A comparison of the responses of benthic invertebrate individuals, populations, and communities to creosote contamination, with emphasis on Chironomidae (Diptera).

George Pardalis
University of Windsor

Follow this and additional works at: https://scholar.uwindsor.ca/etd

Recommended Citation
Pardalis, George., 'A comparison of the responses of benthic invertebrate individuals, populations, and communities to creosote contamination, with emphasis on Chironomidae (Diptera).’ (1997). Electronic Theses and Dissertations. 565.
https://scholar.uwindsor.ca/etd/565
INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6” x 9” black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700  800/521-0600
NOTE TO USERS

The original manuscript received by UMI contains pages with indistinct and/or slanted print. Pages were microfilmed as received.

This reproduction is the best copy available

UMI
A Comparison of the Responses of Benthic Invertebrate Individuals, Populations, and Communities to Creosote Contamination, with Emphasis on Chironomidae (Diptera).

by

George Pardalis

A Thesis Submitted to the
Faculty of Graduate Studies and Research
Through the Department of Biological Sciences
in Partial Fulfillment of the Requirements for the Degree of Master of Science at the
University of Windsor

Windsor, Ontario, Canada

1997

©George Pardalis 1997
All Rights Reserved
The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L’auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

L’auteur conserve la propriété du droit d’auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.
Abstract

Environmental management strategies include the use of aquatic biota for environmental monitoring and assessment. There exists a need for reliable early warning indicators of impending environmental degradation.

This study evaluated the toxicity of creosote as indicated by a 48-h acute toxicity sediment bioassay and various measures of the benthic invertebrate community and chironomid populations in artificial ponds, to which creosote was added. The study also evaluated the potentially useful early warning indicator, chironomid morphological deformities, in determining a toxic response.

In the laboratory, a 48-h spiked-sediment toxicity test was conducted by placing 4th instar *Chironomus riparius* Meigen larvae in formulated sediment (sand, sculptor's clay, potting soil) spiked with creosote (a PAH mixture). The estimated median lethal concentration (LC50) value was 437 μg creosote/g sediment.

A toxicity study was conducted in 10,000-L artificial ponds (mesocosms) located at the Turfgrass Institute, University of Guelph, Guelph, Ontario. Ponds received a single application of creosote to produce a logarithmic series of nominal concentrations ranging from 0.053 μL/L to 100 μL/L water. The zoobenthos were sampled during the summer and early fall of 1995 (4 July: one day before application of creosote and 1, 4, 8, and 12 weeks posttreatment). Chironomidae was the dominant benthic family present on all sampling dates. Of 4,416 chironomids identified, the genera *Procladius, Ablabesmyia, Tanytarsus,* and *Psectrocladius* were relatively common. As creosote concentration increased,
dominance, based on relative abundance, shifted from *Tanytarsus* or *Procladius* to
*Psectrocladius*. Principal Components analysis identified distinct chironomid assemblages
based on the distribution of chironomid taxa and their abundances among the ponds.
*Procladius* and *Ablabesmyia* consistently belonged to one assemblage while *Psectrocladius*
belonged to another.

Four community attributes (total invertebrate density, family richness, chironomid
density, chironomid generic richness) and two population measures (*Procladius* density,
*Psectrocladius* density) were tabulated and regressed against creosote concentration.
Significant changes in all attributes were detected 1 week after treatment. The nominal
concentration of creosote producing measurable effects decreased with time posttreatment.
Log-Log transformations typically produced a negative relationship from which LC50
values (creosote concentration causing 50% reduction relative to values in control ponds)
were obtained. Density of all invertebrates (1 week posttreatment) was the most sensitive
measure of detrimental effect (LC50=0.33 μg/g sediment), and chironomid richness
(number of genera) (12 weeks posttreatment) was the least sensitive (LC50>63 μg/g
sediment). *Procladius* populations were more sensitive (LC50≤0.85 μg/g) than
*Psectrocladius* populations (LC50≤15 μg/g) to creosote contamination.

Combined triplicate samples of *Procladius* larvae from each pond were examined
for deformities of various cephalic structures (ligula, paralabial combs, and mandibles).
The incidence of ligula deformities in the larvae collected from the pond that received a
nominal concentration of 0.97 μL creosote/L water (0.51-2.19 μg creosote/g sediment) was
significantly greater than the incidence of ligula deformities in the larvae from the control
ponds (1-tailed G-statistic Goodness of fit, $G=14.89$, $p<0.001$). However, incidences of deformities at greater creosote concentrations were not greater than the control. The incidences of paralabial comb and mandible deformities were not significantly greater than that of the control ponds at any treatment concentration. Chi-square tests of independence showed that there was a significant association between the incidence of ligula deformities and asymmetry in the number of teeth on the left and right paralabial combs.

According to this study, cephalic deformities in *Procladius* larvae are not as consistent an indicator of sediment contamination as traditionally used community measures. Detrimental effects in the field studies occurred at much lower concentrations than were observed in the laboratory toxicity tests.
Acknowledgements

I would like to thank my family, C. Jones and W. Ray for their constant support and encouragement. Without them, I would not have been able to get through this experience. I also wish to thank Dr. Ciborowski for his patience throughout this project. Special thanks to L. Hudson, L. Cervi, R. Townsend, M. Tran, J. Chan, D. Sinasac and J. Rozic for their assistance in collecting and sorting the samples.

This research was supported by grants from the Canadian Network of Toxicology Centres (Green Plan, Environment Canada), the Environmental Youth Corps (Ontario Ministry of Energy and Environment), NSERC and Jobs for Ontario Youth.
Table of Contents

Abstract ........................................................................................................ iii

Acknowledgements ..................................................................................... vi

List of Tables ............................................................................................. ix

List of Figures ........................................................................................... xi

Chapter I. General Introduction ................................................................. 1
   Biomonitoring ............................................................................................ 1
   Chironomids and Biomonitoring ............................................................... 2
   Using chironomids in Organism Level Biomonitoring .............................. 3
      Biochemical and Cellular Indicators ................................................... 3
      Morphological Deformities ................................................................. 3
      Life-history Responses ....................................................................... 6
   Using Chironomids in Community Level Biomonitoring ......................... 6
   Toxicity Studies ....................................................................................... 7
      Single Species Toxicity Tests .............................................................. 7
      Mesocosm Studies .............................................................................. 8
   Objectives ............................................................................................... 9

Chapter II. The Acute Toxicity of Creosote to the Aquatic Midge, Chironomus riparius Meigen ................................................................. 10
   Introduction ............................................................................................. 10
   Materials and Methods .......................................................................... 11
      Study Organism .................................................................................. 11
      Experimental Design .......................................................................... 12
      Chemical Analysis .............................................................................. 14
      Statistical Analysis ............................................................................ 15
   Results ..................................................................................................... 16
      Test Parameters ................................................................................. 16
      Chemical Analysis .............................................................................. 16
      Recovery .............................................................................................. 16
      Lethal Concentration ......................................................................... 16
   Discussion ............................................................................................... 19

Chapter III. Evaluating Macroinvertebrate Community Changes and Chironomid (Procladius) Deformities Induced by Creosote in Outdoor Aquatic Mesocosms ............................................................. 21
   Introduction ............................................................................................. 21
   Materials and Methods .......................................................................... 24
List of Tables

Table 3.1: Number of chironomids (pooled samples) mounted and identified of each genus from each artificial pond 4 weeks posttreatment (August 1, 1995). ........... 39

Table 3.2: Number of chironomids (pooled samples) mounted and identified of each genus from each artificial pond 8 weeks posttreatment (August 31, 1995). ....... 40

Table 3.3: Number of chironomids (pooled samples) mounted and identified of each genus from each artificial pond 12 weeks posttreatment (September 26, 1995). ....... 41

Table 3.4: Correlation matrix of relative abundances (octaves) of common chironomid genera (replicate samples pooled on each date) in the artificial ponds (N=16) 4 weeks following creosote application (August 1, 1995). ....................... 47

Table 3.5: Correlation matrix of relative abundances (octaves) of common chironomid genera (replicate samples pooled on each date) in the artificial ponds (N=16) 8 weeks following creosote application (August 31, 1995). ....................... 48

Table 3.6: Correlation matrix of relative abundances (octaves) of common chironomid genera (replicate samples pooled on each date) in the artificial ponds (N=16) 12 weeks following creosote application (September 26, 1995). ....................... 49

Table 3.7: Factor loadings determined by principal components analysis from relative abundances of common chironomid genera from the 16 artificial ponds for the August 1, 1995 (+4 weeks), August 31, 1995 (+8 weeks), and September 26, 1995 (+12 weeks) sampling periods. ....................................................... 51

Table 3.8: Correlation matrix of $\log_{10}(x+1.1)$ transformed total abundances (replicate samples pooled on each date) of common chironomid genera in the artificial ponds (N=16) 4 weeks following creosote application (August 1, 1995). ....................... 59

Table 3.9: Correlation matrix of $\log_{10}(x+1.1)$ transformed total abundances (replicate samples pooled on each date) of common chironomid genera in the artificial ponds (N=16) 8 weeks following creosote application (August 31, 1995). ....................... 61

Table 3.10: Correlation matrix of $\log_{10}(x+1.1)$ transformed total abundances (replicate sampled pooled on each date) of common chironomid genera in the artificial ponds (N=16) 12 weeks following creosote application (September 26, 1995). ....................... 62

Table 3.11: Factor loadings determined by principal components analysis from the total abundances (pooled samples) of common chironomid genera in the 16 artificial ponds for the August 1, 1995 (+4 weeks), August 31, 1995 (+8 weeks), and September 26, 1995 (+12 weeks) sampling periods. ....................................................... 63
Table 3.12: Summary of regression analyses of mesocosm data, and LC50 values for community (density and richness) and population (density) responses. .......................... 74

Table 3.13: Incidence of deformities (percent ± 1 SE) in ligulae of Procladius larvae. .... 104

Table 3.14: 1-tailed G-tests for goodness of fit on the incidence of deformed ligulae of Procladius larvae. Data from all dates combined. ........................................ 107

Table 3.15: Incidence of deformities (percent ± 1 SE) in paralabial combs of Procladius larvae. ................................................................. 108

Table 3.16: 1-tailed G-tests for goodness of fit on the incidence of deformed paralabial combs of Procladius larvae. Data from all dates combined. ............... 110

Table 3.17: Incidence of deformities (percent ± 1 SE) in mandibles of Procladius larvae. ................................................................. 112

Table 3.18: 1-tailed G-tests for the goodness of fit on the incidence of deformed mandibles of Procladius larvae. Data for all dates combined. ...................... 114

Table 3.19: Chi-square test of independence of occurrence on deformities in ligulae and paralabial combs of Procladius larvae. ........................................... 115

Table 3.20: Chi-square test of independence of occurrence of deformities in ligulae and mandibles of Procladius larvae. ............................................... 116

Table 3.21: Chi-square test of independence of occurrence of deformities in paralabial combs and mandibles of Procladius larvae. ....................................... 117
List of Figures

Figure 2.1: Percent ± 1 SE of C. riparius recovered from the experimental containers
(N=5 replicates per treatment). ........................................ 17

Figure 2.2: Creosote concentration-mortality relationship for fourth instar
C. riparius larvae. Regression line takes the form: Log_{10}(Percent Mortality)=
-11.21+6.14 Log_{10}(Creosote Concentration). R^2=0.93. ............... 18

Figure 3.1: Artificial pond (mesocosm) prior to addition of water. Plastic planting trays
filled with marshland sediment laid out on bottom of pond. ............... 25

Figure 3.2: Procladius head capsule, ventral view. (Source: Oliver and Roussel 1983). .... 29

Figure 3.3: Deformities in ligula, paralabial combs, and mandibles of Procladius larvae:
(a) normal and (b-h) deformed ligulae; (i) normal and (j-l) deformed paralabial
combs; and (m) normal and (n-q) deformed mandibles. (Source: Warwick 1989). ... 30

Figure 3.4: Relative abundance of invertebrates (percent ± 1 SE) 1 day before creosote
application (July 4, 1995). Numbers above each concentration represent total
number of invertebrates collected in each pond. N_{control}=2 ponds; N_{treatment}=3
Petersen grabs. ............................................................. 33

Figure 3.5: Relative abundance of invertebrates (percent ± 1 SE) 1 week after creosote
application (July 11, 1995). Numbers above each concentration represent total
number of invertebrates collected in each pond. N_{control}=2 ponds; N_{treatment}=3
Petersen grabs. ............................................................. 34

Figure 3.6: Relative abundance of invertebrates (percent ± 1 SE) 4 weeks after creosote
application (August 1, 1995). Numbers above each concentration represent total
number of invertebrates collected in each pond. N_{control}=2 ponds; N_{treatment}=3
Petersen grabs. ............................................................. 35

Figure 3.7: Relative abundance of invertebrates (percent ± 1 SE) 8 weeks after creosote
application (August 31, 1995). Numbers above each concentration represent total
number of invertebrates collected in each pond. N_{control}=2 ponds; N_{treatment}=3
Petersen grabs. ............................................................. 36

Figure 3.8: Relative abundance of invertebrates (percent ± 1 SE) 12 weeks after creosote
application (September 26, 1995). Numbers above each concentration represent
total number of invertebrates collected in each pond. N_{control}=2 ponds; N_{treatment}=
3 Petersen grabs. ............................................................ 38
Figure 3.9: Relative abundance of chironomids (percent ± 1 SE) 1 day before creosote application (July 4, 1995). Numbers above each concentration represent total number of invertebrates collected in each pond. $N_{\text{control}}=2$ ponds; $N_{\text{treatment}}=3$ Petersen grabs.

Figure 3.10: Relative abundance of chironomids (percent ± 1 SE) 4 weeks after creosote application (August 1, 1995). Numbers above each concentration represent total number of invertebrates collected in each pond. $N_{\text{control}}=2$ ponds; $N_{\text{treatment}}=3$

Figure 3.11: Relative abundance of chironomids (percent ± 1 SE) 8 weeks after creosote application (August 31, 1995). Numbers above each concentration represent total number of invertebrates collected in each pond. $N_{\text{control}}=2$ ponds; $N_{\text{treatment}}=3$

Figure 3.12: Relative abundance of chironomids (percent ± 1 SE) 12 weeks after creosote application (September 26, 1995). Numbers above each concentration represent total number of invertebrates collected in each pond. $N_{\text{control}}=2$ ponds; $N_{\text{treatment}}=3$

Figure 3.13: Graphical representation of results of principal components analysis on the basis of relative abundances (pooled samples) of common chironomid genera collected on August 1, 1995 (4 weeks posttreatment). Each point represents an artificial pond. Numbers above each point represent nominal concentrations. Genera listed are those whose relative abundances load heavily on the factor.

Figure 3.14: Factor scores resulting from principal components analysis on basis of relative abundances (pooled samples) of common chironomid genera collected on August 1, 1995 (4 weeks posttreatment) along nominal creosote concentration gradient.

Figure 3.15: Graphical representation of results of principal components analysis on the basis of relative abundances (pooled samples) of common chironomid genera collected on August 31, 1995 (8 weeks posttreatment). Each point represents an artificial pond. Numbers above each point represent nominal concentrations. Genera listed are those whose relative abundances load heavily on the factor.

Figure 3.16: Factor scores resulting from principal components analysis on basis of relative abundances (pooled samples) of common chironomid genera collected on August 31, 1995 (8 weeks posttreatment) along nominal creosote concentration gradient.
Figure 3.17: Graphical representation of results of principal components analysis on the basis of relative abundances (pooled samples) of common chironomid genera collected on September 26, 1995 (12 weeks posttreatment). Each point represents an artificial pond. Numbers above each point represent nominal concentrations. Genera listed are those whose relative abundances load heavily on the factor. 57

Figure 3.18: Factor scores resulting from principal components analysis on basis of relative abundances (pooled samples) of common chironomid genera collected on September 26, 1995 (12 weeks posttreatment) along nominal creosote concentration gradient. 58

Figure 3.19: Graphical representation of results of principal components analysis on the basis of total abundances (pooled samples) of common chironomid genera collected on August 1, 1995 (4 weeks posttreatment). Each point represents an artificial pond. Numbers above each point represent nominal concentrations. Genera listed are those whose relative abundances load heavily on the factor. 64

Figure 3.20: Factor scores resulting from principal components analysis on basis of total abundances (pooled samples) of common chironomid genera collected on August 1, 1995 (4 weeks posttreatment) along nominal creosote concentration gradient. 65

Figure 3.21: Graphical representation of results of principal components analysis on the basis of total abundances (pooled samples) of common chironomid genera collected on August 31, 1995 (8 weeks posttreatment). Each point represents an artificial pond. Numbers above each point represent nominal concentrations. Genera listed are those whose relative abundances load heavily on the factor. 66

Figure 3.22: Factor scores resulting from principal components analysis on basis of total abundances (pooled samples) of common chironomid genera collected on August 31, 1995 (8 weeks posttreatment) along nominal creosote concentration gradient. 67

Figure 3.23: Graphical representation of results of principal components analysis on the basis of total abundances (pooled samples) of common chironomid genera collected on September 26, 1995 (12 weeks posttreatment). Each point represents an artificial pond. Numbers above each point represent nominal concentrations. Genera listed are those whose relative abundances load heavily on the factor. 69

Figure 3.24: Factor scores resulting from principal components analysis on basis of total abundances (pooled samples) of common chironomid genera collected on September 26, 1995 (12 weeks posttreatment) along nominal creosote concentration gradient. 70
Figure 3.25: Mean density (no./225 cm²) of Procladius and Psectrocladius larvae
(±1 SE) 4 weeks posttreatment (August 1, 1995) ........................................... 71

Figure 3.26: Mean density (no./225 cm²) of Procladius and Psectrocladius larvae
(±1 SE) 8 weeks posttreatment (August 31, 1995) ........................................... 72

Figure 3.27: Mean density (no./225 cm²) of Procladius and Psectrocladius larvae
(±1 SE) 12 weeks posttreatment (September 26, 1995) ................................. 73

Figure 3.28: Mean density (no./225 cm²) ± 1 SE of invertebrates among ponds 1 day
before creosote application (July 4, 1995). Ncontrol=2 ponds; Ntreatment=3 Petersen
grabs.  ................................................................................................................ 76

Figure 3.29: Mean richness (no. families/225 cm²) ± 1 SE of invertebrates among
ponds 1 day before creosote application (July 4, 1995). Ncontrol=2 ponds; Ntreatment=3
Petersen grabs.  .................................................................................................... 77

Figure 3.30: Mean (±1 SE) invertebrate density (no./225 cm²) among ponds 1 week
following creosote application (July 11, 1995). Ncontrol=2 ponds; Ntreatment=3
Petersen grabs. Solid point represents control pond. .......................................... 78

Figure 3.31: Mean (±1 SE) invertebrate richness (no. families/225 cm²) among ponds
1 week following creosote application (July 11, 1995). Ncontrol=2 ponds; Ntreatment=
3 Petersen grabs. Solid point represents control pond. Line fitted by eye. ............ 79

Figure 3.32: Mean (±1 SE) invertebrate density (no./225 cm²) among ponds 4 weeks
following creosote application (August 1, 1995). Ncontrol=2 ponds; Ntreatment=3
Petersen grabs. Solid point represents control pond. ........................................... 80

Figure 3.33: Mean (±1 SE) invertebrate richness (no. families/225 cm²) among ponds 4
weeks following creosote application (August 1, 1995). Ncontrol=2 ponds; Ntreatment=
3 Petersen grabs. Solid point represents control pond. Line fitted by eye. ............ 81

Figure 3.34: Mean (±1 SE) invertebrate density (no./225 cm²) among ponds 8 weeks
following creosote application (August 31, 1995). Ncontrol=2 ponds; Ntreatment=3
Petersen grabs. Solid point represents control pond. Line fitted by eye. ............... 82

