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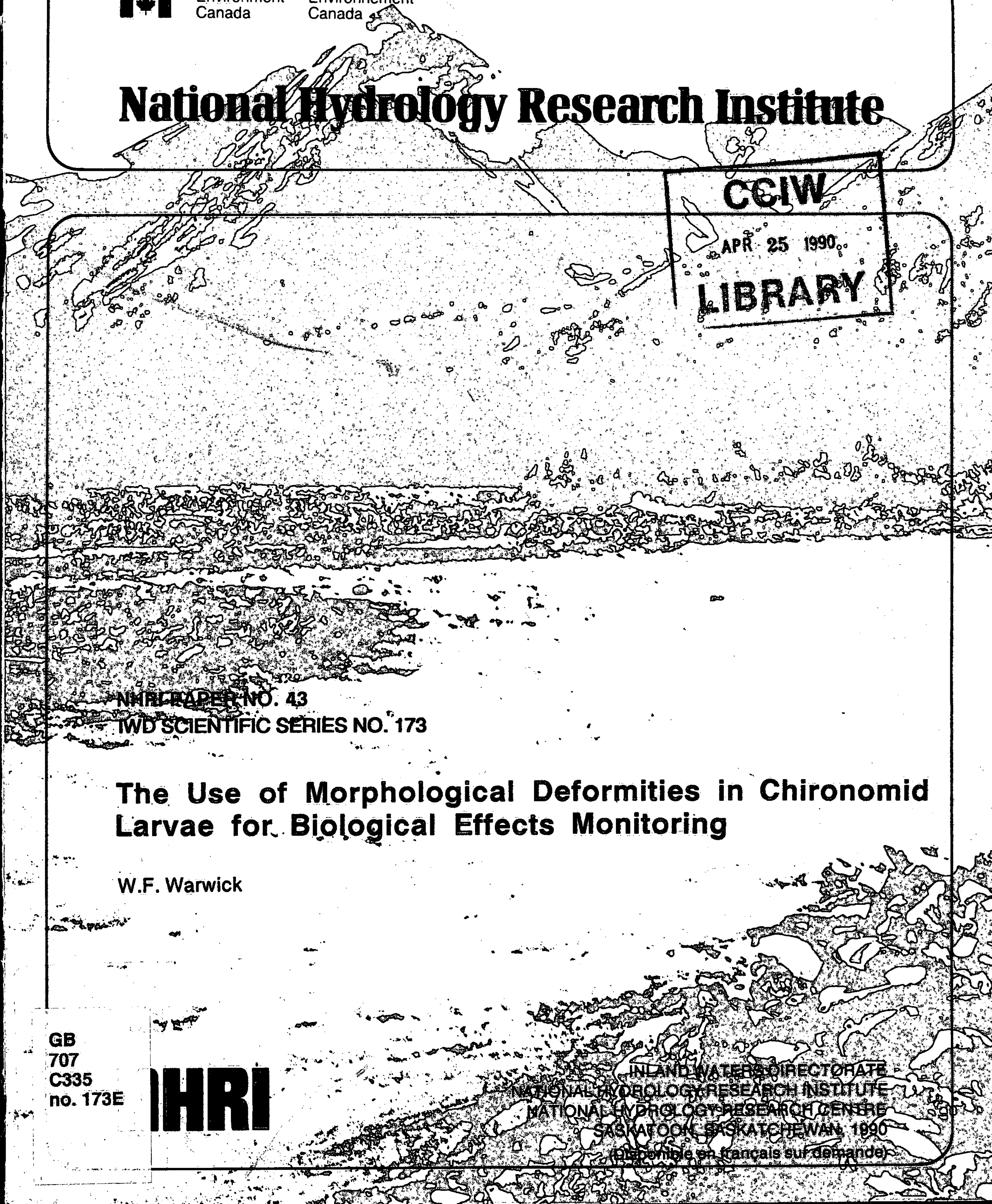

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The Use of Morphological Deformities in Chironomid Larvae for Biological Effects Monitoring

W.F. Warwick

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NATIONAL HYDROLOGY RESEARCH INSTITUTE
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SASKATOON, SASKATCHEWAN, 1990
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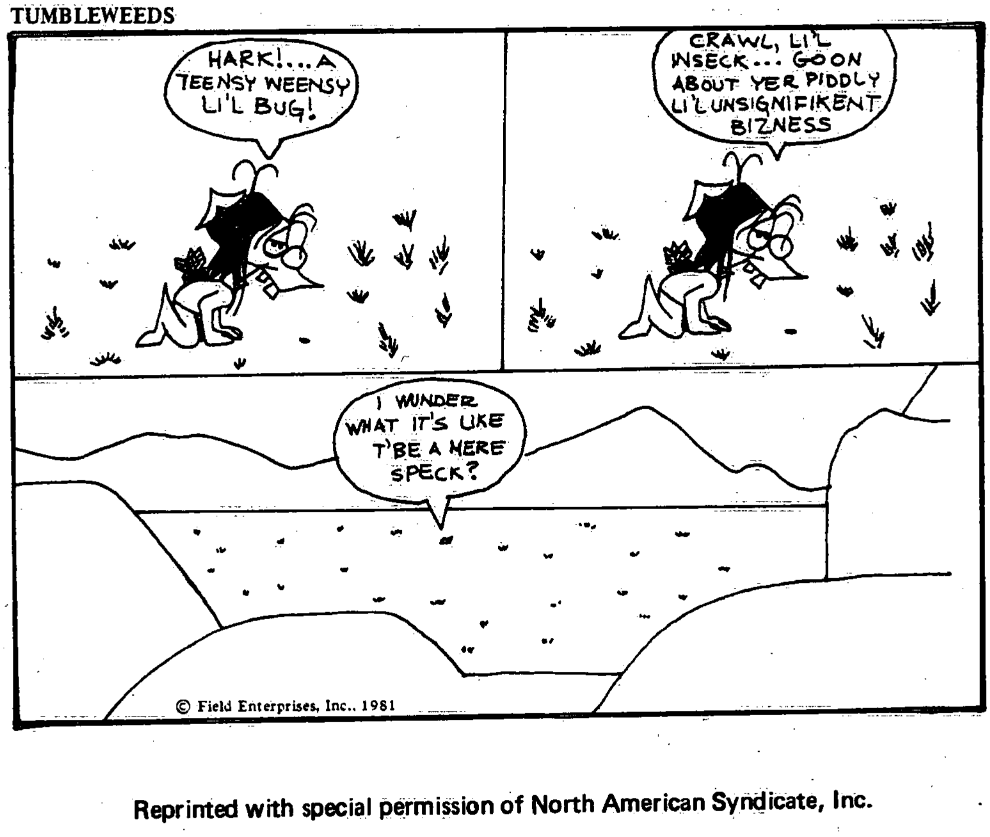

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Abstract

Biological communities provide a direct means of observing the impact of contaminants because they are exposed to, and directly involved in, the transformations that contaminants undergo in freshwater ecosystems. The response of these communities provides a direct measure of the net toxic burden impacting an ecosystem. The purpose of this paper is to review the role of chironomids (Diptera: Chironomidae) in aquatic communities and, in particular, the utility of morphological deformities in chironomid larvae for detecting and assessing the significance of contaminants in freshwater ecosystems.

Résumé

C'est par le biais des communautés biologiques que l'on peut observer directement les effets des contaminants car elles sont exposées aux transformations que subissent les substances dans les écosystèmes d'eau douce et y contribuent directement. Les réactions de ces communautés permettent de mesurer directement la charge nette de substances toxiques agressant un écosystème. L'objet de ce rapport est d'étudier le rôle des chironomidés (Diptera : Chironomidae) dans les communautés aquatiques et, plus particulièrement, d'examiner la possibilité d'avoir recours aux anomalies morphologiques des larves de ces insectes pour déceler la présence des matières contaminantes et évaluer l'importance de leurs incidences sur les écosystèmes d'eau douce.

Executive Summary

Contamination of the environment by chemical wastes poses one of the most serious threats to the quality of life in Canada and the world today. Through use, waste, and discard, an immense amount of chemicals in some form or other is entering the environment and threatening the stability of the ecosphere (Hall and Chant, 1979).

The more traditional toxicology methods have largely proven inadequate to meet the challenge posed by contaminants in freshwater ecosystems (Hall and Chant, 1979). According to NRCC (1985), "there are no methods available that are widely accepted and reliable for determining the degree of impact of [these] chemical contaminants on free-living organisms." More and more, emphasis is shifting towards an ecological approach to measuring the health of freshwater ecosystems where "even the least effective, crudest ecological methods are superior to pipe and technology-based standards for protecting the environment. Ecological methods are the only methods that have a feedback loop from the system being protected, based on ecological qualities" (Cairns, 1986). Without a means of determining environmental response to contaminant stress directly, preservation and control of the quality of freshwater resources will never be effective.

Biological communities provide a direct means of observing the impact of contaminants because they are exposed to, and directly involved in, the transformations that contaminants undergo in freshwater ecosystems. The responses of these communities provide a direct measure of the net toxic burden impacting an ecosystem. The purpose of this paper is to review the role of chironomids (Diptera: Chironomidae) in aquatic communities and, in particular, the utility of morphological deformities in chironomid larvae for detecting and assessing the significance of contaminants in freshwater ecosystems.

Chironomids possess a number of advantages that make them particularly suitable for accessing the highly complex and dynamic world of the freshwater ecosystem. Their advantage lies in the structure and characteristics of the family Chironomidae, which, in large measure, integrates the key features of almost every type of freshwater ecosystem. These advantages can be summarized as follows:

1. *Cosmopolitan distribution*. The worldwide distribution of the family accords them the potential to be a truly international, intercontinental biomonitoring group.
2. *Family size*. The family represents the largest family of aquatic insects, and the genera composing the family seem ubiquitous and similar worldwide. This makes possible comparison of results on both micro-scales (site-specific) and macro-scales (regional, national, or international).
3. *Habitat diversity*. Chironomids inhabit virtually every type and condition of aquatic habitat and represent all functional groups within these habitats.
4. *Environmental sensitivity*. Chironomids display an exceptionally wide range of sensitivity to environmental parameters, which means that the key features of the ecosystem will be integrated either at the individual or family level.
5. *Environmental utility*. Chironomid communities have already been used successfully in the lake classification system to define the trophic state of lakes.
6. *Food chain relationships*. As a primary source of food for fish and ducks, chironomids form a primary link in the transmission of contaminants from sediments to the higher levels of the food chain.
7. *Life history stages*. Chironomids are exposed to contaminants throughout the longest, most critical, and metabolically active stage of their life cycle — the larval stage.
8. *Life cycle length*. The larval stage is long enough (typically about 11 months under Canadian conditions) to provide a good reading of environmental conditions and yet short enough to be manageable in practical terms.
9. *Preservation of larval remains*. The head capsules of larvae preserve well in sediments and leave a permanent record of past environmental conditions in time frames ranging from years to millenia.

10. *Palaeo-reconstruction of environmental change.* Pre-impact conditions can be reconstructed from chironomid subfossil assemblages to provide target goals for mitigation and reclamation procedures.

11. *Culture experiments.* Some species (e.g., *Chironomus tentans* Fabricius) are easily cultured in the laboratory and can be used in dose-response experiments to validate and calibrate responses observed in field populations.

A number of reservations concerning the suitability of the chironomid family as environmental indicators have been voiced. These arise primarily from the daunting size and complexity of the family and the lack of detailed autecological information on individual species. These may not prove as disadvantageous in the long run as might be expected, as evidenced in the following arguments.

1. *Taxonomic complexities.* The size of the chironomid family is truly daunting for the uninitiated, but identification keys are rapidly improving and, as long as distinct types can be separated, an immense amount of useful information can be derived without having to resort to definitive identifications.

2. *Life history and ecological requirements.* Morphological studies of individual responses to contaminants do not immediately require a detailed understanding of these requirements. It is the quality of the environment that is important; all other parameters are already integrated by the animal.

3. *Generic responses.* At the present time, it appears that different genera respond differently to different contaminants. The reason for this is not known, but this fact may, in the long run, turn out to prove advantageous in discriminating between different chemicals or classes of chemical contaminants.

Morphological deformities in individual chironomid larvae possess a number of advantages for detecting and assessing the impact of contaminants in aquatic ecosystems. These are as follows.

1. *Role of the individual.* Individual larvae represent the most basic unit of biological communities. They integrate into a single whole the lower levels of biological organization (molecular, cellular, and organ) and form the building blocks for the higher levels of organization (populations, communities, and ecosystems).

2. *Early warning indicator.* All toxic effects begin with interactions between a contaminant and an individual organism. Because individual responses occur before population and community responses, changes that can be detected at the level of the individual organism are likely to be more sensitive indicators than changes observed at the higher levels of organization.

3. *Permanency of response.* Morphological deformities represent direct physical responses to contaminants that cannot be modified or altered by sampling or other types of stress.

4. *Archivability.* Properly prepared slide-mounted specimens provide a permanent record of contaminant/individual interactions that can be archived for future reference and exchanged between laboratories for interlaboratory comparisons. Permanent collections archived for future review and comparison are particularly important in monitoring programs and in programs where mitigation/reclamation procedures are being implemented.

5. *Preservability.* Chironomid remains preserve well in sediments. Preservation records not only morphological responses to contaminants, but also fundamental long-term changes to population and community structure.

Morphological deformities probably will prove most useful in three areas of biological effects monitoring: detection, assessment, and determination of the success of mitigation. A fourth use — the identification of specific contaminants or classes of contaminants — remains a tantalizing, if not more difficult, possibility at this time.

1. *Detection.* One of the most critical steps in resolving a problem is recognizing that a problem exists. Detection must be done with a broad enough brush to be efficient and yet at a fundamental enough level to be effective for early warning. The chironomid family meets both these criteria.

2. *Assessment*. Where single contaminants are present, very specific tests can be used to determine effect. Unfortunately, it is more often the case that many chemicals are present, interacting with one another in such a way that specific tests are inappropriate, if not impossible, to conduct. Chironomid communities integrate these interactive effects and provide a measure of the "total net toxicity" impacting the ecosystem.

3. *Determination of the success of mitigation*. Chironomid communities are important in this often neglected area of research in two ways. First, conditions prior to defilement by contaminants can be reconstructed, using palaeo-techniques, to provide target goals for remedial work. Because they lack preservability, soft-bodied animals do not offer this advantage. Second, because most first instar larvae are planktonic, chironomid larvae can rapidly recolonize an ecosystem as environmental conditions improve.

4. *Identification*. The ability to identify specific contaminants or classes of contaminants is a highly desirable objective, but one that may be difficult to achieve. There are encouraging signs that the potential is there (e.g., the palmate structures on the antennae of larvae from Tobin Lake), but such specificity will probably be difficult to achieve. Specificity of response will probably be achieved through the mosaic of responses shown by larval communities rather than single, contaminant-specific morphological responses.

Morphological deformities in chironomid larvae show considerable potential for monitoring the biological effects of contaminants, but the techniques for using these responses to contaminants are still very much in the developmental stages. Standard methods for the preparation and mounting of specimen material have been detailed, and a system for numerically quantifying the severity of deformities in larval antennae has been proposed (Warwick, 1985). Analysis of populations from uncontaminated sites and sites of differing levels of contamination is continuing in order to define the range of deformation attainable in other morphological structures of the head capsule. Initial information suggests that, at higher concentrations, the antennae are no longer able to respond and that response shifts to other, perhaps less sensitive, hard parts of the head capsule, such as the teeth and mandibles. The information derived from these structures will be used to refine diagnostic capabilities of the technique and will be included in a comprehensive Index of Total Morphological Response for individual larva.

It is impossible at this point to tell how far the technique can be developed and refined, but there are certain very definitive statements that can be made. Morphological analyses meet, in part or in whole, the three broad criteria listed by NRCC (1985) for biological monitoring tests: relevance, transferability, and refinement and development.

1. *Relevance*. There can be no doubt about the relevance of deformed larvae in the environment. The hypothesis linking morphological deformities with contaminants originated with the observation that deformed larvae occurred in areas receiving industrial and/or agricultural contaminants, but not in areas receiving domestic sewage. The presence of deformed larvae, particularly those displaying massive and grotesque malformation, clearly indicates that environmental conditions have been seriously degraded by chemical contaminants.

2. *Transferability*. Permanent slide mounts of specimen material from different areas are easily exchanged for interlaboratory comparison and calibration. These slide mounts also provide a permanent record of community responses that is archivable and readily available for future reanalysis and comparison.

3. *Refinement and development*. Although there is still considerable work to be done on refining the technique, this in no way detracts from the obvious benefits of starting a pilot program at the present time. Although the full potential of such a program may not be realized immediately, the materials prepared for such a program would not be lost, but would always be available for reanalysis as techniques are further refined. Such a program, even if introduced on a limited scale, would go a long way in redressing the perennial problem of lack of background data. To put it another way, we must start somewhere

and better now than later. The introduction of a pilot program would involve other workers and do much to develop the technical expertise required. It would also greatly speed and assist in expanding the data base on morphological, temporal, and spatial variation and in realizing the full potential of the technique. Field data should also be validated and calibrated by comparison with responses induced in larvae exposed under controlled conditions to chemicals of known teratogenicity.

This paper was prepared in response to a request from management of the National Hydrology Research Institute. It provides information on a number of practical considerations ranging from sampling techniques to analysis and report preparation. Also included are some thoughts and suggestions on staffing requirements, comparisons with other techniques, and directions for future research.

Although the techniques for using these physical responses are still much in the developmental stage, there is no arguing the relevance and environmental importance of morphological deformities and their relationship to contaminants. At its most fundamental, the presence of large numbers of deformed larvae signal that something is radically wrong with their environment. Since chironomid larvae form an integral link in aquatic food chains, and since humans are intimately and ultimately linked to these same systems, it is important that we heed the warning that these morphological responses convey.

Preface

To illustrate the extent and urgency of the problem facing Canadians today by contaminants in their environment, I quote the opening remarks made by Dr. Ian McTaggart-Cowan in Hall and Chant's (1979) report to the Canadian Environmental Advisory Council. His words say it all!

