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**CHIRONOMID LARVAE (DIPTERA:CHIRONOMIDAE) AS
INDICATORS OF SEDIMENT TERATOGENICITY AND
GENOTOXICITY**

by

Lori Ann Hudson

A Thesis
submitted to the
Faculty of Graduate Studies and Research
through the Department of
Biological Sciences in Partial Fulfilment
of the Requirements for the Degree
of Master of Science at the
University of Windsor

Windsor, Ontario, Canada

1994



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ABSTRACT

Chironomids are potentially important indicators of the effects of sediment-bound contaminants. I evaluated mouthpart (mentum) deformities of larval chironomids collected from six locations varying in the amounts and types of organochlorine contaminants. Two thousand and six larvae belonging to 18 genera were collected. The overall incidence of deformities varied from 1.3% at an uncontaminated site (Anchor Bay, Michigan) to 11.4% at a more contaminated site (Peche Island at Windsor/Detroit). Five genera were widely distributed among sites (*Chironomus*, *Cryptochironomus*, *Polypedilum*, *Stictochironomus*, *Phaenopsectra*). The incidence of deformities varied significantly among genera (G-statistic Goodness of fit test, $G=43.68$, $p < 0.001$). *Cryptochironomus*, *Polypedilum*, and *Stictochironomus* showed uniformly low incidences of deformities among sites. The incidences of deformities in *Chironomus* and *Phaenopsectra* varied significantly among sites ($G=43.27$, $p < 0.001$ and $G=10.77$, $p < 0.05$ respectively), and reflected gross levels of sediment contamination.

In a laboratory study, I exposed *Chironomus salinarius* Kieffer larvae to mixtures of contaminated Trenton Channel (near Detroit, Michigan) sediment diluted with uncontaminated, synthetic sediment (sand, sculptor's clay, potting soil). Groups of 20 second instar *C. salinarius* were grown to 4th instar (10 d) in water-filled 1-L jars containing 300 mL of Trenton Channel sediment: synthetic sediment mixture in ratios of 1:0, 1:1, 1:3, 1:7, 1:15, or 0:1. Larval head capsules were examined for mentum deformities. Giant chromosomes, located in the salivary glands were examined for (i) incidence of puffs occurring in atypical locations, a reflection of stress protein production

and (ii) reduction in relative size of the nucleolus, reflecting inhibition of RNA synthesis activity. Incidence of chironomid deformities from control (0:1) sediments (± 1 SE) was $7.9 \pm 1.6\%$ (N=268): $3.9 \pm 1.5\%$ of 178 control larvae examined displayed nucleolus reduction. Incidence both of deformities and of nucleolus reduction increased approximately linearly with each doubling of contaminant concentration, from $16.8 \pm 2.1\%$ (N=313) at 1:15 to $29.0 \pm 4.0\%$ (N=261) at (1:0) for deformities, and from $5.4 \pm 1.8\%$ (N=149) to $24.2 \pm 3.5\%$ (N=149) for puff reductions. Puff reduction in a larva was unrelated to mentum condition (G-statistic test of independence, $G = 1.96$ $p > 0.05$), indicating that these are independent responses to contaminant stress. Chironomids are good indicators of sediment contamination and can be used to identify toxic stress before community effects become visible.

ACKNOWLEDGEMENTS

I wish to thank Dr. Jan J.H. Ciborowski for his tireless support, guidance and advice throughout my research. I would like to thank everyone who helped in my field collections including Todd Leadley, Randee Mayrand, Alene Schincariol, Kari Muir, Tania Edwards, Amanda Plante, Ray Poulin, Jeff Whyte, and Rebecca Boyko. I would like to thank Dr. Peter Adler (Clemson University), Dr. M. Petras and M. Vrzoc for their advice and assistance with chromosome preparations. Thanks also to Glen Bird (AECL, Pinawa) and Thomas Diggins (University of Buffalo) for chironomid cultures and advice on rearing procedures.

Thanks to the members of the lab who helped to preserve my sanity and to Ann Marie and Ron for making me smile. Special thanks to Becky and Ray who were always there for me, through the good times and the bad. Finally, I would like to thank my family for their constant support and encouragement, without them I could not have achieved my goals.

This research was funded by the Natural Sciences and Engineering Research Council of Canada and the Great Lakes University Research Fund through grants to Jan J.H. Ciborowski. I would also like to thank the Natural Sciences and Engineering Research of Canada for scholarship support throughout my research.

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CHAPTER I. GENERAL INTRODUCTION

The Great Lakes

The Great Lakes and their connecting channels support a rich and diverse community ranging from invertebrates such as mayflies and chironomids to many commercially important species of fish (Manny *et al.* 1988). This area is threatened by toxic inputs from the many industries along its shores. The objective of my research was to evaluate the effects of sediments contaminated with toxic organic substances such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), pesticides, heavy metals and other industrial byproducts on the morphological and genetic characteristics of the benthic organisms, chironomids. My research focused particularly on the area that lies between lakes Erie and Huron, which are connected by the St. Clair River, Lake St. Clair and the Detroit River.

The large volume of unpolluted water from Lake Huron that enters the St. Clair and Detroit rivers maintains river quality in a range acceptable for aquatic life (Manny *et al.* 1988). However, the sediments of the Detroit and St. Clair Rivers are contaminated with a variety of persistent toxic substances, including PCBs and heavy metals (Hamdy and Post 1985; Environment Canada and U.S. Environmental Protection Agency (EC & EPA) 1988). In this study, I define a contaminant as a substance foreign to a natural system or a substance present at unnatural concentrations (EC & EPA 1988). Many of these substances are only slightly soluble in water but have accumulated in the sediments

in concentrations that exceed Canadian guidelines for open water disposal of dredged sediments (Thornley and Hamdy 1984; Kauss and Hamdy 1985). Because these substances are loosely bound to the sediments by cation-exchange processes, they may be released if the sediments are disturbed (DePinto *et al.* 1987). Organisms that live in or feed upon these sediments are thus especially susceptible to contaminant uptake and its consequences.

The St. Clair River

The St. Clair River provides a corridor for fish between Lakes Huron and Erie. In addition to providing habitat for fish during sensitive life stages such as spawning and rearing, the St. Clair River forms a riverine delta at its mouth that serves as an important habitat for fish, waterfowl, reptiles, amphibians, fur-bearing mammals, and plant species (EC & EPA 1988). The St. Clair River is a major shipping channel, a recreational resource for boating, fishing and hunting, a food source for native Canadians, a source of drinking water, and it is the source of industrial process water for Canada's largest petro-chemical complex. Unfortunately, it also serves as a receptacle for treated municipal and industrial effluents (EC & EPA 1988).

Contaminant Inputs

The principal contributors of contaminants to the St. Clair River are industries such as The Cole Drain, Polysar, Dow Chemical and Ethyl Canada. Specifically, the St. Clair River sediments are contaminated with PCBs, oil, grease, mercury and many chemicals

that are characteristic of the petrochemical industry including hexachlorobenzene (HCB), octachlorostyrene (OCS), and perchloroethylene (PCE) (EC & EPA 1988). In addition to direct point discharges from industrial and municipal sources (Sarnia, Marine City, and Port Huron Waste Water Treatment Plants), non-point sources could be potential sources of contaminants. These include surface landfill sites, liquid waste disposal zones in deep geological strata ("deep wells"), urban runoff, and agricultural runoff (EC & EPA 1988).

The Detroit River

The Detroit River provides habitat for many aquatic organisms, including at least 82 species of phytoplankton, 31 species of aquatic macrophytes, 300 species of macrozoobenthos, 65 species of fish, and 27 species of waterfowl (Manny *et al.* 1988). Furthermore, the river is a major source of drinking water and a source of process or cooling water for more than 30 industries and power plants (Manny and Kenaga 1991).

Contaminant Inputs

The Detroit River receives contaminant inputs from a number of tributaries and wastewater discharges, which add a myriad of contaminants that accumulate in the sediments of the river. The principal tributaries on the United States side of the Detroit River include the Rouge and Ecorse rivers, the Marsh and Monguagon creeks and the Detroit Waste Water Treatment Plant. On the Canadian side, the principal tributaries are the Little River, Turkey Creek, Marrantette Drain and the Canard River (EC and EPA

1988) (Figure 1).

In addition to effluent from sewage treatment plants, power plants, steel mills, petroleum refineries, salt mines and manufacturers of chemicals, automobiles, and plastics are additional sources of contaminants. These sources are mainly on the U.S. shore. Before 1977, wastes from many industries were pumped directly into the Detroit River. However, since then such wastes have been diverted through municipal sewage treatment plants. As a result, The Detroit Waste Water Treatment Plant discharge in the Detroit River is now the principle source of 15 contaminants of concern including PCBs, hexachlorobenzene (HCB), cadmium, nickel, chromium, zinc, phenol, ammonia, phosphorus, oil and grease and cyanide (EC and EPA 1988). Permitted industrial discharges along the U.S. shore contribute additional oil and grease, ammonia, iron, phosphorous, phenols, cyanide, copper, chromium, cadmium, cobalt zinc, nickel, polycyclic aromatic hydrocarbons (PAHs), PCBs and HCB (Table 1). The 214 combined sewer overflows on the U.S. shore are the primary sources of lead and mercury (Table 1). The 26 combined sewer overflows from the City of Windsor discharge smaller amounts of contaminants (Table 1) (EC & EPA 1988).

Many of these chemicals are persistent (stable and/or hydrophobic). They become adsorbed to the sediment where they are not easily degraded and where they may remain for long periods of time. These chemicals, although persistent, are not necessarily acutely toxic: they may not cause the immediate death of an organism, but instead they may elicit teratogenic or mutagenic effects. A teratogen is an agent that causes developmental malformation (Lincoln *et al.* 1988). Teratogenic effects are a reflection of damage to the

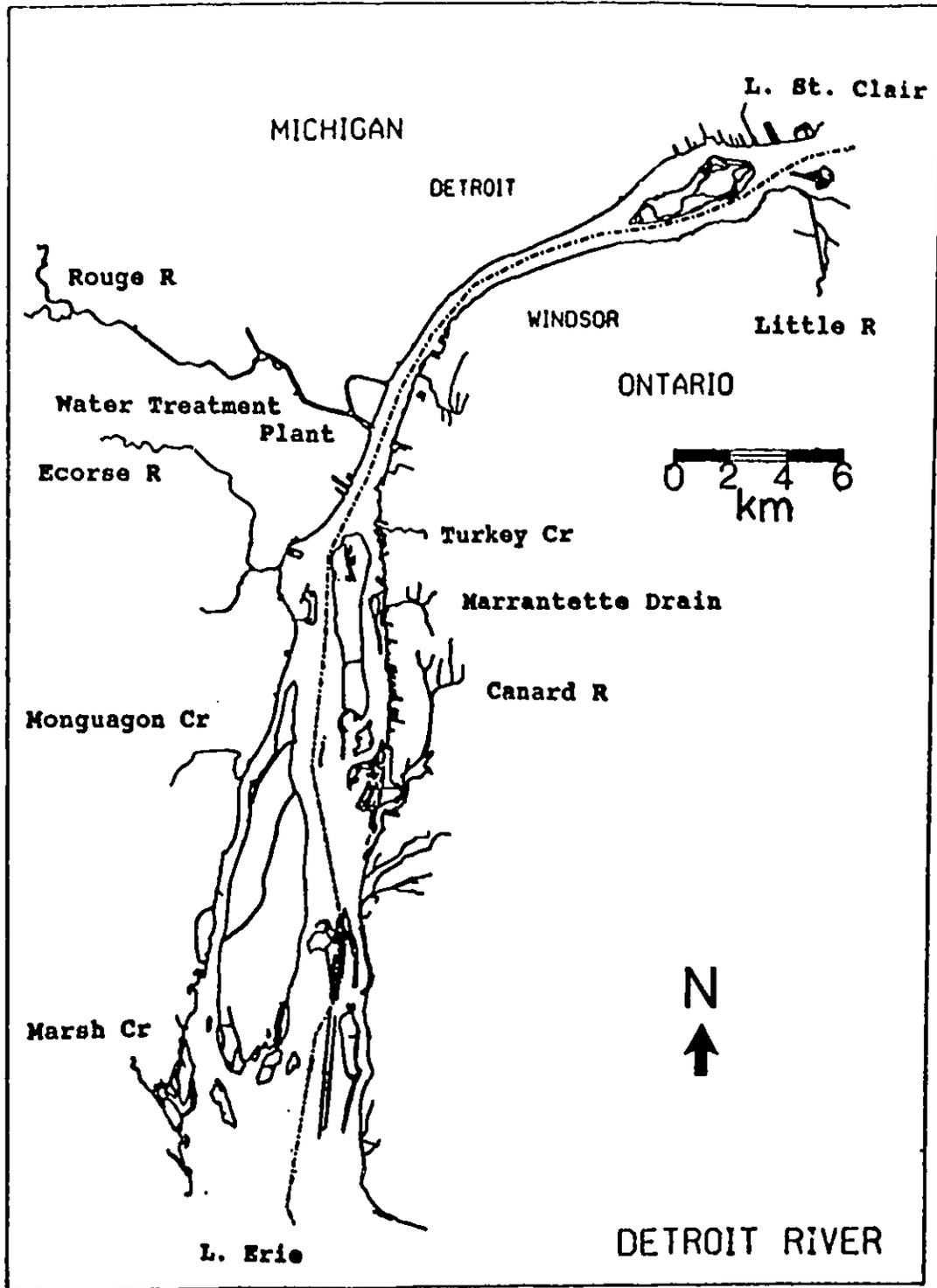


Figure 1. Contaminant inputs to the Detroit River (Source: Manny and Kenaga 1991).

Table 1. Estimated mean annual loadings of contaminants to the Detroit River 1979-1986 (EC & USEPA 1988).

Contaminant	Total measured annual loading (thousands) of kilograms)	Percentage of loading from each source					
		Point sources		Tributaries		Combined overflows	
		Michigan	Ontario	Michigan	Ontario	Detroit	Windsor
Chloride	623,207	21.6	63.9	12.3	1.2	0.2	0.8
Suspended solids	57,608	33.7		50.0	1.9	14.4	
Oil and grease	15,720	77.8	0.4			21.0	0.8
Ammonia	11,693	77.6	5.3	6.2	5.4	5.3	0.2
Iron	1,561	75.7	7.8	2.7	0.1	5.1	8.6
Phosphorus	916	49.4	9.1	16.6	11.3	12.7	1.0
Zinc	316	54.4	19.5	17.0	0.6	6.2	2.3
Nickel	74	49.6	8.4	10.0	24.3	6.6	1.0
Phenol	55	64.0	34.6			1.2	0.2
Lead	49	16.3	22.0	19.4	1.7	31.7	8.8
Cyanide	44	97.9	1.9				0.2
Copper	38	27.4	24.5	24.9	0.5	19.9	2.9
Chromium	16	71.3				28.7	
Cadmium	6	52.6	5.9	13.0	0.1	25.3	3.1
PAHs*	2	82.9	13.3				3.8
Mercury	2	2.4	0.1	1.1	0.1	96.2	0.1
PCBs*	0.20	37.8	5.6	22.1	0.1	33.9	0.6
Cobalt	0.02	99.0	0.5				0.5
Hexachlorobenzene	0.001	96.7				3.3	

* PAHs = polyaromatic hydrocarbons, and PCBs = polychlorinated biphenyls.

organism during the developmental process. In contrast, a mutagen is an agent that causes changes in the genetic structure of organisms that may become immediately evident (e.g. tumours) or that may appear in subsequent generations (Tamarin 1991). Therefore, mutagenic effects may be passed from one generation to the next. However, teratogenic effects appear in that one individual and are passed no further.

Effects of Contaminants

There is circumstantial evidence that many animal populations in the Detroit River have been affected by contaminants. Acute toxicity, which by definition is the mortality obtained by exposing organisms to lethal concentrations of substances within a short period of time (Kosalwat and Knight 1987), has been demonstrated for a broad variety of organisms at various trophic levels (Gilbertson 1988, Kosalwat and Knight 1987). Also important is chronic toxicity, or the effects of prolonged exposure to lower concentrations of chemicals on different plant and animal populations. The lower Detroit River is a major spawning ground for fishes that inhabit the river and western Lake Erie, and these fish deposit their eggs in contaminated sediments (Manny *et al.* 1988). Heavy metals such as chromium that are present in these sediments can kill eggs and larvae of several of the fish species (Eisler 1986).

Contaminated sediments may also negatively affect benthic macroinvertebrates in the river. Growth of *Chironomus tentans* Fabricius in contaminated sediments from the Trenton Channel was slower than in uncontaminated sediments collected elsewhere in the river (Giesy *et al.* 1987). Furthermore, the production of burrowing mayflies (*Hexagenia*

limbata Serville) between April and October 1986 was significantly lower in the Detroit River, where sediment concentrations of oil and metals exceeded established guidelines for disposal of polluted sediments, than in other areas of the upper connecting channels where contaminant concentrations did not exceed the guidelines (Edsall *et al.* 1991). In a laboratory sediment bioassay, Trenton Channel sediments required dilution by a factor of 1:3 with uncontaminated sediment in order to support growth of *Hexagenia* larvae to maturity (Ciborowski *et al.* 1992).

Contaminated sediments also negatively affect fishes in the river. Larval channel catfish (*Ictalurus punctatus* (Rafinesque)) fed significantly more slowly when exposed to contaminated sediments from the Trenton Channel than when exposed to uncontaminated sediments (White *et al.* 1987). Potentially, this could lead to reduced reproduction or a decrease in population size. Laboratory studies confirm the harmful effects of these contaminated sediments. Ali *et al.* (1993) have examined contaminant stress by measuring cyto- and genotoxicity of sediment extracts from the Trenton Channel using established cell cultures of brown bullhead (*Ictalurus nebulosus* (Lesueur)). Based on their DNA repair assay and a dye-uptake cytotoxicity assay, the Trenton Channel sediments were more cytotoxic and genotoxic than extracts from either Turkey Island or Walpole Island.

Another demonstrated effect of contaminants on Great Lakes biota is reproductive dysfunction, which has resulted in decreased population sizes of several bird species, including herring gulls (*Larus argentatus*), bald eagles (*Haliaeetus leucocephalus*) and double-crested cormorants (*Phalacrocorax auritus*) (Gilbertson 1988).

Examples of carcinogenicity include the observed formation of external tumours on fish. Suns *et al.* (1985) found neoplasms on 14.4 % of the bullheads and on 4.8 % of the walleye examined. They also found liver neoplasms in 15.4 % of the bowfin.

Another documented effect of contaminants on the biota is body deformities, and much of the research has dealt with the double-breasted cormorant (Fox *et al.* 1991). Between 1979 and 1987, 31,168 cormorant chicks were examined. Seventy chicks were found to have bill defects. Other anomalies, such as club feet, supernumerary digits, and eye and skeletal abnormalities were also observed (Fox *et al.* 1991).

The organisms for which dramatic developmental or mutagenic effects have been described are typically those at relatively high levels of the food chain. My goal was to evaluate contaminant effects on an organism at the base of the food chain by examining the effects of contaminants on larvae of the benthic organism *Chironomus*. The base of the food chain is important because it provides food for many other organisms, and the changes that occur at this level become magnified at higher trophic levels (e.g. fish and potentially humans). Substances that are harmful to chironomids could also be harmful to the organisms that feed on them.

Objectives

My research comprised two important aspects. First, I examined whether the incidence of morphological abnormalities was correlated with variation in contamination in the field. Secondly, I examined whether these abnormalities could be induced in a predictable manner by exposure to contaminants under controlled experimental

conditions. In Chapter II, I report spatial and taxonomic variation in mentum deformities in chironomids collected from sites varying in contaminant types and concentrations. In Chapter III, I report the results of a laboratory bioassay designed to examine teratogenic effects (mentum deformities) and genotoxic effects (differences in chromosomal structure) in chironomids exposed to a range of contaminant levels or concentrations.

