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Stream microcosm toxicity tests: Colonizing test organisms and predicting the effects of fenvalerate on riffle insect communities

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STREAM MICROCOSM TOXICITY TESTS: COLONIZING TEST ORGANISMS

AND PREDICTING THE EFFECTS OF FENVALERATE

ON RIFFLE INSECT COMMUNITIES

An Abstract of a Thesis

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In Partial Fulfillment

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Dan H. Breneman

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ABSTRACT

Small streams and rivers draining agricultural watersheds are frequently exposed to hazardous chemicals. Unfortunately, chemical registration procedures currently do not include standardized protocols capable of predicting chemical impacts in lotic ecosystems. Three experiments were conducted utilizing artificial streams and rock-filled artificial substrates in an attempt to further standardize stream microcosm test procedures.

In the first experiment stream microcosms were used to predict the effects of a synthetic pyrethroid insecticide on riffle insect communities. Aquatic macroinvertebrates colonized on artificial substrates were placed in artificial streams and dosed in triplicate at 0.0, 0.01, 0.1, 1.0, and 10.0 ug/L fenvalerate (cyano (3-phenoxyphenyl) methyl-4-chloro-alpha-(1-methylethyl) benzeneacetate) for a 30-day period. The stream microcosms dosed at 0.1 ug/L fenvalerate exhibited significant ($p \leq 0.05$) reductions in species richness and total density following the 30-day toxicity test.

Artificial substrates placed in a natural riffle area during the second experiment were randomly sampled at weekly intervals for a seven week period to determine an optimal colonization period and to compare the artificial and natural substrate communities. Riffle insect communities colonizing the artificial substrates reached species

equilibrium and maximum densities by weeks one and four, respectively. Artificial substrates were selectively colonized by collector-filterers, and collector-gatherers were more abundant in the natural substrate.

The final experiment was conducted in an attempt to reduce colonization periods and collect artificial substrate communities more representative of natural riffle insect communities. Macroinvertebrates were colonized in artificial substrates, embedded and unembedded in the natural substrate, that were either uncolonized or precolonized with periphyton. Precolonized-unembedded substrates were colonized by significantly ($p \leq 0.05$) greater densities than the other experimental groups on week one. Unembedded substrate were colonized by riffle insect communities functionally more similar to the natural stream community than the embedded substrates.

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Test Organisms and Predicting the Effects of

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PROLOGUE

The purpose of biological toxicity testing is to evaluate potentially hazardous materials by determining what concentrations cause substantial risk to the environment. The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), first promulgated in 1947, requires pesticide manufacturers to demonstrate product safety in aquatic ecosystems through a series of toxicity test procedures. Prospective agricultural chemicals are subjected to a testing hierarchy designed to evaluate physical/chemical properties, usage patterns, and potential hazards to aquatic ecosystems. Single-species tests, multispecies tests, and field studies constitute the bulk of biological testing required by the United States Environmental Protection Agency (USEPA) in environmental risk assessment programs. Acute, single-species tests constitute the first tear of aquatic toxicity tests and may trigger further chronic test procedures if potential risks to nontarget organisms are demonstrated. Subsequent testing procedures (e.g., microcosm and mesocosm tests) may be required to fulfill higher-level tests when a substantial risk is evident and an ecosystem-level response is desired. At present, there is considerable controversy over which procedures are both cost-effective and good predictors of xenobiotic impacts on aquatic ecosystems.

Single-species toxicity tests are routinely employed for initial screening to determine potential chemical hazards because the procedures involved are simple and inexpensive. Therefore, a large number of chemicals can be rapidly tested. However, single-species toxicity tests do not adequately predict potential hazards to aquatic ecosystems because they: 1) use genetically homogeneous laboratory-stock organisms that may or may not represent the "most sensitive" species (Cairns 1986a); 2) often lack realistic environmental conditions (e.g., nutrient cycling, energy transfer); 3) do not account for interactions between individuals in a community (e.g., succession, competition, predator-prey interactions); and, 4) use organisms that may not be indigenous to potential receiving ecosystems. Single-species toxicity tests have improved water quality, but because of a better understanding of ecosystem complexity and a need for more realistic tests, some workers have recently questioned the predictive utility of single-species tests when evaluating environmental safety and harm (National Research Council 1981, Cairns 1983, Kimball and Levin 1985).

Multispecies toxicity tests are being developed in an attempt to more accurately predict potential hazards in aquatic ecosystems (Cairns 1985, 1986b). Because multispecies tests require more time and expertise to conduct, they are not the logical choice for initial

screening of chemicals. However, for chemicals that prove
toxic during short term toxicity tests and have a potential
for widespread use, multispecies toxicity tests should be
considered. Multispecies tests currently available include
field studies, mesocosm studies, and microcosm studies.

Field studies manipulate natural aquatic ecosystems
and monitor the initial response and potential recovery over
several experimental seasons. Ecosystem-level studies
conducted in the Experimental Lakes Area in Ontario, Canada
expanded our understanding of lake eutrophication (e.g.,
Schindler 1974) and acidification (e.g., Schindler *et al.*
1985). Lotic ecosystem manipulations, conducted on
productively and morphologically similar first order streams
within the Coweeta Hydrologic Laboratory evaluated
macroinvertebrate community response following an
insecticide-induced disturbance (Wallace *et al.* 1986). A
similar study by Kreutzweiser *et al.* (1989) examined stream
invertebrate drift response to a herbicide applied at
various sites within the same stream reach. Field
manipulation studies present researchers with valuable
ecosystem-level responses, but data are site-specific and
often not replicated. In addition, exposure of natural
environments to hazardous materials raises ethical
questions.

In order to increase test replication and maintain an
ecosystem-level response, investigators have recently

employed outdoor lake (e.g., Crossland *et al.* 1987, Larsson and Sodergren 1987), estuarine (e.g., Oviatt *et al.* 1984), marine (e.g., Kuiper 1982), and lotic mesocosms (e.g., Stout and Cooper 1983, Irvine 1985, Clements *et al.* 1989a). Outdoor mesocosms are designed to reflect natural environmental conditions on a smaller-scale by positioning enclosures within the natural ecosystem or transferring a portion of the source ecosystem to terrestrial based chambers. A review by Odum (1984) discussed several mesocosm designs and their contribution to aquatic research on lake and marine ecosystems.

Floating enclosures were used in British Columbia as part of CEPEX (Controlled Ecosystem Pollution Experiment). Ecosystem-level responses were monitored inside plastic floating containers large enough to support a majority of the naturally occurring organisms. In addition, "limno-corrals" have been utilized to evaluate herbicide effects on plankton communities (Hamilton *et al.* 1988) and the effects of mercury and cadmium on several marine trophic levels (Kupier 1982).

The Marine Ecosystem Research Laboratory (MERL) employs terrestrial based mesocosms to determine the effects of environmental stressors such as oil spills on marine and estuarine ecosystems. MERL consists of a series of large, cylindrical test chambers near Narragansett Bay, Rhode Island. Water and organisms are continuously exchanged

between the test chambers and the source ecosystem to simulate seasonal fluctuations and average turnover, thus establishing a self-sustaining system (Oviatt et al. 1984). Although ecosystem-level responses can be monitored continuously, the process of simulating turnover by periodically exchanging the contents of each vessel may discharge potentially hazardous materials directly into the source ecosystem. To reduce potential environmental contamination, Besser and Rabeni (1987) percolated rainwater through test plots and collected the runoff in small vinyl receiving pools stocked with test organisms. This test system examined the bioavailability and toxicity of lead-mine leachates to smaller-scale lentic ecosystems with minimal risk to the environment. In addition, 0.1 to 2-ha pond mesocosms, constructed to comply with standardized criteria, are widely used during the pesticide registration program (Touart 1988, Heinis and Knuth 1992, Lozano et al. 1992, Webber et al. 1992).

Lotic mesocosms may be constructed by partially re-routing a stream to flow through a series of man-made channels. The USEPA research facility located near Monticello, Minnesota utilizes eight experimental streams containing alternating riffles and pools. The experimental channels are approximately one meter wide and more than 500 m long. Water and organisms indigenous to the Mississippi River are diverted into channels, and toxicants are

introduced below the first riffle, providing an upstream
within-channel control (Stout and Cooper 1983, Perry and
Troelstrup 1988). Stream water from source ecosystems has
also been pumped into smaller streamside artificial streams
to study the impact of heavy metals on aquatic
macroinvertebrates (Clements *et al*. 1989b) and to test
methods for analyzing the structure and function of aufwuchs
communities (Clark *et al*. 1980). Utilizing smaller portions
of the lotic environment enables workers to investigate
ecosystem-level parameters, maintain some degree of
investigator control, and in most cases, improve
experimental replication. Unfortunately, mesocosms are
often costly to construct, difficult or impossible to
transport, and are susceptible to climatic, biological, and
anthropogenic perturbations.

Laboratory-based biological models of natural
ecosystems, or microcosms, have recently been introduced to
increase replication and conduct ecosystem-level tests under
controlled conditions. Vinyl tubs or glass aquaria are used
to support communities indigenous to lentic ecosystems.
Test chambers utilized in stream microcosm research are of
several designs including rectangular troughs constructed of
wood or concrete and oval "racetrack" test vessels to
simulate lotic ecosystems. Microcosms have been used to
study ecological aspects of freshwater communities such as:
1) the effects of herbivores on periphytic communities

(e.g., Lamberti *et al.* 1987, Steinman *et al.* 1987, McCormick and Stevenson 1989); 2) the influence of stream sedimentation on macrobenthic distribution (e.g., Brusven and Prather 1974); and, 3) the effects of temperature on aquatic insect drift (e.g., Sherberger *et al.* 1977). However, much of the current interest in microcosm research is focused on predicting chemical influence on aquatic ecosystems.

Aquatic microcosms have been used to determine xenobiotic effects on periphytic assemblages (e.g., Krieger *et al.* 1988, Pratt *et al.* 1988, Cairns *et al.* 1990, Scanferlato and Cairns 1990) and the influence of toxicants on artificial streams stocked with benthic macroinvertebrates (e.g., Hedtke 1984, Lynch *et al.* 1985, Clements *et al.* 1988a; 1989b, Pontasch *et al.* 1989, Pontasch and Cairns 1989; 1991). Microcosm toxicity tests are easily replicated and can predict the influence of hazardous chemicals without harming the natural environment. However, the lack of standardized methods have limited the use of microcosm toxicity tests within the regulatory framework.

Standard methods for the determination of water quality include those published by the EPA (e.g., USEPA 1985), American Society for Testing and Materials (e.g., ASTM 1985), and American Public Health Association (e.g., APHA *et al.* 1985). These protocols are continuously tested against specific guidelines and periodically revised. In order for

methods to be endorsed as "standard," they must be extensively tested and their limitations known. Cairns (1990) indicated that a number of provisional methods utilizing higher levels of biological organization (i.e. microcosm tests) have been proposed, but these methodologies are not yet standard protocols. The hesitancy in incorporating multispecies, microcosm toxicity tests into the regulatory framework is founded on test expense and complexity of interpreting results. If test expense is a decisive factor, and the costs of both over or underprotecting the environment are considered, microcosm tests may be no more expensive than conventional tests (Niederlehner *et al.* 1986; Cairns and Pratt 1987). Environmentally realistic tests may incorporate several ecological processes (e.g., primary production, macrophyte stress response, macroinvertebrate recovery) and often include longer exposure periods to ensure test communities complete partial or entire life cycles. Consequently, tests are more complex and may require a greater level of expertise when interpreting results. However, the ability to examine higher levels of organization provides investigators with an opportunity to make more sound ecological assessments. This two year project is part of a larger effort to develop methods that can reliably and cost-effectively predict chemical hazards to aquatic ecosystems in a multitude of regional settings. Specifically, this

research utilized laboratory-based artificial streams and rock-filled artificial substrates to address the following objectives:

Objectives

1) Conduct a stream microcosm toxicity test to predict the effects of the synthetic pyrethroid insecticide fenvalerate on aquatic insects indigenous to Iowa streams.

2) Determine the minimum colonization period necessary to maximize macroinvertebrate densities and species richness in rock-filled artificial substrates.

3) Test methods designed to decrease macroinvertebrate colonization periods and collect an artificial substrate community more representative of the natural stream community.

Objective one involved exposing artificial substrates colonized by riffle insect communities to selected fenvalerate concentrations in a series of artificial stream microcosms for 30 days. Species-abundances of both adults and immatures per microcosm were used to predict the sensitivity of natural stream communities. Results from this experiment are reported in Chapter One.

Objective two involved randomly sampling artificial substrates colonized by aquatic macroinvertebrates at weekly intervals during a seven week colonization study. Species richness and total density per substrate were used to

determine an optimal colonization period. Results from this experiment are reported in Chapter Two.

Objective three utilized artificial substrates uncolonized and precolonized with periphyton which were embedded or unembedded in the natural stream bottom. Macroinvertebrate densities per substrate at six weekly intervals were used to determine which substrate type reduced the colonization period, yet provided an assemblage of organisms similar to the natural stream community. Results from this experiment are reported in Chapter Three.

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STUDY AREA

The Volga River is a first to third order stream in northeast Iowa that follows a ~120.0 km course from west central Fayette Co. to its confluence with the Turkey River in south central Clayton Co. The Volga River flows from a relatively flat, glaciated landscape to a region of greater topographic relief marked by wooded valleys and streams entrenched in Paleozoic age bedrock. This area in northeast Iowa is noted as the Paleozoic Plateau and the bedrock is dominated by limestone and other carbonate rocks. The combination of riparian vegetation and spring-fed streams produce a habitat rich in plant and macroinvertebrate life.

The specific study sites were located in riffle areas. Riffles contain high levels of dissolved oxygen and numerous microhabitats within the substrate. Therefore, riffles are highly productive areas in a stream. Research conducted during the first experimental season (Chapters 1 and 2) utilized a riffle area adjacent to relatively flat agricultural land 7 km downstream from the Volga River headwaters. During typical flow conditions the stream at this site was 16.2 m wide, 22 cm deep, and current velocity was approximately 77 cm/s. The riffle area was 20 m in length and the substrate was dominated by cobbles (6-13 cm) 50% embedded in pebbles (2-6 cm) with considerable sedimentation by sand and silt.

The study site for the second experimental season (Chapter 3) was located within the city limits of Fayette, Iowa approximately 12 km from the Volga River headwaters. During typical flow conditions, the stream at this riffle area was approximately 20 cm deep, 15 m wide, and current velocity was 66 cm/s. Artificial substrates were placed in a ~20 m riffle area dominated by cobbles (13-25 cm) unembedded in pebbles (2-6 cm) with minimal amounts of sedimentation.

CHAPTER ONE

STREAM MICROCOSM TOXICITY TESTS: PREDICTING THE

EFFECTS OF FENVALERATE ON RIFFLE INSECT COMMUNITIES

ABSTRACT

Stream microcosms were used to predict toxicity of the pyrethroid insecticide fenvalerate (cyano (3-phenoxyphenyl) methyl-4-chloro-alpha-(1-methylethyl) benzeneacetate) to riffle insect communities. Over a 30-d test period stream microcosms were dosed in triplicate at 0.0, 0.01, 0.1, 1.0, and 10.0 ug/L fenvalerate. The relative sensitivities of 12 species were determined by statistically comparing abundances over all concentrations.

Initial exposure resulted in a significant increase in drift in the 1.0 and 10.0 ug/L treatments. After 30 d, several taxa exhibited density reductions at 0.01 ug/L, but this reduction was significant for only one taxon. Densities of most other taxa decreased significantly at 0.1 ug/L. Significant reductions in species richness and total density also were observed at 0.1 ug/L fenvalerate. Overall, the results suggest that at environmental concentrations of 0.1 ug/L, mayflies and stoneflies would be eliminated and riffle beetles, caddisflies and some chironomids would be present in significantly reduced numbers.

Keywords: Stream microcosms, Toxicity, Fenvalerate, Insects

INTRODUCTION

Synthetic pyrethroids have become a commonly used insecticide in the last decade because they combine high insecticidal activity with low avian [1] and mammalian [2] toxicity. Synthetic pyrethroids resemble natural pyrethrins but, due to changes at several sites on the synthetic molecule, possess greater photostability [3,4] and remain active in various soil conditions [5,6]. The enhanced environmental stability and widespread use of synthetic pyrethroids increase the possibility of unintentional exposures in aquatic ecosystems where non-target organisms may be adversely affected. Fenvalerate is a synthetic pyrethroid commonly employed for pest control in a variety of agricultural settings.

In earlier studies using acute exposures, EC_{50}s for freshwater zooplankton exposed to fenvalerate ranged from 0.12 to 5.0 ug/L [7,8]. Chronic tests with *Daphnia galeata mendotae* resulted in reduced production of young at 0.01 ug/L fenvalerate [9]. Acute toxicity to <24-h old *Pimephales promelas* ranged from a 3 h LC_{50} of 5.0 ug/L to a 96 h LC_{50} of 0.85 ug/L fenvalerate [10]. Bradbury *et al.* [11] reported LC_{50}s for 30-31 d old *P. promelas* ranged from 2.06 to 0.75 ug/L fenvalerate following 24 h and 168 h exposures, respectively. Early life stage tests with *Cyprinodon variegatus* resulted in an estimated NOEC of $\leq$0.56 ug/L fenvalerate [12]. Clark *et al.* [13] reviewed the

effects of several synthetic pyrethroids on marine invertebrates and fish and reported similar toxicity ranges. Although most literature concerning the effects of synthetic pyrethroid exposures in aquatic ecosystems is based on single species responses, inadvertent release into aquatic ecosystems will impact entire communities.

The ability of single-species toxicity tests to accurately predict community-level responses has been questioned [14-16]. As a result, multispecies tests have been developed to allow better predictions of community-level responses and reduce uncertainties when extrapolating from the laboratory to the field [17,18]. To simulate potential field situations, Webber et al. [19] exposed pond mesocosms in triplicate to both aerial drift and direct sediment runoff containing esfenvalerate, a fenvalerate isomer with similar physical/chemical properties but demonstrating greater insecticidal activity. Following esfenvalerate application, macroinvertebrate densities and adult emergence were significantly lower in high-rate ponds (0.7 ppb and 56.3 ppb mean aqueous and sediment concentrations, respectively) than in medium, low-rate, and control ponds. Heinis and Knuth [20] reported a two stage distribution of esfenvalerate occurred within littoral enclosures. Water contained the majority of esfenvalerate during the first 2 d, but by 4 d, the major reservoir was sediment and macrophytes.

Pond mesocosm studies are currently employed in the testing hierarchy for pesticide registration when triggered by single-species test results. However, in most regions, small streams are exposed more frequently than lakes and ponds to nonpoint source agricultural pollutants. Unfortunately, limited information is available concerning the sensitivity of stream communities to these inputs. Recently, toxicity tests utilizing naturally occurring assemblages of stream organisms have been introduced [21,22] and field-validated [23,24]. This research employed stream microcosms to predict fenvalerate effects on riffle insect community structure. Test concentrations were selected based on results from previous single-species toxicity tests and expected environmental concentrations.

Materials and Methods

Artificial Streams

The laboratory-based artificial stream system consists of 15 oval artificial streams (1.7 x 0.24 x 0.13 m channel) constructed of molded fiberglass. Each stream is covered by a 1.0 x 0.75 x 0.3 m emergence net (mesh size 1.0 mm). Two 120 cm Durotest Vita-lites[R] over each stream provided a photoperiod corresponding to that on d 15 of the test. A 13 cm standpipe, covered with a 1.0 mm mesh screen to prevent macroinvertebrate escape, maintained a volume of 55 L in each stream. Current (25 cm/s) was provided by a 0.25 hp

electric motor turning paddle wheels attached to an iron rod.

Fenvalerate concentrations, based on the active ingredient (cyano (3-phenoxyphenyl) methyl-4-chloro-alpha-(1-methylethyl) benzeneacetate), were prepared by serial dilution from the 2.4 emulsifiable concentrate of Pydrin[R] [30% a.i.(w/v)]. Fenvalerate stock solutions were stored in 20 L glass carboys covered with aluminum foil. Artificial stream microcosms were pulse dosed to proper concentrations by adding 550 ml of appropriate 0.0, 1.0, 10.0, 100.0 and 1000.0 ug/L fenvalerate stock solutions. The microcosms were then continuously exposed in triplicate at 0.0, 0.01, 0.1, 1.0, and 10.0 ug/L fenvalerate by adding 3 ml/min. of appropriate stock solutions while a headbox system supplied dechlorinated tap water to all artificial streams at 297 ml/min. Turnover of test medium in the artificial streams occurred approximately eight times every 24 h.

Establishing Stream Microcosms

The colonization site was located in a riffle area 7 km downstream from the Volga River headwaters in northeast Iowa. During normal flow conditions the stream was 16.2 m wide, 0.22 m deep, and mean current velocity was 64.4 cm/s. Substrate was dominated by cobbles (6-13 cm) 50% embedded in pebbles (2-6 cm).

To provide a macroinvertebrate food source, periphyton was colonized on 60, 25 cm^3 polyurethane foam units (PFUs)

placed in the source riffle. After 7 d the PFUs were transported in an aerated cooler to the laboratory. The contents were squeezed into a bucket and the slurry strained through a sieve (mesh size 250 microns) to eliminate macroinvertebrates. The periphyton slurry (1.3 L) was then added to each artificial stream and allowed to develop for five weeks prior to initiation of the toxicity test.

Macroinvertebrate colonization occurred in 64 rock-filled plastic containers (10.6 x 10.6 x 8.3 cm) with six circular holes (12 mm diam.) in each side. The artificial substrates were secured to wooden frames previously anchored to the stream bottom with iron rods and concrete blocks. After six weeks the artificial substrates were removed by placing a dip-net (mesh size 350 microns) behind the substrate as it was randomly transferred to one of 32 coolers (7 L capacity) filled with river water. Two substrates were placed in each cooler and transported to the laboratory. During the 2 h transport, temperature and dissolved oxygen were maintained at ambient stream levels (19 °C and 8.7 ppm O_2) by pumping air through a small radiator placed in a cooler of ice. Air was then shunted to airstones in each cooler through a series of valves and hoses. At the laboratory, the contents of two coolers (four substrates) were randomly assigned to each artificial stream. Macroinvertebrate communities were allowed to acclimate for 2 d in the artificial streams before the pulse

dose was administered and peristaltic pumps activated.
After 30 d the test was terminated and the contents of each
microcosm were sampled (see below).

Sampling

Macroinvertebrate drift was measured in each microcosm
1 h after returning the artificial substrates to the
laboratory and 2 d later, following the initial pulse dose.
Insect drift was measured by inserting a 15 X 12 cm dip net
(mesh size 350 um) in the artificial stream for 1 min.
Aquatic insects entering the drift were identified to family
and enumerated in a water-filled enamel pan, then returned
to the artificial stream. Drift data were used to quantify:
1) mortality during transportation, and; 2)
macroinvertebrate avoidance response to initial fenvalerate
exposures. Emergent adult insects were aspirated from
emergence traps every 48 h to 72 h and preserved in 70%
ETOH.

After 30 d the contents of each microcosm were washed
through a sieve (500 um) and preserved in 70% ETOH. Insects
were sorted by hand using a 2X magnification lens and size-
classed as small, medium, large, pupae (for holometabolous
insects), or adult. With the exception of midges
(Chironomidae: Diptera), insects were identified to the
lowest possible taxonomic unit using appropriate references
and the species-abundances of both adults and immatures per
microcosm were determined.

Grab samples (170 ml) from each artificial stream taken
on days 1, 10, 20, and 30 of the test period were extracted
with hexane (30 ml) and analyzed to determine actual
fenvalerate concentrations. In addition, dissolved oxygen,
pH, conductivity, alkalinity, water hardness, temperature,
and current velocity were monitored weekly to ensure that
the stream microcosms reflected those parameters in the
natural source ecosystem.

