

















NORTHERN RIVER BASINS STUDY PROJECT REPORT NO. 140 ASSESSING PULP MILL CONTAMINATION USING MORPHOLOGICAL DEFORMITIES IN CHIRONOMID LARVAE (Diptera Chironomidae) UPPER ATHABASCA RIVER, APRIL 1992













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Prepared for the Northern River Basins Study under Project 2329-D1

by

W. F. Warwick Ecosystem Health Assessment Unit, Ecological Research Division, Environment Canada

NORTHERN RIVER BASINS STUDY PROJECT REPORT NO. 140 ASSESSING PULP MILL CONTAMINATION USING MORPHOLOGICAL DEFORMITIES IN CHIRONOMID LARVAE (Diptera Chironomidae) UPPER ATHABASCA RIVER, APRIL 1992

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PREFACE:

The Northern River Basins Study was initiated through the "Canada-Alberta-Northwest Territories Agreement Respecting the Peace-Athabasca-Slave River Basin Study, Phase II - Technical Studies" which was signed September 27, 1991. The purpose of the Study is to understand and characterize the cumulative effects of development on the water and aquatic environment of the Study Area by coordinating with existing programs and undertaking appropriate new technical studies.

This publication reports the method and findings of particular work conducted as part of the Northern River Basins Study. As such, the work was governed by a specific terms of reference and is expected to contribute information about the Study Area within the context of the overall study as described by the Study Final Report. This report has been reviewed by the Study Science Advisory Committee in regards to scientific content and has been approved by the Study Board of Directors for public release.

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(Dr. Fred & Wrona, Science Director)

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ASSESSING PULP MILL CONTAMINATION USING MORPHOLOGICAL DEFORMITIES IN CHIRONOMID LARVAE (Diptera Chironomidae) UPPER ATHABASCA RIVER, APRIL, 1992

STUDY PERSPECTIVE

Organic contaminants which enter aquatic ecosystems can become associated with particles of organic and inorganic materials in depositional The presence and persistence of zones. contaminants in these sediment depositional zones may constitute a source of toxicity to organisms which live on or near the substrate. As an example, this source of toxicity may have direct impacts on benthic invertebrates (bottom-dwelling organisms) as well as indirect effects on other organisms which use them as food. Chironomid (midge) larvae, in particular, are considered good environmental indicators and have been used in prior research to test the sublethal responses of aquatic invertebrates to nutrient-rich wastes and contaminant pollution in aquatic ecosystems. The purpose of the Northern River Basins Study was to understand and characterize the cumulative effects of development on the water and aquatic environment of the Peace. Athabasca and Slave rivers. Under the Synthesis and Modelling component a project was initiated to look into the number of species and the frequency of morphological deformities in the chironomid community. The Chironomid community of the upper Athabasca River were selected to determine the utility of these biota to assess the cumulative effects of the Hinton Combined Effluent (HCE).

Related Study Questions

- 1a) How has the aquatic ecosystem, including fish and/or other aquatic organisms, been affected by exposure to organochlorines or other toxic compounds?
- 4a) What are the contents and nature of the contaminants entering the system and what is their distribution and toxicity in the aquatic ecosystem with particular reference to water, sediments and biota?
- 13b) What are the cumulative effects of manmade discharges on the water and aquatic environment?
- 14) What long term monitoring programs and predictive models are required to provide an ongoing assessment of the state of the aquatic ecosystems?

The goal of this project was to provide baseline information on the occurrence and spatial distribution of chironomid deformities in depositional areas of the upper Athabasca River. These data were supplemented by identification and counts of individual chironomid taxa. The technique allowed an evaluation of spatial trends in the incidence of deformities relative to known point source effluents in the upper Athabasca River.