Study Organism

The study organism was the larva of a midge (Diptera:Chironomidae). The family Chironomidae is ubiquitous and usually the most abundant insect group in all types of freshwater environments. Chironomids can attain population densities of $10^5/m^2$ (Oliver and Roussel 1983). These organisms have a cosmopolitan distribution, and are the dominant family in the nearctic region. Furthermore, they inhabit a wide range of environments, with some species of this family being terrestrial or semi-terrestrial and others marine (Pinder 1983). There is no reliable estimate of the total number of species in the family. Over 5000 species have been described even though species in large areas such as Asia have not been studied (Oliver 1971). I worked with the genus *Chironomus*, species of which are detritivores that live in soft, often highly organic sediments (Oliver 1971) and are therefore directly exposed to contaminants through their diet.

Chironomus is a holometabolous insect. Thus, it has 4 life stages; egg, larva, pupa and adult. The larvae are red in colour due to the presence of haemoglobin. The possession of haemoglobin is correlated with the ability of *Chironomus* and related larvae to function in environments with low oxygen concentrations (Oliver 1971).

Chironomus, like most other Chironominae, builds a larval case on or within the substrate in which they live. This case consists of particles from the substrate, lined and held together with proteinaceous silk-like threads secreted by the salivary glands (Oliver 1971). Rapid larval settling, and construction of a tube in the sediment are critical for maximum growth because larvae of *Chironomus* grow faster when associated with the sediment (Townsend *et al.* 1981). These salivary glands must therefore carry out rapid protein synthesis for use in tube construction.

Salivary glands have polyploid nuclei, in which the DNA exceeds the normal diploid quantity. The polytene chromosomes are a specific case of polyploidy and are formed by repeated replication of the original chromatids without separation of the resulting strands (Michailova 1989). Therefore, growth in such tissues is due to cell enlargement rather than cell number. Polytene chromosomes can be isolated from different larval tissues including the epithelium of the midgut, hindgut, malpighian tubules and the salivary glands. The salivary glands have provided the best material for chromosomal preparations (Michailova 1989).

Each larva passes through four larval instars. During each successive stage the larva becomes larger and becomes more intensely red. During the larval stage, the chironomid head capsule is extremely complex, providing numerous sclerotized structures that can be examined for morphological deformities (Warwick 1988) (Figure 2).

The pupa is black, and the most obvious changes during the brief pupal stage are respiratory and locomotory in nature. The onset of pupation is evident by the development of an enlarged thorax, wing buds, leg buds and compound eyes (Townsend

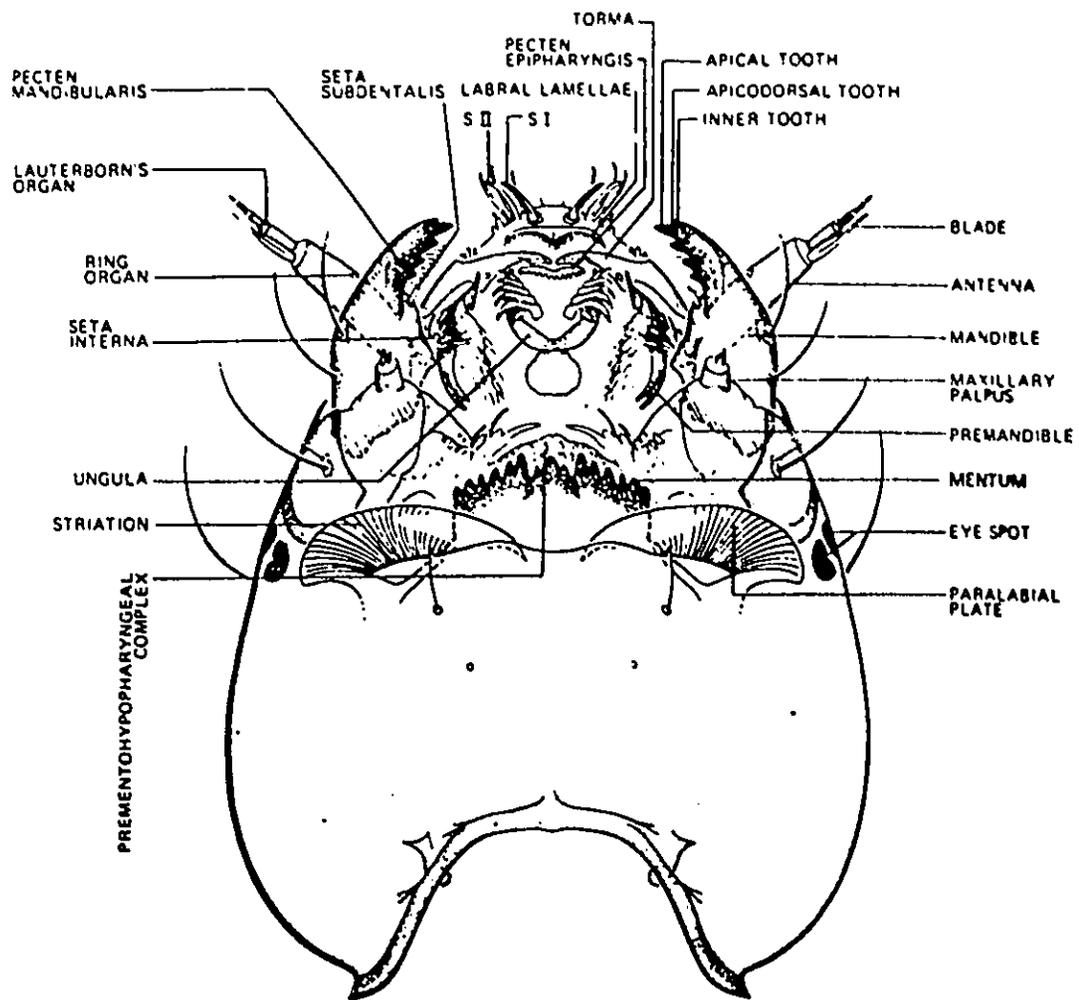


Figure 2. *Chironomus* head capsule, ventral view. (Source: Oliver and Roussel 1983).

et al. 1981).

In the adult stage, sexual dimorphism is striking. The male can be easily distinguished from the female by its large, plumose antennae and by the presence of paired genital claspers on the posterior tip of the abdomen (Townsend *et al.* 1981). The adult stage lasts, at the most, several weeks during which time the reproductive aspects of the life cycle are carried out (Oliver 1971). Mating occurs and within 24-48 hours oviposition takes place. The female coats the eggs with a gelatinous substance produced by the accessory gland. The matrix holds the individual eggs in an egg mass, which the female drops into the water. In *Chironomus*, this egg mass has a characteristic spiral shape (Townsend *et al.* 1981). Adults are attracted to ultraviolet light, making collection of gravid females, necessary for laboratory cultures, relatively easy.

There are several reasons why this organism is especially suitable for studying the effects of genotoxins and teratogens. These can be divided into environmental and practical reasons.

Environmental Aspects

The environmental reasons for using *Chironomus* are quite extensive. These organisms are benthic and are therefore of particular interest because sediments are often reservoirs for pollutants (Pesch *et al.* 1981).

Because chironomids are important primary consumers, providing food for fish and other aquatic predators, they may contribute to the biomagnification of toxicants. Furthermore, *Chironomus* has a relatively short life cycle in comparison to other

organisms that may serve as biological indicators such as fish or birds. Unlike fish or birds, which may only develop observable signs of physical malformation after several years, the morphological responses of chironomid larvae are probably a reasonably accurate reflection of the conditions present in the sediments at the time of sampling (Warwick 1988). Additionally, chironomids in water are exposed to contaminants during the longest and most critical stage of their life cycle, the larval stage. All of the energy required to complete the life cycle is built up in the larval stage because the adults, with few exceptions, do not feed (Oliver 1971). Therefore, it is during this stage that energy storage in the form of body tissues takes place. Because many hydrophobic persistent substances become incorporated into the sediments, bound to detrital particles, chironomids are directly exposed to these substances through their diet (Warwick 1988). Finally, chironomids are relatively sessile and therefore they reflect the conditions in the sediment where they were collected (Oliver 1971).

Practical Aspects

There are also practical reasons for using chironomids as biological indicators. Chironomids have been recognized as standard organisms for sediment bioassays to evaluate acute and chronic toxicity by many Canadian and U.S. government agencies (Bedard *et al.* 1992, Day *et al.* 1994). The development of endpoints that indicate physiological, teratogenic and genotoxic effects would dramatically contribute to the diagnostic value of these bioassays. The complexity of morphological features associated with the sclerotized head provides many opportunities to evaluate morphological

deformities (Hamilton and Saether 1971, Warwick *et al.* 1987, Warwick and Tisdale 1988, Dickman *et al.* 1990, Dermott 1991, Warwick 1985, 1988, 1991). *Chironomus* has a small karyotype (n=4) (Hirvenoja and Michailova 1991). This makes it especially easy to evaluate aspects of individual chromosomes. Furthermore, the large polytene chromosomes of the salivary glands permit direct examination for chromosome aberrations, chromosome inversions, puffing, or chromosomal breaks, which might indicate that the chironomid is under stress. Finally, chironomids are easily cultured (Maier *et al.* 1990) to provide a continuous supply of experimental material, which is essential for laboratory studies. Even under laboratory conditions these organisms can be reared to emergence and will successfully mate (Townsend *et al.* 1981).

Ecological Significance of Chironomids

In general, chironomids are important members of aquatic ecosystems. They have been shown to accumulate PAHs (Dickman *et al.* 1992). This presents a potential problem to organisms that utilize chironomids as a food source. Chironomid larvae are important prey items for many types of fish, contributing 20% by weight to the total diet of brown (*Salmo trutta*) and rainbow trout (*Salmo gairdneri*) (Brown *et al.* 1980). Furthermore, emergent chironomids can account for up to 60% of the diet of many avian species at certain times of the year (Titmus and Badcock 1980). Contamination of this food source could have two different implications. If contaminant levels cause delayed and depressed emergence then this could result in a substantial reduction in food quantity and/or quality for aquatic and terrestrial wildlife. Secondly, there is the possibility that

the contaminants found in chironomids may accumulate in higher organisms, those feeding at successively higher trophic levels, resulting in biomagnification. This is true for both aquatic and terrestrial predators. Adults developing from chironomid larvae exposed to high copper concentrations have higher concentrations of copper (Kosalwat and Knight 1987). Similarly PCB compounds may be concentrated and transferred from the aquatic to the terrestrial environment. Therefore, terrestrial predators feeding on emerging aquatic insects, whose larval stage is in contaminated sediment, are exposed to organochlorine residues (Larsson 1984).

Study Area

This research contributed to aspects of a larger, cooperative study of the toxic effects of sediments from locations in the St. Clair and Detroit rivers (Haffner *et al.* 1993, Ali *et al.* 1993). Five study sites were designated for common study. Each location contained substrate consisting of soft sediment, which is the preferred habitat of *Chironomus* (Pinder 1986). These sites were in the vicinity of Walpole Island, Anchor Bay, Peche Island, Turkey Island and the Trenton Channel (Figure 3). I also designated an external reference site, a pond in Ojibway Park near Windsor, Ontario (Figure 3). These sites allowed me to examine chironomids dwelling in sediments differing in contaminant composition and levels. The relative amounts of contaminants and their overall expected toxicity is summarized in Table 2. A more detailed account of the contaminants in these sediments is presented in Chapter II.

Table 2. Relative amounts of contaminants at the study sites and the overall expected toxicity to the biological community. (Based on contaminant levels, Chapter II)

Site	Petrochemicals	PCBs	PAHs	Overall Expected Toxicity
Anchor Bay	low	low	low	low
Ojibway Pond	low	low	low	low
Walpole Island	moderate	low	moderate	moderate
Peché Island	moderate	moderate	moderate	moderate
Turkey Island	low	low	moderate	moderate
Trenton Channel	high	high	high	high

Study of Morphological Deformities

The first aspect of the investigation deals with the study of deformities as indicators of environmental conditions. Most studies on the abnormalities of freshwater invertebrates have examined the incidence of deformities in Chironomidae (Warwick 1987). Extensive field investigations have documented common and diverse deformities in various structures (Warwick 1985, 1988, 1989, 1990a, 1990b, 1991, Warwick *et al.* 1987, Warwick and Tisdale 1988, Dermott 1991, Lenat 1993, Dickman *et al.* 1992, Van Urk *et al.* 1992, Diggins and Stewart 1993). However, laboratory evidence of a cause-effect relationship with pollutants is lacking. Documenting contaminant effects based on morphological deformities is based mainly on the response of individual larvae. Observing changes at the level of the individual organism may be more useful than changes at the community level because individual responses are likely to be detectable before community responses and therefore may provide an earlier warning of contaminant effects on the biota (Warwick 1985).

Warwick (1988) defined a deformity as "any morphological feature that departs from the normal configuration". This definition is necessarily broad and all-inclusive because the boundaries between normal and abnormal variation have not been established for most morphological structures of chironomids.

The occurrence of deformed chironomids both within and outside of the Great Lakes region has been reported by many researchers (Table 3). Lenat (1993) provided several estimates of background levels of mentum deformities in chironomids grown in uncontaminated sediment (Table 3). The incidence of deformities in chironomids from

differentially contaminated sediments varies widely (Table 3). Some of the deformities that have been identified in specimens of *Chironomus* include a thickened exoskeleton (Hamilton and Saether 1971), a heavily pigmented head capsule (Hamilton and Saether 1971), antennal deformities (Table 3), and various mouthpart deformities (Table 3). I examined the mandible, premandibles and the mentum. Preliminary studies suggested that it would be best to concentrate on the mentum because it is easily observed and it showed elevated incidences of deformities in the presence of elevated contaminant levels. Despite the broadly scattered literature on chironomid deformities, there has been no systematic assessment of how susceptibility to deformities varies across genera.

Study of Genotoxic Effects

Teratogenic effects are only one aspect that can be addressed to examine the influence of contaminated sediments on this organism. It is also possible to look at genotoxic effects or more specifically, mutagenesis i.e. damage to chromosome structure. Several approaches have been used to detect chromosome damage in animals. These include looking at chromosomal breaks (human lymphocytes (Nevstad 1979), Chinese hamster ovary cells (Sudharsan and Heddle 1980)), inversions (chironomids (Michailova and Petrova 1991, Hirvenoja and Michailova 1991, Prevosti 1974, Martin and Wulker 1971, Martin 1962)) and induction or reduction in puffing (Jang 1992, Aziz *et al.* 1991, Bentivegna and Cooper 1993). Additionally, Pesch *et al.* (1981) suggested the *in vivo* application of sister chromatid exchange (SCE analysis) to assess genotoxicity in marine worms. This technique proved relatively unsuccessful in chironomid larvae in earlier

Table 3. Reported incidences of deformities in larval chironomids.

Structure Examined	Incidence of Deformity (%)	Location	Contaminants	Reference
Mouthparts (Mentum)	83 (n=40)	Inner Port Hope Harbour, L. Ontario	Chain radio-nucleotides & trace metals	Warwick et al. 1987
Mouthparts (Mandibles ligula, mentum)	12-30 (n=1500)	St. Clair R. Downstream of Sarnia Industrial Waterfront	Agricultural pesticides, sewage drains petrochemicals	Dermott 1991
Antennae	8 (n=1176)	Tobin Lake, Saskatchewan	5000 chemical compounds make chemical soup (agricultural & industrial)	Warwick 1985
Antennae & Mouthparts (Mentum, mandibles premandibles)	3-50 (n=108)	Lac St. Louis and Laprarie Basins (St. Lawrence R)	PCBs, mirex, heavy metals, HCB PAHs, DDE, industrial wastes	Warwick 1990
Mouthparts (Mentum)	14 (n=14)	Kane Dock (Niagara R.)	coal tar products: benzo (a) pyrene & 15 PAHs	Dickman et al. 1992
Mouthparts (Mentum)	3 (n=739)	King's Bridge (Niagara R.)	reference site	Dickman et al. 1992
Mouthparts (Mentum)	19 (n=150)	Beaver Creek, Ontario	Agricultural chemicals Atrazine Prometron	Dickman et al. 1990
Mouthparts (Mentum)	10-50	River Ijssel Netherlands	PCBs, HCB, Cd, Hg, Pb, Cu	Van Urk et al. 1992
Antennae Mouthparts (Mandibles, premandibles, mentum epipharyngeal pecten)	41 (n=201)	Tobin Lake reservoir on Saskatchewan River	Agricultural & Industrial residues from 270,000 km ² of Canadian prairie provinces	Warwick and Tisdale 1988
Mouthparts (Mentum)	3.6 (n=84) 5.4 (n=296) 5.6 (n=125)	North Carolina Streams	reference sites	Lenat 1993

laboratory studies (Whyte and Ciborowski pers. comm.).

The induction of new puffs or reduction of puffs originally present may be important in identifying organisms that are, in some way, under environmental stress. Chromosome puffs are swellings on chromosomes associated with the local uncoiling of chromatin fibres and rapid RNA synthesis (Pelling 1959). It is possible that organisms exposed to increased contaminant levels may increase RNA synthesis that could lead to the production of cellular stress proteins. Alternatively, characteristic puffs may decrease in size due to inhibition of RNA synthesis caused by some environmental stressor.

The important advantage of studying chironomids is that one can assess morphological deformities and chromosomal differences in the same individual because the chromosomal structure of chironomids can be determined by examining the polytene chromosomes. Therefore, it is possible to examine the relationship between morphological deformities (teratogenesis) and chromosomal changes.

CHAPTER II. SPATIAL AND TAXONOMIC VARIATION IN INCIDENCE OF MOUTHPART DEFORMITIES IN CHIRONOMID LARVAE

Introduction

The Great Lakes and their connecting channels support a rich and diverse community ranging from invertebrates to commercially important fish species (Manny *et al.* 1988). The area is threatened by potentially toxic inputs from the many industries along its shores. This is especially true of the heavily industrialized area of the St. Clair and Detroit rivers, which connect lakes Huron and Erie (Figure 3). The sediments of these rivers are contaminated with a variety of persistent, hydrophobic substances, including PCBs and heavy metals (Hamdy and Post 1985; Environment Canada and Environmental Protection Agency (EC & EPA) 1988). Although these chemicals are not necessarily acutely toxic to all organisms, they may elicit teratogenic effects that result in developmental malformation (Lincoln *et al.* 1988).

There is circumstantial evidence that some animal populations in the Great Lakes have been affected by contaminants. Documented effects of contaminants include reduced growth rates of midge (chironomid) larvae (Giesy *et al.* 1987), reduced feeding in fishes (White *et al.* 1987) and reproductive dysfunction in birds, including herring gulls (*Larus argentatus*), bald eagles (*Haliaeetus leucocephalus*) and double-crested cormorants (*Phalacrocorax auritus*) (Gilbertson 1988).