Data Analysis

Species-abundances of both adults and immatures per
microcosm were determined. Macroinvertebrate taxa with mean
densities $\geq$ 4 in at least one treatment were considered a
core taxon. Total insect densities for each core taxon were
compared over all experimental groups to determine
concentration effects. Data were analyzed by a one-way
ANOVA followed by Fisher's least significant difference
(LSD) procedure for separation of means (see Appendix A).

Results and Discussion

Water chemistry data from the stream microcosms and the
Volga River are reported in Table 1.1. Dissolved oxygen,
conductivity, hardness, alkalinity, temperature, and pH in
the stream microcosms reflected conditions in the source
ecosystem. Fenvalerate concentrations determined from grab
samples taken throughout the test were, in most cases, lower
than targeted concentrations (Table 1.1). Fenvalerate

Table 1.1. Water quality characteristics of the Volga River, Iowa and stream microcosms during a 30-day toxicity test.

	Volga River	0.0 (ug/L)	NOMINAL CONCENTRATIONS 0.01 (ug/L)	0.1 (ug/L)	1.0 (ug/L)	10.0 (ug/L)
TEMPERATURE ($^\circ$C)	22.1	19.3	19.3	19.3	19.3	19.3
s.d. =	3.5	1.0	1.0	1.0	1.0	1.0
n =	6	9	9	9	9	9
DO (mg/L O_2)	8.4	8.9	8.9	8.6	8.7	8.6
s.d. =	0.7	0.0	0.2	0.1	0.3	0.3
n =	5	9	9	9	9	9
CONDUCTIVITY (uMHOS)	572.5	404.7	409.0	407.7	413.0	410.0
s.d. =	29.6	51.3	55.1	54.9	51.4	50.1
n =	6	9	9	9	9	9
pH [H^+]	7.8	8.3	8.2	8.2	8.2	8.2
s.d. =	0.6	0.1	0.1	0.1	0.0	0.1
n =	6	9	9	9	9	9
HARDNESS (mg/L $CaCO_3$)	293.0	246.7	232.3	236.3	245.3	238.0
s.d. =	22.4	8.0	11.9	17.9	9.7	7.5
n =	6	9	9	9	9	9
ALKALINITY (mg/L $CaCO_3$)	168.2	197.0	195.0	196.3	197.2	191.0
s.d. =	6.0	7.8	1.7	4.0	4.2	3.5
n =	5	9	9	9	9	9
FENVALERATE (ug/L)	N.A.	N.D.	0.011	0.058	0.286	2.869
s.d. =	----	----	0.0333	0.0894	0.1389	0.8682
n =	----	9	9	9	9	9

N.A. = Not Available   N.D. = Not-Detected

derivatives have been shown to adsorb to plants and sediments [e.g., 20], and in this study, fenvalerate may have adsorbed to particulate organic matter and sediments deposited within the artificial substrates and/or periphytic growth on the stream walls.

Drift samples taken 1 h after returning the artificial substrates to the laboratory indicated aquatic macroinvertebrate transfer was accomplished with only two individual fatalities. These data support previous research in which there was no significant mortality following a similar transfer [22]. Drift samples taken 1 h after the initial pulse dose revealed a significant drift response in the 1.0 and 10.0 ug/L concentrations (Figure 1.1). Relative to control streams, a slight increase in drifting insects was also observed in the 0.01 and 0.1 ug/L treatments. The insects most frequently entering the drift included Baetidae (Ephemeroptera) and Hydropsychidae (Trichoptera). In addition, several *Isonychia bicolor* (Ephemeroptera: Oligoneuriidae) were observed clinging to the sides of the microcosms in the 1.0 and 10.0 ug/L treatments. Most *I. bicolor* nymphs eventually returned to the artificial substrates.

Other than aquatic insects, Oligochaeta were the only other macroinvertebrates regularly observed in the stream microcosms. Oligochaeta were not found in sufficient numbers to allow for statistical analysis but were observed
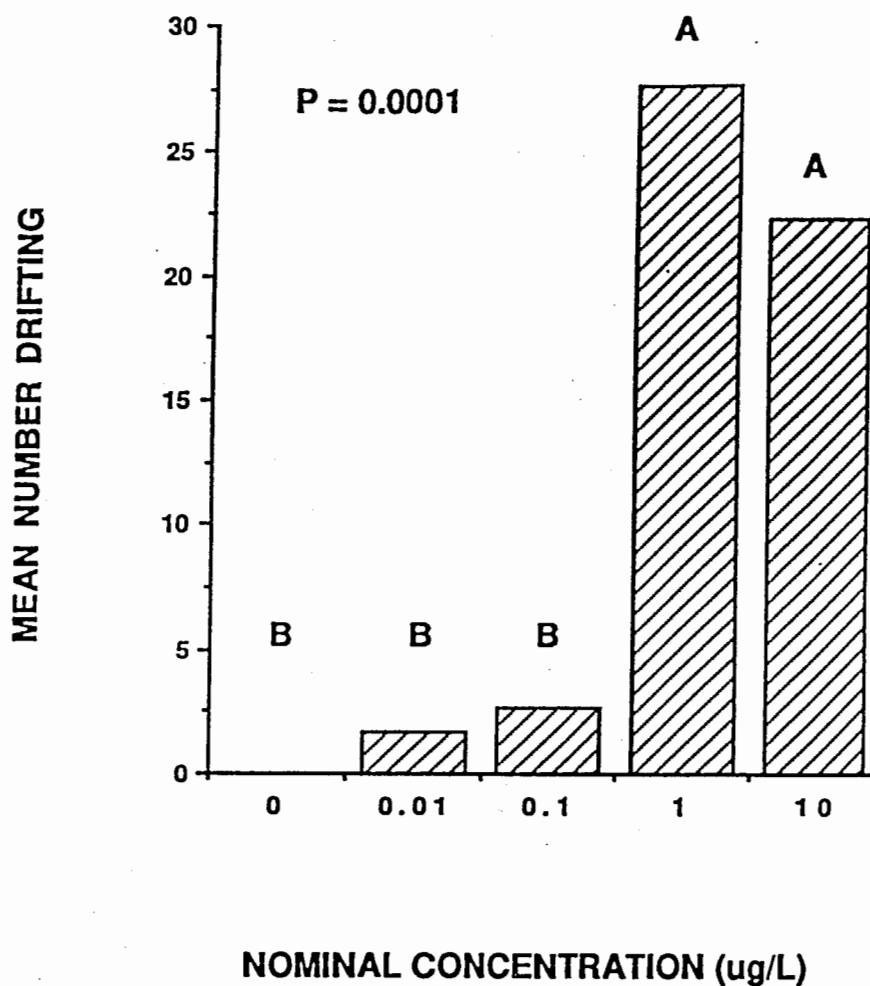
Figure 1.1. Mean number of immature aquatic insects from 1 min. drift samples taken 1 h after the initial fenvalerate pulse dose. P value is from the overall ANOVA. Treatments with the same letter are not significantly different ($p \leq 0.05$)-analyzed by Fisher's LSD procedure.

in all treatments at test termination, suggesting a
tolerance to the fenvalerate concentrations tested. Thirty-
four insect taxa, representing seven orders, were collected
from the stream microcosms during the 30-d test. Community-
level analysis including all 34 taxa revealed a significant
reduction ($p \leq 0.05$) in mean species richness from 19.3 in
the control microcosms to 12.3 taxa in the 0.1 ug/L
treatment. The decrease in mean species richness at 0.1
ug/L was primarily due to a decline in Ephemeroptera,
Plecoptera, and Odonata species. A significant reduction ($p
\leq 0.05$) in total mean density also occurred in the 0.1 ug/L
treatment. Twelve core taxa (mean densities $\geq$ 4 per
treatment) were included in the following analysis.

Most mayflies (Ephemeroptera) were adult insects
collected during the 30-d test period. This finding
supports previous results indicating the importance of
monitoring adult emergence during tests with riffle insect
communities [24,25]. Of the 12 mayfly species collected,
only *Isonychia bicolor* and *Baetis* spp. (Baetidae) were
considered core taxa. *Baetis* spp. included *B. flavistriga*,
B. dubius, and *B. intercalaris*, with *B. intercalaris* being
the dominant species. Due to the lack of keys for adult
females and the difficulty in identifying subimago adults,
Baetis spp. results are reported at the generic level. Both
I. bicolor and *Baetis* spp. were significantly reduced in the
0.1 ug/L treatment (Figures 1.2, 1.3). All organisms

observed at 0.1 ug/L and higher concentrations were adults
that emerged during the first 7 d. The mayflies *Ephoron
leukon* and *Ephoron album* (Polymitarcyidae), *Caenis* sp.
(Caenidae), and *Stenonema* spp. (Heptageniidae) were present
in the stream microcosms but densities were insufficient to
allow statistical analysis. A few adult *Ephoron* sp., *Caenis*
sp., and *Stenonema* spp. emerged early in the test from the
0.0 ug/L and 0.01 ug/L treatments.

The predatory stonefly *Claassenia sabulosa* (Plecoptera:
Perlidae) colonized the artificial substrates in low
numbers. Relative to controls, *C. sabulosa* was
significantly reduced at 0.01 ug/L and eliminated at the 0.1
ug/L concentration (Figure 1.4). All *C. sabulosa* were
nymphs collected at the end of the test.

Riffle beetles (Coleoptera: Elmidae) were represented
by five genera. However, *Stenelmis parva* was the only
species with densities considered sufficient for statistical
analysis. *S. parva* exhibited a significant reduction in the
0.1 ug/L treatment (Figure 1.5). However, mortalities in
the 0.1 and 1.0 ug/L treatments were primarily larvae. The
adults, none of which were observed in the emergence traps,
appeared more tolerant than other core taxa to the lower
concentrations tested. *S. parva* adults were not
significantly affected ($p \leq 0.05$) until the 10.0 ug/L
treatment. Previous research on petroleum hydrocarbon

Figure 1.2. Mean number of *Isonychia bicolor*
(Ephemeroptera) in stream microcosms exposed to fenvalerate.
Analysis is based on combined densities of adults and
immatures. P value is from the overall ANOVA. Treatments
with the same letter are not significantly different ($p \leq$
0.05)-analyzed by Fisher's LSD procedure.
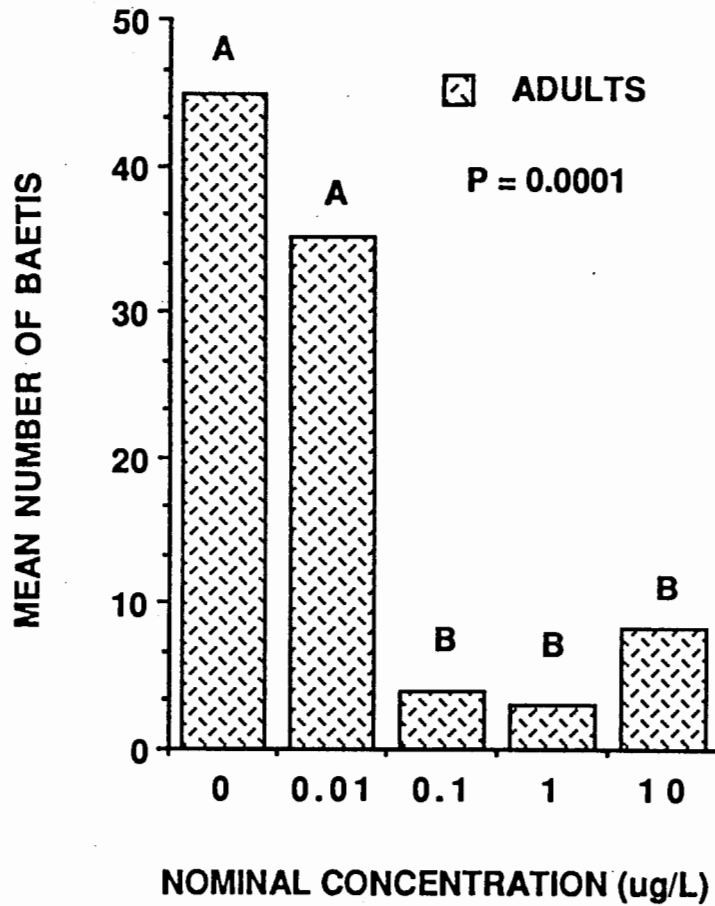
Figure 1.3. Mean number of *Baetis* spp. (Ephemeroptera) in stream microcosms exposed to fenvalerate. Analysis is based on combined densities of adults and immatures. P value is from the overall ANOVA. Treatments with the same letter are not significantly different ($p \leq 0.05$)-analyzed by Fisher's LSD procedure.
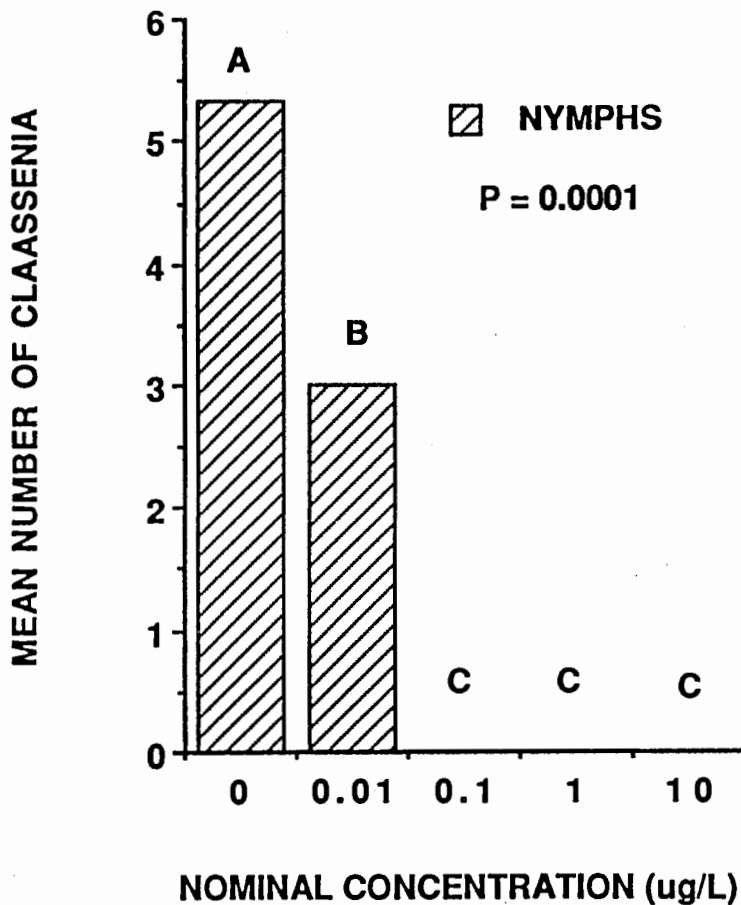
Figure 1.4. Mean number of *Claassenia sabulosa* (Plecoptera) in stream microcosms exposed to fenvalerate. Analysis is based on combined densities of adults and immatures. P value is from the overall ANOVA. Treatments with the same letter are not significantly different ($p \leq 0.05$)-analyzed by Fisher's LSD procedure.
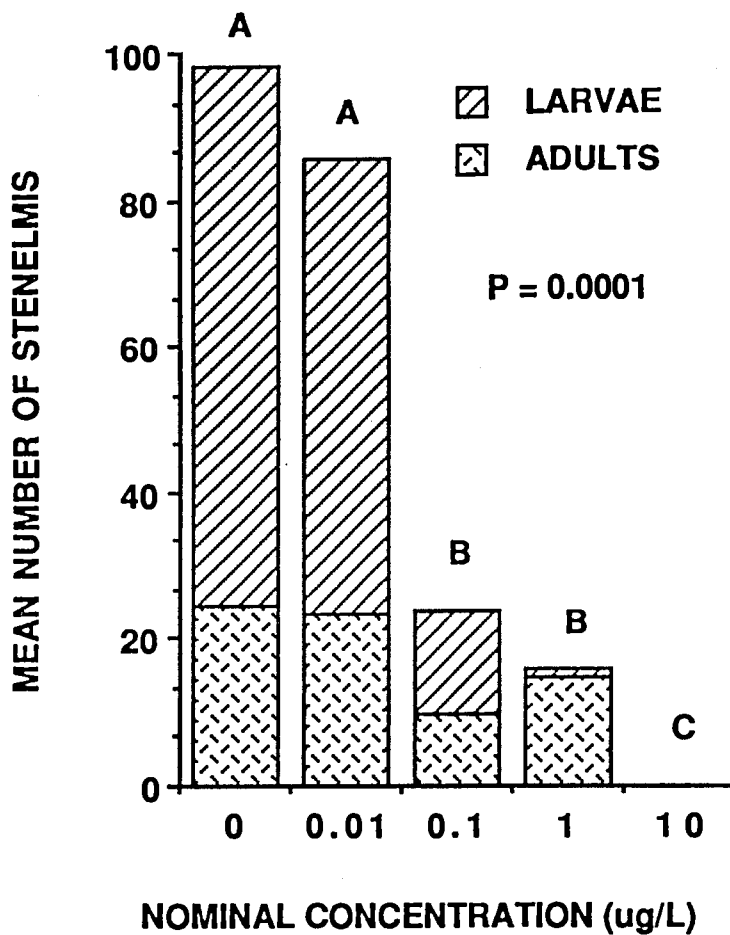
Figure 1.5. Mean number of *Stenelmis parva* (Coleoptera) in stream microcosms exposed to fenvalerate. Analysis is based on combined densities of adults and immatures. P value is from the overall ANOVA. Treatments with the same letter are not significantly different ($p \leq 0.05$)-analyzed by Fisher's LSD procedure.

spills indicate riffle beetles may be more tolerant of some environmental stressors than other stream insects [26,27].

As a group, caddisflies (Trichoptera) had the highest densities in the stream microcosms. Caddisflies exhibited an 8 d mass emergence beginning 13 d after the initial dose. The most abundant caddisfly, *Hydropsyche morosa* (Hydropsychidae), a species known to selectively colonize the artificial substrates used in this research [25], was significantly reduced in the 0.1 ug/L treatment (Figure 1.6). A separate analysis revealed a significant larval density reduction $(p \leq 0.05)$ in the 0.01 ug/L treatment. As presented, holometabolous "larvae" include both early to late instars and pupae. The few *H. morosa* remaining in the 0.1 ug/L and higher concentrations at the end of the test period were nearly all pupae. *Cheumatopsyche* sp. (Hydropsychidae) and *Chimarra* sp. (Philopotamidae) exhibited significant density reductions at 0.1 ug/L (Figure 1.7, 1.8). Separate analysis on *Chimarra* sp. revealed that the significant increase in "larvae" occurring from the 0.01 to 1.0 ug/L treatments were exclusively pupae. In addition, other caddisflies such as *Helicopsyche* sp. (Helicopsychidae) and *Pycnopsyche* sp. (Limnephilidae), present in the 0.1 ug/L and higher treatments, were also in the pupal stage. Holometabolous insects in the pupal stage appear to have been more tolerant to the fenvalerate concentrations tested, but as indicated by an increase in *Chimarra* sp. pupae at the
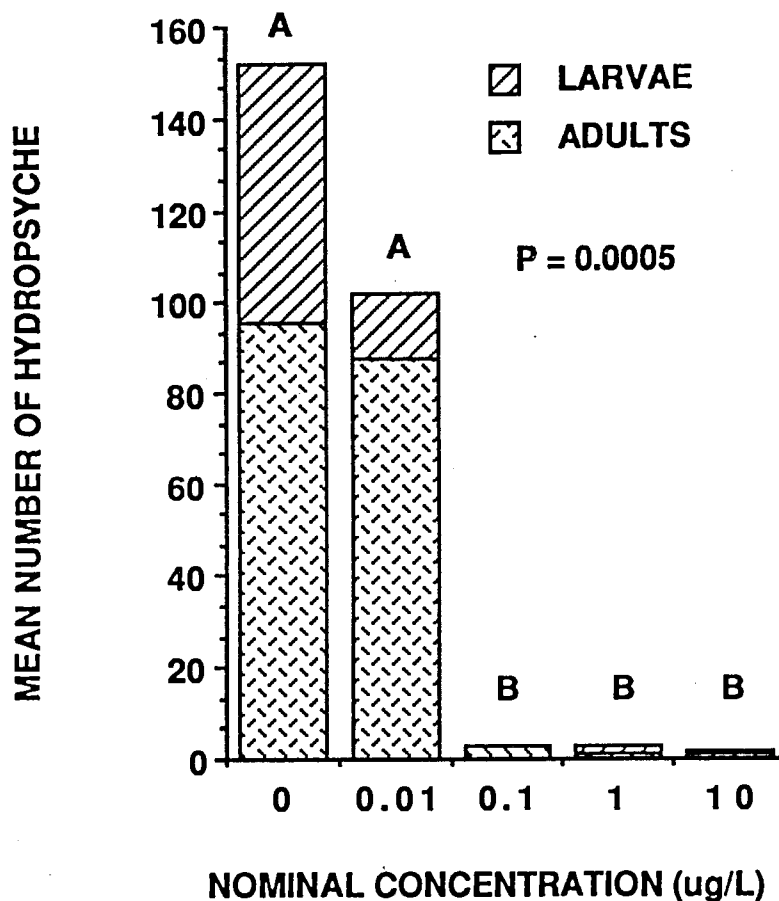
Figure 1.6. Mean number of *Hydropsyche morosa* (Trichoptera) in stream microcosms exposed to fenvalerate. Analysis is based on combined densities of adults and immatures. P value is from the overall ANOVA. Treatments with the same letter are not significantly different (*p* ≤ 0.05)-analyzed by Fisher's LSD procedure.
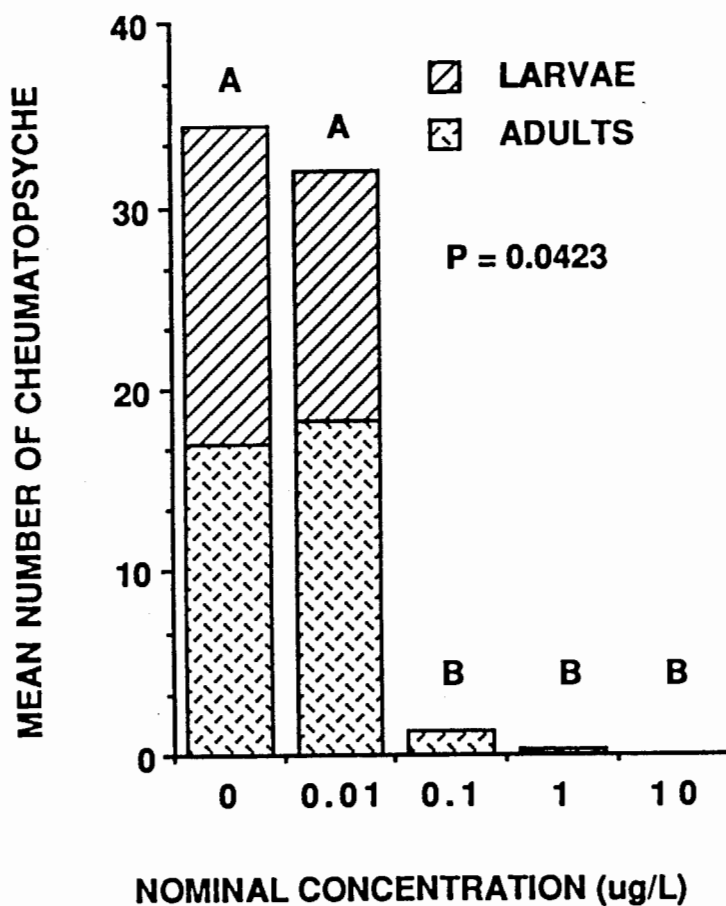
Figure 1.7. Mean number of *Cheumatopsyche* sp. (Trichoptera) in stream microcosms exposed to fenvalerate. Analysis is based on combined densities of adults and immatures. P value is from the overall ANOVA. Treatments with the same letter are not significantly different ($p \leq 0.05$)-analyzed by Fisher's LSD procedure.
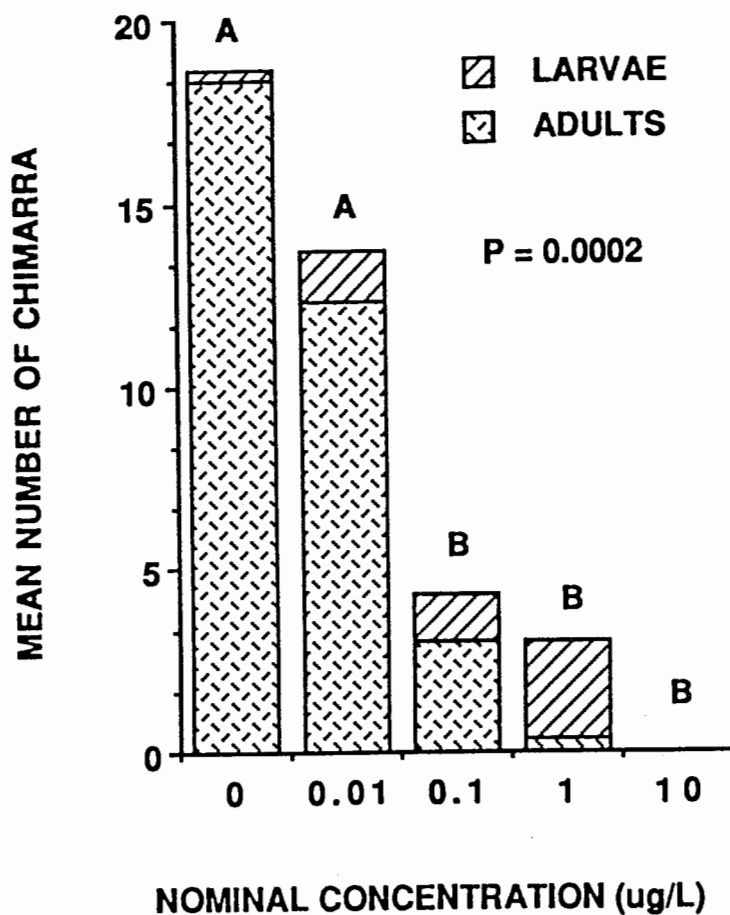
Figure 1.8. Mean number of *Chimarra* sp. (Trichoptera) in stream microcosms exposed to fenvalerate. Analysis is based on combined densities of adults and immatures. P value is from the overall ANOVA. Treatments with the same letter are not significantly different ($p \leq 0.05$)-analyzed by Fisher's LSD procedure.

higher concentrations, adult emergence may have been
inhibited.