> *One of the most daunting environmental problems of our time arises from the flood of man-made chemicals pervading our lives. The products or by-products of our industries, they are in every home in a multitude of forms . . . in variety too numerous to list. The ingenuity of those who have contrived new chemical compounds and devised new ways of inserting them into our economy in useful forms or new processes has had much to do with the improvement of the human state.*
>
> *We have too frequently ignored the other side of the coin. To our distress, we have slowly learned that some of these products are damaging to human health. For some of these, we have developed restrictive legislation which we hope will protect us. But the ultimate fate of every compound is to be discharged via the sewer or the incinerator stack or by accident into the air, the water or onto the land, where singly or in combination they alter the environment. Species are destroyed, lakes and rivers lose their ability to support their normal faunas, vegetation changes; the habitats upon which life forms depend become less suitable as places for plants, animals and man to survive.*
>
> *It is urgent that Canadians clearly grasp the extent and insidiousness of this threat to the livability of our environment. It is imperative that they support the slow, undramatic, costly, perhaps uncomfortable but undeniably essential steps to redress the rapidly accelerating . . . deterioration caused by . . . contaminants in the environment.*

These words were written in 1979. They are as pertinent now as they were then.

The Use of Morphological Deformities in Chironomid Larvae for Biological Effects Monitoring

W.F. Warwick

INTRODUCTION

Ecotoxicity, the defilement of the environment by chemical wastes, is one of the most serious threats to the quality of the Canadian environment today. According to the Canadian Environmental Advisory Council, environmental contaminants pose one of the most serious threats to human and environmental well-being now confronting Canadians. Chemicals and chemical technology dominate all facets of our society, not only through the established chemical industry, but through almost every other industrial and commercial activity. Our technological lifestyles depend heavily on chemicals to the extent that the industry now generates for every inhabitant in North America more than one tonne of chemicals per year, comprising more than 60 000 different substances (Hall and Chant, 1979). Through use, waste, and discard, this immense amount of chemicals eventually ends up in the environment in some form or other. Implicit in society's actions has been the assumption that the environment is infinitely resilient, able to withstand almost any abuse. This assumption is no longer tenable. In recent years, it has become all too apparent that not only is the systematic degradation of the environment undesirable, but the stability of the ecosphere is itself threatened by people's unthinking actions (Goldsmith et al., 1972).

Once in the environment, many chemicals, by their very nature, are rapidly taken up by living organisms. Because of the interrelationships between biological systems, these spread rapidly and pervasively through all living organisms. The spread of toxic chemicals has had a devastating effect on the extremely complex organization of different forms of life and the elaborate mechanisms by which they interact. Many of the more sensitive forms have been reduced or eliminated with consequent effects on the ability of an ecosystem to adjust and maintain its basic features. Toxic chemicals, through their insidious spread and invasive power, have introduced an element of increasing instability into the ecosphere as a whole (Goldsmith et al., 1972).

There is good reason to believe that the more serious aspects of environmental contamination result from the accumulated effects of tens of thousands of contaminants in interaction with one another in the environment. The problem for society is that once a chemical enters the environment, it is impossible to control or contain. Individual chemicals rapidly change their characteristics in the aquatic environment. Interaction among pollutants is more often present than absent. 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 major pollutant (SCEP, 1970); seldom do they act antagonistically to cancel out adverse effects (Regier, 1986). The more serious aspects of environmental contamination, therefore, derive from a multitude of chemicals that interact simultaneously and synergistically, whose effects are long-term, indiscriminate, often irreversible, and that have deleterious effects even at low concentrations.

The effects of subsequent interventions generally are cumulative and degrade the environment further. At present, there are no good means of evaluating cumulative impacts, either from methodological or regulatory points of view (Dayton, 1986). The law generally focuses on project-specific impacts, irrespective of possible cumulative impacts already in effect or that could result from other projects known or projected. Furthermore, most environmental regulations ignore potential additive effects on linked ecosystems over any spatial or temporal scales. A practical result is that people supporting a proposed environmental perturbation can argue that their project would have a negligible effect compared to the background situation, even if the background situation is badly disturbed. Clearly, a more holistic approach is needed.

The adoption of an ecological approach has been encouraged by a number of researchers (Hall and Chant, 1979; Cairns, 1980, 1981, 1983, 1986; Cairns and van der Schalie, 1980; Beanlands and Duinker, 1983; Regier and Grima, 1984). According to Cairns (1986), "even the least effective, crudest ecological methods are superior to pipe and technology-based standards for protecting the environment. Ecological methods are the only methods that have a feedback loop from the system being protected, based on ecological qualities." Without a means of directly determining environmental response to contaminant stress, preservation and control of environmental quality will never be effective.

The purpose of this paper is to review the role of chironomid larvae in aquatic communities and particularly the utility of morphological deformities for detecting and assessing the significance of contaminants in freshwater ecosystems. Biological communities as a whole provide a way to observe the impact of contaminants directly because they are exposed to, and directly involved in, the transformations that contaminants undergo in aquatic environments. Thus, their responses provide a direct measure of the net toxic burden impacting the ecosystem.

There are a wide variety of choices available for a suitable biological indicator, but chironomid larvae possess a number of advantages that make them particularly suitable for accessing the highly complex and dynamic world of the aquatic ecosystem. In addition, morphological deformities in chironomid larvae have shown considerable potential for detecting and assessing the effects of contaminants. The techniques for using these morphological manifestations are still in the developmental stages, but there is no arguing their relevance to environmental contaminants. Even at the most fundamental level, the presence of grotesquely deformed larvae signals that something is fundamentally wrong with their environment. Continuing research has shown that the more subtle morphological responses also correlate with levels of contamination, but these relationships may not always be as straightforward as one might expect.

The purpose of this paper is (1) to present the chironomid as a test animal, (2) to discuss its place and utility in environmental science, (3) to discuss the state of development of the biological screening tool based on morphological deformities, and (4) to assess the prospects for routine application of morphological techniques for assessing the degree and nature of environmental degradation, i.e., the state of health of aquatic environments.

LEVELS OF BIOLOGICAL ORGANIZATION

Biological communities are arranged (Fig. 1) in a hierarchy of seven basic levels of organization to make up the ecosphere: ecosystems, communities, populations, individuals, organs, cells, and molecules. In their potential for ecological monitoring, each level is characterized by inherent strengths and weaknesses.

Figure 1 presents a stress/response matrix (adapted from NRCC, 1985), which shows that

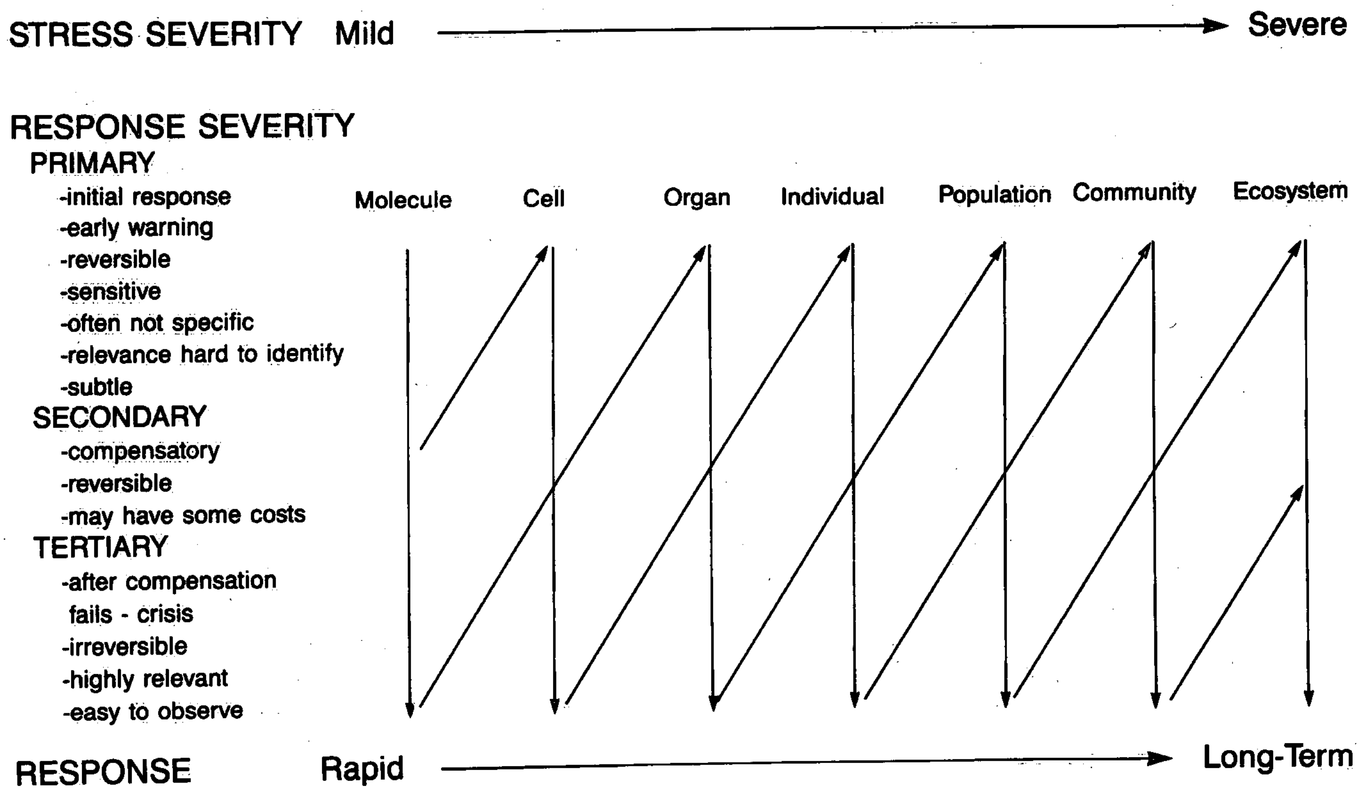

Figure 1. Stress/response matrix. Adapted from NRCC (1985).

(1) a primary response at the individual level will cause few, if any, changes at the population and community level;

(2) a secondary response at the individual level will have some energy cost (e.g., reduced growth rate), which may cause some primary changes in population structure;

(3) a tertiary response at the individual level (e.g., high mortality of a specific age class) may cause significant changes in population structure leading to compensation (e.g., changes in fecundity) and some early changes in community structure (e.g., changes in predator-prey relationships);

(4) a tertiary change in population structure (e.g., extinction of a species) may cause secondary or compensatory community changes (e.g., change in species dominance);

(5) a tertiary change in community structure would represent loss of species and would be equivalent to a complete degradation of the environment (e.g., oligochaete-dominated benthos near sources of organic pollution).

The Ecosystem

At the one extreme, natural ecosystems are complex, highly variable, and regionally differentiated; only they possess the depth and sensitivity of information needed to give a true estimate of the ecological impact of contaminants on the environment. The very complexity of the ecosystem is the stuff and purpose of its stability. Damage to the microcomponents of the system reduce its ability to respond and to recover from chemical degradation. Any sensitive operational tool should be able to determine how many microcomponents of an ecosystem can be damaged before the larger system shows substantive deterioration. An analogy would be: How many rivets can fall out of an airplane before it crashes? Good maintenance is essential if disaster is to be averted. This applies equally well to an airplane and to an ecosystem. We must learn to "inspect all the rivets" if we are to avert environmental disaster.

The difficulty with the ecosystem level is its inherent complexity. Relationships in natural systems are nonlinear, and most are marked by thresholds, limits, and discontinuities. It is small wonder that ecologists have trouble agreeing to unifying themes (Dayton, 1986).

One of the most difficult problems ecologists have is impressing upon legislators, regulators, and others with non-biological backgrounds the difficulties and complexities of natural systems and yet communicating an appreciation for the benefits to be derived from an ecological approach to environmental assessment. As Cairns (1986) states, we seem to err on the one hand by saying that ecosystems are so complex that they defy any meaningful understanding, or that one cannot possibly predict what will happen in an ecosystem as a result of the introduction of a chemical or any particular intervention. On the other hand, we say that one or two simple tests involving a single species will enable us to predict ecosystem health. The "overawed by complexity" attitude is a paralyzing one, inhibiting constructive action, but dependence on a single species or tests with little environmental realism could lead to serious problems when dealing with complex, highly dynamic systems. In that uncomfortable, intellectually unsatisfactory middle ground lies the strategy that will enable us to deal with contaminants in the environment in a satisfactory, responsible manner.

Communities and Populations

Organization at community and population levels is similarly complex and subject to enormous variability. Communities are composed of many populations and both share a number of elementary characteristics (Dayton, 1986). Large numbers of the components (populations and individuals) interact with each other in a variety of ways, including reproduction, intra- and interspecific competition, communication, predation, and mutualism. Sex ratios, age structure, population oscillations, and spatial and temporal relationships, including the degree of environmental heterogeneity and life history patterns, are only a few important variables that affect population and community function.

Traditionally, community/population studies have focused on identifying, enumerating, and listing individual components and have ignored the more complex factors operating at these levels. While descriptive indices are useful, subtle effects on communities and populations may only become evident after long exposure times. Since investigation, diagnosis, and correction of contaminant problems may take even longer, the traditional approach to field studies generally is inadequate to prevent significant environmental damage (NRCC, 1985).

Organ, Cell, and Molecular Levels

At the other extreme, organization at the organ, cell, and molecular levels is similarly complex and highly variable. The number of biochemical responses to stress that could be measured are almost limitless (blood components, enzyme activity, metabolite levels, excretion rates, digestion, neural transmission, etc.), but few have been developed systematically (NRCC, 1985). Physiological, biochemical, and histological measurements presently available have little utility because it has not been possible to link biochemical responses to adverse environmental conditions and the health of populations, communities, and ecosystems, the ultimate yardsticks of response (NRCC, 1985). Biochemical techniques hinge heavily not only on a prior knowledge of the organism's physiology, but also on the chemistry of the contaminant. Both require extensive background information on the chemical and biological structure of the ecosystem, a knowledge of the source and nature of the contaminant, and a knowledge of the other causes of stress on the population.

The Individual

The individual also is a complex entity, subject to enormous variability in its own right and sharing many of the characteristics of populations and communities discussed above. For the purpose of environmental monitoring, however, the individual has a number of advantages. The individual is the first level of life; without the individual, biochemical techniques are of little use and the higher levels of hierarchical arrangement become redundant. The individual is the fundamental component of all life and provides the first point of entry into the living biosphere. Petersen and Petersen (1983) suggested that changes at the level of the individual organism can be more useful than community changes because individual responses occur before community responses. The assumption can be made that all toxic effects on ecosystems begin with some chemical interaction between a contaminant and the individual organism, whether at the organ, cell, or molecular level (NRCC, 1985). Since these interactions are unique and precede all effects at higher levels of organization, response at the level of the individual provides the earliest credible warning of environmental degradation.

BENTHIC COMMUNITIES

Biological communities provide a wide variety of choices for suitable biological indicators of environmental contamination. The challenge remains one of focusing upon those aspects of biological systems that lend some element of order or predictability. Biological communities generally contain critical species or (more importantly) functionally important groups of species that can provide these elements.

While "everything may be connected to everything else," there is abundant evidence from many communities that some species have more important community roles than others (Elton, 1966; Dayton, 1984). In systems where there is evidence that important species exist, they should be studied or at least monitored (Lewis, 1976). There is considerable evidence to support the functional group concept in benthic communities. Dayton (1986) identified a number of functional groups in soft-bottom habitats; these included suspension feeders, deposit feeders, burrowers, and tubicolous organisms. According to Dayton (1986), these groups are not mutually exclusive, but tend to be resistant. By modifying the sedimentary environment, they restrict the recruitment of representatives of other groups and thereby maintain a considerable internal stability. This stability is the key to the successful functioning and well-being of the biosphere.

There is considerable evidence also that soft-bottom benthic communities are exposed to the greater part of the contaminant loading imparted to an aquatic ecosystem. Sediment is a major transport mechanism and ultimate repository for most contaminants. Most chemicals concentrate in the sediments at concentrations many orders of magnitude greater than in the overlying water column. The concentrations of many compounds such as higher molecular weight hydrocarbons (DDT, PCBs, etc.) remain very high in the sediments for years after cessation of input. Because benthic organisms live in the sediments, they are directly exposed to these residues. Contaminants not lethal to these organisms often accumulate in their body tissues and are transferred up the food chain. Because benthic organisms form such a fundamental component of the food chain, they serve as one of the primary links in the transmission of contaminants through the food chain to humans.

Chironomidae as a Functional Grouping

Chironomids form one of the most important functional groups in soft-bottom communities and fulfill all the categories identified by Dayton (1986), including a few additional ones. The exact terminology, based mainly on feeding regime, varies among authors (Oliver, 1971; Smock, 1983; Pinder, 1986), but these categories include (1) deposit feeders, which ingest sediment and detritus (nonliving organic matter, plant or animal, that has begun to be broken down by microconsumers) indiscriminately; (2) omnivores, which selectively ingest detritus, living plant material, and some animals; (3) filter feeders, which live on or in the sediments and plants and which remove algae, detritus, and other suspended matter from the water column; (4) carnivores, which attack and consume other aquatic invertebrates; (5) surface feeders, which feed on materials trapped in the sediment-surface film; (6) leaf-miners, which burrow in and ingest plant materials; and (7) parasitic species, which live symbiotically, commensally, or otherwise on other aquatic invertebrates. Few chironomids appear to be restricted rigidly to a single mode of feeding, but the range of methods is indicative of the functional importance of the family in the soft-bottom environment. Dayton (1986) emphasized the role of soft-bottom communities in cumulative impact assessments because they are exposed to the full impact of contaminants accumulating in the sediments.

Advantages of Chironomidae in the Biological Indicator Role

Chironomidae possess a number of advantages as environmental indicators. Their advantages lie in the structure and characteristics of the family itself, which in large measure integrates the key features of an ecosystem.

1. Cosmopolitan Distribution

The distribution of the family is worldwide. The two species found in Antarctica are the southernmost free-living holometabolous insects known. Chironomids also extend to the northern limits of land and they make up one-fifth to one-half of the total number of species in the Arctic fauna. Between these geographical extremes, they have radiated into nearly every habitat that is aquatic or wet, including peripheral areas of the world's oceans (Oliver, 1971).

Relevance: The cosmopolitan distribution of the Chironomidae gives the family the capacity to be a truly international, intercontinental monitoring group.