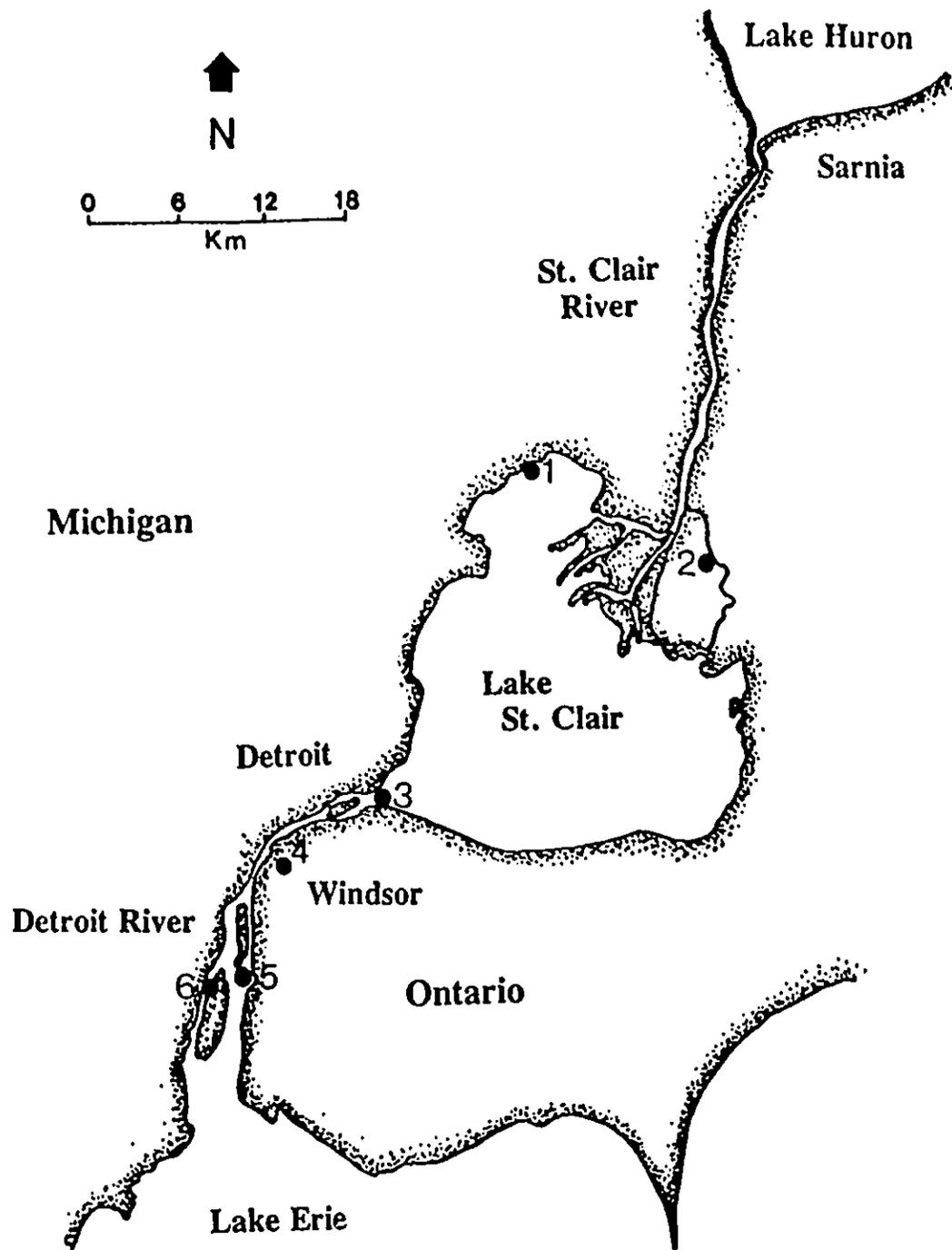


Figure 3. Location of the study sites in the Huron-Erie corridor. 1-Anchor Bay, 2-Walpole Island, 3-Peche Island, 4-Ojibway Pond, 5-Turkey Island, 6-Trenton Channel.

Organisms for which dramatic developmental abnormalities have been described are typically those at relatively high positions of the food chain (birds and fishes). The purpose of this chapter was to evaluate potential contaminant effects on the larvae of benthic chironomids, which are at the base of the food chain.

Chironomid larvae have excellent potential as biomonitor organisms as they live in intimate contact with contaminated sediments, feeding on detritus and algae associated with the sediments (Larsson 1984, Reynolds 1987). These sediments are often reservoirs for pollutants (Pesch *et al.* 1981). Chironomid larvae constitute a major component of the benthic invertebrate biomass associated with freshwater sediments and are often a significant portion of the diet of predatory invertebrates and fishes (Hart and Fuller 1974). Contaminants accumulated in invertebrate tissues are thus transmitted to foraging fish and waterfowl and ultimately, to humans. Therefore, chironomid larvae play a primary role in the bioaccumulation and transport of contaminants. Since chironomids are relatively sessile, they reflect the conditions in the sediment where they were collected (Oliver 1971) to a better extent than vertebrates.

Morphological abnormalities in chironomids have been examined by several researchers who have related them to contaminant gradients. The most conspicuous features of deformed larvae are thickened endoskeletons and head capsules (Hamilton and Saether, 1971) and various antennal (Warwick 1985, 1988, 1989, 1990a, 1990b, 1991, Dermott 1991) and mouthpart deformities (Dickman *et al.* 1992, Dickman *et al.* 1992, Van Urk *et al.* 1992, Diggins and Stewart 1993, Lenat 1993).

My study had two objectives. Firstly, I wanted to document spatial variability in

incidence of chironomid deformities in the St. Clair and Detroit rivers and correlate them with classes or levels of potentially teratogenic substances. Secondly, I evaluated the variability in incidence of deformities among taxa to designate genera especially suitable for indicating sediment teratogenicity.

Study Areas

Larval chironomids were collected from depositional (fine) sediments at six study sites in the vicinity of Anchor Bay, Ojibway Pond (Windsor), Walpole Island, Peche Island, Turkey Island and Trenton Channel (Figure 3). These sites were chosen in order to provide a gradient of contaminant types and concentrations.

Anchor Bay was selected as an internal reference site. This site has soft, muddy sediments similar to the other sites (Griffiths 1987). However, the sediments are relatively uncontaminated (Pugsley *et al.* 1985) because water from the St. Clair River flushes directly from Walpole Island to Peche Island (Leach 1980). Concentrations of PCBs and octachlorostyrene (OCS) in the Anchor Bay area are among the lowest in the Huron-Erie corridor. Pugsley *et al.* (1985) reported total PCBs below the detection limit of 2.3 $\mu\text{g}/\text{kg}$ and octachlorostyrene levels $< 1 \mu\text{g}/\text{kg}$.

An additional reference site (a 0.5 ha partly wooded, roadside pond at Ojibway Park near Windsor, Ontario) was included in the study in order to provide background levels of deformities and document natural variability of mentum structure.

The Walpole Island site is located in the Chenal Ecarte of the St. Clair River approximately 50 km downstream of the Sarnia industrial waterfront (Figure 3).

Sediments and emergent aquatic insects are moderately contaminated primarily with petrochemical byproducts including hexachlorobenzene (HCB), octachlorostyrene (OCS), and pentachlorobenzene (QCB) (Kovats and Ciborowski 1989 and Table 4).

The Peche Island site was at the head of the Detroit River (Figure 3). In the mid-1980s concentrations of sediment-bound petrochemicals such as HCB, were less in this area than at the St. Clair River mouth. This suggests that some of these contaminants are retained, degraded, or volatilized in Lake St. Clair and also that some are carried through to the Detroit River (Environment Canada & U.S. Environmental Protection Agency [EC & USEPA], 1988). More recent analysis of sediments from this area suggests that this is no longer the case (Table 4). Peche Island sediments had relatively higher concentrations of several contaminants including PCBs and petrochemicals (Table 4) than the Walpole Island site located upstream of Lake St. Clair.

The final two sites, Trenton Channel and Turkey Island, were located downstream of the other sites in the Detroit River. The sites were chosen to allow me to look at local differences in the Detroit River sediments. Thornley and Hamdy (1984), Kauss and Hamdy (1985) and IJC (1987) had previously reported considerably higher sediment PCB concentrations on the U.S. side of the Detroit River (Trenton Channel site) than on the Canadian side (Turkey Island). In 1983, the highest levels of PCBs in the Detroit River were at the mouth of the Trenton Channel. Once again, recent data support this (Table 4).

I expected that Anchor Bay and Ojibway Park, the two reference sites, would yield larvae with the lowest incidences of deformities. Walpole Island and Turkey Island were

Table 4. Contaminant concentrations (mg/kg) in sediments from Walpole Island, Peche Island, Turkey Island and Trenton Channel.

Compound	*Walpole Island (mg/kg)	**Peche Island (mg/kg)	*Turkey Island (mg/kg)	*Trenton Channel (mg/kg)
OCCs				
	WI1		TI1	TR1
QCB	0.03	0.12	0.02	0.30
HCB	0.24	0.41	0.05	1.72
OCS	0.13	0.21	0.01	0.37
pp'-DDE	0.02	1.00	0.02	1.02
Aroclor Mix	0.23	0.94	0.27	12.53
PAHs				
	WI2		TI2	
Napthalene	ND	NA	0.70	ND
Acenapthylene	ND	NA	ND	1.70
Acenapthene	ND	NA	ND	ND
Flourine	0.36	NA	ND	3.65
Phenanthrene	2.58	NA	2.40	35.25
Anthracene	ND	NA	ND	11.00
Flouranthene	2.75	NA	4.15	84.35
Pyrene	3.50	NA	4.25	77.85
Benzo (a) Anthracene	3.25	NA	3.70	52.85
Chrysene/Triphenylene	2.65	NA	3.80	56.00
Benzo (a) flouranthrene	3.30	NA	6.45	67.80
Benzo (k) flouranthrene	1.00	NA	0.35	27.60
Benzo (a) Pyrene	1.95	NA	3.90	29.40
Indeno (1,2,3,-cd) Pyrene	ND	NA	ND	25.55
Dibenzo (a,h,) Anthracene	ND	NA	ND	13.40
Dibenzo (g,h,i) Perylene	ND	NA	ND	34.80

PCBs presented as 1:1 mixture of 1254/1260. ND, not detected.
NA, not available.

*Source: Ali et al. 1993.

** Source: Haffner et al. 1993.

expected to support organisms displaying elevated but similar incidences of deformities, as these sites had sediments with similar contaminant types and concentrations (Table 4). Incidences of deformities in organisms from the Peche Island site were expected to be higher than the aforementioned sites. Finally, based on the detected contaminant levels, I anticipated that Trenton Channel organisms would display the greatest incidence of deformities.

Materials and Methods

Field Collection and Sorting

Between May 1991 and May 1993, the six study sites were sampled using either a Ponar grab sampler or a D-frame kick net, depending upon the water depth at the site being sampled. Samples were taken at points that ranged from 1 to 20 m from shore and at depths of 1 to 15 m. Upon collection, sediments were transferred to and rinsed through a sieve bucket (mesh size 0.600 mm). At this time, red chironomids were readily visible. They were removed from the sieve bucket with forceps, blotted on paper towelling to remove excess water and preserved in vials of chilled Carnoy's fluid (3:1 v/v absolute ethanol:glacial acetic acid). Each site was visited 3 times, and an estimated 250 sediment samples were collected during each of these visits.

Mounting and Evaluation

In the laboratory, larvae were individually slide-mounted for taxonomic identification and morphological examination. Only third and fourth-instar larvae were mounted

because smaller individuals cannot reliably be identified to the generic level (Oliver and Roussel 1983).

The head capsule of each larva was removed and placed ventral side up on a microscope slide in a drop of CMC-9AF[®] aqueous mounting medium (Master's Chemical Company, Des Plaines, Illinois). The corresponding body of each individual was stored in a labelled shell vial containing Carnoy's fluid for later analysis of polytene chromosome structure (Hudson and Ciborowski, unpublished). A cover slip was placed on the slide and gentle pressure was applied to the slip to separate the mouthparts and properly orient the head capsule. The cover slip was then ringed with clear nail polish and allowed to dry and clear for at least 7 d. Chironomids were identified to the generic level using the keys of Oliver and Roussel (1983), Wiederholm (1983) and Coffman and Ferrington (1984).

Each head capsule was examined for the presence of mentum deformities. A mentum was classified as either "normal" or "deformed" based on the criteria of Dickman *et al.* (1992). A mentum that exhibited blunt or chipped teeth was judged to be "worn" (normal) but not deformed. Similarly, a mentum tooth with jagged edges was considered to be "broken" and also was classified as normal. Only misshapen teeth on the mentum that had smooth edges were considered to be "deformed". Other characteristics of the mentum that resulted in a classification of "deformed" included fused teeth, crossed teeth, extra teeth, missing teeth or teeth of aberrant shape or size. Asymmetry of the mentum was also an indication of deformity. Representative head capsules from the Detroit River, indicating deformed mouthparts are shown in Figure 4. Upon completion



(a)



(b)



(c)



(d)



(e)



(f)

Figure 4. *Chironomus* head capsules with various deformities. (a) and (b) normal mentum structure, 3 median teeth with 6 lateral teeth on either side. (c) deformed, gap-5 right lateral teeth and 4 median teeth (d) deformed, 5 left lateral teeth, 5 median teeth (e) deformed, 7 left lateral teeth, 8 right lateral teeth, 4 median teeth (f) deformed, 1 left lateral tooth, 5 right lateral teeth. (400 X magnification).

of examination for deformities in all chironomids, each "deformed" specimen was re-examined to confirm that it had been categorized correctly.

Statistical Methods

The incidence of deformed larvae from the different study sites was expressed as the proportion \pm 1 SE of larvae at each site that displayed deformities. Standard errors were calculated according to the binomial theorem *i.e.*, $SE = \sqrt{pq}^k$ where p = deformed specimens, q = undeformed specimens, and k = sample size (Sokal and Rohlf 1981). I used the G-statistic goodness of fit test (Sokal and Rohlf 1981) to test for differences in the incidence of deformities among sites (H_0 = Incidence of deformities is equal at all sites).

Results

I identified and assayed 2,006 red chironomids, belonging to 18 genera (all Chironomini) collected from the 6 designated study sites (Table 5). Representatives of only six of these genera (*Chironomus*, *Crytochironomus*, *Microtendipes*, *Polypedilum*, *Stictochironomus*, and *Phaenopsectra*) were collected in large enough numbers at one or more sites to be considered 'common' (40 or more individuals), and hence potentially suitable for biomonitoring purposes. Because all of these genera are largely detrital feeders, (Coffman and Ferrington 1984) they are all exposed to hydrophobic contaminants in the sediment through their diet in addition to direct exposure through contact with overlying and pore water.

Table 5. Number of organisms of each genus collected at the different study sites.

Genus	Sites					
	Anchor Bay	Ojibway Pond	Walpole Island	Pече Island	Turkey Island	Trenton Channel
<i>Chironomus</i>	49	283	26	305	79	0
<i>Phaenopsectra</i>	118	0	11	24	133	45
<i>Stictochironomus</i>	174	0	126	3	17	0
<i>Polypedilum</i>	1	0	230	35	19	0
<i>Cryptochironomus</i>	29	0	43	63	68	2
<i>Microtendipes</i>	0	0	1	40	0	0
<i>Endochironomus</i>	0	20	0	0	0	0
<i>Harnischia</i>	0	0	14	0	0	0
<i>Demicryptochironomus</i>	8	0	3	0	0	0
<i>Einfeldia</i>	3	4	0	1	2	0
<i>Dicrotendipes</i>	0	0	0	1	9	0
<i>Pseudochironomus</i>	8	0	0	0	0	0
<i>Glyptotendipes</i>	0	4	0	0	1	0
<i>Cladopelma</i>	0	0	0	2	0	0
<i>Nilodorum</i>	0	0	2	0	0	0
<i>Paratendipes</i>	0	0	1	0	1	0
<i>Nilothauma</i>	1	0	0	0	0	0
<i>Parachironomus</i>	0	0	0	1	0	0
Total	391	317	457	475	329	47

Microtendipes was common at only a single site. The remaining common genera occurred at four or more locations. Only two genera (*Cryptochironomus* and *Phaenopsectra*) were found at the heavily contaminated Trenton Channel site. Few invertebrates of any description except for oligochaetes were collected from this location.

There was significant heterogeneity in the overall incidence of mentum deformities among the 5 widespread, common genera (G-statistic Goodness of fit test, $G=43.68$, $p<0.001$). *Cryptochironomus*, *Polypedilum* and *Stictochironomus* exhibited a low overall incidence of deformities, ranging from 0 to 2.5 percent (Table 6). The other two abundant genera, *Chironomus* and *Phaenopsectra* appeared to be more prone to deformities, with overall incidences of 9.3 and 5.7 percent, respectively. There was significant among-site variation in the incidence of mentum deformities for the two genera displaying the greatest overall frequency of deformities (G-statistic Goodness of fit test, $G=43.27$, $p<0.001$; and $G=10.77$, $p<0.05$ for *Chironomus* and *Phaenopsectra*, respectively). For *Chironomus*, deformities were most common in populations from Walpole and Peche Island sites (Figure 5) and least common in the two reference site populations. No specimens were collected from Trenton Channel. The Walpole Island *Phaenopsectra* population also displayed maximum deformity incidences (Figure 6). No deformities were detected in individuals from Peche Island, but relatively few individuals were collected from this site. *Phaenopsectra* was absent from the Ojibway Pond reference site.

Table 6. Incidences of Deformities (percent \pm 1 SE) of the most abundant genera at all of the study sites combined.

Least Susceptible to Deformities:

Genus	Incidence	N
<i>Cryptochironomus</i>	0.0 \pm 0.0	205
<i>Polypedilum</i>	1.4 \pm 0.7	285
<i>Stictochironomus</i>	2.5 \pm 0.9	320

Most Susceptible to Deformities:

Genus	Incidence	N
<i>Phaenopsectra</i>	5.7 \pm 1.3	331
<i>Chironomus</i>	9.3 \pm 1.1	742

Overall incidence of deformities was genus specific (G-statistic Goodness of fit test, $G=43.678$, $p < 0.001$).
 N = Sample size.

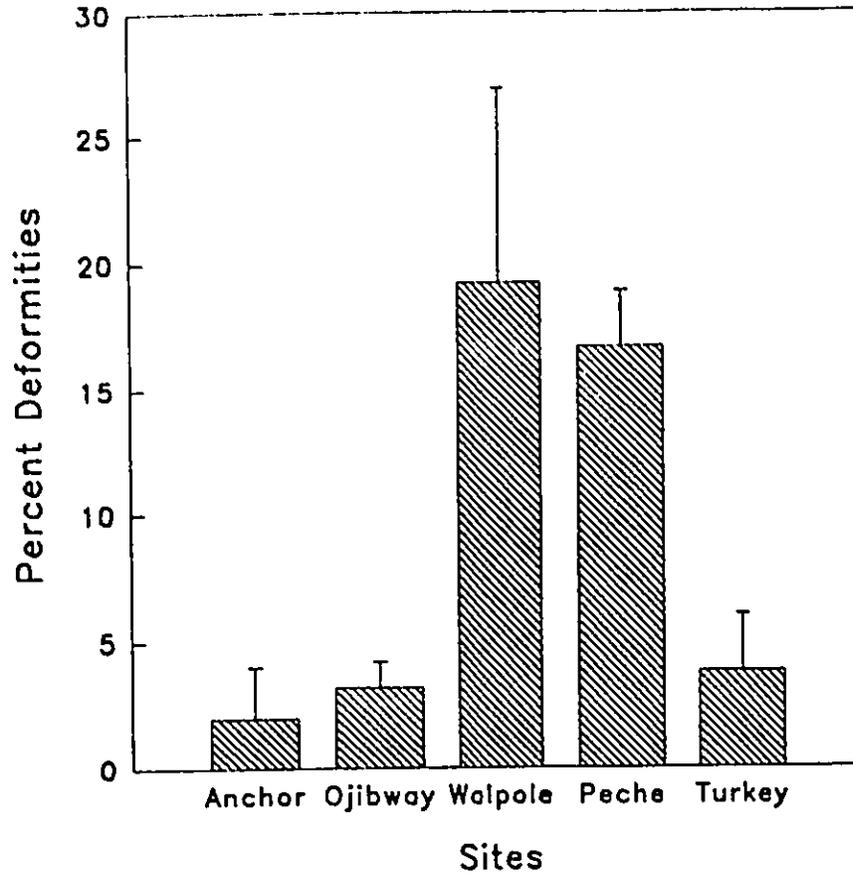


Figure 5. Incidence of deformities ($\% \pm 1$ SE) in *Chironomus* larvae from five study sites. No *Chironomus* larvae were collected from the Trenton Channel. Anchor Bay N=49, Ojibway Pond N=283, Walpole Island N=26, Peche Island N=305, Turkey Island N=79. N = Number of organisms examined.

