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Use of Three New Zealand Chironomids, *Chironomus zealandicus*, *Chironomus* sp. a and *Polypedilum pavidus* (Insecta: Diptera) as Biotoxicity Test Organisms

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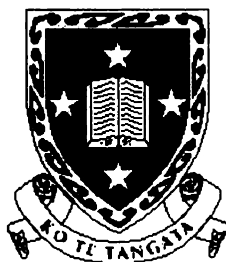
Jeyasingham Kanapathippillai



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Abstract

Three species of New Zealand chironomid, *Chironomus* sp. a, *Chironomus zealandicus* and *Polypedilum pavidus* were studied for their potential as biological indicators of freshwater environmental pollution and for use as laboratory bioassay species.

A standard methodology was developed to successfully culture these three chironomids in the laboratory in order to obtain all larval stages of each species for laboratory bioassays.

Head capsule deformities and physical wear were examined for *C. zealandicus* collected from four study sites in summer and winter from 1994 to 1996, and from larvae reared for two successive generations in a control substrate. Results showed that the incidence of head capsule deformity was influenced by both substrate type and season. The cause of this deformation was not identified although sediment chemistry, genetics and other physico-chemical factors are collectively implicated. Significantly greater frequency of physical wear of head capsule structures in larvae collected from Hamurana Stream and a progressive decline of wear in larvae reared on a paper towel control substrate indicated that feeding activity on a relatively coarse substrate may have caused the wear.

A field survey indicated that, although all these species were present at every site, they show particular site preference. A life cycle test was performed to investigate the substrate preference of *C.* sp. a and *C. zealandicus* on sediments collected from the four study sites, Hamilton Lake, Lake Ngaroto and Hamurana Stream and Sulphur Point of Lake Rotorua. Results showed that Lake Ngaroto sediment and Sulphur Point sediment favour *C.* sp. a and *C. zealandicus* respectively.

The acute toxicity of arsenite (As^{3+}), arsenate (As^{5+}), and copper (Cu^{2+}) under different conditions was investigated. Although *C. zealandicus* and *C.* sp. a are closely related, they showed considerable difference in sensitivity to arsenic and copper. A number of

biological (age), chemical (valency state), and physical (season, temperature) factors were observed to affect the toxicity of arsenic and copper to the test chironomid species.

Chronic sublethal effects of arsenic-spiked sediments from Hamilton Lake and Lake Ngaroto and a paper towel substrate on *C. zealandicus* and *C.* sp. a were also studied under laboratory conditions. This study showed that the sensitivity of the chironomid species varied depending on the length of exposure to the spiked sediment, the number of endpoints examined, and other laboratory testing conditions such as renewal of dilution water. This experiment also indicated that *C. zealandicus* was more resistant to arsenic spiked sediment than *C.* sp. a. A protocol was developed for conducting standard sediment toxicity tests.

The results of this work imply that although one or more of the chironomid species used in this study may be used as a bioindicator in monitoring freshwater lakes, use of these species as indicator organisms should be carefully assessed based on their occurrence in the location concerned. Although these three species may be used in laboratory bioassays, *C. zealandicus* appeared to be more convenient because of its continuous abundance at study sites and tolerance to physical handling.

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List of abbreviations

AAS	Atomic absorption spectroscopy
AET	Apparent effect threshold
ARCS	Assessment and remediation of contaminated sediments
As	Arsenic
As^{3+}	Arsenite (trivalent arsenic)
As^{5+}	Arsenate (pentavalent arsenic)
ASTM	American society for testing and materials
Cr	Chromium
Cu	Copper
Cu^{2+}	Cupric ion
DDE	2,2-Bis (4-chlorophenyl)-1,1-dichloroethene
DDT	1,1-Bis (4-chlorophenyl)-2,2,2-dichloroethene
EC_{50}	Median effective concentration
FAAS	Flame atomic absorption spectroscopy
GFAAS	Graphyte furnace atomic absorption spectroscopy
LC_{50}	Median lethal concentration
MATC	Maximum acceptable toxicant concentration
PAH	Poly-aromatic hydrocarbons
PCB	Polychlorinated biphenyl
SEC	Sediment effective concentration
SE	Standard error
TIE	Toxicity identification evaluation
USEPA	United states environmental protection agency

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General introduction

1.1. INTRODUCTION

One of the most serious threats to the quality of an aquatic environment is contamination by persistent chemicals. Contaminated sediments are a major source of pollution in aquatic ecosystems and represent a potential threat to all components in it (Sorensen et al. 1977; Landrum and Robbins 1990). Sediments are a repository for organic and inorganic contaminants that can accumulate to high concentrations (Shimp et al. 1971; Oschwald 1972; Medine and McCutcheon 1989). It has become increasingly important to directly determine environmental responses to contaminant stress in order to monitor and regulate environmental quality.

The use of natural bioindicators to assess water quality and the health of freshwater ecosystems offers a number of advantages over chemical and/or bioassay approaches alone. Organisms are good indicators of aquatic conditions because they are intimately bound to their environment and are directly subject to the sum total of all chemical, physical and biological processes influencing that environment over the length of their life cycles. On the other hand, chemical measurements, and to a lesser degree bioassay measurements, are biased toward short-term conditions that exist only at the time of sampling or experimentation (Warwick 1990a). Chemical analysis, in itself, provides little useful information on the effects of contaminants, especially when they are present in the water column at concentrations below the limits of analytical detection, and gives no information of their bioavailability. In addition, complete chemical characterizations are time-consuming, expensive and most often require specialized equipment (Giesy and Hoke 1989). Certitude that all toxic compounds in a sample are identified may never be achieved and, hence, chemical measurements may lead to an underestimation of the total toxicity (Vermulen 1995). Because of synergistic and antagonistic interactions, the combination of chemicals may result in an enhanced or reduced toxicity, further complicating the interpretation of chemical analyses (Giesy et al. 1988a, 1998b; Warwick 1988; Giesy and Hoke 1989). This has lead to increased interest in biological

methods for assessing the impact of contaminants on water quality and the general health of aquatic ecosystems.

Sediment toxicity testing is a relatively new approach used in ecological risk assessments. The US Army Corps (USEPA-USACOE 1977) developed the first sediment tests because of concerns in the late 1960s and early 1970s over contamination of dredged material and its suitability for open-water disposal (USEPA 1994a). There was relatively little testing until the 1980s, with a dramatic increase in the latter part of that decade (Burton 1991). Increasing interest has focused on developing and applying methods for assessing the toxicity of contaminated sediments. Nebeker et al. (1984) first described general methods for conducting toxicity tests with freshwater sediments using amphipods, midges, mayflies, and cladocerans. This initial publication stimulated research on developing more detailed testing procedures. The science has progressed at a comparatively fast rate because of the similarities to, and the earlier development of, the water column and effluent toxicity tests. The USEPA has been developing approaches for managing contaminated sediments and method standardization that will result in a greater amount of sediment testing and research in the near future (Southerland et al. 1992; USEPA 1994a).

To evaluate the toxicity of a substance to an organism, it is essential to test the effect of the substance both in the laboratory and in the field. Laboratory sediment toxicity tests are increasingly being recommended and used in the determination and identification of sediment-associated contaminants. Laboratory tests with field-collected sediment have been used to determine spatial and temporal distribution of the toxicity of a substance in the sediment (Swartz et al. 1984). In addition, sediment toxicity tests with complex contaminant mixtures are an important tool for making decisions about the extent of remedial action needed for contaminated aquatic sites. These provide strong complementary evidence of the degree of pollution-induced degradation in aquatic communities (Chapman 1986; Barrick et al. 1988).

A standard protocol is essential to conduct toxicity tests with an organism to evaluate its sensitivity to various toxicants and to compare its sensitivity with that of the same species of other regions and other taxa. A standard testing protocol is usually developed

depending on several aspects of the test animal (e.g., biology, behaviour, morphology, ecology) and it is not necessarily applicable for all the species of the same family or even genus. However, available standard protocols for conducting toxicity tests with benthic macroinvertebrates are mostly concerned with Northern Hemisphere species. Many benthic macroinvertebrates of the Southern Hemisphere, which may be used as successful bioindicators, have been rarely used in ecotoxicology.

Although the initial development of sediment toxicity test methodology emphasized acute tests, there has been recognition that chronic endpoints such as growth and reproduction are more sensitive and discriminatory measurements of biological effects.

The necessity of studying the sensitivity of an organism to a toxicant under as many biological (e.g., different larval instars), chemical (species of chemical) and physical (different temperature) conditions as possible is well recognized by environmental biologists. The sensitivity of three common New Zealand chironomids to sediment toxicity has been investigated under a number of laboratory conditions in the present study. Although the specific sediment effect concentrations (SEC) were not determined for individual toxicants, protocols developed in this study may be used to evaluate the toxicity of chemicals to chironomids in the future.

1.2. CHIRONOMIDAE AND BIOMONITORING

Benthic invertebrates are closely associated with surficial sediments and therefore may be exposed to contaminants in the sediments. Many aquatic organisms are exposed to contaminant mixtures during their entire life cycle or at least throughout the most important parts of it. For this reason, combination effects have been widely recognized as an important aspect of the ecotoxicological assessment of chemicals (Murphy 1980; EIFAC 1987; Enserink et al. 1991).

Many invertebrates including crustaceans, molluscs, and insects have been used as bioindicators in aquatic pollution. However, the most widespread organisms used in biological monitoring of aquatic ecosystems have been genera of the family

Chironomidae (Insecta: Diptera) (Clarke et al. 1995). Rosenberg (1992) summarized the advantages and difficulties of using freshwater benthic macroinvertebrates as well as Chironomidae in biomonitoring.

1.2.1. Advantages of using Chironomidae for biomonitoring

1. Ubiquitous occurrence of Chironomidae in many different types of aquatic systems, and in habitats within those waters which could be used to monitor the effects on those habitats.

2. Chironomids are, perhaps, the most diverse of benthic macroinvertebrates (Coffman and Ferrington 1984). Availability of a large number of species offers a spectrum of responses to environmental stresses.

3. Relatively long life cycles compared with other biotic groups allow elucidation of temporal changes due to perturbations. Life cycles can be, on the other hand, relatively short in warm waters (Benke et al. 1984).