2. Family Size

The family Chironomidae is the largest family of aquatic insects. Although there is no reliable estimate of the total number of species in the family, Oliver (1971) estimated that over 5000 species had been described by 1971. According to Ashe (1983), the species within the family Chironomidae are currently divided into 10 subfamilies and 24 tribes. Within these, the genera seem ubiquitous and very similar worldwide.

Relevance: The distribution of species with similar attributes throughout the world makes it possible to compare biological responses on microscales (site-specific) and macroscales (regional, national, or international). According to Cairns (1986), extrapolation between different scales represents a component of the problem of extrapolation from one level of biological organization to another. Justification for extrapolating from a small region to a larger region is difficult enough even when the ecosystem is homogeneous.

3. Habitat Diversity

The range of habitats occupied by the Chironomidae is unparalleled among other insect groups. Chironomids occupy virtually every type and condition of aquatic habitat including the littoral and benthic regions of lakes, ponds, temporary pools, waterfalls, glacial meltwater, fast- and slow-flowing rivers, hot and cold springs, water-filled axils of plants, tree holes, and the water-filled flowers of insectivorous plants. A number of species are also found in marine, brackish water and even in such terrestrial habitats as cow dung. Chironomids are truly ubiquitous and frequently form the most abundant group of insects in freshwater environments.

Relevance: The extraordinary ecological range of the Chironomidae ensures that they will have representatives in all functional groups within their communities (Dayton, 1986).

4. Environmental Sensitivity

As a family, chironomids display an exceptionally wide range of sensitivity to environmental parameters such as dissolved oxygen concentrations, pH, salinity, substrate, water current, depth, food, temperature, dehydration, freezing, and pollution by organic wastes, heavy metals, and contaminants. Some species have narrow ranges of tolerance to certain conditions, while others are very broad. *Protanypus morio* requires high levels of dissolved oxygen, whereas *Chironomus plumosus* can withstand anoxic conditions up to 120 days (Nagell and Landahl, 1978). *Stenochironomus gibbus* is narrowly confined to burrowing in submerged rotten wood, whereas *Chironomus riparius* is tolerant of heavy organic pollution, possibly because of its potentially rapid rate of development (Pinder, 1986). *Polypedilum vanderplankii* can tolerate complete dehydration and remain viable for months (Oliver, 1971). Hard rock and gravels are inhabited predominantly by members of the Orthocladiinae and Diamesinae, whereas members of the Chironominae and Tanypodinae predominate in sands and silts (Pinder, 1986). On the basis of preliminary information, species of *Chironomus* appear to be very sensitive to environmental contaminants, whereas species of *Cryptotendipes* and *Heterotrissocladius* apparently are not (Warwick, 1988).

Relevance: The exceptional range of sensitivity means that the key features of the whole ecosystem will be integrated either at the individual or family level. Regier (1986) suggested that species (or suites of species) that integrate many or most of the biotic and abiotic features and processes of an ecosystem would make good indicators of the relative well-being of that ecosystem.

5. Environmental Utility

The usefulness of chironomid communities in environmental assessment has already been demonstrated in

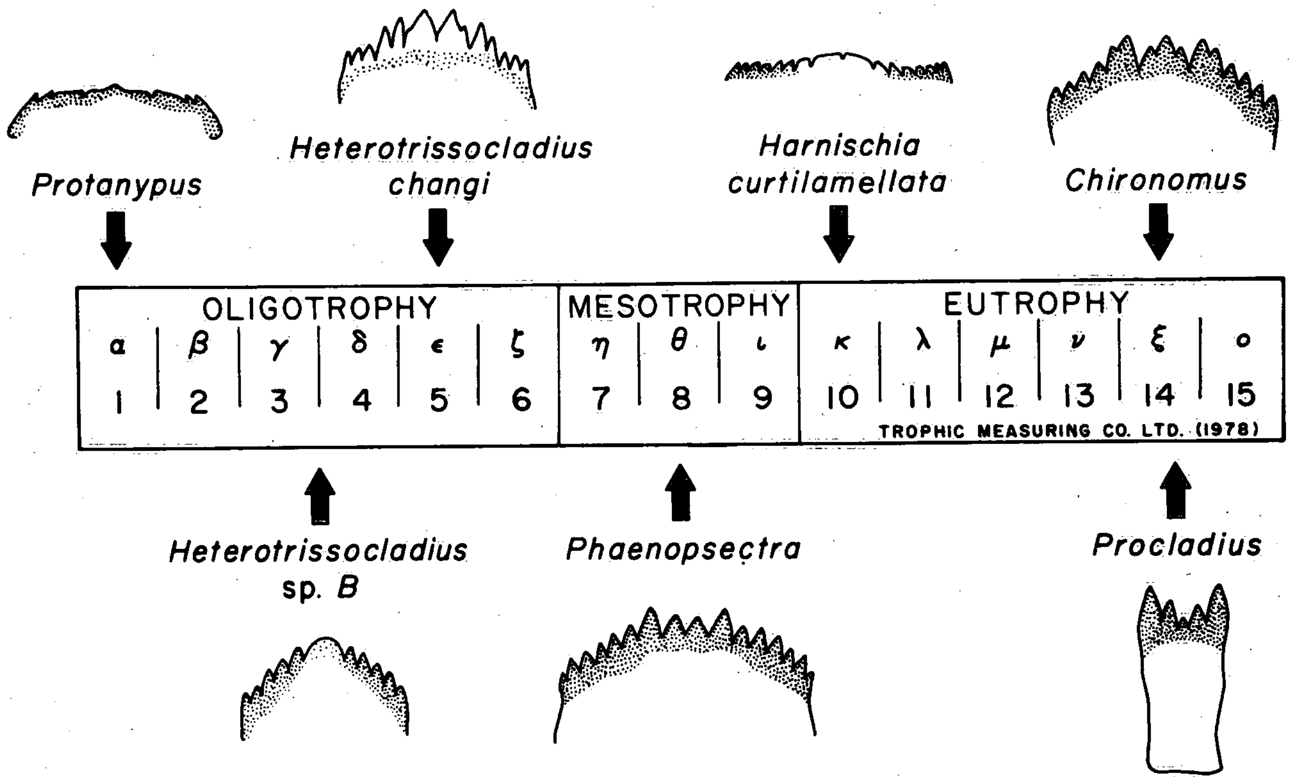

Figure 2. Pictorial representation of the lake classification system based on chironomid community assemblages. After Warwick (1980).

the lake classification system. The development of this system began with the pioneering works of Thienemann (1913, 1922, 1931) and Naumann (1917, 1929) and evolved (Brundin, 1949; Saether, 1975, 1979; Wiederholm, 1976, 1980; Warwick, 1980a) into a relatively sophisticated system for classifying trophic conditions in lakes (Fig. 2). The system is based primarily on the response of chironomid communities to changes in oxygen supply, food availability, temperature, sedimentation patterns, and other environmental parameters that effect changes in the composition and relative abundance of species in the community (Warwick, 1988). Brundin (1956) showed that lake typology based on chironomid communities probably has worldwide validity. Although relatively new to North America, the lake classification system is widely used in Europe to assess and monitor trophic conditions in lakes.

Relevance: The lake classification system demonstrates the operational utility of the Chironomidae in environmental assessment, in particular, the assessment of trophic conditions.

6. Food Chain Relationships

Chironomid larvae play a primary role in the accumulation and transmission of contaminants because they function at a fundamental level in the food chain. Many chironomid larvae are primary grazers and feed directly on organic detritus, on microbially conditioned detritus, or on the microbial communities themselves (Pinder, 1986). Chironomid larvae, in turn, form an integral part of the food base for fish and dabbling or diving ducks.

Relevance: Since the majority of contaminants in sediments are bound to organic materials, chironomid larvae are directly exposed to the effects of contaminants through their food base. Contaminants accumulated in their tissues are quickly transmitted to foraging fish and ducks and, ultimately, to humans. Because of their physical size, chironomid larvae also represent one of the most basic levels of the food chain to be easily studied.

7. Life History Stages

The greater part of the life cycle of chironomids (Fig. 3) is spent in the larval stage, which typically lasts about 11 months in the mid-latitudes of Canada. All energy required to complete the life cycle is built up in the form of body tissue during the larval stage because, with only few exceptions, the adult does not feed. The intermediate pupal stage lasts only a few days, while adults live only from a few days to a few weeks at most (Oliver, 1971). Breeding takes place during the adult stage and the female lays her eggs to begin the cycle again.

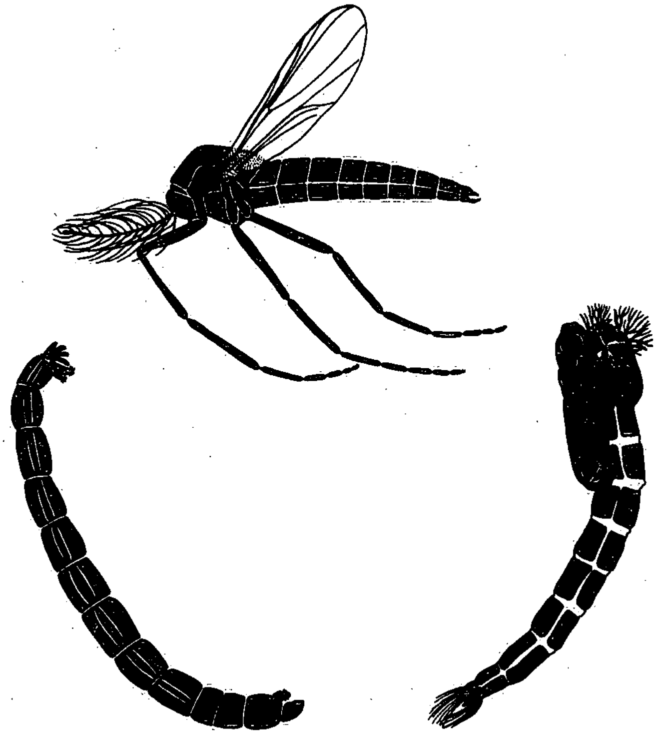

Figure 3. Larva, pupa, and imago (adult) of the family Chironomidae (counterclockwise from lower left).

Relevance: Chironomid larvae are exposed to contaminants throughout the longest, most metabolically active and critical stage of their life cycle.

The eggs are laid in a protective gelatinous matrix, usually close to the female's original emergence site. There is not a great deal of precise information available about the duration of the egg stage. It lasts from a few days to a few weeks and probably is temperature-dependent and related to the overall length of the life cycle of individual species. Upon hatching, larvae develop through four distinct larval instars (five have been reported in some Tanypodinae), which are easily separable by the width or length of the head capsule. First instar larvae usually remain planktonic until a suitable substrate is found.

Relevance: Evidence has shown that contaminants like DDT can be transmitted from the female to the egg with consequent effects on succeeding generations (Derr and Zabik, 1972). The incremental growth of succeeding instars is an important factor in determining the stage of impact and the variability induced by environmental stress (Odum et al., 1979). The planktonic phase is important in the dispersal of species that are not normally free-swimming and in the recolonization of areas where recovery, through natural processes or mitigative intervention, is occurring (Dayton, 1986).

In a suitable habitat, first or second instars establish a mode of life that usually continues throughout the larval stages. Burrowing larvae usually penetrate only the top few centimetres of substrate, whereas surface-dwelling larvae can move freely over the substrate surface. A few species, like *Phaenopsectra*, undertake migration into the water column on a diel cycle (Oliver, 1971).

Relevance: Chironomid larvae, unlike fish, are non-migratory (NRCC, 1985); what movement is undertaken, assists in dispersal rather than avoidance of unsuitable conditions. Since most larvae live in direct contact with the sediments, exposure to recent cumulative contaminant loadings is direct and the effects immediate.

8. Life Cycle Length

The life cycle of chironomids is comparatively short. In temperate latitudes, many species are uni- or bivoltine, but the occurrence of three or more annual generations is not uncommon (Pinder, 1986). Species inhabiting the profundal of deep lakes may require more than a year to complete larval development, whereas, in the high latitudes of the north, larvae may require from two to seven years to develop. Under optimum culture conditions, *Chironomus tentans* larvae require 30-33 days from hatching to reach the prepupal larval stage. Compared to the time scales of other biological species, the length of the life cycle of chironomids represents a manageable time frame for environmental assessment work.

Chemical analyses represent the quality of water at the instant of sampling (Fig. 4). Phytoplankton and zooplankton integrate conditions within the water column over the span of a few days to a few weeks or months.

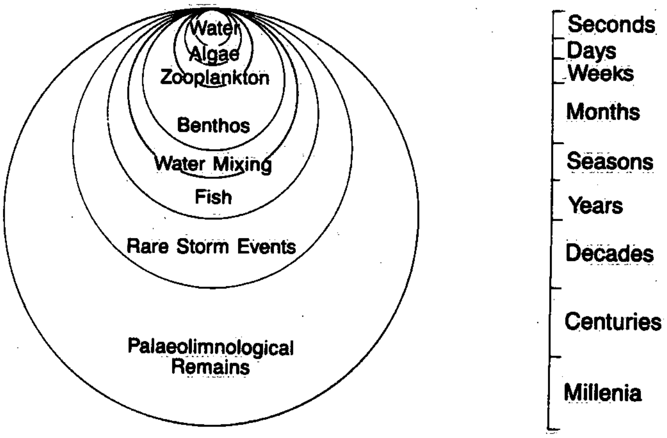

Figure 4. Comparison of temporal cycles involved in physical, chemical, and biological monitoring schemata.

Chironomid larvae integrate conditions in the bottom sediments over periods of months in southerly and intermediate latitudes and annually in more northerly latitudes. The life span of fish is considerably longer and, depending on the species, can span decades. The response time, therefore, is correspondingly long [for example, the development of spinal curvature in some fish species as a result of contaminants may take 5–10 years to become apparent (NRCC, 1985)] and too often these populations are at grave risk even before the problem is identified.

Relevance: The length of the chironomid life cycle is short enough to be manageable for practical purposes, yet rapid enough for good definition and long enough to get an adequate "fix" on environmental conditions.

9. Preservation of Larval Remains

The head capsule of chironomid larvae is composed of chitin. This material is the second hardest known biological material, second only to the enamel of vertebrate teeth. As a result, the larval head capsule is virtually impervious to normal degradation processes and is incorporated into the sedimentary record more or less intact. These remains may represent larvae that perished before completing the life cycle, remains that were voided in the excreta of predators such as fish, or remains that were cast after pupation and emergence. Whatever the source, these remains are incorporated into the annual deposit of sediments to form a permanent historical record of the chironomid community. This record extends from the present back into geologic history. Ashe (1983) records 23 genera that are known only from the fossil record.

Relevance: Analysis of the fossil record using palaeolimnological techniques to reconstruct the changes in freshwater ecosystems, both natural and anthropogenic, introduces an element of time that is approachable by no other biological or chemical means. Time scales ranging from years to millenia may be investigated to place historical changes in proper perspective.

10. Palaeo-reconstruction of Environmental Change

Warwick (1980a, 1980c) demonstrated the usefulness of palaeolimnological techniques in deciphering and reconstructing the impact of colonization and the development of modern society on the Bay of Quinte, Lake Ontario. He was able to show (Fig. 5) that severe degradation of the bay had begun as early as 1800, that three processes – sedimentation, eutrophication, and contamination – were involved in the degradation process, and that even earlier aboriginal populations had had their effect on the bay. Particularly noteworthy was the decline in the number of chironomid taxa living in the bay from 158 prior to European colonization to 18 in the modern sediments. Following the airplane analogy, there obviously are a lot of "rivets" missing from the Bay of Quinte ecosystem! The study clearly demonstrates how far human intervention has altered the Bay of Quinte ecosystem; from its original pristine condition, where light penetration in the water column was in excess of 22 m, conditions in the bay have degraded to the point where heavy algal growth and turbidity limit light penetration to only a few metres.

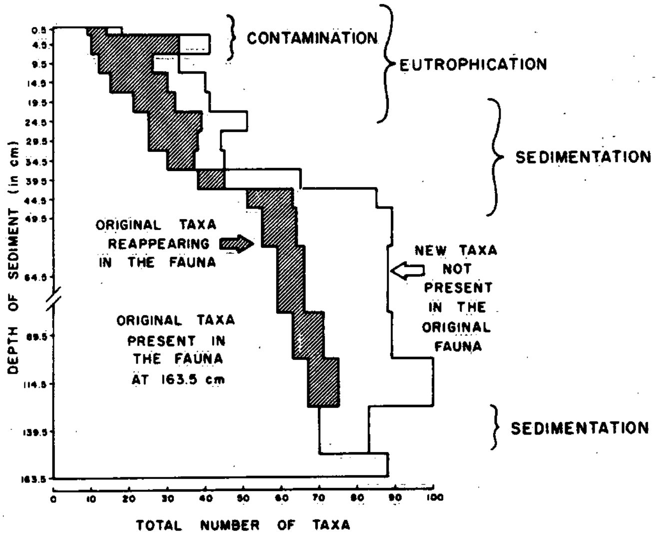

Figure 5. The effect of the major impact processes on the numbers of chironomid taxa inhabiting Bay of Quinte sediments. Original taxa reappearing in the fauna are indicated by the crosshatched area. From Warwick (1980c).

Relevance: Stratigraphic analysis and interpretation of the remains of chironomid larvae in sediments provide one of the best methods for the examination of long-term changes, both natural and anthropogenic, in freshwater ecosystems. The techniques are sensitive and immediate to the point where even the impact of small aboriginal populations can be discerned.

Relevance: An understanding of the relevant scales of natural variability – the scales of historical, prevailing, and potential future dominating processes – is one of the most important underpinnings of cumulative impact assessment (Cornford, 1986). It is essential to be able to separate natural from anthropogenic changes and to prevent the recognition of the considerable natural background variability from clouding the identification of cumulative impacts (Dayton, 1986). According to Cairns (1986), it is "quite difficult to work out the causality of a single cultural intervention (if relatively severe), because it will likely act in a non-continuous manner through time and space. To sort out the causality of two types of cultural interventions initiated concurrently, perhaps interacting with each other and with the natural background of turbulence, may strain any research budget because of the complexity of the problem. Three or more cultural interventions, acting concurrently at a level of some severity, might better be treated as a simple problem since it will likely be intractable if treated as a complex problem. To sort out the separate effects of these various interventions with scientific rigour, after the fact and in terms of causal mechanisms, is now virtually impossible." In spite of operating concurrently, however, the effects of three major processes involved in the degradation of the Bay of Quinte ecosystem were identifiable from analysis of the information contained in nature's diary — the fossil record. The effects of erosion (sedimentation), pollution (eutrophication), and contamination were evident in the changes in the chironomid fossil assemblages from a Tanytarsini-dominated fauna, to a mesotrophic *Phaenopsectra* fauna, to a eutrophic fauna of *Chironomus* exhibiting morphological deformities.