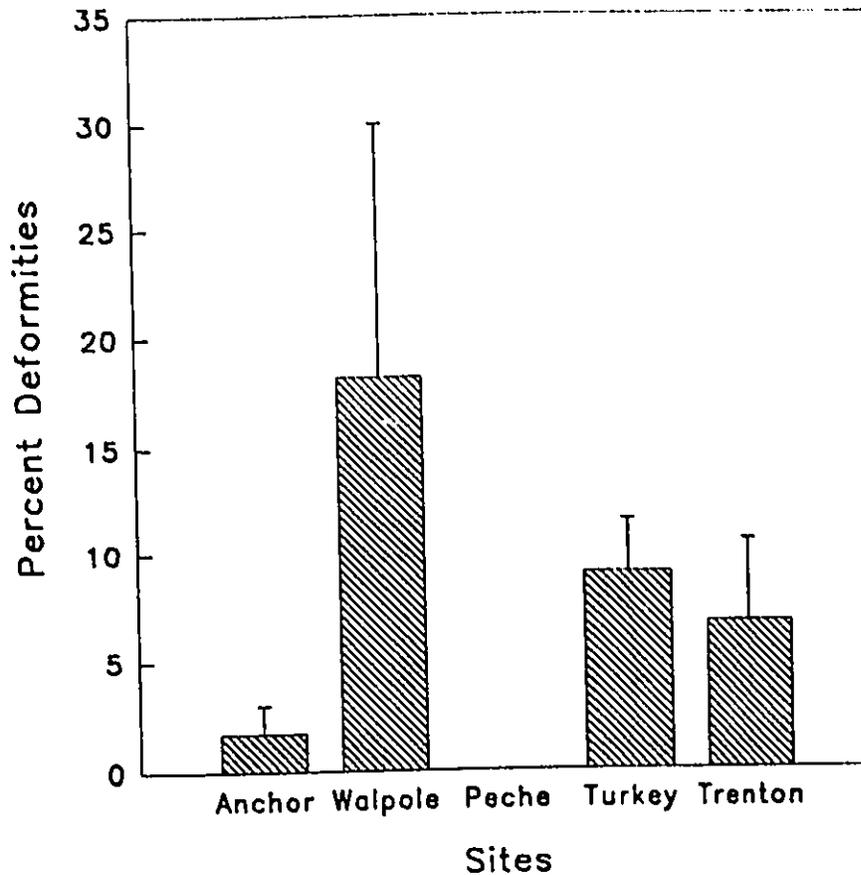


Figure 6. Incidence of deformities ($\% \pm 1$ SE) in *Phaenopsectra* larvae from five study sites. No *Phaenopsectra* larvae were collected from Ojibway Pond. Anchor Bay N=118, Walpole Island N=11, Peche Island N=24, Turkey Island N=133, Trenton Channel N=45. N = Number of organisms examined.

Discussion

In order to be useful as a field biomonitor, a taxon should be both widespread and sensitive to changes in environmental quality. The ubiquity of Chironomidae among aquatic habitats makes them especially promising invertebrates for such studies. My collection of visually distinctive ('red') chironomids yielded eighteen taxa, five of which were abundant enough to warrant further consideration. Five of these genera occurred at all but one of the Detroit/St. Clair river sampling sites. These genera have previously been recognized as being contaminant-tolerant. Dickman *et al.* (1992) reported that *Chironomus*, *Endochironomus* and *Polypedilum* maintain populations at sites with elevated contaminant levels where other genera cannot survive. Dermott (1991) reported that *Phaenopsectra (Tribelos) jucundus* and *Cryptochironomus* also appeared to be pollution tolerant in that they occurred in areas degraded by contaminants. I collected only *Cryptochironomus* and *Phaenopsectra* at all sites, including the most heavily contaminated one (Trenton Channel). The low susceptibility of these genera, and of *Cryptochironomus* to mentum deformities in this study attests to their ability to withstand contaminated conditions. However, this resistance reduces their suitability as biomonitors. Wiederholm (1984a) noted that in areas having a high incidence of deformity in some genera, cohabiting genera did not display deformities. Hare and Carter (1976) suggested that taxa that do not display physical responses to contaminants may respond in other, more subtle (physiological or behavioural) ways.

Chironomus and *Phaenopsectra* were the spatially widespread genera that showed the

greatest incidences of mentum deformities. Dermott (1991) also reported elevated incidences of deformities for *Chironomus* and *Phaenopsectra* (30% and 9%, respectively) from some locations in the St. Clair River. Dermott (1991) and Warwick and Tisdale (1988) suggested that the susceptibility of *Chironomus* to deformities (10-15X greater than the pollutant-tolerant, predatory genus *Procladius*) might be attributable to *Chironomus* feeding directly on contaminant-laden detritus.

More recent studies indicate that there may be a linear response to increased contaminant levels along a gradient. Van Urk *et al.* (1992) found that *Chironomus* cf. *plumosus* larvae occurred at lower population densities and with higher frequencies of deformities as contamination levels increased. Similarly, Dickman *et al.* (1992) found a significantly higher frequency of mentum deformities in coal tar contaminated sediments in the Niagara River than at a reference site, King's Bridge Park located near the confluence of the Welland and Niagara rivers.

I expected that organisms collected from the highly contaminated sediments of the Trenton Channel would display the greatest incidences of deformities. However, it was the Walpole Island and Peche Island sites that yielded organisms with the highest incidence of mentum deformities. This may be related to one or more factors. The most severely affected animals may have died and were therefore not collected (Warwick, 1990). The small numbers of certain genera collected were not sufficient to permit me to observe the deformity levels expected.

My results are inconclusive for *Phaenopsectra* and, to an extent, for *Chironomus*, because of small sample sizes at some sites. Clearly, the ability to detect potential

differences in incidence of deformities among sites (statistical power) depends on an adequate sample size from each location. In order to detect a doubling in the incidence of deformities over 3% background levels, at least 125 larvae from each population must be collected (sample size determined from binomial theorem, Sokal and Rolf 1981). This can be a daunting task when one is confronted with a sample site that is so heavily contaminated that impacts are already evident in reduced diversity and altered community structure. This was the case in Trenton Channel, where the benthic community was made up almost exclusively of oligochaetes. Perhaps use of a subtle biomonitoring procedure, such as examining mentum deformities is unnecessary in such grossly affected regions. However, the greatest utility of surveys such as a deformity census is to provide evidence of subtle environmental degradation before gross effects at the community level become obvious.

The Relevance of Mentum Deformities

Benthic invertebrates, including chironomid larvae accumulate PAHs and numerous other persistent hydrophobic organochlorine compounds in their tissues (Knight 1984, Larsson 1984, Rosiu *et al.* 1989). Dickman *et al.* (1992) found that deformed chironomids had higher PAH concentrations in their tissues relative to undeformed chironomids from the same site. They suggested that these PAHs acted as teratogens. Teratogenic abnormalities appear to result from the alteration of developing cells, which may lead to improper functioning of these cells or interference with differentiation so that proper cell types do not form or do not form in the proper number or location (Kamrin

1988). If this is the case, then the elevated incidences of deformities at the different study sites indicate a biologically significant contaminant burden to the organisms in these areas. This could ultimately lead to a decrease in population sizes, resulting in a change in food availability to organisms at higher levels of the food web. Furthermore, if the increased contaminant levels do not result in the death of the organism then this could result in bioaccumulation of these contaminants in the organisms that feed on chironomids. This could affect fish stocks and ultimately humans that consume the fish.

My study considered only mentum deformities in the screening process. Much of the detailed work on morphological deformities has involved examining the antennae. Although Warwick (1985) elegantly demonstrated the merits of using antennal deformities in chironomid larvae as a sensitive index of pollution, Dermott (1991) identified several difficulties of dealing with such a delicate morphological feature. The antennae are more easily damaged during sampling, or distorted during mounting than are hardened mouthparts. Furthermore, examination of antennae requires tremendous accuracy of identification at much higher magnifications than is necessary for mentum studies (Dermott 1991). Dermott (1991) recognized the demanding nature of such work and reported that a week was required to collect, sort and mount 300 larvae, after which examination of each specimen required about 3 min. In contrast, I was able to collect, sort and mount a comparable number of larvae in approximately 4 d; examination of each specimen required approximately 1 minute. Therefore, examining only the mentum saves time and allows greater sample sizes to be examined, thereby increasing confidence in the data obtained.

Saether (1970) suggested that deformed individuals may represent physical evidence that such organisms are near the limit of their "ecological range". Therefore, one can utilize the presence of these deformities to identify unfavourable ecological conditions before entire populations are eliminated from an area. These areas can then be identified as areas of concern and steps can be taken to restore the area to health so that it may maintain a diverse assemblage.

In summary, I have identified both spatial and taxonomic variation in relation to contaminant levels and with respect to taxa. I recommend *Chironomus* and *Phaenopsectra* as species of choice in such studies. Given the difference between background incidence of deformities in reference areas, Anchor Bay and Ojibway Pond (3%) and frequencies in moderately contaminated areas (6% or more), sample sizes from each site should be approximately 125 or greater to assure adequate power (80%) to detect a significant elevation (doubling) of deformities over background. However, laboratory experiments that demonstrate quantitative responses to known concentrations of contaminants are necessary to definitively establish the usefulness of mentum deformities. A laboratory experiment by Hamilton and Saether (1971) produced only negative results, with no deformed chironomid larvae being produced after exposure to insecticides, herbicides and PCBs. Their culture of *Chironomus tentans* was either eliminated or drastically reduced with exposure to higher concentrations of each of these chemicals. Clearly, further experiments (see thesis Discussion) designed specifically to determine a quantitative relationship between deformities and sediment contamination are needed to conclusively evaluate these organisms as potential biomonitors.

CHAPTER III. TERATOGENIC AND GENOTOXIC RESPONSES OF LARVAL *CHIRONOMUS* (DIPTERA:CHIRONOMIDAE) TO CONTAMINATED SEDIMENT

Introduction

One of the most serious threats to the quality of the environment is contamination by persistent chemicals. Our dependence on chemicals for lifestyle enhancement has reached the extent that industry now generates more than one tonne of chemicals for every inhabitant of North America (Hall and Chant 1979). These chemicals ultimately end up in the environment in one form or another. People have assumed that the environment is infinitely resilient and able to withstand almost any abuse. However, this is no longer an acceptable opinion (Warwick 1990a). It has become increasingly important to directly determine environmental responses to contaminant stress in order to control environmental quality. Biological communities provide a way to observe the impact of contaminants directly, and their responses provide a direct measure of the net toxic burden on the ecosystem (Warwick 1990a).

Chironomids have been recognized as suitable biological indicators of aquatic systems (Warwick 1990a). Investigators have identified that morphological structures such as the antennae, mentum, mandibles and epipharyngeal pecten may be developmentally altered in the presence of chemicals. Furthermore, researchers have reported an increase in the incidence of such morphological deformities in areas contaminated with a variety of substances (Hamilton and Saether 1971, Warwick 1985; 1987; 1990a; 1990b; 1991,

Warwick and Tisdale 1988, Dermott 1991, Dickman *et al.* 1990, Dickman *et al.* 1992, Van Urk *et al.* 1992, Lenat 1993).

Fewer researchers have examined the possible mutagenic effects of these environmental contaminants on chironomids. The relatively large size of chironomid larvae, the structural and functional peculiarities of their chromosomes and the high degree of polyteny in larval tissues such as the salivary glands, make chironomids good prospective subjects for genetic, cytogenetic and molecular biological studies. Polytene chromosomes are formed by the repeated replication of the original chromatids without separation of the resulting strands (Michailova 1989). They are easily observed under a light microscope at 400X magnification because at full size these giant chromosomes are almost 100 times thicker and 10 times longer than the chromosomes of typical cells. These chromosomes are found in well-differentiated organs that are engaged in vigorous metabolic activity that grow by an increase in cell size rather than cell number (Michailova 1989). Michailova (1989) found that the most suitable materials for analysis were the polytene chromosomes of the salivary glands.

As early as 1952, Beerman (1952) examined polytene chromosomes in chironomid larvae. Much of the work on these chromosomes has been in order to provide additional information that can help to separate species where conventional taxonomy is difficult ("cytotaxonomy") (Michailova 1989). Michailova (1989) has worked extensively with chironomid larvae, developing chromosomal maps for different species and identifying inversions characteristic of different populations. An extension of this work has identified structural and functional abnormalities of the polytene chromosomes, including

inversions, asynapsis, and differences in the degree of polyteny due to the presence of lead (Michailova and Belcheva 1990). Michailova and Belcheva (1990) also identified malformations of the mandible and submentum in chironomids grown in lead-contaminated sediments.

In general, there is a lack of laboratory experiments examining morphological and genetic abnormalities in chironomids. Kosawalt and Knight (1987) observed an increase in the incidence of deformities of epipharyngeal plates in chironomid larvae reared in copper-contaminated sediment. Hamilton and Saether (1971), in contrast, found an inverse relationship between antennal deformities and the concentration of DDE in laboratory reared chironomid larvae. However, to this point, no one has simultaneously investigated morphological deformities and chromosomal modifications in the same organism.

This laboratory experiment was designed to examine the morphological and genetic responses of chironomid larvae across a contaminant gradient, and to determine whether they exhibit a dose-dependent response. Morphological response was examined by identifying deformities in the mentum (reviewed in detail in Chapter II). Chromosomal responses were examined based on differences in the polytene chromosome structure of the salivary glands. I looked for additional puffs, reduction in puff size and reduction in the size of the nucleolus. Finally, I determined whether there was a correlation between mentum deformities and abnormalities in chromosome structure of individual larvae.

Polytene Chromosome Structure

One can potentially examine at least three aspects of chironomid polytene chromosome structure to investigate the organism's response to contaminants.

Puff Induction

Firstly, chromosomes frequently exhibit puffs, which are "swellings" on chromosomes that are associated with the local uncoiling of chromatin fibres and rapid RNA synthesis (Pelling 1959). Karim and Thummel (1992) found that, in *Drosophila*, specific puffs, activated by the hormone ecdysone, encode regulatory proteins that function to repress their own expression and activate a large set of late secondary response genes. It may be possible to examine unique regions of puffing along the chromosome to see if increased RNA synthesis is associated with exposure to increased contaminant levels, and to associate this increased gene activity with the production of heat shock proteins (hsps) or stress proteins. The induction of such proteins by stressors is a response system involving the production of new proteins that may confer resistance against environmental stressors (Hightower 1993). Potentially, such molecular biomarkers could bridge the gap between chemical analyses for the presence of toxicants and impairments of organismal physiology leading to community-level effects (Hightower 1993). Accumulation of stress proteins in cells has been shown to be proportional to the severity of the stress, i.e., the concentration of the stressor (DiDomenico *et al.* 1982). Based on these previous findings, I wished to examine chromosomes for additional puffs that would be indicative of stress.

Puff Regression

A second response involving the chromosome puffs is their regression. Compounds that inhibit RNA synthesis at the level of transcription cause puffs to regress (Beerman 1971). Bentivegna and Cooper (1993) suggested using this regression as a biomarker because it represents a potentially deleterious effect - reduced RNA synthesis. They induced reduction of puff size at sites of giant puffs (Balbiani rings) using known inhibitors of RNA synthesis. Beerman and Clever (1964) found that the RNA in one puff differs from the RNA made in another puff. Therefore, measuring the size of particular Balbiani rings would examine a mechanism in which specific contaminants might cause inhibition of RNA of a particular kind associated with a precise locus on the chromosome.

Nucleolus Reduction

Finally, the nucleolus may have potential as an indicator of overall RNA synthesis inhibition. The nucleolus is a specialized part of the chromosome that is composed of RNA and protein (Beerman and Clever 1964). The DNA of the nucleolus includes multiple copies of the genes from which ribosomal RNA (rRNA) is transcribed. After this rRNA is synthesized, it combines with proteins and the resulting complex leaves the nucleus and enters the cytoplasm where it becomes part of the ribosomes. Thus, the nucleolus is responsible for manufacturing and exporting to the cytoplasm the precursors of ribosomes on which proteins will be synthesized. These nucleoli tend to be small in cells that do not carry out protein synthesis (Keeton and Gould 1986). Cyclohexamide

(CHM), a known inhibitor of protein synthesis induces nucleolar "segregation", which severely reduces the size of the nucleolus and has also been shown to cause a loss of material (Diez *et al.* 1977). Reduction in the size of the nucleolus is indicative of overall decreased RNA synthesis, which could prove to be harmful to the chironomids.

Materials and Methods

Sediment

Sediment for the bioassay was collected from the Trenton Channel of the Detroit River (site 107 of Furlong *et al.* 1988). Trenton Channel sediment is contaminated with a variety of persistent organic contaminants and metals including polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated naphthalenes (PCNs), and polychlorinated terphenyls (PCTs) (Furlong *et al.* 1988, Chapter 2, Table 4). Furthermore, these sediments also contain pesticides, octachlorostyrene, pentachlorobenzene, and hexachlorobenzene (Furlong *et al.* 1988). There is evidence that many animal populations in this area have been affected by these contaminants. Researchers have documented death of eggs of fish species (Eisler 1986), slowed growth of benthic invertebrates (Giesy *et al.* 1987), reproductive dysfunction resulting in decreased population sizes of birds (Gilbertson 1988), and carcinogenicity in the observed formation of external tumours on fish (Suns *et al.* 1985). In an earlier survey performed during the summers of 1991 and 1992, very few chironomids were found to inhabit the sediments of this area. The samples were dominated by oligochaetes (Chapter II).

The purpose of the experiment was to examine a response along a contaminant

gradient. In order to develop an exposure gradient, the Trenton Channel sediment was diluted using a synthetic sediment composed of garden soil, sculptor's clay and silica (quartz) sand (Hanes *et al.* 1991). Dilutions were performed so that there were six different treatments, each consisting of a different proportion of Trenton Channel sediment, with 14 replicates of each treatment. Sediment was collected in August 1992 using a Petersen grab sampler and held in cold storage (4°C) until the summer of 1993 when the laboratory experiment was performed. Previously, Othoudt *et al.* (1991) demonstrated that there was no effect of storage time of the sediment on toxicity. The sediment mixtures were stored for 1 year.

The experimental containers were 1-L hexane-rinsed, Universal[®] glass jars. Jars were covered with plastic lids that had an insert of hexane-rinsed aluminum foil. The jars were filled with dechlorinated water and 300 mL of Trenton Channel: synthetic sediment mixture in ratios of 1:0, 1:1, 1:3, 1:7, 1:15 or 0:1 v/v. Containers were individually aerated and were allowed to aerate gently, clear and equilibrate for 7 d prior to the addition of chironomids.

Organism Collection and Maintenance

Gravid female *Chironomus salinarius* were collected from a pond at Ojibway Park, near Windsor, Ontario (Chapter II). This was an external reference site for a field study which indicated that chironomids from the pond in this area had relatively low levels of mentum deformities, suggesting that the sediments in this pond are relatively uncontaminated. Although *Chironomus* displays mouthpart deformities, it is relatively

pollution tolerant (Chapter II).

Adult chironomids were collected at night using an ultraviolet light source to attract individuals (Kovats and Ciborowski 1989). Organisms attracted to the light settled on plastic portions of the apparatus or on the white cloth placed under the trap. Female adults were collected individually into 20-mL scintillation vials. A small amount of dechlorinated water added to the vial induced oviposition by gravid females. The egg mass deposited by each female was then transferred to its own rearing chamber. The rearing chambers were 25 x 15 cm translucent, polyethylene containers containing a 1 cm depth of silica (quartz) sand and a 4 cm depth of aerated, dechlorinated tap water at room temperature (20-22°C). Containers were continually aerated, and the developing chironomids received 1 mL of a Tetra Min^R/dechlorinated water mixture (approximately 40 mg) every second day.