Figure 3.35: Mean (±1 SE) invertebrate richness (no. families/225 cm²) among ponds 8
weeks following creosote application (August 31, 1995). Ncontrol=2 ponds;
Ntreatment=3 Petersen grabs. Solid point represents control pond. ................. 83

Figure 3.36: Mean (±1 SE) invertebrate density (no./225 cm²) among ponds 12 weeks
following creosote application (September 26, 1995). Ncontrol=2 ponds; Ntreatment=3
Petersen grabs. Solid point represents control pond. ....................................... 85
Figure 3.37: Mean (±1 SE) invertebrate richness (no. families/225 cm²) among ponds 12 weeks following creosote application (September 26, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. Solid point represents control pond. .......................... 86

Figure 3.38: Mean density (no./225 cm³) ± 1 SE of chironomids among ponds 1 day before creosote application (July 4, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. ............................................ 87

Figure 3.39: Mean richness (no. genera/225 cm²) ± 1 SE of chironomids among ponds 1 day before creosote application (July 4, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. ............................................ 88

Figure 3.40: Mean (±1 SE) chironomid density (no./225 cm²) among ponds 1 week following creosote application (July 11, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. Solid point represents control pond. ..................... 89

Figure 3.41: Mean (±1 SE) chironomid density (no./225 cm²) among ponds 4 weeks following creosote application (August 1, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. Solid point represents control pond. ..................... 90

Figure 3.42: Mean (±1 SE) chironomid richness (no. genera/225 cm²) among ponds 4 weeks following creosote application (August 1, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. Solid point represents control pond. Line fitted by eye. .......... 91

Figure 3.43: Mean (±1 SE) chironomid density (no./225 cm²) among ponds 8 weeks following creosote application (August 31, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. Solid point represents control pond. Line fitted by eye. .......... 92

Figure 3.44: Mean (±1 SE) chironomid richness (no. genera/225 cm²) among ponds 8 weeks following creosote application (August 31, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. Solid point represents control pond. .......... 94

Figure 3.45: Mean (±1 SE) chironomid density (no./225 cm²) among ponds 12 weeks following creosote application (September 26, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. Solid point represents control pond. Line fitted by eye. .......... 95

Figure 3.46: Mean (±1 SE) chironomid richness (no. genera/225 cm²) among ponds 12 weeks following creosote application (September 26, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. Solid point represents control pond. .......... 96

Figure 3.47: Mean (±1 SE) Procadius density (no./225 cm²) among ponds 4 weeks following creosote application (August 1, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. Solid point represents control pond. .......... 97
Figure 3.48: Mean (±1 SE) *Psectrocladius* density (no./225 cm2) among ponds 4 weeks following creosote application (August 1, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. Solid point represents control pond. Line fitted by eye. 99

Figure 3.49: Mean (±1 SE) *Procladius* density (no./225 cm2) among ponds 8 weeks following creosote application (August 31, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. Solid point represents control pond. Line fitted by eye. 100

Figure 3.50: Mean (±1 SE) *Psectrocladius* density (no./225 cm2) among ponds 8 weeks following creosote application (August 31, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. Solid point represents control pond. Line fitted by eye. 101

Figure 3.51: Mean (±1 SE) *Procladius* density (no./225 cm2) among ponds 12 weeks following creosote application (September 26, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. Solid point represents control pond. Line fitted by eye. 102

Figure 3.52: Mean (±1 SE) *Psectrocladius* density (no./225 cm2) among ponds 12 weeks following creosote application (September 26, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. Solid point represents control pond. Line fitted by eye. 103

Figure 3.53: Incidence of deformities (percent ± 1 SE) in ligulae of *Procladius* larvae. Numbers above data points represent sample sizes (all sampling dates combined). Level of significance: * p<0.05. 106

Figure 3.54: Incidence of deformities (percent ± 1 SE) in paralabial combs of *Procladius* larvae. Numbers above data points represent sample sizes (all sampling dates combined). 109

Figure 3.55: Incidence of deformities (percent ± 1 SE) in mandibles of *Procladius* larvae. Numbers above data points represent sample sizes (all sampling dates combined). 113
Chapter I. General Introduction

Biomonitoring

Natural ecosystems are complex systems that are simultaneously exposed to many natural and anthropogenic stresses. Biomonitoring can be defined as "the systematic use of biological responses to evaluate changes in the environment...these changes are often due to anthropogenic sources..." (Matthews et al. 1982, p. 129 cited in Rosenberg 1992). The biological responses used are referred to as bioindicators or indicators.

An indicator is "a characteristic of the environment that, when measured, quantifies the magnitude of stress, habitat characteristics, degree of exposure to stressor, or degree of ecological response to the exposure" (Hunsaker and Carpenter 1990). The number of potential indicators is infinite because every measurable variable in the environment may be an indicator of some aspect of the environmental conditions. However, it is impossible to measure all environmental variables in a cost and time efficient manner so indicators must be selected that are judged appropriate to meet management goals.

There are three general types of indicators: compliance indicators, diagnostic indicators and early warning indicators (Cairns et al. 1993). Compliance indicators (e.g., indicator species) are those used to assess the restoration and maintenance of undegraded environmental conditions. Diagnostic indicators (e.g., sediment bioassays) provide information regarding the cause of noncompliance, and early warning indicators (e.g., physiological/biochemical attributes of an organism) anticipate unacceptable conditions.
before they take effect. Biomonitoring programs are most effective when several different indicators among biotic groups are used together with physical and chemical data.

**Chironomids and Biomonitoring**

Chironomids belong to the family Chironomidae (Diptera) which contains over 5,000 species (Oliver 1971). They are a common insect group in aquatic environments and are an important functional group in benthic communities (Warwick 1990a).

Chironomids have four distinct life stages: egg, larva, pupa and adult. During development, a larva passes through four instars. First instar larvae are planktonic. Second instar larvae settle to the sediment, and the larvae live in or on the sediment until pupation (Oliver 1971). It is during the larval stage that chironomids are exposed to contaminants because the pupal life stage is very short compared to the larval stage, and adult chironomids generally do not feed (Warwick 1988).

There are several advantages to using chironomids for the assessment of environmental conditions. Because chironomids are exposed to contaminants during the larval stage, the longest stage in a short life cycle compared to other species like fish, they represent the culmination of effects over a relatively short period of time. Also, their sedentary nature and their diets place them in direct contact with toxic substances bound to the detrital portion of the sediment.

Due to the abundance and taxonomic richness of this common insect group in the natural environment, Chironomidae are frequently used in the biomonitoring of freshwater bodies (Rosenberg and Resh 1993). They have been used for the classification of water
bodies (Saether 1979), as sentinels (Dickman et al. 1992), and as test organisms in bioassays (Hill et al. 1993).

Using Chironomids in Organism Level Biomonitoring

Biochemical and Cellular Indicators

The effects of pollutants are usually first manifested at the molecular and biochemical levels where the functioning of important biochemical pathways can be affected (McCarthy and Shugart 1990). This disruption in function may, after a period of time, be expressed by decreases in an organism’s ability to grow, to reproduce or to survive. Therefore the measurement of biochemical or cellular parameters has the potential for early warning of adverse effects (Dyer et al. 1993).

Examples of biochemical and cellular parameters include changes in energy metabolism, DNA, RNA and protein synthesis (see Appendix 1) (Huggett et al. 1992). The use of these biochemical indicators in benthic invertebrates, especially chironomids, is limited because there is a lack of basic knowledge of these processes in these organisms (Rosenberg 1992).

Morphological Deformities

Warwick (1990a) defined a deformity as “any morphological feature that departs from the normal configuration”. Among aquatic invertebrates, morphological abnormalities have been reported in tubificid worms (Chapman and Brinkhurst 1987), stonefly larvae (Donald 1980), and mayfly larvae (Pescador and Rasmussen 1994), but the literature is dominated
by reports of deformities in chironomid larvae. Morphological deformities in chironomids range from mildly abnormal mouthparts to the thickening and fusing of body parts (Warwick 1988). Deformities have been observed in the antennae, epipharyngeal pecten, labial lamellae, mentum/ligula, mandible, paraglossa, paralabial combs, premandibles and the ventromentum (Warwick 1988).

Brinkhurst et al. (1968, reviewed in Warwick 1988) found three deformed chironomid larvae in the western basin of Lake Erie from an area exposed to industrial effluents. Cook and Veal (1968, reviewed in Warwick 1988) also reported a high incidence of deformities in *Chironomus* spp. collected from Port Hope Harbour, Lake Ontario, in an area near the cooling water discharge from a uranium refining plant. Deformed mouthparts in the larvae of *Chironomus* spp. were reported in areas contaminated with industrial and agricultural chemicals, but not in areas receiving only domestic effluent (Hamilton and Saether 1971). A detailed review is found in Warwick (1990b). Subsequent field studies have reported antennal (Warwick 1985), mouthpart (Hare and Carter 1976, Koehn and Frank 1980, Warwick 1980a, 1980b, 1980c, Wiederholm 1984, Warwick et al. 1987, Pettigrove 1989, Dickman et al. 1990, 1992, Janssens de Bisthoven et al. 1992, van Urk et al. 1992, Diggins and Stewart. 1993, Lenat 1993, Hudson and Ciborowski 1996a) or both antennal and mouthpart deformities (Warwick and Tisdale 1988, Warwick 1989, 1990a, 1990b, 1991, 1992, Dermott 1991, Bird 1994) due to trace metal, PAH, PCB and other types of sediment contamination. However, many of these studies were observational in nature, so much of the evidence is largely circumstantial (Vermeulen 1996). Laboratory studies have provided a causal link between deformities and trace metals (Kosalwat and Knight 1987,

It appears that different deformities may be caused by specific chemicals (Warwick 1988). However, the mechanisms through which these chemicals are able to cause deformities are not clear. The proposed mechanisms vary from phenotypic (Hare and Carter 1976) to enzymatic (Frank 1981) to hormonal (Warwick 1988).

Mouthpart deformities have been reported in a number of chironomid genera (Warwick 1988, 1990b, Hudson and Ciborowski 1995, Vermeulen 1996), but many of the studies have focused on the genus Chironomus (Diggins 1997). It appears that this genus is very susceptible to deformities across a broad range of contaminants (Warwick 1990b). The genus Procladius has also been commonly reported in deformity studies (Tennessee and Gottfried 1983, Warwick 1989, 1990a, Pettigrove 1989, Dermott 1991, Diggins and Stewart 1993).

Petersen and Petersen (1983) suggested that changes at the level of the individual would be more useful as early warning indicators because individual responses occur before changes at the community level. Cervi (1996) found that the incidence of deformities was positively correlated to pupal size indicative of slowed development. The presence of a deformity also had a significantly negative effect on adult emergence. These results indicate that morphological deformities are related to the fitness of an individual. Therefore, morphological deformities may serve as an early warning indicator of potentially harmful effects from contaminants.
Life-history Responses

Life-history attributes commonly measured in laboratory toxicity studies include survival or mortality, growth or development, and reproduction or emergence (Rosenberg and Resh 1993). However, the reliance on mortality as an indicator of overall system health may be underprotective because other life-history attributes (growth and reproductive success) may be detrimentally affected well before mortality occurs, while the sublethal responses may be overprotective (Thomas et al. 1994).

Chironomids are recognised as a useful test species in acute and chronic sediment toxicity studies (Bedard et al. 1992, Day et al. 1994), and detailed test protocols have been developed. The life-history responses most frequently used in chironomid toxicity tests are mortality/survival and growth (Bedard et al. 1992, Hill et al. 1993). These tests are usually conducted with *Chironomus decorus*, *C. riparius*, *C. tentans* or *Tanytarsus dissimilis* (Rosenberg 1992). Results obtained from these tests must be carefully interpreted because every species has a different sensitivity to a specific chemical (Hill et al. 1993).

Using Chironomids in Community Level Biomonitoring

Traditionally, community studies focus on identifying, enumerating and listing taxonomic groups present in collected samples (Warwick 1990a). Freshwater benthic macroinvertebrate surveys have been used extensively to evaluate habitat, sediment and water quality (Davis and Lathrop 1992). This approach has been used to document crude-oil exposure (Rosenberg and Wiens 1976, Cushman 1984, Cushman and Goyert 1984) and trace metal exposure (Wiederholm 1984). It has been used in biomonitoring for many years
and has been combined with sediment chemistry and toxicity tests in an approach called the "Sediment Quality Triad Approach" (Chapman et al. 1987).

Chironomids as a single group have been used primarily for the biological classification of the trophic status of lakes (Saether 1979, Wiederholm 1980). They have also been used to monitor organic richness in streams (Ferrington and Crisp 1989) and acidified lakes (Raddum and Saether 1981). Together with oligochaetes, they dominate the most severely degraded habitats and are useful in pinpointing localized areas of pollution (Saether 1979).

Toxicity Studies

Single Species Toxicity Tests

There are two basic types of single species toxicity tests: single species acute, and single species chronic (Rosenberg 1992).

Single species toxicity tests have shown that contaminants in sediments affect the survival of benthic species (Hill et al. 1993). These tests are the backbone of many biomonitoring programs and part of the sediment quality triad. Acute single species toxicity tests are short-term (between 48 and 96 h). Mortality is a common life-history response measured. Chronic single species toxicity tests may last for the entire life cycle of an organism. Commonly measured responses are mortality, growth and reproduction (Buikema and Voshell 1993). However, laboratory toxicity tests have been criticized for their lack of environmental realism because toxicity is influenced not only by the amount of toxicant available to the organism but also by oxygen concentration, temperature, and food availability (Harkey et al. 1994).
**Mesocosm Studies**

Buikema and Voshell (1993) defined mesocosms as “experimental containers holding >10 m³” and Odum (1984) described mesocosms as “middle-sized worlds falling between laboratory microcosms and large, complex, real world macrocosms.” The term *mesocosm* generally refers to a controlled, intermediate-sized artificial pond or flume that is used to simulate natural systems (Touart and Slimak 1989, Touart 1988). Artificial ponds usually contain representative pond biota such as flora and invertebrate fauna. Boyle (1983) proposed that mesocosms should also be capable of supporting reproducing populations of fish. These assemblages of organisms, together with appropriate substrates, should be as complex as natural communities and in theory should respond to stressors very similarly to natural systems.

Mesocosm studies evolved from the farm pond studies of the 1970’s and early 1980’s (Shaw *et al.* 1994). Mesocosm studies were developed to mitigate many of the problems associated with farm pond studies. They have more environmental realism than standardized laboratory tests because physical, chemical and biological interactions are present (Johnson *et al.* 1994, Lucasen and Leeuawangh 1994), but their responses are not as variable as the natural ecosystem (Shaw and Kennedy 1996).

The mesocosm study was part of the US EPA’s testing protocol for pesticide registration. It was a final-tier field test used to negate any presumptions of hazard that had been shown in the lower-tiered testing (Touart and Slimak 1989). In 1992, the mesocosm study was removed from the testing protocol because the assumption of risk was too difficult to cost-effectively falsify due to the variability of aquatic communities (Shaw and
Kennedy 1996). The role mesocosm studies can play in biomonitoring programs has yet to be decided.

Objectives

The purpose of this research was to compare laboratory toxicity test and benthic invertebrate community responses to the potentially useful early warning indicator, chironomid morphological deformities. I expected individuals (by exhibiting deformities) would show the detrimental effects of creosote before community effects became apparent. Also, because organisms in natural environments do not exist under ideal abiotic conditions, I expected effects in the field to occur at lower concentrations than in the laboratory.

In a mesocosm study, I determined whether there was a dose-response relationship between creosote concentrations and the incidence of morphological deformities in Procladius, the dominant chironomid genus colonizing the study ponds. Several community level measures of environmental degradation (e.g., abundance and richness) were also determined for comparative purposes.

Using a sediment bioassay, I determined the acute toxicity of creosote on fourth instar larvae of Chironomus riparius.

This research was part of a larger study to develop and validate the performance of early warning indicators to assess the toxicity of a mixed PAH source (creosote) to experimental aquatic ecosystems (Harris et al. 1996).
NOTE TO USERS

Page(s) not included in the original manuscript and are unavailable from the author or university. The manuscript was microfilmed as received.

10

This reproduction is the best copy available.

UMI
Bioaccumulation of PAHs by benthic invertebrates has been demonstrated by Varanasi et al. (1985). Leversee et al. (1982), Clements et al. (1994), and Harkey et al. (1994) have reported that chironomids are capable of bioaccumulating and metabolizing PAHs.

A spiked-sediment toxicity test is used to establish cause-and-effect relationships between chemicals and adverse effects. In general, test organisms are exposed to sediments that have been inoculated with known quantities of toxic chemicals. After a specified period of time, the test organisms are examined for adverse effects. This test assumes that the lab results of a given sediment are representative of effects from sediment in the field and that the chemical dynamics are similar in spiked sediment and natural sediment (Lamberson and Swartz 1992).

The objective of this chapter is to determine the 48-h median lethal concentration (the creosote concentration where 50% of the test organisms die within 48 h) of creosote to fourth-instar C. riparius. This LC50 value will be compared with field-based community and individual responses.

Materials and Methods

Study Organism

The chironomid species used in this toxicity study was Chironomus riparius Meigen. Chironomids are widely distributed in North America and Europe and colonise any available water body (Oliver 1971). Chironomus riparius has been used in acute and chronic laboratory toxicity tests to determine the toxicity (in terms of growth and survival) and bioavailability of contaminants in the sediments of aquatic systems (Williams 1985,

Continuous cultures of *C. riparius* have been maintained at the University of Windsor since 1994. The stock animals were originally obtained from the Canada Centre for Inland Waters (Burlington, ON). After the cultures were initiated, further animals were obtained from the State University of New York, Buffalo. The organisms were reared in 20-L continually aerated aquaria containing 2 cm depth of silica sand and 10 cm depth of carbon-filtered, dechlorinated City of Windsor tapwater. The organisms were fed a ground Tetra Min<sup>R</sup>/dechlorinated water mixture *ad libitum*. Due to temperature fluctuations in the culture room (18-26°C), time to emergence was unpredictable. In order to ensure a regular supply of larvae in adequate numbers for toxicity studies, egg masses were collected from the stock cultures and placed individually in 2-L glass jars containing 2 cm of silica sand and 8 cm of carbon-filtered, dechlorinated water. The jars were placed in a controlled environmental chamber at a temperature of 23±1°C with a photoperiod of 16 h light: 8 h dark and gently aerated. The feeding regime was similar to that of the stock cultures. The majority of the larvae would consistently emerge 16-18 d after inoculation.

**Experimental Design**

An acute toxicity test (48 h in duration) was conducted on early fourth-instar *C. riparius* midge larvae (Powlesland and George 1986, Williams et al. 1986) randomly selected from the stock colony reared in the environmental chamber. Groups of 15 larvae were used for each replicate. There were five replicates for each of five test concentrations
and the control. Because of the physical nature of creosote, acetone was used as a carrier (Borthwick and Patrick 1982). A carrier (acetone only) control was also included in the design. The control and test concentration containers were randomly arranged in the environmental chamber. Dissolved oxygen levels and pH were measured at the beginning and end of the test.

The toxicity test was conducted in a controlled environmental chamber in the dark (to avoid PAH photodegradation) with the temperature maintained at 23±1°C. The experimental containers were 2-L hexane-rinsed glass jars containing formulated sediment composed of silica sand, garden soil and sculptor’s clay (Hanes 1992) and dechlorinated, carbon-filtered water to achieve a 4:1 (v/v) water:sediment ratio (Bedard et al. 1992). In order to reduce evaporation of water and volatilization of the toxicant, hexane-rinsed aluminum foil-covered plastic lids were placed on the jars. Each lid had a small hole cut in it to allow for the introduction of capillary tube air lines which provided continuous aeration during the study. The larvae were not fed during the test.