4. Only simple inexpensive equipment is required for sampling.

5. Relatively well-known taxonomy and keys for identification are available, even though this is not true for many groups of New Zealand Chironomidae.

6. Responses of many common species to different types of pollution have been established. Although these are proportionately less sensitive for chironomids than for some other benthic macroinvertebrates, responses are quite clear and sharp (e.g., mouthpart deformities and cuticle thickening).

7. Sedentary nature, bright red colour, and the characteristic feature of the figure eight movement (Fisher 1986) make for easy detection of response in bioassay tests.

8. Unlike many other benthic macroinvertebrates, various life stages (e.g., egg, larva, pupa and adult) and various parts in a particular life stage (e.g., structures of the head

capsules of larvae) of chironomids could be used to observe responses to the environmental impact. In addition to the detection of impact, severity of the impact also could be assessed by indexing responses (e.g., multiple deformities of head capsule structures).

9. More than a single aspect such as biological (life cycle; Benoit et al. 1997), physiological (haemoglobin studies; Thomson 1975), morphological (deformity; Warwick 1985, 1988), and behavioural (oviposition, feeding, flight; Williams et al. 1985) responses could be studied for a single species.

1.2.2. Disadvantages of using Chironomidae for biomonitoring

Although chironomids possess a number of advantages for use as indicator organisms, as for many other benthic macroinvertebrates, they have disadvantages too.

1. They do not respond to all impacts. Therefore, a monitoring programme using other organisms together with Chironomidae is needed (Rosenberg 1992).

2. They sometimes respond to factors other than water quality conditions such as current velocity, or the nature of the substrate. Ecological knowledge of the species involving careful experimental design is needed to carry out bioassays to assess water quality.

3. The abundance and distribution of chironomids are highly influenced by the season. This may pose sampling problems during specific periods or in specific habitats.

4. Drift behaviour may carry invertebrates into habitats in which they do not normally occur. This may give false information on the abundance and distribution of the animal.

The incidence of chironomid abnormalities has been suggested as a potential biological contaminant marker, applicable *in situ*, to environments as diverse as major harbours, drainage canals, rivers, streams, ponds, and the profundal and littoral zones of most lakes (Warwick and Tisdale 1988; Diggins and Stewart 1992). Although not an indicator of specific contaminants, the occurrence of abnormal chironomid larvae may

serve as an economically and biologically relevant first step evaluator and long term monitor of the benthic environment, and can suggest where more intensive bioassay and chemical testing would be most effectively employed.

Head capsule deformity in chironomids that were sampled from contaminated sites showed positive relationships with the level of pollution. However, it has always been difficult to establish a contaminant specific head capsule deformity relationship in *in situ* studies. There may be several possible reasons for this: 1. Sediments often contain complex mixtures of contaminants. The possible effect of a specific contaminant may be altered in sediment due to the synergistic and/or antagonistic effect of other contaminants present. 2. Lack of baseline deformity data for a particular sediment makes comparison of deformities from reference and contaminated sites difficult. 3. The influence of physico-chemical factors on head capsule deformity other than the contaminant concerned may be difficult to avoid. 4. There may be difficulties in distinguishing developmental deformities from physical wear in natural sediments. Laboratory bioassays, on the other hand, may avoid all these possible complications and confirm the role of a contaminant in inducing head capsule deformity in chironomids. Under controlled conditions, even a clear dose-response relationship and the type of deformity for a contaminant may be established.

Although chironomids are used as test organisms for a wide range of toxicants in aquatic environments, their use to test heavy metal pollution is considerably more common.

1.3. HEAVY METALS IN THE AQUATIC ENVIRONMENT IN NEW ZEALAND

Various sources such as geothermal activity, weathering, erosion, urban runoff, municipal wastes, refuse tips, agriculture, and industries contribute to pollution of the aquatic environment by heavy metals in New Zealand.

New Zealand's main geothermal areas are centred in the north and central regions of the North Island and the north of the South Island (Smith 1986). For large water bodies, the deposition of metals by the atmosphere may constitute a high proportion of the total input. However, very few data are available regarding such deposition in New Zealand. Weathering of rock is one of the main sources of background metal levels in the aquatic environment, and in areas containing metaliferous ore bodies, water and sediment metal levels may be elevated. Erosion of soils is a source of suspended solid material in waters that contributes to aquatic environmental pollution. Levels of heavy metals in New Zealand soil vary with region (Smith 1986).

Urban storm water in New Zealand is generally separated from domestic and industrial sewer systems and the untreated storm runoff is usually discharged directly into the closest water body, be it fresh, estuarine, harbour, or coastal. Treatment of industrial discharges is usually followed by dilution before diversion into sewage systems or waterways (Rajendram 1992). Although runoff usually contains low metal concentrations, large volumes are usually discharged leading to proportionately large loads into enclosed water bodies (Smith 1986).

Industries in New Zealand dispose of their waste water to municipal sewers, to surface water, on land, or to commercial treatment plants. Smith (1986) summarized possible industrial sources of heavy metals, including arsenic (As), mercury (Hg), copper (Cu) and chromium (Cr). Agriculture, electronics, engineering and soldering, leather, metal finishing, metal mining, paints and pigments, petroleum refining, printing, rubber, storage batteries, textiles, and the timber industry are major industrial sources of heavy metal pollutants. Most of the agricultural chemicals registered in New Zealand for agricultural use are organic, but several registered compounds contain heavy metals, such as copper (Cu) or zinc (Zn) (Smith 1986). Mining, especially base metal mining, has the potential to discharge heavy metals unless wastes are treated (Lawrence and Smith 1983). The concentration of heavy metals in waters from mining operations may be increased by the frequent presence of sulfide minerals. The timber industry in New Zealand also contributes considerably to aquatic environmental pollution. The aquatic environment is polluted by both waste water of paper and pulp mills and preservatives (copper - chrome - arsenate) used in timber preservation. As, Cu, and Cr are the main

ingredients of timber preservatives used in timber treatment plants, where effluent discharge containing these metals have been known to contaminate streams.

Arsenic has been chosen for this study because it is one of the more important metals in the New Zealand environment and is continuously released into waterways in the central North Island due to natural geothermal activity. Although arsenic is generally regarded as highly toxic to all aquatic communities, its toxicity has rarely or never been studied in New Zealand aquatic invertebrates. The toxicity of arsenic to three species of chironomid has been studied in this work. Sediments often contain complex mixtures of heavy metals. However, it is impractical to study all at once. Therefore, Cu and Cr were considered as reference heavy metals for As in this study and compared wherever appropriate.

1.4. AIMS AND OBJECTIVES

The first objective of this study was to investigate the relationship between the degree of chironomid head capsule deformity, and the presumptive degree of impact at four quite different lake sites.

Two Waikato lakes (Lake Rotoroa - commonly known as Hamilton Lake, and Lake Ngaroto), and two Lake Rotorua sites (Hamurana Stream and Sulphur Point) (Fig 2.1) were selected for this study in the belief that these sites represented differing levels of natural and anthropogenic impact. Study sites are described in Chapter 2.

Chironomid larvae are common components of the benthos at all sites. However, preliminary observations indicated that three different chironomid species, *Chironomus zealandicus, Chironomus* sp. *a,* and *Polypedilum pavidus* dominated different sites (Chapter 3). Studying only one species as a bioindicator may not always be useful in biomonitoring ecosystems such as the lake sites of this study. Using more than one species of an organism may be more reliable. Therefore, these three species were used for further laboratory bioassays.

Although the species selected for the present study are the major chironomid species in many lakes in the central North Island of New Zealand, their abundance is not uniform throughout the year and is considerably influenced by seasonal and other environmental factors. In some cases, one species dominates the benthic macroinvertebrate community while other two are completely lacking. Nevertheless, a continuous supply of test organisms is essential for laboratory bioassays and to this end cultures of each species were established and this represents the second objective of the thesis.

The third objective was to test the toxicity of site sediments under laboratory conditions with chironomids as indicator organisms.

The fourth objective was to investigate chironomid species as indicator organisms to study the toxicity of As under laboratory conditions.

The fifth objective of this study was to develop standard testing protocols using three New Zealand chironomid species and to evaluate the potential of test species as indicator organisms.

Characterization of study sites

2.1. INTRODUCTION

Four sites chosen for this study were presumed to show differing levels of pollution. All study sites are situated in the central North Island (Fig. 2.1). The biotic and abiotic properties of some of the study sites have been studied by Fish (1975), Irwin (1975), Waipa County Council (1978), Waikato Valley Authority (1985), and Department of Lands and Survey (1986) and are briefly summarized below.

The objective of this chapter is to describe the distribution and relative abundance of benthic macroinvertebrates at each study site and to relate physico-chemical characteristics of the environment they inhabit. A monthly survey of benthic macroinvertebrate communities was carried out for twelve months from June 1996 to May 1997. Measurements of physico-chemical properties at sampling sites and of sediment samples collected from each site were also carried out by appropriate methods described below.

2.1.1. Hamilton Lake (Lake Rotoroa)

Both Hamilton Lake and Lake Ngaroto are situated on the margin of the Rukuhia peat bog. Hamilton Lake is a small shallow peat lake (37° 48' S; 175° 16' 30" E, area = 55 ha and maximum depth < 6 m) situated within the Hamilton City boundaries and is surrounded by an established urban catchment composed of residential and parkland areas. The lake bottom is composed of fine sticky mud and decaying vegetation.