Six sediment depositional areas were examined, and 14 of 51 chironomid taxa showed some form of observed morphological deformities in response to the HCE. The frequency of deformities among the groups increased significantly below the HCE outfall at the Weldwood Bridge, but declined rapidly at the next downstream site (Obed Mountain Coal Bridge), and then increased steadily to the farthest downstream site (Windfall Bridge). The trend toward increasingly severe deformities, particularly in the mouth parts, suggests that the effects of contaminants are more apparent downstream. Chironomid community diversity increased notably downstream of the HCE, and gradually decreased to reference (upstream Hinton) values with distance downstream. A possible explanation for the trends seen may be that the initial affects of contaminants are masked by high nutrient inputs and increased productivity, but as the influence of this component declines with distance downstream, the effect of the contaminant component becomes more apparent.

Although the rates of morphological deformities were low, results from this research indicate that certain groups of chironomid larvae have been affected to some degree by contaminant and nutrient input into the upper Athabasca River. Morphological deformities in chironomid larvae appear to be a sensitive tool for monitoring sublethal contaminant effects in riverine environments. This information will be used to prepare a report on cumulative impacts and could be used to support development of biomonitoring programs and guidelines for ecosystem health in northern rivers.

REPORT SUMMARY

The objective of Northern River Basins Study Project 2329-D1 was to (a) "obtain baseline information on the occurrence and spatial distribution of chironomid deformities in depositional areas of the upper Athabasca River; and (b) using [these] baseline data, assess the potential for using chironomid deformities as an early indicator of anthropogenic stress in the upper Athabasca River."

The Chironomidae possess a number of advantages as indicators of aquatic conditions (Warwick 1990) and have been used successfully in the lake classification system to categorize lakes according to trophic state (Brundin 1949; Saether 1975; Warwick 1980). As an **indicator community**, chironomids obviate many of the limitations of single **indicator species** because they represent all functional groups within their environment. The interpretation of morphological responses to contaminants is regarded as a direct extension of the lake classification system (Pinder 1986).

As an analytical tool, the response of chironomid communities combine the ability to analyse both contaminant and trophic processes, and their interaction, simultaneously (Warwick 1992). Chironomid communities are evolutionarily pre-adapted to changes in trophic level, whereas they have no inherent mechanisms to counter the toxic effects of contaminants. The ability of chironomids to monitor trophic changes (community analysis) and contaminant impacts (deformity analysis) simultaneously is quite unique among bioindicators and allows for "in situ" assessments of these processes and their interactions.

Chironomid community diversity increased dramatically below the Hinton Combined Effluent outfall in response to increased nutrient loads and levels in primary production, and possibly increased particulate food resources, issuing from the domestic waste components of the effluent. The impact of increased trophy gradually declined downstream as this component degraded and selfcleansing of the river proceeded. The frequency of morphological deformities increased significantly immediately below the effluent outfall, fell sharply again, then increased progressively downstream. Similarly, the severity of deformities increased progressively downstream, with the deformities in fauna from the furthest downstream station at Windfall Bridge (Site 6) being much more severe than those from Weldwood Bridge (Site 2) immediately below the effluent outfall.

The hypothesis is put forward that the impact of contaminants is immediately substantial, but largely masked by the effects of the domestic waste component of the effluent. However, as this component begins to degrade downstream through natural biodegradation processes and the masking effect is removed, the effects of the contaminant component is expressed more fully. This phenomenon appears to be a function of the differential breakdown rates of the two components of the combined effluent introduced at Hinton.

The data show that all six sites in the Hinton-Whitecourt reach of the upper Athabasca River, including the reference site near Entrance (Site 1), have been impacted to some degree by contaminant and nutrient inputs to the riverine environment. Under such circumstances, the early warning capability of the technique might be difficult to assess, but the definition shown even by the low frequencies of deformation presented here, indicate that the technique is very sensitive to environmental perturbation.

Morphological deformities have not been previously described for many of the taxa found in this study; these are new to the literature.

This project has elucidated some of the basic principles involved in the interactions between nutrients and contaminants in riverine ecosystems and macroinvertebrate responses to these interactions.