Diptera were represented by five core taxa. Chironomid
midges from the subfamilies Chironominae and Orthocladiinae
were the most abundant Diptera. Chironominae and
Orthocladiinae densities were significantly reduced in the
1.0 ug/L and 0.1 ug/L treatments, respectively (Figure 1.9,
1.10). Tanypodinae (Chironomidae), *Simulium* sp.
(Simuliidae), and *Atherix Iantha* (Athericidae) were present
in low numbers and, because of high variability within
treatments, did not show a significant response to the
concentrations tested. However, other than a few adult
Simulium sp. and Chironomidae collected during the first 6 d
of testing, all Diptera were completely eliminated in the
10.0 ug/L treatments.

Previous research examining bioavailability of
synthetic pyrethroids to aquatic species reported that test
organisms accumulate greater levels when allowed to enter
sediments than when placed in the water column above the
sediments [e.g., 3]. The persistence of esfenvalerate in
littoral sediments was determined by Heinis and Knuth [20]
utilizing pond mesocosms. Sediment core samples contained
38.2 ng/goc esfenvalerate 1 d after application and 5.59
ng/goc after 354 d. Although benthic sediments in lentic
ecosystems are somewhat more stable than in lotic
ecosystems, the two habitats share somewhat similar benthic
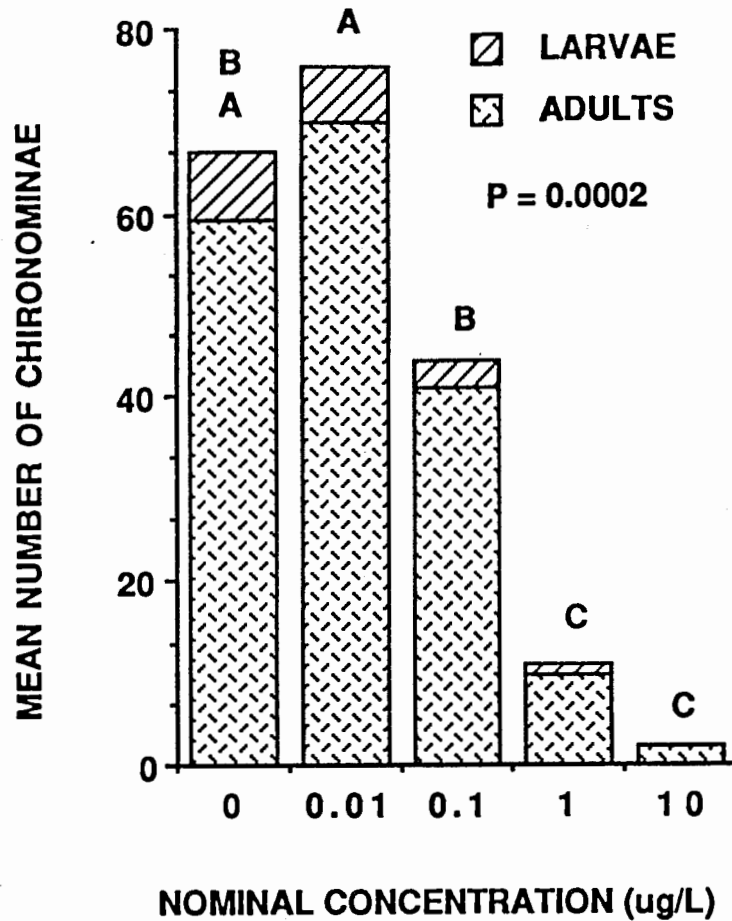
Figure 1.9. Mean number of Chironominae (Diptera) in stream microcosms exposed to fenvalerate. Analysis is based on combined densities of adults and immatures. P value is from the overall ANOVA. Treatments with the same letter are not significantly different ($p \leq 0.05$)-analyzed by Fisher's LSD procedure.
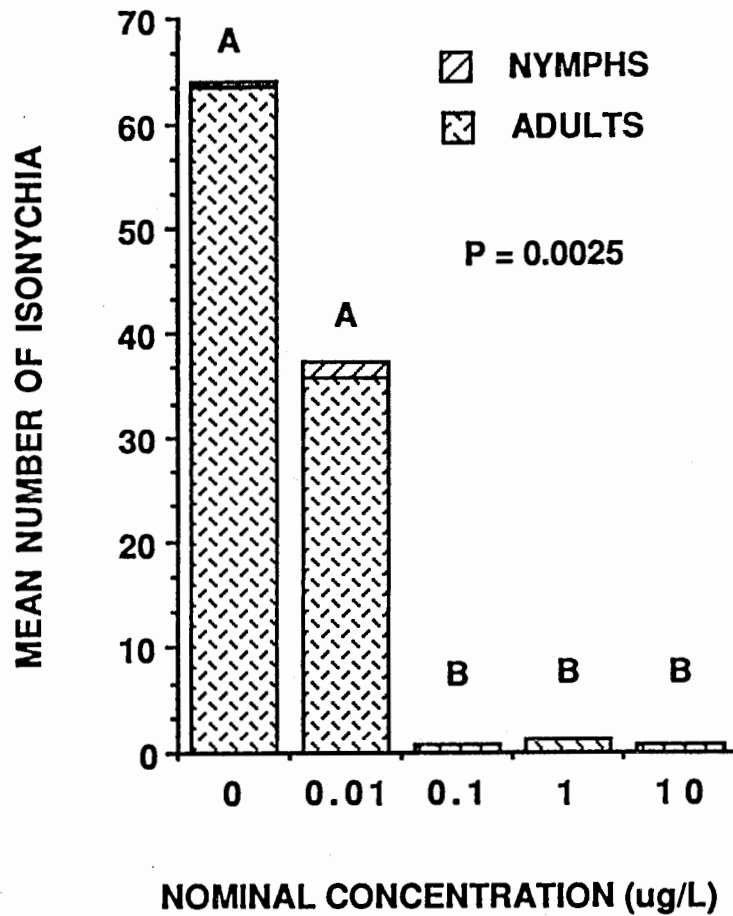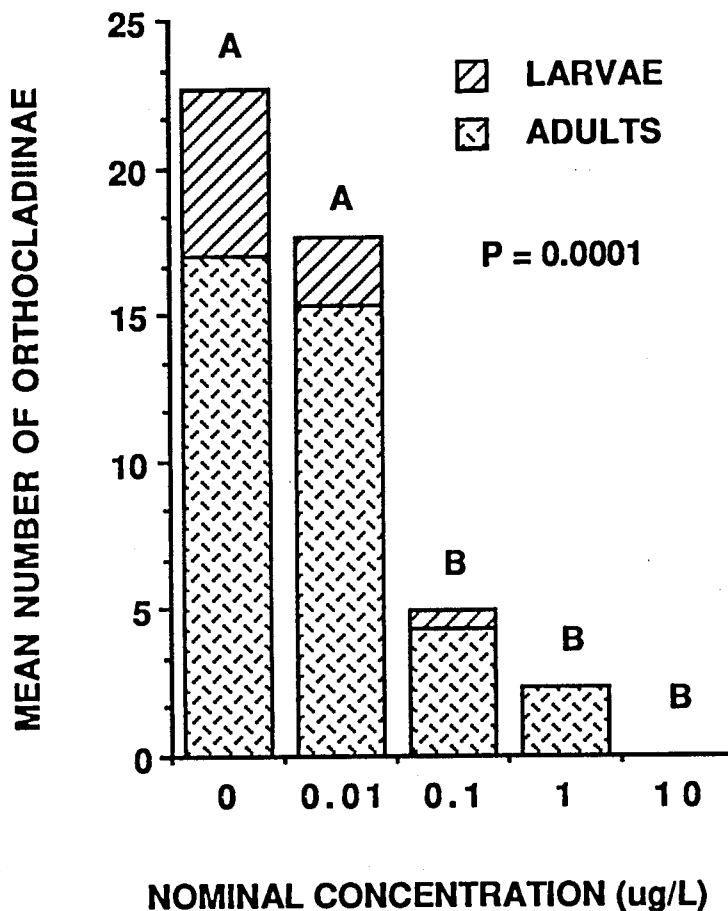
Figure 1.10. Mean number of Orthocladiinae (Diptera) in stream microcosms exposed to fenvalerate. Analysis is based on combined densities of adults and immatures. P value is from the overall ANOVA. Treatments with the same letter are not significantly different ($p \leq 0.05$)-analyzed by Fisher's LSD procedure.

and detrital communities. Therefore, the adsorption of fenvalerate by plants and sediments in pond mesocosms [19,20,28] may also occur in streams and rivers. As riffle insect communities utilize contaminated sediments and organic matter for case-building materials or nutrients, the ability of fenvalerate to biotransfer, directly or indirectly from these materials to subsequent trophic levels, becomes an important long-term environmental concern.

Conclusions

The impact of fenvalerate on lotic ecosystems depends on the magnitude and duration of exposure. Under actual field conditions, riffle insect communities would be exposed to fenvalerate during agricultural runoff, aerial drift, and accidental spills. Previous studies reported runoff from experimental plots contained concentrations ranging from 0.2 to 39.7 ug/L fenvalerate [29]. Aqueous concentrations in tidal creeks reached 0.106 ug/L fenvalerate following a 48 h low-intensity rainfall on cultivated fields [30].The initial fenvalerate pulse dose during this study simulated short-term exposures and indicated that concentrations above 1.0 ug/L fenvalerate will significantly increase drift from impacted areas.

Because most field exposures would be episodic events, the continuous fenvalerate exposures during this 30-d test

subjected riffle insect communities to worst case conditions. The difference between nominal and actual concentrations in the stream microcosms may have resulted from the chemical partitioning to organic matter or sediments present in the microcosms [c.f., 20,30]. Therefore, the riffle insect communities actually exhibited slightly greater sensitivity to fenvalerate than suggested by the nominal concentrations used in the discussion above. Following the 30-d test period density reductions were significant for *C. sabulosa* in the 0.01 ug/L treatment, making a community-level, no observable effect concentration (NOEC) for fenvalerate impossible to determine. However, as concentrations increased from 0.01 to 0.1 ug/L fenvalerate, eight core taxa experienced significant density reductions and *C. sabulosa* was completely eliminated. Except for a significant decrease in Chironominae densities, core taxa appeared unaffected by an increase from 0.1 to 1.0 ug/L fenvalerate. With the exception of a two *H. morosa* pupae, and solitary *S. parva*, *Pycnopsyche* sp., and Limoniinae (Diptera: Tipulidae) larvae remaining in the 10.0 ug/L microcosms following the 30-d test period, riffle insect communities were completely eliminated as nominal concentrations increased from 1.0 to 10.0 ug\L fenvalerate.

Streams and rivers are influenced by xenobiotic disturbances from the point of origin and continue downstream, essentially linking the entire system. Because

the type, intensity, and duration of pesticide exposures can potentially influence a multitude of biotic and abiotic factors, toxicity testing protocols for lotic ecosystems will be difficult to standardize. Toxicity tests that are applicable on a regional basis, provide environmentally realistic results, and are not economically inappropriate for the pesticide registration program must be considered. Laboratory-based, stream microcosm tests provide replicable, community-level predictions that are conveniently applied to site-specific communities under a variety of exposure conditions.

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CHAPTER TWO

AQUATIC MACROINVERTEBRATE COLONIZATION OF ARTIFICIAL SUBSTRATES FOR USE IN STREAM MICROCOSM TESTS

ABSTRACT

Colonized artificial substrates are utilized to stock stream microcosms with aquatic macroinvertebrates during multispecies toxicity tests. We investigated the minimum time period necessary for artificial substrates to reach equilibrium in terms of abundances and kinds of colonizing organisms.

Macroinvertebrate communities were colonized in rock-filled plastic containers secured to wooden frames previously anchored to the natural stream bottom. Five substrates were sampled weekly for seven weeks to determine abundances and number of taxa present.

Maximum species richness was reached by week one. Total density significantly increased ($p \leq 0.05$) during each of the first four sampling periods. A major flood event in week five reduced both species richness and total density. Eleven of fifteen core taxa reached density equilibrium on or before week three, suggesting a three week period is sufficient for colonizing an assemblage of macroinvertebrates for use in stream microcosm tests.

Keywords: Artificial substrates, Colonization, Streams, Macroinvertebrates

INTRODUCTION

The need to accurately predict aquatic ecosystem response to chemical exposure prompted the development of multispecies toxicity tests utilizing communities indigenous to potential receiving ecosystems (c.f., Cairns 1985, 1986). Artificial substrates previously colonized by riffle insect communities are often used to stock stream microcosms during multispecies toxicity tests (e.g., Clements *et al.* 1988; Pontasch *et al.* 1989; Pontasch and Cairns 1989, 1991). Rosenberg and Resh (1982) described several artificial substrate types and listed advantages and disadvantages of their use in benthic macroinvertebrate studies. Two disadvantages that limit the usefulness of artificial substrates in obtaining test organisms for multispecies toxicity tests include: 1) lengthy colonization periods increase the possibility of vandalism, spate, drought or burial; and 2) the time periods required for populations to reach equilibrium levels are generally unknown. An understanding of macroinvertebrate colonization dynamics is necessary to reduce colonization periods while providing an assemblage of organisms similar to natural stream communities. In addition, macroinvertebrate densities sufficient to statistically determine treatment responses are essential during stream microcosm toxicity tests.

Despite differences in total number of macroinvertebrates colonizing rock trays placed at the same

site during consecutive seasons, Clements *et al.* (1989)
observed macroinvertebrate densities leveling off within two
to three weeks each year and suggested that longer periods
would simply increase the possibility of sampling device
disturbance. Similarly, DePauw *et al.* (1986) found that a
three week colonization period was adequate for collecting
both early and late colonizers. However, Shaw and Minshall
(1980) reported that although the total number of colonizing
invertebrates leveled-off 32 days after artificial substrate
introduction, maximum densities of some taxa may not be
reached during a 30-day period. In addition, certain taxa
often selectively colonize artificial substrates (e.g.,
Minshall and Minshall 1977, Pontasch and Cairns 1989)
causing differences in proportional abundance for some taxa
when compared to natural substrate communities.

Variability in substrate complexity, current velocity,
and allochthonous inputs within lotic ecosystems causes
differences in biotic and abiotic conditions (e.g., Perry
and Schaeffer 1987), and influences riffle insect community
structure and function (Rabeni and Minshall 1977, Reice
1980). Therefore, optimal colonization periods for
artificial substrates at a given site are difficult to
predetermine. The purpose of this study was to: 1)
determine the minimum colonization period necessary to
maximize species richness and achieve equilibrium densities;
and, 2) compare the proportional contribution of various

functional feeding groups to determine if artificial
substrate communities reflect the natural stream community.

Materials and Methods

Study Area

The Volga River is a first to third order stream in
northeast Iowa that follows a ~120.0 km course to its
confluence with the Turkey River. During normal flow
conditions the stream at the colonization site was ~16.2 m
wide, ~22 cm deep, and current velocity was approximately 77
cm/s. Substrate was dominated by cobbles (6-13 cm) 50%
embedded in pebbles (2-6 cm). A major flood event occurred
during the fifth week of colonization. Although anchoring
techniques prevented artificial substrate loss, a
considerable amount of coarse sand and small pebbles (0.2-1
cm) were deposited in and around the artificial substrates.

Macroinvertebrate Colonization

Macroinvertebrates were colonized in rock-filled (2-6
cm) plastic containers (10.6 x 10.6 x 8.3 cm) with six
circular holes (12 mm dia.) in each side. The artificial
substrates were secured to wooden frames previously anchored
to the stream bottom with iron rods and concrete blocks.
Five substrates were randomly removed weekly for seven
consecutive weeks by placing a dip-net (mesh size 350
microns) behind the substrate as it was transferred to a
sampling bucket. The artificial substrate contents were

then strained through a sieve (mesh size 500 microns) and the remaining macroinvertebrates and debris were preserved in 70% ETOH. Natural substrate samples (Hess sampler) were also taken on week six to determine species richness and abundance of natural benthic organisms. The contents of both sample types were sorted by hand using a 2X magnification lens. With the exception of Chironomidae (Diptera), aquatic insects were identified to the lowest possible taxonomic unit using appropriate keys, and species-abundances per sample were determined. In addition, water chemistry (pH, conductivity, dissolved oxygen, hardness, alkalinity) and other stream parameters (temperature, depth, width, current velocity) were monitored at weekly intervals.

Data Analysis

Taxa with mean densities greater than three per artificial substrate in any week were considered a core taxon. Density differences among weeks for each core taxon were analyzed by one-way ANOVA followed by Fisher's least significant difference procedure (LSD) for the separation of means (see Appendix B). Species-abundance data from Hess samples were not directly compared to artificial substrate samples because of differences between surface area and volume of the two sample types. However, comparisons based on proportional contribution to total numbers by taxon and functional feeding group were examined using the functional classification described in Merritt and Cummins (1984).

Weekly functional group percentages were analyzed by one-way ANOVA, and Fisher's LSD procedure was employed to determine differences among weeks.

Results and Discussion

Artificial Substrate Colonization

Similar conditions existed in the stream throughout the seven week colonization period (Table 2.1). The artificial substrates were colonized by 28 insect taxa representing six orders. Species richness reached equilibrium by week one, was reduced in week five following the flood, and peaked in week six (Figure 2.1). Wise and Molles (1979) reported species accrual in duplicate wire baskets was most rapid during the first day and appeared to reach equilibrium by day nine. Clements et al. (1989) conducted two colonization studies during similar seasons in consecutive years. In the first study species equilibrium was reached by week two, but the following year species equilibrium was not reached until day 28. These studies suggest riffle insect communities can quickly reach species equilibrium, but colonization dynamics are subject to various site-specific and seasonal fluctuations. Due to the flood event during week five, the time period necessary for total macroinvertebrate abundance to reach equilibrium (i.e. maintain maximum densities in two consecutive weeks) could not be determined. Total macroinvertebrate abundance significantly increased each

Table 2.1. Mean current velocity, depth, and water chemistry data collected weekly from the Volga River, Iowa.

Parameter	Colonization Period (week)						
	1	2	3	4	5	6	7
Water Temp. (^{o}C)	24.0	26.0	23.0	22.0	18.5	19.0	21.0
DO (mg/L O_2)	8.9	N.A.	7.2	8.8	8.4	8.7	8.6
Conductivity (uMHOS)	600	550	525	580	600	580	600
pH [H^+]	8.0	8.3	8.0	6.6	8.0	8.0	8.0
Hardness (mg/L $CaCO_3$)	295	258	286	286	323	310	281
Alkalinity (mg/L $CaCO_3$)	N.A.	162	165	176	165	173	157
Mean Depth (cm)	21.7	20.3	20.0	21.2	45.6	26.7	18.3
Mean C.V. (cm/s)	73.3	44.1	50.5	54.9	85.3	78.3	64.7

N.A. = Not Available
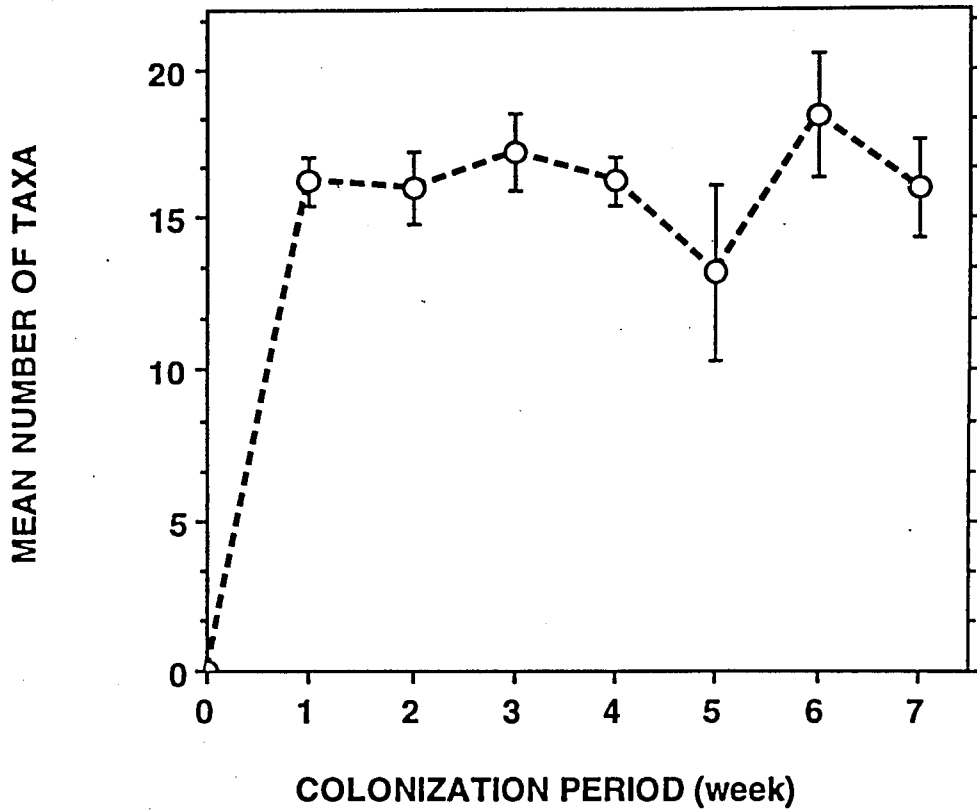
Figure 2.1. Mean number of taxa per artificial substrate during a seven week colonization study. Each point represents the mean ± standard deviation.

week for the first four sampling periods, but the flood event reduced total numbers below week one densities (Figure 2.2). The riffle insect community recolonized the artificial substrates in weeks six and seven to numbers similar to those obtained in weeks one and two, respectively. Fifteen core taxa from four orders were included in the statistical analysis. The densities of three core taxa apparently stabilized in week one and an additional eight core taxa reached density equilibrium by week three. Of the remaining core taxa, three taxa had not reached density equilibrium but were well established by week three. The final core taxon reached density equilibrium by week six. These findings suggests a three week artificial substrates colonization period is adequate in obtaining macroinvertebrate densities for use in stream microcosm tests. Separate analyses for each core taxa are listed in Table 2.2, and are discussed below.