In 1959, eleven thousand litres of the aquatic herbicide sodium arsenite ($NaAsO_2$) were sprayed over about 39 ha of Hamilton Lake to control the problem of aquatic weed growth. Lake sediments maintain high levels of arsenic decades after the herbicidal application. Thirty two years after the application of sodium arsenite the mean

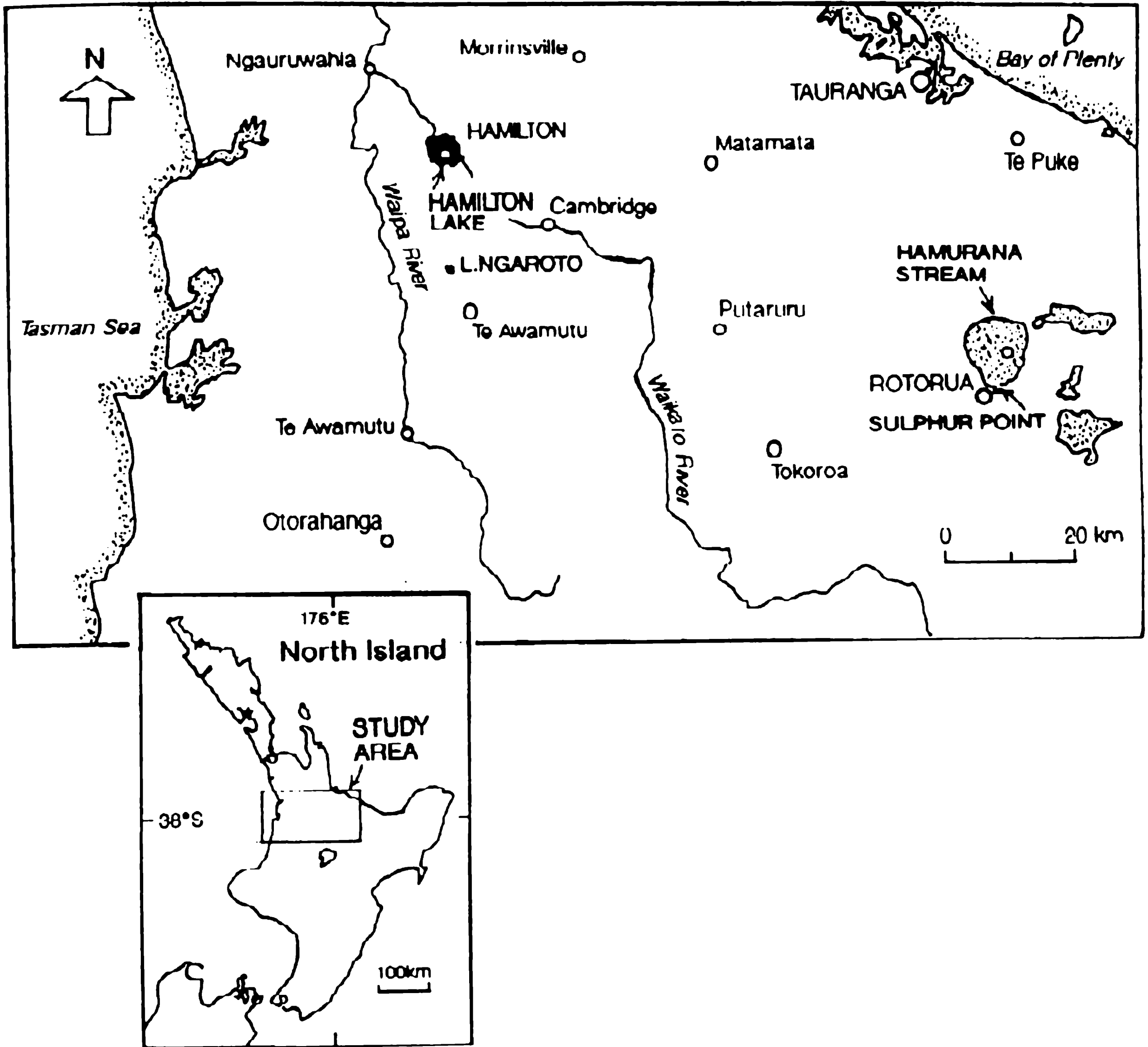


Fig.2.1. Map showing sampling sites.

concentration of all surficial sediments was 184 mg/kg with a range of 12-900 mg/kg (Rajendram 1992). Arsenic in core fractions ranged from 7 to 1080 mg/kg. Tanner and Clayton (1990) also reported elevated sediment As concentrations of 540 - 780 mg/kg for four sites within Hamilton Lake. In addition, there is patchy contamination with other heavy metals such as Pb, Zn, and Cu, believed to enter the lake via storm water inflows (Rajendram 1992).

2.1.2. Lake Ngaroto

Lake Ngaroto (37° 58' 30" S; 175° 17' 30" E) is also a small shallow peat lake (area =108 ha and maximum depth 4 m), probably of very similar age and geographical origin to Hamilton Lake (Irwin 1975) but surrounded by a wholly rural catchment. The lake bottom is composed of fine sticky mud. This lake also has a history of herbicidal weed control designed to improve the lake for boating users. In 1973, the weed *Elodea* had become a problem for lake users and was controlled by an application of herbicide. Subsequently, these weeds were kept in check by water turbidity and a herbicidal spraying programme carried out under the supervision of the Waipa County Council (W.C.C. 1978). In all cases, however, the herbicide applied was organic rather than arsenic as in the case of Hamilton Lake.

Although Hamilton Lake and Lake Ngaroto are similar in geographical origin, age, and structure, abiotic factors such as temperature (both bottom and surface), suspended solids, turbidity, total phosphorus, and total Kjeldhal nitrogen, as well as biotic factors such as chlorophyll - a and phytoplankton were found to be higher in Lake Ngaroto than in Hamilton Lake by the Waikato Valley Authority (1985). This indicates a higher degree of eutrophication in Lake Ngaroto at that time, although Hamilton Lake has recently suffered a collapse of macrophytes and a change to a phytoplankton dominated system very similar to the situation that prevails in Lake Ngaroto.

2.1.3. Lake Rotorua

Two sites were chosen at Lake Rotorua (38° 6' S; 176° 16' E). The lake is large (81 km^2) and shallow (mean depth 10.5 m). All areas within the Rotorua caldera have been influenced by geothermal activity throughout recent history.

Sulphur Point

Sulphur Point (38° 8' 30" S; 176° 15' 30" E) is adjacent to the Rotorua municipal area and receives effluent discharges via the Puarenga Stream from industrial and municipal waste sites. The entire area is also heavily influenced by geothermal activity. The substrate at this site is composed of sticky clay, sand, and rocks. Chironomid larvae seem to be the predominant benthic fauna throughout the year. Water temperatures are usually elevated by geothermal activity (Fish 1975).

Hamurana Stream

Hamurana Springs (38° 2' 20" S; 176° 15' E) is a large cold water spring of very high quality potable water with a discrete channel entering Lake Rotorua that contributes approximately 17% of the lake's daily inflow (Fish 1975). The site chosen for study in Lake Rotorua was adjacent to the Hamurana Stream and is heavily influenced by this inflow. The substrate is composed mainly of coarse sand. The water temperature of the Hamurana Stream is lower than that at Sulphur Point and varies from 11 to 13°C throughout the year (Fish, 1975). The major benthic fauna comprises chironomids, amphipods, and oligochaetes. This site is not known to receive any anthropogenic or geothermal contaminant inputs.

2.2. BIOLOGICAL AND NON-BIOLOGICAL PROPERTIES OF THE STUDY SITES

It is generally not appropriate to study individual components of an ecosystem when making assessments of sediment toxicity, since aquatic ecosystems are composed of

interdependent trophic levels (Burton 1991). Complete ecological assessments of sediment toxicity usually require the use of resident biota as indicators of sediment quality. For the assessment to be successful, closely integrated biological, chemical, and physical data are required. Since sediments tend to integrate historical water quality conditions, the spatial and temporal distribution of resident organisms can reflect the degree to which chemicals in the sediments are toxic. Field surveys of benthic invertebrates provide an essential component of biological assessments of the toxicity associated with contaminated sediments.

There are many factors that need to be considered when sampling sediments for benthic invertebrates that differ from the considerations required for sampling sediments for toxicity testing. Benthic invertebrate distributions are strongly influenced by abiotic factors in the absence of contaminants (Resh 1979; La Point et al. 1984; Pettigrove 1990; La Point and Fairchild 1992) and, in some cases, effects due to abiotic factors can mask the effects of contaminants. Important abiotic characteristics (e.g., sediment grain size, sediment organic content, sediment nutrient content, water quality, current velocity, water depth) at a study site should therefore be evaluated so that the potential confounding effects of these characteristics can be accounted for when the data are analyzed and interpreted.

The collection methods for benthic invertebrate samples depend on the type of habitat to be sampled such as rocky substrate, fine-grained substrate, heavily vegetated areas and the type of system such as flowing or standing water from which the samples are to be taken. Many of the benefits and limitations of different sampling devices have been described in several publications (Resh 1979; Downing 1984; Klemm et al. 1990). Habitats in the present study were devoid of heavy vegetation and rocky substrate and consisted mainly of fine-grained substrate. All sampling at these sites employed an Ekman grab.

Grab samplers are usually designed as a box with a set of jaws, or a rotating bucket, that takes a wedge-shaped bite out of the surface sediment. Grab samplers are designed to take discrete "bites" of the sediments that are representative of a fixed area and are therefore the preferred method for collecting sediments for the quantitative assessment

of benthic infauna. Grab sampling has a number of advantages over other sampling methods in assessing benthic macroinvertebrate community structure. They are easy to use and the smaller grab samplers allow hand deployment. Grab samplers also do not disturb the surface sediment significantly unless they overpenetrate. Disadvantages of the grab sampler include the uncertainty of the depth of sediment penetration and the loss of sample integrity when the sampler is opened. Penetration depth of remotely deployed grab samplers in deep water can be highly variable, depending on sampler design and sediment composition. The depth of water at sampling sites in this study, however, never exceeded about 1.2 m and the sampler was deployed by hand to minimise sampling variance.

To determine which indices to evaluate in studying benthic macroinvertebrate communities, it is necessary to determine the kind of information required for the planned data analysis. Some analytical methods are amenable only to a qualitative assessment of benthic invertebrate communities. However, the more desirable goal of a study to characterize a sediment substrate is the quantitative assessment of invertebrates (USEPA 1994a). Several metrics are available for qualitative and quantitative assessments of benthic communities (Merritt and Cummins 1984; Pennak 1989; Plafkin et al. 1989; Klemm et al. 1990). Community structure measurements can be divided into four broad categories (USEPA 1994b): 1. numbers of individuals and standing crop; 2. multivariate analyses; 3. diversity and similarity indices; 4. indicator organisms. Benthic community health can be assessed by determining the structure (e.g., taxa richness), community balance (e.g., percent dominant taxa), functional feeding group (e.g., percent scrapers, percent filter feeders) and composition of the macroinvertebrate community (Plafkin et al. 1989). Most of these metrics are quantitative, although the use of indicator organisms tends to be more qualitative.