Experimental Conditions

Chironomid larvae were allowed to develop until early 2nd instar (approximately 10-12 days after egg mass collection). At this time, 20 chironomids were added to each treatment jar. Individuals were randomly chosen from different egg masses to add to the treatment jars. The experiment was allowed to continue for 10 d, by which time most individuals had reached 4th instar. Experimental conditions remained constant, 16:8-h light:dark photoperiod and 21°C. The experimental containers were covered for the duration of the experiment, which minimized evaporation and made addition of water during the experiment unnecessary. Chironomids were fed 1 mL of the

TetraMin/dechlorinated water mixture every second day.

After 10 d, the sediment was sieved and individual chironomids were removed, blotted on paper towelling and placed in three successive changes of cold (4°C) Carnoy's fluid (3:1 v/v absolute ethanol:glacial acetic acid). This preserved the chironomids until analysis for mentum deformities and polytene chromosome structure.

Examination of Mentum Deformities

The heads of individual chironomids were removed and slide-mounted in CMC-9AF^R aqueous mounting medium. Gentle pressure was applied with the cover slip to ensure separation of the various mouthparts so that the mentum was clearly visible. At this time, the body was placed in a labelled vial of Carnoy's fluid and stored refrigerated until further processing. Slides were allowed to clear for one week before each head was examined for deformities in the structure of the mentum (asymmetry, missing teeth, additional teeth or gnarled or twisted teeth). Each mentum was examined twice to ensure the correct designation of mentum condition was indicated. The designation of "deformed" was not given to those chironomids which displayed worn or chipped teeth.

Polytene Chromosome Structure

The bodies of the chironomids were used in the analysis of polytene chromosome structure. Chironomids were stained using a modification of the acid fuchsin technique of Rothfels and Dunbar (1953) (P.H. Adler, Clemson Univ., pers. comm). Chironomid salivary glands were exposed by slitting the abdomen and thorax along the ventral

surface. Larvae were then placed in deionized water for 20 min. Then, they were transferred into 2 mL of 1.0 N HCl preheated to approximately 70°C. They were placed in an oven and maintained at this temperature. After 10 min, the HCl was drawn off and replaced with 2 mL of Feulgen stain (a DNA specific stain). The larvae were stained for 1 h. The stain was siphoned off and the chironomids were transferred to sulphur water (200 mL deionized water, 1 g potassium metabisulfite, and 10 mL HCl). After 9 min the sulphur water was removed and replaced with cold tap water. The salivary glands were then excised and placed in a drop of 50% acetic acid on a microscope slide. The glands were then macerated and a coverslip was applied with gentle pressure to squash the preparation. Because this preparation fades quickly upon exposure to air and light, the slide was ringed with clear nail polish and examined within 2 h. The polytene chromosome structure was viewed at 1000X magnification using a Leitz Diaplan microscope attached to a Panasonic^R high-resolution camera. A Targa^R 64 computer video digitizing card was used to digitize the video image and display it on a computer screen. The bands and the nucleolus were clearly visible, and measurements were taken utilizing the JAVA^R image analysis computer package. Each chironomid preparation was examined for the occurrence of additional puffs, and the size of the nucleolus was measured.

Measurements of Chromosomal Puffing

Puffing was measured at the nucleolus on Chromosome I (Figure 7). The diameters of the nucleolus and its chromosome were measured using the JAVA computer package. Bentivegna and Cooper (1993) found that in control organisms the diameter of a puff was

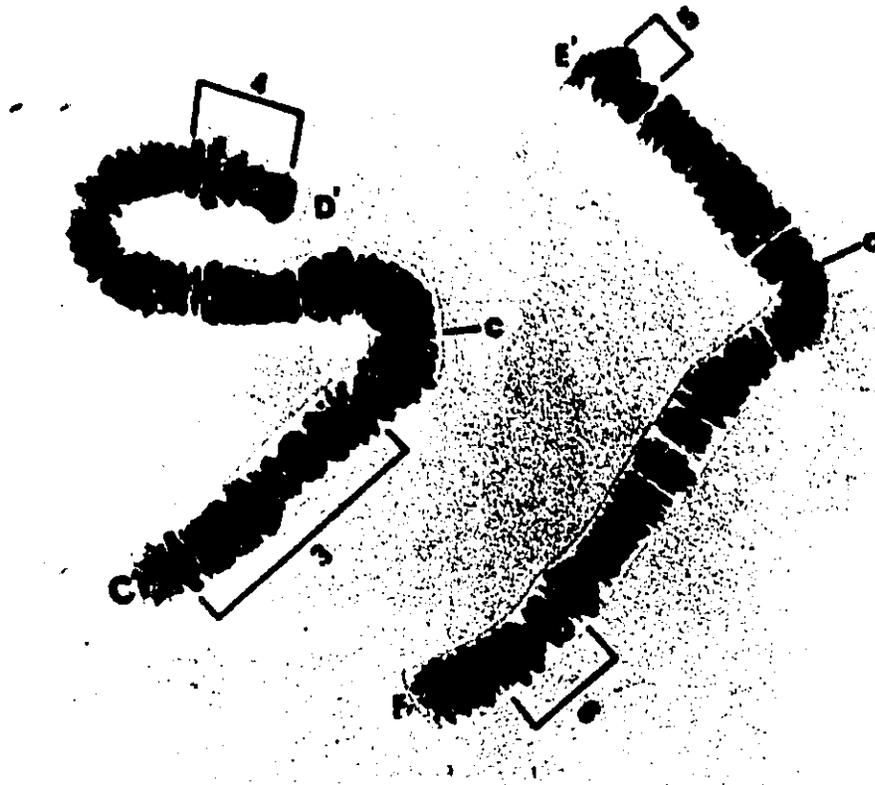


Figure 7. Polytene chromosome structure of *Chironomus salinarius*. (From: Michailova 1989). A' B', C' D', E' F', and G' denote Chromosomes I, II, III, and IV respectively. Numbers 1 - 6 denote marker sequences. C=Centromere, N=Nucleolus, BR₁=Balbiani ring 1, BR₂=Balbiani ring 2.

proportional to that of the chromosome width at the same locus. Furthermore, chromosome diameters corresponded to the sizes of the organisms. Therefore, measurements of both nucleolus and band diameter allow data from different-sized larvae to be standardized. The relative nucleolus size was determined utilizing the equation based on these relationships which was developed by Bentivegna and Cooper (1993).

$$\text{Nucleolus Size} = \frac{\text{Nucleolus Diameter} - \text{Chromosome Diameter}}{\text{Chromosome Diameter}}$$

A total of three nuclei were measured in each individual chironomid larva and each measurement was taken twice in order to obtain an accurate estimate of nucleolus size. The standard nucleolus size of a control organism was approximately 1.0. A nucleolus was classified as reduced if it was decreased in size by 25% or more (Figure 8).

Statistical Methods

The incidence of deformities and its standard error for each treatment was calculated from the binomial theorem in the same manner as outlined in Chapter II. Similarly, the incidence of reduced nucleolus from the different treatments was expressed as the overall proportion \pm 1 SE of larvae from each treatment that showed a reduction in the size of the nucleolus. I used a G-statistic goodness of fit test to test for differences in the incidence of deformities and differences in the incidence of reduced nucleolus among the different treatments. I used regression analysis to examine the relationship between mean recovery (percent per replicate) of chironomids and treatment (proportion

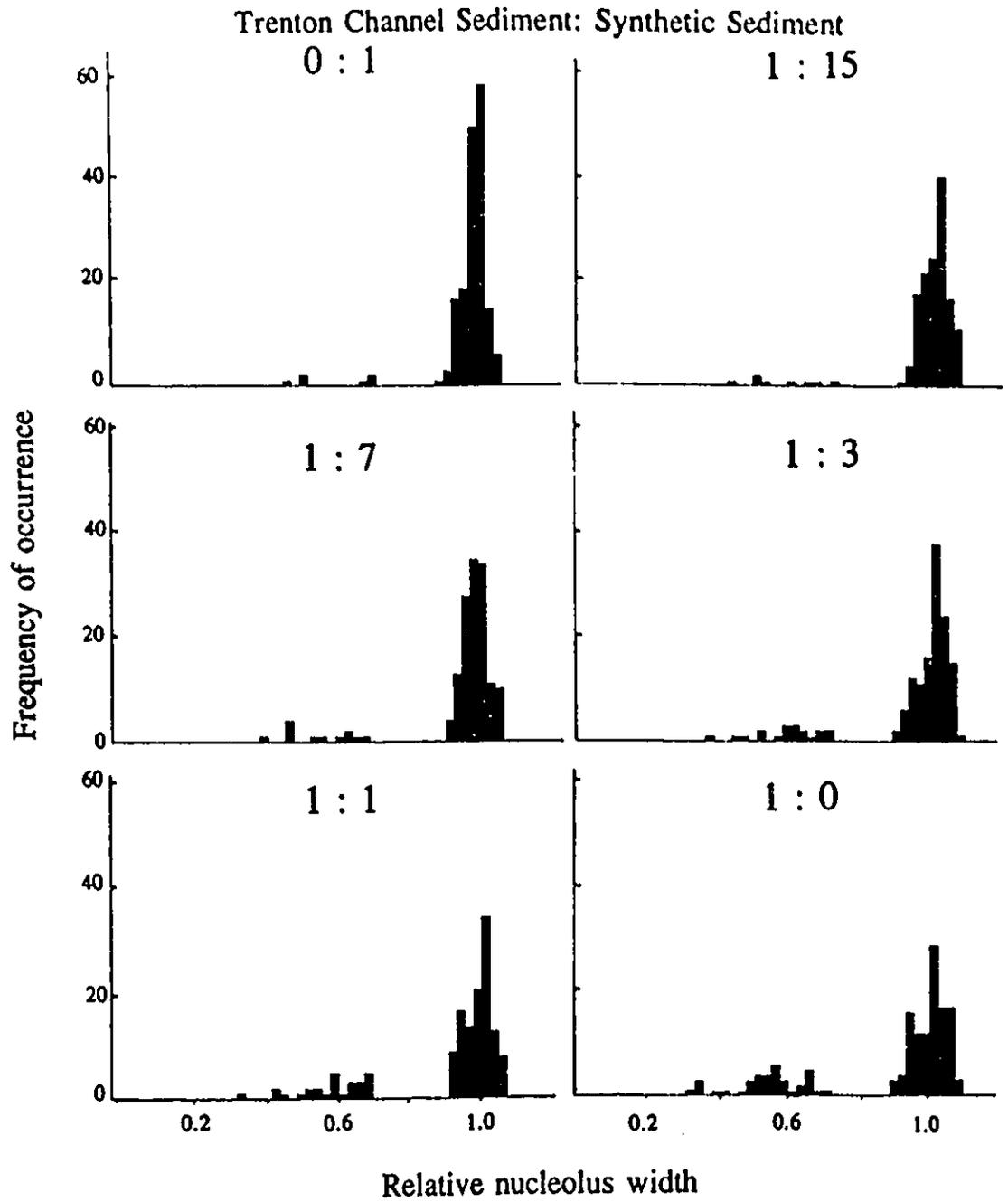


Figure 8. Frequency distribution of the relative nucleolus width of *Chironomus salinarius* exposed to sediment dilutions.

of Trenton Channel sediment). Regression analysis was used to examine the relationship between the degree of nucleolus reduction and the proportion of Trenton Channel sediment. Finally, I used a G-statistic of independence (Sokal and Rohlf 1981) to examine if mentum condition was independent of nucleolus reduction.

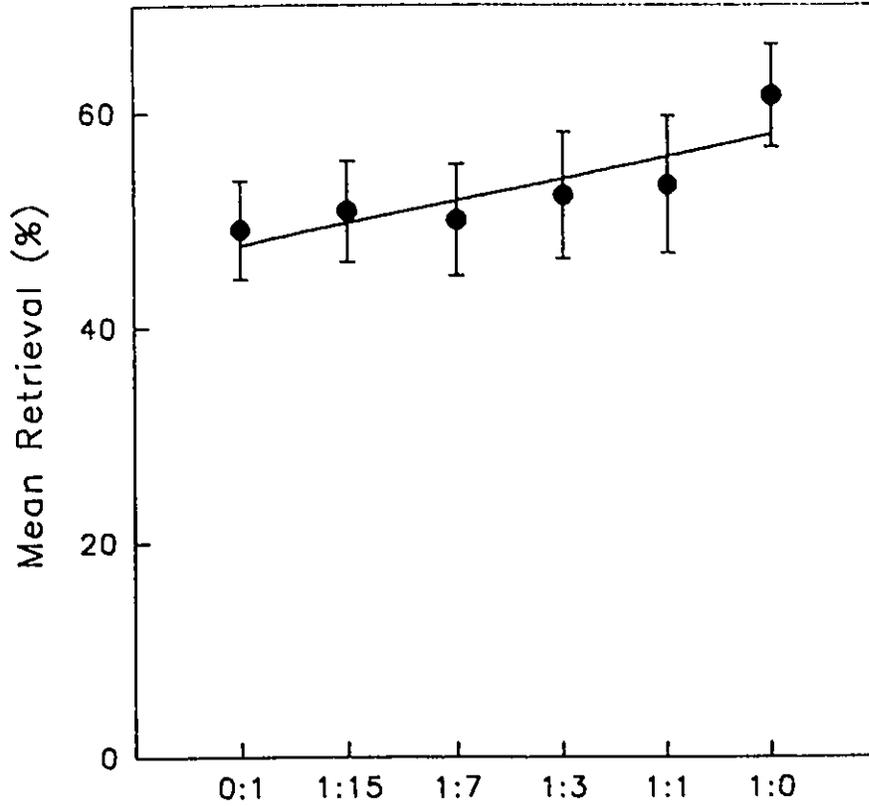
Results

Recovery

During the experiment, many chironomids emerged before the completion (10 d). For this reason, retrieval of chironomids from the experimental jars varied due to both emergence and mortality. Mean recoveries from replicates ranged from 49.1% \pm 4.6 (N=14) in the 0:1 sediment to 61.6% \pm 4.8 (N=14) in the 1:0 (Trenton Channel) sediment. Other recovery rates were 50.8% \pm 4.7 (N=14) in 1:15, 50.0% \pm 5.2 (N=14) in 1:7, 52.3% \pm 6.0 (N=14) in 1:3, and 53.3% \pm 6.4 (N=14) in the 1:1 treatment. Recovery rate was related to the proportion of Trenton Channel sediment (Figure 9. Simple Regression $F[1,4] = 47.8, p > 0.05$). The retrieval of chironomids from 1:0 Trenton Channel:synthetic sediments was higher than from other treatments. This was due to higher emergence in the less contaminated sediments, presumably due to faster growth.

Deformities

Initially, the mentum of each chironomid was assayed for deformities. A total of 1702 chironomids were examined. Common deformities included extra or missing teeth,



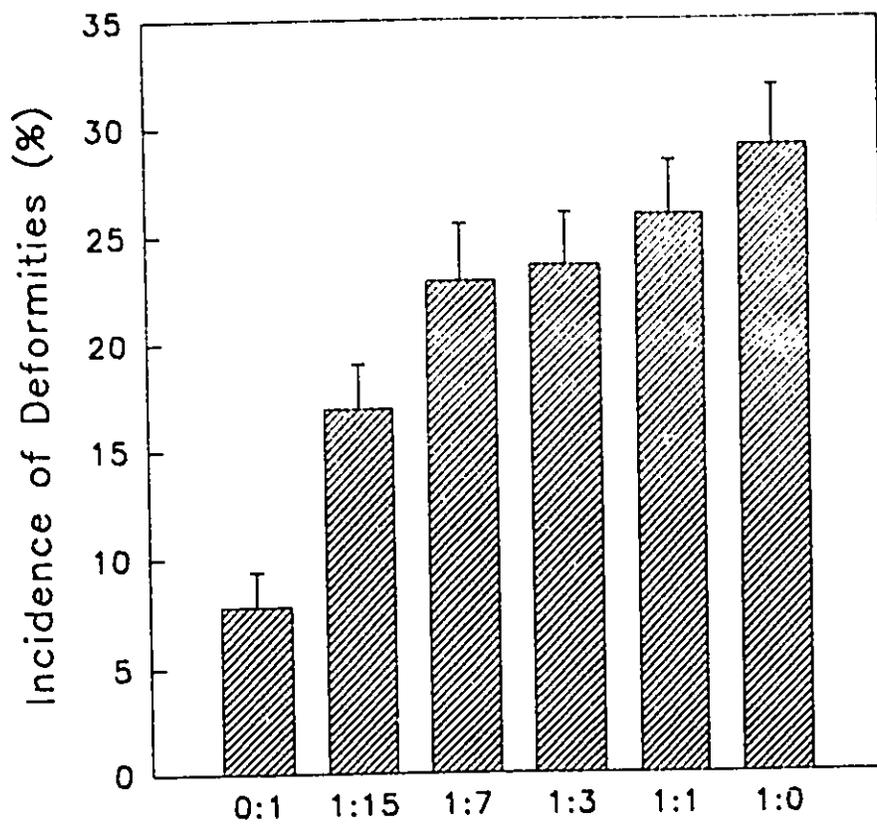
Trenton Channel Sediment:Synthetic Sediment

Figure 9. Percent \pm 1 SE of chironomid larvae retrieved from experimental containers (N = 14 per treatment). Regression line takes the form (Percent Retrieved) = 45.62 + 2.07 (Proportion of Trenton Channel Sediment). $R^2 = 0.721817$.

asymmetry and various degrees of gnarled or twisted teeth. Köhn gaps, a deformity characterized by a large gap in the mentum that may or may not include the loss of some teeth (Köhn and Frank 1980, Warwick 1988, Warwick and Tisdale 1988) were also identified. However, this deformity only occurred in the more heavily contaminated treatments (1:0 and 1:1 dilutions) and they accounted for only 2% of the total incidence of deformities at these treatment levels. Other than this particular abnormality, there was no indication that those sediments with the highest proportions of Trenton Channel sediment produced larvae that were more severely deformed than larvae exhibiting deformities in other treatments. These treatments produced more chironomids with mentum deformities but the severity of the deformity was relatively uniform, with extra teeth and asymmetry present in larvae from the control sediments as well as in those from the Trenton Channel sediment mixtures.

The lowest incidences of mentum deformities were found in larvae from the control (0:1) sediments, and the proportion of larvae with mentum deformities increased approximately linearly with each doubling of contaminant concentration from $16.8 \pm 2.1\%$ (N=313) at 1:15 to a high of $29.0 \pm 4.0\%$ (N=261) at 1:0, which was the undiluted Trenton Channel sediment (Figure 10). There was significant among-treatment variation in the incidence of mentum deformities (G-statistic Goodness of fit test, $G=44.64$, $p<0.001$).

The frequency of deformities in larvae from the control (0:1) sediments was $7.9 \pm 1.6\%$, which is considerably higher than reference or baseline values found earlier in field-collected larvae (Chapter II) and also higher than levels considered to be



Trenton Channel Sediment: Synthetic Sediment

Figure 10. Incidence of deformities ($\% \pm 1$ SE) in *Chironomus salinarius* larvae from a laboratory sediment dilution experiment. 0:1 (N=268), 1:15 (N=313), 1:7 (N=266), 1:3 (N=297), 1:1 (N=297), 1:0 (N=261). N = Number of organisms examined.