The mayfly *Isonychia bicolor* (Ephemeroptera: Oligoneuriidae), a filter-feeder known to selectively colonize the substrates used in this research (Pontasch *et al.* 1989, Pontasch and Cairns 1989), significantly increased in numbers during each of the first four sampling periods. After near elimination from the artificial substrates in week five, *I. bicolor* steadily recolonized the substrates but had not regained preflood densities by week seven. Mayfly core taxa also included *Stenonema* spp.
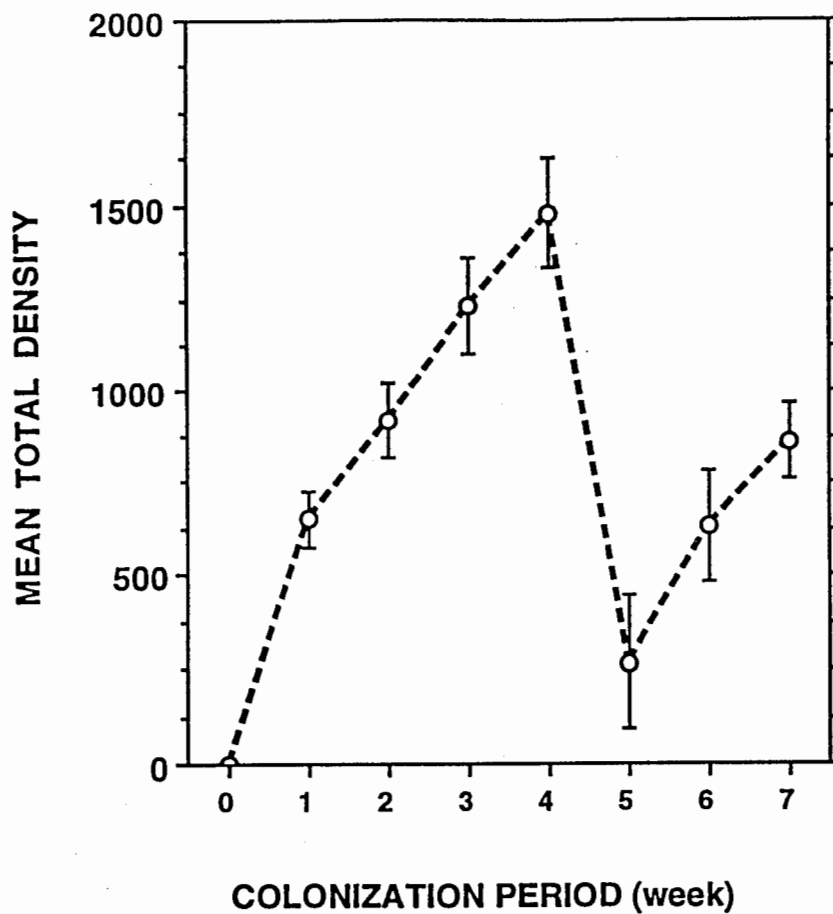
Figure 2.2. Total macroinvertebrate abundance per artificial substrate during a seven week colonization study. Each point represents the mean ± standard deviation.

Table 2.2. Core taxa colonizing artificial substrates at weekly intervals. Data are means followed by standard error. P value is from the overall ANOVA. Means with the same letter are not significantly different ($p \leq 0.05$) based on Fisher's LSD procedure. CF = collector filterers; CG = collector-gatherers; SC = scrapers; PR = predators.

Taxon				Colonization Period (week)				
Functional Group		1	2	3	4	5	6	7
P value								
Isonychia bicolor	DE	C	B	A	E		CD	B
(CF)	0.0001	15 ± 3.14	60 ± 6.59	152 ± 10.1	235 ± 13.6	5.0 ± 5.6	46 ± 10.3	121 ± 24.4
Stenonema spp.	D	CD	AB	A	D		CD	BC
(SC)	0.0002	4.4 ± 1.33	23 ± 5.70	61 ± 10.9	68 ± 9.59	2.4 ± 2.43	11 ± 2.02	37 ± 15.3
Baetis spp.	B	B	B	A	B		A	A
(CG)	0.0001	63 ± 10.1	155 ± 7.98	204 ± 11.9	238 ± 44.2	25 ± 5.94	193 ± 28.6	231 ± 28.6
Ephoron spp.	BC	A	AB	BC	C		C	C
(CG)	0.0007	8.8 ± 2.50	29 ± 5.84	20 ± 9.37	6.8 ± 3.07	1.4 ± 0.87	2.0 ± 0.71	2.0 ± 1.10

Table 2.2   cont.

Taxon Functional Group P value	Colonization Period (week)						
	1	2	3	4	5	6	7
Caenis tardata	D	CB	A	AB	CD	CD	D
(CG) 0.0001	7.6±1.67	30±7.04	51±12.4	44±5.22	13±4.74	15±1.76	8.4±2.14
Tricorythodes sp.	B	A	A	A	B	B	B
(CG) 0.0001	2.2±0.49	9.6±1.81	12±2.11	11±2.36	4.2±0.80	2.2±0.80	0.0±0.0
Stenelmis parva	BC	BC	B	BC	B	A	A
(CG) 0.0001	2.6±0.75	2.4±0.69	0.6±0.60	4.2±1.46	4.6±1.47	8.6±2.16	11±0.93
H. morosa	B	B	B	A	C	BC	B
(CF) 0.0001	199±21.4	240±5.25	281±49.4	431±48.8	54±27.1	106±17.0	201±15.3
Cheumatopsyche sp.	C	BC	AB	A	D	D	C
(CF) 0.0001	97±12.3	124±8.62	143±21.3	174±2.14	19±15.7	40±8.87	95±10.3
Chimarra sp.	C	BC	AB	A	BC	C	BC
(CF) 0.0388	4.8±2.22	9.8±2.18	19±6.02	23±9.00	9.8±2.96	3.8±0.37	6.0±2.19
Hydroptila sp.	A	B	BC	BC	D	CD	BCD
(SC) 0.0001	37±8.81	20±4.41	13±2.83	14±3.97	0.2±0.20	3.4±0.81	9.2±1.50

Table 2.2   cont.

Taxon Functional Group P value	Colonization Period (week)						
	1	2	3	4	5	6	7
Chironominae	BC	BC	A	ABC	BC	AB	BC
(CG) 0.0255	95±9.43	118±21.3	187±35.2	140±21.7	99±19.1	149±19.4	85±13.6
Orthocladiinae	A	A	A	A	B	B	AB
(CG) 0.0127	45±7.31	43±10.2	39±3.81	46±5.19	22±3.81	21±4.68	30±1.67
Tanypodinae	B	A	A	B	B	B	B
(PR) 0.0009	10±1.69	25±6.28	31±8.26	9.6±3.19	4.4±0.87	10±2.93	6.0±1.26
Simulium sp.	A	BC	BCD	AB	CD	CD	BCD
(CF) 0.0008	57±9.89	31±9.52	19±6.79	35±10.9	1.2±0.58	12±4.44	16±7.40

(Heptageniidae), a common scraper, represented by *S. terminatum* and *S. modestum*. Both species achieved equilibrium densities by week three, but were significantly reduced in week five and had not regained preflood numbers by week seven.

Collector-gathering mayflies common to the artificial substrates included *Baetis* spp. (Baetidae), *Ephoron* spp. (Polymitarcyidae), *Caenis tardata*. (Caenidae), and *Tricorythodes* sp. (Tricorythidae). The majority of *Baetis* spp. were *B. intercalaris*, but *B. flavistriga* and *B. armillatus* were also present. All *Baetis* spp. reached equilibrium densities by week four, but were significantly reduced by the flood the following week. *Baetis* spp. quickly recolonized the artificial substrates and regained density equilibrium by week six.

Burrowing mayflies including *Ephoron leukon* and *Ephoron album* reached density equilibrium by the second week of colonization, but numbers then began to steadily decline. *Ephoron* spp. densities following the flood were significantly lower than those recorded in weeks two and three, and remained in low numbers during subsequent sampling periods. *Tricorythodes* sp. and *Caenis tardata* reached density equilibrium in weeks two and three, respectively, and were significantly reduced by the flood event. Following the flood, *C. tardata* remained in low numbers but *Tricorythodes* sp. densities continued to decline

and no organisms were present in the artificial substrates by week seven. *Ephoron* spp., *C. tardata*, and *Tricorythodes* sp. are known to inhabit benthic silts and sediments (Merritt and Cummins 1984, Williams 1984, Hilsenhoff 1991), but successfully colonized artificial substrates containing minimal amounts of sediment at the beginning of this study. These taxa were then collected in reduced numbers following the flood when a habitat apparently more suitable for their existence was created as sediment was deposited in and around the substrates. The reduction in *Tricorythodes* sp. numbers in the artificial substrates may have been due to adult emergence; nymphs develop rapidly during the spring and early summer (Hilsenhoff 1991). *C. tardata* life cycles are poorly understood and explanations for their density reduction remain uncertain. However, the flood event may have reduced both *C. tardata* and *Tricorythodes* sp. densities in the natural substrate to levels that could not be reestablished by downstream drift or immigration from the hyporheic zone during subsequent sampling periods. The reduction in *Ephoron* spp. densities prior to the flood may have resulted from adult emergence or an inability to compete for nutrients and interstitial space in the artificial substrates. The later explanation appears more probable due to the large number of early instar nymphs collected in weeks two and three.

Riffle beetles (Coleoptera) were represented by a single core taxa, *Stenelmis parva* (Elmidae). Both larval and adult *S. parva* were present in low numbers during initial sampling periods and did not significantly increase until after the flooding event. Riffle beetles prefer the hyporheic zone (Williams 1984) and partial burial of the artificial substrates by sand and small pebbles provided this type of habitat. Greater riffle beetle densities due to partial burial of substrates was also reported by Pontasch and Cairns (1989).

The group of aquatic insects colonizing the artificial substrates in the highest densities were caddisflies (Trichoptera). *H. morosa* (Hydropsychidae) and *Cheumatopsyche* sp. (Hydropsychidae) reached equilibrium densities in weeks four and three, respectively. Both genera were significantly reduced by the flood in week five to densities below those achieved in week one. The hydropsychids steadily recolonized the substrates and by week seven densities were similar to those in week one. Pontasch and Cairns (1989) previously reported abundant hydropsychid colonization of rock-filled artificial substrates. *Chimarra* sp. (Philopotamidae), another collector-filtering trichopteran, was well established by week three and significantly reduced by the flood event in week five. *Chimarra* sp. numbers continued to decline and never regained preflood densities. A herbivorous caddisfly,

Hydroptila sp. (Hydroptilidae), reached equilibrium densities by week one and then steadily declined in subsequent weeks. The apparent success of hydroptilids as early colonizers in this study may have resulted from an abundance of early instars present in the drift. The first four hydroptilid instars are free-living and have no cases; the sedentary fifth instars construct retreats attached to stable, smooth-surface substrates (McAuliffe 1984). Therefore, early instar *Hydroptila* sp. are more likely to enter the drift, increasing the possibility of recolonization at downstream locations, and the *Hydroptila* sp. colonizing the artificial substrates during this study were primarily early instars. The significant reduction of Hydroptilid densities in week two may have been caused by the abundance of net-spinning hydropsychids competing for attachment sites for their retreats. *Hydroptila* sp. densities remained low throughout subsequent sampling periods.

The most abundant dipterans colonizing the artificial substrates were the chironomid subfamilies Chironominae, Orthocladiinae, and Tanypodinae (Chironomidae). Chironominae reached density equilibrium in week three, were significantly reduced by the flood, but regained preflood densities in week six. Orthocladiinae reached equilibrium densities in week one. Following a significant density reduction in week five, Orthocladiinae regained preflood

densities by week seven. Tanypodinae were well established by week two but a significant reduction occurred in week four prior to the flood. Tanypodinae remained in low numbers during subsequent sampling periods. Densities of another dipteran, the common black fly larvae *Simulium* sp. (Simuliidae), peaked by week one but densities were variable thereafter. *Simulium* sp. did experience a significant reduction in densities following the flood. Erman and Chouteau (1979) suggested that competition for substrate attachment between larger Simuliidae larvae and earlier instars results in the displacement of smaller organisms. Gersabeck and Merritt (1979) reported blackfly larvae colonized clean artificial substrates for approximately two weeks, then apparently vacated the substrates because accumulating materials (e.g., sediments, detritus, periphyton) hindered larval attachment. *Simulium* sp. larvae in this study were equally size classed in week one, but as densities decreased in subsequent weeks a majority of the remaining organisms were larger instars.

Natural and Artificial Substrate Comparisons

Natural and artificial substrate comparisons are based on the proportional abundances of four functional feeding groups including collector-filterers (CF), collector-gatherers (CG), scrapers (SC), and predators (PR). Hess sample data from week six suggest the artificial substrates did not provide a representative sample of some taxa in

terms of proportional abundances (Table 2.3). However, the
number and kinds of species collected from both types of
samples were identical. Collector-filterers from the
natural substrate in week six represented 34±8.05% of total
numbers, but mean CF percentages in the artificial
substrates over weeks one to four reached 55.0±5.11% (Table
2.3). Collector-gatherers in the natural substrate achieved
percentages (mean of 59±8.35%) substantially greater than in
the artificial substrates (mean over weeks 1-4 of
37.4±4.62%). Scraper percentages from the artificial
substrates were comparable to Hess sample data, with the
exception of weeks five and six, when densities were
significantly reduced ($p \leq 0.05$) in the artificial
substrates. Predators collected from the artificial
substrates remained in low numbers throughout the study
period and were not significantly different from natural
substrate samples.

Prior to the flood event, artificial substrates
projected into the water column and created habitats
structurally different from the natural substrate which was
dominated by small cobbles. Therefore, the artificial
substrates were probably exposed to greater quantities of
filterable organisms and detritus compared to the natural
substrate, and consequently, CF selectively colonized the
artificial substrates.

Table 2.3. Comparison of proportional contribution to total numbers by functional group. Data are mean percentages followed by standard deviation. P value is from the overall ANOVA. Mean percentages with the same letter are not significantly different ($p \leq 0.05$) based on Fisher's LSD procedure. CF = collector-filterers; CG = collector-gatherers; SC = scrapers; PR = predators.

P value Functional Group	1	2	3	4	5	6	7	Hess Sample
				Colonization Period (week)				
0.0001	AB	AB	B	A	C	C	AB	C
CF %	57±6.1	51±6.0	50±10.3	61±2.2	28±13.3	32±9.4	51±6.5	34±8.1
0.0001	C	C	C	C	A	AB	C	BC
CG %	34±6.6	42±4.5	41±7.9	32±4.3	69±12.9	63±8.7	42±7.8	59±8.4
0.0099	A	AB	A	A	C	C	A	AB
SC %	7±2.8	5±2.0	6±1.5	6±2.1	1±1.1	2±0.6	6±3.4	5±3.3
0.1076								
PR %	2±0.3	2±1.3	3±1.6	1±0.6	2±0.9	3±1.8	1±0.6	2±1.2

The partial burial of the artificial substrates by sediments deposited by the flood concealed the substrate interstices to some degree, thus reducing the amount of suspended nutrients available to silken nets or filtering appendages. Therefore, following the flood event, the artificial substrates were no longer selectively colonized by CF and resulted in substrate communities functionally more similar to the natural stream community.

Conclusions and Recommendations

Workers utilizing artificial substrates to obtain aquatic macroinvertebrates for testing purposes should consider shorter colonization periods to reduce potential disturbances. Although three dominant core taxa (*Baetis* spp., *I. bicolor*, and *H. morosa*) reached maximum densities after week three, all were well established by weeks one and two. Results from this study suggest artificial substrates colonized for three weeks will achieve maximum species richness and densities sufficient for determining treatment responses during a multispecies toxicity test. In addition, a three week colonization period may improve toxicity test results by colonizing taxa like *Simulium* sp., *Hydroptila* sp., and *Ephoron* sp., not abundant after longer periods, with densities sufficient for statistical analysis. However, utilizing short exposure periods will not safeguard artificial substrates from being selectively colonized by

certain taxa. Habitat conditions created by sediments deposited in and around the artificial substrates during the flood more closely simulated the natural substrate structure and significantly reduced selective colonization by CF. Riffle beetles numbers significantly increased following the flood, probably due to sediment deposition. This indicates that other CG insects common to the hyporheic zone (i.e. caenids, trichorythids) may be attracted to artificial substrates that more closely simulate natural benthic conditions. Artificial substrates colonized by aquatic macroinvertebrates will be more useful for stream microcosm studies when artificial substrate communities and natural stream communities achieve similar functional feeding group proportions. This research suggests that embedding artificial substrates in the natural stream bottom may result in more representative samples of the naturally occurring aquatic community and improve the predictive utility of stream microcosm tests.

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CHAPTER THREE

COLONIZING RIFFLE INSECT COMMUNITIES FOR STREAM

MICROCOSM STUDIES: DECREASING LOGISTICAL CONSTRAINTS

AND IMPROVING ENVIRONMENTAL REALISM

ABSTRACT

We compared macroinvertebrate colonization of embedded
and unembedded artificial substrates to determine which
technique results in proportional abundances most similar to
natural stream communities. In addition, artificial
substrates precolonized with periphyton were compared to
uncolonized substrates to determine if macroinvertebrate
colonization periods could be reduced.

Riffle insect communities were colonized in rock-filled
plastic containers with six circular holes in each side that
were either uncolonized or precolonized with periphyton.
These substrates were secured to embedded (~10 cm) and
unembedded trays previously anchored in a riffle area of the
Volga River, Iowa. Five substrates from each experimental
group were sampled weekly for six weeks.

Species equilibrium was reached by week one and
remained similar among all experimental groups in subsequent
weeks. Unembedded substrates precolonized with periphyton
had higher densities than uncolonized-unembedded substrates,
but the difference was only significant on week one.
Embedded substrates reduced selective colonization by
collector-filterers, but, because substrates placed in the

water column better simulated the large cobbles which dominated the colonization site, unembedded substrates colonized riffle insect communities functionally more similar to the natural stream community.

INTRODUCTION

The need for more accurate predictions of xenobiotic impacts on aquatic ecosystems prompted the development of multispecies toxicity tests, capable of modeling ecological processes such as succession, immigration, and nutrient cycling (e.g., Cairns, 1985; 1986). Currently, pond mesocosm studies are becoming standardized and may be required by the U.S. Environmental Protection Agency (US EPA) for registration of agricultural chemicals when single-species toxicity tests indicate potential hazards to aquatic ecosystems (Touart, 1988; SETAC, 1992). However, development and standardization of multispecies test procedures utilizing communities indigenous to streams and rivers has not progressed as rapidly. Artificial stream test systems currently available include outdoor mesocosms and indoor microcosms (c.f. Odum, 1984). Mesocosms are often expensive to construct, difficult to transport (therefore site-specific), and are susceptible to biological and climactic perturbation (Gillett, 1988). Laboratory-based microcosms provide a higher degree of investigator control and are easily adapted to evaluate various chemicals and/or particular trophic levels. However, laboratory-based systems require an assemblage of organisms derived from potential receiving ecosystems.

Riffle insects communities are frequently colonized in artificial substrates for use in stream microcosm toxicity

tests (e.g., Clements *et al.*, 1988; Pontasch & Cairns, 1991; Chapter 1). Colonization often requires lengthy exposure periods, increasing the possibility of natural and anthropogenic perturbation. In addition, selective colonization by some species can result in test communities that are not representative of natural communities (e.g., Rosenberg & Resh, 1982; Pontasch & Cairns, 1989; Chapter 2); thereby, reducing the applicability of test results.

One objective of this research was to determine if macroinvertebrate colonization periods could be reduced by precolonizing the artificial substrates with periphyton. Periphyton communities are of major importance to some macroinvertebrate functional feeding groups (Cummins, 1973; 1974; Minshall, 1978; Lamberti & Moore, 1984). Artificial substrates, initially placed in a sampling location, are probably not good habitats for aquatic insects because sufficient periphytic food resources are not present. Pratt *et al.* (1989) reported periphyton assemblages achieved species equilibrium on polyurethane foam artificial substrates within 7 to 21 d. Consequently, the time period required for periphytic colonization of artificial substrates may inhibit initial aquatic macroinvertebrate colonization, and therefore, lengthen colonization periods.

A second objective of this research was to determine if selective colonization by some functional feeding groups could be reduced by embedding the artificial substrates in

the natural substrate. Aquatic insects have adapted specialized feeding mechanisms to process nutrients available in freshwater ecosystems (c.f., Sweeney, 1984). For example, caddisfly larvae of the family Hydropsychidae, mayflies of the family Oligoneuriidae, and black fly (Simuliidae) larvae are collector-filtering insects common in North American streams and rivers (Merritt & Cummins, 1984). Collector-filtering insects utilize specialized body parts or construct silken nets to filter food from the water column. Collector-filtering insects are often abundant when both quality and quantity of seston are high, such as in lake outlets and below impoundments (e.g., Wallace & Merritt, 1980). Tricorythid and caenid mayflies (Ephemeroptera), riffle beetles (Coleoptera: Elmidae), and several midges (Diptera: Chironomidae), are collector-gathering aquatic insects common to North American streams (Merritt & Cummins, 1984). Collector-gatherers are often abundant in the hyporheic zone where detritus accumulates in substrate interstices.

Benthic community trophic structure is potentially sensitive to environmental stressors and alterations in macroinvertebrate functional feeding groups are often used to detect disturbances (e.g., Wallace *et al.*, 1986; Clements *et al.*, 1988). Community-level effects may not be detected when utilizing artificial substrates that are selectively colonized by tolerant organisms or if sensitive species are

collected in low densities. Previous research suggests that detrital content (Culp *et al.*, 1983) and artificial substrate positioning (Mason *et al.*, 1973) influences macroinvertebrate colonization. Artificial substrates are usually positioned in the water column, exposing substrate interstices to higher current velocities and suspended detrital particles (Rabeni & Minshall, 1977). The passage of filterable organisms and detritus through the sampling device attracts collector-filterers and limits colonization by collector-gatherers common to the hyporheic zone (Benfield *et al.*, 1974; Minshall & Minshall, 1977; Shaw & Minshall, 1980; Pontasch & Cairns, 1989). Deposition of sediments in and around artificial substrates during riffle insect colonization has been shown to alter community structure, reducing collector-filterers and attracting taxa associated with the hyporheic zone (Pontasch & Cairns, 1989; Chapter 2). It was hypothesized that embedding the artificial substrates would decrease the amount of filterable nutrients passing through the sampling device and reduce selective colonization by collector-filterers. In addition, an increase in detrital accumulation in the substrate interstices should improve colonization by collector-gatherers.