The most frequently used and simplest metric, numerical abundance, was used to interpret the results of this survey. This index has the advantage of being easily measured and is highly sensitive to the physico-chemical characteristics of sites (Sheehan and Winner 1984; van Hassel et al. 1988).

2.2.1. METHODS

2.2.1.a. Collection of sediment samples

Monthly sampling of sediment and measurements of physico-chemical parameters were made on the same day at Hamilton Lake and Lake Ngaroto while on the following day at the Lake Rotorua sites. Sediment samples were collected from each of the four study sites using an Ekman grab (150 x 150 mm). Samples were taken in shallow water close to shore to reduce confounding effects related to deep water stratification. Depth of the water at sampling sites varied from 0.7 m to 1.2 m depending on lake levels. Four replicate sediment samples from each site were pooled together in a clean bucket, mixed well and a subsample of about 500 g was transferred into a clean polythene bag, returned to the laboratory on ice and frozen at -20°C. Samples collected for metal and total organic matter analysis were pooled together before taking a subsample in order to minimize, mainly, the time and cost of analysis. Frozen samples were freeze-dried as soon as they were brought to the laboratory and were stored at room temperature. All sediment samples were analyzed for heavy metals within six weeks from sampling. Another 100 g of the pooled sediment subsample from each site was oven dried at 100°C within 24 hours for measurement of moisture content and total organic matter. A third set of subsamples from each site was collected in a clean polythene container for determination of sediment particle size distribution.

Four replicate benthic grab samples were collected at each site to study the macroinvertebrate community structure at each of the randomly selected stations where chironomid specimens were collected for culture and toxicity tests. Each sample was sieved through a 250 μm brass screen. Site water was used for rinsing. Material retained by the sieve was collected in bags containing 10% formalin and kept on ice until returned to the laboratory.

Physico-chemical parameters of water measured at the sampling sites were dissolved oxygen (YSI model 55 oxygen meter - Yellow Springs Instrument), conductivity and temperature (YSI model 30 conductivity, salinity and thermometer - Yellow Springs Instrument) and pH (pH M20 standard pH meter - Radiometer Copenhagen). Sediment

particle size distribution was determined at the beginning of this study while moisture content, total organic matter and heavy metals were measured as needed for specific experiments.

2.2.1.b. Sediment particle size, moisture content and total organic matter determination

The particle size distribution of sediment samples from the four sites was determined by a Malvern Instruments MASTERSIZER (Malvern Instrument Ltd., UK). System details are given in Appendix 1 (Table A.1).

The moisture content of sediment samples was measured by drying. Subsamples were thoroughly mixed in their original container. Three replicates of approximately 25 to 30 g of whole sediment were taken into pre-weighed 30 mL glass vials and accurately weighed. Moisture content was determined by leaving the sediment vials in an oven at 100°C until dried to a constant weight. The weight of vials was recorded every 12 hours. The difference in weight of a sample before and after drying in the oven gave the moisture content.

Another three replicates of thoroughly mixed whole sediment were oven dried at 100°C to a constant weight. These were then ignited in a pre-heated furnace for an hour at 600°C to determine the total organic matter. The difference in weight of a sediment sample before and after ignition in the furnace gave the total organic matter.

2.2.1.c. Analysis of heavy metals

Total arsenic (As), chromium (Cr), and copper (Cu) were measured from freeze-dried sediment samples collected at sites in summer (December) 1994, 1995, and 1996 and winter (June) 1995, 1996 and 1997 (Chapter 3).

2.2.1.d. Heavy metal measurement in natural sediment

Freeze-dried sediment samples were acid digested as follows and As, Cr, and Cu were measured by either flame atomic absorption spectrometry (FAAS) or graphite furnace atomic absorption spectrometry (GFAAS).

All glassware was cleaned by soaking in 20% nitric acid (laboratory grade) for 24 hours, rinsed in MILLI-Q water not less than 5 times to remove any traces of acid, and dried in an oven at 60°C before being used for metal determination.

Each freeze-dried sediment sample was thoroughly mixed and 2 g was digested in a mixture of 15 mL of concentrated nitric acid (HNO_3) and 5 mL of hydrogen peroxide (H_2O_2). The acid sediment mixture was boiled in a 100 mL beaker covered by a watch glass for about 1 to $1^1/_2$ hours on an electric hot plate and then allowed to cool to room temperature. The solution was then filtered through acid hardened filter paper (Whatmann # 541) into a 100 mL volumetric flask. The final volume of each sediment extract was made up to the mark of the volumetric flask with MILLI-Q water.

To determine As content of the samples, another set of sediment samples was acid digested as above and 0.4 mL of potassium iodide (KI) was added from a stock solution of 50 g/L to the acid digested samples to have a final concentration of 0.2 g/L. Potassium iodide was added to the acid digested mixture to reduce pentavalent arsenic to the trivalent state because the response of trivalent arsenic is higher than that of pentavalent arsenic in FAAS (Driehaus and Joekel 1992). Acid digested samples were stored at 4°C until analysis. All samples were analyzed within three weeks from digestion.

2.2.1.e. Preparation of standard solutions for AAS

The atomic absorption spectrometer (AAS) was calibrated against a blank (MILLI-Q water and acid mixture) and As, Cu, and Cr standard solutions at different concentrations. Standards of each metal were prepared by diluting 1 g/L stock solutions with MILLI-Q water. The equivalent amount of acid mixture used to digest samples

was also added to the standards. In addition, the required volume of KI solution was also added to As standard solutions to give a final concentration of 0.2 g/L.

2.2.1.f. Determination of heavy metals by flame AAS

A GBC 909 atomic absorption spectrometer was used to determine Cu and Cr in the sediment extracts. Arsenic determination required a hydride generator (GBC HG 3000 - GBC Scientific Equipment Pty Ltd, Australia) in addition to the atomic absorption spectrometer. Hollow cathode lamps composed of a tungsten anode and a cylindrical cathode were used as radiation sources. The cylinder was made of the corresponding element for each heavy metal tested. An air-acetylene mixture was used for fuel and oxidation supply to the flame.

The method used for As was slightly different from the method used to determine Cu and Cr. In As determinations, samples and standards were mixed with sodium boro-hydride ($NaBH_4$) solution and concentrated hydrochloric acid (HCl) in the reaction chamber of a hydride generator to produce arsenic hydride. Other analysis parameters for FAAS are given in Appendix 1 (Table A.2).

2.2.1.g. Determination of heavy metal concentration by graphite furnace AAS

Depending on the concentration of heavy metals in samples, a GBC 905 graphite furnace AAS with a GBC GF 3000 graphite furnace was used to determine As, Cr, or Cu concentration. Sediment samples and standards were prepared as for flame AAS. Analysis parameters for GFAAS are given in Appendix 1 (Table A.3).

2.2.1.h. Invertebrate Community structure

Before sorting in the laboratory, samples were rinsed thoroughly with tap water to remove excess formalin, silt and/or mud. Each sample was placed in a 2 L glass beaker and agitated with tap water so that the lighter detrital material floated while the snails, bivalves, and heavier material remained on the bottom of the beaker. Sorting times ranged from approximately 3 to 6 hours per sample.

A binocular dissecting microscope with a magnifying power of 4x-12x was used to sort samples. Organisms were sorted and enumerated into the following taxonomic groups: Oligochaeta, Hirudinea, Trichoptera, Coleoptera, Chironomidae, Amphipoda, Bivalvia, and Gastropoda. These samples were used to estimate macroinvertebrate numerical abundance (individuals/m^2).

2.2.2. RESULTS

Physico-chemical parameters measured monthly at the sampling sites from June 1996 to May 1997 are given in Table 2.1. Between the two Waikato lakes, no major differences were observed in dissolved oxygen, pH, or temperature. However, conductivity was always slightly higher in Lake Ngaroto than in Hamilton Lake. Between the two sites in Lake Rotorua, dissolved oxygen and pH were higher while conductivity and temperature were lower at Hamurana Stream than at Sulphur Point.

The particle size distribution, moisture content and total organic matter of sediment samples is given in Table 2.2. The sediments of Hamilton Lake and Lake Ngaroto are composed of silt and fine sand with relatively higher moisture content while Lake Rotorua sediments are primarily composed of coarse sand. Total organic matter in Lake Ngaroto and Hamilton Lake sediments is 5 to 7 times higher than in sediments from the Lake Rotorua sites. Arsenic (As), copper (Cu), and chromium (Cr) concentrations in sediments from sites over 6 seasons are given in Table 2.3. No clear relationship was observed for metals between seasons in any lake. However, overall metal concentration was relatively higher in the Waikato Lakes than at Rotorua sites, particularly for Cu and Cr.

