELECYPODA										
Pisidium sp.	•	•	•	•	•	•				•
Sphaerium striatinum				•		•				•
Alasmidonta calceolus										•
Strophitus undulatus										•
Elliptio dilatata										•
Lasmigona complanata										•
Actinonais ellipsiformes										•
Pyganodon grandis										•

APPENDIX ONE (CONTINUED)

TAXA	1	2*	3*	4	5*	6	7	8*	9	10
Pleurobema coccineum										•
Corbicula fluminea										•
GASTROPODA										
Ferrissia rivularis	•	•	•	•	•	•	•	•	•	•
Helisoma sp.										•
Planorbella sp.			•	•			•			•
Armiger crista	•									•
Valvata tricarinata										•
Valvata bicarinata										•
Valvata sincera				•						•
Fossaria sp.	•			•						•
Goniobasis livescens						•			•	•
Physa sp.	•	•	•	•			•		•	•
Amnicola limosa										•
Viviparus georgianus										•
Campeloma decisum										•
HYDRACARINA	•	•	•	•		•	•	•	•	•
AMPHIPODA										
Gammarus pseudolimnaeus	•	•	•	•	•	•	•	•	•	•
Hyalella azteca	•			•						•
ISOPODA										
Caecidotea intermedius	•	•	•	•	•	•	•	•	•	•
DECAPODA										
Orconectes propinquus	•		•	•	•	•				•
COLLEMBOLA				•		•				•
EPHEMEROPTERA										
Stenonema sp.							•		•	•
Stenonema terminatum	•	•	•	•		•	•		•	•
Stenonema exiguum				•			•			•
Stenonema fuscum	•			•	•	•	•	•	•	•
Stenacron interpunctatum	•	•	•	•		•	•	•	•	•
Baetis levitans						•	•		•	•
Baetis brunneicolor						•	•			•
Baetis sp.	•		•	•	•	•	•	•	•	•
Pseudocloeon sp.	•								•	•
Ephemera simulans			•			•				•
Ephemeralla lata						•				•
Caenis sp.	•	•	•	•		•	•	•	•	•
Leptophlebia sp.	•		•				•			•
Paraleptophlebia sp.	•									•
Tricorythodes sp.	•					•			•	•
ODONATA										

APPENDIX ONE (CONTINUED)

TAXA	1	2*	3*	4	5*	6	7	8*	9	10
Calopteryx maculatum	•		•		•					•
Aeshna sp.			•							•
Gomphus sp.										•
Enallagma sp.										•
Argia sp.			•					•		•
PLECOPTERA										
Taeniopteryx sp.	•			•	•				•	•
HEMIPTERA										
Plea sp.										•
Ranatra fusca										•
Aquarius sp.										•
Gerris sp.										•
Belostoma flumineum										•
Notonecta sp.										•
MEGALOPTERA										
Sialis sp.	•		•							•
Nigronia serricornis										•
TRICHOPTERA										
Hydropsyche sp.	•	•	•	•	•	•	•	•	•	•
Cheumatopsyche sp.	•		•	•	•	•	•	•	•	•
Helicopsyche borealis				•	•	•	•		•	•
Oecetis sp.	•			•			•			•
Nectopsyche diarina	•		•							•
Lype diversa	•		•							•
Brachycentrus numerosus				•		•				•
Psychomyia flavida				•		•			•	•
Pycnopsyche guttifer			•	•	•					•
Pycnopsyche luculenta				•						•
Neophyllax sp.				•						•
Glossosoma nigrior						•			•	•
Hydroptila sp.	•		•		•	•	•	•		•
Molanna sp.										•
Phryganea sp.										•
COLEOPTERA										
Macronychus glabratus	•		•	•	•			•		•
Stenelmis sp.			•	•	•	•	•		•	•
Optioservus fastiditus	•		•	•	•	•	•	•	•	•
Dubiraphia sp.	•	•	•	•	•	•	•			•
Ancyronyx variegata	•							•		•
Ectopria nervosa					•	•				•
Peltodytes sp.				•	•					•
Laccophilus sp.										•
Agabus sp.										•

APPENDIX ONE (CONTINUED)

TAXA	1	2*	3*	4	5*	6	7	8*	9	10
Tropisternus sp.										•
Sperchopsis sp.										•
Hydroporus sp.										•
Gyrinus sp.										•
DIPTERA										
Tipula sp.			•	•	•					•
Eriocera sp.	•			•						•
Antocha sp.	•		•	•		•	•	•	•	•
Chrysops sp.				•						•
Hemerodromia sp.	•	•	•	•	•	•	•	•	•	•
Simulium vittatum	•		•	•	•	•	•		•	•
Simulium tuberosum	•		•	•		•				•
Anopheles sp.										•
Ceratopogonidae	•	•	•	•						•
Chironomidae	•	•	•	•	•	•	•	•	•	•