This study tested four artificial substrate experimental groups including: 1) precolonized-embedded

(PE); 2) precolonized-unembedded (PU); 3) uncolonized-embedded (UE); and 4) uncolonized-unembedded (UU).

Materials and Methods

Colonization Site

Periphyton and macroinvertebrate communities were colonized on artificial substrates placed in a riffle area of the Volga River, Iowa. The Volga River is a first to third order stream in northeast Iowa that follows a ~120.0 km course. During normal flow conditions the stream at the colonization site was 20 cm deep, 15 cm wide, and current velocity was 66 cm sec^{-1}. Water chemistry parameters were periodically monitored (mean values: DO = 9.4 mg l^{-1}, pH = 7.9, hardness = 258 mg l^{-1} CaCO$_3$, alkalinity = 155 mg l^{-1} CaCO$_3$, conductivity = 490 uS cm^{-1}) and remained stable throughout the study. The natural substrate, composed of limestone and other carbonate rocks, was dominated by large cobbles (13-25 cm) unembedded by pebbles (2-6 cm).

Periphyton Precolonization

The artificial stream system used for periphyton precolonization consisted of 15 oval artificial streams (1.7 x 0.24 x 0.13 m channel) constructed of molded fiberglass. Two 120 cm Durotest Vita-lites^R supplied daylight-equivalent lighting. A headbox system supplied water at 300 ml min^{-1}. and a 13 cm standpipe maintained a 59 l volume in each artificial stream. Current (25 cm s^{-1}) was provided by a

0.25 hp electric motor turning paddlewheels attached to an iron rod.

Periphyton was colonized in the field on 150, 25 cm^3 polyurethane foam units (PFUs). Following a 7 d colonization period, the PFUs were transported in an aerated cooler to the laboratory, their contents squeezed into a bucket, and the slurry strained through a sieve (mesh size 125 microns) to eliminate macroinvertebrates. The periphyton slurry (2 l) was added to each artificial stream and allowed to colonize rock (2-6 cm) filled plastic containers (10.6 x 10.6 x 8.3 cm) with six circular holes (18 mm dia.) in each side for six weeks.

Periphytic growth on cobbles in the natural source ecosystem was sampled throughout the precolonization period to determine natural periphytic biomass. In addition, five periphyton samples were collected weekly throughout the six-week precolonization period from initially sterile cobbles placed in the artificial streams. All periphyton samples were collected by scraping a 1.77×10^{-4} m^2, 9.62×10^{-4} m^2, or 1.96×10^{-3} m^2 surface area with a bristle brush and then filtering the contents onto glass-fiber filter paper. The surface area collected depended on periphytic growth and ability to filter samples. Filter papers were placed in glass vials and frozen until analyzed. Chlorophyll a concentrations were determined with one-half of the filter paper and biomass (ash-free dry weight) determined from the

other. Analyses for chlorophyll *a* content and biomass were conducted as described in *Standard Methods for the Examination of Water and Wastewater* (APHA *et. al*, 1985).

Macroinvertebrate Colonization

Five sets of two wooden trays placed on the stream bottom were alternately positioned between five sets of two trays embedded (~8-10 cm deep) in the natural stream substrate (Figure 3.1). Unembedded wooden trays resembled those used in previous research (c.f., Clements *et al.*, 1989; Pontasch & Cairns, 1991; Chapters 1 & 2). Embedded trays consisted of plastic containers (10.6 cm x 10.6 cm x 8.3 cm) with six circular holes (18 mm dia.) in each side attached to a board (10 cm x 120 cm). The embedded designed allowed rock-filled sampling baskets to be positioned inside identical containers previously embedded in the stream bottom. Ten embedded and ten unembedded wooden trays were anchored to the stream bottom with concrete blocks and iron rods.

Artificial substrates precolonized with periphyton were transported to the natural stream in aerated coolers. A total of 70 precolonized and 70 uncolonized artificial substrates were systematically placed in the embedded and unembedded wooden trays to ensure proper retrieval. Five substrates from each experimental group (PE, PU, UE, and UU) were randomly removed from the river at weekly intervals for six weeks by placing a dip-net (mesh size 200 microns)
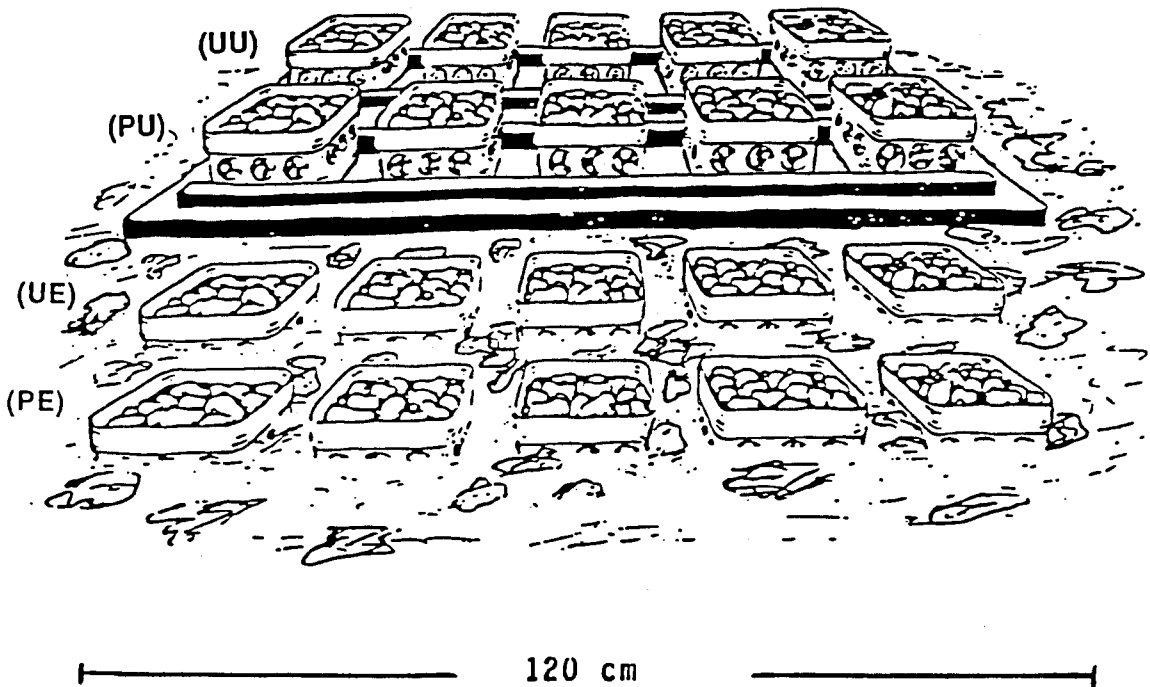
Figure 3.1. Schematic diagram of the four artificial substrate experimental groups: 1) precolonized-embedded (PE); 2) precolonized-unembedded (PU); 3) uncolonized-embedded (UE); and 4) uncolonized-unembedded (UU).

behind the substrate as it was transferred to a sampling
bucket. Artificial substrate contents were strained through
a sieve (mesh size 500 microns) and macroinvertebrates and
remaining debris were preserved in 70% ETOH. Six natural
substrate samples (Hess sampler) were taken on days 1 and 42
of colonization to determine abundance of benthic organisms
in the natural substrate. All samples were sorted by hand
using a 2X magnification lens. With the exception of
Chironomidae (Diptera), which were identified to subfamily,
aquatic insects were identified to genus or species, size
classed, and enumerated.

Data Analysis

Taxa with mean densities greater than four in any
experimental group on any sampling date were considered a
core taxon. ANOVA was used to determine density differences
between experimental groups. Fischer's least significant
difference (LSD) procedure was employed for the separation
of means (see Appendix C).

Species-abundance data from Hess samples were not
directly compared to artificial substrate samples because of
differences in surface area and volume of the two sample
types. However, comparisons based on proportional
contribution to total numbers by functional group were
examined using the functional group classification described
in Merritt & Cummins (1984). Differences in functional

group proportions were determined by ANOVA followed by Fisher's LSD test for the separation of means.

Results and Discussion

Periphytic Biomass and Chlorophyll *a*

The artificial streams seeded with periphyton were not capable of producing periphytic biomass and chlorophyll *a* content equal to the natural source ecosystem. Following the six-week precolonization period, periphyton appeared well established in the stream microcosms, but, a significant difference ($p \leq 0.05$) remained between the two periphytic communities. The natural periphytic community contained a mean chlorophyll *a* content and ash-free dry weight (AFDW) of 331.2 $\pm$ 133.6 and 25,806.5 $\pm$ 13,709.1 mg m^{-2}, respectively. Periphyton sampled from the artificial streams contained a mean chlorophyll *a* content of 3.7 $\pm$ 2.0 mg m^{-2} and an AFDW of 1,201.9 $\pm$ 684.8 mg m^{-2}. Although the artificial streams did not establish periphyton biomass equal to the natural source ecosystem, the periphyton precolonized on the artificial substrates appeared to have provided a sufficient food source for riffle insects (see below).

Macroinvertebrate Colonization

Species equilibrium. Species equilibrium in all artificial substrate types was reached by week one (Figure 3.2). PE substrates were colonized by a slightly greater number of taxa toward the end of the study, but the
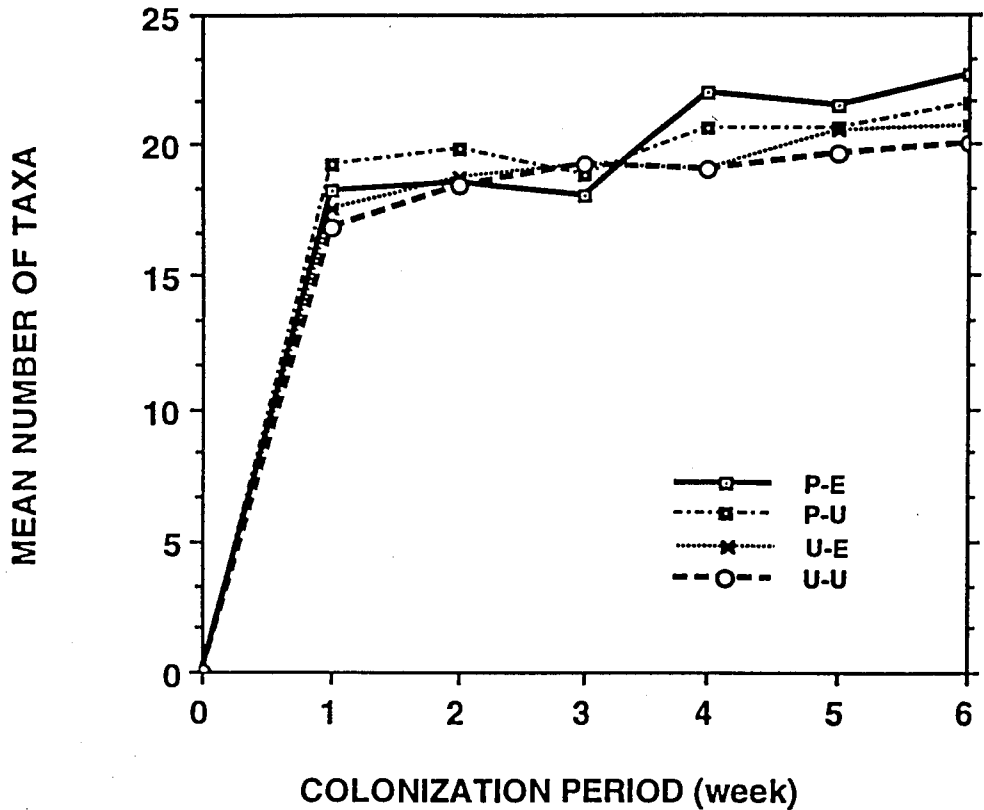
Figure 3.2. Mean number of taxa per substrate colonizing each experimental group over a six week colonization period. PE = precolonized-embedded; PU = precolonized-unembedded; UE = uncolonized-embedded; UU = uncolonized-unembedded.

difference was only significant ($p \leq 0.05$) in week four, and only between the two substrate types not precolonized with periphyton.

Effects of precolonizing substrates. During the six week macroinvertebrate colonization period, 43 taxa representing nine orders were collected from the artificial substrates. Total macroinvertebrate density in the PU substrates was consistently higher than the UU substrates. However, the difference was significant ($p \leq 0.05$) only for week one (Figure 3.3). In week one, precolonized periphyton apparently provided the PU substrates with a food source capable of attracting macroinvertebrate colonists. By week two, natural periphytic communities appeared to be well established on the UU substrates and macroinvertebrates reached densities similar to the PU substrates. These results indicate precolonizing substrates with periphyton may be beneficial if a one week macroinvertebrate colonization period is desired. No significant differences in total macroinvertebrate density occurred between the PE and UE substrates throughout the study. Eighteen taxa reached densities sufficient to be considered core taxa (mean $\geq$ 4 individuals per substrate), and separate analyses for each are reported below.

The mayflies (Ephemeroptera) were represented by eight core taxa (Table 3.1). PU substrates were consistently colonized by greater *Isonychia bicolor* (Oligoneuriidae)
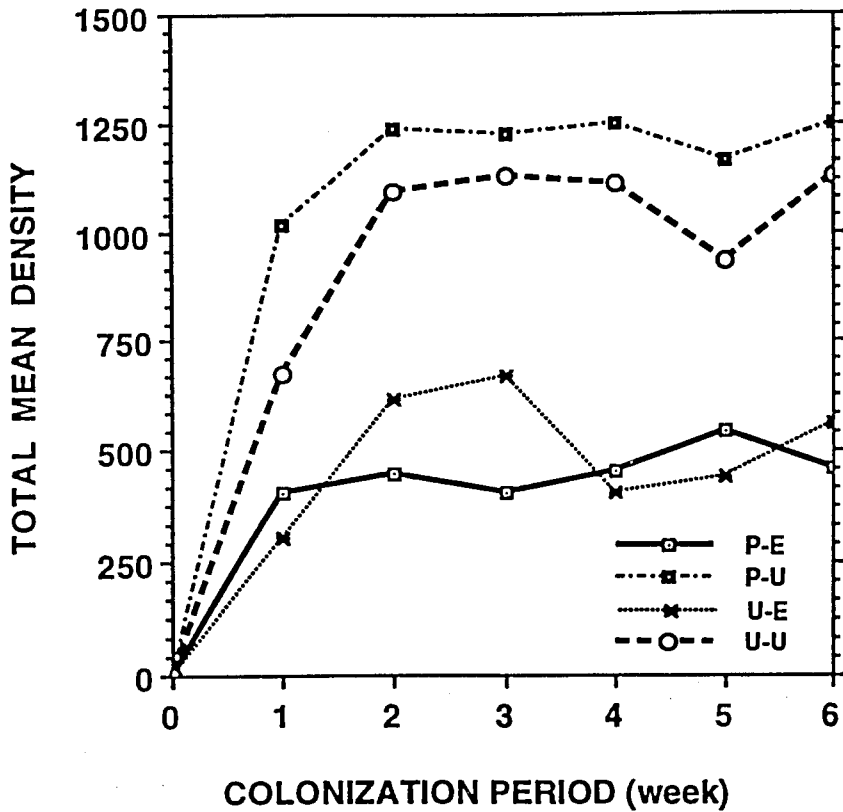
Figure 3.3. Mean macroinvertebrate abundance per substrate from each experimental group over a six week colonization period. PE = precolonized-embedded; PU = precolonized-unembedded; UE = uncolonized-embedded; UU = uncolonized-unembedded.

Table 3.1. Ephemeroptera core taxa colonizing four artificial substrate types at weekly intervals. Data are means followed by standard error. Feeding groups: CF = collector filterers; CG = collector-gatherers; SC = scrapers. Substrate types: PU = precolonized unembedded; UU = uncolonized unembedded; PE = precolonized embedded; UE = uncolonized embedded.

Taxon	Substrate type	Colonization Period (week)					
Feeding Group		1	2	3	4	5	6
Isonychia	PU	108±9.22	144±31.6	139±4.93	220±37.1	222±29.7	211±46.5
bicolor	UU	74±13.7	77±11.1	157±8.56	197±17.1	178±27.6	205±23.6
(CF)	PE	55±16.8	58±15.1	27±4.73	46±21.3	53±26.1	45±20.6
	UE	66±20.6	119±30.9	94±28.4	56±37.4	38±14.5	68±36.1
Baetis	PU	156±19.1	238±31.6	156±4.93	138±37.1	46±29.7	27±46.5
intercalaris	UU	102±18.2	185±29.9	154±33.1	122±20.4	35±12.2	33±6.39
(CG)	PE	51±11.9	44±15.2	11±4.40	20±5.00	12±5.00	5.8±2.46
	UE	24±3.40	78±9.44	29±10.8	14±5.81	6.3±2.10	4.8±1.44

Table 3.1 cont.

Taxon Feeding Group	Substrate Type	Colonization Period (week)					
		1	2	3	4	5	6
Baetis	PU	23±5.33	5.3±0.48	6.0±1.82	3.0±1.91	4.3±1.31	17±7.55
armillatus	UU	14±3.61	4.8±2.50	5.5±1.26	7.3±1.89	5.5±2.22	13±2.22
(CG)	PE	7.3±1.56	4.3±2.84	3.8±1.80	5.8±1.89	5.0±1.68	8.8±1.49
	UE	0.8±0.48	4.0±1.35	2.5±0.96	3.3±1.03	4.5±1.89	3.3±1.70
Baetis	PU	9.0±1.87	2.0±0.41	1.0±1.00	15±3.85	6.0±2.16	0.3±0.25
flavistriga	UU	7.5±4.63	5.8±0.85	12±3.07	11±5.00	11±7.77	8.0±3.56
(CG)	PE	3.5±1.55	0.5±0.50	0.3±0.25	5.5±3.52	1.0±0.58	2.3±0.25
	UE	2.0±1.35	1.8±1.44	3.3±1.60	2.8±1.38	3.3±2.25	2.3±1.31
Stenonema	PU	14±4.13	25±8.56	105±17.0	145±15.8	138±21.6	182±40.4
terminatum	UU	14±2.40	35±9.65	136±20.1	154±25.6	154±29.3	184±24.6
(SC)	PE	35±3.23	43±10.7	72±7.05	91±17.0	111±29.0	82±19.2
	UE	23±4.67	45±7.34	120±26.1	65±24.5	93±14.3	92±22.4

Table 3.1   cont.

| Taxon Feeding Group | Substrate Type | Colonization Period (week) ||||||
		1	2	3	4	5	6
Stenonema	PU	3.5±0.65	14±8.46	14±6.80	21±5.07	46±12.0	48±3.16
modestum	UU	4.3±1.18	3.5±0.96	10±2.17	34±5.54	35±10.3	36±10.6
(CG)	PE	18±3.75	15±3.90	23±2.90	43±4.43	39±6.36	46±4.59
	UE	8.0±1.08	16±2.83	40±7.14	27±8.59	34±4.84	32±7.89
Caenis	PU	7.5±1.94	31±1.49	43±14.4	14±3.78	8.5±3.30	4.0±2.12
tardata	UU	5.0±1.78	23±6.49	34±6.83	20±4.77	8.5±3.40	8.0±2.94
(CG)	PE	33±7.98	57±8.77	50±11.0	28±6.56	16±2.53	7.8±1.55
	UE	21±5.96	30±4.02	59±7.31	17±2.25	11±2.04	8.3±2.32
Tricorythodes	PU	2.5±0.65	6.0±0.91	11±4.12	3.8±1.03	1.8±1.18	1.8±0.48
sp.	UU	2.3±0.48	10±4.64	4.0±2.04	0.0±0.0	0.8±0.48	0.3±0.25
(CG)	PE	10±1.96	20±8.29	8.3±1.65	5.3±0.63	2.0±0.71	2.5±1.50
	UE	3.8±2.06	11±4.53	6.0±2.16	4.8±1.25	2.5±0.65	1.3±0.48

densities than UU substrates. However, the differences were not statistically significant. *Baetis* spp. (Baetidae) were dominated by *Baetis intercalaris*, but also included *Baetis armillatus* and *Baetis flavistriga*. PU substrates were colonized by greater *B. intercalaris* densities than the UU substrates, but the difference was significant only for week one. Scraping mayflies included *Stenonema terminatum* and *Stenonema modestum* (Heptageniidae). *S. terminatum* colonized the PE substrates with significantly greater densities than the UE substrates in week one, but numbers were similar in subsequent weeks. *S. modestum* was not significantly influenced by the periphyton precolonization. By week two, *Caenis tardata* (Caenidae) densities in the PE substrates were significantly greater than UE substrates, but no differences among substrate types occurred in subsequent weeks. *Tricorythodes* sp. (Tricorythidae) numbers in the PE substrates were greater than the UE substrates for the first three sampling periods, although the differences were significant only in week one.

Stoneflies (Plecoptera) were present in low numbers and the only core taxon, *Claassenia sabulosa* (Perlidae), showed no significant response to periphyton precolonization (Table 3.2).

Stenelmis parva (Coleoptera: Elmidae) densities increased gradually throughout the six-week colonization period (Table 3.2). Previous research indicated a steady

Table 3.2. Plecoptera, Coleoptera, and Trichoptera core taxa colonizing four artificial substrate types at weekly intervals. Data are means followed by standard error. Feeding groups: CF = collector filterers; CG = collector-gatherers; SC = scrapers; PR = predators. Substrate types: PU = precolonized unembedded; UU = uncolonized unembedded; PE = precolonized embedded; UE = uncolonized embedded.

Taxon Feeding Group	Substrate type	Colonization Period (week)					
		1	2	3	4	5	6
Claassenia	PU	1.3±0.75	2.0±0.91	5.5±0.96	6.8±2.39	4.3±0.95	9.3±3.28
sabulosa	UU	0.5±0.29	4.0±1.78	5.0±1.29	4.8±1.11	7.8±1.11	7.5±2.40
(PR)	PE	1.8±1.44	4.3±1.25	4.5±1.32	2.3±0.85	3.8±1.25	1.5±0.29
	UE	2.8±0.63	3.8±0.63	5.3±2.69	1.8±0.75	2.3±1.11	2.8±0.75
Stenelmis	PU	0.0±0.0	0.0±0.0	1.8±1.03	2.3±1.11	3.0±1.08	3.3±1.70
parva	UU	0.0±0.0	0.8±0.48	2.0±1.15	2.5±0.96	4.3±1.89	4.5±2.33
(CG)	PE	2.0±0.71	1.8±0.85	4.5±0.96	9.8±4.40	14±3.20	11±1.03
	UE	0.5±0.50	1.3±0.63	8.5±2.18	6.8±3.09	8.8±2.75	9.3±2.75

Table 3.2 cont.

Taxon Feeding group	Substrate Type	Colonization Period (week)					
		1	2	3	4	5	6
Hydropsyche	PU	370±26.5	466±50.1	392±90.6	331±77.3	341±62.6	321±35.7
morosa	UU	153±42.3	307±77.4	300±82.7	313±87.2	328±99.7	235±50.6
(CF)	PE	93±37.6	62±22.5	71±6.41	58±12.5	72±26.7	67±16.6
	UE	71±14.5	153±51.7	104±29.3	65±12.2	68±28.2	76±30.0
Cheumatopsyche	PU	63±11.8	83±6.42	121±32.1	138±11.9	165±23.1	242±24.3
sp.	UU	56±11.9	79±4.85	101±12.5	114±14.9	139±21.7	195±16.5
(CF)	PE	39±14.0	41±18.0	58±14.7	39±10.6	109±36.0	92±28.3
	UE	32±6.60	58±12.6	97±15.0	43±6.12	83±22.2	138±48.3
Chimarra	PU	12±2.39	37±6.42	33±17.1	66±20.8	74±31.3	83±15.2
sp.	UU	7.3±3.45	11±4.60	28±9.39	35±6.94	33±9.13	58±15.3
(CF)	PE	4.3+2.50	5.0±2.80	4.8±2.84	11±9.31	20±11.7	6.5±2.75
	UE	3.3±1.11	5.0+3.11	14±6.66	12±10.8	7.0+4.14	32±24.5

Table 3.2 cont.