ACKNOWLEDGEMENTS

I thank Gregg Babish, Environment Canada, Regina, SK, for preparing the map in Fig. 1 and Dr. F. J. Wrona for his comments and suggestions. The project was funded by the Northern River Basins Study group and Environment Canada.

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1.0 INTRODUCTION

The Athabasca River is a large, unregulated northern river which originates near Jasper, Alberta, flows across the province in a northeasterly direction, and empties into the southwestern corner of Lake Athabasca. Seasonal and longitudinal trends in river water quality are considerably modified by inputs from relatively large tributaries as well as anthropogenic sources such as municipal, pulp mill and oil extraction effluents (Hamilton et al. 1985).

Weldwood of Canada Ltd., Hinton Division, is the uppermost of a series of pulp mills to influence the reaches of the Athabasca River. It introduces effluent into the Athabasca River mixed with domestic sewage from the town of Hinton, Alberta (Fig. 1). The mill is a bleached kraft mill which, at the time of sampling (1992), still used molecular chlorine for bleaching. The mill upgraded to 100% chlorine dioxide substitution in mid-1993 (Golder Associates, 1994).

To test the impact of the mill on the quality of the river ecosystem, benthos was collected from above the point of introduction of the combined effluents to a distance approximately 170 km downstream of that point. These fauna were analysed for the presence of morphological deformities in chironomid larvae to determine biological responses to pulp-mill effluent.

2.0 <u>METHODS</u>

Macroinvertebrate samples were collected from depositional areas of the Hinton-Whitecourt reach between 7-14 April 1992 by R. L. & L. Environmental Services Ltd., Edmonton, Alberta. These were collected using an Ekman dredge and sorted into basic taxonomic categories for further processing (R. L. & L. Environmental Services Ltd. 1993). This work was carried out under Northern River Basins Study Contract 2371 and 2521-B1 (Dunnigan 1993). The chironomid larvae collected by R. L. & L. were forwarded to this investigator for taxonomic identification and morphological assessment and interpretation. All chironomid larvae were permanently mounted on glass slides for taxonomic identification and morphological evaluation following Warwick (1985). Community structure was described using Shannon-Wiener Diversity Indices. Taxonomy generally follows Wiederholm (1983) and evaluations follow the definition and procedures put forward in Warwick (1985). Sample site relationships were tested using Chi-square according to Gad and Weil (1982). Unfortunately, protocols have not yet been devised for assessing the severity of deformities for the taxa encountered in this type of environment.

3.0 <u>RESULTS</u>

3.1 Productivity Changes

The presence of taxa such as *Abiskomyia* and *Monodiamesa* above the outfall (Site 1) indicates that the river entering the Hinton-Whitecourt Reach was moderately oligotrophic (Saether 1979, Warwick 1980). The impact of nutrient inputs on the chironomid community (Fig. 2) is reflected in the increase in population size and diversity below the outfall at Weldwood Bridge (Site 2). Further downstream, these declined until, at Windfall Bridge (Site 6), community diversity had returned to approximately pre-impact values. Community structure was considerably altered, however, toward a fauna more characteristic of the later phases of oligotrophy (Warwick 1980).

The Ohio E.P.A. (1988) ranks the biological quality of stream environments according to diversity indices as follows: >3.5 is exceptional, 2.9-3.5 is good, 2.3-2.9 is fair and <2.3 is poor. According to these criteria, the only site with "good" environmental quality was the Weldwood Bridge site (Site 2) while the other 5 sites ranked only "fair".

3.2 Deformities

A total of 1642 chironomid larvae were identified and assessed for morphological deformities. Of the 51 taxa encountered, 14 showed responses to contaminants in the river in the form of observed morphological deformities. (The response of the taxon, *Paracladopelma winnelli* Jackson, remains ambiguous at this point.) The most responsive taxa appear to be three species of *Orthocladius* and two species of *Polypedilum*. Other responding taxa included *Monodiamesa, Hydrobaenus*, cf. *Abiskomyia*, cf. *Parakiefferiella*, and two unidentified types of Orthocladiinae, two of Chironomini, and one of Tanytarsini.