Benthic macroinvertebrate samples from Hamilton Lake exhibited a narrow range of total numerical abundance throughout the year (Tables 2.4, 2.8 and 2.9). Although chironomids were the most abundant organisms, representatives from the groups Oligochaeta, Hirudinea, Bivalvia, and Gastropoda were commonly collected in low numbers. Among chironomids, *Chironomus zealandicus* was usually the most abundant

Table 2.1. Monthly physico-chemical parameters measured at four lake sites from June 1996 to May 1997. (S - Surface water, B - Bottom water)

	Hamilton					Ngaroto					Hamurana					Sulphur Point				
Month	Dissolved oxygen (%)		Conductivity (µS/cm)	pH	Temperature (°C)	Dissolved oxygen (%)		Conductivity (µS/cm)	pH	Temperature (°C)	Dissolved oxygen (%)		Conductivity (µS/cm)	pH	Temperature (°C)	Dissolved oxygen (%)		Conductivity (µS/cm)	pH	Temperature (°C)
	S	B				S	B				S	B				S	B			
June	73.5	74.4	116	6.9	9	73.5	73.5	154	5.8	9	87.2	87.2	118	6.5	12	22.3	10.9	573	3.3	15
July	50.1	50.1	116	6.8	9	52.8	51.0	129	5.9	12	85.2	85.2	177	7.1	11	31.7	29.8	409	4.1	15
August	87.0	78.9	99	6.9	11	60.7	53.5	124	6.0	11	87.2	87.2	129	6.5	12	68.2	68.2	370	3.3	12
September	103.4	99.2	81	6.7	17	103.3	101.3	123	6.2	16	93.1	87.3	113	7.1	14	41.2	41.2	426	4.1	18
October	99.2	95.0	93	6.9	18	120.4	122.5	124	6.5	18	124.1	123.1	109	7.2	17	16.8	16.8	476	3.2	21
November	109.8	108.8	97	7.0	18	109.9	109.9	132	6.8	19	103.3	107.4	155	7.6	16	101.4	101.4	202	3.1	18
December	96.4	94.1	97	6.9	21	112.2	107.8	130	6.9	20	107.8	107.8	187	7.3	20	55.0	55.0	515	3.5	20
January	96.7	96.7	98	7.4	23	72.4	70.0	144	6.9	24	111.0	103.4	166	7.9	19	11.9	11.9	235	6.5	19
February	75.5	70.9	106	6.6	22	47.8	47.8	159	6.6	23	132.9	122.4	189	7.9	23	4.2	4.2	493	2.9	25
March	91.8	89.8	101	6.9	18	76.0	76.0	139	6.2	18	121.6	122.6	152	6.8	16	44.2	44.2	331	3.7	19
April	95.2	88.3	85	6.7	15	62.0	62.0	160	6.6	14	113.2	112.2	136	8.1	12	82.4	82.4	420	5.3	18
May	77.6	69.8	109	6.5	14	38.8	38.8	157	6.6	14	121.4	121.4	116	6.4	13	16.9	15.9	425	3.4	15

Table 2.2. Particle size distribution (percentage of the total), moisture content and total organic matter of four lake sediment substrates used in the emergence test (Chapter 4).

	Hamilton	Ngaroto	Hamurana	Sulphur Point
Clay (0.050 µm - 2.00 µm)	3.5	3.3	0	0
Silt (2.00 µm - 63.00 µm)	34.4	47.9	2.0	8.9
Fine Sand (63.00 µm - 250.00 µm)	45.8	32.6	13.0	24.9
Coarse sand (250 µm - 1000 µm)	16.6	15.6	65.0	61.8
Very coarse sand (1000 µm - 2000 µm)	0.0	0.0	17.6	4.5
Moisture content (%)	31.0	44.0	7.0	11.0
Total Organic Matter (%)	10.6	14.9	1.9	2.5

Table 2.3. Concentration of As, Cu and Cr (in mg/kg dry weight) in the sediments sampled at four lake sites during six seasons.

Season	Metal	Hamilton	Ngaroto	Hamurana	Sulphur Point
Summer-94 (December)	As	1.6	0.2	4.0	0.9
	Cu	4.8	14.5	4.9	1.2
	Cr	3.9	3.8	0.2	0.7
Winter-95 (June)	As	7.5	10.7	8.9	8.3
	Cu	20.9	27.1	4.9	1.3
	Cr	6.0	3.9	0.2	1.3
Summer-95 (December)	As	10.9	7.9	9.3	2.6
	Cu	27.9	15.0	0.6	0.1
	Cr	17.2	5.0	0.2	0.6
Winter-96 (June)	As	11.0	6.0	13.0	10.6
	Cu	34.0	12.9	0.2	2.1
	Cr	17.8	8.3	0.2	1.9
Summer-96 (December)	As	12.7	8.9	11.4	8.1
	Cu	27.9	23.8	6.5	6.0
	Cr	12.7	4.3	1.2	0.9
Winter-97 (June)	As	13.2	7.1	8.0	11.3
	Cu	33.6	22.8	4.8	11.6
	Cr	13.5	5.5	1.5	1.1

Table 2.4. Percentage contribution of major taxa to the total number of taxa collected in grab samples from June 1996 to May 1997 from Hamilton Lake. Each value is a mean of 4 replicates with standard errors in parentheses. Spaces with no data indicate particular taxon was not found in sediment samples.

	Jun-96	Jul-96	Aug-96	Sep-96	Oct-96	Nov-96	Dec-96	Jan-97	Feb-97	Mar-97	Apr-97	May-97
Chironomidae	100.0 (38.4)	73.0 (21.5)	86.4 (14.9)	48.2 (29.9)	79.0 (19.2)	83.2 (10.0)	93.3 (6.3)	57.2 (5.9)	67.3 (46.0)	78.4 (27.9)	21.1 (6.0)	48.0 (14.4)
Trichoptera												
Coleoptera												
Oligochaeta		26.8 (5.6)	13.6 (5.6)	39.4 (14.0)	14.6 (7.1)	7.4 (3.1)	6.6 (2.2)	42.7 (4.9)	31.5 (11.0)	14.0 (8.7)	35.3 (11.1)	31.5 (3.7)
Hirudinea				9.0 (6.8)	1.7 (0.9)							1.5 (1.2)
Bivalvia					3.3 (1.2)	9.3 (0.9)					7.0 (2.5)	5.0 (1.7)
Gastropoda				3.3 (1.2)	1.7 (1.0)				1.2 (13.3)	7.6 (10.0)	36.0 (12.0)	14.0 (4.4)
Amphipoda												
Mean abundance of all benthic organisms (number/m^2)	156 (64)	631 (121)	324 (123)	396 (192)	759 (156)	1671 (469)	3360 (345)	807 (117)	1129 (300)	573 (62)	629 (274)	888 (103)
Taxa richness	1	2	2	4	5	3	2	2	3	3	4	5

Table 2.5. Percentage contribution of major taxa to the total number of taxa collected in grab samples from June 1996 to May 1997 from Lake Ngaroto. Each value is a mean of 4 replicates with standard errors in parentheses. Spaces with no data indicate particular taxon was not found in sediment samples.

	Jun-96	Jul-96	Aug-96	Sep-96	Oct-96	Nov-96	Dec-96	Jan-97	Feb-97	Mar-97	Apr-97	May-97
Chironomidae	90.0 (8.5)	83.7 (13.5)	88.2 (13.0)	86.8 (11.0)	85.0 (9.1)	83.9 (12.0)	85.7 (5.6)	97.8 (3.6)	95.9 (1.6)	87.9 (9.5)	97.3 (28.7)	93.5 (7.2)
Trichoptera												
Coleoptera	2.0 (0.5)	3.9 (1.1)	0.8 (0.8)	0.8 (0.1)	0.6 (0.4)	0.6 (0.5)						
Oligochaeta	2.0 (1.3)		7.9 (5.5)	7.3 (5.3)	1.9 (0.6)	2.0 (0.9)	0.7 (0.4)	1.3 (0.9)	1.5 (1.7)	3.3 (1.4)		
Hirudinea	3.0 (0.8)	7.2 (3.2)	1.4 (1.3)	1.3 (1.0)	4.8 (1.5)	5.1 (2.4)	2.6 (0.4)		1.1 (1.9)	3.9 (1.4)		
Bivalvia	2.0 (1.0)	3.9 (1.7)	1.4 (1.3)	1.3 (1.0)	1.9 (0.6)	2.0 (1.1)				3.0 (1.0)	1.4 (0.3)	2.9 (0.2)
Gastropoda	1.0 (1.6)	1.2 (1.1)	0.3 (1.0)	2.6 (0.9)	5.8 (0.6)	6.2 (1.4)	11.0 (1.1)	0.9 (0.4)	1.5 (0)	1.8 (1.1)	1.2 (0.3)	3.5 (0.5)
Amphipoda												
Mean abundance of all benthic organisms (number/m^2)	1982 (223)	1114 (146)	1563 (468)	1705 (610)	2301 (49)	2145 (210)	3084 (84)	9635 (819)	7413 (483)	2027 (890)	4761 (1158)	3502 (501)
Taxa richness	6	5	6	6	6	6	4	3	4	5	3	3

Table 2.6. Percentage contribution of major taxa to the total number of taxa collected in grab samples from June 1996 to May 1997 from Hamurana Stream. Each value is a mean of 4 replicates with standard errors in parentheses. Spaces with no data indicate particular taxon was not found in sediment samples.

	Jun-96	Jul-96	Aug-96	Sep-96	Oct-96	Nov-96	Dec-96	Jan-97	Feb-97	Mar-97	Apr-97	May-97
Chironomidae	21.6 (2.0)	18.8 3.3)	20.4 (2.0)	12.7 (3.8)	14.5 (2.0)	8.2 (2.8)	6.0 (2.0)	33.0 (3.5)	46.6 (2.7)	42.0 (6.0)	34.8 (9.0)	22.4 (1.6)
Trichoptera	12.0 (0.4)	12.3 (1.4)	13.3 (1.4)	2.4 (1.2)	5.5 (1.4)	9.0 (2.2)	5.4 (1.0)	7.0 (1.0)	1.0 (0.2)	2.0 (0.7)		3.0 (0.4)
Coleoptera	0.7 (0.1)	0.7 (0.1)	0.2 (0.2)									0.3 (0)
Oligochaeta	4.0 (0.4)	12.3 (2.2)	3.8 (0.8)	11.3 (5.2)	1.4 (1.6)	4.0 (1.3)	16.2 (3.0)	10.0 (1.0)	3.2 (0.9)	4.6 (0.4)	20.4 (1.6)	·21.4 (1.0)
Hirudinea	0.4 (0.1)	1.0 (0.1)	2.2 (1.3)	2.0 (0.7)	0.2 (0.1)		0.7 (0.3)					0.5 (0.1)
Bivalvia	2.8 (1.3)	0.3 (0.3)	4.8 (1.3)	4.3 (3)	9.8 (1.5)	7.7 (0.3)	4.9 (1.0)	15.6 (2.5)	2.3 (0.2)	2.0 (0.3)	2.6 (0.7)	0.5 (0.1)
Gastropoda	42.0 (2.0)	37.5 (2.2)	42.6 (1.3)	48.0 (4.2)	59.4 (11.3)	64.0 (2.9)	54.0 (2.2)	9.7 (1.4)	21.6 (1.9)	39.6 (4.7)	37.8 (4.9)	40.1 (5.8)
Amphipoda	16.0 (0.4)	17.0 (1.3)	12.6 (0.1)	19.0 (3.7)	9.0 (2.8)	7.0 (3.4)	12.6 (2.8)	24.8 (2.0)	25.3 (1.8)	9.8 (4.3)	4.5 (0.7)	11.7 (2.0)
Mean abundance of all benthic organisms (number/m^2)	6281 (125)	6501 (232)	6702 (656)	5097 (750)	11280 (1182)	6936 (485)	4937 (540)	5381 (366)	17724 (1599)	19103 (684)	10906 (1395)	16369 (935)
Taxa richness	8	8	8	7	7	7	7	6	6	6	5	8

Table 2.7. Percentage contribution of major taxa to the total number of taxa collected in grab samples from June 1996 to May 1997 from Sulphur Point. Each value is a mean of 4 replicates with standard errors in parentheses. Spaces with no data indicate particular taxon was not found in sediment samples.