Relevance: Too often, participants in slow processes of major change, like environmental degradation, are not aware of the extent to which change has taken place around them, and even less of the extent to which their behaviour as individuals and groups has accommodated to that change (Vallentyne, 1978). In the case of the Bay of Quinte, it was not until the mid-1940s that concern over the quality of the Bay of Quinte ecosystem was voiced (Tucker, 1948). Almost 20 years later, however, McCombie (1967) still was unable to demonstrate conclusively any difference between phytoplankton concentrations measured in 1963-64 and those measured in 1945 by Tucker. And yet, palaeolimnological analysis of changes in chironomid community structures (Warwick, 1980a) showed that serious degradation of the Bay of Quinte ecosystem had begun over 200 years previously!

Reconstruction of the impact of these anthropogenic interventions show how far the Bay of Quinte ecosystem had been degraded. As part of the plan under Project Quinte to halt further degradation and institute mitigative measures, this study provided a clear picture of the former condition of the bay and put remedial-action targets into a meaningful context.

Relevance: Palaeolimnological analysis can provide the type of baseline information required to determine the success of mitigation measures. Cairns (1986) identified a number of issues in this important area.

1. How does one determine when the degradation caused by a cumulative impact has been arrested?
2. If the stress is removed, will the ecosystem return to its original dynamic equilibrium condition without further intervention? If not, what form should this intervention take?
3. If restoration of a damaged ecosystem to its original condition is not possible, how does one select an alternative ecosystem that will be compatible with the other ecosystems to which it is linked?
4. If a successful process similar to the one a damaged ecosystem had in its original stages of development is under way, should all further intervention cease?

These questions can be approached logically only when the condition of the original ecosystem and the extent to which it has been degraded are known. More often than not, however, this kind of information is not available from contemporary monitoring tools. The health of ecosystems usually becomes an issue only *after* they have been degraded, and usually, little or no background information on original conditions is available upon which to make sound decisions for remedial action.

11. Culture Experiments

Certain species of chironomids can be maintained easily in culture for experimental purposes. The easiest to maintain in the laboratory probably is *Chironomus tentans* Fabricius. Unlike most species, *C. tentans* does not swarm, but mates on contact. This allows cultures to be maintained almost indefinitely under laboratory conditions. The life history stages of *C. tentans* are well known, and optimal conditions for maintaining the species in culture have been described (Lawrence, 1981).

Relevance: Single species culture experiments to determine biological response to chemicals of concern have recently fallen into disfavour because these tests have been used inappropriately to predict the cumulative effects of chemicals in the environment without relevance to field conditions. According to Cairns (1986), there is a danger that indicator species come to be seen as an end unto themselves and not, rather, as imperfect surrogates of the whole ecosystem. This unfortunate trend can be fostered if an additional step is taken in which the impact on the test species in a controlled, stereotyped, laboratory setting is taken as sufficient indication of what the impact will be on the whole ecosystem. The trend toward excessive reliance on laboratory species as surrogates of integrated ecosystem features may be an example of overemphasis on a criterion of legal relevance and statistical precision over the criteria of comprehensiveness and realism in order to satisfy requirements of the legal process (Regier, 1986).

Single species tests can be used to advantage to supplement field studies, however, *as long as their limita-*

tions are fully acknowledged. Single species tests in which laboratory cultures are exposed to measured concentrations of known chemical stressors or chemicals of concern under controlled conditions can be used to calibrate the responses observed in field populations, to determine the mode of action, and to refine assessment capabilities. In this sense, *Chironomus tentans* is ideal; the sensitivity of the species to toxic chemicals has already been demonstrated under laboratory conditions and the sensitivity of the genus as a whole has been demonstrated in the field (Warwick, 1980a, 1980b, 1985, 1988). Also, species of *Chironomus* are widely distributed and tolerant of a broad range of ecological conditions (Pinder, 1986). Since it is particularly important that field and laboratory measurements are made at the same level of biological organization (Cairns, 1986), the responsiveness of the genus *Chironomus* to chemicals, its broad range of tolerance, and the ease with which *C. tentans* can be cultured greatly facilitate comparison and extrapolation between laboratory measurements and a wide range of field situations.

The length of time between hatching and development of fourth instars of *Chironomus tentans* is about 33 days. This represents not only a manageable period for test purposes, but also represents the greater proportion of the total life cycle. Experimental conditions can be established with a high degree of experimental realism, and population parameters such as brood size, age of maturation, survivorship, and growth rates can be easily monitored.

Relevance: With proper experimental design, these characteristics meet many of the criteria for test species outlined by Cairns (1986) and Dayton (1986). Results at the population level would also provide a useful window into the responses of other less well-known species that do not show overt morphological responses to contaminants.

Disadvantages of Chironomidae in the Biological Indicator Role

Chironomidae possess a number of disadvantages as environmental indicators; these lie primarily in the daunting size and complexity of the family and the lack of detailed autecological information on individual species.

1. Taxonomic Complexities

The family Chironomidae is the most widely distributed and frequently the most abundant group of insects in freshwater environments. An enormous amount has been written about their biology, and yet most faunistic works either ignore the Chironomidae or deal with them superficially (Pinder, 1986). The primary reason for such deficiencies has been the lack of readily available "taxonomic keys" for their identification coupled with the large number of species frequently encountered within even small bodies of water. Until very recently, taxonomic information has not been readily available, but has been spread throughout a wide and diffuse literature. These "keys" have also concentrated largely on the adult fly, which has necessitated rearing the larval stage through to the adult stage for proper identification.

Relevance: Recent publications that have summarized generic diagnoses and keys to larvae and pupae of the Holarctic region (Wiederholm, 1983, 1985; Ashe, 1983; Oliver and Roussel, 1983) have done much to bring together the large amount of taxonomic information available and present it in a practical, workable form. In the strict taxonomic sense, the difficulties in identifying immature stages will result in some loss of definition in some traditional measures of community response (diversity, biomass, abundance, etc.). However, in an operational sense, as long as larvae can be separated reliably into taxonomic types or "taxa," the loss of definition can be kept within acceptable limits. Each type should be defined carefully to permit comparison with other work and a permanent slide record kept to permit future re-analysis as taxonomic questions are resolved. Even if precise and complete identifications cannot be made at present, there is still a great deal of excellent information and natural history available to make reasonable use of such community data (Dayton, 1986).

2. Life History and Ecological Requirements

Although the broad environmental requirements of the various subfamilies are relatively well known (Oliver, 1971), the detailed ecology and life cycles of the great majority of species are not (Pinder, 1986). In the profundal of deep lakes, where conditions are relatively stable and predictable, chironomid population dynamics and community structures may be explained relatively easily. In rivers, and indeed in the littoral of lakes, conditions are considerably more variable and the chironomid community correspondingly more complex.

Relevance: Morphological studies, particularly the response of individual larvae to contaminant stress, do not require a detailed understanding of the life history and ecological requirements of each species for immediate application. What is required is an understanding of the range of physical variation within a species type. Life history and ecological information will certainly be advantageous in later stages of development to extrapolate from the level of the individual to higher levels of biological organization (i.e., population, community, and ecosystem levels), but this in no way detracts from morphological techniques for detection or mitigation monitoring.

Relevance: This same comment applies as well to river studies where attempts have only recently been made to classify rivers using chironomid associations in a way similar to the lake classification concept (Morris and Brooker, 1980). In general, rivers support a much more diverse community than the profundal zone of lakes, and they present a wider range of environmental conditions so that taxonomic and sampling difficulties are much more severe. Morphological studies of individuals may circumvent some of the difficulties imposed by rapid and substantial changes in the quality and quantity of faunal components characteristic of riverine systems. Multivoltine species, like the Orthocladiinae, that have overlapping generations and continuous recruitment may prove of greater significance under these circumstances than species that have only one or two discrete generations (Pinder, 1986).

3. Generic Responses

Certain chironomid genera appear to be more susceptible to morphological deformities than other genera (Hare and Carter, 1976; Wiederholm, 1984; Van Urk et al., 1985; Warwick, 1988). There appears to be no common link between those species that do manifest response and those that do not. Hare and Carter (1976) suggested that taxa that do not display any physical response to contaminants may respond in other, more subtle ways, perhaps physiological or behavioural in nature.

Relevance: Although the reason for it is not yet known, this seemingly selective response may in the long run prove to be an advantage rather than a disadvantage. There is evidence that some species (or some life history stages of some species) may be very sensitive to some toxicants and resistant to others (Cairns, 1986). Warwick (unpubl. data) found that, although 18% of the *Chironomus* larvae from the southern (contaminated) end of Last Mountain Lake were deformed, less than 1% of the co-existing *Cryptochironomus* sp. were deformed. In Maskwa Lake, however, where no known sources of contamination exist, the incidence of deformities in *Cryptochironomus* specimens found in very shallow shore samples was about 19%. The only obvious difference between the two sites was the difference in water depth and temperature, although UV radiation and salinity may also be factors (Warwick, 1988). The possibility of different responses in different species to different stimuli becomes important in developing a mosaic of species responses to determine causality, since it is highly unlikely that any one response will be specific for a specific contaminant (NRCC, 1985).

Morphological Deformities in Individual Larvae

Morphological deformities represent an extension of the more traditional and successful methods of using chironomid larvae for biological assessment of water quality (Hamilton and Saether, 1971). Deformities represent "real" responses to contaminants and have the potential for providing a credible early warning signal for environmental degradation by chemical contaminants. In general terms, all toxic effects begin with an interaction between a contaminant and some biochemical receptor in an individual (NRCC, 1985). The physical deformation of one or more morphological structures represents a response in the individual to that interaction. The assumption, then, is that the morphological deformities representing the contaminant/individual interaction reflect the toxic effect of these contaminants in the environment. According to NRCC (1985), observation of such "real" responses in larvae exposed in the real environment offers a highly profitable strategy for environmental assessment.

Morphological deformities represent overt responses to contaminants in the environment. As such, they meet the guidelines set forth by NRCC (1985) for developing sensitive early warning indicators of ecosystem health: (1) morphological deformities are induced during the most sensitive stage of the chironomid life cycle; (2) there are species native to all aquatic ecosystems of interest; (3) representatives of eury- and stenotopic species cover a wide range of biotic and abiotic factors that modify the effects of contaminants in the environment; and (4) the length of the larval stage and its direct exposure to contaminants in the bottom habitat are appropriate to demonstrate the relevance of primary responses to tertiary ones. Petersen and Petersen (1983) suggested that responses at the level of the individual are more useful than responses at the community level because they occur before those at the community level and can thus provide an earlier warning of environmental change. Since subtle effects on populations, communities, and ecosystems may be evident only after long exposure, and since investigation, diagnosis, and correction may take even longer, investigation at these higher levels of organizations are inadequate to prevent significant environmental damage (NRCC, 1985).

The frequency and severity of morphological deformities in chironomid larvae reflect the amount and type of biologically available contaminant to which they are exposed. The amount of contaminant available to biological communities frequently bears little relationship to the total contaminant loading in the environment (Warwick, 1986). Frequently too, synergistic effects magnify the potency of small amounts of chemical contaminants beyond their individual toxicity when mixed together (SCEP, 1970). Testing for specific contaminants may yield only negative results because many species may have the ability to metabolize target chemicals into their metabolites, which do not show up on the tests, but which are still toxic, mutagenic, and carcinogenic (Dayton, 1986). Chemical tests generally are highly specific for the intended target chemical; if this chemical has been transformed to a metabolite or its chemistry altered through synergistic combination, the test will give a false negative. Since few chemical combinations react antagonistically to produce a less toxic compound, a negative test result may be misleading, and, from the point of view of the environment, may be far more serious than if the target chemical had been present because of the false sense of security it produces. Chemical analyses of body burdens can also be misleading if concentration data are not transcribed into absolute numbers or if life history factors are not taken into account. Morphological responses in individual organisms are not specific in the same sense. Because the individual is directly exposed, the effect of the total number of chemicals present is integrated, synergistic effects are automatically taken into account, and response is based on the net contaminant burden.

Morphological analyses also permit a number of life history responses to be included in the analysis to increase discrimination and sensitivity. Chironomid larvae increase in size in discrete increments of approximately 60%. The 60% rule makes the separation of instar stages comparatively easy. This allows sample homogeneity (stratified sampling to reduce error by making samples more directly comparable) to be increased and the susceptibility of different instar stages to be studied. The effects of sublethal stress on growth rates and survivorship can also be determined from physical size data.

Measurements of physical size also permit the question of distinguishing between contaminant trends and natural variability to be addressed. Odum et al. (1979) noted that an increase in variability is one of the frequent responses to stress, yet even ecologists have discarded certain field measurements because they are thought to be too highly variable. In fact, differences in variability rather than differences in averages or means might be the best measure of stress in natural systems (Cairns, 1986).

Morphological characteristics are not affected by sampling stress in the same way as biochemical responses. Sampling fish, for example, involves stress ranging from mild (anesthesia) to severe (gill netting). In fact, fish often die from sampling stress (NRCC, 1985). In order to conduct biochemical tests, fish need to be held undisturbed in situ until their physiological responses return to baseline levels. These expensive and time-consuming techniques are not necessary when morphological responses are used.

Morphological deformities in chironomid larvae have been reported from a number of localities throughout the world. Warwick (1988) listed reports from the St. Lawrence Great Lakes, the Canadian prairies, the interior of British Columbia, Europe, Scandinavia, and the continental United States. In keeping with the worldwide distribution of the chironomid family, reports of morphological deformities in indigenous fauna will undoubtedly accrue as interest in, and appreciation of, this area of research increase.

Descriptions of Morphological Deformities

Morphological deformities range from mildly abnormal mouthparts (Fig. 6) to the grotesque thickening and fusing of all body structures (Fig. 7). Deformities in the mentum range from mild asymmetry in the number of lateral teeth, to overlapping in the outer lateral teeth, or to overlapping of lateral and median teeth (Fig. 6c). More extensive deformation may include the addition of count-

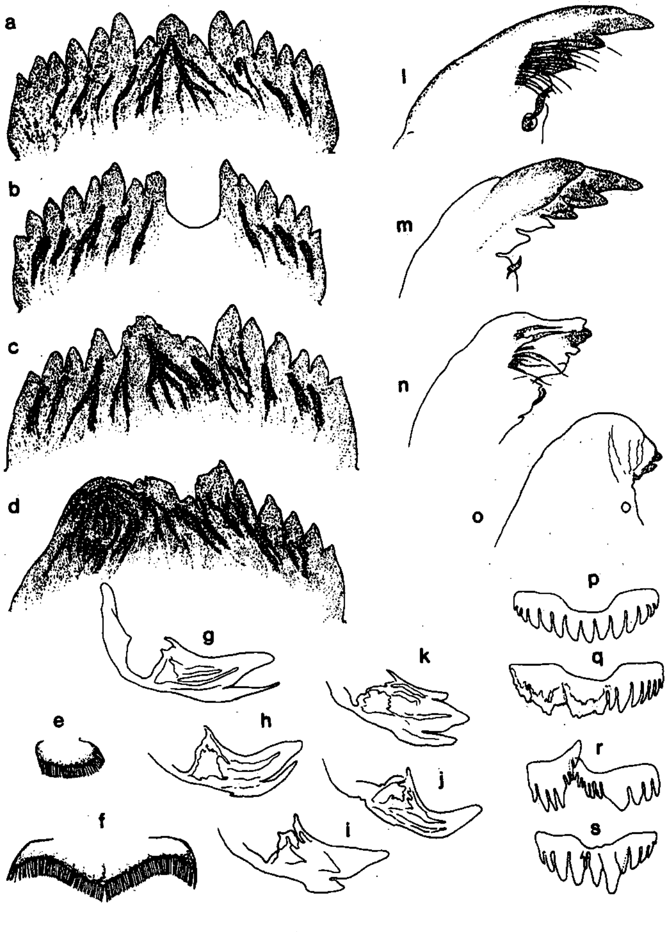

Figure 6. Morphological deformities in the mouthparts of *Chironomus* Meigen: (a) normal mentum; deformed menta showing (b) Köhn gap, (c) central/lateral overlap, and (d) extensive lateral disorganization; (e) deformed and (f) normal labral lamellae; (g) normal and (h-k) deformed premandibles; (l) normal and (m-o) deformed mandibles; and (p) normal and (q-s) deformed epipharyngeal pecten. Specimen (b) is from the Teltowkanal; specimen (c) is from Last Mountain Lake; all other specimens are from Tobin Lake.