Taxon Feeding group	Substrate Type	Colonization Period (week)					
		1	2	3	4	5	6
Hydroptila	PU	22±8.72	6.3±2.78	11±5.92	18±2.66	17±3.68	31±3.04
sp.	UU	19±4.12	8.8±4.11	16±5.91	19±5.92	14±3.99	18±2.71
(SC)	PE	1.5±0.87	1.3±0.48	0.8±0.48	2.5±0.65	7.0±4.08	6.8±1.38
	UE	2.0±0.41	1.0±0.71	2.8±1.11	5.0±1.87	11±5.11	15±6.59

increase in elmid beetle densities also occurred throughout
a 64-d colonization period (Shaw & Minshall, 1980).
Although collected in low numbers, *S. parva* densities in the
PE substrates were slightly higher than the UE substrates in
week one, but densities were similar in subsequent weeks.

Four core taxa represented the caddisflies
(Trichoptera). *Hydropsyche morosa* (Hydropsychidae) and
Chimarra sp. (Philopotamidae) densities in the PU substrates
were consistently greater than the UU substrates. However,
the differences were significant only in week one for *H.
morosa* and week two for *Chimarra* sp. (Table 3.2).
Cheumatopsyche sp. (Hydropsychidae) numbers were not
significantly affected by periphyton precolonization.
Hydroptila sp. (Hydroptilidae) densities in the PU
substrates were consistently greater than the UU substrates,
but the difference was significant only on week six.

Diptera were represented by five core taxa and did not
respond significantly to periphyton precolonization (Table
3.3). Overall, precolonizing substrates with periphyton
increased total macroinvertebrate densities in week one, and
did not appear to result in selective colonization by any
functional feeding groups. By week two, apparently due to
the colonization of all substrate types by natural
periphytic communities, precolonized substrates no longer
attracted greater macroinvertebrate abundances.
Consequently, the advantages associated with substrates

Table 3.3. Diptera core taxa colonizing four artificial substrate types at weekly intervals. Data are means followed by standard error. Feeding groups: CF = collector filterers; CG = collector-gatherers; SC = scrapers; PR = predators. Substrate types: PU = precolonized unembedded; UU = uncolonized unembedded; PE = precolonized embedded; UE = uncolonized embedded.

Taxon	Substrate type	Colonization Period (week)					
Feeding Group		1	2	3	4	5	6
Chironominae	PU	37±7.49	117±14.1	70±6.08	82±14.4	35±5.12	29±8.01
(CG)	UU	32±10.7	122±18.8	75±6.07	90±22.7	24±2.25	26±3.49
	PE	25±5.12	40±8.98	23±11.6	35±11.3	32±10.9	25±5.49
	UE	16±1.97	46±8.63	34±6.75	37±9.41	26±5.52	18±4.91
Orthocladiinae	PU	16±4.19	31±5.17	54±12.4	27±3.10	8.8±1.80	12±2.75
(CG)	UU	17±1.04	27±8.61	41±3.07	24±4.77	8.0±2.12	13±2.29
	PE	18±6.24	34±4.33	25±4.63	23±3.47	17±2.50	14±1.68
	UE	18±3.90	28±5.79	33±7.31	17±4.84	12±4.14	15±2.78

Table 3.3 cont.

Taxon Feeding Group	Substrate Type	Colonization Period (week)					
		1	2	3	4	5	6
Tanypodinae	PU	4.8±1.03	22±5.88	34±6.16	42±3.81	22±5.99	39±7.77
(PR)	UU	6.8±1.49	26±8.04	28±5.23	45±6.55	13±1.11	39±4.71
	PE	8.8±4.52	13±3.19	8.8±4.01	24±7.56	24±13.9	28±3.50
	UE	3.5±0.87	7.3±0.25	16±5.11	26±6.41	26±7.72	38±12.2
Simulium	PU	160±10.0	59±20.8	12±10.1	4.8±2.43	1.8±0.85	4.8±2.56
sp.	UU	175±60.5	83±58.2	8.5±3.80	2.5±0.87	1.0±0.41	10±8.94
(CF)	PE	1.0±0.71	0.3±0.25	7.3±7.25	0.3±0.25	0.0±0.0	0.0±0.0
	UE	6.0±5.02	4.5±4.17	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

precolonized with periphyton are only applicable during exposure periods of one week or less.

Effects of embedding substrates. Total macroinvertebrate densities in the unembedded substrates were significantly higher ($p \leq 0.05$) than embedded substrates on each sampling date (Figure 3.3). However, densities of most taxa in the embedded substrates were sufficient by week one to determine treatment responses during stream microcosm studies. Comparisons between core taxa colonizing embedded and unembedded substrates were analyzed separately and the results are listed below.

Isonychia bicolor colonized the PU and UU substrates with significantly greater densities than the PE and UE substrates on weeks four through six (Table 3.1). *Baetis intercalaris* colonized the PU and UU substrates with greater densities than the PE and UE substrates throughout the study, but the difference was significant only during the first four sampling periods (Table 3.1). *Stenonema terminatum* had colonized the PU and UU substrates with significantly greater numbers than the PE and UE substrates by week four, and by week six PU and UU densities were double those in the PE and UE substrates (Table 3.1). *Stenonema modestum* colonized the PE and UE type substrates with greater densities than the PU and UU substrates (Table 3.1), but the difference was significant only on weeks one and three. *Caenis tardata* and *Tricorythodes* sp. possess

morphological characteristics advantageous for interstitial, hyporheic habitats (Williams, 1984). However, density differences between the embedded and unembedded substrates were significant only in week one as PE substrates achieved greater densities than the unembedded substrates (Table 3.1).

Claassenia sp. densities in the PU and UU substrates were greater than the PE and UE substrates during the last three sampling periods (Table 3.2). However, the difference was significant only in week five between the UU substrates and those substrates embedded in the stream bottom.

Riffle beetles are common hyporheic inhabitants (Merritt & Cummins, 1984; Williams, 1984) and, although collected in low numbers, *Stenelmis parva* colonized the PE and UE substrates in consistently greater numbers than the PU and UU substrates (Table 3.2).

The four caddisfly core taxa (*Hydropsyche morosa, Cheumatopsyche* sp., *Chimarra* sp., and *Hydroptila* sp.) colonized the PU and UU substrates with greater densities than the PE and UE substrates (Table 3.2). However, *H. morosa* was the only caddisfly to colonize the unembedded substrates with significantly greater densities than the embedded substrates throughout the entire study. The selective colonization of unembedded substrates by net-spinning caddisflies has been reported in previous research (Pontasch & Cairns, 1989; 1991).

Midge larvae from the family Chironomidae were the most abundant Diptera colonizing the artificial substrates. Chironominae in the PU and UU substrates maintained significantly greater densities than in the PE and UE substrates until week four (Table 3.3). Similarly, Tanypodinae showed a preference for the PU and UU substrates, but the difference was only significant in week three. Densities of Orthocladiinae were not significantly different in the embedded and unembedded substrates. The response of black fly larvae (Simuliidae) was more variable in the PU and UU substrates than in the PE and UE substrates (Table 3.3). *Simulium* sp. densities in the unembedded substrates were significantly greater than the embedded substrates in weeks one and two, and larval densities in all substrate types were reduced in subsequent weeks.

Overall, taxa known to selectively colonize artificial substrates (i.e. Hydropsychidae, Oligoneuriidae, Simuliidae) were collected in lower densities in the embedded substrates than in the unembedded substrates, and taxa associated with the hyporheic zone were collected in slightly greater densities than in the unembedded substrates.

Core Taxa Reaching Equilibrium Densities

Core taxa maintaining maximum densities for two consecutive weeks were considered to have reached density equilibrium. The cumulative number of core taxa reaching equilibrium densities were enumerated at weekly intervals to

determine an optimal colonization period. Core taxa in the embedded substrates reached equilibrium densities slightly faster than in the unembedded substrates (Figure 3.4), but this was probably caused by significantly lower macroinvertebrate abundances in the embedded substrates. Thirteen core taxa in the PE substrates reached equilibrium on week one and all 18 core taxa in the PE and UE substrates reached equilibrium by week four. Fourteen core taxa reached equilibrium in the PU and UU substrates by week three. However, all core taxa in the unembedded substrates did not reach equilibrium densities until week six. As indicated by species richness, total macroinvertebrate abundance, and the cumulative number of core taxa reaching equilibrium densities, a three week colonization period appears optimal for colonizing riffle insect communities for stream microcosm studies.

Functional Group Comparisons

Because total macroinvertebrate abundance, species richness, and cumulative number of core taxa reaching equilibrium densities all indicate that a three week period is optimal for colonization, discussion of functional group comparisons will be limited to week three data. In addition, only PE and PU substrate data are used to compare substrate positioning. Natural and artificial substrate functional group comparisons from week three revealed PE substrates were colonized by significantly lower
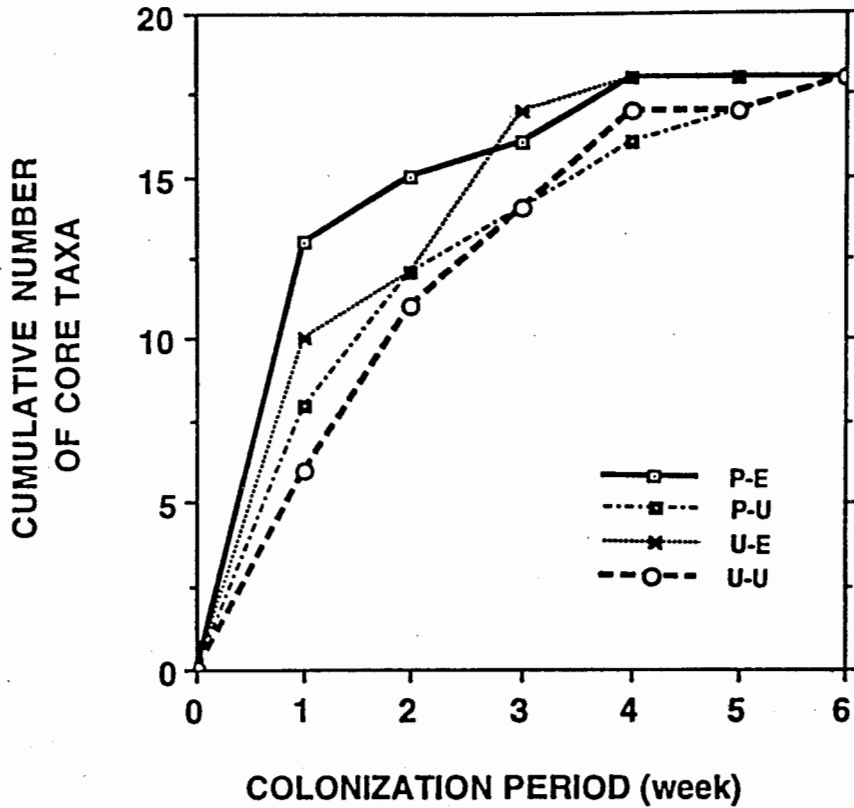
Figure 3.4. Cumulative number of core taxa reaching equilibrium densities per substrate from each experimental group over a six week colonization period. PE = precolonized-embedded; PU = precolonized-unembedded; UE = uncolonized-embedded; UU = uncolonized-unembedded.

collector-filterer (CF) percentages than those observed in
the natural substrate (Figures 3.5, 3.6). The PE substrates
may have been exposed to lower current velocities, resulting
in greater sediment deposition within substrate interstices
and limiting the availability of suspended food particles to
CF. Consequently, PE substrates did not provide a habitat
favorable for abundant CF colonization. However, CF
colonized the PU substrates with percentages similar to
those in the natural community (Figures 3.6, 3.7). No
significant differences in collector-gatherer (CG)
percentages occurred among the natural and artificial
substrates in week three. Scrapers (SC) colonized the PE
substrates with percentages significantly greater than the
natural and unembedded substrates, which obtained similar
percentages. Predator (PR) percentages were similar in all
substrate types throughout the colonization period.
Shredder (SH) percentages in the natural substrate community
were consistently higher than all artificial substrate
types, but extremely low percentages in both substrate types
made the differences unimportant.

Although embedded substrates reduced selective
colonization by CF, the unembedded substrates were colonized
by a riffle insect community functionally more similar to
the natural stream community. The colonization site was
dominated by large cobbles (13-25 cm) unembedded in pebbles
(2-6 cm), creating a riffle with the majority of the

P-E ARTIFICIAL SUBSTRATE
(week three)
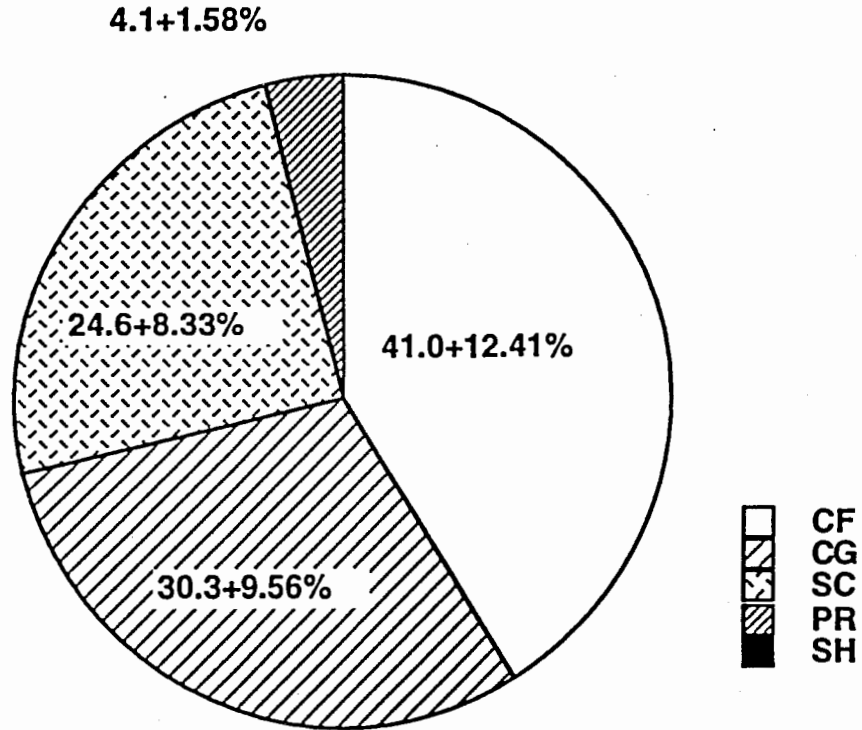
Figure 3.5. Functional group percentages from precolonized-embedded artificial substrates following a three week colonization period. Comparisons are based on proportional contribution to total numbers by functional group. CF = collector-filterer; CG = collector-gatherer; SC = scraper; PR = predator; SH = shredder. Data are mean percentages ± standard deviation.

NATURAL SUBSTRATE
(Hess Sampler)
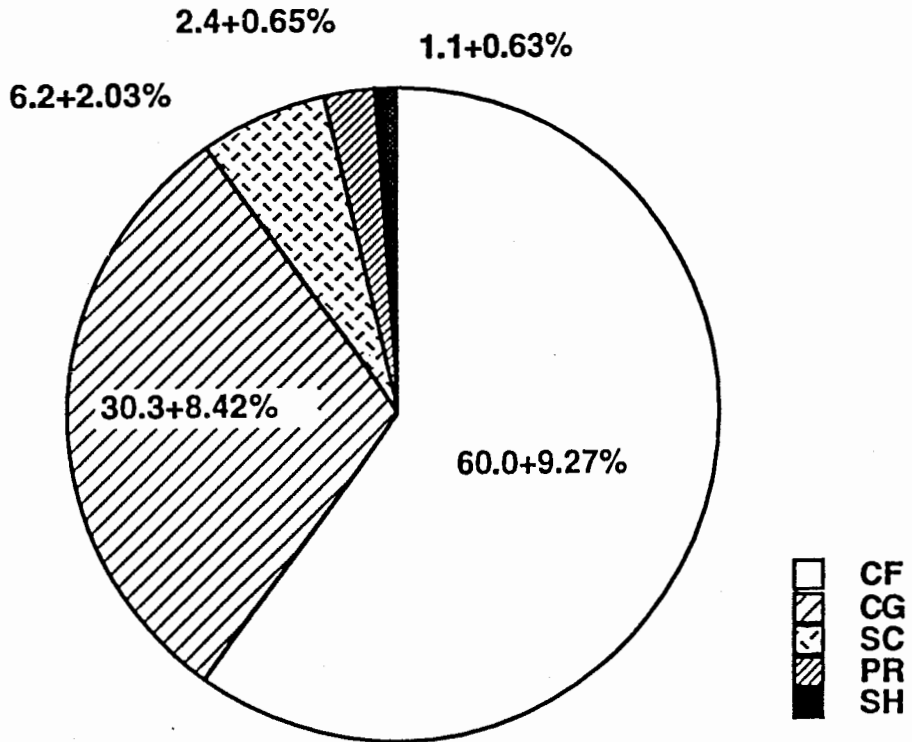
Figure 3.6. Functional group percentages from the natural substrate (Hess sampler). Comparisons are based on proportional contribution to total numbers by functional group. CF = collector-filterer; CG = collector-gatherer; SC = scraper; PR = predator; SH = shredder. Data are mean percentages ± standard deviation.

P-U ARTIFICIAL SUBSTRATE
(week three)
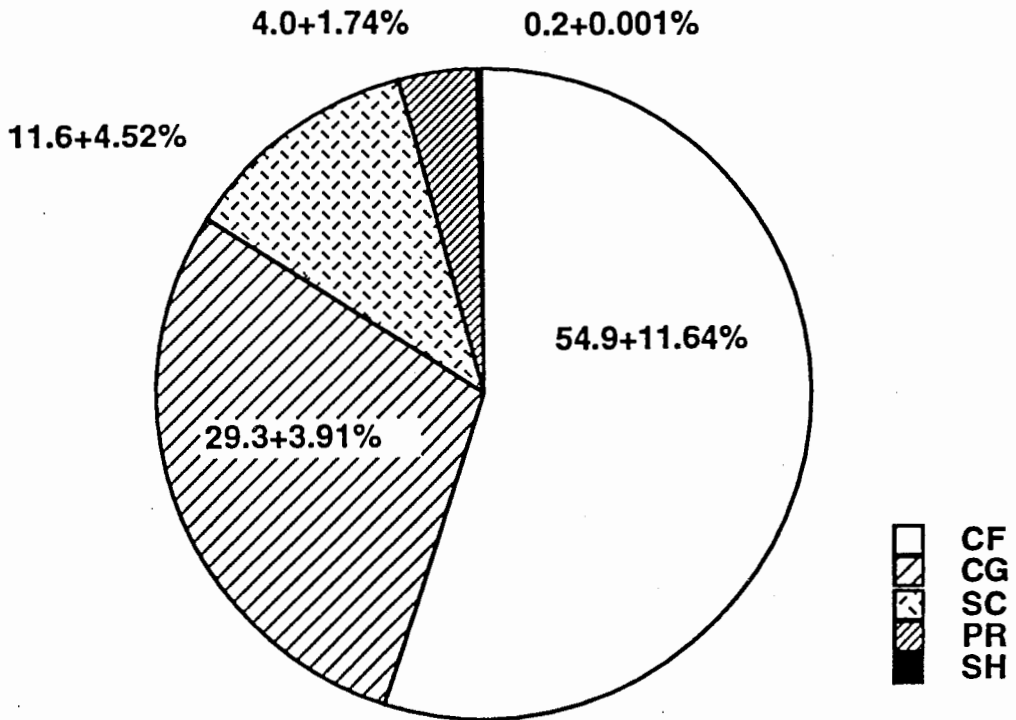
Figure 3.7. Functional group percentages from precolonized-unembedded artificial substrates sampled following a three week colonization period. Comparisons are based on proportional contribution to total numbers by functional group. CF = collector-filterer; CG = collector-gatherer; SC = scraper; PR = predator; SH = shredder. Data are mean percentages ± standard deviation.

large cobbles projecting into the water column. Unembedded substrates closely simulated natural substrate position, potentially exposing the sampling devices to higher current velocities and suspended filterable nutrients. Consequently, the unembedded substrates were colonized by CF, as well as the remaining feeding groups, in proportions functionally similar to the natural community.

A previous colonization study conducted at an upstream site reported CF insects selectively colonized artificial substrates identical to the UU design (Chapter 2). Riffle structure at the upstream colonization site was dominated by small cobbles (6-13 cm) 50% embedded in pebbles (2-6 cm) with considerable sedimentation by sand. The riffle area at the upstream site did not contain large cobbles projecting into the water column, thus the artificial substrates placed on the streambed created habitats structurally different from the natural substrate. Artificial substrates at the upstream site were apparently exposed to greater amounts of filterable organisms and detritus than the natural substrate. This difference in nutrient availability resulted in CF densities in the artificial substrates nearly double those in the natural substrate (c.f., Figures 3.8, 3.9). In comparison, the natural substrate structure at the upstream site probably accumulated detritus and other nutrients in the substrate interstices, providing CG insects with easier access to an abundant food source.

ARTIFICIAL SUBSTRATE
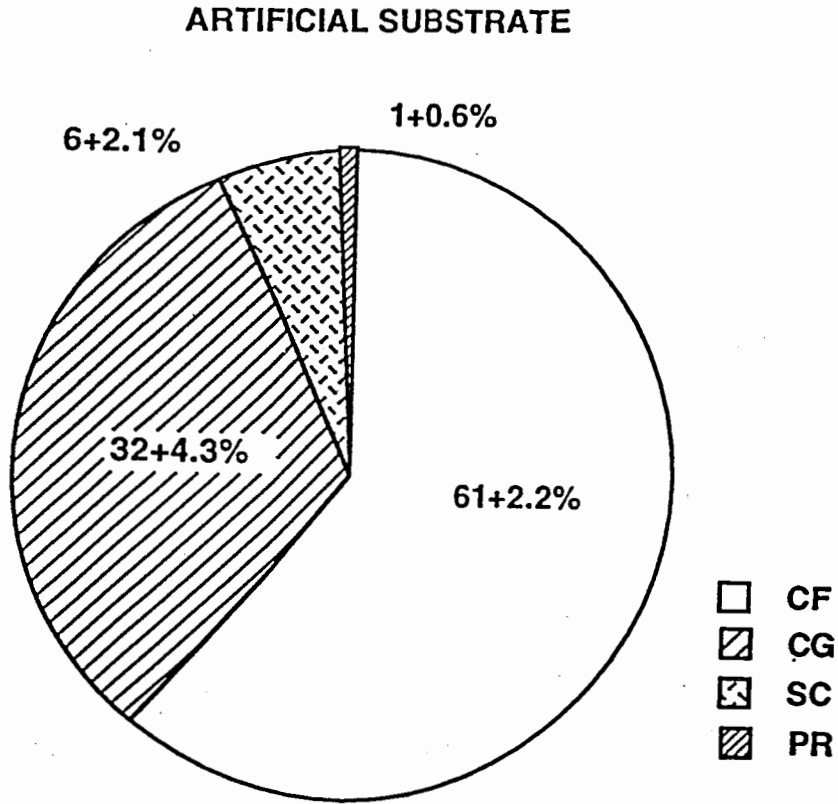
Figure 3.8.   Functional group percentages from artificial
substrates sampled at an upstream site (Chapter 2).
Comparisons are based on proportional contribution to total
numbers by functional group.   CF = collector-filterer; CG =
collector-gatherer; SC = scraper; PR = predator; SH =
shredder.   Data are mean percentages ± standard deviation.