The test concentrations of creosote were dissolved in 100 mL of acetone and this mixture was added directly to the dry artificial sediment of each replicate. Six replicates were prepared for each of the test concentrations and controls. The sediment was manually mixed until all of the sediment was visibly wet. The sediment was again manually mixed for another 10 min. All of the replicate jars, controls and treatments, were placed in a darkened fume hood until the acetone had evaporated (approximately 48 h). The sediment was mixed again in order to break up clumps. The water was then gently added and the test containers were placed in the environmental chamber one week prior to the commencement of the
toxicity test in order for the toxicant concentration to equilibrate between the water and sediment (Hudson 1994). Midge larvae were added to five of the six replicates. The sixth replicate was included in the test design as a blank. The sediment in these jars was analysed to determine the sediment PAH concentrations.

At the end of the test, the sediment and water of each replicate was passed through a 500-µm sieve, and the number of live and dead larvae enumerated. Death was defined as the absence of movement in response to probing with forceps. The sediment and water from the blank jars were stored separately in hexane-rinsed amber jars and bottles, respectively. The lids for these containers were covered in hexane-rinsed aluminum foil. The water and sediment samples were stored at 7°C and -20°C respectively until chemical analysis.

Chemical Analysis

Only the sediment was analysed for its PAH concentrations. The methodology used was a hybrid of the protocols used by the Centre for Toxicology, University of Guelph (Bestari et al. 1997) and the Great Lakes Institute for Environmental Research, University of Windsor (Lazar 1995). Briefly, the sediment samples were thawed at room temperature in the dark. Approximately 10 g of wet sample was dried using a vacuum filtration apparatus. Approximately 5 g of dry sample was weighed out and its mass was recorded. The sample was mixed with 25 g of sodium sulphate and the mixture was transferred to an extraction thimble. The samples were extracted, using hexane, in a group of six using six Soxhlet extractors. The extraction lasted 16 h. The volumes of sediment extract were reduced in a rotary evaporator, and this concentrated extract was poured into a separatory funnel containing Nanopure® grade water and extracted with hexane. The hexane extract
was rotaevaporated to 50 mL and poured through a chromatographic column containing anhydrous sodium sulphate. The dried extract was then rotaevaporated to 2 mL and passed through a florisil column. The extract was eluted with hexane. Activated copper powder was added to the extract. The prepared extracts were sent to the Centre for Toxicology, University of Guelph for analysis by a HPLC-fluorescence detector (Bestari et al. 1997). The PAHs analyzed were: naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo-(a)-anthracene, chrysene, benzo-(b)-fluoranthene, benzo-(k)-fluoranthene, benzo-(a)-pyrene, dibenzo-(a,h)-anthracene, benzo-(g,h,i)-perylene, and indeno-(1,2,3-cd)-pyrene. These PAHs are on the US EPA’s Priority List of Pollutants (Gurprasad et al. 1995) and typically make up about 30% of the total volume of creosote (Bestari et al. 1997).

Water content was also determined for each sediment sample. A known mass of sediment sample, placed in a preweighed aluminum weighing boat, was oven-dried at 106°C for 24 h. The loss in mass was the mass of the water.

**Statistical Analysis**

To make the dose-response relationship linear, the concentrations of creosote were Log\(_{10}\) transformed and the *Chironomus* mortality values were transformed to the probit scale (Finney 1971). A linear regression was conducted to relate probit-transformed mortality to Log\(_{10}\) creosote concentration. The LC50 value was determined by interpolating the creosote concentration that corresponded to 50% mortality from the regression line.
Results

Test Parameters

The temperature remained constant at 23±1°C during the test. Dissolved oxygen (>80% saturation) and pH (6.0-6.5) values did not change from change from the beginning to the end of the test.

Chemical Analysis

The total PAH concentrations (sum of the concentrations of the 15 PAHs analyzed) for the sediment samples were 0.1684 µg/g, 158.91 µg/g, 182.14 µg/g, 356.65 µg/g, 457.82 µg/g and 785.27 µg/g for the carrier control and the five test concentrations respectively. The control replicate was not analyzed.

Recovery

The mean (± 1 standard error) percentage of larvae recovered (alive and dead) from the control, carrier control and the five test concentrations were: 89.3±3.39%, 90.7±5.00%, 89.3±4.00%, 96.0±1.64%, 93.3±3.65%, 100±0.00%, and 92.0±3.88% respectively (Figure 2.1). The remaining test organisms had either developed into pupae or adults.

Lethal Concentration

Figure 2.2 shows that mortality of C. riparius was significantly and positively related to increasing creosote concentration (R²=0.93, p<0.01). Many of the surviving larvae collected from the 457 µg/g and 785 µg/g test containers were discoloured after the 48 h period, indicating metabolic impairment and breakdown of hemoglobin.
Figure 2.1: Percent ± 1 SE of *C. riparius* recovered from the experimental containers (N=5 replicates per treatment).
Figure 2.2: Creosote concentration-mortality relationship for fourth instar *C. riparius* larvae. Regression line takes the form: \( \log_{10}(\text{Percent Mortality}) = -11.21 + 6.14 \log_{10}(\text{Creosote Concentration}) \). \( R^2 = 0.93 \).
In this static, spiked-sediment test, the 48-h LC50 value (based on sediment concentrations) of creosote to fourth instar *C. riparius* larvae was 437 µg/g.

**Discussion**

This particular toxicity test design was originally chosen in order to determine the stress protein 70 accumulation to creosote concentration relationship. Stress protein 70 may accumulate in the cells of organisms undergoing development (Sanders 1990). In order to negate this potentially confounding variable, an acute toxicity test with fourth instar larvae was conducted instead of a chronic toxicity test that allowed second instar larvae to develop into fourth instar larvae. Although single species acute toxicity tests were common a decade ago for the evaluation of potentially harmful substances, chronic toxicity tests are currently used (Pascoe *et al.* 1989) because detrimental effects may be related to changes in growth, reproduction, and other functions that do not necessarily result in early death (Kosalwat and Knight 1987).

The evaluation of creosote toxicity to aquatic species is difficult because creosote is not readily dissolved in water. Different approaches to toxicity testing have resulted in different toxicity results which are not readily comparable. For example, Millemann *et al.* (1984) reported 48 hr LC50 values of 2.81 mg/L and 0.49 mg/L for naphthalene and phenanthrene, respectively, for fourth-instar *Chironomus tentans*. These values convert to 2,810 µg/L and 490 µg/L but a direct comparison to the sediment values in this study is not possible because the test concentrations were prepared differently.
Gurprasad *et al.* (1995) examined the PAH content of out-of-service railroad ties, telephone ties and marine pilings in Western Canada. The analysis focussed on those PAHs identified on the US EPA's Priority Pollutants List. They reported a total average PAH concentration of 3,126 μg/g for each item examined. Creosote-derived phenanthrene and benzo(a)pyrene have been reported in river sediments at concentrations of 231 μg/g and 16 μg/g (Black 1982). Berard and Tseng (1986) reported sediment concentrations in Thunder Bay Harbour, near a wood preservation facility, at 26,388 mg/kg, and Environment Canada (1993) has measured total PAH concentrations of up to 39,630 mg/kg. Concentrations of fluoranthene of 181 μg/g have been reported in *C. riparius* tissue (Clements *et al.* 1994).

Ciborowski and Corkum (unpubl.) evaluated the toxicity of field-collected creosote-laden sediment by determining the 21-day survival of *Hexagenia* larvae. They suggested the LC50 value was approximately 242 μg/g sediment. Based on this result and the concentrations of PAHs found in aquatic sediments, the LC50 value determined in this study appears to be a reasonable ballpark estimate of the acute effects of creosote on *C. riparius*. However, a single 48-h acute toxicity test can not accurately evaluate the toxicity of a compound. Long-term studies under realistic conditions are needed to accomplish this goal. The use of outdoor aquatic ponds is one method employed to examine the toxicity of compounds under realistic conditions (Touart and Slimak 1989).
Chapter III. Evaluating Macroinvertebrate Community Changes and Chironomid (*Procladius*) Deformities Induced by Creosote in Outdoor Aquatic Mesocosms.

**Introduction**

In aquatic systems, many benthic macroinvertebrates spent most of their life cycle living in the sediment or feeding upon detritus associated with the sediment (Rosenberg and Resh 1993). Because of this association, they may be used to identify potential sediment contamination. Benthic macroinvertebrate community composition has been used as a regulatory tool by governments for years (Davis and Lathrop 1992). Benthic invertebrate surveys have been used to monitor contaminant effects in aquatic systems (Krieger 1984, Thornley 1985, Hart *et al.* 1986, Pinel-Alloul *et al.* 1996). Community level responses to sediment contamination may include: a reduction in the number of organisms present, a reduction in taxonomic richness, the elimination of intolerant populations or a change in the relative abundance of dominant taxa (Ciborowski *et al.* 1995).

Due to the complexity of factors affecting the responses at the community level, specific causative relationships are difficult to identify in actual field tests (Cairns *et al.* 1993). Surrogate field testing, in the form of application of stress to artificial ponds or mesocosms, permits the determination of dose-response relationships under reasonably realistic conditions (Shaw *et al.* 1994). Once changes at the community level become apparent (i.e., loss of organisms or taxa), significant damage to the aquatic system will have already taken place and will probably be difficult to reverse. However, sublethal changes observed in the health of individuals may serve as early warning indicators of imminent deleterious effects at the community level because individual responses must precede
community responses (Petersen and Petersen 1983). The incidence of morphological deformities in chironomid larvae may serve as such an early warning indicator of potentially harmful effects from sediment contamination (Warwick 1988). Warwick (1990a) suggested that a morphological deformity was a clear indication that something was fundamentally wrong with the environment.

The longest developmental stage in the chironomid life cycle is the larval stage, and chironomid larvae are closely associated with sediment (Oliver 1971). During this period, chironomids are often exposed to persistent hydrophobic contaminants, many of which are accumulated in their tissues. Dickman et al. (1992) reported that deformed chironomid larvae from a contaminated canal had higher PAH concentrations in their tissues than did undeformed chironomids from the same area. They suggested that PAHs acted as teratogens.

Warwick (1990a) believed that as contaminant concentrations increased, observable effects (morphological deformity) shifted from easily deformed structures (e.g., antennae) to less sensitive structures (e.g., mandibles). Based upon extensive observation, he ordered the following morphological structures from most to least susceptible to deformity: antennae, epipharyngeal pectin, mentum, mandible, premandible and labral lamellae.

The occurrence of polynuclear aromatic hydrocarbons in aquatic environments is well documented (Helfrich and Armstrong 1986, Marcus et al. 1988, Swartz et al. 1988, Krantzberg and Boyd 1992, Pham et al. 1993, Huntley et al. 1995). The combustion of fuels is a major PAH source (Helfrich and Armstrong 1986), and these emissions enter aquatic systems primarily through atmospheric deposition (Pham et al. 1993).
Contamination with PAHs from wood-treatment plant wastewater may also be significant in some areas (Borthwick and Patrick 1982). Once in the aquatic environment, PAHs accumulate in the sediment (Marcus et al. 1988) because of their hydrophobic nature and their strong affinity for organic matter (Huntley et al. 1995). Reports of sediment PAH concentrations have ranged from 0.2 µg/g to 6.2 µg/g for sediments collected from Southern Lake Michigan (Helfrich and Armstrong 1986) to as high as 26,388 µg/g in sediments from Thunder Bay Harbour (Environment Canada 1993). The survival of many fish and invertebrate species is adversely affected by moderate levels of PAH contamination (Neff and Anderson 1981, Catallo and Gambrell 1987). However, studies have shown that PAHs may enhance the abundance (Bunch 1987) or alter the activity (Bauer and Capone 1985) of sedimentary bacteria. As well, several researchers have reported an increase in the growth of microalgae at both low (Carman et al. 1995) and high concentrations of PAHs (Chan and Chiu 1985).

The objective of this study was to: (1) tabulate incidences of deformities of various cephalic features in chironomids collected from artificial ponds treated with various concentrations of creosote; (2) determine if a relationship existed between the incidence of morphological deformities in cephalic structures of the common chironomid genera and creosote (a mixed PAH source) concentrations; and, (3) to compare the sensitivity these individual responses to the community (density and taxonomic richness) and population level responses of the benthic macroinvertebrates that had naturally colonized the artificial ponds (mesocosms). I expected that as creosote concentration increased, observable effects at the community and population levels would include a decrease in the number of
organisms and a loss of taxa. I also expected that there would be an increase in the incidence of deformities at lower creosote concentrations than that which community level effects occurred.

Materials and Methods

Mesocosm Study Design

The experiment was conducted using 16 10,000-L mesocosms. The details of the construction of the artificial ponds are described in Harris et al. (1996). Artificial ponds (Figure 3.1), 1 m deep and 4 m in diameter, are located at the Turfgrass Institute of the University of Guelph in an enclosed area. They were lined with food grade polyvinyl chloride (PVC) that is nontoxic to aquatic life. Plastic planting trays (52.1x25.4x5.7cm deep) were filled with dry sediment, excavated from a local marsh, to a depth of approximately 5 cm. The organic content of the sediment was 5% (Bestari et al. 1997). In May 1995, planting trays were placed on the floor of each pond so that 50% of the bottom surface area was covered. After the addition of the sediments, the ponds were filled with water pumped from a nearby holding pond (62x62x4m). During the pretreatment period (approximately 3 weeks in duration), the water from the artificial and holding ponds was allowed to continually intermix through a pump and standpipe system. Plankton and benthos communities colonized the ponds naturally. Potted macrophytes (*Myriophyllum* spp.) and six breeding pairs of fathead minnows (*Pimephales promelas*) were added to each artificial pond (Harris et al. 1996).
Figure 3.1: Artificial pond (mesocosm) prior to addition of water. Plastic planting trays filled with marshland sediment laid out on bottom of pond.
On 5 July 1995, creosote was added to each pond by sub-surface injection, and the water was then thoroughly mixed (Harris et al. 1996). Fourteen ponds each received a single application of creosote to produce a logarithmic series of nominal concentrations ranging from 0.053 $\mu$L/L to 100 $\mu$L/L water. Two ponds that did not receive creosote were designated as controls.

Sample Collection and Sorting

Researchers and technicians from the University of Guelph regularly collected water quality and residue chemistry data. Phytoplankton, periphyton, zooplankton, macrophyte and fish samples were also collected (Harris et al. 1996).

The benthic communities of the artificial ponds were sampled 1 day prior to creosote application (4 July) and 1 (11 July), 4 (1 August), 8 (31 August) and 12 (26 September) weeks posttreatment. Triplicate mini-Petersen grabs (225 cm$^2$; Ward's Scientific Ltd.) were taken from randomly selected planting trays in each pond from a platform extended across the pond. Using a stainless steel putty knife, a subsample (1/3 of a 167 mL jar) from each of the collected sediment samples was placed in an amber jar. This subsample was analyzed by the Centre for Toxicology, University of Guelph for concentrations of 15 PAH compounds (Bestari et al. 1997). The remaining sample was rinsed in a sieve bucket (mesh size 600 $\mu$m), and the material remaining in the bucket was preserved in Kahle's solution. In the laboratory, samples were rinsed through a graded series of sieves (4.00, 1.00, 0.500, 0.250, 0.125 mm). Material retained on each sieve was sorted at low and medium power with a dissection microscope (Ciborowski 1991). Animals removed from each sieve category were preserved and stored separately in 70% ethanol. The macroinvertebrates
(≥500 μm) were enumerated and identified to the lowest practical level. For consistency, except for chironomid genera, I will refer to invertebrate designations at the family taxonomic level in this thesis.

**Chironomid Identification**

All chironomid larvae from the 4-mm and 1-mm sieve fractions were slide-mounted for taxonomic identification and morphological evaluation. The slide-mounting procedure is outlined in Hudson (1994). The larva was positioned so that its head capsule was ventral side up. If the larvae was large, the head capsule and body were mounted under separate cover slips on the same slide. The chironomid was placed on a microscope slide in a few drops of CMC-9AF® aqueous mounting medium (Master's Chemical Company, Des Plaines, Illinois). A glass cover slip was placed over the larva and gentle pressure was applied in order to separate the mouthparts. After approximately 24 h, the coverslip was ringed with nail polish. The specimen was allowed to clear for several days before being examined with a compound light microscope at 400X magnification. All larvae were identified to genus using the key of Oliver and Roussel (1983). Voucher specimens are archived at the University of Windsor.

**Morphological Evaluation**

All slides containing *Procladius* specimens were selected for detailed morphological evaluation. Each specimen was assigned a unique, random number and the slides were placed in numerical order. Another unique, random number was assigned and the slides were again numerically ordered. This double-blind evaluation should have been sufficient to
prevent any bias associated with prior knowledge of the level of contamination of the pond with which each larvae was associated.

The ligula, paralabial combs (=paralabial teeth) and the mandibles of *Procladius* larvae (Figure 3.2) were examined for deformities. Deformities of the ligula, paralabial combs and mandibles were categorized based on Dermott’s (1991) and Warwick’s (1989) frameworks (Figure 3.3). Potential ligula deformities included: asymmetry, absence of teeth, overlapping of teeth, presence of accessory teeth, and the presence of forked teeth. Possible deformities in the paralabial combs included: asymmetry in the number of teeth per comb, reduction in size of teeth, and absence of teeth. Wrinkled outer edge, fused, or bifid teeth were possible mandible deformities.

**Statistical Analysis**

*Community Characterization*

The relative abundances of the dominant taxa in the overall invertebrate and chironomid communities were calculated for each sampling date (pretreatment and 1, 4, 8, and 12 weeks posttreatment). The chironomid community, 1 week posttreatment, was not analyzed because the data were not available. A Principal Components Analysis (PCA) was conducted on the chironomid communities to determine similarities in chironomid density ($\log_{10} x + 1.1$ transformed) and relative abundance ($\log_{2} x + 0.5$ transformed) among the ponds for each of the posttreatment sampling periods (1 August, 31 August, and 26 September). Only chironomid genera that were designated as “common” (occurred in $\geq 25\%$ of the ponds and comprised $\geq 10\%$ of the chironomid community in at least one pond) were used in the analyses.
Figure 3.2: *Proclaius* head capsule, ventral view. (Source: Oliver and Roussel 1983).
Figure 3.3: Deformities in ligula, paralabial combs, and mandibles of *Procladius* larvae: (a) normal and (b-h) deformed ligulae; (i) normal and (i-l) deformed paralabial combs; and (m) normal and (n-q) deformed mandibles. (Source: Warwick 1989) .
Community (Density and Taxa Richness) and Population (Density) Responses to Creosote Contamination

Total invertebrate and total Chironomidae density and taxa richness values were calculated for each pond for the pretreatment and 1, 4, 8, 12 weeks posttreatment sampling periods. Chironomid generic richness, 1 week posttreatment, was not calculated because the data were not available.

Linear regression analysis was used to determine the sediment creosote concentration-community/population response relationship. Both the dependent (density, richness) and independent (actual PAH concentration in sediment) variables were Log_{10} transformed prior to performing regression analysis. Median lethal concentration (LC50) values were determined for each community response for the three posttreatment sampling dates. The LC50 value was the estimated PAH concentration in the sediment that reduced the community response to 50% of the control value.