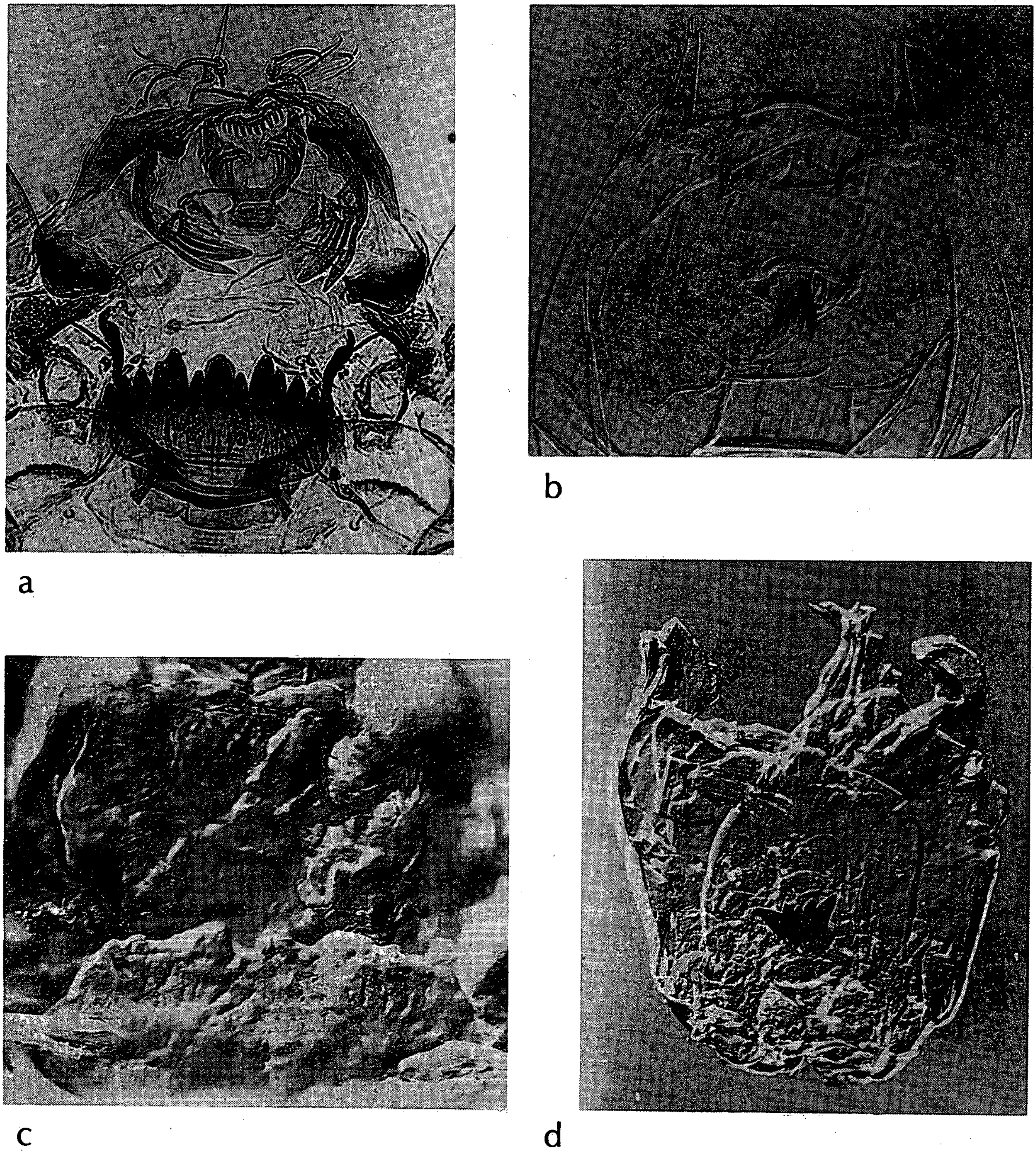

Figure 7. Thickening and fusing in the head capsule of massively deformed larvae of *Chironomus* Meigen and *Procladius* Skuse: buccal area of (a) normal and (c) massively deformed *Chironomus* larvae and head capsules of (b) normal and (d) massively deformed *Procladius* larvae.

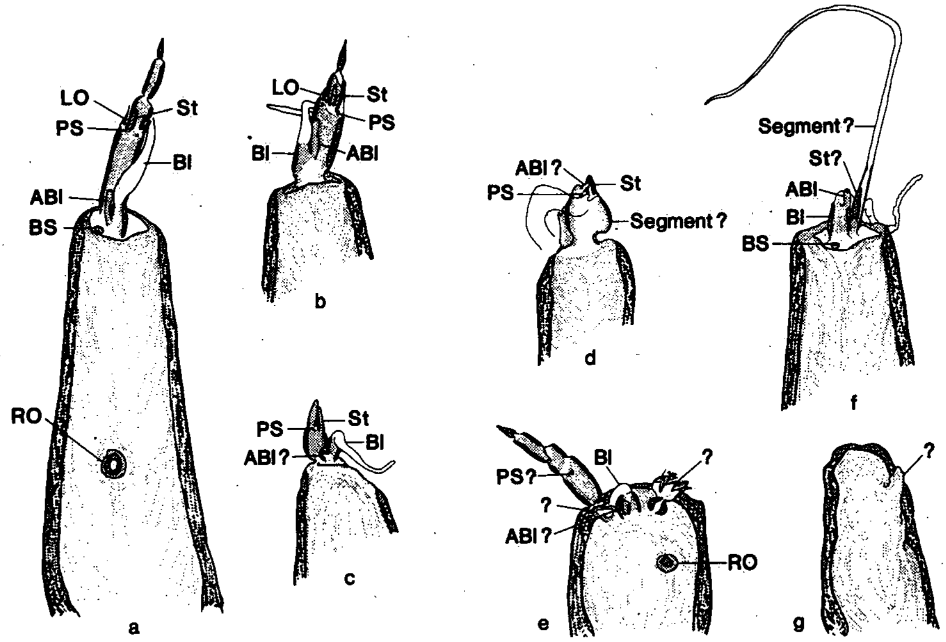

Figure 8. Deformed antennae of *Chironomus* Meigen: (a) normal antenna; deformed antennae showing (b,c) loss of distal segments, (d) questionable second segment, (e) displacement of blade (Bl) and accessory blade (ABl) complex, structures of unknown homology and reduction in length, (f) fusing of apical segments, and (g) fusing of apex of basal segment, loss of ring organ (RO) and structure of unknown homology. Other structures indicated include the organs of Lauterborn (LO), style (St), and the basiconic sensillum (BS). Nomenclature after Warwick (1985).

less numbers of teeth to the lateral or median areas of the mentum (Fig. 6d) or the loss of many of its teeth (Fig. 6b). Deformed mandibles often have apical teeth added or deleted (Figs. 6l, 6m), fused (Fig. 6n), or stunted (Fig. 6o). The sub-apical teeth of premandibles are sometimes shorter than the primary tooth (Fig. 6h), but occasionally they are much reduced in length (Fig. 6i), absent (Fig. 6j), or accompanied by additional teeth (Fig. 6k). The teeth of the epipharyngeal pecten often are fused (Fig. 6q), separated and disorganized (Fig. 6r), or stunted and cramped (Fig. 6s). Deformities in the labral lamellae (Fig. 6e), ventromentum, and other less obvious structures have also been observed, but little information is available at this time to provide an adequate description of the range of deformation possible in these structures.

Antennal deformities range from mild shortening of the basal segment to the more severe reduction in length of one or all antennal segments (Fig. 8e). The more obvious deformities involve the loss of individual segments (Figs. 8b, 8c), the fusion of one or more adjacent segments (Fig. 8f), or the presence of segments of questionable equivalence (Fig. 8d). In severe cases, only the fused remnant of the basal segment may remain (Fig. 8g). In some cases, sensory organs such as the blade/accessory blade complex or the peg sensillum may be displaced from their normal positions at the apices of the first (basal) or second segments, respectively. The ring organ on the basal segment may also be displaced or entirely lacking (Fig. 8g). In some cases, structures ranging from simple lumps and bumps to more complex trident- or palmate-shaped structures (Fig. 8e) are present for which no homology is known.

More severely deformed larvae display massive thickening of the body and head capsule walls and disorganization of the structures of the head capsule. These may range from thickening and fusing of individual structures (Fig. 9) to the complete loss of structural integrity in the head (Fig. 10). The thickened structures of massively deformed larvae characteristically are laminated (Figs. 9b, 9d), and the outer body wall is often sheathed with a loosely attached layer of thin integument or "sloughed skin" (Figs. 9a, 9b). The chitinous claws of the posterior parapods often are reduced to pudgy, finger-like protuberances that appear seemingly devoid of any physical strength (Figs. 9c 9d). It is difficult to comprehend how the massively deformed larvae in Figure 10 were able to survive to reach their apparent stage of "development."

Indexing Morphological Responses

The potential for abnormal variation in an animal as complex as a chironomid larva is immense, and the number of possible expressions of response correspondingly high. At present, I regard any morphological feature that departs from the normal configuration as a deformity. I have deliberately kept the definition of a deformity simple until such time as we are able to distinguish between normal and abnormal variations with a higher degree of certainty. Both the frequency and the severity of deformities are important in assessing the impact of contaminants on an ecosystem.

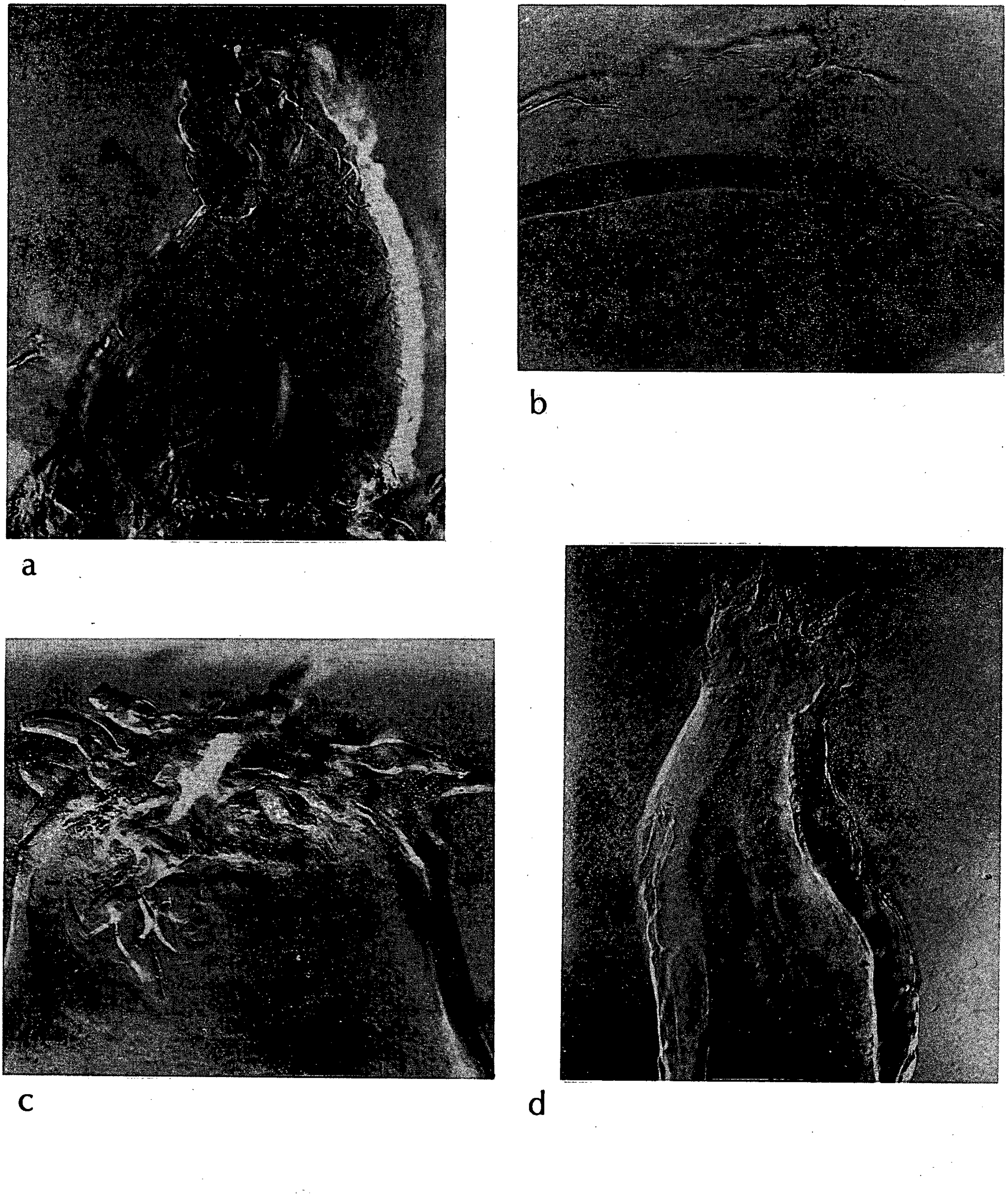

Figure 9. Deformities in other body structures of *Chironomus* Meigen: (a) deformed mandible showing gnarled apical teeth, massive thickening, and loosely attached outer covering, (b) "sloughed" skin attached to outer body wall, and (c,d) deformed claws and massive thickening of posterior parapod or proleg.

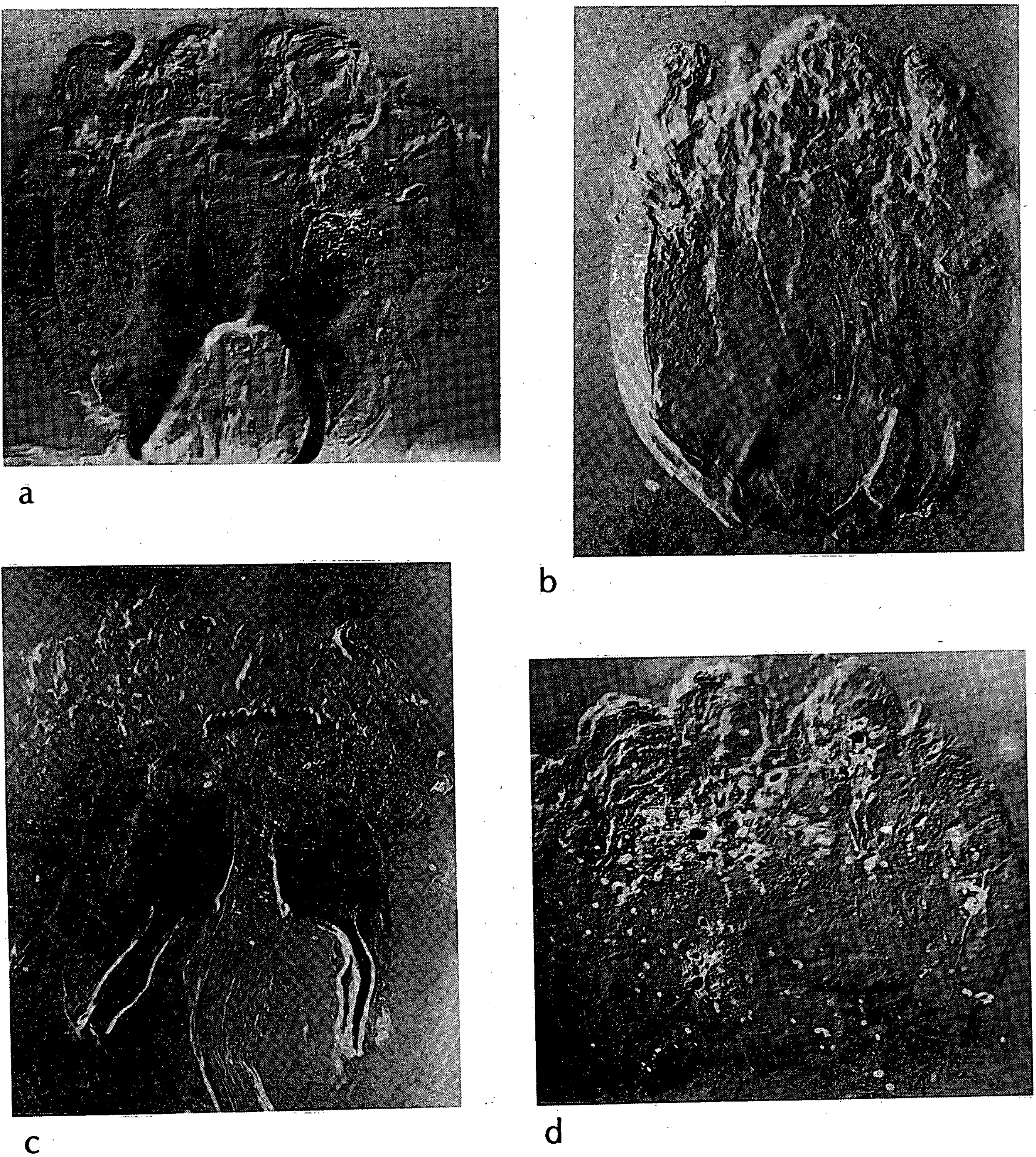

Figure 10. Head capsules of massively deformed larvae of *Chironomus* Meigen from Pasqua Lake, southeastern Saskatchewan.

The first quantitative numerical index devised to assess contaminants based on morphological parameters was based on the larval antennae (Warwick, 1985) because, as receptor organs, antennae probably are more sensitive to low levels of contamination. In addition, the number of antennal segments provide a readily quantifiable index of response.

Index of Severity of Antennal Deformation (ISAD)

Warwick (1985) presented an indexing scheme for classifying the severity of antennal deformities in the genus *Chironomus* as a preliminary step in developing a biological screening tool. The antennae of *Chironomus* were chosen over other morphological features as a starting point for several reasons: (1) intuitively, as a receptor organ, antennae may be expected to be more sensitive to contaminants; (2) the effects of contaminants would be more readily quantifiable because antennae possess a quantifiable number of segments; (3) the genus *Chironomus* is one of the most common, widely distributed, and well-documented chironomid genera; (4) recognition of abnormal specimens within a particular genus requires a high degree of familiarity with the genus to be able to distinguish between normal and abnormal variation; and (5) deformities are known to occur in other genera, but they appear to be more common in detritivores like *Chironomus*.

The degree of severity of deformation in an individual antenna is assessed according to the ranking system summarized in Table 1. Ranking of severity is carried out according to a sequence of steps, each of which is assigned a separate scale according to the type of deformation involved. In Step 1, antennae are assigned to basic categories depending on the number of genuine and/or questionable segments present. Deformed antennae with five segments are assigned a value of 1; antennae lacking segments are assigned 4 additional points for each missing segment or 2 points where the presence of a segment is questionable. Thus, a deformed antenna having two genuine and one questionable segment (i.e., 3? segments) would be assigned a value of 1 plus 8 (two missing segments) plus 2 (one questionable segment) for a value of 11. In Step 2, the first 10% variation in antennal length is regarded within normal or "zero" variation, but a value of 1 is assigned for each additional 10% reduction in length. In Step 3, values of 1 and 2 are assigned, respectively, if the ring organ has either been displaced or lost. In Steps 4 and 5, values of 2 are assigned if the apex of the basal segment has been completely fused over or if any of the accessory sensory organs have been displaced. In Step 6, values of 1 or 2 are assigned, respectively, for the presence of simple (minor) or more complex (major) structures for which no homology is known. The values assigned at each of these steps are then summed to provide IMR – the Index of Morphological Response – for the antennae of individual larvae. Individual IMR values are then summed to provide a measure of the morphological response of a chironomid population to toxic stress. The Index of Severity of Antennal Deformation (ISAD) is then defined as:

$$\text{ISAD} = \Sigma \frac{\text{IMR}}{n}$$

where IMR represents the total of the indices of morphological response describing the degree of severity of deformation in individual antennae and n is the total number of specimens examined in a particular taxon or population. The procedures and more complex interpretive aspects of the scheme are detailed in Warwick (1985).