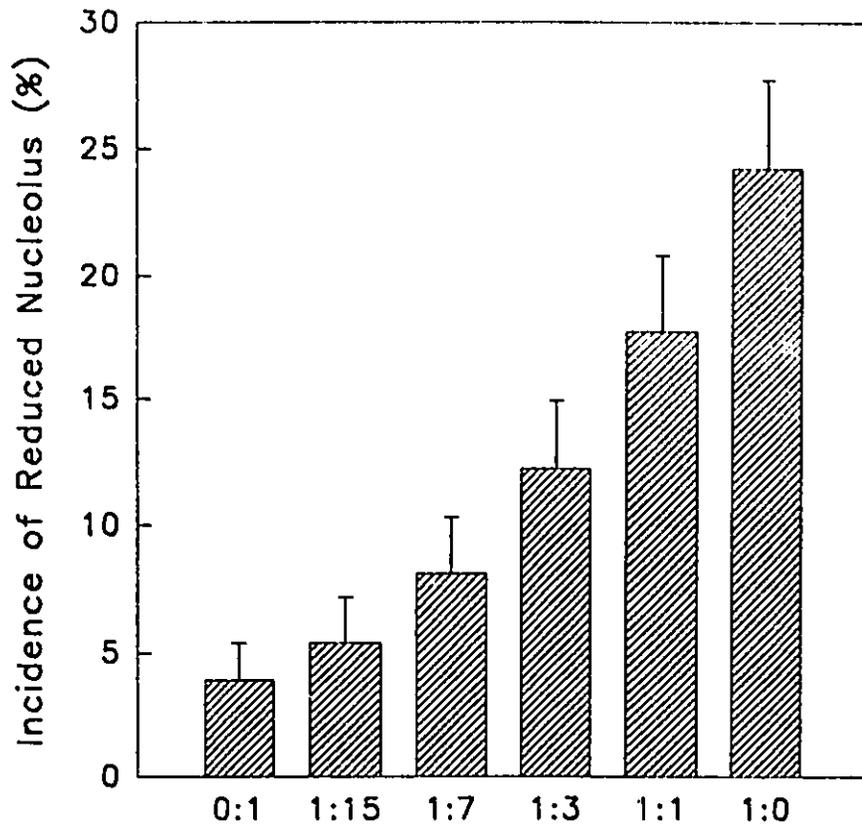
background reported by other authors (Lenat 1993, Dickman *et al.* 1992).

Polytene Chromosomes

For most of the treatments, except the control (N=178), 150 larvae were examined for abnormalities in chromosome structure. The chromosomes were examined for extra puffs, indicative of the production of stress proteins. None of the organisms from any treatment had extra puffs evident. The two Balbiani rings, normally located on Chromosome IV, were not well differentiated in any of the chironomids. Therefore it was inappropriate to measure these in an attempt to identify inhibition of RNA synthesis.

The nucleolus, located on chromosome I, was measured in each individual. There was a direct relationship between the proportion of Trenton Channel sediment and response (reduction in nucleolus size). The proportion of larvae that displayed reduced nucleolus size was lowest in control (0:1) sediments ($3.9 \pm 1.5\%$, N=178), and the proportion of larvae showing this response increased approximately linearly with each doubling of contaminant concentration from $5.4 \pm 1.8\%$ (N=149) at 1:15 to a high of $24.2 \pm 3.5\%$ (N=149) at 1:0 (Figure 11). There was significant among-treatment variation in the incidence of reduced nucleolus (G-statistic Goodness of fit test, $G=40.17$, $p<0.001$).

The frequency distribution of the relative nucleolus diameter for chironomids from the different treatments is summarized in Figure 8. The degree of nucleolus reduction in affected individuals was not related to the proportion of Trenton Channel sediment in which they were reared (Regression Analysis $F_{[1,103]} = 1.05$, $p > 0.05$).



Trenton Channel Sediment:Synthetic Sediment

Figure 11. Incidence of polytene chromosome nucleolus reduction ($\% \pm 1$ SE) in *Chironomus salinarius* from a laboratory sediment dilution experiment. 0:1 (N=178), 1:15 (N=149), 1:7 (N=149), 1:3 (N=147), 1:1 (N=147), 1:0 (N=149). N = Number of organisms examined.

Finally, nucleolus reduction in a larva was unrelated to mentum condition (G-statistic of independence, $G = 1.96$, $p > 0.05$), indicating that deformities and nucleolus reduction in individual larvae were independent responses to contaminant stress.

Discussion

Very few studies have examined the contaminant stress response of chironomids in the laboratory under controlled conditions. I found that the incidence of mentum deformities increased with increasing contaminant levels. To my knowledge, this is the first laboratory documentation of a clear dose-response relationship of deformities to sediment contamination. Recent field studies are consistent with these findings that there may be a linear response to increased contaminant levels along a gradient. Van Urk *et al.* (1992) found that *Chironomus cf. plumosus* larvae occurred at lower population densities and with higher frequencies of deformities as contamination levels increased. Dickman *et al.* (1992) found a significantly higher frequency of mentum deformities in coal tar contaminated sediments than at reference sites. I also found that sites with elevated contaminants had increased incidences of deformities (Chapter II). Laboratory studies also support my findings. Michailova and Belcheva (1990) exposed *Glyptotendipes barbipes* to two lead concentrations and found that there was an increase in malformations of the teeth of the mandible and submentum and in the shape of genitalia and wings of emergent adults. In another laboratory study, Kosalwat and Knight (1987) demonstrated that copper-contaminated sediment caused deformities in the epipharyngeal plate of larval mouth parts.

Incidence of Mentum Deformities

The incidence of mentum deformities in the control (0:1) sediments of the laboratory study was approximately 3.0-4.0 percentage points higher than in reference sites reported for most field studies. This suggests that something in the control sediments was affecting the chironomids. It is also possible that the Tetra Min^R/water food mixture was contaminated. However, it still remains that the incidence of deformities in the control treatment was significantly lower than those incidences in the treatments containing Trenton Channel sediment.

Incidence of Nucleolus Reduction

As the proportion of contaminated Trenton Channel sediment increased a greater proportion of organisms displayed a reduction in the size of the nucleolus. The nucleoli are large deposits of RNA and protein that are formed in all types of cells by chromosomal regions known as nucleolar organizers (Kecton and Gould 1986). They function in the formation of ribosomes which are the sites of protein synthesis in the cytoplasm (Beerman and Clever 1964). Diez *et al.* (1977) demonstrated that a 24 h treatment of the carcinogen cyclohexamide (CHM) caused nucleoli to become segregated and to show a reduction in material. They observed a separation of the fibrillar and granular parts of the segregated nucleolar organizing region (NOR) caused by contraction of the NOR which also involves a condensation of the fibrillar material associated with it (Unuma *et al.* 1973). The NOR in polytene chromosomes can be considered a special type of puff (Wolstenholme 1965, Pelling and Beerman 1966). Therefore, contraction of

the NOR may represent a reduction in puffing state caused by a decrease of rRNA (Diez *et al.* 1977). Reduction in the activity of the NOR has also been demonstrated in the presence of copper (Aziz *et al.* 1991). It has been suggested that CHM may be involved in the inhibition of factors involved in the initiation of RNA polymerase activity (Gross and Pogo 1974). More specifically, the reduced puffing in *Chironomus nucleoli* by CHM has been attributed to inhibition of RNA polymerase I which specifically synthesizes nucleolar RNA (Horgen and Griffin 1971, Timberlake *et al.* 1972).

Balbani rings

Although, *Chironomus salinarius* has two Balbani rings on chromosome IV (Michailova 1989), these giant puffs were not clearly visible in any of my preparations. These puffs are composed of RNA and it is possible that this population was exposed to some substance that inhibited RNA synthesis and resulted in a reduction in the size of the puffs. Researchers have found that chemicals such as actinomycin D (Act D), benzo[e]-pyrene (BeP) and dimethylnitrosamine (DMN) cause chromosomal puffs (Balbani rings) to regress in size (Bentivegna and Cooper 1993). This reduced chromosomal puffing has been particularly associated with inhibition of RNA synthesis (Bentivegna and Cooper 1993). More specifically, chemicals such as Act D and a-amanitin inhibit RNA polymerase II (non-nucleolar RNA polymerase) (Horgen and Griffin 1971), which results in chromosomal puffs regressing in size (Clever 1967, Beerman 1971). Therefore, regression in these puffs would involve a different type of inhibition than the generalized inhibition of the nucleolus that I observed.

Puff Induction

Examination of the polytene chromosome structure included searching for supernumerary puffs. Such puffs are visible regions of highly active transcription on dipteran polytene chromosomes (Pelling 1964). These puffs have been studied extensively in *Drosophila*. Many puffs have been identified and associated with the transcription of messenger RNA (mRNA) from genes and translated to produce proteins. Specifically, Crowley and Meyerowitz (1984) associated a puff, 68C, with the region along the chromosome that contains the structural genes for three salivary gland glue proteins in *Drosophila*. The region is puffed during the stage in which the glue proteins are being produced. Ashburner (1972) indicated that the function of puffs may be to code for a number of proteins and enzymes which themselves are of little relevance to the physiology of the larval salivary gland itself, but which will be released into the body fluid on the breakdown of the gland and be used later in life for histogenesis or for other functions.

Based on these ideas, puffing could be useful as an indication that a specific gene or genes along the chromosome become active, producing RNA that codes for proteins that could possibly fall into the category of cellular stress proteins based upon the fact that they can be induced by heat, heavy metals, hypoxia, and other environmental challenges (Hightower 1993). This would allow one to perform initial steps to associate specific chemicals with changes at individual genes (bands) through increased RNA synthesis and then at a later stage examine how this mRNA is translated to produce proteins.

Hightower (1980) proposed that most, if not all inducers of cellular stress proteins

are united by their ability either to damage cellular proteins directly or to cause cells to produce abnormal proteins biosynthetically. The use of molecular biomarkers such as stress proteins has the potential to bridge the gap between chemical analysis for the presence of toxicants and impairments of organismal physiology leading to community-level effects (Hightower 1993). These stress proteins are actually a way of looking at the organism's induction pathways leading to enhanced gene expression and they have the ability to register the actual impact of a stressor on the cells and tissues of organisms (Hightower 1993).

I did not detect any obvious additional puffing along the chromosomes (indeed the giant Balbiani rings were difficult to locate). However, my staining method was not designed to produce high-contrast resolution of puff formation. Therefore, I cannot definitively state that there was no increase in RNA levels, especially RNA levels associated with the production of cellular stress proteins that might confer resistance against environmental stressors. Puffs vary from small, in which a particular band simply loses its sharp contour and presents a diffuse out-of-focus appearance, to very large puffs or Balbiani rings, in which bands may look as if they have "exploded" into a large ring of loops around the chromosome (Beerman and Clever 1964). Many times the chromosomes were coiled upon each other, making regions of them unrecognizable. Thus, a small puff could have easily been overlooked. Chromosome puffs contain significant amounts of RNA, whereas normal unpuffed bands chiefly contain DNA and histone (Beerman and Clever 1964). Pelling (1959) demonstrated that the rate of RNA synthesis is closely correlated with the relative size of puffs. Perhaps using a

metachromatic dye such as toluidine blue, which simultaneously stains RNA red-violet and DNA a shade of blue (Becerman and Clever 1964) might better indicate the formation of puffs that are indicative of active genes along the chromosome.

Other Indicators of Genetic Damage

Other alternatives would involve looking at additional chromosomal abnormalities. Michailova and Belcheva (1990) exposed *Glyptotendipes barbipes* to different lead concentrations and found that replication activity was inhibited and there was extensive asynapsis or unpairing of the homologues which was probably caused by differences at the molecular level that did not affect the banding pattern. A proportion of the specimens also had inversions. The occurrence of such abnormalities could prove to be extremely useful as additional indicators of genetic damage to an organism.

My results indicate that there was no relationship between puff reduction and mentum condition in a larva (G-statistic test of independence, $G = 1.96$ $p > 0.05$) indicating that these are independent responses to contaminant stress. Little work has examined the relationship between the genetic and morphological (teratogenic) abnormalities in chironomids. Michailova and Belcheva (1990) examined the effects of lead on the external morphology and the polytene chromosome structure of *G. barbipes*. Lead caused both morphological and genetic abnormalities in these organisms. However, no attempt was made to examine both in the same larva. Kalter (1971) found that pathological effects of such teratogenicity were not clearly correlated with the genetic toxicity. However, a review by Alia *et al.* (1992), considering the effects of pesticides on certain

species of mammals, indicated that when a chemical compound showed mutagenicity there was a high likelihood for this compound to be teratogenic. In my study, there was no association between reduction in the size of nucleolus and teratogenic effects within individuals, although the two were clearly related at the population scale.

The finding of such a response to contaminants could have tremendous ecological implications. It would be important to examine how these two responses, chromosomal differences and mentum deformities relate to the organism's overall fitness. If mentum deformities reduce feeding efficiency or if changes in the genetic structure affect development or reproduction then this could have serious ecological ramifications. Because chironomids make up a considerable proportion of the benthic biomass, they are a valuable food source for predatory invertebrates and benthivorous fishes. Any change in their population could affect the food web found in that area.

In this study I exposed chironomids to a bulk sediment that contained many different pollutants. Future studies should be designed to examine single chemicals and their teratogenic or genotoxic effects on chironomids in an attempt to identify the chemicals that cause biological effects.

I am still unsure as to the suitability of examining polytene chromosome structure of organisms collected in the field. Michailova (1985) has shown that the best chromosome preparations are produced using larvae bred in the laboratory. However, the chromosome preparations from field collected chironomids may be useful as the bands of the chromosomes are visible. One possible drawback of working with field populations has to do with the great amount of diversity in the family Chironomidae. The field study

revealed that at a single site fifteen different genera were collected (Chapter II). In order to determine additional puffs at specific locations it would require detailed knowledge of the diversity in the chromosome structure of each species.

I have shown that chironomids appear to be good indicators of sediment quality. To my knowledge, this was the first laboratory study to demonstrate a dose-response relationship in which the incidence of mentum deformities increased across a contaminant gradient. Furthermore, I have shown that there is an increase in the incidence of nucleolus reduction with increasing sediment contamination, suggesting the possible inhibition of overall RNA synthesis by such contaminants. My study examined teratogenic and genetic responses in the same individual. In this study, my results indicated that genetic abnormalities (reduced puffing) and mentum deformities are unrelated. This research provides a foundation upon which further studies can be based in order to increase our knowledge of the effects of contaminants on biological components of the environment.

CHAPTER IV. GENERAL DISCUSSION

The Study of Biological Effects

One of the major goals of the discipline of ecotoxicology is to understand and document the effect of sediment-bound contaminants on components of aquatic ecosystems. The traditional approach of measuring the concentrations of individual contaminants can provide information on the degree to which a site is contaminated with a particular chemical. However, it does not provide any information on how these contaminants affect the overall health of the ecosystem. Therefore, it is necessary to determine the effects of contaminants on organisms directly. By measuring the contaminants effects on individuals, it is possible to determine the net toxic burden influencing the system. Benthic organisms are exposed to and are directly involved in the changes that chemicals undergo in aquatic environments. Additionally, since toxic effects begin as a chemical interaction between a contaminant and the individual, examining the developmental, physiological and ecological responses of the individual provides the earliest warning of environmental degradation. I have selected larvae of chironomids as such an indicator.

The field study was designed to look at spatial and taxonomic variation in incidences of deformities at sites along the Huron-Erie corridor. I expected that sites with higher relative amounts of chemicals would have chironomids with relatively higher incidences of mentum deformities. I found that genera differ in their sensitivity to contaminants and that the incidence of deformities does generally increase with increased

contaminant levels.

The laboratory study exposed *C. salinarius* to a contaminant gradient generated from Trenton Channel sediments. As I expected, chironomids exposed to greater proportions of contaminated sediment showed an increase in the incidence of mentum deformities. The incidence of nucleolus reduction also increased with increased contaminant levels. Additionally, I looked at teratogenicity (deformities) and genotoxicity (nucleolus reduction) in the same organism. These two measures were independent of one another in my study.

Advantages of the Study

In situ biological methods as well as chemical and laboratory bioassay methods are needed to assess water quality and the general health of freshwater ecosystems. Community-level approaches tend to focus on the response of the ecosystem to different environmental impacts (Warwick 1991). At the other extreme, chemical methods and laboratory bioassays provide data amenable to a regulatory approach, focusing on specific pollutants with an aim towards establishing specific rules for their discharge into the environment (Ohio Environ. Protect. Agency, 1987). The limitation of such chemical measurements and to some extent, bioassay measurements lies in the fact that they are based on short-term conditions existing at the time of sample collection (Warwick 1990a).

Neither biological nor chemical/bioassay methods should be used exclusively, but instead any well balanced study would use them in conjunction with one another. The

environment of an individual organism is more complicated than dealing with single pulses of single chemicals for short periods of time. Warwick (1991) identified three primary processes that appear to determine the response in bottom-dwelling organisms. These include trophic and sedimentation processes that determine the availability of contaminants and also the contaminant process, which encompasses contaminant inputs, their by-products and the products of synergistic and antagonistic interaction. The interaction between contaminants and their bioavailability can be examined in terms of faunal response at any level of organization (Warwick 1991). Therefore, benthic organisms are good candidates as indicators of aquatic conditions because they are intimately bound to their environment and are directly subject to the sum total of all chemical, physical, and biological processes influencing that environment over the length of their life cycle (Warwick 1991). This is one important strength of my study. The field data allow me to see the combined effects of a myriad of contaminants on chironomids. The laboratory study component used a bioassay approach to demonstrate a cause-effect relationship between mentum deformities or reduced nucleolus puffing and the degree of sediment contamination.

One of the advantages of a study such as this one is that it examines chronic toxicity. Most bioassay evaluations are concerned with acute toxicity i.e. the mortality obtained by exposing organisms to high levels of substances for a short period of time, usually 96 h or less (Kosalwat and Knight 1987). These evaluations provide information that is used to determine the sensitivity of an organism to a toxicant. However, their usefulness is limited by the fact that they may be difficult to interpret and to extrapolate to field

conditions. Death is a crude index of environmental stress. Measures of chronic toxicity are more informative because the effects of many substances may not be evident for longer periods of time. Effects may be related to changes in appetite, metabolism, morphology, growth, reproduction, development of sex products, maturation, hatching, survival of different life stages, deformities, behaviour or other vital functions that do not result in early death (Kosalwat and Knight 1987). I examined the response of chironomids over the longest and most sensitive stage of their life cycle, the larval stage. These organisms spent their entire larval stage in sediments that differed in contaminant type and concentration.

Another strength of the study is that it examined the response of the individual. Pollution stresses extend from effects on the individual organism and can be important in determining the quantity and quality of a species in an ecosystem (Kosalwat and Knight 1987). Petersen and Petersen (1983) suggested that monitoring changes at the level of the individual organism can be more useful than looking for community changes because individual responses occur before community responses. However, cases where strong community effects are clearly evident may make monitoring at the individual or population level redundant or even impossible. This was demonstrated in my results from the Trenton Channel field site where very few individuals could be collected. Thus, monitoring the response of the individual was no longer valid.

Many studies, including this one, have shown that chironomids are good field indicators of sediment contamination. A good biomonitor must be abundant and ubiquitous, it must be able to survive poorer environmental conditions and it must show

sensitivity to contaminants e.g. deformities or a change in the polytene chromosome structure (Warwick 1990a). *Chironomus* incorporates all of these properties. *Chironomus* exhibits deformities in the range of 20% of individuals examined in the areas studied in the Huron-Erie corridor. This particular genus feeds on fine detritus, which exposes these organisms to a broad variety of hydrophobic contaminants including volatile organics (Hare and Carter 1976) and insecticides (Hamilton and Saether 1971, Warwick 1985) and makes them even more suitable as a biomonitor. My field survey indicated that *Chironomus* was both the most abundant genus, and the genus most sensitive to contaminants; the greatest incidences of deformities were found at sites that were most contaminated, except for the Trenton Channel. *Phaenopsectra* shows a similar trend and should also be considered in future studies.