Incidence of Deformities

Samples from the three posttreatment dates were pooled for each pond. The relative frequency of deformities was expressed as a percentage (100 x the ratio of the number of actual to possible occurrences) ± 1 SE (Warwick and Tisdale 1988). The standard errors were calculated using the binomial formula: \( \sqrt{pq/k} \) where p=proportion of deformed specimens, q=proportion of undeformed specimens and k=sample size (Sokal and Rohlf 1995). The 1-tailed G-statistic goodness of fit test was used to determine if differences in the incidence of deformities in the test concentrations were significantly greater than the control (Sokal and Rohlf 1995).
Results

Invertebrate Community Composition

The invertebrate communities in the artificial ponds were dominated by the family Chironomidae (Figures 3.4-3.8) on all sampling dates. Other common taxa present were mayflies (Caenis: Caenidae: Ephemeroptera), cladocerans (Daphnidae), and oligochaete worms (Naididae and Tubificidae). During the pretreatment sampling period, chironomids composed between 75.4±14.21% and 99.2±0.12% of the total benthic invertebrate numbers in each pond (Figure 3.4). One week posttreatment (11 July), Daphnidae (47.2±10.9%-58.1±5.1%) and Chironomidae (46.7±4.8%-49.2±11.3%) were the co-dominant invertebrate groups in the ponds with nominal creosote concentrations ≤0.10 μL/L. At nominal concentrations ≥0.17 μL/L, Chironomidae (63.9±17.2%-100±0.0%) was the dominant invertebrate group in each pond (Figure 3.5).

Four weeks following creosote application, 45±4.82% of the benthic community in the control ponds was composed of chironomids (Figure 3.6). Caenis mayflies made up 21.2±1.03% of the benthic community in the control ponds. The percentage of the community that consisted of chironomid midges ranged from 33.3±33.3%, at 56 μL/L, to 97.0±3.03%, at 9.83 μL/L, for this sampling period. Chironomidae was the most dominant insect taxon in every pond except in the pond that received a nominal creosote concentration of 17.6 μL/L (Daphnidae was dominant).

Chironomidae composition ranged from 51.3±3.21% (control) to 93.9±6.07% (100 μL/L) eight weeks posttreatment (Figure 3.7). Mayflies comprised 30.1±4.02% of the control invertebrate community but their dominance was reduced as the creosote
Figure 3.4: Relative abundance of invertebrates (percent ± 1 SE) 1 day before creosote application (July 4, 1995). Numbers above each concentration represent total number of invertebrates collected in each pond. \( N_{\text{control}} = 2 \) ponds; \( N_{\text{treatment}} = 3 \) Petersen grabs.
Figure 3.5: Relative abundance of invertebrates (percent ± 1 SE) 1 week after creosote application (July 11, 1995). Numbers above each concentration represent total number of invertebrates collected in each pond. $N_{control}=2$ ponds; $N_{treatment}=3$ Petersen grabs.
Figure 3.6: Relative abundance of invertebrates (percent ± 1 SE) 4 weeks after creosote application (August 1, 1995). Numbers above each concentration represent total number of invertebrates collected in each pond. N_{control}=2 ponds; N_{treatment}=3 Petersen grabs.
Figure 3.7: Relative abundance of invertebrates (percent ± 1 SE) 8 weeks after creosote application (August 31, 1995). Numbers above each concentration represent total number of invertebrates collected in each pond. \( N_{\text{control}} = 2 \) ponds; \( N_{\text{treatment}} = 3 \) Petersen grabs.
concentration increased. Other invertebrate families comprised between 0% and 20.2±11.66% in their respective ponds.

Twelve weeks posttreatment (Figure 3.8), the percentage of the benthic community composed of chironomids ranged from 34.8±2.81% in the control ponds to 91.8±1.65% in the pond with a nominal creosote dose of 56 μL/L. Caenis individuals comprised between 5.3± 0.21% and 39.9±4.81% (control) of the benthic pond communites. The family Chironomidae was not as dominant in these samples as it had been during previous sampling periods.

Overall, Chironomidae was the dominant invertebrate family in each pond among all of the sampling dates. Generally, at low creosote concentrations, chironomids consisted of a smaller proportion of the benthic community, but the overall number of invertebrates was high. As the nominal concentration of creosote increased, the number of individuals in other invertebrate taxa was reduced, and consequently, chironomids comprised a larger proportion of the invertebrate community because their relative numbers were not reduced as much.

**Chironomid Community Composition**

I identified 3,222 chironomids belonging to 14 different genera collected during the three posttreatment sampling dates (Tables 3.1-3.3). Another 1,194 chironomids were identified from the pretreatment ponds. Although numbers varied among dates and ponds, the genera Procladius, Ablabesmyia, Tanytarsus and Psectrocladius were relatively common and the genera Cryptotendipes, Endochironomus, Dicrotendipes and Cladotanytarsus were relatively rare.
Figure 3.8: Relative abundance of invertebrates (percent ± 1 SE) 12 weeks after creosote application (September 26, 1995). Numbers above each concentration represent total number of invertebrates collected in each pond. $N_{\text{control}}=2$ ponds; $N_{\text{treatment}}=3$ Petersen grabs.
Table 3.1: Number of chironomids (pooled samples) mounted and identified of each genus from each artificial pond 4 weeks posttreatment (August 1, 1995).

<table>
<thead>
<tr>
<th>Genus</th>
<th>0</th>
<th>0</th>
<th>0.05</th>
<th>0.10</th>
<th>0.17</th>
<th>0.30</th>
<th>0.54</th>
<th>0.97</th>
<th>1.73</th>
<th>3.08</th>
<th>5.51</th>
<th>9.83</th>
<th>17.5</th>
<th>36</th>
<th>56</th>
<th>100</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tanytarsus</td>
<td>64</td>
<td>34</td>
<td>45</td>
<td>30</td>
<td>30</td>
<td>62</td>
<td>21</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>294</td>
</tr>
<tr>
<td>Procladius</td>
<td>15</td>
<td>17</td>
<td>27</td>
<td>10</td>
<td>9</td>
<td>12</td>
<td>16</td>
<td>95</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>209</td>
</tr>
<tr>
<td>Ablabesmyia</td>
<td>14</td>
<td>25</td>
<td>19</td>
<td>24</td>
<td>8</td>
<td>20</td>
<td>25</td>
<td>5</td>
<td>6</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>157</td>
</tr>
<tr>
<td>Psectrocladius</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>14</td>
<td>7</td>
<td>14</td>
<td>16</td>
<td>7</td>
<td>34</td>
<td>35</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>149</td>
</tr>
<tr>
<td>Micropsectra</td>
<td>12</td>
<td>2</td>
<td>13</td>
<td>6</td>
<td>0</td>
<td>9</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>53</td>
</tr>
<tr>
<td>Lenzziella</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>6</td>
<td>23</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>48</td>
</tr>
<tr>
<td>Chironomus</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>21</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>Cryptochironomus</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>21</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>Cladotanytarsus</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Polypedilum</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Endochironomus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Cryptotendipes</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>87</td>
<td>109</td>
<td>77</td>
<td>135</td>
<td>87</td>
<td>123</td>
<td>40</td>
<td>58</td>
<td>45</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>964</td>
<td></td>
</tr>
<tr>
<td>Genus</td>
<td>0</td>
<td>0.05</td>
<td>0.10</td>
<td>0.17</td>
<td>0.30</td>
<td>0.54</td>
<td>0.97</td>
<td>1.73</td>
<td>3.08</td>
<td>5.51</td>
<td>5.93</td>
<td>9.83</td>
<td>17.5</td>
<td>31.5</td>
<td>56</td>
<td>100</td>
<td>Total</td>
</tr>
<tr>
<td>-------------</td>
<td>-----</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>-----</td>
<td>----</td>
<td>-----</td>
<td>-------</td>
</tr>
<tr>
<td>Procladius</td>
<td>79</td>
<td>50</td>
<td>70</td>
<td>39</td>
<td>43</td>
<td>20</td>
<td>40</td>
<td>32</td>
<td>43</td>
<td>16</td>
<td>10</td>
<td>13</td>
<td>9</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>522</td>
</tr>
<tr>
<td>Psectrocladius</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Alkalbemiania</td>
<td>16</td>
<td>14</td>
<td>15</td>
<td>8</td>
<td>3</td>
<td>6</td>
<td>9</td>
<td>14</td>
<td>25</td>
<td>14</td>
<td>14</td>
<td>13</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>184</td>
</tr>
<tr>
<td>Tanylarbus</td>
<td>9</td>
<td>9</td>
<td>5</td>
<td>11</td>
<td>11</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>8</td>
<td>6</td>
<td>32</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>106</td>
</tr>
<tr>
<td>Polypedilum</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Chromonomus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Cryptochironomus</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Lanzella</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Microspectra</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Chiodotanyarns</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Dirctolindipes</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Stictochironomus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3.2: Number of chironomids (pooled samples) mounted and identified of each genus from each artificial pond 8 weeks posttreatment (August 31, 1995).
Table 3.3: Number of chironomids (pooled samples) mounted and identified of each genus from each artificial pond 12 weeks posttreatment (September 26, 1995).

<table>
<thead>
<tr>
<th>Genus</th>
<th>0</th>
<th>0</th>
<th>0.05</th>
<th>0.10</th>
<th>0.17</th>
<th>0.30</th>
<th>0.54</th>
<th>0.97</th>
<th>1.73</th>
<th>3.08</th>
<th>5.51</th>
<th>9.83</th>
<th>17.5</th>
<th>31</th>
<th>56</th>
<th>100</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procladius</td>
<td>120</td>
<td>56</td>
<td>83</td>
<td>109</td>
<td>60</td>
<td>42</td>
<td>45</td>
<td>71</td>
<td>38</td>
<td>47</td>
<td>19</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>705</td>
</tr>
<tr>
<td>Psectrocladius</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>42</td>
<td>45</td>
<td>71</td>
<td>38</td>
<td>47</td>
<td>19</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Ablabesmyia</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>38</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>54</td>
</tr>
<tr>
<td>Tanytarsus</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Lenzilla</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Cryptochironomus</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Chironomus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Polypedilum</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Cryptotendipes</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cladotanytarsus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Dicrotendipes</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Endochironomus</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>140</td>
<td>65</td>
<td>97</td>
<td>116</td>
<td>70</td>
<td>45</td>
<td>46</td>
<td>79</td>
<td>46</td>
<td>77</td>
<td>111</td>
<td>64</td>
<td>32</td>
<td>57</td>
<td>6</td>
<td>1101</td>
<td></td>
</tr>
</tbody>
</table>
On 4 July, 1 day prior to the application of creosote, *Procladius* (7.1%±1.87% to 78.5% ±2.11%) and *Tanytarsus* (12.9%±6.57% to 31.8%±5.55%) dominated the chironomid communities, while *Psectrocladius* (≤ 6.9%±3.67% for all ponds) was rare (Figure 3.9).

On 1 August, 4 weeks following creosote application, the relative abundances of *Procladius* and *Tanytarsus* were greater than *Psectrocladius* in ponds that received nominal concentrations less than 1.73 μL/L (Figure 3.10). However, in ponds that received nominal concentrations greater than 1.73 μL/L, the relative abundance of *Psectrocladius* was greater than *Procladius* and *Tanytarsus*. This pattern of relative abundance, switching between *Procladius* (dominant at concentrations <5.51 μL/L) and *Psectrocladius* (dominant at concentrations ≥5.51 μL/L) persisted 8 and 12 weeks posttreatment (31 August and 26 September, respectively) as well (Figures 3.11 and 3.12). *Tanytarsus* was not abundant on either of these two sampling dates.

**Multivariate Analysis of Chironomid Taxa**

On 1 August, 4 weeks posttreatment, there were significant, positive correlations among the relative abundances of *Procladius*, *Ablabesmyia*, *Tanytarsus* and *Lenziella*, and between *Tanytarsus* and *Micropsectra* (Table 3.4). Eight weeks posttreatment (31 August), *Psectrocladius* was negatively correlated with *Procladius* and *Tanytarsus* (Table 3.5). The correlation coefficients were -0.851 (p<0.01) and -0.521 (p<0.05), respectively. On 26 September (12 weeks posttreatment), *Psectrocladius* was negatively correlated with *Procladius* and *Ablabesmyia* while, *Procladius* and *Ablabesmyia* and, *Lenziella* and *Chironomus* were positively correlated (Table 3.6).
Figure 3.9: Relative abundance of chironomids (percent ± 1 SE) 1 day before creosote application (July 4, 1995). Numbers above each concentration represent total number of invertebrates collected in each pond. $N_{\text{control}}=2$ ponds; $N_{\text{treatment}}=3$ Petersen grabs.
Figure 3.10: Relative abundance of chironomids (percent ± 1 SE) 4 weeks after creosote application (August 1, 1995). Numbers above each concentration represent total number of invertebrates collected in each pond. Ncontrol=2 ponds; Ntreatment=3 Petersen grabs.
Figure 3.11: Relative abundance of chironomids (percent ± 1 SE) 8 weeks after creosote application (August 31, 1995). Numbers above each concentration represent total number of invertebrates collected in each pond. \( N_{\text{control}} = 2 \) ponds; \( N_{\text{treatment}} = 3 \) Petersen grabs.
Figure 3.12: Relative abundance of chironomids (percent ± 1 SE) 12 weeks after creosote application (September 26, 1995). Numbers above each concentration represent total number of invertebrates collected in each pond. N\text{control} = 2 ponds; N\text{treatment} = 3 Petersen grabs.
Table 3.4: Correlation matrix of relative abundances (octaves) of common chironomid genera (replicate samples pooled on each date) in the artificial ponds (N=16), 4 weeks following creosote application (August 1, 1995).

<table>
<thead>
<tr>
<th></th>
<th>Ablabesmyia</th>
<th>Procladius</th>
<th>Lenziella</th>
<th>Micropsectra</th>
<th>Tanytarsus</th>
<th>Chironomus</th>
<th>Psectrocladius</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ablabesmyia</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procladius</td>
<td>0.602*</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lenziella</td>
<td>0.332</td>
<td>0.519*</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Micropsectra</td>
<td>0.351</td>
<td>0.322</td>
<td>0.583*</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tanytarsus</td>
<td>0.547*</td>
<td>0.574*</td>
<td>0.770**</td>
<td>0.615*</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chironomus</td>
<td>0.114</td>
<td>-0.018</td>
<td>-0.257</td>
<td>0.116</td>
<td>0.026</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Psectrocladius</td>
<td>0.151</td>
<td>-0.105</td>
<td>0.045</td>
<td>0.118</td>
<td>0.034</td>
<td>0.305</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Significance levels: * (p<0.05); ** (p<0.01)
Table 3.5: Correlation matrix of relative abundance (octaves) of common chironomid genera (replicate samples pooled on each date) in the artificial ponds (N=16), 8 weeks following creosote application (August 31, 1995).

<table>
<thead>
<tr>
<th></th>
<th>Ablabesmyia</th>
<th>Procladius</th>
<th>Tanytarsus</th>
<th>Psectrocladius</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ablabesmyia</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procladius</td>
<td>0.467</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tanytarsus</td>
<td>0.395</td>
<td>0.380</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Psectrocladius</td>
<td>-0.363</td>
<td>-0.851**</td>
<td>-0.521*</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Significance levels: * (p<0.05); ** (p<0.01)
Table 3.6: Correlation matrix of relative abundances (octaves) of common chironomid genera (replicate samples pooled on each date) in the artificial ponds (N=16), 12 weeks following creosote application (September 26, 1995).

<table>
<thead>
<tr>
<th></th>
<th>Ablabesmyia</th>
<th>Procladius</th>
<th>Lenziella</th>
<th>Tanytarsus</th>
<th>Chironomus</th>
<th>Psectrocladius</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ablabesmyia</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procladius</td>
<td>0.562*</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lenziella</td>
<td>0.170</td>
<td>-0.482</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tanytarsus</td>
<td>-0.105</td>
<td>-0.495</td>
<td>0.175</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chironomus</td>
<td>0.281</td>
<td>-0.369</td>
<td>0.795**</td>
<td>0.225</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Psectrocladius</td>
<td>-0.523*</td>
<td>-0.781**</td>
<td>0.481</td>
<td>0.290</td>
<td>0.490</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Significance levels:  * (p<0.05);  ** (p<0.01)
Principal Components Analysis of the correlation matrix of the relative abundances (octaves) of common (present in 25% of the ponds and comprising ≥10% of one pooled sample) chironomid genera extracted two, one and two factors for the 4, 8, and 12 week posttreatment sampling dates, respectively (Table 3.7).

For the data 4 weeks posttreatment, *Procladius, Ablabesmyia, Tanytarsus, Lenziella* and *Micropsectra* loaded strongly on Factor 1, which accounted for 44.5% of the variance in the data set, whereas, *Psectrocladius* and *Chironomus* loaded more strongly on Factor 2, which accounted for 20.1% of the variance (Table 3.7). A bivariate plot of the taxa factors shows that three chironomid taxa groups of different composition co-occurred in ponds containing low (0-0.54 µL/L), intermediate (1.72-5.51 µL/L), and high (31-56 µL/L) nominal concentrations of creosote (Figure 3.13). The factor scores were plotted against nominal creosote concentrations (Figure 3.14). Factor 1 decreased with increasing creosote concentration. Factor 2 increased significantly at a nominal concentration of 1.73 µL/L and steadily decreased at greater concentrations. Generally, at low creosote concentrations, *Procladius, Ablabesmyia, Tanytarsus, Lenziella,* and *Micropsectra* dominated the invertebrate community, whereas, at intermediate creosote concentrations, *Psectrocladius* and *Chironomus* became the dominant chironomid genera. At creosote concentrations ≥36 µL/L, neither of these chironomid assemblages were dominant.

At 8 weeks posttreatment, one factor was extracted from the data set and it explained 63% of the variance. *Procladius, Ablabesmyia* and *Tanytarsus* loaded positively while *Psectrocladius* loaded negatively (-0.895) (Table 3.7). Based on their factor scores, two complementary chironomid assemblages, characterizing ponds receiving 0-3.08 µL/L, and
Table 3.7: Factor loadings determined by Principal Component Analysis from relative abundances of common chironomid genera from the 16 artificial ponds for the August 1, 1995 (+4 weeks), August 31, 1995 (+8 weeks) and September 26, 1995 (+12 weeks) sampling periods.

<table>
<thead>
<tr>
<th>Genus</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sampling Period</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+4 weeks</td>
<td>+8 weeks</td>
<td>+12 weeks</td>
<td>+4 weeks</td>
<td>+8 weeks</td>
<td>+12 weeks</td>
</tr>
<tr>
<td></td>
<td>Factor 1</td>
<td>Factor 2</td>
<td>Factor 1</td>
<td>Factor 1</td>
<td>Factor 1</td>
<td>Factor 2</td>
</tr>
<tr>
<td>Psectrocladius</td>
<td>0.060</td>
<td>0.755</td>
<td>-0.895</td>
<td>-0.839</td>
<td>0.343</td>
<td></td>
</tr>
<tr>
<td>Chironomus</td>
<td>-0.033</td>
<td>0.830</td>
<td>N/A</td>
<td>-0.163</td>
<td>0.932</td>
<td></td>
</tr>
<tr>
<td>Procladius</td>
<td>0.761</td>
<td>-0.107</td>
<td>0.882</td>
<td>0.905</td>
<td>-0.277</td>
<td></td>
</tr>
<tr>
<td>Ablabesmyia</td>
<td>0.699</td>
<td>0.242</td>
<td>0.668</td>
<td>0.810</td>
<td>0.488</td>
<td></td>
</tr>
<tr>
<td>Tanytarsus</td>
<td>0.904</td>
<td>0.011</td>
<td>0.702</td>
<td>-0.480</td>
<td>0.222</td>
<td></td>
</tr>
<tr>
<td>Lenziella</td>
<td>0.837</td>
<td>-0.221</td>
<td>N/A</td>
<td>-0.245</td>
<td>0.887</td>
<td></td>
</tr>
<tr>
<td>Micropsectra</td>
<td>0.723</td>
<td>0.176</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

N/A = chironomid genera not used in PCA analysis
Figure 3.13: Graphical representation of results of principal components analysis on the basis of relative abundances (pooled samples) of common genera collected on August 1, 1995 (4 weeks posttreatment). Each point represents an artificial pond. Numbers above each point represent nominal concentrations of creosote. Genera listed are those whose total abundances load heavily on the factor.
Figure 3.14: Factor scores resulting from principal components analysis on basis of relative abundances (pooled samples) of common chironomid genera collected on August 1, 1995 (4 weeks posttreatment) along nominal creosote concentration gradient.
9.84-31 μL/L creosote, respectively) were present (Figure 3.15). When the factor scores were plotted against nominal creosote concentration, Factor 1 decreased between 0.97-1.73 μL/L (Figure 3.16). At low creosote concentrations, Procladius, Ablabesmyia and Tanytarsus dominated the chironomid community, whereas, at creosote concentrations ≥ 9.84 μL/L, Psectrocladius became the dominant chironomid genus.