Table 1. Summary of the Values Assigned to Individual Types of Deformities in Antennae of *Chironomus* spp. Larvae [Adapted from Warwick (1985)]

Step	Feature	Indices of Morphological Response (IMR)
1.	Basic classification categories	
	a. loss of genuine segments	1, 5, 9, 13, 17, and 21
	b. presence of questionable segments	3, 7, 11, 15, and 19
2.	Reduction in antennal length	1 – 9
3.	Displacement or loss of ring organ	1 – 2
4.	Apical fusing of basal segments	2
5.	Displacement of sensory organs	2
6.	Structures of unknown homology	
	a. major structures	2
	b. minor structures	1

Although developed originally for *Chironomus* species, ISAD can easily be adapted to other chironomid species because the indexing system is open-ended. Chironomid species with different numbers of segments in their antennae can be accommodated simply by adjusting the range of basic classification categories.

Application of ISAD

The severity of antennal deformations in *Chironomus tentans* larvae exposed to DDE by Hamilton and Saether (1971) were analyzed by ISAD after the original specimens were remounted using our techniques. Unexpectedly, the results showed an inverse relationship between ISAD and the concentration of DDE (Fig. 11). Although based on small sample populations (n = 253), regression analysis for the line described by the equation

$$y = 1.29 - 0.05x$$

indicated that the inverse relationship was highly significant ($r = 0.971$ where $r_{0.01} = 0.959$ for df 1,3).

The frequency of antennal deformities for the same data set also decreased as the concentration of DDE increased (Fig. 11). The inverse relationship described by the equation

$$y = 24.01 - 1.05x$$

was significant between 95% and 99% probability ($r = 0.946$ where $r_{0.05} = 0.878$ for df 1,3).

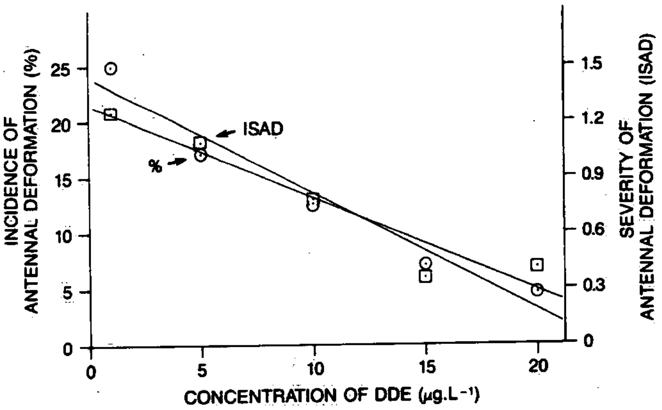

Figure 11. Dose-response relationships between the frequency (%), severity (ISAD), and concentration (µg/L) in *Chironomus tentans* Fab. larvae exposed to DDE. Highly significant regression equation for the severity of deformation (ISAD) is $y = 1.29 - 0.05x$; significant regression equation for frequency of deformation (%) is $y = 24.01 - 1.05x$. From Warwick (1988).

The reason for the inverse relationship between antennal deformities and the concentration of DDE is not immediately clear. As Lugo (1978) and Warwick (1985) suggested, the inverse relationships probably represent an isolated portion of the total response of antennae to DDE (Fig. 12). Quantal dose-response curves may be regarded as a graded response with the familiar bell-shaped distribution if, as in ISAD, the entire population of antennae is regarded as an individual (Chan et al., 1984). Individual members of such a population respond differently to a particular stimulus such as a contaminant. Some members will be highly sensitive, while others will be very resistant. In these experiments, the range of DDE concentrations used probably spans a range of antennal response in which resistance to DDE is increasing. If this hypothesis is correct, the range of greatest antennal sensitivity probably is less than the equivalent of 1 µg DDE/L.

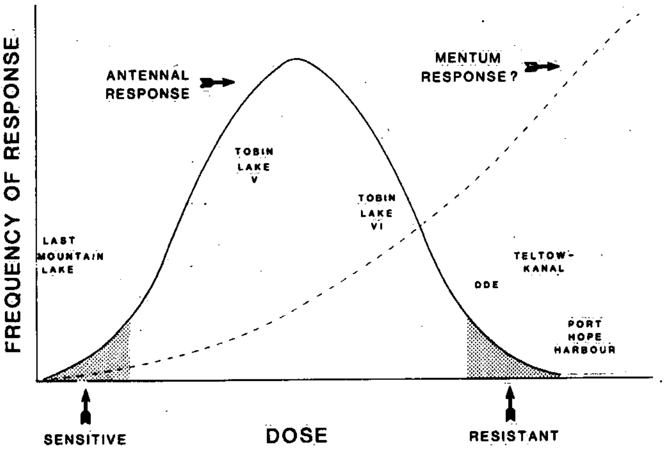

Figure 12. Interpretation of deformities in antennae and menta in terms of the quantal dose-response relationship (Chan et al., 1984). Placement of sampling locations tentative; for illustration purposes only. From Warwick (1988).

This hypothesis is supported by evidence from several field sites. In Tobin Lake, Saskatchewan, both the frequency (%) and severity (ISAD) of antennal deformation, contrary to expectation, declined along the hydraulic gradient established between the inflow of the Saskatchewan River and the Squaw Rapids dam (Fig. 13). Comparison of data sets from Sites V and VI show that ISAD declined (Table 2) from 1.64 to 1.19 between the two sites, respectively. The incidence of antennal deformities similarly decreased from 8.4% to 7.0% between the two sites. According to the relationship shown in the DDE experiments, Site VI, located 5.6 km farther downstream, should be the more severely contaminated of the two sites.

Three observations suggest that Site VI is indeed the more severely contaminated site. Reservoir hydraulics are such that, as hydraulic energy dissipates, waterborne particulates of decreasing density are deposited into the sediments. Because different chemical species tend to bind

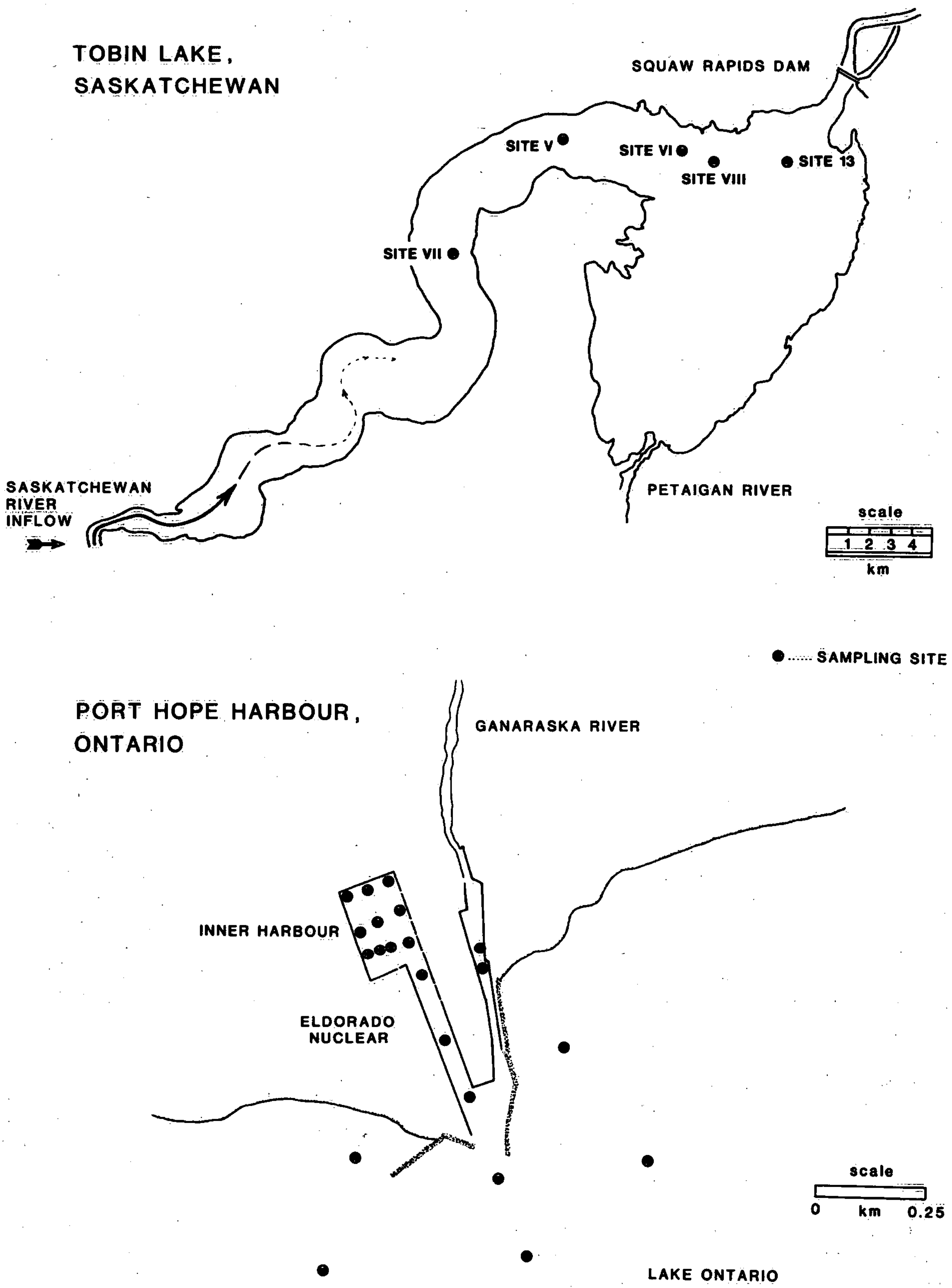

Figure 13. Sampling locations in Tobin Lake and Port Hope Harbour, Lake Ontario. Adapted from Birkholz et al. (1980) and Hart et al. (1986), respectively. From Warwick (1988).

Table 2. Comparison of the Frequency (%) and Severity (ISAD) of Antennal Deformation and the Frequency (%) of Menta and Total (%) Deformation in *Chironomus* spp. Larvae

Sample designation	Last Mountain Lake* II	Tobin Lake† V	Tobin Lake† VI	Teltowkanal‡	Port Hope Harbour§ (all sites)
Depth of water (m)		14.3	23.5		
Number of larvae examined	935	690	488	83	251
Number of *Chironomus* larvae	837	688	488	83	48
Number of deformed antennae	10	58	34	2	0
Incidence of deformed antennae (%)	1.2	8.4	7.0	2.4	0
Number of evaluated antennae (d)	9	58	34	2	0
Total IMR	99	1126	582	38	0
ISAD	0.12	1.64	1.19	0.46	0
Frequency of deformed menta (%)	1.0	4.8	13.1	28.9	47.9
Frequency of deformities (%) (all types)	7.2	15.1	26.0	34.9	70.8

Source: Warwick (1988).
* Comparatively uncontaminated.
† Contaminated.
‡ Highly contaminated.
§ Very highly contaminated.

selectively to different waterborne fractions, the hydraulic sorting process should establish a gradient of contaminants between the inflow area and the dam (Fig. 13). Samoiloff et al. (1983) established that a chemical gradient does exist along this line. They found that, out of 52 potentially toxic compounds isolated from sediments from Sites VII and VIII (located one on either side of Sites V and VII), only 5 were common to both sites. Evidence for a concentration gradient was found even further downstream. In recent work, massively deformed larvae of *Chironomus anthracinus* type were found at Site 13 (Warwick, unpubl. data). At the present time, the presence of massively deformed larvae is believed to indicate a greater degree of contamination. And finally, the sediments in the deeper water areas also contain large quantities of light, fibrous organic materials believed to be pulp-fibre wastes from pulp mills located along the Saskatchewan River. The presence of these materials is important, not only because it indicates hydraulic energy is negligible near the dam, but also because many chemicals selectively bind with organic materials (Karickhoff, 1981; Carter and Suffet, 1982). Interpreted in this manner, these field data agree with the pattern suggested by the experimental data.

The hypothesis that larval antennae become increasingly unresponsive at higher concentrations of contaminants is also supported by data from the Teltowkanal (Table 2). Only two larvae out of 83 *Chironomus thummi* specimens from this highly contaminated canal had deformed antennae despite major deformities in other morphological structures. The ISAD value for these antennae was only 0.46. This compared more closely with the value for comparatively uncontaminated Last Mountain Lake (0.12) than either of the Tobin Lake sites, and yet the canal is acknowledged to be highly contaminated (Köhn and Frank, 1980).

This trend is even more apparent in the data from Port Hope Harbour, where no deformed antennae were found among the 48 *Chironomus* spp. examined (Table 2). The harbour is very highly contaminated with radionuclide wastes, heavy metals, and elevated water temperatures (Warwick et al., 1988). There is a very high rate of serious deformities, and particularly multiple deformities, in the greater proportion of these fauna (>84%), and yet there is little indication of deformities in the larval antennae. The only observation to this effect, but as yet not quantified, is that the physical dimensions of the antennae seem unusual. This may be a function of species differences, but it may also reflect growth problems in Port Hope *Chironomus* populations (Wentsel et al., 1977).

The relationships between the field sites are shown in Figure 12 in relation to the quantal dose-response curve. Their positioning is tentative at this time, but the figure serves to show graphically the line of reasoning being followed.

Morphological Deformities as a Biological Screening Tool

Design-uses of the Tool

Morphological deformities probably will prove most useful in three areas of biological effects monitoring:

detection, assessment, and determination of the success of mitigation. Specificity on the identification of specific contaminants or classes of contaminants (NRCC, 1985) remains a tantalizing yet elusive fourth area of possible use.

Detection of Contaminants

One of the most critical steps in resolving a problem (Fig. 14) is recognizing that a problem exists (NRCC, 1985). In Hamilton Harbour, Lake Ontario, the impact of cyanides, phenolics, polynuclear aromatic hydrocarbons, BOD, COD, etc., might be anticipated from a knowledge of the steel industry bordering the bay and its effluents (NRCC, 1985). In Pasqua Lake, southeastern Saskatchewan, however, where the sources of contaminants are farther removed and possibly more diffuse, the recognition of a contamination problem may be less easily made. The presence of a remnant fauna of *Chironomus* (a high proportion of which were deformed) and the absence of any other members of the benthic community demonstrate unequivocally that the latter ecosystem has been degraded and a contamination problem does exist (Warwick, 1980b). Now that the problem has been identified, logical steps should be taken to identify the contaminants and their source, and remedial action undertaken. Green (1979) clearly identified the importance of problem identification in the progress of any environmental study in the sequence of steps: problem → question → hypothesis → sampling design → statistical analysis → tests of hypothesis → interpretation and presentation of results. Without the knowledge that a problem exists, the cost of the shotgun approach of routinely analyzing for each and every potential contaminant and its degradation products (even if these are known) would be haphazard and costly.

Detection must be done with a broad enough brush to be efficient, and yet at a fundamental enough level to be effective. In this sense, the "brush" represented by the chironomid family is certainly broad enough to be efficient; by virtue of its size and ecological ubiquity, the family represents virtually every type and condition of aquatic habitat likely to be encountered. Chironomids also represent a fundamental level in the food chain; response at their level clearly will precede responses at the higher levels of organization. In contrast, by the time problems of spinal curvature or dermal papillomas become noticeable in fish populations, the problem is already serious and too far advanced to meet the criterion of an early warning indicator of contamination.

Morphological deformities at the level of the individual occur before those at the population or community level (Petersen and Petersen, 1983). Preliminary evidence indicates that the larval antenna is particularly sensitive to environmental contaminants. Antennae appear to be capable of responding to very low levels of contamination, and deformities in these morphological structures probably will provide an excellent measure of low-level, chronic exposure (Warwick, 1985). As such, they have the potential to provide an excellent early warning signal for detecting contaminants in the environment.

Assessment of Contaminants

The tests used to assess the health of an ecosystem must be appropriate to the expected contaminant (NRCC, 1985). Where an ecosystem is impacted by only a single intervention (chemical or other), very specific tests can be applied to determine effect. Unfortunately, the more usual case involves a number of different interventions emanating from different sources and operating for a considerable period of time. Regier (1986) states that, under such circumstances, a "general degradation syndrome" (GDS) comes to be exhibited by the impacted ecosystem. This is similar to Selye's (1974) "general adaptation syndrome" (GAS), but, where GAS is focused at the organismal level, GDS is focused at the ecosystem level. Where human intervention is already severe, it may be difficult to determine the impact of new interventions, but by and large, these will act cumulatively to drive the ecosystem further into GDS. To sort out the separate effects of these various interventions with scientific rigour, after the fact and in terms of causal mechanisms, may be extremely difficult, if not impossible (Cairns, 1986).

Few ecosystems of concern are defined before damage has been done; unfortunately, most have already been degraded by multiple contaminant exposures before concern is realized. Faced with this fact, some attempt must be made to unravel cause and effect relationships to determine the best means of stopping or reversing ecosystem degradation. The assumption must be made in biological monitoring that a given measurement reflects the level of a specific stress or response. Four general levels of response (adapted from NRCC, 1985) are as follows.

1. Generalized stress responses. These will indicate exposure to the net toxicity impacting the ecosystem.
2. Structural or biochemical pathology. These will indicate exposure to one or more members of a class of compounds that are chemically dissimilar, but that have a common mode or site of toxicity. These begin to narrow the focus of the search.
3. General contaminant responses. These will indicate exposure to one or more members of a class of chemically similar compounds. These continue to narrow the focus of the search.