	Jun-96	Jul-96	Aug-96	Sep-96	Oct-96	Nov-96	Dec-96	Jan-97	Feb-97	Mar-97	Apr-97	May-97
Chironomidae	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)
Trichoptera												
Coleoptera												
Oligochaeta												
Hirudinea												
Bivalvia												
Gastropoda												
Amphipoda												
Mean abundance of all benthic organisms (number/m^2)	2844 (130)	667 (43)	2133 (460)	800 (372)	2400 (404)	26889 (1469)	28844 (2207)	29289 (2893)	16356 (4255)	18622 (2230)	13867 (1042)	10662 (1015)
Taxa richness	1	1	1	1	1	1	1	1	1	1	1	1

Table 2.8. Mean abundance (number/m^2) of chironomids collected in grab samples from four sampling sites from June 1996 to May 1997. Each value is a mean of 4 replicates with standard errors in parentheses. Spaces with no data indicate particular taxon was not found in sediment samples.

	Jun-96	Jul-96	Aug-96	Sep-96	Oct-96	Nov-96	Dec-96	Jan-97	Feb-97	Mar-97	Apr-97	May-97
Hamilton Lake												
C. zealandicus	111	102	89	124	533	1022	2711	267	36	378	111	213
	(38)	(11)	(31)	(64)	(46)	(270)	(304)	(31)	(21)	(126)	(29)	(66)
C. sp. a		124	13	22	22	156	236		58	13		13
		(61)	(11)	(22)...	(20)	(97)	(49)		(42)	(11)		(11)
P. pavidus	44	236	178	44	44	213	191	436	667	58	22	200
	(26)	(49)	(54)	(42)	(41)	(98)	(86)	(90)	(75)	(21)	(13)	(59)
Other chironomids												
Total Chironomids	155	462	280	190	599	1391	3138	703	761	449	133	426
	(64)	(121)	(97)	(102)	(65)	(443)	(370)	(117)	(72)	(122)	(18)	(76)
Lake Ngaroto												
C. zealandicus				13			22			13	13	13
				(11)			(22)			(11)	(11)	(11)
C. sp. a	1067	756	1022	1067	933	1556	2400	8889	7111	1600	3689	5689
	(154)	(84)	(279)	(112)	(80)	(245)	(126)	(729)	(461)	(827)	(1222)	(756)
P. pavidus	711	178	356	400	1022	244	222	533		124	933	1067
	(69)	(63)	(65)	(57)	(117)	(106)	(85)	(126)		(33)	(118)	(369)
Other chironomids												22
												(13)
Total Chironomids	1778	934	1378	1480	1955	1800	2644	9422	7111	1737	4635	6791
	(223)	(232)	(104)	(150)	(51)	(262)	(65)	(811)	(461)	(842)	(1141)	(1018)
Hamurana Stream												
C. zealandicus				13		13	44				22	44
				(11)		(22)	(26)				(58)	(44)
C. sp. a	200	111	124	102	222	267	124	347	3122	3111	156	489
	(29)	(38)	(52)	(58)	(48)	(63)	(52)	(90)	(506)	(240)	(83)	(77)
P. pavidus	1156	1122	1244	533	1378	267	133	1422	5166	5156	6444	3133
	(97)	(193)	(51)	(96)	(164)	(107)	(26)	(263)	(537)	(170)	(1113)	(187)
Other chironomids					36	22						36
					(21)	(13)						(21)
Total Chironomids	1356	1233	1368	648	1636	569	301	1769	8288	8267	6622	3702
	(130)	(232)	(104)	(166)	(179)	(55)	(86)	(307)	(1027)	(1112)	(106)	(187)
Sulphur Point												
C. zealandicus	2844	667	2133	800	2400	26889	28844	29289	16356	18622	13867	10662
	(130)	(42)	(459)	(372)	(404)	(1468)	(2206)	(2892)	(4257)	(2230)	(1042)	(934)
C. sp. a												
P. pavidus												
Other chironomids												
Total Chironomids	2844	667	2133	800	2400	26889	28844	29289	16356	18622	13867	10662
	(130)	(42)	(459)	(372)	(404)	(1468)	(2206)	(2892)	(4257)	(2230)	(1042)	(934)

Table 2.9. Relative abundance of three test species of chironomid to the total number of chironomids collected in grab samples from June 1996 to May 1997 from four sampling sites. Each value is a mean of 4 replicates with standard errors in parentheses. Spaces with no data indicate particular taxon was not found in sediment samples.

	Jun-96	Jul-96	Aug-96	Sep-96	Oct-96	Nov-96	Dec-96	Jan-97	Feb-97	Mar-97	Apr-97	May-97
Hamilton Lake												
C. zealandicus (%)	71.6 (19.2)	22.0 (5.0)	31.8 (8.2)	65.3 (18.8)	89.0 (12.0)	73.4 (3.1)	86.4 (2.5)	38.0 (2.5)	4.7 (2.2)	84.1 (22.1)	83.5 (3.4)	50.0 (6.8)
C. sp. a (%)		27.0 (7.7)	4.6 (1.7)	11.6 (6.7)	3.7 (3.8)	11.3 (8.2)	7.5 (1.5)		7.6 (2.7)	3.0 (1.5)		3.0 (1.3)
P. pavidus (%)	28.4 (9.2)	51.0 (8.8)	63.6 (4.6)	23.1 (5.0)	7.3 (3.7)	15.3 (3.8)	6.1 (2.3)	62.0 (5.4)	87.7 (10.0)	12.9 (4.2)	16.5 (2.5)	47.0 (6.1)
Other chironomids (%)												
Total chironomids (number/m^2)	155 (64)	462 (121)	280 (97)	190 (102)	599 (65)	1391 (443)	3138 (370)	703 (117)	761 (72)	449 (122)	133 (18)	426 (76)
Lake Ngaroto												
C. zealandicus (%)				0.9 (0.6)			0.8 (0.8)			0.8 (0.8)	0.3 (0.1)	0.2 (0:1)
C. sp. a (%)	60.0 (4.2)	81.0 (7.7)	74.0 (8.1)	72.1 (8.8)	47.7 (4.2)	86.4 (7.6)	90.8 (2.0)	94.3 (2.1)	100 (1.6)	92.1 (6.6)	79.6 (14.5)	83.8 (6.9)
P. pavidus (%)	40.0 (4.3)	19.0 (5.8)	26.0 (4.7)	27.0 (1.6)	52.3 (4.9)	13.6 (4.7)	8.4 (1.3)	5.7 (1.4)		7.1 (2.1)	20.1 (13.9)	15.7 (6.9)
Other chironomids (%)												0.3 (0.3)
Total chironomids (number/m^2)	1778 (223)	934 (232)	1378 (104)	1480 (150)	1955 (51)	1800 (262)	2644 (65)	9422 (811)	7111 (461)	1737 (842)	4635 (1141)	6791 (1018)
Hamurana Stream												
C. zealandicus (%)				2.0 (0.2)		2.3 (0.1)	14.6 (0.4)				0.3 (0.5)	1.1 (0.2)
C. sp. a (%)	14.7 (0.5)	10.0 (0.7)	9.1 (0.7)	15.7 (0.8)	13.6 (0.4)	46.9 (0.8)	41.2 (1.0)	19.6 (0.9)	37.6 (1.6)	37.6 (1.0)	2.4 (1.0)	13.2 (0.4)
P. pavidus (%)	85.3 (1.4)	90.0 (2.4)	90.9 (1.3)	82.3 (2.8)	84.2 (1.5)	46.9 (1.0)	44.2 (0.6)	80.4 (2.6)	62.3 (1.0)	62.4 (5.0)	97.3 (7.5)	84.6 (0.1)
Other chironomids (%)					2.2 (0.2)	3.9 (0.1)						0.9 (0.1)
Total chironomids (number/m^2)	1356 (130)	1233 (232)	1368 (104)	648 (166)	1636 (179)	569 (55)	301 (86)	1769 (307)	1769 (1027)	8267 (1112)	6622 (106)	3702 (187)
Sulphur Point												
C. zealandicus (%)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)
C. sp. a (%)												
P. pavidus (%)												
Other chironomids (%)												
Total chironomids (number/m^2)	2844 (18)	667 (42)	2133 (459)	800 (372)	2400 (404)	26889 (1468)	28844 (2206)	29289 (2892)	16356 (4257)	18622 (2230)	13867 (1042)	10662 (934)

species at this site although *P. pavidus* sometimes dominated. The maximum taxon richness of 5 was recorded from this site in two sampling months.

Greater than 80% of the benthic macroinvertebrates in grab samples from Lake Ngaroto were chironomids. *Chironomus* sp. a was the predominant species of chironomid throughout the sampling period although *P. pavidus* was also common (Tables 2.5, 2.8 and 2.9). *Chironomus zealandicus* was rare in Lake Ngaroto. The maximum taxon richness of 6 was recorded from this site on six sampling seasons.