Two factors were extracted from the 12 weeks posttreatment data set. Procladius and Ablabesmyia loaded positively, and Psectrocladius and Tanytarsus loaded negatively on Factor 1, which explained 49.4% of the variance. Chironomus and Lenziella loaded more strongly on Factor 2, which explained an additional 27.8% of the variance. Three chironomid assemblages at low (0-0.97 μL/L), intermediate (5.51-31 μL/L), and high (56-100 μL/L) nominal concentrations of creosote were present (Figure 3.17). At nominal concentration 3.08 μL/L, Factor 1 decreased significantly and at creosote concentration 9.83 μL/L, Factor 2 increased significantly (Figure 3.18). Chironomid dominance switched from Procladius and Ablabesmyia (low creosote concentrations) to Chironomus (intermediate creosote concentrations) to Psectrocladius and Tanytarsus (high creosote concentrations).

**Multivariate Analysis of Chironomid Densities**

Correlations on the Log₁₀(x+1.1) transformed total abundance (all sampling dates combined) of common chironomid genera revealed that Ablabesmyia, Procladius, Lenziella, Micropsectra and Tanytarsus were significantly correlated, 4 weeks posttreatment (1 August) (Table 3.8). On 31 August, 8 weeks following creosote application, Ablabesmyia, Procladius and Tanytarsus were significantly correlated, and Procladius and Psectrocladius
Figure 3.15: Graphical representation of results of principal components analysis on the basis of relative abundances (pooled samples) of common chironomid genera collected on August 31, 1995 (8 weeks posttreatment). Each point represents an artificial pond. Numbers above each point represent nominal concentrations. Genera listed are those whose relative abundances load heavily on the factor.
Figure 3.16: Factor scores resulting from principal components analysis on basis of relative abundances (pooled samples) of common chironomid genera collected on August 31, 1995 (8 weeks posttreatment) along nominal creosote concentration gradient.
Figure 3.17: Graphical representation of results of principal components analysis on the basis of relative abundances (pooled samples) of common genera collected on September 26, 1995 (12 weeks posttreatment). Each point represents an artificial pond. Numbers above each point represent nominal concentrations of creosote. Genera listed are those whose total abundances load heavily on the factor.
Figure 3.18: Factor scores resulting from principal components analysis on basis of relative abundances (pooled samples) of common chironomid genera collected on September 26, 1995 (12 weeks posttreatment) along nominal creosote concentration gradient.
Table 3.8: Correlation matrix of $\log_{10}(x + 1.1)$ transformed total abundance (replicate samples pooled on each date) of common chironomid genera in the artificial ponds (N=16), 4 weeks following creosote application (August 1, 1995).

<table>
<thead>
<tr>
<th></th>
<th>Ablabesmyia</th>
<th>Procladius</th>
<th>Lenziella</th>
<th>Micropsectra</th>
<th>Tanytarsus</th>
<th>Chironomus</th>
<th>Psectrocladius</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ablabesmyia</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procladius</td>
<td>0.795**</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lenziella</td>
<td>0.704**</td>
<td>0.708**</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Micropsectra</td>
<td>0.702**</td>
<td>0.495*</td>
<td>0.622*</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tanytarsus</td>
<td>0.920**</td>
<td>0.757**</td>
<td>0.832**</td>
<td>0.734**</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chironomus</td>
<td>0.128</td>
<td>-0.098</td>
<td>-0.293</td>
<td>0.014</td>
<td>-0.061</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Psectrocladius</td>
<td>0.340</td>
<td>0.344</td>
<td>0.258</td>
<td>0.216</td>
<td>0.213</td>
<td>0.426</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Significance levels: * (p<0.05); ** (p<0.01)
had a significant negative correlation (Table 3.9). *Procladius* was also negatively correlated to *Lenziella* and *Psectrocladius*, 12 weeks posttreatment (26 September) (Table 3.10). Positive correlations occurred between *Procladius* and *Ablabesmyia*, and among *Lenziella*, *Chironomus* and *Psectrocladius*.

Principal Components Analysis on the variance/avariance matrix of the total abundance (all sampling dates combined) of common chironomid genera extracted two factors for the data set of 1 August. *Tanytarsus*, *Procladius*, *Ablabesmyia*, *Lenziella* and *Micropsectra* loaded more strongly on Factor 1, which explained 66.4% of the variance (Table 3.11). *Psectrocladius* and *Chironomus* loaded more strongly on Factor 2, which explained 15.2% of the variance. The plotted factor scores indicated that three distinct chironomid communities existed in the artificial ponds at low (0-0.97 μL/L), intermediate (1.73-5.51 μL/L), and high (17.6-100 μL/L) nominal concentrations of creosote (Figure 3.19). Factor 1 decreased as creosote concentration increased and Factor 2 increased to a peak of 3.08 μL/L, and then decreased (Figure 3.20). At low creosote concentrations, *Tanytarsus*, *Procladius*, *Ablabesmyia*, *Lenziella* and *Micropsectra* were dominant, and at intermediate creosote concentrations, *Psectrocladius* and *Chironomus* dominated the chironomid community. At high creosote concentrations, no chironomid genera were dominant.

*Psectrocladius* loaded on Factor 1 (64.9% variance explained) and *Procladius* loaded on Factor 2 (25.9% variance explained), 8 weeks posttreatment (Table 3.11). Common chironomid communities were present at nominal concentrations 0-1.73 μL/L, and 9.83-31 μL/L (Figure 3.21). Factor 1 increased from 0.97 μL/L to 9.83 μL/L, and then decreased at higher concentrations (Figure 3.22). Factor 2 decreased from 0 μL/L to 0.30 μL/L,
Table 3.9: Correlation matrix of $\log_{10}(x + 1.1)$ transformed total abundance (replicate samples pooled on each date) of common chironomid genera in the artificial ponds (N=16), 8 weeks following creosote application (August 31, 1995).

<table>
<thead>
<tr>
<th></th>
<th>Ablabesmyia</th>
<th>Procladius</th>
<th>Tanytarsus</th>
<th>Psectrocladius</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ablabesmyia</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procladius</td>
<td>0.576*</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tanytarsus</td>
<td>0.679*</td>
<td>0.595*</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Psectrocladius</td>
<td>-0.166</td>
<td>-0.543*</td>
<td>-0.250</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Significance levels: * (p<0.05); ** (p<0.01)
Table 3.10: Correlation matrix of $\log_{10}(x + 1.1)$ transformed total abundance (replicate samples pooled on each date) of common chironomid genera in the artificial ponds (N=16) 12 weeks following creosote application (September 26, 1995).

<table>
<thead>
<tr>
<th></th>
<th>Ablabesmyia</th>
<th>Procladius</th>
<th>Lenziella</th>
<th>Tanytarsus</th>
<th>Chironomus</th>
<th>Psectrocladius</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ablabesmyia</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procladius</td>
<td>0.703**</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lenziella</td>
<td>0.055</td>
<td>-0.561*</td>
<td>0.263</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tanytarsus</td>
<td>-0.163</td>
<td>-0.090</td>
<td>0.805**</td>
<td>0.252</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Chironomus</td>
<td>0.002</td>
<td>-0.494</td>
<td>0.517*</td>
<td>0.222</td>
<td>0.537*</td>
<td>1.000</td>
</tr>
<tr>
<td>Psectrocladius</td>
<td>-0.279</td>
<td>-0.601*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significance levels: * (p<0.05); ** (p<0.01)
Table 3.11: Factor Loadings determined by Principal Components Analysis from total abundances (pooled samples) of common chironomid genera in the 16 artificial ponds for the August 1, 1995 (+4 weeks), August 31, 1995 (+8 weeks) and September 26, 1995 (+12 weeks) sampling periods.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Sampling Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+4 weeks</td>
</tr>
<tr>
<td></td>
<td>Factor 1</td>
</tr>
<tr>
<td>Psectrocladius</td>
<td>0.130</td>
</tr>
<tr>
<td>Chironomus</td>
<td>-0.043</td>
</tr>
<tr>
<td>Tanytarsus</td>
<td>0.721</td>
</tr>
<tr>
<td>Procladius</td>
<td>0.513</td>
</tr>
<tr>
<td>Ablabesmyia</td>
<td>0.499</td>
</tr>
<tr>
<td>Lemiella</td>
<td>0.344</td>
</tr>
<tr>
<td>Micropsectra</td>
<td>0.344</td>
</tr>
</tbody>
</table>

N/A = chironomid genera not used in PCA analysis
Figure 3.19: Graphical representation of results of principal components analysis on the basis of total abundances (pooled samples) of common genera collected on August 1, 1995 (4 weeks posttreatment). Each point represents an artificial pond. Numbers above each point represent nominal concentrations of creosote. Genera listed are those whose total abundances load heavily on the factor.
Figure 3.20: Factor scores resulting from principal components analysis on basis of total abundances (pooled samples) of common chironomid genera collected on August 1, 1995 (4 weeks posttreatment) along nominal creosote concentration gradient.
Figure 3.21: Graphical representation of results of principal components analysis on the basis of total abundances (pooled samples) of common genera collected on August 31, 1995 (8 weeks posttreatment). Each point represents an artificial pond. Numbers above each point represent nominal concentrations of creosote. Genera listed are those whose total abundances load heavily on the factor.
Figure 3.22: Factor scores resulting from principal components analysis on basis of total abundances (pooled samples) of common chironomid genera collected on August 31, 1995 (8 weeks posttreatment) along nominal creosote concentration gradient.
increased from 0.97 μL/L to 5.51 μL/L, and then decreased at higher creosote concentrations. At low creosote concentrations, *Tanytarsus*, *Ablabesmyia* and *Procladius* were dominant, and at intermediate creosote concentrations, *Psectrocladius* dominated the chironomid community.

For the data collected 26 September, *Psectrocladius* loaded on Factor 1, which explained 68.6% variance, and *Procladius* loaded on Factor 2, which explained 17.8% variance (Table 3.11). Ponds 0-1.73 μL/L and ponds 9.83-56 μL/L had common chironomid assemblages (Figure 3.23). Factor 2 decreased as the nominal concentration of creosote increased, and Factor 1 increased until 5.51 μL/L, and decreased at higher concentrations (Figure 3.24). At low creosote concentrations, *Procladius* was dominant in the chironomid community, whereas, at higher creosote concentrations, *Psectrocladius* became dominant. *Procladius* and *Psectrocladius* densities were plotted along an increasing creosote concentration gradient (Figures 3.25-3.27). The dominance of these genera, based on densities, switched at approximately 1.73 μL/L, 9.83 μL/L, and 3.08 μL/L for 4, 8, and 12 weeks posttreatment, respectively.

**Community Response (Density and Richness) to Creosote Contamination**

Community responses for the pretreatment sampling date (4 July) were plotted against nominal concentrations of creosote (μL/L) whereas, community responses for the posttreatment (11 July, 1 August, 31 August, 26 September) were plotted against sediment concentrations of creosote (μg/g). The linear regression parameters and median lethal concentration values are summarized in Table 3.12.
Figure 3.23: Graphical representation of results of principal components analysis on the basis of relative abundances (pooled samples) of common genera collected on September 26, 1995 (12 weeks posttreatment). Each point represents an artificial pond. Numbers above each point represent nominal concentrations of creosote. Genera listed are those whose total abundances load heavily on the factor.
Figure 3.24: Factor scores resulting from principal components analysis on basis of total abundances (pooled samples) of common chironomid genera collected on September 26, 1995 (12 weeks posttreatment) along nominal creosote concentration gradient.
Figure 3.25: Mean density (no./225 cm²) of Procladius and Psectrocladius larvae (±1 SE) 4 weeks posttreatment (August 1, 1995).
Figure 3.26: Mean density (no./225 cm²) of *Procladius* and *Psectrocladius* larvae (±1 SE) 8 weeks posttreatment (August 31, 1995).
Figure 3.27: Mean density (no./225 cm$^3$) of *Procladius* and *Psectrocladius* larvae (±1 SE) 12 weeks posttreatment (September 26, 1995).
Table 3.12: Summary of regression analyses of mesocosm data, and LC50 values for community (density and richness) and population (density) responses.

<table>
<thead>
<tr>
<th></th>
<th>slope (SE)</th>
<th>intercept (SE)</th>
<th>R²</th>
<th>p</th>
<th>LC50 (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Density</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>All Invertebrates</em></td>
<td>pretreatment</td>
<td>-0.02 (0.04)</td>
<td>2.23 (0.04)</td>
<td>0.03</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>+1 week</td>
<td>-0.82 (0.16)</td>
<td>1.62 (0.12)</td>
<td>0.66</td>
<td>&lt;0.001</td>
<td>0.33</td>
</tr>
<tr>
<td>+4 weeks</td>
<td>-1.04 (0.10)</td>
<td>1.83 (0.08)</td>
<td>0.89</td>
<td>&lt;0.001</td>
<td>0.88</td>
</tr>
<tr>
<td>+8 weeks</td>
<td>non-linear</td>
<td>1.97 (0.04)</td>
<td>0.77</td>
<td>&lt;0.001</td>
<td>27*</td>
</tr>
<tr>
<td>+12 weeks</td>
<td>-0.35 (0.05)</td>
<td>2.02 (0.03)</td>
<td>0.03</td>
<td>&gt;0.05</td>
<td>1.28</td>
</tr>
<tr>
<td><strong>All Chironomids</strong></td>
<td>pretreatment</td>
<td>-0.02 (0.03)</td>
<td>2.02 (0.03)</td>
<td>0.03</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>+1 week</td>
<td>-0.71 (0.14)</td>
<td>1.46 (0.97)</td>
<td>0.67</td>
<td>&lt;0.001</td>
<td>0.53</td>
</tr>
<tr>
<td>+4 weeks</td>
<td>-1.16 (0.09)</td>
<td>1.67 (0.11)</td>
<td>0.90</td>
<td>&lt;0.001</td>
<td>1.37</td>
</tr>
<tr>
<td>+8 weeks</td>
<td>non-linear</td>
<td>1.97 (0.04)</td>
<td>0.77</td>
<td>&lt;0.001</td>
<td>30*</td>
</tr>
<tr>
<td>+12 weeks</td>
<td>non-linear</td>
<td>2.02 (0.03)</td>
<td>0.03</td>
<td>&gt;0.05</td>
<td>35*</td>
</tr>
<tr>
<td><strong>Procladius</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+4 weeks</td>
<td>-0.31 (0.18)</td>
<td>0.65 (0.12)</td>
<td>0.22</td>
<td>&gt;0.05</td>
<td>&gt;63*</td>
</tr>
<tr>
<td>+8 weeks</td>
<td>-0.38 (0.07)</td>
<td>1.11 (0.06)</td>
<td>0.69</td>
<td>&lt;0.001</td>
<td>0.85</td>
</tr>
<tr>
<td>+12 weeks</td>
<td>-0.67 (0.09)</td>
<td>1.13 (0.06)</td>
<td>0.81</td>
<td>&lt;0.001</td>
<td>0.53</td>
</tr>
<tr>
<td><strong>Psectrocladius</strong></td>
<td>+4 weeks</td>
<td>non-linear</td>
<td></td>
<td></td>
<td>4.5*</td>
</tr>
<tr>
<td>+8 weeks</td>
<td>non-linear</td>
<td></td>
<td></td>
<td></td>
<td>15*</td>
</tr>
<tr>
<td>+12 weeks</td>
<td>non-linear</td>
<td></td>
<td></td>
<td></td>
<td>5*</td>
</tr>
<tr>
<td><strong>Richness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Total Invertebrates</em></td>
<td>Pretreatment</td>
<td>-0.01 (0.01)</td>
<td>0.78 (0.05)</td>
<td>0.07</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>+1 week</td>
<td>non-linear</td>
<td></td>
<td></td>
<td></td>
<td>0.80*</td>
</tr>
<tr>
<td>+4 weeks</td>
<td>non-linear</td>
<td></td>
<td></td>
<td></td>
<td>1.25*</td>
</tr>
<tr>
<td>+8 weeks</td>
<td>-0.17 (0.04)</td>
<td>0.64 (0.03)</td>
<td>0.58</td>
<td>&lt;0.001</td>
<td>5.66</td>
</tr>
<tr>
<td>+12 weeks</td>
<td>-0.17 (0.04)</td>
<td>0.70 (0.03)</td>
<td>0.62</td>
<td>&lt;0.001</td>
<td>18.98</td>
</tr>
<tr>
<td><strong>Total Chironomids</strong></td>
<td>Pretreatment</td>
<td>0.01 (0.01)</td>
<td>0.52 (0.04)</td>
<td>0.18</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>+4 weeks</td>
<td>non-linear</td>
<td></td>
<td></td>
<td></td>
<td>3.25*</td>
</tr>
<tr>
<td>+8 weeks</td>
<td>-0.07 (0.06)</td>
<td>0.54 (0.05)</td>
<td>0.09</td>
<td>&gt;0.05</td>
<td>&gt;63*</td>
</tr>
<tr>
<td>+12 weeks</td>
<td>-0.01 (0.06)</td>
<td>0.45 (0.04)</td>
<td>0.00</td>
<td>&gt;0.05</td>
<td>&gt;63*</td>
</tr>
</tbody>
</table>

*a* LC50 values estimated from line fit 'by eye'

*b* LC50 value is greater than the highest sediment concentration

*c* LC50 value estimate 'by eye' from linear part of concentration-response curve
All Invertebrates

On 4 July, one day prior to treatment, there was no apparent trend for total invertebrate density (Figure 3.28) and family richness (Figure 3.29), but both of these community measures decreased with increasing creosote contamination during the 4, 8 and 12 week posttreatment sampling dates (Figures 3.30-3.37).

On 11 July, 1 week posttreatment, the control pond values were 207.3±66.6 individuals/225 cm² and 3.7±0.2 families/sample for the mean density and mean richness, respectively. Log₁₀ density and Log₁₀ creosote concentration had a significant linear relationship (p<0.001) (Figure 3.30) whereas, the relationship between Log₁₀ richness and Log₁₀ creosote concentration was not linear (Figure 3.31). There was a significant decline in richness between the nominal concentrations of 0.6 µL/L and 1.0 µL/L (from 3.7±0.2 to 1.3±0.3 families/sample). Linear regression of Log₁₀ density (4 weeks posttreatment) against Log₁₀ creosote concentration in the sediment was highly significant (p<0.001) (Figure 3.32). There was a significant decrease in family richness between 1.19 µg/g and 1.50 µg/g (Figure 3.33) and this relationship was not linear (Repeated-values Regression Analysis, F=2.92, p>0.05). The mean density and richness of the total invertebrates in the control ponds 4 weeks posttreatment were 154±13.23 individuals/225 cm² and 4.8±0.17 families/sample, respectively.