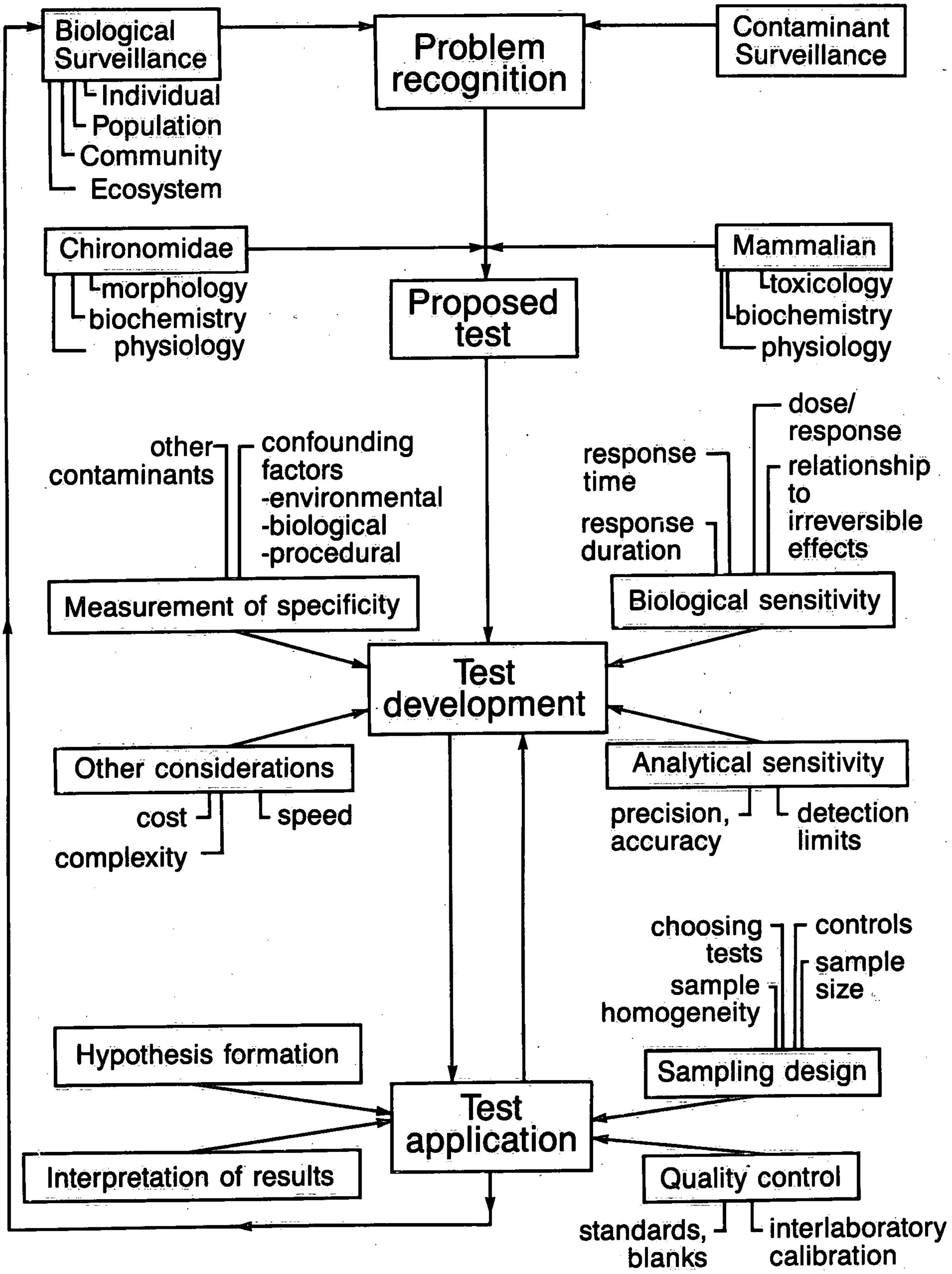

Figure 14. Stages in the development and application of diagnostic tests. Adapted from NRCC (1985).

4. Specific contaminant responses. These will indicate exposure to specific contaminants and provide a definitive diagnosis.

Few tests are highly specific for any one contaminant, and even positive test results may not provide a well-defined diagnosis of cause and effect. For example, the biochemical test for the inhibition of the enzyme ALA-D is very specific for lead. However, zinc can displace lead from the sulfhydryl binding sites of ALA-D and "activate" the enzyme, thus reversing inhibition and causing a false negative test result for lead exposure (NRCC, 1985). Since it is likely that no one test will suffice to diagnose ecosystem health, a combination of indicators probably will be required to identify or eliminate possible stressors. The pattern formed by the mosaic of responses, rather than individual responses, will probably provide a more functional basis of analysis and identification.

There can be no doubt that deformed chironomid larvae represent a generalized response to contaminant stress in freshwater ecosystems (Warwick, 1985). Even at the most fundamental level, the presence of grotesquely deformed larvae signals that something is radically wrong in their environment. At a more subtle level, the patterns of response in different morphological structures vary considerably between sites impacted by different forms of contamination. The patterns of morphological deformities in the former Rat Lake basin in north-central Manitoba differ considerably from those in Tobin Lake in east-central Saskatchewan. Mercury is generally understood to be the primary cause for concern in Rat Lake (Bodaly et al., 1984), whereas, in Tobin Lake, as many as 5000 chemical compounds may make up the "chemical soup" to which the chironomid larvae are exposed (D.A. Birkholz, 1983, Environmental Protection Service, Edmonton, Alberta, pers. comm.). There are also differences between the Tobin Lake data sets, which suggests some specificity of response. Although a definitive statement would be premature, the presence of characteristic types of deformities, particularly the development of structures of unknown homology, provides encouraging evidence that specific responses can and will be found. The palmate- and trident-shaped structures of unknown homology appearing on the deformed antennae of larvae from Tobin Lake Sites V and VI are examples (Warwick, 1985). Continuing research has shown that the relationship between morphological responses and contaminants may not be as straightforward as one might anticipate. In the broader context, the fact that some species of chironomids may respond, whereas others may not, may form another integral component of the diagnostic mosaic.

As a final point, assessment of the severity of morphological responses also plays an important role in the assessment procedure (NRCC, 1985). Percent response or frequency may show changes over space and time when "mean response" does not. However, much information may be lost by condensing data to this extent. For example, two sites may show only very slight differences in the incidence of deformities, suggesting equivalent exposure. However, the exposure at one site may be much greater, a fact indicated by the much more severe deformities occurring at that site. Warwick (1985) showed that, at this stage of development at least, both indices should be used together, and with great care, in the assessment procedure.

Determination of the Success of Mitigation

Cairns (1986) identified a number of issues in this important area.

1. How does one determine when the degradation caused by cumulative impacts has been arrested?
2. If the stress is removed, will the ecosystem return to its original dynamic equilibrium condition without further intervention?
3. If the ecosystem cannot return to its original condition without further intervention, what form should this intervention take?
4. If restoration of a damaged ecosystem to its original condition is not possible, how does one select an alternative ecosystem that will be compatible with the other ecosystems to which it is linked?
5. If a successful process similar to the one a damaged ecosystem had in its original stages of development is under way, should all further intervention cease?

Natural ecosystems have an amazing capacity to recover, given time and alleviation of the stresses impacting a system. Numerous examples exist in the literature, perhaps the most notable being the progress made in the reclamation of the River Thames.

Many questions arise in reclamation work. The degradation of an ecosystem may not cease immediately when discharges into it, or stress on it, are eliminated. The disequilibrium produced by a variety of stresses may persist for a substantial period of time or may even be permanent. If improvement is anticipated, how long should one wait for evidence to confirm this?

Evidence from the Bay of Quinte shows that there may be a considerable lag between the time stress is alleviated and the ecosystem begins to recover. For example, demands from Great Britain for naval timber from Canada increased dramatically in 1806–08 after Napoleon's conquest of Europe severed Britain from her traditional sources of Baltic timber. This increase in the activity in timbering operations was reflected almost immediately in the accumulation of fine erosion materials in the sediments of the bay. Once started, however, accumulation of these materials did not cease when timbering activity abruptly came to a halt during the War of 1812, but persisted, albeit at a slower rate, for some time. Once set in motion, a process like erosion takes time to stabilize and attain equilibrium after perturbation ceases.

Chironomid larvae can play an important part in measuring the success of mitigation through their ability to quickly recolonize an ecosystem. Early instars are planktonic and can rapidly move from refugia into an ecosystem when conditions permit. Because of their fundamental role in the food chain, they will necessarily precede higher level consumer groups.

Chironomid larvae can also play an important role in determining the success of mitigation measures by defining conditions both before (palaeo-fauna) and after (contemporary fauna) degradation of the ecosystem. Very few biological indicator groups have this dual capability because very few are preserved in the sediments. Many soft-bodied communities like protozoa or oligochaetes are potentially useful for monitoring environmental conditions, but lack preservable hard structures. Definition of the chironomid palaeo-community provides a direct target or goal for mitigative measures. Without such a target, predictions for the success of such measures may be inaccurate or overly optimistic. According to Cairns (1986), more substance is badly needed in our predictive capability for determining the rate of return to original conditions or the direction and rate toward a new equilibrium condition.

PRACTICAL CONSIDERATIONS

The Mechanics of Sampling and Analysis

The procedures used for collecting, processing, and analyzing biological samples, particularly the Chironomidae, are summarized below. The account is based largely on Warwick and Casey (1982), Oliver and Roussel (1983), and Warwick (1985).

Sampling and Collecting

Methods for collecting chironomid larvae differ according to habitat and whether qualitative or quantitative samples are required. Quantitative techniques for lotic habitats are described in Brinkhurst (1974) and for lentic habitats in Cummings (1962) and Hynes (1970). General collecting techniques, including illustrations of some apparatus are found in Needham and Needham (1941), Pennak (1953), and Martin (1977). More detail is provided by Welch (1948), Macan (1958, 1970), and Edmondson and Winberg (1971).

Dip nets, hand picking, nets pushed through the substrate, or a net capturing larvae dislodged by disturbing the substrate upstream from the net (kick method) are all suitable in habitats that can be approached from shore or by boat. Brundin (1966) captured larvae drifting with the current by half-immersing nets in streams for a period of time. In deeper waters, scuba diving, grabs, or dredges and corers may be used. The latter types sample and retain a portion of the substrate as the sampler is raised to the water surface. Many types of samplers have been devised (Welch, 1948; Edmondson and Winberg, 1971), but the Ekman dredge is most commonly used for sampling soft substrates. Artificial substrates are used where substrate type (e.g., rock or cobble), water depth, or current velocity make conventional sampling difficult. These are made of wire baskets or trays filled with stones, concrete blocks, webbing, or plates of different materials (Anderson and Mason, 1968; Hilsenhoff, 1969; Simmons and Winfield, 1971; Rosenberg and Wiens, 1976) placed in the habitat and left for a period of time to allow colonization.

Sorting

Samples from soft-bottom substrates are concentrated by sieving through a screen or net (Warwick and Casey, 1982). Coarser materials, such as rocks, can be rinsed or scrubbed with a soft brush into a net suspended in water or a water-filled pail. Common mesh sizes range from 200 to 400 microns, but sizes down to 73 microns can be used if very small species are encountered. Except for early instars, 200-micron mesh nets are a good compromise and probably will retain most larvae. After concentration, the sample is placed in a white tray with water and larvae and other members of the benthic community picked out with forceps or a pipette. Examination of the sample bit by bit under a binocular microscope will ensure complete removal, but this is very time-consuming. A number of techniques using density flotation (sugar or salt solutions), heat (Berlese funnel traps), or aeration have been devised to aid in removing larvae from fine material (Anderson, 1959; Kajak et al., 1968), but most do not work well when large amounts of organic debris are present. Sorting is best done live; movements by the living animal greatly assist sight recognition against background debris. Sorting preserved material is considerably easier if larvae are differentially stained for higher visibility with dyes like Rose Bengal.

Preservation and Storage

Larvae may be killed directly in a preservative, but killing in 95% ethanol or in water slowly heated to near boiling relaxes the mouthparts. Larvae are best preserved in 70%-80% ethanol. Formalin and lacto-phenol have been used previously, but besides tending to make larval specimens brittle, they have a number of other unpleasant characteristics that make their use inadvisable. Preservative in which larvae have been killed should be replaced within one or two days. If larvae are to be stored for lengthy periods of time, the preservative should be replaced from time to time with fresh preservative. Larvae should be stored in sealed containers to prevent loss of preservative. Three-dram vials with neoprene stoppers (Martin, 1977) or straight-sided shell vials stoppered with cotton-wool plugs and stored in museum jars are among the best containers available. The latter take up less space, but specimens are less readily available than those stored in three-dram vials set in racks (Martin, 1977). Identification labels printed on stable card labels with non-running India ink are placed inside each specimen vial before sealing.

Preparation and Mounting

Canada balsam is the preferred material for mounting larval material for microscopic examination because the permanency of slides made from this medium is well established. Also, if for any reason the specimen needs to be remounted, it can easily be recovered by softening the balsam in xylene vapour for removal. Other mounting media (e.g., Euparol, Permount, Hoyer's CMC 10, ACS mountant) are easier to use, in that specimens may be mounted directly from ethanol or water, but may not be as permanent.

Warwick (1985) proposed standardized preparation and mounting techniques based on those described by Warwick (1980a, 1980b) and Warwick and Casey (1982). These are essentially the same as the methods described by Schlee (1966) and Saether (1969), except that the head capsule and body of the larvae are fully flattened in the mounting process. Larvae are cleared initially by digesting in either warm or cold 8% KOH. The time required for clearing in warm KOH is largely dependant on the size of the individual specimen. Clearing with cold KOH requires a longer period of time, but can be used effectively to control the rate at which specimens are processed. The gut and body contents are removed by piercing the body wall, removing the gut tract and flushing out solubilized fats and oils. Cleared larvae are then passed through successive baths of glacial acetic acid, anhydrous ethanol, ethanol layered over cedarwood oil, and cedarwood oil. The head and bodies of medium to large larvae are generally mounted separately; smaller larvae are mounted intact. Dissection of the head capsule normally is carried out on the glass slide in Canada balsam rather than at the cedarwood oil stage to prevent loss of parts during manipulation. The head capsule is placed ventral side up and the mandibles snapped back to prevent obscuring the antennae. A coverslip is put in place and the head capsule flattened by exerting increasing pressure to the coverslip with a pair of forceps. A rotary motion can be applied to the coverslip during the flattening process to spread or shift mouthparts that threaten to obscure other diagnostic features. The body is similarly flattened. Using this method, a skilled technician can prepare and mount an estimated 50 to 100 larvae per day depending on the size and condition of the specimens.

The technique differs only slightly from traditional methods in that the structures of the head capsule and body are fully flattened and spread out. This is done to permit the examination of fine morphological detail and measurement of individual structures using high-power, oil immersion lenses. Some traditional measurements may be precluded by flattening, but these can be replaced by other measurements. For example, head capsule width could be replaced by mentum width.

Specimens with worn teeth on the mentum or mandibles are easily distinguished from those with deformed teeth. The margins of worn teeth appear crinkly or pebbled at high power magnifications because the dorsal and ventral plates of the mentum wear or break asynchronously. In contrast, the margins of unworn, normally shaped teeth or deformed teeth appear smooth because the two plates are properly fused together.

The importance of good slides cannot be overemphasized. It is especially important that the labral setae (SI and SII) and epipharyngeal pectin be visible and that the mandibles do not overlie and obscure each other. Well-prepared and well-mounted specimens not only greatly aid identification and evaluation, but will also serve as a permanent record of environmental conditions for many years. Permanent slides are readily transportable and provide a ready vehicle for interlaboratory comparisons of specimen materials from different sites.

Identification and Evaluation

Larval identification is done using a compound research microscope and appropriate taxonomic keys.

Until very recently, most of the required taxonomic information was spread throughout a wide and diffuse literature, focused largely on the adult fly, and ignored the immature stages. Recent publications have done much to remedy this situation and summaries of generic diagnoses and keys to larvae and pupae of the Holarctic region (Wiederholm, 1983, 1985; Ashe, 1983; Oliver and Roussel, 1983) have done much to bring together the large amount of taxonomic information available and present it in a practical, workable form. These keys are continuously being perfected, but as long as larvae can be separated reliably into taxonomic types or "taxa," the loss of information is limited. Each such type should be carefully described, however, to facilitate comparison with other works. Maintenance of a permanent slide record also means that taxonomic problems can always be reviewed as new information becomes available. Maintaining a permanent record for future review is particularly important in monitoring programs and determining the effects of mitigation.

Evaluation of the severity of morphological deformation in the different mouthparts of chironomid larvae is still in the developmental stages. As described above, an initial system for numerically quantifying the severity of deformities in the larval antennae has already been proposed (Warwick, 1985). The data bases for deformities in the other morphological structures is continuously being expanded to explore their full range of potential variation. Estimates of severity, at whatever stage of sophistication, add an important dimension to the evaluation of response that less sophisticated mathematical determinations, such as frequency or percent response, may mask. The importance of the dimension of severity cannot be denied.

Data Analysis and Reporting

It is unlikely that any one scientist will possess the expertise to deal with all aspects of the research required for data analysis and reporting, particularly in view of the very complexity of the problems being faced today (NRCC, 1985). Backing for individual scientists in the form of access to ancillary expertise (e.g., statistical, computer analyses, graphics) and formation of multidisciplinary teams to explore the complex relationships between contaminants and morphological responses and responses at the higher levels of biological organization is vital if progress is to be made.

Personnel Requirements

The staff required for any program will depend largely on the purpose and scope of that program. Since these, will of necessity be highly variable, I propose to discuss staff requirements in terms of "units" – the basic building blocks by which program requirements can be met. Many factors will act to determine the number and complement of the units required. The composition of a research unit will differ from that of an operational unit because the focus and intent are different. The size, urgency, and intent of a project will also determine how many "units" are required to meet program objectives.

Research Units

The size of a research unit will vary depending on its orientation and purpose. Field-oriented units tend to be larger than laboratory-based units, in large measure because of logistic requirements. The minimum complement of a research unit (Table 3) typically might include a research scientist (RES) supported by two technicians (EG) and temporary or summer student staff as required. The maximum complement might include a research scientist (RES) supported by a biologist (BI), three technicians (EG), and temporary or summer student staff as required. Smaller units involved in research tend to function at a higher level of intensity, whereas larger units tend to operate on a broader level. The operation of individual units may range from the development of practical research tools to the resolution of taxonomic problems according to their leader's orientation and/or program requirements.