The laboratory study showed that there is a linear response in the incidence of mentum deformities with increasing proportion of contaminated sediment. To my knowledge, this is the first laboratory study to show such a linear response over a contaminant gradient. Previously, Warwick (1985) found that the severity of deformities was inversely proportional to exposure to DDE, and suggested that there was a graded response at lower concentrations, but increased resistance or mortality occurring at higher concentrations. Warwick examined antennal deformities whereas my study focused on mentum deformities. The hardened mouthparts may be more suitable structures for accurate identification of deformities. Due to their sensory function, antennae may be the first structures to detect and be affected by toxic substances. They may react readily, but then become saturated at higher concentrations of contaminants. Examination of antennae

requires detailed, time consuming methods of slide mounting and fine, accurate examination for deformities at greater magnifications (Dermott 1991). Furthermore, the antennae are more easily damaged during sampling or distorted during mounting than the hardened mouthparts. The mentum shows sensitivity to contaminant levels and it requires less intensive methods, which reduces mounting and screening effort. Therefore, it appears to serve as an excellent indicator of contaminant stress.

My laboratory experiment also attempted to examine morphological deformities and genetic differences in the same individual. There was no association between these two effects. It is possible that by only examining the reduction in the size of the nucleolus that some other genetic anomaly, such as increased RNA synthesis along the chromosome was overlooked. The two assays, morphological and genetic, serve as independent measures of the effects of sediment contamination. Used in conjunction they provide a broad measure of different forms of toxicity.

Limitations of the Study

Sample Size

One problem with field studies and the power of the results I obtained stems from the facts that chironomids have a patchy distribution and there is vast diversity within the family. Therefore, it takes a large sampling effort to obtain sufficient numbers of chironomids to precisely document incidence of deformities in an individual genus. However, my data clearly show that individual genera must be tabulated independently. Furthermore, some sites were polluted to the extent that very few individuals were

collected, indicating that the community was affected to the point of decreased population sizes, making the use of deformities as an indicator of contaminant stress inappropriate. Small sample sizes result in large standard errors and make finding significant differences in incidence of deformities between sites difficult.

In addition to these problems, field studies have proven difficult in attempting to correlate effects of contaminants on field populations (Warwick 1991). This is an advantage of laboratory studies, which can lend themselves to testing for a direct cause-effect relationship, albeit at the expense of loss of realism.

Incidence vs. Index of Severity

Warwick's work on antennal deformities yielded an index of severity of deformity. I evaluated only the incidence of deformities. Careful examination of mentum deformities suggested that there was no association between the degree of contamination and the severity of mentum deformities. Extremely deformed menta were observed in organisms in the reference sediments as well as those with much higher levels of contaminants. A second concern with indicating severity stems from the fact that there is no substantial information on what these deformities represent to the fitness of the organism affected. Therefore, it would seem unnecessary to index severity until we know whether more deformed larvae have a lower fitness. This is a missing component in the research at this time. It has been suggested that severe deformities can be expected to reduce the survival of larvae afflicted, especially considering the reorganization of tissues and sequence of

events necessary for the larvae to molt through the four instars (Dermott 1991). Cushman (1984) suggested that the mouthpart deformities may affect feeding and consequently growth. Van Urk *et al.* (1992) suggested that there may be a lower mortality rate for deformed larvae because they are less active and consequently less susceptible to predation. Experiments are necessary to examine whether individuals with these deformities experience lower fitness through lower numbers reaching adult stage, differences in reproductive capacities or reduced growth. The presence of these deformities may be an index of overall chemical stress affecting developmental organization. If mentum deformity is simply a surrogate measure of overall developmental or physiological malformation, then it need not itself have a fitness cost. There may be other abnormalities that have a more direct fitness cost, but may be more difficult to detect.

One potential limitation of the laboratory study involved the contaminated sediment chosen to create a gradient. I collected sediment from the Trenton Channel, a site known to contain high levels of a suite of contaminants. This design did not allow me to examine the specific effects of individual chemicals. However, the method I used takes into account the total contaminant burden and the synergistic and antagonistic effects of the various components on the organism. Such responses may be impossible to estimate except by direct observation of a sediment's overall effect on the aquatic ecosystem. Individual chemicals rapidly change their characteristics in the aquatic environment, and interaction among pollutants is common. Individual chemicals are modified and integrated through physical, chemical and biological processes to the extent that the total effect of

a large number of minor pollutants may be as great as, or greater than, that of a single major pollutant (Study of Critical Environmental Problems [SCEP] 1970). It is essential to recognize that organismal responses are the result of many chemicals interacting simultaneously and synergistically. Alternatively, chemicals may compete for binding sites such that less toxic chemicals may ameliorate the effects of more toxic compounds (Vamvakas *et al.* 1992).

Limitations of Methodology

Deformities

There are some problems involved in the proper identification of mentum deformities. Inflated incidences of deformities can result from misidentifying deformities when in actuality it is wear or damage to the structure (breaks) or simply natural variability in morphology. Worn mouthparts result from feeding in coarse sediments. This wear was not identified as a deformity in my studies. Furthermore, abrupt breaks due to damage during mounting or collection were identified as such and therefore reported incidences of deformities are considered to be conservative. Broken teeth with jagged edges were readily distinguishable from deformed structures. Lenat (1993) found that there were only slight differences in final tabulations of deformities when identifying these as Class I deformities (slightly deformed / chipped teeth). Individual variation is also a potential problem. With the addition of an extra, reference site, individual variation was included in the screening process. It is important to document natural or background levels of deformities because deformed individuals do occur in natural

populations living under relatively unstressed environmental conditions.

Chromosomes

The staining technique that I employed limited my ability to examine different aspects of chromosomal structure such as additional puffs (areas of increased RNA synthesis) that may have been produced in response to stress. As a result, reduction in nucleolus size served as my measure of genotoxic effect.

Initially, I was unsuccessful at obtaining good chromosomal preparations from field collected chironomids. Laboratory reared chironomids have been deemed most suitable for such studies (Michailova 1989). However, depending upon the application and the amount of resolution required, field collected chironomids could be useful. It would be fruitful to develop a reliable method of preparing chromosomes from field collected larvae.

One of the reasons that chironomids are attractive biomonitors is the ease with which they are cultured to provide a continuous supply of experimental material. Although my initial intent was to develop self-sustaining populations from the various field sites, I was unable to maintain cultures beyond the first generation. Presumably, this was due to local conditions (air and water supplies) since cultures are apparently easily maintained elsewhere. Cultures received from other laboratories also quickly went extinct, failing to survive beyond one generation. For this reason, larvae for the laboratory studies came from egg masses that were collected 1-2 weeks prior to the initiation of the laboratory experiment. Ideally, I wanted to start with one egg mass and

culture these animals so that individuals were genetically identical. However, after several setbacks this method was deemed impractical.

Options For Future Research

There are alternatives to the use of mentum deformities as indicators of environmental contamination. Some of these alternatives should be considered to complement future studies examining contaminant effects. Growth is one of the best indicators of an organism's response to pollutants since it represents an integration of all physiological processes (Kosalwat and Knight 1987). Several studies have shown that midges reared in heavy metal contaminated sediment show a significant decrease in growth when compared to those in reference sediment (Kosalwat and Knight 1987, Wentsel *et al.* 1977). This type of response is a general one and it would be the earliest, albeit most generalized, indication of contaminant stress. It would be informative to employ a multifactorial approach of a bioassay using *Chironomus*. After exposure to a contaminant gradient, it would be possible to simultaneously examine survival, growth, deformities, and chromosomal differences. These measures would be based on different endpoints, providing additional information, and the result would be a broader indication of contaminant effects.

Field Survey Improvements

Improvements to the field survey would provide a more complete picture of the effects of contaminants on chironomid communities. In addition to *Chironomus*,

Procladius has been identified as a tolerant genus. Warwick (1990b) also reports that there has been a decline in *Chironomus: Procladius* ratios in Laprairie Basin, Quebec since 1969 that coincided with the increase in mentum deformities. This is more of a general survey approach and it would provide information in those areas where few individuals of the genus *Chironomus* were collected, making incidences of deformities inappropriate due to small sample sizes. It may also be informative to examine community diversity in different areas. I measured the incidence of deformities in chironomid genera as an indication of the effects of contaminants on individuals that are apparent before community effects become discernable. The abundant chironomid genera provide an accurate estimate of contaminant stress through incidence of deformities. However, inclusion of rare taxa may prove to be important in areas where community-level effects are already occurring. The loss of rarer taxa instead provide an indication of the successional changes being forced by contaminant stress.

Successional changes could also be determined using the remains of different chironomid genera recovered from the sediments, no longer appearing in the contemporary community (Warwick 1990b). Different genera have different levels of tolerance to chemicals so that those with lower tolerances are eliminated at low contaminant levels. This would allow me to census those genera eliminated from the community and see if more contaminated sites had a larger reduction in the diversity of the original community. Comparing population densities could be another indicator of toxic stress. It would be possible to look at the abundance of individual taxa and not just the elimination of them. The initial stages of degradation are usually indicated by a loss

of some species and a dramatic increase in densities of an already common taxa (Wiederholm 1984b). Finally, a general survey could include looking at the relative abundance of oligochaetes at different study sites. Saether (1970) reported that a decrease in the chironomid fauna was accompanied by an increase in the number of oligochaetes.

Laboratory Study Improvements

Mentum Deformities

Extensive laboratory tests exploring individual chemicals and their effects are instrumental in determining what causes chironomid deformities and at what concentrations of these chemicals chironomids begin to show signs of contaminant stress. It may be possible to examine whether certain classes of compounds produce teratogenic effects, genotoxic effects or both. It may also be useful to examine whether chemicals work synergistically to cause higher incidences of deformities or perhaps it might be more interesting to examine the possibility that chemicals may work in an antagonistic way, thereby preventing some chemicals from being available to produce effects in organisms.

Chromosomal Abnormalities

Laboratory studies dealing with the effects of contaminants on the chromosomal material are also of great importance. There are a number of responses that could provide information on the effect of contaminants on the polytene chromosome structure. In this study, I examined a reduction in the size of the nucleolus. I consider this to be

a relatively general response because it is associated with the production of ribosomal RNA (rRNA). More rigorous laboratory experiments would examine not only nucleolus reduction but also reduction in the size of giant puffs (Balbiani rings). Reduction in the size of these puffs has been attributed to inhibition of RNA synthesis at the level of transcription. A more suitable species to use would be *Chironomus tentans*, which has two nucleoli and one Balbiani ring, all located on separate chromosomes. This species has been used in numerous acute and chronic toxicity tests and the positions of Balbiani rings and the nucleolus have already been described.

One very important area of research that this study did not address is that of the induction of heat-shock and related proteins (hsps). Inducers of this response can either damage cellular proteins directly or they may cause cells to produce stress proteins biosynthetically. Hence, the heat-shock response is actually a response to protein damage or proteotoxicity (Hightower 1993). This shares some similarities with inducible DNA-repair responses in which proteins are induced by stressors that cause DNA damage, genotoxicity (Hightower 1993). Therefore, genotoxic chemicals do not necessarily induce hsps unless they also damage proteins. Future laboratory studies should expose a single species to individual known contaminants followed by a method capable of identifying the induction of heat-shock proteins through increased RNA synthesis. This freshly synthesized RNA could be labelled utilizing *in vivo* autoradiographic methods described by Diez *et al.* (1977) and Ali *et al.* (1993) which use ³H-Uridine. This would readily delineate the newly synthesized areas or puffs of RNA along the chromosome and it would provide the opportunity to link the effect of a specific contaminant to a specific

locus on the chromosome. Another method of labelling could utilize the *in vitro* method of Bentivegna and Cooper (1993) who used methyl green and pyronine, which stains DNA green and RNA pink. The acid-fuschin staining method that I used in this study did not allow me to detect small puffs along the chromosome, which are the result of the uncoiling of a single band.

It may also be possible to perform electrophoretic analysis to identify these newly synthesized proteins directly rather than indirectly through increased RNA synthesis. Then it would be possible to see if the production of specific molecular weight proteins is associated with different stressors. One protein, Hsp 70kD, has been used as an indicator of the toxic effects of hazardous substances in soil invertebrates (Kohler *et al.* 1992).

Caution should be used when choosing the components of the heat shock response to use as molecular biomarkers because some of the components of the response are relatively short-lived. A number of the major hsp's have half-lives of the order of two hours (Hightower 1993), which may be useful for rapid bioassays in a controlled laboratory setting. However, hsp's usually have half-lives of the order of days and would be more appropriate for use as biomarkers in tissue samples from native animal populations. Extensive pilot studies would be essential. It is also important that the same life stages of the organism are used, since certain puffs have been associated with specific stages of development.

Another alternative to such methods would involve looking at chromosome polymorphism, which reflects genetic heterogeneity within and among populations

(Michailova and Petrova 1991). The methods previously discussed look at transient changes. However, such polymorphisms represent changes that occurred in the past and are passed to subsequent generations. When a change in the environment occurs, the population can be preserved because of genotype heterogeneity which ensures viability of individuals under various conditions (Dobzhansky 1970). The study of chromosome polymorphism in differentially stressed populations would clarify the extent to which such polymorphism ensures species adaptation and how far it can produce differentiation of populations. Theoretically, it would be possible to choose a species of holarctic distribution and abundance in different contaminant levels. Michailova and Belcheva (1991) indicated that the resultant polymorphous system in *C. plumosus* can be regarded as a biological system which varies according to the environment. Populations with a high degree of polymorphism will thus have attained considerable adaptiveness and specialization in the varied ecological niches of their environment. At the same time it would prove useful to look for heterozygous inversions, asynapsis or unpairing of homologues probably owing to differences at a molecular level that are not reflected in the banding pattern. It would also be possible to look at the degree of polyteny, which differs as a result of an inhibitory effect of certain contaminants on the replication activity of homologues. It would be possible to look at different populations from differentially stressed areas and determine if there is an increase in the number of inversions or if some populations are characterized by specific inversions.

Another interesting idea would involve looking at the possibility of chironomids having resistance to contaminants. In a laboratory study, one could induce heat shock

proteins with low concentrations of a known inducer and see if survival and growth are increased and deformities and genetic damage are decreased in populations exposed to initial low levels followed by much higher doses. This might give insight into how these organisms deal with stressors in their environment and if the presence of the hsp's confer resistance to chironomids. This has been shown to increase survival in harsh conditions where a mild heat-shock rendered *Chironomus* larvae resistant to subsequent heat exposure which would have otherwise been lethal (Carretero *et al.* 1991). These heat-shock proteins seem to be involved with the acquisition of long-term survival.

Synopsis

I have shown that chironomids are excellent indicators of sediment contamination. They represent a suitable integration of the effects of the biophysical conditions and anthropogenic stresses. Their position at the base of the food chain is such that important changes that occur at this level are likely to be of consequence in levels of community health and structure at higher trophic levels. There does not appear to be a relationship between the mentum deformities (teratogenic) and the reduction of the nucleolus (genotoxic) in the same organism. Therefore, these assays are useful independent measures of the deleterious nature of sediment contamination. The study also identifies that there are many more areas that need to be explored in order to reach a fuller understanding of how these organisms deal with chemicals in their environment.

REFERENCES

- Ali, F., R. Lazar, D. Haffner, K. Adeli. 1993. Development of a rapid and simple genotoxicity assay using a brown bullhead fish cell-line: application to toxicological surveys of sediments in the Huron-Erie corridor. *J. Great Lakes Res.* 19:342-351.
- Alia, M.E., E. Laborda and F.A. Anton. 1992. Mutagenicity-Carcinogenicity as related to teratogenic activity. *Bull. Environ. Contam. Toxicol.* 48:275-281.
- Ashburner, M. 1972. Puffing patterns in *Drosophila melanogaster* and related species, p. 101-151. In W. Beerman [ed.] *Developmental Studies On Giant Chromosomes*. Springer, NY.
- Aziz, J.B., N.M. Akrawi and G.A. Nassori. 1991. The effect of chronic toxicity of copper on the activity of Balbiani rings and nucleolar organizing region in the salivary gland chromosomes of *Chironomus ninevah* larvae. *Environ. Pollut.* 69:125-130.
- Bedard, D., A. Hayton, and D. Persoud. 1992. Ontario Ministry of the Environment Laboratory Sediment Biological Testing Protocol. Water Resources Branch, Ontario Ministry of the Environment. Queen's Printer for Ontario. 26 p.
- Beerman, W. 1952. Chromomeren Konstanz und spezifische Modifikationen der Chromosomenstruktur in der Entwicklung und Organdifferenzierung von *Chironomus tentans*. *Chromosoma (Berlin)* 5: 139-198.
- Beerman, W., and Clever, U. 1964. Chromosome puffs. *Scientific American* 210: 50-58.

- Beerman, W. 1971. Effect of a-amanitin on puffing and intranuclear RNA synthesis in *Chironomus* salivary glands. *Chromosoma* 34: 152-167.
- Bentivegna, C.S. and K.R. Cooper. 1993. Reduced chromosomal puffing in *Chironomus tentans* as a biomarker for potentially genotoxic substances. *Environ. Toxicol. & Chem.* 12:1001-1011.
- Brown, A. E., R.S. Oldham, and A. Warlow. 1980. Chironomid larvae and pupae in the diet of brown trout (*Salmo trutta*) and rainbow trout (*Salmo gairdneri*) in Rutland water, Leicestershire, p. 323-329. In D. A. Murray [ed.] *Chironomidae: Ecology, Systematics, Cytology, and Physiology*. Pergamon Press, NY.
- Carretero, M.T., M.J. Carmona, and J.L. Diez. 1991. Thermotolerance and heat shock proteins in *Chironomus*. *J. Insect Physiol.* 37:239-246.
- Ciborowski, J.J.H., E.C. Hanes and L.D. Corkum. 1991. Standardized materials and procedures for *Hexagenia*, a benthic bioassay organism: application to 21-day sediment bioassays. *Proc. 1991 Ontario Ministry of the Environment Technology Transfer Conference on Environmental Research*. Toronto, ON. II:676-684.
- Ciborowski, J.J.H., E.C. Hanes, and L.D. Corkum. 1992. Standardized rearing materials and procedures for *Hexagenia*, a benthic aquatic bioassay organism: serial dilution bioassay for sediment toxicity testing and organism calibration testing. *Pres. 1992 Ontario Ministry of the Environment Technology Transfer Conference on Environmental Research*. Toronto, Ontario.
- Clever, U. 1967. Control of chromosomal puffing, p. 161-186. In L. Goldstein [ed.]. *The Control of Nuclear Activity*. Prentice-Hall, Englewood Cliffs, NJ.