Benthic macroinvertebrate samples from the Hamurana Stream site exhibited a fairly wide range of total numerical abundance (Table 2.6) and a higher degree of diversity. In addition to chironomids, trichopterans, oligochaetes, bivalves, gastropods and amphipods were also recorded from this site on all 12 sampling occasions. This is also the only site from which trichopterans and coleopterans were recorded in grab samples. Chironomids were less abundant in September to December than in January to April. Although *C.* sp. a and *P. pavidus* were present throughout the year in these sediments there are seasonal fluctuations in the abundance of both species. *Polypedilum pavidus* is the predominant species at the site and it accounts for more than 80% of the total chironomid population in 8 out of 12 samples (Tables 2.6, 2.8 and 2.9).

Chironomus zealandicus was the only macroinvertebrate collected in grab samples from Sulphur Point throughout the sampling period although they were always extremely abundant (Tables 2.7, 2.8 and 2.9). Abundance was relatively higher in summer months than in winter.

2.2.3. Discussion

The two Waikato peat lakes are clearly more eutrophic than the two sites in Lake Rotorua. Hamilton Lake and Lake Ngaroto contain fine sediment with high organic content whereas Sulphur Point and Hamurana Stream contain relatively coarse sediment with low organic content.

Because of a relatively high total organic content in sediments from Hamilton Lake and Lake Ngaroto, many heavy metal ions may have been bound with sediments. This is the probable cause why concentrations of As, Cu, and Cr were generally higher in sediments from Hamilton Lake and Lake Ngaroto than at Hamurana Stream and Sulphur Point.

Heavy metal concentrations were higher in winter than in summer in Hamilton Lake and at Sulphur Point. No such relationship was observed with Lake Ngaroto or Hamurana Stream. Accumulation and release of heavy metal between sediment and the water column in an aquatic ecosystem depend on several physico-chemical factors (USEPA 1980). Metal ions are readily absorbed by sediments under conditions of high organic content, low pH, low temperature and low mineral content (Thanabalasingham and Pickering 1986). Temperature may have a direct effect on the release of metals from sediments or exert an indirect influence through other variables such as redox condition. For example, Stuart and Kim (1995) observed higher concentrations of arsenic in summer than in winter in Waikato River water. Relatively high temperatures in summer at sampling sites may have favoured the release of heavy metals from sediment to the water column. Heavy metal concentrations reported in Hamilton Lake sediment were relatively lower than those reported in a previous study (Rajendram 1992). This may be because the sediment samples were collected only from shallow water, and not from deep water in this study.

Field surveys of macroinvertebrate communities are an essential tool for assessing the toxicity associated with contaminated sediments and have several advantages over chemical analyses and laboratory toxicity tests (Swift et al. 1996). Winner et al. (1980) hypothesized that heavily polluted habitats are dominated by midges, moderately polluted habitats by midges and caddisflies, and minimally polluted or unpolluted habitats by caddisflies and mayflies. They also found that there was a direct relationship between the fraction of a community composed of chironomids and the degree of heavy metal pollution, and suggested that the percentage of chironomids in samples may be a useful index of heavy metal pollution. However, results of all field surveys do not fully agree in ranking sites according to their environmental quality as defined chemically (Pinel-Alloul et al. 1996). Bailey et al. (1995) found that the relationship between field

and laboratory estimates of biological structure provides strength for the argument for using both structural (community) and functional (bioassay) measures of ecological integrity. Therefore, the simultaneous use of both laboratory and field approaches is more relevant in assessing contaminated sediment.

In this study, grab samples collected substantially different benthic communities at the four sampling locations. Chironomids dominate the macroinvertebrate community at all sites except for Hamurana Stream although the dominant species varied between sites. Sulphur Point and Hamilton Lake have the lowest diversity and Hamurana Stream the highest. If chironomids dominate low diversity communities in stressed environments as discussed above, then these results confirm Sulphur Point and Hamilton Lake as the most stressed sites confirming the original premise that these two sites are more impacted. However, community diversity and chironomid abundances do not seem to corelate with the concentrations of heavy metals measured in the sediments, implying that other physico-chemical characteristics of the sediment may be involved.

The macroinvertebrate community living in and on the bottom of aquatic systems is determined to a large extent by the physical characteristics of the substrate (Hynes 1970; Merritt and Cummins 1984). The bottom of an aquatic ecosystem is characterized by sediment consisting of either heavier material including sand, gravel and cobble or, typically, of silt and sand mixed with organic material. Both of these habitats have characteristic benthic communities made up of animals adapted to the physical conditions found there (Hynes 1970). The benthic community in fine particulate sediment typically includes oligochaete worms, leaches, chironomid larvae and bivalves (Swift et al. 1996). Sediments of Hamilton Lake and Lake Ngaroto are of this type. However, numerical abundance or taxon richness of samples from Hamilton Lake was not as high as from Lake Ngaroto. As mentioned earlier in this chapter, arsenic contamination of Hamilton Lake sediment may be a reason for this although measured As levels in Hamilton Lake were not unusually high. Sand, gravel, and cobble substrate provides habitat for a more diverse benthic community including a wide variety of aquatic insects, crustaceans and snails (Swift et al. 1996). Sediments of Sulphur Point and Hamurana Stream of Lake Rotorua are of this type. However, taxon richness is much higher at Hamurana Stream than at Sulphur Point. Differences in physico-

chemical characters such as dissolved oxygen, temperature, pH, and conductivity between these two sites throughout the year may be a reason for this.

Studies have shown that chironomid species richness may be low with increased abundance of a few tolerant species in areas with lower pH due to oligotrophication (Lindergaard 1995). However, Cranston et al. (1997) found higher species richness at low pH. These authors attributed this to the presence of a large tropical (Australian and South-east Asian) pool of species tolerant of naturally acidic aquatic habitats. According to Stark (1989), *C. zealandicus* can survive a temperature maximum of about 34°C and a recorded pH minimum of 1.8 in geothermal waters. During this survey, the lowest pH and highest temperature were recorded at Sulphur Point and *C. zealandicus* was the only macroinvertebrate that was reported from Sulphur Point. *Chironomus zealandicus* would appear to be the only species that can survive the conditions prevailing at Sulphur Point.

Taxonomy and laboratory culture of test species

3.1. INTRODUCTION

The evaluation of sediment bound pollutants requires a suitable bioassay organism with a known life history (Anderson 1980). One of the basic characteristics of an ideal bioassay organism is that it must be easily cultured in the laboratory so that organisms are available for testing throughout the year (Maier et al. 1990). In addition to continuous supply, a well-established laboratory culture would provide test organisms free of contamination by any known pollutant, in particular by the substance to be tested. Furthermore, the laboratory culture of test organisms would avoid a possible difference in sensitivity of wild-collected test animals with differing season and sampling sites.

Using Chironomidae as test organisms in toxicity tests has several advantages (Chapter 1). The organism can be cultured in the laboratory and can be maintained in the laboratory under test conditions. A relatively short life cycle and a high fecundity give a large number of organisms in laboratory cultures. Chironomids are ecologically important (USEPA 1994a) and tolerant to a wide range of natural sediment physico-chemical conditions and water quality conditions.

Adult chironomids lay eggs immediately following copulation after which the female soon dies. The whole process may be completed in 10 minutes or in an hour at the most. In many species, oviposition appears to be triggered by changing light intensity, especially at dusk. According to Nolte (1993), most chironomids deposit their eggs on firm substrate such as macrophytes, stones or leaf litter, close to the water's edge.

The larvae have the longest life span of all other life stages. There are four larval stages recognized and usually distinguished on the basis of the head capsule width. The larvae build and live in tubes and swim violently when disturbed, thrashing the body in a figure-of-eight movement. The first instar larva moults within the tube which is then

vacated for a new one. The tube of the second instar larva is usually at a deeper level than the previous one. The third and fourth instars progressively enlarge the same tube to accommodate their increase in size.

Pupal life span is shorter than the other life stages. Three main pupal functions are ecdysis from the larval cuticle, providing sufficient respiratory oxygen, and moving to the water surface for adult eclosion.

Adult chironomids are short lived and their behaviour is concerned largely with reproduction. Mating requires spatial and temporal synchronization of emergence and subsequent adult location. Emergence is rapid and the adult is able to fly almost immediately.

The test species used in this study were not all continuously available in the required number from the selected sites. Although *C. zealandicus* was continuously available at Sulphur Point, the abundance was higher in early summer months (December and January) than throughout the rest of the year. Conversely, *P. pavidus* was more abundant in winter months than in summer months at Hamurana Stream where it appears to dominate in the benthic community. In Hamilton Lake and also Lake Ngaroto, chironomid populations showed fluctuations with season. Therefore, to obtain all life stages of *C. zealandicus, C.* sp. a and *P. pavidus* for laboratory bioassays, cultures of these chironomids were created and maintained.

3.2. TAXONOMY OF TEST SPECIES

Three New Zealand chironomid species, *C. zealandicus*, *C.* sp. a., and *P. pavidus*, were chosen. These three organisms are the common components of the benthos at the sites selected for this study.

Freeman (1959) recognized two species of *Chironomus* in New Zealand, *C. zealandicus* Hudson and *C. analis* Freeman, while two further species, *C. antipodensis* and *C. subantarcticus,* were described by Sublette and Wirth (1980). However, it has been

recognized for many years that there were additional species in both the North and South Islands. Studies on *C. zealandicus* (Robb 1966; Forsyth 1971) have indicated that adults corresponding to *C. zealandicus* were produced by two different larval types; one a thummi-type that possesses a pair of ventral tubes on the 8th abdominal segment, and the other a salinarius-type that does not possess ventral tubes. While Robb (1966) considered this difference to be of environmental origin, depending upon the salinity of the water in which the larvae developed, this result has not been confirmed by other work. Cytological studies indicate that the different larval types have a different banding pattern in the polytene chromosomes and different complements of inversion polymorphisms (Jon Martin, pers. com.). Forsyth (1978) subsequently allocated the name *C. zealandicus* Hudson to the form with a thummi-type larva and referred to the common form with a salinarius-type larva as *C.* sp. a. *Polypedilum pavidus* is a widespread and locally distributed chironomid species. The larvae have been found in shallow lakes and ponds predominantly along the West Coast of the North Island of New Zealand (Forsyth 1971)

Three chironomid species used in this study belong to the following taxanomic groups:

Order: Diptera
Superfamily: Chironomoidea
Family: Chironomidae
Subfamily: Chironominae
Tribe: Chironomini

Taxonomy and life stages of *Chironomus zealandicus* (Hudson, 1892)

Genus: *Chironomus*
Species: *zealandicus*

The dark brown ovoid eggs of *C. zealandicus* are contained in an elongate, slightly crescentic, gelatinous capsule and are arranged in a herring-bone fashion in a spiral within its thick walls. A holdfast at one end anchors the mass to a solid object near the

surface of the water. The numbers of eggs per mass range from 300 to 1600 (Forsyth 1971) (Fig. 3.1 A1).