The frequency of deformities among these fauna increased rapidly below the effluent outfall at Weldwood Bridge (Site 2), declined almost equally rapidly at Obed Mountain Coal Bridge (Site 3), then increased steadily again to Windfall Bridge (Site 6; Fig. 6). The increase in the frequency of deformities immediately below the effluent outfall was highly significant compared to the upstream reference site (Site 1), as was its decline toward Obed Mountain Coal Bridge site. The chi-square values increase successively towards Windfall Bridge (Site 6) where the differences are not significant at traditional levels ($X^2_{(0.05)} = 3.8410$), but did achieve significance above 90%.

The severity of deformities among the 14 responding taxa also increased downstream towards Windfall Bridge (Site 6) approximately 170 km downstream. At Weldwood Bridge (Site 2), deformities in cf. *Orthocladius* sp. 3 generally involved the loss or gain of a single tooth on the mentum, whereas at Windfall Bridge (Site 6), several specimens with 'keyhole' deformities - so called because of the characteristic curling of the teeth into a circular pattern - were found in both menta and mandibles (Fig. 4).

The trend towards increasingly severe deformities indicates that the effects of contaminants are becoming more severe downstream. This same trend is shown in the sequential Chi-square values in Table 1, particularly in the comparisons with reference Site 1 near Entrance, and in the

	Weldwood Bridge	Obed Mtn. Coal Bridge	Emerson Lakes	Knight Bridge Bridge	Windfall
Entrance	8.85431**	0.03590	1.27404	1.86418	3.17269
Weldwood		8.27312**	1.73133	3.10396	2.92154
Obed Mtn.			0.94905	1.44420	2.65309
Emerson				0.00155	0.10200
Knight					0.13490

Table 1.Sequential Chi-square values for downstream site comparisons, Hinton-
Whitecourt reach, Athabasca River, 1992.
(** denotes highly significant values)

Values: $X_{(0.10)}^2 = 2.71; X_{(0.05)}^2 = 3.8410; X_{(0.005)}^2 = 7.8790$

decline in community diversities towards Windfall Bridge (Site 6). If the severity of these deformities could be quantified numerically and the types of deformities taken into account, the pattern of response would undoubtedly become more readily apparent than simply using frequency data alone. Unfortunately methods for scaling the severity of deformities in these taxa are not yet available.

4.0 **DISCUSSION**

The effects of the introduction of nutrient-rich domestic wastes are evident in the responses of the chironomid community. Based on these results, the reach can be divided into 'zones of pollution' using the terminology of Whipple and Fair (1948) (Fig. 5). These terms are used in the descriptive sense only and do not connote zones of pollution in the sense of the original authors. The 'zone of degradation' immediately below the Hinton Combined Effluent is followed by 'zones of active

decomposition' and 'recovery' as domestic waste products are assimilated downstream. This process is probably rapid as these wastes are treated and well oxygenated before release into the river.

The effects of the introduction of contaminants are less easily interpreted. The 'zone of impact' immediately below the outfall (Fig. 3) is identified by a sharp oscillation marked by the rapid increase in the frequency of deformities in chironomid larvae followed by an almost equally rapid decline at Obed Mountain Coal Bridge (Site 3). From this point, the system generally stabilizes and the frequency of deformities begins to increase steadily downstream. Although deformities have been described for very few of the responding taxa and criteria to quantify the severity of these deformities are not yet available, observation clearly shows (Fig. 4) that severity increases steadily downstream with the most seriously deformed larvae appearing at the bottom of the reach at Windfall Bridge (Site 6) near Whitecourt.

The data support the hypothesis that the domestic sewage component of the combined effluent initially masks the effect of the contaminant component near the outfall, but because this process begins to break down more rapidly following normal bio-degradation processes, the effects of the contaminant component become increasingly apparent (Fig. 5). The differential in the apparent rates of breakdown of the two effluent components - one for which chironomid `communities are evolutionarily pre-adapted and the other for which they are not (Cairns and Niederlehner 1989) - become increasingly apparent as the unmasking process proceeds.