For samples collected 8 weeks posttreatment, the mean density and richness values, for the control ponds, were 132.3±8.41 individuals/225 cm² and 6.5±0.58 families/sample, respectively (Figure 3.34 and 3.35). Invertebrate density decreased but there was significant deviation from linearity of the transformed data (Repeated-values Regression Analysis,
Pretreatment

Figure 3.28: Mean density (no./225 cm²) ± 1 SE of invertebrates among ponds 1 day before creosote application (July 4, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs.
Figure 3.29: Mean richness (no. families/225 cm²) ± 1 SE of invertebrates among ponds 1 day before creosote application (July 4, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs.
Figure 3.30: Mean (± 1 SE) invertebrate density (no./225 cm²) among ponds 1 week following creosote application (July 11, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. Solid point represents control pond.
Figure 3.31: Mean (± 1 SE) invertebrate richness (no. families/225 cm²) among ponds 1 week following creosote application (July 11, 1995). \( N_{\text{control}}=2 \) ponds; \( N_{\text{treatment}}=3 \) Petersen grabs. Solid point represents control pond. Line fitted by eye.
Figure 3.32: Mean (± 1 SE) invertebrate density (no./225 cm²) among ponds 4 weeks following creosote application (August 1, 1995). N_{control} = 2 ponds; N_{treatment} = 3 Petersen grabs. Solid point represents control pond.
Figure 3.33: Mean (± 1 SE) invertebrate richness (no. families/225 cm²) among ponds 4 weeks following creosote application (August 1, 1995). $N_{control}=2$ ponds; $N_{treatment}=3$ Petersen grabs. Solid point represents control pond. Line fitted by eye.
Figure 3.34: Mean (± 1 SE) invertebrate density (no./225 cm²) among ponds 8 weeks following creosote application (August 31, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. Solid point represents control pond. Line fitted by eye.
Figure 3.35: Mean (± 1 SE) invertebrate richness (no. families/225 cm²) among ponds 8 weeks following creosote application (August 31, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. Solid point represents control pond.
F=3.16, p>0.05) between 16.8 \mu g/g and 63.3 \mu g/g, but richness decreased linearly (p<0.001) with increasing creosote concentrations.

Twelve weeks posttreatment, mean invertebrate density was 181.7±28.47 and mean richness was 6.0±0.88 in control ponds. Both Log_{10} density and Log_{10} richness declined linearly with increasing Log_{10} creosote concentration (p<0.001, Figure 3.36, and p<0.01, Figure 3.37).

All Chironomids

For the pretreatment sampling date, chironomid density was variable (Figure 3.38) and generic richness exhibited no trend (Figure 3.39) but these chironomid community measures decreased with increasing creosote concentrations (Figures 3.40-3.46). On 11 July, 1 week posttreatment, there was a significant linear relationship between Log_{10} chironomid density and Log_{10} creosote concentration (p<0.001) (Figure 3.40). The mean density in the control ponds was 90.3±16.5 chironomids/sample.

Four weeks posttreatment, Log_{10} chironomid density decreased linearly (p<0.001) (Figure 3.41) while the Log_{10} generic richness decline was non-linear, decreasing between 2.07 \mu g/g and 7.64 \mu g/g (Figure 3.42). Values in the control ponds were 69.0 ± 10.02 chironomids/225 cm² for density and 5.2 ± 0.33 genera/sample for richness.

The regression line for Log_{10} chironomid density, 8 weeks posttreatment, and Log_{10} creosote concentration was significant (p<0.01) (Figure 3.43). The abundance of chironomids in the controls was 65.7±4.81 individuals/sample. The linear relationship was not significant (p>0.05) for Log_{10} chironomid richness (8 and 12 weeks posttreatment)
Figure 3.36: Mean (± 1 SE) invertebrate density (no./225 cm²) among ponds 12 weeks following creosote application (September 26, 1995). $N_{\text{control}}=2$ ponds; $N_{\text{treatment}}=3$ Petersen grabs. Solid point represents control pond.
Figure 3.37: Mean (+ 1 SE) invertebrate richness (no. families/225 cm²) among ponds 12 weeks following creosote application (September 26, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. Solid point represents control pond.
Figure 3.38: Mean density (no./225 cm²) ± 1 SE of chironomids among ponds 1 day before creosote application (July 4, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs.
Figure 3.39: Generic richness (no./225 cm\(^2\)) ± 1 SE of chironomids among ponds 1 day before creosote application (July 4, 1995). \(N_{control}=2\) ponds; \(N_{treatment}=3\) Petersen grabs.
Figure 3.40: Mean (± 1 SE) chironomid density (no./225 cm²) among ponds 1 week following creosote application (July 11, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. Solid point represents control pond.
Figure 3.41: Mean (± 1 SE) chironomid density (no./225 cm²) among ponds 4 weeks following creosote application (August 1, 1995). $N_{\text{control}}=2$ ponds; $N_{\text{treatment}}=3$ Petersen grabs. Solid point represents control pond.
Figure 3.42: Mean (± 1 SE) chironomid richness (no. genera/225 cm²) among ponds 4 weeks following creosote application (August 1, 1995). $N_{\text{control}}=2$ ponds; $N_{\text{treatment}}=3$ Petersen grabs. Solid point represents control pond. Line fitted by eye.
Figure 3.43: Mean (± 1 SE) chironomid density (no./225 cm²) among ponds 8 weeks following creosote application (August 31, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. Solid point represents control pond. Line fitted by eye.
(Figures 3.44 and 3.46), but it was significant for Log$_{10}$ chironomid density (p<0.01), 12 weeks following creosote application (Figure 3.45).

Median lethal concentration values were determined for all linear relationships that were significant and estimated, by eye, for the non-linear relationships (Table 3.12). Density of all invertebrates (1 week posttreatment) was the most sensitive, with an LC50 value of 0.33 μg/g. Chironomid richness (8 and 12 weeks posttreatment) was the least sensitive (LC50 > 63 μg/g). Invertebrate density LC50 values ranged from 0.88 μg/g to approximately 27 μg/g while invertebrate richness LC50 values ranged from approximately 0.80 μg/g to 18.98 μg/g. Chironomid density LC50 values ranged from 0.53 μg/g (1 week posttreatment) to approximately 35 μg/g (12 weeks posttreatment). Chironomid richness LC50 values ranged from approximately 3.25 μg/g (4 weeks posttreatment) to >63 μg/g (8 and 12 weeks posttreatment).

**Chironomid Population Responses (Density) to Creosote Contamination**

Log$_{10}$ Procladius and Psetrocladius densities were plotted against Log$_{10}$ creosote concentrations. The LC50 values for Procladius were determined as described previously. The LC50 values for Psetrocladius were estimated by fitting a line, by eye, along the negative part of the concentration-response curve, and using the maximum mean density (on this negative slope) as a starting point. The linear regression parameters and the LC50 values are summarized in Table 3.12.

Four weeks posttreatment, 1 August, the relationship between Log$_{10}$ density and Log$_{10}$ concentration for Procladius was not significant (p > 0.05) (Figure 3.47) and consequently, the LC50 value was >63 μg/g. The maximum density for Psetrocladius was 12.8±4.6
Figure 3.44: Mean ($\pm$ 1 SE) chironomid richness (no. genera/225 cm$^2$) among ponds 8 weeks following creosote application (August 31, 1995). $N_{\text{control}}=2$ ponds; $N_{\text{treatment}}=3$ Petersen grabs. Solid point represents control pond.
Figure 3.45: Mean (± 1 SE) chironomid density (no./225 cm²) among ponds 12 weeks following creosote application (September 26, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. Solid point represents control pond. Line fitted by eye.
Figure 3.46: Mean (± 1 SE) chironomid richness (no. genera/225 cm²) among ponds 12 weeks following creosote application (September 26, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. Solid point represents control pond.
Figure 3.47: Mean (± 1 SE) *Procladius* density (no./225 cm²) among ponds 4 weeks following creosote application (August 1, 1995). N<sub>control</sub>=2 ponds; N<sub>treatment</sub>=3 Petersen grabs. Solid point represents control pond.
individuals/sample (Figure 3.48). The LC50 value was estimated to be approximately 4.5 μg/g sediment.

Eight weeks posttreatment, the mean *Procladius* density was 27.4±4.9 individuals/225 cm² in the control ponds. *Procladius* Log₁₀ density decreased linearly with increasing Log₁₀ creosote concentration (p<0.001) (Figure 3.49) and the LC50 value was 0.85 μg/g. The maximum density of *Psectrocladius* was 33.1±9.3 individuals/sample (Figure 3.50). The LC50 value was approximately 15 μg/g sediment.

Twelve weeks posttreatment, Log₁₀ *Procladius* density had significantly declined with increasing Log₁₀ creosote concentrations (p<0.001) (Figure 3.51). The mean density in the control ponds was 41.1±1.8 individuals/sample and the LC50 value was 0.53 μg/g. The LC50 value for *Psectrocladius* was approximately 5 μg/g and its maximum density was 26.8±4.4 individuals/225 cm² (Figure 3.52).

Generally, the LC50 values for *Procladius* were less than that of *Psectrocladius*, indicating that *Psectrocladius* larvae were more tolerant to creosote contamination than *Procladius*.

**Incidence of Deformities**

*Ligula*

In normal *Procladius*, the ligula is a bisymmetrical five-toothed structure (Pettigrove 1989). The most common deformity of this structure in the 1,444 *Procladius* larvae examined was the absence of the median tooth of the ligula, resulting in the appearance of only four teeth. The incidence of ligula deformities in the larvae (all sampling dates combined) collected posttreatment ranged from 0.0±0.0% to 14.3±13.2% (Table 3.13,
Figure 3.48: Mean (± 1 SE) *Psectrocladius* density (no./225 cm²) among ponds 4 weeks following creosote application (August 1, 1995). \( N_{\text{control}} = 2 \) ponds; \( N_{\text{treatment}} = 3 \) Petersen grabs. Solid point represents control pond. Line fitted by eye.
Figure 3.49: Mean (± 1 SE) Procladius density (no./225 cm²) among ponds 8 weeks following creosote application (August 31, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. Solid point represents control pond.
Figure 3.50: Mean (± 1 SE) *Psectrocladius* density (no./225 cm²) among ponds 8 weeks following creosote application (August 31, 1995). N = 2 ponds; N = 3, *P* < 0.05.
Figure 3.51: Mean (± 1 SE) Procladius density (no./225 cm²) among ponds 12 weeks following creosote application (September 26, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. Solid point represents control pond.
Figure 3.52: Mean (± 1 SE) *Psectrocladius* density (no./225 cm²) among ponds 12 weeks following creosote application (September 26, 1995). N_{control}=2 ponds; N_{treatment}=3 Petersen grabs. Solid point represents control pond. Line fitted by eye.
Table 3.13: Incidence of deformities (percent ± 1 SE) in ligula of *Procladius* larvae.

<table>
<thead>
<tr>
<th>Nominal Concentration (µL/L)</th>
<th>Time Since Treatment (weeks)</th>
<th>All Dates Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>0</td>
<td>11.7±7.8 (17)</td>
<td>0.0±0.0 (50)</td>
</tr>
<tr>
<td>0.05</td>
<td>7.4±5.0 (27)</td>
<td>5.7±2.8 (70)</td>
</tr>
<tr>
<td>0.10</td>
<td>2.6±1.8 (78)</td>
<td>2.5±1.4 (120)</td>
</tr>
<tr>
<td>0.17</td>
<td>7.4±5.0 (39)</td>
<td>5.7±2.8 (70)</td>
</tr>
<tr>
<td>0.30</td>
<td>2.6±2.5 (39)</td>
<td>3.7±1.8 (107)</td>
</tr>
<tr>
<td>0.54</td>
<td>0.0±0.0 (18)</td>
<td>1.7±1.7 (59)</td>
</tr>
<tr>
<td>0.97</td>
<td>6.3±2.5 (11)</td>
<td>5.0±4.9 (20)</td>
</tr>
<tr>
<td>1.73</td>
<td>2.3±2.3 (43)</td>
<td>2.4±2.4 (42)</td>
</tr>
<tr>
<td>3.08</td>
<td>6.3±2.5 (95)</td>
<td>5.0±4.9 (20)</td>
</tr>
<tr>
<td>5.51</td>
<td>3.8±2.7 (52)</td>
<td>8.6±3.4 (68)</td>
</tr>
<tr>
<td>9.83</td>
<td>3.8±2.7 (52)</td>
<td>8.6±3.4 (68)</td>
</tr>
<tr>
<td>17.5</td>
<td>5.3±3.6 (38)</td>
<td>3.6±2.0 (89)</td>
</tr>
<tr>
<td>31</td>
<td>18.2±11.6 (11)</td>
<td>0.0±0.0 (3)</td>
</tr>
<tr>
<td>56</td>
<td>0.0±0.0 (9)</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0.0±0.0 (2)</td>
<td>25.0±21.7 (4)</td>
</tr>
</tbody>
</table>

() = Sample Size

104
Figure 3.53). The incidence of ligula deformities in the larvae collected from the pond that received a nominal creosote concentration of 0.97 μL/L water (0.51-2.19 μg/g sediment) was significantly greater than the incidence of deformities in the larvae from the control ponds (1-tailed G-statistic Goodness of fit test, $G=14.89$, $p<0.001$; Table 3.14). The incidences of deformities were $6.5\pm1.7\%$, $2.4\pm1.4\%$, and $2.3\pm1.0\%$ for the 0.97 μL/L pond and the two control ponds, respectively (all sampling dates combined). The larvae from ponds that received nominal creosote concentrations $\geq 9.83$ μL/L were pooled because individual sample sizes were small. The incidence of ligula deformities in this pooled sample of larvae was significantly greater (5.7%) than control levels (1-tailed G-statistic Goodness of fit test, $G=3.73$, $p<0.05$; Table 3.14).

*Paralabial Combs*

The paralabial combs are a paired set of 5-9 triangular-shaped teeth on the prementohypopharyngeal complex (Warwick and Tisdale 1988). The most common paralabial comb deformity in the 1,331 *Procladius* larvae examined was asymmetry between the paired paralabial combs. The number of teeth was not the same for both combs. The incidence of deformities (all sampling dates combined) ranged from $0.0\pm0.0\%$ to $16.7\pm15.2\%$ (Table 3.15). The incidence of paralabial comb asymmetries in the two control ponds were $10.1\pm2.2\%$ and $14.8\pm3.4\%$, respectively (Table 3.15). There was no significant increase in the incidence of paralabial deformities in the larvae from the treated ponds when compared to the control ponds (Figure 3.54, Table 3.16).
Figure 3.53: Incidence of deformities (percent ± 1SE) in ligulae of Procladius larvae. Numbers above data points represent sample sizes (all sampling dates combined). Level of significance: * p<0.05
Table 3.14: 1-tailed G-tests for goodness of fit on the incidence of deformed ligula of Procladius larvae. Data from all dates combined.

<table>
<thead>
<tr>
<th>Nominal Concentration (μL/L)</th>
<th>Average % deformed</th>
<th>Number normal</th>
<th>Number deformed</th>
<th>Total</th>
<th>df</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.4</td>
<td>120</td>
<td>3</td>
<td>123</td>
<td>1</td>
<td>0.0042</td>
</tr>
<tr>
<td>0</td>
<td>2.3</td>
<td>208</td>
<td>5</td>
<td>213</td>
<td>1</td>
<td>0.0000</td>
</tr>
<tr>
<td>0.05</td>
<td>4.5</td>
<td>171</td>
<td>8</td>
<td>179</td>
<td>1</td>
<td>2.7809</td>
</tr>
<tr>
<td>0.10</td>
<td>3.3</td>
<td>148</td>
<td>5</td>
<td>153</td>
<td>1</td>
<td>0.5018</td>
</tr>
<tr>
<td>0.17</td>
<td>1.5</td>
<td>134</td>
<td>2</td>
<td>136</td>
<td>1</td>
<td>0.5277</td>
</tr>
<tr>
<td>0.30</td>
<td>1.1</td>
<td>91</td>
<td>1</td>
<td>92</td>
<td>1</td>
<td>0.7969</td>
</tr>
<tr>
<td>0.54</td>
<td>1.4</td>
<td>71</td>
<td>1</td>
<td>72</td>
<td>1</td>
<td>0.3390</td>
</tr>
<tr>
<td>0.97</td>
<td>6.5</td>
<td>203</td>
<td>14</td>
<td>217</td>
<td>1</td>
<td>10.8558</td>
</tr>
<tr>
<td>1.73</td>
<td>3.6</td>
<td>86</td>
<td>3</td>
<td>89</td>
<td>1</td>
<td>0.3569</td>
</tr>
<tr>
<td>3.08</td>
<td>0.0</td>
<td>81</td>
<td>0</td>
<td>81</td>
<td>1</td>
<td>1.9262</td>
</tr>
<tr>
<td>5.51</td>
<td>0.0</td>
<td>36</td>
<td>0</td>
<td>36</td>
<td>1</td>
<td>0.8561</td>
</tr>
<tr>
<td>9.83...</td>
<td>5.7</td>
<td>50</td>
<td>3</td>
<td>53</td>
<td>1</td>
<td>1.8256</td>
</tr>
</tbody>
</table>
Table 3.15. Incidence of deformities (percent ± 1 SE) in paralabial combs of *Procladius* larvae.

<table>
<thead>
<tr>
<th>Nominal Concentration (μL/L)</th>
<th>Time Since Treatment (weeks)</th>
<th>All Dates Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>0</td>
<td>0.0±0.0 (14)</td>
<td>9.2±2.8 (109)</td>
</tr>
<tr>
<td>0.05</td>
<td>0.0±0.0 (20)</td>
<td>9.1±3.3 (77)</td>
</tr>
<tr>
<td>0.09</td>
<td>0.0±0.0 (5)</td>
<td>9.9±3.0 (101)</td>
</tr>
<tr>
<td>0.17</td>
<td>23.5±10.3 (17)</td>
<td>14.5±4.8 (55)</td>
</tr>
<tr>
<td>0.30</td>
<td>11.1±10.5 (9)</td>
<td>5.3±3.6 (38)</td>
</tr>
<tr>
<td>0.54</td>
<td>0.0±0.0 (7)</td>
<td>15.6±5.4 (45)</td>
</tr>
<tr>
<td>0.97</td>
<td>3.3±1.9 (92)</td>
<td>7.5±3.2 (67)</td>
</tr>
<tr>
<td>1.73</td>
<td>33.3±27.2 (3)</td>
<td>20.0±6.9 (34)</td>
</tr>
<tr>
<td>3.08</td>
<td>50.0±35.4 (2)</td>
<td>16.3±5.6 (43)</td>
</tr>
<tr>
<td>5.51</td>
<td>0.0±0.0 (1)</td>
<td>17.6±9.2 (17)</td>
</tr>
<tr>
<td>9.83</td>
<td>—</td>
<td>16.7±15.2 (6)</td>
</tr>
<tr>
<td>17.5</td>
<td>—</td>
<td>0.0±0.0 (3)</td>
</tr>
<tr>
<td>31</td>
<td>—</td>
<td>0.0±0.0 (1)</td>
</tr>
<tr>
<td>56</td>
<td>—</td>
<td>0.0±0.0 (5)</td>
</tr>
<tr>
<td>100</td>
<td>0.0±35.4 (2)</td>
<td>100.0±0.0 (1)</td>
</tr>
</tbody>
</table>

( ) = Sample Size
Figure 3.54: Incidence of deformities (percent ± 1SE) in paralabial combs of Procladius larvae. Numbers above data points represent sample sizes (all sampling dates combined).
Table 3.16: 1-tailed G-tests for goodness of fit on the incidence of deformed paralabial combs of *Procladius* larvae. Data from all dates combined.