Operational Units

The complement of an operational unit will be governed largely by the size of its program. The unit may range from a biologist (BI) supported by two technicians to

Table 3. Personnel Complements for Research and Operational Units

Personnel	Research units		Operational units	
	Minimum complement	Maximum complement	Minimum complement	Maximum complement
Research scientist (RES)	1	1		1
Biologist (BI)		1	1	2–4
Technician (EG)	2	3	2	4–8
Temporary or summer student staff	as required	as required		

a research scientist (RES) supported by two or four biologists (BI) and four to eight technicians (EG). The smaller unit could probably handle the monitoring operations on a system the size of the Qu'Appelle River basin quite adequately, whereas a considerably larger unit would be needed to handle similar operations on a system the size of the Saskatchewan River basin.

Basin Teams

I would anticipate that both research and operational units would be integrated with other types of units operating in a basin to form mutually supporting and complementary teams to share resources and maximize return for effort. Thus, research units might support operational units in selecting proper sampling sites, assisting in the resolution of taxonomic problems, and interpretating data. Operational units involved in biological monitoring should operate alongside, and in conjunction with, units measuring physiochemical parameters. Units involved in historical and socioeconomic developments within the watershed could also provide useful information for determining the sources and origins of de facto or potential problems. Backed by the necessary support infrastructure, such a "basin team" would ensure the cross-fertilization of ideas and data necessary for a holistic approach to environmental problems.

Level of Training

Unit research scientists (RES) should be trained primarily in the field of limnology, with particular expertise in aquatic entomology, but also with backgrounds in other areas of limnology and supporting disciplines such as chemistry, statistics, and computer programming. Biologists (BI) and technicians (EG) should have similar backgrounds, but at lesser levels of expertise. In the design of any unit or team complement, attention should be given to complementing and supplementing the strengths and talents of the individual members.

Project Development

In the research environment, perhaps more than in operational applications, the demands made on individual unit members change as a project develops (Table 4).

Sampling and Collecting

It is important that the research scientist be directly involved in the initial stages of sampling and collecting, even if support staff are eminently qualified to carry out the actual operation themselves. Direct involvement in field operations allows the scientist to modify or change program parameters or adapt to, or take advantage of, situations presented in the field. Some decisions are best made on the spot rather than from behind a desk. Direct involvement also provides a less tangible but very important "feel" for the ecosystem being studied, which may prove beneficial and even crucial to understanding the results of an investigation. Direct involvement is less crucial in the laboratory setting, where conditions are controlled and less variable. It is still highly desirable, however.

Sorting

Separating living or preserved organisms from the coarse bottom debris remaining after sieving is both tedious and time-consuming. It does not require a high level of technical qualification, but does require a high level of patience and thoroughness. Temporary or summer student staff under the supervision of technical staff have been used successfully to perform this task. In-house training is usually provided in basic techniques, the use of microscopes and taxonomic keys, and data enumeration at levels commensurate to the task. First- or second-year university students with a science background have been employed satisfactorily at this task.

Table 4. Level of Involvement of Unit Members at Various Stages of Program Development

Personnel	Sampling and collecting	Sorting	Preparation and mounting	Identification and evaluation	Analysis and reporting
Research scientist (RES)	***	*		***	***
Biologist (BI)	**	*	*	**	**
Technician (EG)	*	***	***	*	*
Temporary or summer student staff		***	*		

* Tertiary involvement.
** Secondary involvement.
***Primary involvement.

Preparation and Mounting

Preparation and mounting are most important and require a high degree of technical training. This is usually provided in-house and, after basic instruction, simply requires time and patience to develop the necessary skills to perform the job at a satisfactory level. The importance of these skills cannot be denied. Dr. O.A. Saether once stated that "a poor slide is worse than no slide at all!" Poorly mounted material may not only cause a great deal of aggravation for the person subsequently trying to work with it, but may actually lead to erroneous conclusions. The importance of the proper preparation and mounting of specimen material cannot be emphasized enough. The task requires a high degree of skill that comes only with patience and practice.

Identification and Evaluation

Identification and evaluation require a fundamental knowledge of systematics, particularly as it applies to chironomid taxonomy, and a thorough knowledge of the morphology of individual taxa. This type of training is most likely achieved at the postgraduate level. Access to the scientific community involved in chironomid research is also important.

Data Analysis and Reporting

Data analysis and reporting usually require postgraduate training. Access to ancillary support services is also important, as no one person can be expected to be expert in all facets of such a program.

Comparison with Other Techniques

Some practical aspects of using different biological systems for biological monitoring are compared below.

Collection Methods

The collection of benthic samples is a comparatively simple operation, and the apparatus required are simple and inexpensive. Compared to fish collection, there is no net-setting and the catch per unit effort is usually high. If it is not, as in the case of Pasqua or Echo lakes, this in itself is an indication that all is not well in the ecosystem. Chironomid larvae are generally robust, and since the tool is based on morphological (physical) characteristics, capture shock does not pose a serious problem nor are expensive live-holding facilities required, such as those for fish caught for biochemical analysis (NRCC, 1985). Unlike bacteriological or chemical sampling, benthic sampling does not require the ultra-clean or sterile equipment that is so difficult to maintain under field conditions. Benthic samples can be maintained live comparatively easily for return to the laboratory, or they can be preserved directly in the field. Unlike fish, chironomids cannot migrate to avoid exposure to difficult environmental conditions. Samples collected for chironomid larvae will also include other components of the benthic community. These will not only provide additional information on the health of the ecosystem, but will also facilitate transition to, and integration at, higher levels of biological organization (that is, at population, community, and ecosystem levels) with consequent spin-offs to classical work.

Equipment

The initial costs of equipment used in benthic work are generally low. Dredges and grabs are inexpensive and easily maintained compared to the nets and holding facilities required to handle live fish. The costs of chemical reagents and the glassware required for preparation and mounting larvae are minimal compared to the costs incurred by a chemical/analytical laboratory. The cost of stereo or compound microscopes is generally low compared to those for advanced chemical laboratory instrumentation such as chromatographic or spectrographic instrumentation. If properly cared for, these microscopes should depreciate minimally, and obsolescence is unlikely because light microscopes generally are at or near the limits of technical development. (The same comment may not apply to electron microscope equipment.)

Operations

The cost of collecting benthic samples is comparatively low, particularly when these can be prorated over a period of time. For example, Vollenweider has stated that a ten-year mean was required to get an accurate estimate of the phosphorus loading in the Laurentian Great Lakes. The cost of collecting and analyzing water samples on a biweekly(?) basis for ten years to get one number must be truly staggering. I suspect that sampling the benthic communities in these lakes two or three times in the same ten-year period could be done more cheaply and provide the same, if not more, information and a permanent archivable record for future reference and comparison.

Analysis

The cost of morphologically analyzing 1000 chironomid larvae is probably far less than that of analyzing a similar number of fish. The number of morphological features to be screened is finite (about 12), whereas the number of biochemical tests that could be made are legion (NRCC, 1985). To determine the appropriate biochemical tests to be used to screen for contaminants would require an extensive knowledge of background conditions, e.g., sources of suspected contaminants and biotic and abiotic features (NRCC, 1985). Similarly, analysis for lists of priority pollutants may fail entirely to detect or even approximate the net toxic burden impacting an ecosystem. In contrast, chironomid larvae are directly exposed to the net contaminant burden and directly integrate the interaction between contaminants and abiotic factors. Morphological deformities are not affected by the trauma of sampling or other sampling/preparation procedures. Sampling fish involves stress ranging from mild (anesthesia) to severe (gill netting) and may even be severe enough to cause death. Under stress, biochemical changes are often instantaneous and ephemeral; to obtain the persistence of response required to get accurate biochemical readings, newly captured fish must be held undisturbed in cages until their physiological responses stabilize and return to baseline levels (NRCC, 1985). The persistence of chemical species in the water column can also be ephemeral, and chemical analyses may or may not detect their presence, particularly in flowing waters. Because of their life span and direct exposure, chironomids integrate conditions in their environment over a measurable period of time.

Statistics

The ability to measure a response must be closely matched by its size (NRCC, 1985). When responses are large relative to controls, there is little requirement for accuracy (how closely the estimated value approaches the true value), for precision (repeatability), or for low detection limits. As the size of the response decreases (e.g., at low levels of exposure typical of the natural environment), the effects of analytical error on the ability to detect a difference from "normal" or control levels increase. The ease with which chironomid larvae may be collected usually ensures that there is a sufficiently large data base available for good statistical analyses. Sample homogeneity may also be increased easily by using measuring techniques to reduce sample variability (e.g., separate specific instar groups) and thus to improve statistical results.

Storage and Handling

Prepared and mounted larval specimens represent a permanent record that can be stored and reused at will. Slide-mounted specimens are easily and compactly stored in boxes containing 100 slides. Slide boxes are roughly the size of a standard library book and stack in the same manner. Permanently mounted larval specimens are highly portable and can readily be exchanged between laboratories for interlaboratory comparisons and calibration. Preserved larvae can also be maintained almost indefinitely, as long as their storage containers are tightly sealed and preservative levels maintained. Dessicated materials can also be successfully recovered by rehydration in sodium orthophosphate solution.

Historical Connections

The preservation of chironomid remains in the sediments allows comparisons between current (degraded) community structures and those that existed before degradation of the environment took place. This connection is very important, particularly for targeting goals for rehabilitation measures and determining the success of mitigation procedures. Bacterial communities, communities of soft-bodied animals, and even some animals with more fragile chitinous exoskeletons are rapidly degraded and leave no trace of their communities in the sediments for future comparisons. Under most circumstances, chironomid remains stay permanently buried in the sediments where they were deposited; the events they record, unlike chemical elements, remain unaltered by remobilization and redeposition processes.

DIRECTIONS FOR FUTURE RESEARCH

Field Studies

NRCC (1985) suggested that tests used in biological monitoring should meet three broad criteria:

1. The test must be relevant. It should measure a response related to the toxic effects of contaminants and the ultimate well-being of the individual, population, community, or ecosystem.

2. The test must be transferable. Different laboratories must be able to produce comparable results on check samples.

3. The test must be adequately refined and developed.

Relevance

There can be no doubt about the relevance of deformed chironomid larvae in the environment — the first and most important point in developing biological monitoring tools. Even at the most fundamental level, the presence of grotesquely deformed larvae signals the fact that something is dramatically wrong in their environment. Saether (1970) speculated that grotesquely deformed larvae represented larvae living at or near the limits of their ecological range, but dismissed the theory of edge effect because these larvae were so conspicuous that they would surely have been described in the literature before Brinkhurst et al. (1968) described them. Warwick (1985) has subsequently presented experimental evidence that confirms the linkage between this type of deformity and chemical contaminants; in very preliminary experiments, massively deformed larvae were induced in cultures exposed to chemicals like DDE, a breakdown product of DDT. Warwick (unpubl. data) has subsequently found similar massively deformed larvae in two sites in Saskatchewan, Pasqua Lake and Tobin Lake.

Warwick (1980a) also presented evidence that connected the phenomenon of morphological deformities with the introduction of chemical waste products into the environment. Using palaeolimnological techniques, he was able to correlate the appearance of morphologically deformed chironomids in the Bay of Quinte with the introduction of waste chemicals from an industrial complex built in Belleville, Ontario. The plant, which introduced phenols and other waste products into the bay, was built in 1949, and deformed larvae began to appear about two years later. This unusually close correspondence leaves little doubt about the source of the problem and strengthens the relationship between cause and effect.

Transferability

Test results are also readily transferable. As NRCC (1985) states, it is more than likely that further biases, ambiguities, and problems will arise after a procedure has been introduced into service that will require continuous feedback and test development to further refine the method. Because morphological deformities represent a permanent response to contaminants, they cannot be altered by sampling or preparation procedures. Properly prepared, permanently mounted specimens of deformed larvae are highly portable and can be exchanged readily between laboratories for comparison.

Development and Refinement

The third point, development and refinement, examines the "robustness" of the test. At present, the relationship between morphological deformities and contaminants is in the early stages of refinement. As indicated earlier, Warwick (1985) proposed a method for indexing the severity of deformities in the larval antennae. This method is viewed as a first step towards developing a more comprehensive index of total morphological response using the deformities in all morphological structures. In the antennae, initial information shows that dose-response relationships are not linear but bell-shaped, indicating a quantal dose-response relationship (Fig. 12). Initial information also suggests that as antennal response is overwhelmed at higher concentrations of toxicity, response shifts to other, less sensitive morphological structures like the menta (teeth) and mandibles. Continued research is being directed at (1) developing an adequate data base on which to establish similar indices of severity for each of the other morphological structures for inclusion in an Index of Total Morphological Response and (2) examining the relationship between the morphological deformities in these structures and the concentrations of toxic chemicals. One can only speculate on the form these relationships may take, but they may range from interlocking, equally spaced response curves (Fig. 15a) to unequally spaced curves differing in shape and symmetry (Fig. 15b). One way to develop these relationships is to continue to develop data bases for differentially impacted ecosystems and gradually fill in the "morphological templates" using the antennal indices as a starting point.

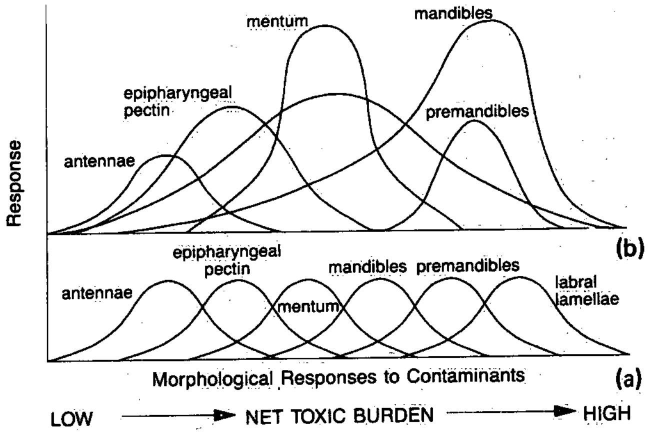

Figure 15. **Possible scenarios for relationships between quantal dose response relationships in different morphological structures. Sequences and relationships hypothetical; for illustration purposes only.**

Other Field Parameters

The biological monitoring capabilities of morphological deformities might also be usefully refined and expanded by field studies that focused on the relationships between contaminants and such life history factors as emergence patterns, generation differences, instar susceptibility, community age, physiological predisposition, and inter- and intraspecific differences. These studies should include the more traditional measures of abundance, biomass, and species diversity in accompanying benthic communities to aid in extrapolation between the higher levels of biological organization. In the long run, the inclusion of this information in a comprehensive index could greatly improve the usefulness and accuracy of biological monitoring.

Laboratory Culture Experiments

The exposure of cultures of chironomid larvae to chemical agents of known toxicity or "reference" contaminants can be usefully employed to calibrate responses observed in field populations. With the long-range transport of airborne pollutants (LRTAP), increased use of pesticides and herbicides in agriculture, and increasing industrialization, it is more likely that the degradation of an ecosystem will be the result of a mixture of chemicals rather than a single contaminant. The net effect of these mixtures probably will bear little or no resemblance to its component parts. A yardstick based on a set of reference contaminants would provide a basis for determining the net effect of such mixtures without having to identify their component parts. Such a yardstick, based on a number of responsive species, each characteristic of a different aquatic ecosystem, would greatly improve the measuring and detection capabilities of the technique for operational use.

Culture experiments could also be employed to examine the theory that deformation in the larval head capsule shifts from the more sensitive structures like the antennae to harder structures like the menta and mandibles as the concentration of contaminant increases. It might be logical to start with DDE since some documentation already exists on its effects. The original single-dose experiments should also be expanded to include continuous concentration exposures and multiple generation exposures and to answer questions concerning bioaccumulation effects and the importance of exposure time in culture testing.

Similar experiments could also be used to determine the significance of massive deformities. Currently, these are thought to signify a response to higher concentrations of contaminants, but it may be that they can occur at any point on a concentration gradient. The mechanisms or processes through which chemicals exert their effects should also be examined. A number of mechanisms have been proposed, which vary from phenotypic (Hare and Carter, 1976) to enzymatic (Frank, 1981) and hormonal (Slàma and Williams, 1965). From the evidence available so far, it seems likely that larvae may respond to different chemicals in different ways. If this is the case, such specificity of response may be important in identifying chemicals or classes of chemicals in contaminated environments. Such information would add immeasurably to the diagnostic value of the tool. The effects of chemicals on chromosomal structure and histochemical makeup should also be investigated, particularly in view of their emerging use in taxonomic work. The evolutionary aspects of contaminants in the environment have largely been ignored (Policansky, 1986).

Experiments to establish the relationship between the sensitivity of naturally exposed organisms and laboratory cultures become more meaningful and accurate the closer laboratory conditions mirror conditions in the ecosystem. According to Cairns (1986), better laboratory results are obtained when (1) both field and laboratory measurements are made at the same level of biological organization; (2) a high degree of environmental realism exists for certain attributes (e.g., water quality); and (3) the test species survive for the duration of the test. In approaching environmental realism, the effects of such biotic factors as temperature, oxygen regime, salinity, food type and availability, and substrate conditions could be examined (Warwick, 1988). Such information would greatly improve the precision with which extrapolations could be made between experimentally exposed and naturally exposed organisms.

CONCLUSIONS

Although the techniques for using these physical responses are still much in the developmental stage, there is no arguing the relevance and environmental importance of morphological deformities and their relationship to contaminants. At its most fundamental, the presence of large numbers of deformed larvae signal that something is radically wrong with their environment. Since chironomid larvae form an integral link in aquatic food chains, and since humans are intimately and ultimately linked to these same systems, it is important that we heed the warning these morphological responses convey.

In conclusion, I repeat Dr. McTaggart-Cowan's admonition: "It is urgent that Canadians clearly grasp the extent and insidiousness of this threat to the livability of our environment. It is imperative that they support the slow, undramatic, costly, perhaps uncomfortable but undeniably essential steps to redress the rapidly accelerating . . . deterioration caused by . . . contaminants in the environment" (Hall and Chant, 1979).

There are those who believe there is a quick, cheap, and easy "fix" for our environment's problems. I doubt very much this will prove to be the case. It is going to take determination, perserverence, and hard work before any progress will be made. Without this acknowledgment and the will to accept the challenge, we will get nowhere!

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