The bio-availability of contaminants to bottom-dwelling communities is largely governed by the sedimentary milieu into which they are deposited (Warwick 1980,1991; Burton 1992). Under normal circumstances, trophic state determines the ratio of organic and mineral components in sediments which, in turn, govern binding and remobilization processes. However, under abnormal circumstances where rates of accumulation of the mineral components become excessive, these processes can be modified or halted completely (Warwick 1980). Examples of masking by mineral sediments have been shown in the Southern Indian Lake study and by the release of untreated domestic sewage in the Laprairie basin of the St. Lawrence River (Warwick 1990).

This study provides evidence for the real-time interaction between the major processes of eutrophication and contamination. The active degradation of domestic wastes along the course of the Athabasca River - a natural process - has lead to the unmasking and exposure of the second process in the downstream reaches. Unfortunately, the compounding effects of the Alberta Newsprint Company and Millar Western Pulp Ltd. mills above and below Whitecourt, Alberta, respectively, probably will further complicate interpretation of these already complex interactions.

5.0 <u>CONCLUSIONS</u>

The frequency of deformities in chironomid larvae increases significantly at the point of contaminant introduction, but the severity of deformities is low which is hypothesized as due to the masking effect of nutrients inputs in the form of domestic sewage.

The rate at which the effects of domestic sewage are assimilated in normal downstream recovery are more rapid than the assimilation or degradation of contaminants.

As the masking effect of domestic wastes declines, the effects of contaminants becomes more apparent in the increasing severity of deformities in downstream chironomid communities.

The occurrence of the most seriously deformed larvae 170 km downstream from the point of introduction gives some indication of the longevity of pulp-mill contaminants in oligotrophic riverine systems.

6.0 **RECOMMENDATIONS**

 re-sample the Hinton-Whitecourt reach, and extend sampling well downstream of Whitecourt, to (a) address the apparent problems in the present data set apparent in reference parameters, sample size, site location, preservation and spatial range, and (b) examine the influence of additional pulp mill effluents on the river system which by just above Whitecourt has begun to recover from the effects of domestic waste pollution, if not contaminant pollution, from Hinton;

- enquire into the availability of pre-impact collections of chironomid larvae from this and other reaches of the Athabasca River and prepare them for comparison with the present data set;
- further examine the interaction between trophic processes and contamination processes further, particularly with regard to the masking or suppression effects of organic waste pollution;
- examine the interplay between sedimentation processes and contaminants further, particularly with regard to masking or suppression effects;
- 5) examine the chironomid fauna in the Hinton municipal sewage lagoons and outfall area to (a) determine the origins of the species occurring immediately downstream (ie Are these indigenous species stimulated by increased nutrient levels or have they been released into the riverine environment?), and (b) check the lagoon fauna for deformities to determine lagoon toxicity;
- 6) re-appraise the deformity analyses outlined here which are conservative estimates at best;
- 7) prepare a taxonomic manual, including an identification text and drawings of normal and deformed chironomid specimens, to assist future research and assessment of the Hinton-Whitecourt reach of the Athabasca River;

7

8) prepare a slide index to accompany the microscope slides prepared for the Hinton-Whitecourt reach of the world-class Athabasca River, and deposit these and the accompanying slides in a secure repository where they can be made available to future workers.

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Changes in the frequency of deformities in chironomid larvae following the introduction of mixed pulp-mill and domestic waste effluents. Figure 3:







Zones of pollution following the introduction of the domestic waste component and the resulting increased productivity. Figure 5:



Zones of interaction between the nutrient component and the contaminant component of the mixed pulp-mill, domestic-waste effluent. Figure 6:

APPENDIX A: TERMS OF REFERENCE

NORTHERN RIVER BASINS STUDY

SCHEDULE A - TERMS OF REFERENCE

Project 2329-C1/D1: Chironomid Deformities as an Indicator of Anthropogenic Stress in the Athabasca River - Reach Specific Study

I. Background

The incidence of deformities in chironomid mouth parts and antennae is generally higher is areas contaminated by wastewater effluents than at background control sites. Therefore, chironomid deformities may be an indicator of anthropogenic stress in aquatic ecosystems.