- Coffman, W.P. and L.C. Ferrington. 1984. Chironomidae, p. 551-652. In R.W. Merrit and K.W. Cummins [eds.]. An introduction to the aquatic insects of North America. 2nd ed. Kendall Hunt Publ. Co., Dubuque, Iowa.
- Crowley, T.E., and E.M. Meyerowitz. 1984. Steroid regulation of RNAs transcribed from the *Drosophila* 68C polytene chromosome puff. *Developmental Biology* 102:110-121.
- Cushman, R.M. 1984. Chironomid deformities as indicators of pollution from a synthetic coal-derived oil. *Freshwater Biology* 14:179-182.
- Day, K.E., R.S. Kirby, and T.B. Reynoldson. 1994. Sexual dimorphism in *Chironomus riparius* (Meighen): impact on interpretation of growth in whole-sediment toxicity tests. *Environ. Toxicol. & Chem.* 13:35-39.
- DePinto, J.V., T.L. Theis, T.C. Young, D. Vanetti, M. Waltman and S. Leach. 1987. Exposure and biological effects of in-place pollutants: sediment exposure potential and particle-contaminant interactions. Clarkson Univ., Env. Eng., Rep. No. 87-9. 75 pp. + appendices.
- Dermott, R.M. 1991. Deformities in larval *Procladius* spp. and dominant Chironomini from the St. Clair River. *Hydrobiologia* 219:171-185.
- DiDomenico, B.J., G.E. Bugaisky, and S. Lindquist. 1982. The heat shock response is self-regulated at both the transcriptional and posttranscriptional levels. *Cell* 31: 593-604.
- Dickman, M., I. Brindle and M. Benson. 1992. Evidence of teratogens in sediments of the Niagara River watershed as reflected by chironomid (Diptera: Chironomidae)

- deformities. J. Great Lakes Res. 18:467-480.
- Dickman, M., Q. Lan and B. Matthews. 1990. Teratogens in the Niagara River watershed as reflected by chironomid (Diptera: Chironomidae) labial plate deformities. Water Pollution Research Journal of Canada 24:47-79.
- Diez, J.L., M. C. Santa-Cruz and P. Aller. 1977. Effects of cyclohexamide on RNA synthesis in *Chironomus* polytene chromosomes. Chromosoma (Berl.) 61:369-379.
- Dobzhansky, T. 1970. Genetics of the Evolutionary Process. Columbia University Press. New York, NY. 505 p.
- Edsall, T.A., B.A. Manny, D.A. Schloesser, S.J. Nichols and A.M. Frank. 1991. Production of *Hexagenia limbata* nymphs in contaminated sediments in the Upper Great Lakes connecting channels. Hydrobiologia 219:353-361.
- Eisler, R. 1986. Chromium hazards to fish, wildlife, and invertebrates: A synoptic review. U.S. Fish and Wildlife Serv., Patuxent Wildl. Res. Center, Laurel, Md 20708. Biol.Rep.85 (1.6).
- Environment Canada and U.S. Environmental Protection Agency. 1988. Upper Great Lakes connecting channels study. Final Report, Volume II. U.S. Env. Protect. Agency, Chicago, IL. 591 p.
- Fox, G.A., B. Collins, E. Hayakawa, D.V. Weseloh, J.P. Ludwig, T.J. Kubiak and T.C. Erdman. 1991. Reproductive outcomes of colonial fish-eating birds: a biomarker for developmental toxicants in Great Lakes food chains. II Spatial variation in the occurrence and prevalence of bill defects in young double-crested cormorants in

- the Great Lakes, 1979-1987. *J. Great Lakes Res.* 17:158-157.
- Furlong, E.T., D.S. Carter and R.A. Hites. 1988. Organic contaminants in sediments from the Trenton Channel of the Detroit River, Michigan. *J. Great Lakes Res.* 14:489-501.
- Giesy, J.P., R.L. Graney, J.L. Newsted and C.J. Rosiu. 1987. Toxicity of in-place pollutants to benthic invertebrates. Interim Rep. to U.S. Env. Protect. Agency, Large Lakes Res. Sta., Grosse Ile, Mich. and Mich. State Univ., East Lansing, MI.
- Gilbertson, M. 1988. Epidemics in birds and mammals caused by chemicals in the Great Lakes, p.133-152. In M.S. Evans, [ed.] *Toxic Contaminants and Ecosystem Health: A Great Lakes Focus*. Wiley and Sons. New York, NY.
- Griffiths, R.W. 1987. Environmental quality assessment of Lake St. Clair in 1983 as reflected by the distribution of benthic invertebrate communities. Aquatic Ecostudies, Ltd. Kitchener, Ontario. Prep for Ontario Ministry of the Environment. 35p.
- Gross, K.J. and A.O. Pogo. 1974. Control mechanism of ribonucleic acid synthesis in eukaryotes. *J. Biol. Chem.* 249:568-576.
- Haffner, G.D., M. Tomczak, and R. Lazar. 1993. Organic contaminant exposure in the Lake St. Clair food web. *Hydrobiologia* 0:1-9.
- Hall, R.H. and D.A. Chant. 1979. Ecotoxicity: responsibilities and opportunities. *Can. Environ. Advisory Council Rep. No. 8*. 33 pp.
- Hamdy, Y. and L. Post. 1985. Distribution of mercury, trace organics and other heavy

- metals in Detroit River sediments. *J. Great Lakes Res.* 11: 353-365.
- Hamilton, A.C., and O.A. Saether. 1971. The occurrence of characteristic deformities in the chironomid larvae of several Canadian lakes. *Can. Ent.* 103:363-368.
- Hanes, E.C., J.J.H. Ciborowski and L.D. Corkum. 1991. Standardized rearing materials and procedures for *Hexagenia*. A benthic aquatic bioassay organism. Semi-annual Report to the Research Advisory Committee, Ontario Ministry of the Environment. 42p.
- Hare, L., and J.C.H. Carter. 1976. The distribution of *Chironomus (s.s)? cucini (salinarius group)* larvae (Diptera:Chironomidae) in Parry Sound, Georgian Bay, with particular reference to structural deformities. *Can. J. Zool.* 54:2129-2134.
- Hart, C.N. and S.L.H. Fuller [ed.]. 1974. *Pollution Ecology of Freshwater Invertebrates*. New York: Academic Press. 389 p.
- Hightower, L.E. 1980. Cultured animal cells exposed to amino acid analogs or puromycin rapidly synthesize several polypeptides. *J. Cell Physiol.* 102: 407-428.
- Hightower, L.E. 1993. A brief perspective on the heat-shock response and stress proteins. *Marine Environmental Research* 35:79-83.
- Hirvenoja, M. and P. Michailova. 1991. *Chironomus melanotus* Keyl and its taxonomical and ecological relations to *C. riihimakiensis* Wulker (Diptera: Chironomidae) *Spixiana* 14:309-320.
- Horgen, P.A. and D.H. Griffin. 1971. Specific inhibitors of the three RNA polymerases from the aquatic fungus *Blastocladiella emersonii*. *Proc. Natl. Acad. Sci. USA.* 68:338-341.

- International Joint Commission. 1987. Guidance on characterization of toxic substances problems in areas of concern in the Great Lakes basin. Windsor, Ontario. 179 p.
- Jang, E.B. 1992. Heat shock proteins and thermotolerance in cultured cell line from the Mediterranean fruit fly, *Ceratitis capitata*. Archives of Insect Biochem. Physiol. 19:93-103.
- Kalter, H. 1971. Correlation between the teratogenicity and mutagenicity effects of chemicals in mammals. Chem. Mutagens. 1:57.
- Kamrin, M.A. 1988. Toxicology. Lewis Publ., Chelsea, MI.
- Karim, F.D., and C.S. Thummel. 1992. Temporal coordination of regulatory gene expression by the steroid hormone, ecdysone. The EMBO Journal. 11:4083-4093.
- Kauss, P.B. and Y. Hamdy. 1985. Biological monitoring and organochlorine contaminants in the St. Clair and Detroit Rivers using introduced clams *Elliptio complanatus*. J. Great Lakes Res. 11:247-263.
- Keeton, W.T., and J.L. Gould [ed.] Biological Science, 4th edition. 1986. W.W. Norton & Company Inc., New York, NY. 1175p.
- Knight, A.W. 1984. The evaluation of contaminated sediments employing selected benthic freshwater invertebrates. Final Report of the USEPA Cooperative Agreement No. CR-808424, University of California, Davis, CA. USEPA, Environmental Research Laboratory, Corvallis, OR.
- Köhn, T. and C. Frank. 1980. Effect of thermal pollution on the chironomid fauna in an urban channel, p. 187-194. In D.A. Murray [ed.] Chironomidae: Ecology, Systematics, Cytology and Physiology. Pergamon Press, Oxford, U.K. 354 p.

- Kohler, H.R., R. Trielskorn, W. Stocker, P.M. Kloetzel, and G. Alberti. 1992. The 70 kD heat shock protein (hsp 70) in soil invertebrates: a possible tool for monitoring environmental toxicants. *Arch. Environ. Contam. Toxicol.* 22:334-338.
- Kosalwat, P. and A.W. Knight. 1987. Chronic toxicity of copper to a partial life cycle of the midge *Chironomus decorus* *Arch. Environ. Contam. Toxicol.* 16:283-90.
- Kovats, Z.E., and J.J.H. Ciborowski. 1989. Aquatic insect adults as indicators of organochlorine contamination. *J. Great Lakes Res.* 15:623-634.
- Larsson, P. 1984. Transport of PCBs from aquatic to terrestrial environments by emerging chironomids. *Environ. Pollution* 34:283-289.
- Leach, J.H. 1980. Limnological sampling intensity in Lake St. Clair in relation to distribution of water masses. *J. Great Lakes Res.* 6:141-145.
- Lenat, D.R. 1993. Using mentum deformities of *Chironomus* larvae to evaluate the effects of toxicity and organic loading in streams. *J. N. Am. Benthol. Soc.* 12:265-269.
- Lincoln, R.J., G.A. Boxshall, and P.F. Clark. 1988. *A Dictionary of Ecology, Evolution and Systematics.* Cambridge University Press, NY. 298 p.
- Maier, K.J., P. Kosalwat and P.W. Knight. 1990. Culture of *Chironomus decorus* (Diptera: Chironomidae) and the effect of temperature on its life history. *Environ. Entomol.* 19:1681-1688.
- Manny, B.A., T.A. Edsall and E. Jaworski. 1988. The Detroit River, Michigan: an ecological profile. *U.S. Fish. Wildl. Serv. Biol. Rep.* 85(7.17). 86p.

- Manny, B.A. and D. Kenaga. 1991. The Detroit River: Effects of contaminants and human activities on aquatic plants and animals and their habitats. *Hydrobiologia* 219:269-279.
- Martin, J. 1962. Interrelation of inversion systems in the midge *Chironomus interinctus* (Diptera: Nematocera). *Austral. J. Biol. Sci.* 15:666-73.
- Martin, J. and W. Wulker. 1971. Inversion polymorphism in *Chironomus staegeri* Lundbeck. *Can. J. Cytol.* 13:306-321.
- Michailova, P. 1985. Method of breeding the species from the family Chironomidae, Diptera in experimental conditions. *C. R. Acad. Bulgare Sci.* 38:1179-1181.
- Michailova, P. 1989. The polytene chromosomes and their significance to the systematics and phylogeny of the family Chironomidae, Diptera. *Acta. Zool. Fennica* 186: 1-107.
- Michailova, P. and N. Petrova. 1991. Chromosome polymorphism in geographically isolated populations of *Chironomus plumosus* L. (Chironomidae: Diptera). *Cytobios* 67:161-175.
- Michailova, P. and R. Belcheva. 1990. Different effect of lead on external morphology and polytene chromosomes of *Glyptotendipes barbipes* (Staeger) (Diptera: Chironomidae). *Folia Biol. (Krakow)* 38: 83-88.
- Near, J.C., D.P. Easton, D.S. Rutledge, D.P. Dickinson and R. Spotila. 1990. Heat shock protein 70 gene expression in intact salamanders (*Eurycea bislineata*) in response to calibrated heat shocks and to high temperature encountered in the field. *J. Exp. Zool.* 256:303-314.

- Nevstad, P.N. 1979. Sister chromatid exchanges and chromosome aberrations induced in human lymphocytes by the cytostatic adriamycin in vivo and in vitro. In Genetic Damage in Man Caused by Environmental Agents. Academic Press Inc. Ohio Environmental Protection Agency. 1987. Biological criteria for the protection of aquatic life. Vol. 1. The role of biological data in water quality assessment. Division of Water Quality Monitoring and Assessment, Surface Water Section, Columbus, Ohio. 44p.
- Oliver, D.R. 1971. Life history of the Chironomidae. Ann. Rev. of Entomol. 16:211-230.
- Oliver, D.R. and M.E. Roussel. 1983. The insects and arachnids of Canada. Part II. The genera of larval midges of Canada. Diptera: Chironomidae. Agric. Can. Publ. 1746: 263pp.
- Othoudt, R.A., J.P. Giesy, K.R. Grzyb, D.A. Verbugge, R.A. Hoke, J.B. Drake, and D. Anderson. 1991. Evaluation of the effects of storage time on the toxicity of sediments. Chemosphere 22:801-807.
- Pelling, C. 1959. Chromosomal synthesis of ribonucleic acid as shown by the incorporation of uridine labelled with tritium. Nature 184: 655-656.
- Pelling, C. 1964. Ribonukleinsäure-synthese der riesenchromosomen. Autoradiographische untersuchungen an *Chironomus tentans*. Chromosoma 15: 71-122.
- Pelling, C., and W. Beerman. 1966. Diversity and variation of the nucleolar organizing regions in chironomids. Nat. Cancer Inst. Monog. 23: 393-408.

- Pesch, G.G., C.E. Pesch and A.R. Malcolm. 1981. *Neanthes arenaceodentata*, a Cytogenic Model for Marine Genetic Toxicology. *Aquat. Toxicol* (1981):301-311.
- Petersen, L.B.M., and R.C. Petersen. 1983. Anomalies in hydropsychid capture nets from polluted streams. *Freshwater Biol.* 13:185-191.
- Pinder, L.C.V. 1983. The larvae of Chironomidae (Diptera) of the holarctic region - Introduction. *Ent. Scand. Suppl.* 19:7-10.
- Pinder, L.C.V. 1986. Biology of freshwater Chironomidae. *Annu. Rev. Entomol.* 31:1-23.
- Prevosti, A. 1974. Chromosome inversion polymorphism in the Southwestern range of *Drosophila subobscura* distribution area. *Genetica* 45:111-124.
- Pugsley, C.W., P.D.N. Hebert, G.W. Wood, G. Brotea and T.W. Obal. 1985. Distribution of contaminants in clams and sediments from the Huron-Erie corridor. I-PCBs and Octachlorostyrene. *J. Great Lakes Res.* 11: 275-289.
- Reynoldson, T.B. 1987. Interactions between sediment contaminants and benthic organisms. *Hydrobiologia* 149: 53-66.
- Rothfels, K.H., and R.W. Dubar. 1953. The salivary gland chromosomes of the black fly *Simulium vittatum* Zett. *Can. J. Zool.* 31:226-241.
- Rosiu, C.J., J.P. Giesy, and R.G. Kreis Jr. 1989. Toxicity of sediments in the Trenton Channel, Detroit River, Michigan to *Chironomus tentans* (Insecta: Chironomidae). *J. Great Lakes Res.* 15:570-580.
- Saether, O.A. 1970. A survey of the bottom fauna in lakes of the Okanagan Valley, British Columbia. *Fish. Res. Bd. Can. Tech. Rep.* 196: 1-33.

- Sokal R.R, and J. Rohlf. 1981. Biometry. W. H. Freeman and Company, NY. 859 p.
- Study of Critical Environmental Problems (SCEP). 1970. Man's Impact on the Global Environment. Institute of Technology Press. Boston, MS. 319 p.
- Sudharsan, R.J. and J.A. Heddle. 1980. Simultaneous detection of chromosomal aberrations and sister chromatid exchanges. Experience with DNA intercalating Agents. Mutation Res. 78:253-260.
- Suns, K., G. Crawford and D. Russell. 1985. Organochlorine and mercury chloride residues in young-of-the-year spottail shiners from the Detroit River, Lake St. Clair, and Lake Erie. J. Great Lakes Res. 11:347-352.
- Tamarin, R. 1991. Principles of Genetics. William C. Brown Publishers, Dubuque, IA. 607p.
- Thornley, S., and Y. Hamdy. 1984. An Assessment of the bottom fauna and sediments of the Detroit River. Ontario Min. Environ., Toronto. 48 p.
- Timberlake, W.E., L. McDowell and D.H. Griffin. 1972. Cyclohexamide inhibition of the DNA-dependent RNA polymerase I of *Achlya bisexualis*. Biochem. Biophys. Res. Comm. 46: 942-947.
- Titmus, G., and R.M. Badcock. 1980. Production and emergence of chironomids in a wet gravel pit. p.299-305. In D.A. Murray [ed.] Chironomidae: Ecology, Systematics, Cytology, and Physiology. Pergamon Press, NY.
- Townsend, B.E., S.G. Lawrence and J.F. Flannagan. 1981. *Chironomus tentans Fabricius*, p. 109-142. In S.G. Lawrence [ed.] Manual for the culture of selected freshwater invertebrates. Department of Fisheries and Oceans, Ottawa.

- Unuma, T., R. Senda, M. Muramatsu. 1973. Mechanism of nucleolar segregation differences in effects of actinomycin D and cyclohexamide on nucleoli of rat liver cells. *J. Elect. Microscopy* 22: 205-216.
- Van Urk, G., F.C.M. Kerkum, and H. Smit. 1992. Life cycle patterns, density, and frequency of deformities in *Chironomus* larvae (Diptera: Chironomidae) over a contaminated sediment gradient. *Can. J. Fish. Aquat. Sci.* 49:2291-2299.
- Warwick, W.F. 1980. Paleolimnology of the Bay of Quinte, Lake Ontario: 2800 years of cultural influence. Fisheries and Oceans Canada, *Can. Bull. Fish. Aquat. Sci.* 206:1-117.
1985. Morphological abnormalities in Chironomidae (Diptera) larvae as measures of toxic stress in freshwater ecosystems: indexing antennal deformities in *Chironomus* Meigen. *Can J. Fish. Aquat. Sci.* 42:1881-1914.
1988. Morphological deformities in Chironomidae (Diptera) larvae as biological indicators of toxic stress, p.281-320. In M.S. Evans (ed.) *Toxic Contaminants and ecosystem health: a Great Lakes focus*. Wiley and Sons, New York, NY.
1989. Morphological deformities in larvae of *Procladius* Skuse (Diptera: Chironomidae) and their biomonitoring potential. *Can J. Fish. Aquat. Sci.* 46:1255-1271.
- 1990a. The use of morphological deformities in chironomid larvae for biological effects monitoring. *Environ. Can. Inland Waters Dir. Sci. Publ. Ser.* No.173:1-34.
- 1990b. Morphological deformities in Chironomidae (Diptera) larvae from

- Lac St. Louis and Laprairie Basins of the St. Lawrence River. *J. Great Lakes Res.* 16: 185-208.
1991. Indexing deformities in ligulae and antennae of *Procladius* larvae (Diptera:Chironomidae): Application to contaminant-stressed environments. *Can. J. Fish. Aquat. Sci.* 48:1151-1166.
- Warwick, W.F., J.Fitchko, P.M. McKee, D.R. Hart, and A.J. Burt. 1987. The incidence of deformities in the *Chironomus* Spp. from Port Harbour, Lake Ontario. *J. Great Lakes Res.* 13:88-92.
- Warwick, W.F., and N.A. Tisdale. 1988. Morphological deformities in *Chironomus*, *Cryptochironomus*, and *Procladius* larvae (Diptera: Chironomidae) from two differentially stressed sites in Tobin Lake, Saskatchewan. *Can. J. Fish. Aquat. Sci.* 45:1123-1144.
- Wentzel, R., A. McIntosh, and G. Atchison. 1977. Sublethal effects of heavy metal contaminated sediment on midge larvae (*Chironomus tentans*). *Hydrobiologia* 56:153-156.
- White, D.S., J.Bowers, D.Jude, R. Moll, S. Hendricks, P. Mansfield, and M. Flexner. 1987. Exposure and biological effects of in-place pollutants (bioassays). Interim Rep. to the U.S. Env. Protect. Agency, Large Lakes Res. Sta., 9311 Groh Road, Grosse Ile, MI 48138, USA.
- Wiederholm, T. [ed.]. 1983. Chironomidae of the holarctic region. Keys and diagnoses. Part I. Larvae. *Entomol. Scand. Suppl.*, 19: 457pp.
- Wiederholm, T. 1984a. Incidence of deformed chironomid larvae (Diptera:

Chironomidae) in Swedish lakes. *Hydrobiologia* 109:243-249.

Wiederholm, T. 1984b. Responses of Aquatic Insects to Environmental Pollution, p. 508-557. In V.H. Resh and D.M. Rosenberg [ed.] *The Ecology of Aquatic Insects*. Praeger Publishers, New York, NY.

Wolstenholme, D.R. 1965. The distribution of DNA and RNA in salivary gland chromosomes of *Chironomus tentans* as revealed by fluorescence microscopy. *Chromosoma* 17: 219-229.

Vamvakas, A.,A. Dosescu-Pascalau, G.D. Haffner, and M. Dufresne. 1992. Comparison of aryl hydrocarbon hydroxylase induction by polychlorinated biphenyls in human and murine hepatoma cells. Pres. 1992 International Association of Great Lakes Research (IAGLR).

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