NATURAL SUBSTRATE
(Hess Sampler)
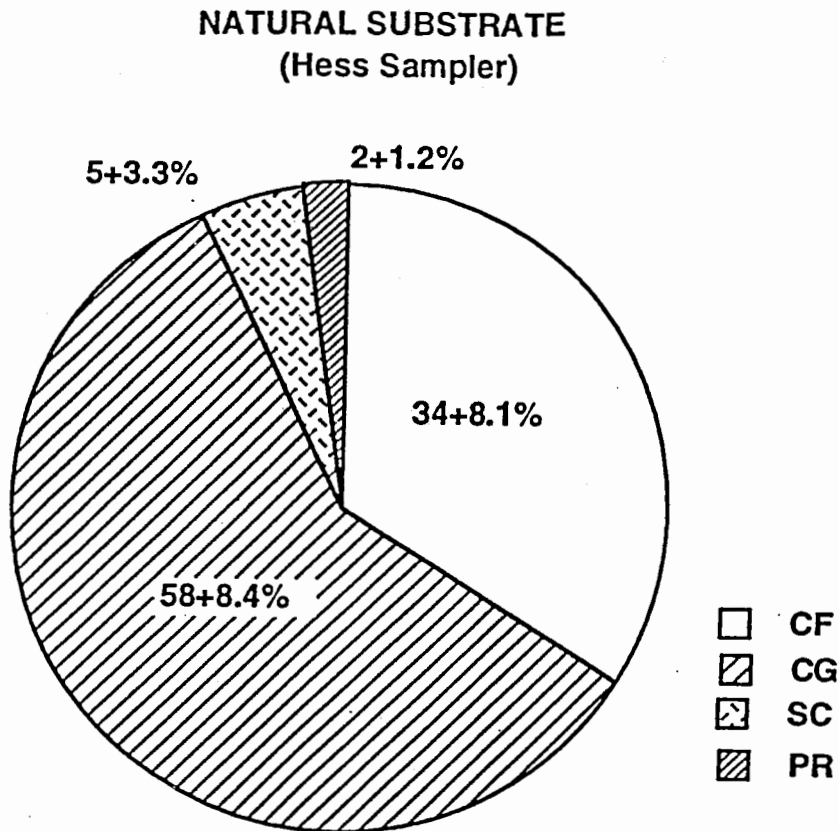
Figure 3.9. Functional group percentages from the natural substrate (Hess sampler) taken from a riffle area at an upstream site (Chapter 2). Comparisons are based on proportional contribution to total numbers by functional group. CF = collector-filterer; CG = collector-gatherer; SC = scraper; PR = predator; SH = shredder. Data are mean percentages $\pm$ standard deviation.

Consequently, the natural substrate community at the upstream site consisted of CG densities nearly two times greater than CG densities achieved in the artificial substrates (c.f., Figures 3.8, 3.9).

Conclusions

Substrate complexity (i.e. size and intersticial space availability) is an important factor in determining riffle insect community structure and function (Minshall, 1984). The results from the functional feeding group comparisons indicate artificial substrates are capable of collecting riffle insect communities functionally similar to natural communities, provided substrate composition (i.e. particle size, food resources) and positioning simulate the natural substrate. As indicated by this study, unembedded substrates probably better simulate the natural substrate structure when placed in riffle areas containing minimal amounts of sedimentation and an abundance of large cobbles or boulders. However, in riffles containing small cobbles and pebbles, or where sedimentation is extensive, embedding substrates should minimize differences caused by positioning artificial substrates in the water column.

Although periphytic biomass on the artificial substrates following the precolonization period was substantially lower than periphytic biomass in the natural stream, total macroinvertebrate density in the precolonized

substrates was significantly greater than the uncolonized substrates on week one. These results indicate that artificial substrates precolonized with periphyton then colonized by macroinvertebrates for one week should reduce the risk of sampling device disturbance, yet provide densities and species richness sufficient for use in stream microcosm tests. However, the advantages of slightly longer colonization periods (e.g., natural periphytic growth, greater detrital deposits, presence of both early and late colonizers, and attainment of equilibrium densities) should be considered when establishing environmentally realistic test systems. Numerous studies employing artificial substrates (e.g., Cairns 1982) have provided a wealth of information regarding ecological organization of benthic communities and should be considered prior to establishing standardized colonization procedures for obtaining test organisms for multispecies research.

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EPILOGUE

Population and community-level responses are the basis of multispecies research (Cairns 1983, Kimball and Levin 1985, Maciorowski 1988) and ecotoxicologists evaluating environmental pollution problems examine a multitude of natural processes (e.g., primary productivity, invertebrate recovery, alterations in trophic structure). Because laboratory-based systems provide a high degree of investigator control, microcosms are adaptable to a wide variety of experimental objectives associated with aquatic toxicology. However, until microcosm research is incorporated into the regulatory framework, additional studies to further standardize test procedures would be beneficial. The cumulative results of this research should provide valuable information concerning riffle insect community colonization of artificial substrates and the effects of fenvalerate on aquatic insects indigenous to Iowa streams.

The stream microcosm toxicity test in Chapter 1 predicted the following riffle insect community responses to fenvalerate:

1) Acute exposures exceeding 1.0 ug/L fenvalerate will significantly increase drift by riffle insects communities.

2) Fenvalerate concentrations reaching 0.01 ug/L will significantly reduce mayfly and stonefly numbers. At

fenvalerate concentrations of 0.1 ug/L, mayfly

densities will be reduced further, stoneflies will be

eliminated, and caddisflies, chironomids, and riffle

beetles will be present in significantly reduced

numbers.   Environmental concentrations reaching 10.0

ug/L fenvalerate will eliminate a majority of the

riffle insect community.

Significant density reductions for some core taxa occurred

at fenvalerate concentrations that would probably go

undetected during routine chemical monitoring in the field.

Recent pond mesocosm studies on esfenvalerate indicate that

although the chemical rapidly leaves the water column,

persistence in benthic sediments may cause significant

reductions in littoral communities (Heinis and Knuth 1992,

Lozano et al. 1992).   Small streams and rivers draining

agricultural watersheds are periodically subjected to

nonpoint source pollutants through surface runoff and

contaminated sediments.   Unfortunately, little information

is available on the persistence of fenvalerate residues in

benthic sediments in streams and rivers.   Further research

focusing on sediment toxicity in lotic ecosystems would be

beneficial when assessing insecticidal impact on riffle

insect communities.

Successfully protecting aquatic habitats on a large

scale will depend on evaluating our current pest management

practices and better understanding their environmental

impact. Unfortunately, concerns centered around profitability and a demand for high quality agricultural products inhibit cooperative progress toward minimizing chemical application. The long-term outcome involving economic and environmental safety tradeoffs is one that will have a major impact on the future of sustainable agriculture and the preservation of aquatic habitats.

Artificial substrates colonized by aquatic macroinvertebrates play an important role in stream microcosm studies, and the following conclusions can be drawn from the research presented in Chapter 2:

1) Species equilibrium and maximum densities were reached in weeks one and four, respectively. However, results suggest that a three week period collected an assemblage of organisms sufficient for use during stream microcosm tests.

2) Artificial substrates were selectively colonized by collector-filtering aquatic insects. However, partial burial of the artificial substrates following a flood decreased collector-filterer densities and increased colonization by organisms common to the hyporheic zone. Current colonization techniques are useful for examining riffle insect community colonization dynamics and obtaining test organisms for multispecies toxicity tests. Artificial substrates not only provide a non-destructive means of sampling aquatic habitats but are easily utilized by non-

experts and improve sample replication. These qualities are essential if stream microcosm tests are to become standardized procedures within the regulatory framework. However, selective colonization by certain organisms, as indicated by artificial and natural substrate comparisons, provides artificial substrate communities functionally different from natural stream communities. Consequently, test results utilizing artificial substrates colonized by an assemblage of organisms not representing naturally occurring communities remain useful, but with limited applicability in predicting ecosystem-level responses.

The results from Chapter 3 suggest that manipulating artificial substrate conditions (i.e. periphyton precolonization, substrate positioning) can influence macroinvertebrate colonization dynamics. Specifically, the following conclusions can be drawn:

1) Precolonizing artificial substrates with a periphytic food source increased macroinvertebrate colonization during the first week but did not influence total density in subsequent weeks.

2) Embedding artificial substrates in the stream bottom decreased selective colonization by collector-filterers. However, unembedded substrates simulated the natural substrate at the colonization site and collected a riffle insect community functionally more

similar to the natural substrate community than the
embedded substrates.

Multispecies tests are not designed to simultaneously
conduct several single-species tests but, rather, examine
community-level interactions. The roles aquatic insects
play in processing stream nutrients are often studied to
assess community-level effects following disturbances (e.g.,
Wallace 1986, Clements *et al.* 1988). The predictive
capabilities of stream microcosm tests will improve when
riffle insect communities colonizing artificial substrates
are functionally similar to natural communities. Results
from this study suggest that functionally similar
communities can be collected when artificial substrate
positioning creates habitats similar to the natural
substrate structure.

Currently, recommendations are being considered to
develop standardized procedures that incorporate microcosms
into the testing hierarchy during pesticide registration
(Cairns 1992, SETAC workshop report 1992). Because
environmental protection is a widespread concern, testing
should include protocols capable of predicting responses of
resident communities in all types of aquatic habitats (i.e.
lentic, lotic, estuarine, marine) without creating test
systems that are economically and logistically
inappropriate. Microcosm test systems are easily
constructed and are adaptable to test a variety of site-

specific communities. In addition, microcosm tests would provide an intermediate test no less informative than mesocosm studies but logistically less complex.

Regardless of the test system, investigators must understand the limitations of their test system when simulating environmental conditions and interpret results based on sound ecological judgement. For applications in the pesticide registration process, simplistic test systems alone do not provide sufficient evidence to determine environmental safety and harm, and overly sophisticated test systems would not be applicable to the large number of chemicals produced annually. Therefore, test systems capable of producing reliable, applicable, and cost-effective answers should be considered. Employing acute and chronic single-species toxicity tests with subsequent laboratory-based multispecies tests (e.g., Larsen *et al.* 1986, Pontasch *et al.* 1989) will combine quick, inexpensive screening with more realistic, yet replicable environmental predictions.

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APPENDIX A.   Statistical Analysis System (SAS) program used in Chapter 1 to analyze the effects of fenvalerate on riffle insect communities.

```
TITLE 'PYDRIN EXPERIMENT OVERALL ANALYSIS';
DATA BUGS;
INPUT CONC STR REP  TYPE $ ISO BAE CLA STE HYD CHE CHM CHI ORT TAN ATH
SIM;
CARDS;
0.0   1   1 A  35   40   0  31  104  23  18  62  18   0  0  1
0.0   1   1 L   2    0   6  76   70  21   1  10   9   2  2  0
0.0   2   2 A  94   52   0  19   81  19  22  67  16   0  0  5
0.0   2   2 L   0    0   5  79   45  15   0   8   5   6  2  1
0.0   3   3 A  61   43   0  23  101   9  15  49  17   0  0  1
0.0   3   3 L   0    0   5  67   55  16   0   4   3   1  2  0
0.01  4   1 A  61   18   0  34  156  43  19  95  20   0  0  1
0.01  4   1 L   3    0   4  52   27  29   1   9   4   7  1  0
0.01  5   2 A  29   43   0  27   61   6  11  57  13   0  0  4
0.01  5   2 L   2    0   3  73   12   8   1   7   1   2  7  0
0.01  6   3 A  17   44   0   9   46   6   7  58  13   0  0  9
0.01  6   3 L   0    0   2  62    4   4   2   2   2   5  2  0
0.1   7   1 A   0    6   0   8    0   2   1  33   4   0  0  6
0.1   7   1 L   0    0   0  10    0   0   3   1   1   3  3  0
0.1   8   2 A   2    1   0  18    6   1   8  60   5   0  0  3
0.1   8   2 L   0    0   0   7    1   0   0   1   0   7  5  0
0.1   9   3 A   0    5   0   3    2   1   0  30   4   0  0  3
0.1   9   3 L   0    0   0  25    0   0   1   6   1   4  2  0
1.0  10   1 A   1    2   0  10    1   0   1  15   0   0  0  4
1.0  10   1 L   0    0   0   1    3   0   2   0   0   1  1  0
1.0  11   2 A   2    5   0  16    0   1   0   5   2   0  0  2
1.0  11   2 L   0    0   0   0    0   0   2   2   0   0  1  0
1.0  12   3 A   1    2   0  18    2   0   0   9   5   0  0  3
1.0  12   3 L   0    0   0   2    2   0   4   2   0   0  2  0
10.0 13   1 A   0    5   0   0    2   0   0   1   0   0  0  1
10.0 13   1 L   0    0   0   0    2   0   0   0   0   0  0  0
10.0 14   2 A   0    5   0   0    0   0   0   1   0   0  0  1
10.0 14   2 L   0    0   0   0    0   0   0   0   0   0  0  0
10.0 15   3 A   2   15   0   0    2   0   0   4   0   0  0  6
10.0 15   3 L   0    0   0   1    0   0   0   0   0   0  0  0
PROC PRINT;
PROC SORT;
BY CONC;
PROC MEANS;
BY CONC;
VAR ISO BAE CLA STE HYD CHE CHM CHI ORT TAN ATH SIM;
OUTPUT OUT=TWO MEAN=ISOM BAEM CLAM STEM HYDM CHEM CHMM CHIM ORTM TANM ATHM
SIMM;
PROC PRINT;
PROC SORT DATA=TWO;
BY CONC;
PROC CHART DATA=TWO;
VBAR CONC / DISCRETE SUMVAR=ISOM SUBGROUP=TYPE;
PROC CHART DATA=TWO;
VBAR CONC / DISCRETE SUMVAR=BAEM SUBGROUP=TYPE;
PROC CHART DATA=TWO;
VBAR CONC / DISCRETE SUMVAR=CLAM SUBGROUP=TYPE;
```

```
PROC CHART DATA=TWO;
VBAR CONC / DISCRETE SUMVAR=STEM SUBGROUP=TYPE;
PROC CHART DATA=TWO;
VBAR CONC / DISCRETE SUMVAR=HYDM SUBGROUP=TYPE;
PROC CHART DATA=TWO;
VBAR CONC / DISCRETE SUMVAR=CHEM SUBGROUP=TYPE;
PROC CHART DATA=TWO;
VBAR CONC / DISCRETE SUMVAR=CHMM SUBGROUP=TYPE;
PROC CHART DATA=TWO;
VBAR CONC / DISCRETE  SUMVAR=CHIM SUBGROUP=TYPE;
PROC CHART DATA=TWO;
VBAR CONC / DISCRETE SUMVAR=ORTM SUBGROUP=TYPE;
PROC CHART DATA=TWO;
VBAR CONC / DISCRETE SUMVAR=TANM SUBGROUP=TYPE;
PROC CHART DATA=TWO;
VBAR CONC / DISCRETE SUMVAR=ATHM SUBGROUP=TYPE;
PROC CHART DATA=TWO;
VBAR CONC / DISCRETE SUMVAR=SIMM SUBGROUP=TYPE;
PROC SORT DATA=BUGS;
BY STR;
PROC MEANS DATA=BUGS;
BY STR;
VAR ISO BAE CLA STE HYD CHE CHM CHI ORT TAN ATH SIM;
ID CONC;
OUTPUT OUT=THREE SUM = ISOS BAES CLAS STES HYDS CHES CHMS CHIS ORTS TANS
ATHS
SIMS;
PROC SORT DATA=THREE;
BY CONC;
PROC PRINT;
PROC ANOVA DATA=THREE;
CLASS CONC;
MODEL ISOS BAES CLAS STES HYDS CHES CHMS CHIS ORTS TANS ATHS SIMS = CONC;
MEANS CONC/LSD TUKEY SCHEFFE;
MEANS CONC/DUNCAN;
```

APPENDIX B. Statistical Analysis System (SAS) program used in Chapter 2 to analyze artificial substrate colonization by riffle insect communities over a seven week period.

```
TITLE 'ARTIFICIAL SUBSTRATE COLONIZATION';
DATA CRITTERS;
INPUT WEEK $ REP $ ISO EPH BAE PSU CAE TRI STM CLA STL
HYD CHE CHM HYT HEL CHI ORT TAN SIM ATH;
MAY = ISO + EPH + BAE + PSU + CAE + TRI + STM;
PLE = CLA;
COL = STL;
CAD = HYD + CHE + CHM + HYT + HEL;
DIP = CHI + ORT + TAN + SIM + ATH;
*baes = bae + psu;
TOT = MAY + PLE + COL + CAD + DIP;
CARDS;
WK1 A   9  5  38  24  6  3  5 0  4 184 126  2 22 1 103 51 12 41 0
WK1 B  11 13  47  13  9  3  4 2  4 195 117 13 18 1 127 61 13 91 0
WK1 C  11 14  43  22  3  3  9 0  0 143  80  6 37 0  94 36 13 37 1
WK1 D  24 11  72  11  7  1  1 0  2 197  59  2 39 0  75 56 10 49 1
WK1 E  22  1   9  35 13  1  3 0  3 275 105  1 68 0  78 21  4 66 0
WK2 A  43 17  54  80 25 12 20 0  2 252 102 12 18 0 202 77 35 66 0
WK2 B  45 41  37 101 37 14 23 0  1 250 153 15 16 0 108 25 26 13 0
WK2 C  66 14  46  73 12  7 17 0  2 242 131  9 16 0  93 23 17 16 0
WK2 D  76 30  82 107 22  4 12 0  2 236 116  2 12 0  89 36  5 33 0
WK2 E  68 42  68 129 53 11 45 1  5 223 117 11 37 0  97 55 40 25 1
WK3 A 184  8  84  98 34  8 42 1  3 248 226 32 24 0 306 40 34 44 2
WK3 B 146 33  69 120 76 13 99 3  0 219 131  9  8 0 204 37 50 10 0
WK3 C 125 50  72 160 84 10 68 1  0 183 116  5 10 1 195 43 47  9 0
WK3 D 163  3  52 150 20 20 38 1  0 293 109 13 12 0 117 49  9 23 0
WK3 E 141  4 123  92 41 10 59 1  0 466 132 34 11 0 114 26 15  9 0
WK4 A 264  2 196  58 34 12 78 0  6 382 174  4 18 0 116 32 14 42 0
WK4 B 224 12 188  36 56  6 34 0  3 336 180 14  8 0 150 52 16 26 4
WK4 C 270 16  82  28 54  5 90 1  9 344 170 14 28 0 122 40 14 74 0
WK4 D 202  0 356   0 30 12 62 0  1 518 168 26 12 0  92 62  4 18 0
WK4 E 214  4 174  74 46 18 76 1  2 576 176 56  6 2 218 42  0 14 0
WK5 A   0  4  13   2 17  4  0 0  2  27   3 13  0 1 104 15  7  1 0
WK5 B   0  0  12   1  5  2  0 0  2   9   0  6  0 0  37 13  3  0 0
WK5 C   7  3  18   0  5  3  0 0  4  32   4  5  1 1  80 20  5  3 0
WK5 D  13  0  42   0 30  6  8 2  5 160  82 20  0 0 146 33  5  0 0
WK5 E   5  0  33   3  9  6  4 2 10  41   7  5  0 4 128 28  2  2 0
WK6 A  18  2  82   3 15  1  3 1  2  56  11  3  1 1 140  7 14  2 3
WK6 B  37  0 186  13 18  2 13 4 15  96  51  3  5 0 170 14 20 25 1
WK6 C  39  1 224  23 19  4 13 1  8  95  29  4  5 2 213 32  8  7 1
WK6 D  60  3 247  12  9  0 12 5 11 125  53  5  2 0 122 29  4 20 0
WK6 E  78  4 164  12 14  4 14 1  7 158  58  4  4 0 102 24  6  6 0
WK7 A  54  0 262  18  8  0 12 2 14 224 102  4 12 0 134 26 10 12 2
WK7 B 122  2 182  60 12  0 26 2 13 156  88  4  8 0  62 34  4 16 0
WK7 C 108  0 116  10  6  0 26 1 10 200 122 12 10 0  66 26  8 10 0
WK7 D 206  6 168  28 14  0 98 0 12 184  60 10  4 0  68 32  4  0 0
WK7 E 116  2 264  46  2  0 26 0  9 244 104  0 12 0  96 32  4 44 0
PROC PRINT;
PROC SORT DATA = CRITTERS;
BY WEEK REP;
PROC MEANS;
BY WEEK;
```

```
VAR ISO EPH BAE PSU CAE TRI STM CLA STL HYD CHE CHM HYT HEL  CHI ORT TAN
SIM ATH MAY PLE COL CAD DIP TOT;
PROC CHART DATA=CRITTERS;
VBAR WEEK / TYPE=MEAN SUMVAR=ISO;
PROC CHART DATA=CRITTERS;
VBAR WEEK / TYPE=MEAN SUMVAR=EPH;
PROC CHART DATA=CRITTERS;
VBAR WEEK / TYPE=MEAN SUMVAR=BAE;
PROC CHART DATA=CRITTERS;
VBAR WEEK / TYPE=MEAN SUMVAR=PSU;
PROC CHART DATA=CRITTERS;
VBAR WEEK / TYPE=MEAN SUMVAR=CAE;
PROC CHART DATA=CRITTERS;
VBAR WEEK / TYPE=MEAN SUMVAR=TRI;
PROC CHART DATA=CRITTERS;
VBAR WEEK / TYPE=MEAN SUMVAR=STM;
PROC CHART DATA=CRITTERS;
VBAR WEEK / TYPE=MEAN SUMVAR=CLA;
PROC CHART DATA=CRITTERS;
VBAR WEEK / TYPE=MEAN SUMVAR=STL;
PROC CHART DATA=CRITTERS;
VBAR WEEK / TYPE=MEAN SUMVAR=HYD;
PROC CHART DATA=CRITTERS;
VBAR WEEK / TYPE=MEAN SUMVAR=CHE;
PROC CHART DATA=CRITTERS;
VBAR WEEK / TYPE=MEAN SUMVAR=CHM;
PROC CHART DATA=CRITTERS;
VBAR WEEK / TYPE=MEAN SUMVAR=HYT;
PROC CHART DATA=CRITTERS;
VBAR WEEK / TYPE=MEAN SUMVAR=HEL;
PROC CHART DATA=CRITTERS;
VBAR WEEK / TYPE=MEAN SUMVAR=CHI;
PROC CHART DATA=CRITTERS;
VBAR WEEK / TYPE=MEAN SUMVAR=ORT;
PROC CHART DATA=CRITTERS;
VBAR WEEK / TYPE=MEAN SUMVAR=TAN;
PROC CHART DATA=CRITTERS;
VBAR WEEK / TYPE=MEAN SUMVAR=SIM;
PROC CHART DATA=CRITTERS;
VBAR WEEK / TYPE=MEAN SUMVAR=ATH;
PROC CHART DATA = CRITTERS;
VBAR WEEK / TYPE = MEAN SUMVAR=MAY;
PROC CHART DATA = CRITTERS;
VBAR WEEK / TYPE = MEAN SUMVAR=PLE;
PROC CHART DATA = CRITTERS;
VBAR WEEK / TYPE = MEAN SUMVAR=COL;
PROC CHART DATA = CRITTERS;
VBAR WEEK / TYPE = MEAN SUMVAR=CAD;
PROC CHART DATA = CRITTERS;
VBAR WEEK / TYPE = MEAN SUMVAR=DIP;
PROC CHART DATA = CRITTERS;
VBAR WEEK / TYPE = MEAN SUNVAR=TOT;
PROC ANOVA DATA = CRITTERS;
CLASS WEEK;
MODEL ISO EPH BAE PSU CAE TRI STM CLA STL HYD CHE CHM HYT HEL CHI ORT
TAN SIM ATH MAY PLE COL CAD DIP TOT = WEEK;
MEANS WEEK/LSD;
```