<table>
<thead>
<tr>
<th>Nominal Concentration (μL/L)</th>
<th>Average % deformed</th>
<th>Number normal</th>
<th>Number deformed</th>
<th>Total</th>
<th>df</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10.1</td>
<td>177</td>
<td>20</td>
<td>197</td>
<td>1</td>
<td>0.3913</td>
</tr>
<tr>
<td>0.05</td>
<td>14.8</td>
<td>92</td>
<td>16</td>
<td>108</td>
<td>1</td>
<td>0.6923</td>
</tr>
<tr>
<td>0.10</td>
<td>10.4</td>
<td>146</td>
<td>17</td>
<td>163</td>
<td>1</td>
<td>0.2105</td>
</tr>
<tr>
<td>0.17</td>
<td>9.9</td>
<td>128</td>
<td>14</td>
<td>142</td>
<td>1</td>
<td>0.5294</td>
</tr>
<tr>
<td>0.30</td>
<td>7.4</td>
<td>75</td>
<td>6</td>
<td>81</td>
<td>1</td>
<td>0.0667</td>
</tr>
<tr>
<td>0.54</td>
<td>7.1</td>
<td>62</td>
<td>8</td>
<td>70</td>
<td>1</td>
<td>0.0000</td>
</tr>
<tr>
<td>0.97</td>
<td>11.4</td>
<td>194</td>
<td>15</td>
<td>209</td>
<td>1</td>
<td>6.1668</td>
</tr>
<tr>
<td>1.73</td>
<td>14.3</td>
<td>66</td>
<td>11</td>
<td>77</td>
<td>1</td>
<td>0.4444</td>
</tr>
<tr>
<td>3.08</td>
<td>12.0</td>
<td>66</td>
<td>9</td>
<td>75</td>
<td>1</td>
<td>0.0000</td>
</tr>
<tr>
<td>5.51</td>
<td>11.1</td>
<td>32</td>
<td>4</td>
<td>36</td>
<td>1</td>
<td>0.0000</td>
</tr>
<tr>
<td>9.83</td>
<td>13.3</td>
<td>13</td>
<td>2</td>
<td>15</td>
<td>1</td>
<td>0.0000</td>
</tr>
<tr>
<td>17.5...</td>
<td>7.4</td>
<td>25</td>
<td>2</td>
<td>27</td>
<td>1</td>
<td>0.5000</td>
</tr>
</tbody>
</table>
**Mandibles**

The mandibles of *Procladius* are sickle-shaped, with a dark apical tooth and one blunt inner auxiliary tooth (Dermott 1991). Many of the 1,345 *Procladius* larvae examined had a reduced or missing auxiliary tooth. The incidences of such mandible deformities in the control ponds were 0.5±0.5% and 1.7±1.2% (Table 3.17). The percentage of deformities in the larvae (all sampling dates combined) ranged from 0.0±0.0% to 14.2±13.2% (Table 3.17). The incidence of deformities observed in the larvae from the treated ponds was not significantly different from that expected based on the average control percentage (Figure 3.55, Table 3.18).

**Associations between Cephalic Structure Deformities**

Chi-square tests of independence showed that there was a highly significant association between the incidences of ligula and paralabial comb deformities in the head capsule of the same individual larvae ($\chi^2_{obs} = 13.89$, $p < 0.001$) (Table 3.19). There were no such associations between the incidences of ligula and mandible deformities, and paralabial comb and mandible deformities, respectively (Tables 3.20 and 3.21).

**Discussion**

**Benthic Community Characterization**

*Caenis* mayfly larvae were subdominant benthic organisms in the control ponds. Their relative abundance decreased as the concentration of creososte increased, indicating that this invertebrate taxon is sensitive to PAH contamination. Ort *et al.* (1995) similarly concluded that the PAH content in crude oil increased mayfly mortality.
Table 3.17: Incidence of deformities (percent ± 1 SE) in mandibles of *Procladius* larvae.

<table>
<thead>
<tr>
<th>Nominal Concentration (μL/L)</th>
<th>Time Since Treatment (weeks)</th>
<th>All Dates Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>0</td>
<td>9.1±8.7 (11)</td>
<td>0.0±0.0 (119)</td>
</tr>
<tr>
<td>0.05</td>
<td>5.9±5.7 (17)</td>
<td>1.9±1.9 (54)</td>
</tr>
<tr>
<td>0.05</td>
<td>0.0±0.0 (23)</td>
<td>0.0±0.0 (74)</td>
</tr>
<tr>
<td>0.10</td>
<td>0.0±0.0 (6)</td>
<td>2.9±1.7 (103)</td>
</tr>
<tr>
<td>0.17</td>
<td>0.0±0.0 (16)</td>
<td>0.0±0.0 (54)</td>
</tr>
<tr>
<td>0.30</td>
<td>0.0±0.0 (10)</td>
<td>0.0±0.0 (40)</td>
</tr>
<tr>
<td>0.54</td>
<td>0.0±0.0 (5)</td>
<td>0.0±0.0 (39)</td>
</tr>
<tr>
<td>0.97</td>
<td>0.0±0.0 (83)</td>
<td>1.5±1.5 (68)</td>
</tr>
<tr>
<td>1.73</td>
<td>0.0±0.0 (3)</td>
<td>0.0±0.0 (37)</td>
</tr>
<tr>
<td>3.08</td>
<td>0.0±0.0 (2)</td>
<td>0.0±0.0 (46)</td>
</tr>
<tr>
<td>5.51</td>
<td>0.0±0.0 (1)</td>
<td>0.0±0.0 (19)</td>
</tr>
<tr>
<td>9.83</td>
<td>—</td>
<td>0.0±0.0 (6)</td>
</tr>
<tr>
<td>17.5</td>
<td>—</td>
<td>0.0±0.0 (3)</td>
</tr>
<tr>
<td>31</td>
<td>—</td>
<td>0.0±0.0 (1)</td>
</tr>
<tr>
<td>56</td>
<td>—</td>
<td>0.0±0.0 (3)</td>
</tr>
<tr>
<td>100</td>
<td>50.0±35.4 (2)</td>
<td>0.0±0.0 (1)</td>
</tr>
</tbody>
</table>

( ) = Sample Size
Figure 3.55: Incidence of deformities (percent ± 1SE) in mandibles of *Procladius* larvae. Numbers above data points represent sample sizes (all sampling dates combined).
Table 3.18. 1-tailed G-tests for the goodness of fit on the incidence of deformed mandibles of *Procladius* larvae. Data for all dates combined.

<table>
<thead>
<tr>
<th>Nominal Concentration (μL/L)</th>
<th>Average % deformed</th>
<th>Number normal</th>
<th>Number deformed</th>
<th>Total</th>
<th>df</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.5</td>
<td>206</td>
<td>1</td>
<td>207</td>
<td>1</td>
<td>0.735</td>
</tr>
<tr>
<td>0</td>
<td>1.7</td>
<td>115</td>
<td>2</td>
<td>117</td>
<td>1</td>
<td>0.377</td>
</tr>
<tr>
<td>0.05</td>
<td>0.0</td>
<td>137</td>
<td>0</td>
<td>137</td>
<td>1</td>
<td>1.500</td>
</tr>
<tr>
<td>0.10</td>
<td>2.7</td>
<td>141</td>
<td>4</td>
<td>147</td>
<td>1</td>
<td>3.600</td>
</tr>
<tr>
<td>0.17</td>
<td>0.0</td>
<td>130</td>
<td>0</td>
<td>130</td>
<td>1</td>
<td>1.400</td>
</tr>
<tr>
<td>0.30</td>
<td>0.0</td>
<td>85</td>
<td>0</td>
<td>85</td>
<td>1</td>
<td>0.900</td>
</tr>
<tr>
<td>0.54</td>
<td>1.6</td>
<td>63</td>
<td>1</td>
<td>64</td>
<td>1</td>
<td>0.129</td>
</tr>
<tr>
<td>0.97</td>
<td>0.5</td>
<td>202</td>
<td>1</td>
<td>203</td>
<td>1</td>
<td>0.655</td>
</tr>
<tr>
<td>1.73</td>
<td>2.3</td>
<td>81</td>
<td>2</td>
<td>83</td>
<td>1</td>
<td>1.344</td>
</tr>
<tr>
<td>3.08</td>
<td>1.3</td>
<td>77</td>
<td>1</td>
<td>78</td>
<td>1</td>
<td>0.011</td>
</tr>
<tr>
<td>5.51</td>
<td>2.4</td>
<td>40</td>
<td>1</td>
<td>41</td>
<td>1</td>
<td>0.500</td>
</tr>
<tr>
<td>9.83...</td>
<td>1.8</td>
<td>52</td>
<td>1</td>
<td>53</td>
<td>1</td>
<td>0.267</td>
</tr>
</tbody>
</table>
Table 3.19: Chi-square Test of Independence of Occurrence of Deformities in Ligula and Paralabial Combs of *Procladius* Larvae.

<table>
<thead>
<tr>
<th></th>
<th>Ligula, Undeformed</th>
<th>Ligula, Deformed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paralabial Combs,</td>
<td>observed = 1103</td>
<td>observed = 27</td>
</tr>
<tr>
<td>Undeformed</td>
<td>expected = 1095</td>
<td>expected = 34.7</td>
</tr>
<tr>
<td>Paralabial Combs,</td>
<td>observed = 127</td>
<td>observed = 12</td>
</tr>
<tr>
<td>Deformed</td>
<td>expected = 134.7</td>
<td>expected = 4.3</td>
</tr>
</tbody>
</table>

\[ \chi^2_{0.05} = 3.844 \]
\[ \chi^2_{obs} = 13.89 \text{ (p}<0.001) \]
Table 3.20: Chi-square Test on Independence of Occurrence of Deformities in Ligula and Mandibles of *Procladius* Larvae.

<table>
<thead>
<tr>
<th></th>
<th>Ligula, Undeformed</th>
<th>Ligula, Deformed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mandibles,</td>
<td>observed = 1299</td>
<td>observed = 41</td>
</tr>
<tr>
<td>Undeformed</td>
<td>expected = 1298.4</td>
<td>expected = 40.3</td>
</tr>
<tr>
<td>Mandibles,</td>
<td>observed = 13</td>
<td>observed = 1</td>
</tr>
<tr>
<td>Deformed</td>
<td>expected = 13.2</td>
<td>expected = 0.4</td>
</tr>
</tbody>
</table>

\[
\chi^2_{0.05} = 3.844 \\
\chi^2_{obs} = 0.915 \ (p>0.05)
\]
Table 3.21: Chi-square Test of Independence of Occurrence of Deformities in Paralabial Combs and Mandibles of *Procladius* Larvae.

<table>
<thead>
<tr>
<th></th>
<th>Paralabial Combs, Undeformed</th>
<th>Paralabial Combs, Deformed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mandibles,</td>
<td>observed = 1127</td>
<td>observed = 131</td>
</tr>
<tr>
<td>Undeformed</td>
<td>expected = 1128.4</td>
<td>expected = 132.5</td>
</tr>
<tr>
<td>Mandibles,</td>
<td>observed = 11</td>
<td>observed = 3</td>
</tr>
<tr>
<td>Deformed</td>
<td>expected = 12.6</td>
<td>expected = 1.5</td>
</tr>
</tbody>
</table>

\[ \chi^2_{0.05} = 3.844 \]
\[ \chi^2_{obs} = 1.722 \ (p > 0.05) \]
Chironomidae comprised the largest fraction of the invertebrate taxa found in the experimental ponds on all of the sampling dates. Overall, the trend was that as creosote concentration increased, chironomids made up an increasingly large proportion of the benthos. Chironomids, as a family group, are considered a pollution-tolerant taxon. Because there is such great ecological diversity within the family, at least some species are able to tolerate conditions unsuitable for most other taxa. The presence of chironomids in even highly polluted environments makes them a useful field biomonitor.

The taxonomic composition of chironomid communities has been used to identify the trophic status of lakes (Saether 1979, Winnell and Carter 1985). Saether (1979) stated that the presence of Chironomus, Polypedilum, Cryptochironomus, Procladius and Ablabesmyia indicated that a system was eutrophic. Nalepa and Thomas (1976), Warwick (1989), Dickman et al. (1992) also regard these chironomid taxa as pollution-tolerant because they are found in contaminated and eutrophic sites. At lower creosote concentrations, Chironomus, Polypedilum and Cryptochironomus were relatively rare in this study, whereas Procladius was common. Tanytarsus was present in the mesocosms, but only at low concentrations of creosote, indicating that this genus is pollution-intolerant. This is consistent with the findings of Krieger (1984) who studied benthic communities of Lake Erie. In this study, Procladius was numerically dominant in low to moderately contaminated ponds. Procladius has been reported to dominate heavily contaminated areas such as Port Hope, Ontario (Hart et al. 1986, Warwick et al. 1987), and Lac St. Louis and Laprairie Basins of the St. Lawrence River (Warwick 1990a). In the more contaminated mesocosms, Psectrocladius was the most abundant chironomid genus, indicating that it is even more
tolerant of pollution that *Procladius*. *Psectrocladius'* dominance reflected both its increasing numbers as well as a reduction in the numbers of other chironomid taxa.

In this study, chironomid genera tend to co-occur to produce distinct faunal groups among ponds. *Procladius* and *Ablabesmyia*, both belonging to the subfamily Tanypodinae, were consistently co-dominant or absent, while *Psectrocladius* (Orthocladiinae) was consistently in a second, independent group. *Chironomus* (Chironomini: Chironomidae) was associated with *Psectrocladius* on some sampling dates, and *Tanytarsus* (Tanytarsini: Chironomidae) was occasionally associated with the Tanypodinae. However, *Procladius*, *Ablabesmyia* and *Psectrocladius* appear to be the underlying influence for the formation of distinct chironomid groups. If only these genera are considered, the chironomid assemblages can be described based on their feeding strategies. The Tanypodinae are predacious while many Orthocladiinae feed on detritus (Oliver and Roussel 1983). The decrease in the numbers of predaceous species at the highest creosote concentrations may be related to changes in the kinds of food available.

**Community and Population Responses**

Enumerations and richness measures are commonly used to assess the environmental quality of an area (Resh et al. 1995). Contaminated areas are typically characterized by a decrease in the number of individuals and taxa. In this study, the overall trend was a decrease in the mean density and richness for both total invertebrates and chironomids as the concentration of creosote increased, but an absolute increase in *Psectrocladius* within the intermediate creosote concentrations. In general, mean density reflected this trend more
clearly than mean richness (i.e., abundance was reduced at lower creosote concentrations than was required to decrease richness).

The median lethal concentration (LC50) usually refers to the chemical concentration that causes 50% mortality in a population (Chan et al. 1984). For this portion of the study, a field-based LC50 was defined as the creosote concentration that reduced the mean density or richness of all benthos, or of chironomids to 50% of that observed in the control ponds. By determining the LC50 for the four community attributes (invertebrate density and richness, chironomid density and richness), it was possible to compare the responses. The slope of the exposure-response curve is also important because it indicates the relationship between the change in concentration and the change in response. A steep slope indicates that the compound is acutely toxic but a shallow slope is indicative of chronic toxicity (Chan et al. 1984).

The slopes of the creosote-density relationships for invertebrates and chironomids 4 weeks posttreatment were greater than those 8 and 12 weeks posttreatment. This indicates that as time passed, the creosote was not as toxic to the benthic community. Large increases in creosote concentration resulted in small increases in community response. Because chironomids comprised such a large fraction of the benthic community, the slopes of the chironomid dose-response curves for both density and richness did not differ greatly from those of the benthos overall. The relationship between the increase in creosote concentration and decrease in abundance/richness did not differ greatly 8 or 12 weeks posttreatment, indicating the benthic communities for these two dates responded similarly to the toxic effects of creosote. Some community attributes also exhibited a step-function concentration
response curve in at least one sampling period, indicating that the low concentrations of creosote were not toxic. LC50 calculations for two-phase responses can be determined from piecewise regression.

Overall, the LC50 values for community or population measured, increased for each successive sampling period. This would seem to indicate that the creosote effect was becoming less pronounced or the invertebrate communities were acclimating to the exposures. Based on creosote concentrations in the sediment, I believe that the creosote concentrations in the ponds over time, generally, follow a "bell-shaped" curve. This would support the explanation that creosote toxicity was decreasing because the organisms' exposures had decreased.

Based on the LC50 values, the mean density of all invertebrates was the most sensitive parameter in that total invertebrate density was reduced at a lower concentration of creosote than any other community measure. It has proved to be a rapid method for assessing the toxic effects of creosote because it requires substantially less effort and taxonomic expertise than determining richness measures.

**Morphological Deformities**

In this study, samples of *Procladius* larvae, pooled across dates, provided relatively large sample sizes over the largest range of creosote concentrations. Therefore, I focused on this genus for the evaluation of morphological deformities.

*Psectrocladius*, abundant at a few higher creosote concentrations, has a mentum made up of two broad, medially-crowned teeth and five lateral teeth (Oliver and Roussel 1983). Warwick (1990) reported one deformed *Psectrocladius* larva (of 28 examined) and the
deformity was a reduced lateral tooth. Since *Psectrocladius* was not abundant in the pretreatment or control ponds, baseline deformity levels could not be determined so this genus was not examined for abnormalities.

The ligula, paralabial combs and mandibles of *Procladius* were examined for deformities. Dermott (1991) reported the incidence of deformities in these structures, from Lake Superior (9 reference sites), to be 4%, 6.5%, and 2.0%, respectively. Tennessen and Gottfriéd (1983) also reported a baseline level of 3.9% for ligula deformities in *Procladius*, and Warwick (1988) reported a baseline level of 2.1%. In this study, the posttreatment incidences of ligula, paralabial combs, and mandibles were 2.3-2.4%, 10.1-14.8%, and 0.5-1.7%, respectively, in the control ponds. These control levels are reasonably similar to those reported in the literature. Differences in the incidences of deformities in the ligula and paralabial combs between this study and the literature can be attributed to possibly different perceptions of what constitutes “reduced tooth size”.

Warwick (1988) speculated that the dose-response relationship between the incidence of deformities and contamination level should follow a quantal (bell-shaped) dose-response curve. He also believed that different cephalic structures would vary in sensitivity to chemically induced stress (Warwick 1990b). Since a broad range of creosote applications was used in field study, it was possible to address these hypotheses.

The incidences of ligula, paralabial comb and mandible deformities did not follow the quantal dose-response curve. The incidences of paralabial comb and mandible deformities were not significantly greater than the mean incidences in the control ponds at any of the creosote concentrations, indicating that they were not sensitive to PAH contamination.
Dermott (1991) stated that increased exposure to contaminants did not necessarily result in higher incidences of deformities. The ligula may be more sensitive to the induction of deformities than the other two structures since there was an increased incidence at one concentration of creosote (0.51-2.19 µg/g sediment) pond. Since the ponds were given a logarithmic series of nominal creosote concentrations, a dose-response relationship was expected. The results seem to indicate that this relationship does not exist for the cephalic structures of *Procladius* when exposed to PAHs.

Different chironomid genera display different levels of tolerance to contaminants (Wiederholm 1984). Hudson and Ciborowski (1996a) noted that different chironomid genera were not similarly susceptible to morphological deformities when exposed to contaminants. The reported incidence of deformed larvae in chironomid genera has been higher than in the genus *Procladius* (Wiederholm 1984). Many deformed *Chironomus* larvae have been found in contaminated areas where very few deformed *Procladius* larvae existed (Warwick 1989, 1991, Dermott 1991, Diggins and Stewart 1993). The genus *Chironomus*’ susceptibility to mentum deformities appears to make it a more useful tool for assessing subtle changes due to pollution levels than the more tolerant *Procladius* genus (Dermott 1991).

According to the results of this study, change in invertebrate density is a more sensitive indicator of the detrimental effects of creosote on the invertebrate community than cephalic deformities of *Procladius*. 

123
Chapter IV. General Discussion

The mesocosm study allowed me to compare the relative sensitivity of various components of the invertebrate community of the artificial ponds to creosote stress. I looked at changes in: overall invertebrate community numbers and richness; chironomid community numbers and richness; patterns of relative and absolute chironomid community composition; changes in the abundance of two key chironomid genera (*Procladius* and *Psectrocladius*); and the sublethal stress (deformities) in one key chironomid genus (*Procladius*). I expected that individuals would show the effects of creosote contamination before community effects were seen but this was not the case. Change in the overall invertebrate community numbers was the most sensitive and consistent indicator of creosote contamination.