Most studies of chironomid deformities have been conducted in lakes or slow flowing rivers. In contrast, relatively little is known about the incidence of chironomid deformities in fast flowing impacted and unimpacted rivers.

In spring 1992, five Ekman dredges were taken from each of six depositional areas in the Hinton-Whitecourt area of the Athabasca River. These locations are (see attachments):

- upstream of Hinton, near Entrance
- Weldwood Bridge (i.e., downstream of Hinton Combined Effluent)
 - Obed Mountain Coal Bridge
 - Near Emerson Lakes
- Knight Bridge
- Windfall Bridge

Invertebrates in these samples have been sorted, enumerated, and identified. Chironomids have been identified to the sub-family level only; they have not been mounted and are currently in separate vials.

II. General Objectives of Pilot Study

- 1) Obtain baseline information on the occurrence and spatial distribution of chironomid deformities in depositional areas of the upper Athabasca River.
- 2) Using the baseline data, assess the potential for using chironomid deformities as an early indicator of anthropogenic stress in the upper Athabasca River.

III. Requirements

Chironomid samples will be supplied to the contractor by the Northern River Basins Study. The Component Coordinator should be notified at once if the number and/or taxonomic composition of chironomids in the samples (see attachments) appear to be insufficient or otherwise unsuitable to carry out the pilot project outlined below.

- Mount chironomids using procedures which will facilitate detailed morphological examination.
- Provide detailed identifications (species levels where possible) and counts for individual chironomid taxa.
- Examine specimens provided and record type and severity of deformities.
- Provide a description (photograph or drawing) of key-type deformities.
- Evaluate spatial trends in the incidence of chironomid deformities relative to known point sources in the study area. Compare the incidence of chironomid deformities with reports from other areas. Assess the potential value of using chironomid deformities in the reach as an indicator of anthropogenic stress in the upper Athabasca River.

IV. Reporting Requirements

- 1) The contractor is to prepare a microscopic assessment of the quality of the NRBS chironomid samples with a one page preliminary report to be provided by March 31, 1994.
- 2) Ten copies of the draft final report are to be submitted to the Component Coordinator by December 1, 1994.
- 3) Three weeks after the receipt of review comments the consultant is to submit ten cerlox bound copies and two unbound, camera-ready originals of the final report to the Component Coordinator. The report is to be printed in a 12 point Times Roman font. If photographs are to be included with the report they should be high contrast black and white. An electronic copy of the report, in Word Perfect 5.1 format, is to be submitted to the Component Coordinator at the same time as the final report. Data presented in the tables, figures, appendices, etc. in the final report are also to be submitted in electronic form (Quattro Pro spreadsheets preferred) to the Component Coordinator. The final report is to contain a table of contents, list of figures, list of tables, acknowledgements, executive summary and an appendix containing Terms of Reference for this contract. All sampling locations presented in the report and electronic spreadsheets are to be geo-referenced (latitude/longitude preferred).

IV. Project Administration

This project is being coordinated by the Contaminants, and Synthesis and Modelling Components of the Northern River Basins Study. Dr. Anne-Marie Anderson, Alberta Environmental Protection, Edmonton (phone: (403) 427-5893, fax (403) 422-9714) is the Scientific Authority on this project and matters of a technical nature should be referred to her. Greg Wagner (Office of the Science Director, Northern River Basins Study, 690 Standard Life Centre, 10405 Jasper Avenue, Edmonton, Alberta T5J 3N4 - phone: (403) 427-1742, fax (403) 422-3055) will act as the Component Coordinator for the project on behalf of the Northern River Basins Study. Matters relating to contract administration should be referred to him.

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