APPENDIX C.   Statistical Analysis System (SAS) program used
in Chapter 3 to analyze aquatic macroinvertebrate colonization
of four artificial substrate types over a six week period.

```
TITLE 'IMPROVED COLONIZATION STUDY ANALYSIS';
DATA BUG;
INPUT WEEK $ TYPE $ REP $ ISO POT BIN BAR BFL TER
MOD STN CAE TRY CLA PTE PER STE
DUB MAC MOR CHU CHM HYD DOL HEL CHI
TAN ORT SIM EMP ATH TIP CEO GOP COR PET HEB;
*PE=PRECOLONIZED-EMBEDDED,PU=PRECOLONIZED-UNEMBEDDED;
*UE=UNCOLONIZED-EMBEDDED,UU=UNCOLOINIZED-UNEMBEDDED;
EPH=ISO+POT+BIN+BAR+BFL+TER+MOD+STN+CAE+TRY;
PLE=CLA+PTE+PER;
COL=STE+DUB+MAC;
TRI=MOR+CHU+CHM+HYD+DOL+HEL;
DIP=CHI+TAN+ORT+SIM+EMP+ATH+TIP;
ODO=CEO+GOP;
TOT=EPH+PLE+COL+TRI+DIP+ODO;
IF REP = C THEN DELETE;
CARDS;
WK1 UU  A  38   0  74  12   5  17   6   0   7   3   0   0   0   0   0  0
    125  51   0  14   0   0  22   6  14 123   0   0   0   0   0   0  0  0
WK1 UU  B 101   0 153  25  21  11   1   0   0   1   0   0   0   0   0  0
    167  77   9  26   0   0  63   8  17 270   0   0   0   0   0   0  0  0
WK1 UU  C  71   0  54   8  13  17   3   0  16   0   2   0   0   0   0  0
    161  67  10   5   0   0  83   4  27  91   0   0   0   0   0   0  0  0
WK1 UU  D  88   0 101  10   0   8   6   0   8   2   1   0   0   0   0  0
    260  71  16  26   0   0  26  10  19 278   0   0   0   0   0   0  0  0
WK1 UU  E  70   0  78  10   4  18   4   0   5   3   1   0   0   0   0  0
     58  24   4  10   0   0  15   3  16  28   0   0   0   1   1   0  0  0

WK1 UE  A 121   0  15   0   1  34  10   1  38   1   3   0   0   0   0  0
     94  49   5   2   0   0  17   3  17   0   0   0   0   0   0   0  0  2
WK1 UE  B  73   0  27   0   6  13   9   0  10   0   4   0  96  31   4  3
      0   0  10   2   7  21   0   0   0   0   0   1   0   0   0   0  0  0
WK1 UE  D  27   0  31   2   1  18   5   0  20   5   1   0   0   2   0  0
     56  17   0   2   0   0  17   6  23   2   1   0   0   0   0   0  0  0
WK1 UE  E  42   0  24   1   0  27   8   6  17   9   3   0   0   0   0  0
     37  29   4   1   0   0  19   3  24   1   0   0   0   0   0   1  0

WK1 PU  A 120   0 168  23  13  13   5   0  13   1   3   0   0   0   0  0
    305  46   6  13   0   0  49   7  19 157   0   0   0   0   0   2  0  1
WK1 PU  B 122   0 192  10   9  16   3   0   6   2   2   0   0   0   0  1
    424  54  14  16   0   0  34   3   5 150   1   0   0   0   0   2  0  1
WK1 PU  C  66   0 176   7  12  13   6   0  30  11   1   0   0   2   0  0
    351  64  15  23   0   0  89   6  31 145   0   0   0   1   0   2  0  0
WK1 PU  D 110   0 102  36   4  24   4   0   4   4   0   0   1   0   0  0
    400  98  10  48   0   0  48   6  25 189   2   1   0   1   0   0  0  0
WK1 PU  E  82   0 161  21  10   4   2   0   7   3   0   0   0   0   0  0
    350  54  17  11   0   0  17   3  16 144   0   0   0   0   0   0  0  0

WK1 PE  A  38   0  57  18   7  37  16   0  17  15   0   0   0   2   0  0
     31  12   5   1   0   0  37  22   2   0   0   0   0   0   0   0  0  0
```

```
WK1 PE   B   25    0  39    1    5  27  10    0  29  11    6    0    0    1    0    0
    90   26    0    0    0    0  12    3  15    1    0    0    0    0    0    0    0    0
WK1 PE   D  102    0  81    8    2  32  19    2  55    8    0    0    0    1    0    0
   199   77   11    4    0    0  26    7  29    3    1    1    0    0    1    0    0    0
WK1 PE   E   54    0  26    2    0  42  28    2  30    6    1    0    0    4    0    0
    50   41    1    1    0    0  24    3  27    0    1    0    0    1    0    0    1    0

WK2 UU   A  102    0 171    1    8  43    2    0  31    5    7    0    0    0    0    0
   199   82    4    4    1    0 154  43    7  20    0    0    0    1    0    0    0    0
WK2 UU   B   88    0 252   12    6   8    6    0   6    2    0    0    0    0    0    0
   534   90   24   21    0    0 115  37   26 256    0    0    0    0    0    1    0    0
WK2 UU   C   95    0 287    8   17  19    7    0   3    4    1    0    0    0    0    0
   482   86   10    7    0    0 155   6    6 224    0    0    0    0    0    1    0    0
WK2 UU   D   67    0 205    2    5  53    2    0  35   23    2    0    0    1    0    0
   276   76   11    4    0    0 146  13   49  47    0    0    0    0    0    1    0    1
WK2 UU   E   52    0 110    4    4  35    4    0  20   11    7    0    0    2    0    0
   219   67    5    6    0    0  71  12   25   9    0    0    0    0    0    0    0    0

WK2 UE   A  184    0  74    3    1  31  20    0  27    9    5    0    0    1    0    0
   161   71    4    0    0    0  34   7   20    0    0    0    0    0    0    0    0    1
WK2 UE   B  156    0  99    3    6  36   8    0  25    3    2    0    0    0    0    0
   287   79   14    3    0    0  71   7   45  17    0    0    0    0    0    1    0    0
WK2 UE   D   88    0  84    8    0  48  16    1  42   24    4    0    0    3    1    0
   128   61    2    0    4    0  44   8   23   1    0    0    0    0    0    0    0    1
WK2 UE   E   49    1  54    2    0  64  20    3  26    8    4    0    0    1    0    0
    37   22    0    1    1    0  35   7   23   0    0    0    0    0    0    0    0    0

WK2 PU   A  189    0 190    4    3   0  39    3  30    5    4    0    0    0    0    0
   336   85   34    2    0    0  76  16   22   5    0    0    0    2    0    1    1    0
WK2 PU   B  196    0 272    5    1  31   9    0  28    7    3    0    0    0    0    0
   549   84   54   12    0    0 122  19   35 106    0    1    0    1    0    2    0    0
WK2 PU   C  110    0 185    4    2  25   3    0  52   26    3    0    0    1    0    0
   370   82   10    3    0    0  89  12   30  13    0    0    0    1    0    1    0    0
WK2 PU   D  130    0 283    6    2  32   6    0  32    8    0    0    0    0    0    0
   437   78   36    1    0    0 135  13   43  57    1    0    0    0    0    0    0    0
WK2 PU   E   60    0 208    6    2  38   2    1  35    4    1    0    0    0    0    0
   540   84   23   10    0    0 136  39   22  66    0    1    0    1    1    2    0    0

WK2 PE   A   21    0  13   12    0  35  14    2  70   43    3    0    0    0    0    0
    24    6    1    0    2    0  40  14   32   0    1    0    0    0    1    0    0    0
WK2 PE   B   44    0  23    5    2  32  25    0  70   19    3    0    0    4    0    0
    43   44    0    1    1    0  39  15   44   1    0    0    0    0    1    0    0    0
WK2 PE   D   75    0  72    0    0  30   6    0  33   10    3    0    0    2    0    0
   127   90   12    2    2    0  61  19   35   0    1    0    0    2    9    0    0    0
WK2 PE   E   88    0  68    0    0  75  14    2  54    6    8    0    0    1    0    0
    54   25    7    2    0    0  17   4   23   0    0    0    0    1    0    0    0    0

WK3 UU   A  140    0 146    8   12 134  12    0  36   10    4    0    0    4    0    0
   248  118   12   14    0    0  74  36   46   9    1    0    0    0    0    0    0    0
WK3 UU   B  162    0 181    6   19  98  11    0  20    1    2    0    0    0    0    0
   496  120   50   32    1    0  92  38   35  19    1    0    0    1    0    2    0    0
WK3 UU   C  226    0  96    2    0 192  29    2  52    2    8    0    0    6    0    0
   288   94    8    4    2    0 102  30   44   0    0    0    0    0    0    0    0    0
WK3 UU   D  179    0 222    6   12 192  14    0  52    3    8    0    0    0    0    0
   350   98   36   12    1    0  72  18   47   4    0    0    0    0    0    0    0    0
WK3 UU   E  148    0  66    2    4 120   4    0  28    2    6    0    0    4    0    0
   104   66   12    4    4    0  63  20   37   2    0    0    0    2    0    0    0    0

WK3 UE   A  122    2  14    4    2  90  56    0  48    8   12    0    0    6    0    0
    84   94    4    2    2    0  54  30   48   0    0    0    0    0    0    0    0    0
WK3 UE   B   37    0  34    4    1  70  22    0  48    6    7    0    0    6    0    0
    91   93   11    2    0    1  25   7   13   0    0    0    0    1    0    0    0    0
```

```
WK3 UE   D 160   0  57   0   8 188  44   0  59   0   0   0   0   7   0   1
          188 137   6   6   0   0  27  10  33   0   1   0   0   0   1   0   0   0
WK3 UE   E  58   1  10   2   2 133  36   0  79  10   2   0   0  15   1   0
           52  64  33   1   0   1  30  16  37   0   1   0   0   2   0   0   0   0

WK3 PU   A 128   0 174   8   4  86   8   0  72   4   4   0   0   3   0   0
          353 129  16   4   0   0  64  32  35   3   1   0   0   0   1   1   0
WK3 PU   B 134   0 280   4   0  68   0   0   8   4   8   0   0   0   0   0
          652 208  84  28   0   0  84  20  40  42   2   0   0   0   0   0   0   0
WK3 PU   C 184   0 136   4   4 130   8   0  18  12   8   0   0   0   0   0
          328 130  14  14   4   0 172  44  62   2   0   0   0   4   0   8   0   0
WK3 PU   D 144   0  64  10   0 128  14   0  60  20   6   2   0   0   0   0
          232  66  12   2   6   0  74  50  90   2   0   0   0   0   0   0   0   0
WK3 PU   E 150   0 106   2   0 140  32   0  32  16   4   0   0   4   0   0
          330  80  20  10   0   0  56  34  52   0   2   0   0   6   0   0   2   0

WK3 PE   A  33   0   7   9   0  70  29   0  80   8   3   0   0   5   0   0
           76  74   3   0   1   0  54   9  38   0   0   0   0   0   3   0   0   0
WK3 PE   B  13   0   6   3   0  92  24   2  47  12   8   0   0   3   0   0
           53  16   0   0   0   0  12   3  22   0   0   0   0   4   0   0   0   0
WK3 PE   D  33   0  24   2   1  65  15   0  47   9   5   0   0   3   0   0
           83  82   3   2   2   0  26   3  21   0   1   0   1   0   0   1   0   0
WK3 PE   E  28   0   6   1   0  60  22   0  27   4   2   0   0   7   1   0
           71  58  13   1   0   0   0  20  17  29   0   0   0   0   0   1   0   0

WK4 UU   A 223   0 170   8  24 227  44   0  25   0   2   0   0   4   0   0
          257 104  23  13   2   0 157  57  26   3   1   0   0   2   0   2   0   0
WK4 UU   B 178   0 139  11   8 114  20   0   6   0   6   0   0   0   0   0
          572 156  52  36   0   0  62  36  11   3   0   0   0   1   0   5   0   0
WK4 UU   C 104   0  67   0   8 116   6   0  14   0   3   1   0   1   0   0
          177  92   8   8   6   0 105  30  15   3   0   0   0   0   0   1   0   0
WK4 UU   D 160   0  78   2   0 123  31   0  26   0   7   0   0   2   0   0
          202  86  24  15   3   0  78  56  23   0   0   0   0   2   0   2   1   0
WK4 UU   E 230   0 100   8  12 152  42   0  24   0   4   0   0   4   0   0
          220 110  40  10   2   0  62  32  34   4   0   0   0   0   0   0   0   0

WK4 UE   A  29   0   2   5   1  31  35   0  13   8   1   0   0   4   0   0
           51  28   0   1   5   0  51  41  20   0   0   0   0   0   1   0   0   0
WK4 UE   B  14   0   8   1   0  34   8   0  22   4   4   0   0   4   1   0
           46  43   1   5   3   0  14  20   9   0   0   0   0   0   0   0   0   0
WK4 UE   D  14   1  15   5   4  58  17   0  13   5   1   0   0   3   1   0
           63  58   2   4   2   0  53  32   9   0   1   0   0   0   1   0   0   0
WK4 UE   E 168   0  29   2   6 136  46   0  19   2   1   0   0  16   0   0
          100  43  44  10   0   0  28  12  29   0   0   0   0   0   0   2   0   0

WK4 PU   A 116   0 150   8  23 147  30   0   8   2   1   0   0   1   0   0
          111 110  21  26   0   1 122  38  32   2   3   0   0   0   3   1   0
WK4 PU   B 290   0 148   0  10 189   8   0  20   5   9   0   0   3   0   0
          472 141 115  17   0   0  82  53  30   2   2   0   0   0   4   0   0
WK4 PU   C 216   0  84  22   4 154  26   0  10   1   3   1   0   1   0   0
          228 154  10  18   2   0 111  53  25   0   1   0   0   1   0   0   0   0
WK4 PU   D 248   0 122   4  20 124  28   0   6   2  12   2   0   0   0   0
          382 168  44  16   0   0  64  36  18  12   0   0   0   0   2   0   0
WK4 PU   E 226   0 132   0   7 120  18   1  20   6   5   0   0   5   0   0
          358 134  83  14   0   0  58  41  26   3   0   0   0   0   6   0   0

WK4 PE   A   8   0   5   9   1  47  34   0  16   7   4   0   1   5   0   0
           53  31   0   2   2   2  63  45  17   0   1   0   0   0   1   0   0   0
WK4 PE   B  45   0  22   9   3  83  54   1  27   5   0   0   0   2   1   0
           40  23   1   1   0   2  31  20  20   0   1   0   0   2   0   1   0   0
WK4 PE   D 106   0  26   2  16 127  38   1  46   4   2   0   1  10   0   0
           94  70  39   3   0   0  39  22  33   1   0   0   0   1   1   2   0   0
```

```
WK4 PE   E  26   0  26   3   2 104  46   0  21   5   3   0   0  22   1   0
    43  31   5   4   6   0   8   9  23   0   0   0   0   2   0   1   1   0

WK5 UU   A 198   0  35   8   0 121  30   0   6   1   7   0   0   5   0   0
   258 112  43  13   2   1  20  12   5   1   1   0   0   2   0   3   0   0
WK5 UU   B 247   0  69   0  34 170  34   0   2   0   5   0   0   0   0   0
   616 181  46  23   0   0  30  10   5   2   2   0   0   0   0   2   0   0
WK5 UU   C  71   0  24   1   2 144  11   0  13   1   8   0   0   3   0   0
   149  91   9  23   5   0  45  45  17   0   3   0   0   1   0   0   0   0
WK5 UU   D 135   0  13   4   6  95  12   0   8   0  10   0   0   9   0   0
   282 171  35  17   0   0  23  15   8   1   1   0   1   0   0   0   0   0
WK5 UU   E 132   0  23  10   4 228  62   0  18   2   9   0   0   3   0   0
   157  93   6   4   4   0  21  14  14   0   1   0   0   0   0   1   0   0

WK5 UE   A  33   0   6   2   1  94  43   0   7   2   0   0   0  10   0   0
    47  64   1   8   2   1  39  47  17   0   3   0   0   2   2   1   0   0
WK5 UE   B  80   0  12   2  10 132  36   0  14   1   5   0   0  10   0   0
   149 140  19  26   0   0  26  25  21   0   3   0   0   0   0   0   0   0
WK5 UE   D  20   0   2  10   1  65  35   0   8   3   3   0   0   1   0   0
    55  93   2   3   2   0  12  11   4   0   1   0   0   0   0   0   0   0
WK5 UE   E  18   2   5   4   1  81  20   0  15   4   1   0   0  14   0   0
    19  36   6   7   5   2  25  19   6   0   1   0   0   1   0   1   0   0

WK5 PU   A 261   0  76   8  12 153  30   0  18   5   3   0   0   0   0   0
   345 109  26  25   1   0  22  15  11   2   2   0   0   2   0   4   0   0
WK5 PU   B 134   0  17   3   2  74  21   0   4   0   7   0   0   5   0   0
   488 201 158  14   0   0  47  38  12   4   0   0   0   0   0   1   0   0
WK5 PU   C 186   0  24  18   2 247  41   0  10   3   2   0   0   1   0   0
   307 174  14  49   0   2  92  34  14   0   2   0   2   2   0   0   0   0
WK5 PU   D 252   0  48   4   6 162  70   0   8   0   3   1   0   3   0   0
   182 144  26   8   1   0  34  11   8   1   2   0   0   2   0   0   1   0
WK5 PU   E 242   0  44   2   4 164  62   0   4   2   4   2   0   4   0   0
   348 204  84  20   0   0  36  24   4   0   4   0   0   2   0   4   2   0

WK5 PE   A  15   0   5   9   0  76  38   0  10   4   4   0   0  19   1   0
    39  57   4   5   2   4  64  65  22   0   6   2   0   0   2   1   0   0
WK5 PE   B 120   0  24   4   2 193  57   0  20   1   7   0   0   5   0   0
    85 156  53  19   0   0  25   8  18   0   2   1   1   0   1   2   0   0
WK5 PE   D  70   0  15   6   2 111  35   0  20   1   1   0   0  18   0   0
   142 185  21   3   1   0  18  11  17   0   2   0   0   5   0   1   0   0
WK5 PE   E   9   0   2   1   0  65  27   0  13   2   3   0   0  13   0   0
    23  39   3   1   0   1  19  10  10   0   1   0   0   4   0   0   0   0

WK6 UU   A 168   0  28  18  18 162  32   0  14   1   3   0   0   0   0   0
   123 203  19  20   6   0  27  40  10   3   4   0   0   1   0   2   0   0
WK6 UU   B 217   0  37  10   2 128  17   0   0   0   8   2   0   1   0   0
   364 155  86  22   0   0  17  50   8  37   4   3   0   0   0   2   0   1
WK6 UU   C 203   0  12   2   1 208  22   0  11   0  11   0   0   5   0   1
   297 318  42  53   8   1  26  13  23   1   3   0   0   0   0   0   0   0
WK6 UU   D 167   0  18   8   4 240  28   0  10   0  14   0   0   9   0   0
   200 186  78  20   4   0  26  39  18   0   0   0   0   0   0   2   1   0
WK6 UU   E 266   0  48  14   8 206  66   0   8   0   5   0   0   8   0   0
   252 234  48  10   2   0  34  27  15   1   0   0   0   0   0   2   0   0

WK6 UE   A  75   0   1   8   0 144  53   0  10   1   2   1   0  14   0   0
    54 174  12  29   0   0  30  72  23   0   3   0   0   1   0   1   0   0
WK6 UE   B  20   1   5   3   0  51  18   0   5   2   4   0   0  13   1   0
    54 114   6   5   0   1  17  40  15   0   2   1   0   1   1   0   0   0
WK6 UE   D 170   0   8   2   4 114  35   0  14   2   4   0   0   8   0   0
   164 245 105  24   0   1  17  21  13   0   0   0   0   0   0   0   0   0
WK6 UE   E  14   0   5   0   5  58  22   0   4   0   1   0   0   2   0   0
    30  17   4   3   1   2   6  20  10   0   1   0   0   3   0   0   0   0
```

```
WK6 PU  A 132    0  20  36    0 156  46   0   6   2   1   0   0   2   0  0
    310 302  68  38    0    0  53  62  17  12   6   1   1   0   0   3   2  0
WK6 PU  B 130    0  42  20    1 188  52   0   0   3  15   3   0   0   0   0  0
    424 222  50  28    0    0  18  32   5   4   2   2   0   0   C   3   0  0
WK6 PU  C 203    0   8   5    1 126  24   0   5   1   6   1   0   1   0   0  0
    291 291  55  35    3    1  45  37  22   0   3   0   0   2   0   2   2  0
WK6 PU  D 281    0  12  12    0  97  40   0   1   1   7   0   0   3   0   0  0
    264 188  96  24    3    0  24  34   9   3   0   0   0   2   0   1   0  0
WK6 PU  E 302    0  33   0    0 290  54   0   9   1  14   1   0   8   0   0  0
    285 256 119  33    0    0  22  28  15   0   1   0   0   2   0   0   0  1

WK6 PE  A  11    0   4   8    3  65  48   1   6   4   1   0   0  14   0  0
     69  43   3   4    2    1  19  20  12   0   1   1   0   0   1   0   0  0
WK6 PE  B  10    1   2   8    2  41  33   0   8   6   2   0   1  11   1  0
     27  44   1   5    1    0  28  24  12   0   2   0   1   0   0   1   0  0
WK6 PE  D  92    1  13  13    2 131  54   1   5   0   2   0   1  11   0  0
    108 150  13  10    0    0  14  30  13   0   0   0   2   1   2   1   0  0
WK6 PE  E  67    0   4   6    2  89  50   0  12   0   1   0   0·  9   1  0
     64 131   9   8    1    0  39  36  19   0   4   1   0   1   0   0   1  0
```

```
*PROC PRINT;
PROC SORT;
  BY type;
PROC MEANS;
  BY type week;
*  var EPH PLE COL TRI DIP ODO COR PET HEB;
 var tot;
PROC SORT DATA = BUG;
  BY type;
PROC ANOVA DATA = BUG;
  BY type;
  CLASS week;
*MODEL EPH PLE COL TRI DIP ODO COR PET HEB = week;
MODEL TOT = WEEK;
MEANS week / LSD;
MEANS week / DUNCAN;
*PROC CHART DATA = BUG;
*vbar week / type = mean sumvar = eph group = type;
*PROC CHART DATA = BUG;
*vbar week / type = mean sumvar = ple group = type;
*PROC CHART DATA = BUG;
*vbar week / type = mean sumvar = col group = type;
*PROC CHART DATA = BUG;
*vbar week / type = mean sumvar = tri group = type;
*PROC CHART DATA = BUG;
*vbar week / type = mean sumvar = dip group = type;
*PROC CHART DATA = BUG;
*vbar week / type = mean sumvar = odo group = type;
PROC CHART DATA = BUG;
  VBAR WEEK / TYPE = MEAN SUMVAR = TOT GROUP = TYPE;

*  MODEL EPH PLE COL TRI DIP ODO COR PET HEB = WEEK TYPE WEEK*TYPE;
*PROC GLM;
*  CLASS WEEK TYPE;
*  MODEL EPH PLE COL TRI DIP ODO COR PET HEB = WEEK TYPE WEEK*TYPE;
*PROC MEANS;
*  BY WEEK;
*  ID TYPE;
*  VAR EPH PLE COL TRI DIP ODO COR PET HEB;
*  OUTPUT OUT = NEW  MEAN = EPHM PLEM COLM TRIM DIPM ODOM CORM PETM HEBM;
*PROC PLOT;
*  PLOT EPHM*WEEK = TYPE;
```