This study also found that at low creosote concentrations, chironomids comprised a smaller proportion of the overall invertebrate community when invertebrate community numbers were high. As creosote concentrations increased, chironomids comprised a larger proportion of the invertebrate community because the number of individuals from other taxa was reduced while chironomid numbers were not reduced to the same extent.

Different chironomid assemblages based on the level of creosote concentration were also observed. Generally, *Ablabesmyia* and *Procladius* consistently co-occurred while *Psectrocladius* was consistently in another independent assemblage. Overall, *Procladius* larvae were more sensitive to creosote contamination than *Psectrocladius* larvae.
Laboratory versus Field-based Toxicity Tests

Field studies usually involve monitoring effects that have already occurred in the environment due to contamination. Because a natural environment is complex, there are many variables over which a researcher has no control. These studies tend to be qualitative and it is often difficult to determine specific causes for biological changes in the environment.

Single-species laboratory toxicity tests involve the exposure of organisms, under constant and optimal growth conditions, to known concentrations of contaminants in order to determine the potential toxicity to the chemical (deNoyelles et al. 1994). The primary advantage of these tests is that they allow the comparison of different effects of contaminants or different endpoints of a specific contaminant. Because the variables of these tests are rigidly controlled and do not take into account complex environmental interactions, it is difficult to use these findings to predict environmental responses (Cairns and Pratt 1986).

Field-based mesocosm, or microcosm, studies represent a compromise between field and laboratory studies. Experimental conditions and chemical concentrations can be controlled while major features of natural communities are simulated (Touart 1988).

As part of this research project, creosote toxicity to chironomids was described by laboratory- and field-derived LC50 values. The laboratory-based LC50 value of 437 µg/g was much larger than any of the LC50 values obtained from the community measurements. The difference ranged from approximately 7X greater than the
chironomid richness (8 and 12 weeks posttreatment) LC50 value, the least sensitive indicator, to 825X greater than the most sensitive community-based LC50 value, chironomid density (1 weeks posttreatment). The laboratory-based LC50 value greatly underestimated the detrimental effect creosote had on the benthic chironomid community. It is not uncommon to find results from laboratory studies which do not correspond to field-based toxicity tests. Tagatz et al. (1983) reported that in field and laboratory studies, creosote reduced the abundance and taxa richness of mollusks at different concentrations (844 µg creosote/g sediment in the field and by 4,420 µg creosote/g sediment in the laboratory). These researchers explained that some of the differences in creosote toxicity between the field and laboratory results may have been due to differences in species’ sensitivities to creosote. Although this may have been a plausible explanation for their study, in this study, I do not believe that Chironomus riparius is more tolerant of creosote than Procladius and Psectrocladius. Several reports in the literature state that Procladius larvae are found in heavily polluted areas where Chironomus spp. cannot survive (Warwick 1985, Dermott 1991). Other environmental factors such as dissolved oxygen may have amplified the toxic effects of creosote but many of the ponds, except at the highest concentrations, had dissolved oxygen levels above 8.0 mg/L. This level of dissolved oxygen should comfortably be within chironomids’ level of tolerance (Pinder 1986).

The route of exposure may have influenced the laboratory and field responses. In the laboratory, creosote was mixed throughout the sediment whereas, in the field it was sprayed into the water and settled to the sediment surface. Clearly, attempting to
extrapolate field responses from my laboratory results could lead to erroneous conclusions.

**Limitations of the Study**

**Invertebrate Colonization**

Initially, the artificial ponds were allowed to naturally colonize, primarily by water recirculation with the holding pond. This colonization period lasted for approximately 22 days before the creosote treatments were applied (July 5). Ferrington *et al.* (1994) believed that it would take one to several years to develop a benthic invertebrate community that contained representative proportions of the natural environment. When mesocosms were part of the pesticide registration protocol, new ponds were allowed to "season" for 6 months if they were lined with sediment from established ponds or 12 months if they were lined with topsoil (Christman *et al.* 1994). If dispersal is the sole method for invertebrates to colonize the artificial ponds, non-flying organisms are at a disadvantage and are usually present in ponds at much lower densities than would naturally be expected (Touart 1988). This may have been the case for oligochaete worms and amphipods in the artificial ponds in this study. Because the time allotted for colonization was so short compared to what is recommended in the literature, the artificial ponds should have been seeded with representative taxa from the area in order to ensure homogeneous, "natural" benthos in each pond. Another recommendation is the use of artificial substrate samplers to collect the benthos. Christman *et al.* (1994) reported that these samplers were used to reduce variation among samples and to shorten processing time in the laboratory.
Mesocosm Experimental Design

A regression approach (i.e., single treatments of many concentrations across a broad range of contamination) was chosen as the mesocosm experimental design in this study (Harris et al. 1996). This approach is not normally used to test specific hypotheses, instead, it is used to determine dose-response curves in toxicity tests (Shaw et al. 1994). Because the focus of this research was the benthic invertebrate community, creosote concentrations were expressed in terms of sediment mass (µg/g) to generate dose-response curves. However, the creosote was added to the water as a mist, and the differences between nominal concentrations were so small for many of the treatments, sediment concentrations only poorly reflected the equal-interval creosote concentration gradient expected from the nominal concentrations applied (Bestari et al. 1997). On all sampling dates, 2-4 of the treatment ponds had sediment concentrations less than those found in the control pond sediments. Instead of having creosote concentrations equally spaced along a logarithmic scale, the sediment concentrations clustered around various concentrations resulting in concentration-response curves being determined by as few as five sediment concentrations (12 weeks posttreatment). This limitation probably altered the precision of the dose-response curves considerably.

Chironomid Taxa Analysis

A primary objective of this project was to assess the relative usefulness of cephalic morphological deformities as indicators of environmental degradation. Consequently, all of the chironomid larvae in the 4-mm and 1-mm sieve fractions were slide-mounted and identified to genus. This approach yielded an adequately large sample size to permit
me to analyze deformities in *Procladius*. Smaller chironomid larvae (those retained in the 500-μm sieve fraction) were not identified or assessed for deformities. This fraction contained between 20% and 65% of chironomid numbers in each sample, depending on the pond and sampling date. This may have biased the chironomid generic richness measures, if susceptibility to creosote is size dependent. In the pretreatment samples, the number of chironomid genera in the 4-mm and 1-mm sieve fractions ranged from 5-7. The number of chironomid genera in the 500-μm pretreatment sieve fraction was between 5-9, with all of the genera of the larger chironomids accounted for. Although ignoring the chironomids in the 500-μm sieve fraction did introduce size bias, the chironomid richness measures were all determined in a similar manner so they are comparable within this study.

**Future Research**

Deformities in chironomids seem to be a useful tool to augment more traditional benthic invertebrate measures of environmental pollution. It is often difficult to obtain the necessary sample sizes of the desired chironomid genus necessary for analysis from simply sampling the resident chironomid community. Of all the chironomid genera in which deformities have been reported, *Chironomus* seems to be the best suited as a bioindicator because it is most susceptible to deformities. However, this genus may not be available in the desired study area. In order to resolve this problem, I suggest that an *in situ* sediment bioassay be developed using cultured *Chironomus* larvae. This bioassay, coupled with the regression approach of this mesocosm study, can be useful
for determining the relationship between degree of contamination and incidence of deformities. A linear relationship was not observed during this study. *Procladius* deformities do not appear to be consistently inducible by creosote contamination. If morphological deformities are to be useful early-warning indicators, then linear concentration-response curves must be documented and linked to changes in the benthic community. Perhaps the *in situ* bioassays could be used for actual field testing and documentation of morphological deformities.
Literature Cited


invertebrates for monitoring contaminated sediments. Presentation to Eighth 
Annual Technical Information Workshop, 43rd Annual Meeting of the North 
American Benthological Society.

transfer of fluoranthene and benzo(a)pyrene in *Chironomus riparius* and *Lepomis 

Report to the Ministry of the Environment.

Credland, P.F. 1973. A new method for establishing a permanent laboratory culture of 

Cushman, R.M. 1984. Chironomid deformities as indicators of pollution from a 


structure and function. In *USEPA Sediment Classification Methods Compendium*, 
D.C.

riparius* (Meigen): impact on interpretation of growth in whole-sediment toxicity 

mesocosms in ecologocal effects testing: detecting direct and indirect effects of 
Lewis, Boca Raton, FL, pp. 577-603.


Dickman, M., I. Brindle and M. Benson. 1992. Evidence of teratogens in sediments of 
the Niagara River Watershed as reflected by chironomid (Diptera: Chironomidae) 


Pham, T., K. Lum and C. Lemieux. 1993. Sources of PAHs in the St. Lawrence River (Canada) and their relative importance. Chemosphere 27: 1137-1149.


Powlesland, G. and J. George. 1986, Acute and chronic toxicity of nickel to larvae of Chironomus riparius (Meigen). Environ. Pollut. (Series A) 42: 47-64.


Introduction

Many contaminants entering aquatic environments often persist in the sediment. Contaminated sediment can be directly toxic to aquatic life or can be a source for bioaccumulation in the food chain (Ingersoll and Nelson 1990). The assessment of sediment quality requires the use of bioassessment tests to estimate the biological damage caused when aquatic organisms are exposed to the contaminated sediments. The standard responses of organisms for chronic (slow response) toxicity tests are changes in survival, growth and reproductive potential (Day *et al.* 1994) but at this point the deleterious effects of a contaminant have already been manifested in the organism.

Generally, the effects of contaminants are manifested first at the molecular and biochemical pathways where the functioning of important processes can be affected and, over time, these effects may be expressed by an organism's decreased ability to grow, to reproduce or to survive. These sensitive indicators of physiological stress, referred to as biomarkers, relate quantifiable cellular events to a dysfunction that a contaminant has produced (McCarthy and Shugart 1990). This approach has been proposed as an early warning system for higher biological level effects. However, the disadvantage of using biomarkers as indicators of stress is that it is often difficult to relate these biochemical and cellular responses to the health of an organism and to adverse effects on a population. But this disadvantage can be overcome by selecting as biomarkers the cellular and biochemical
events that are involved in protecting the cell from environmental insults. Therefore, the cellular stress response, which is characterized by altered gene activity and protein synthesis (Lewis et al. 1975), has the potential to be an effective biomarker because it is activated as soon as the cell has been exposed to any environmental stressor.

The Cellular Stress Response

The response of the cell to chemical and physical insults that result in changes in the pattern of gene activity at both the chromosomal and protein synthesis levels is referred to as the cellular stress response. This response is universal and has been found in many diverse organisms (Schlesinger 19986). When this response is elicited, it protects the cell from environmental stress. Environmental stressors include temperature, UV light and xenobiotics. Upon exposure to a stressor, there is a parallel induction of chromosomal puffs and synthesis of proteins (Lewis et al. 1975).

Protein Induction

Under conditions of environmental stress, proteins are synthesized that are involved in the protection of protein targets and the repair of denatured proteins (Sanders et al. 1992). There are five universal stress protein families-stress 90, stress 70, chaperonin 60, low molecular weight stress proteins and ubiquitin.

Of these protein families, stress protein 70 has the greatest potential as a general biomarker because it is highly conserved and it indirectly accounts for much of the transcriptional activity in stressed cells (Linquist 1986). Also, under stressful conditions the synthesis of the proteins in this family is greatly increased (Sanders 1993). The relationship between the concentration of stress 70 and the concentration of PAHs will be examined.
Objectives

The objective of this study is to develop a protein based assay as a biomarker of general stress to polynuclear aromatic hydrocarbon (PAH) exposure in the benthic invertebrate- *Chironomus riparius* Meigen (Diptera: Chironomidae).

I propose to:

1) establish a laboratory dose-response relationship between various PAH (creosote) concentrations and the stress response in *C. riparius*, and identify any correlations between the stress response and ecologically relevant effects such as survival;

2) conduct an *in situ* study to evaluate the biomarkers' effectiveness under realistic environmental conditions.

Materials and Methods

Laboratory Study

*Creosote Toxicity Test*

The design for this study has been described in Chapter II (this thesis). The chironomid larvae that were recovered alive were immediately stored in liquid nitrogen and then transferred to an ultra-cold freezer until stress protein analysis.

*Heat Shock Study*

Fourth instar larvae of *C. riparius* were reared at 23°C under constant aeration. Twenty-five larvae, in groups of five, were subjected to a heat shock of 35°C for 1 h. Heating was preformed by immersion of tubes containing one group each into a precision controlled waterbath (±1°C). Another twenty-five larvae, in groups of five, were left at
room temperature for 1 h. After the allotted time period, the larvae were immediately placed in liquid nitrogen and then they were transferred to an ultra-cold freezer until stress protein analysis.

Field Study

An in situ exposure was conducted in the artificial ponds at the Turfgrass Institute, University of Guelph, Guelph, Ontario. Three plastic Rubbermaid® containers (26.5x16x6cm deep) were randomly placed on the bottom of each artificial pond prior to the addition of the water. Each container was filled with approximately 3 cm of sediment that was also used to fill the plastic planting trays (see Chapter III-Materials and Methods: Mesocosm Study Design). The containers were covered with a lid that had a 15cm x 12 cm opening covered with a 136 μm Nitex® cloth.

At 1 d pretreatment, the containers’ lids were removed and then replaced three days after the application of creosote to the ponds. At 9 wk posttreatment, each container was inoculated with 50 laboratory-reared fourth instar larvae of C. riparius. After 48 h, the containers were removed from the ponds and the sediment from each container was rinsed through a 500 μm sieve. The larvae recovered alive were placed in liquid nitrogen and transported to the laboratory where they were transferred to an ultra-cold freezer until stress protein analysis.

Stress Protein Analysis Protocol

Sample Preparation

Five chironimid larvae were placed in a test tube containing 60 μL of lysis buffer (containing: 1% TritonX-100, 1 μg/mL leupeptin, and 1 mM phenylmethylsulfonyl
fluoride). Glass beads (0.25-0.32 mm) were added to the mixture and it was vortexed for 5 min. The supernatant was collected, mixed with 5X sample buffer (containing: 250 mM Tris-HCl (pH 6.8), 50% glycerol, 10% sodium-dodecysulphate (SDS), 25% beta-mercaptoethanol (BME) and 0.25% bromophenol blue). The mixture was boiled for 4 minutes and then clarified by centrifugation at 13,000g for 5 min. The supernatant was stored at -20°C until needed.

Soluble protein in the samples was quantified using the BioRad Protein Assay. Protein standards were made using bovine serum albumin (BioRad). Five microlitres of each standard and sample solution were pipetted into separate microtiter plate wells and 200 µL of dye reagent was added to each well. The reagent and sample were thoroughly mixed and allowed to incubate for 5 min. Each well was read on a spectrophotometer with a 595 nm filter.

**Gel Electrophoresis**

The procedure used for the separation of proteins by electrophoresis is described in the BioRad Mini-PROTEAN® II Electrophoresis Cell Instruction Manual. In all cases, 30 µg of protein was separated electrophoretically in 12% sodium dodecyl sulfate (SDS) polyacrylamide gels. The 12% separating gel (containing: 4.0 mL 30% bis-acrylamide (BioRad), 2.5 mL 1.5M Tris-HCl (pH 8.8), 100 µL 10% SDS, 3.35 mL ddH₂O, 50 µL 10% ammonium persulfate (APS), 10 µL N,N,N′,N′-tetramethylethylenediamine (TEMED)) polymerized in gel formers after approximately 45 min. After polymerization, a 4% stacking gel (containing: 1.5 mL 30% bis-acrylamide (BioRad), 2.5 mL 0.5M Tris-HCl
(pH 6.8), 5.9 mL ddH2O, 100 μL SDS, 50 μL 10% APS, 10 μL TEMED) was formed above the separating gel. It was allowed to polymerize for approximately 45 min.

Once the samples were loaded onto the gel, the electrophoretic chamber was immersed in running buffer (pH 8.3) (containing: 9.0 g Tris base, 43.2 g glycine, 3.0 g SDS in 600 mL ddH2O). The gel was run at a constant voltage of 60 V, at room temperature, until the bromophenol marker dye had travelled through the stacking gel. A constant voltage of 100 V, at room temperature, was then applied until the dye had reached approximately 1 cm from the bottom of the glass plates. Prestained SDS-PAGE standards (BioRad) and hsp 70 from bovine brain (Sigma) were run in parallel lanes on each gel. The molecular weight markers were used to determine the relative weights of proteins of interest and the 70 kDa protein served as a control for the antibody’s quality during the Western Blotting process.

**Western Blotting**

The proteins were transferred from the polyacrylamide gels to nitrocellulose membranes in a BioRad Mini Trans-Blot® electrophoretic transfer cell using ice cold blotting buffer (pH 8.3) (containing: 25 mM Tris base, 193 mM glycine and 20% v/v methanol). The protein profiles were transferred at a constant voltage of 100 V for 60 min.

The nitrocellulose blots were blocked with buffer containing 9 g of Carnation® powdered milk and 100 mL Tris Buffered Saline (TBS) (pH 10.3) (containing: 6.06 g Tris, 8.76 NaCl, 100 mL ddH2O) for 2 h. The blocked membranes were incubated with monoclonal anti-heat shock protein 70 (mouse ascites fluid) (Sigma) at a 1:5,000 dilution for 1.5 hr. Alkaline phosphatase conjugated goat anti-mouse IgG, a secondary antibody, at 1:3,000 dilution for 1.5 hr was used to detect the anti-hsp 70 antibody. Between each
incubation, the membranes were washed three times (approximately 5 min each) with Tween Tris Buffered Saline (pH 10.2) (containing: 0.5 g 2% w/v Tween 20 in 1000 mL TBS) and once for five minutes with TBS. An Alkaline Phosphatase Conjugate Substrate Kit (Biorad) was used to visualize blots corresponding to hsp 70.

After the protein profile was transferred from the gel to nitrocellulose, the gel was stained with Coomassie Brilliant Blue R-250 stain in order to observe the efficiency of the transfer.

Results

Stress Protein Analysis

Creosote Toxicity Test

The primary antibody recognised a protein in the lanes that contained samples of the larvae used in the toxicity test control, carrier control and creosote-contaminated treatments. The apparent molecular weight of the protein was approximately 80 kDa. However, the appearance of the blots was inconsistent from one membrane to another. All membranes had at least one blot in a lane that corresponded to a sample collected from the toxicity test, but the corresponding lane was not the same in all the runs. As well, the colour intensity of the blots was random. The colour intensity did not increase or decrease as the creosote concentrations increased.
Heat Shock Study

No blots were observed in any of the lanes that corresponded to either control or heat shocked larvae. A blot was visible in the lane onto which the commercially purchased hsp 70 was loaded. The results were similar when other groups were analysed.

Field Study

These samples were not analysed because the results from the toxicity test were inconclusive.

Discussion

Carretero et al. (1986) found that fourth instar larvae of Chironomus thummi exposed to 35°C for 1 h produced an optimal heat shock response. These researchers reported bands corresponding to 86, 76, 74, 70, 28, and 26 kDa immediately after the heat treatment. Since researchers use the chromosomal maps of C. thummi for C. riparius, then C. riparius is capable of producing hsp 70 under the appropriate stresses. Because there were no blots from the temperature-treated trials and the results from the toxicity test were inconsistent, I concluded that the commercially purchased anti-hsp 70 antibody (Sigma) did not recognise hsp 70 produced in C. riparius larvae.

As well, D. Sinasac (University of Windsor 1996) reported that the monoclonal anti-hsp 70 antibody (Sigma) did not recognize temperature-induced hsp 70 in chironomids although hsp 70 protein was recognized in heat-shocked Drosophila that was run on the same gel.
Literature Cited


Vita Auctoris

Name: George Pardalis

Place of Birth: Windsor, Ontario

Date of Birth: 30 October 1968

Education:
Walkerville C.I.
Windsor, Ontario
1982-1987

Lakehead University
Thunder Bay, Ontario
1988-1993
H.B.Sc.

University of Windsor
Windsor, Ontario
1994-1997
M.Sc.