C. zealandicus larvae are red, of the thummi type, and 4th larval instars are up to 20 mm long (Fig. 3.1 B1). The mentum consists of 15 teeth. There are usually two pairs of long finger-like tubuli situated ventrally on the eighth segment of the abdomen (Fig. 3.2, A). The length of the ventral tubuli may vary but are usually at least as long as the eighth segment. There are paired preanal papillae on the dorsal ninth segment. Two pairs of anal tubuli are also present but are less than half the length of the eighth segment.

The pupa of *C. zealandicus* is up to 15 mm long, and the thorax and abdomen are dark brown. Pupal exuviae have 8 - 9 spines on the postero-lateral spur (Fig. 3.3 A).

Adult *C. zealandicus* live from 4 to 8 days at 22°C (Forsyth 1971). Abdominal tergites of male adults have broad dark bands covering most of the surface, leaving only a narrow pale band at the posterior edge as illustrated by Hudson (1892) and described by Hutton (1902).

Taxonomy and life stages of *Chironomus* sp. a

Genus: *Chironomus*
Species: a

Life cycle and life stages are the same as for *C. zealandicus* described above with little difference in morphology of larvae, pupae and adults.

The egg mass is similar to that of *C. zealandicus* but differs in that eggs are loosely arranged within the gelatinous envelope in *C.* sp. a while compactly arranged in *C. zealandicus* (Fig. 3.1). The egg mass of *C.* sp. a does not have a definite shape.

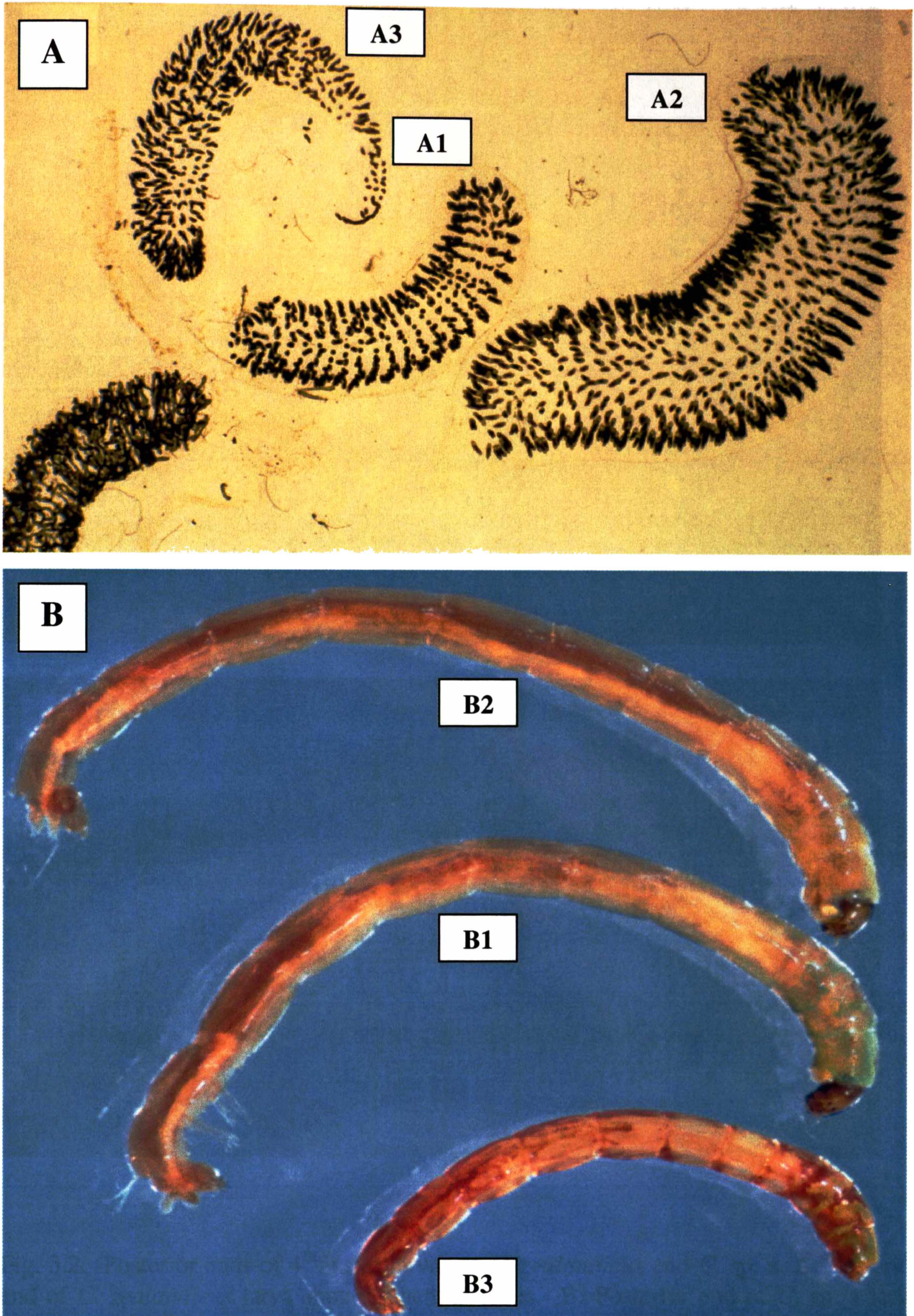


Fig. 3.1. Egg masses (A) and 4th instar larvae (B) of test chironomid species. A1, A2, and A3 are egg masses of *C. zealandicus, C.* sp. a and *P. pavidus* and B1, B2 and B3 are 4th larval instars of *C. zealandicus*, *C.* sp. a and *P. pavidus* respectively.

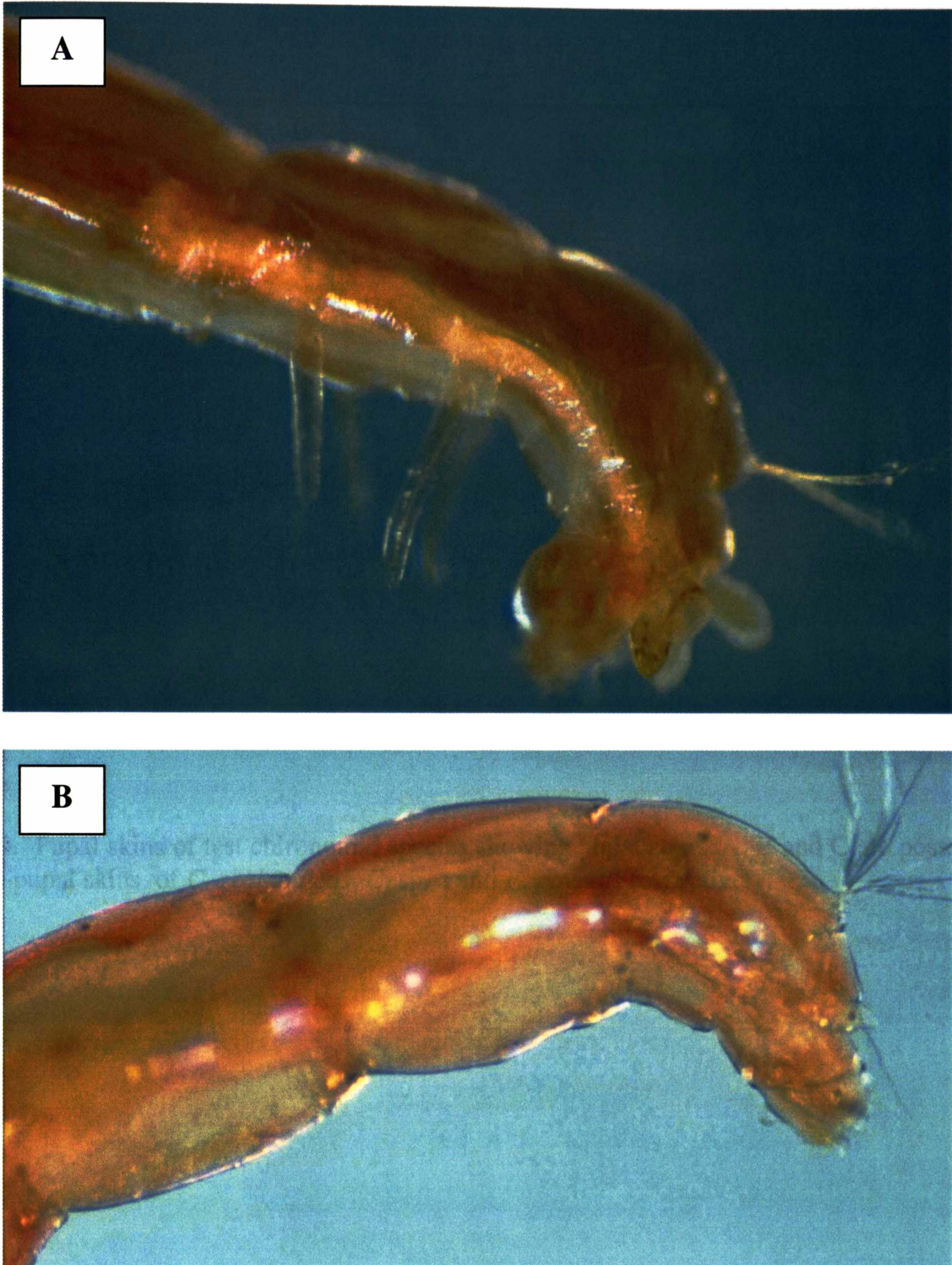


Fig. 3.2. Posterior ends of 4th instar larvae of *C. zealandicus* and *C.* sp. a. A: Posterior end of *C. zealandicus* larva showing ventral tubes. B: Posterior end of *C.* sp. a larva with no ventral tubes present

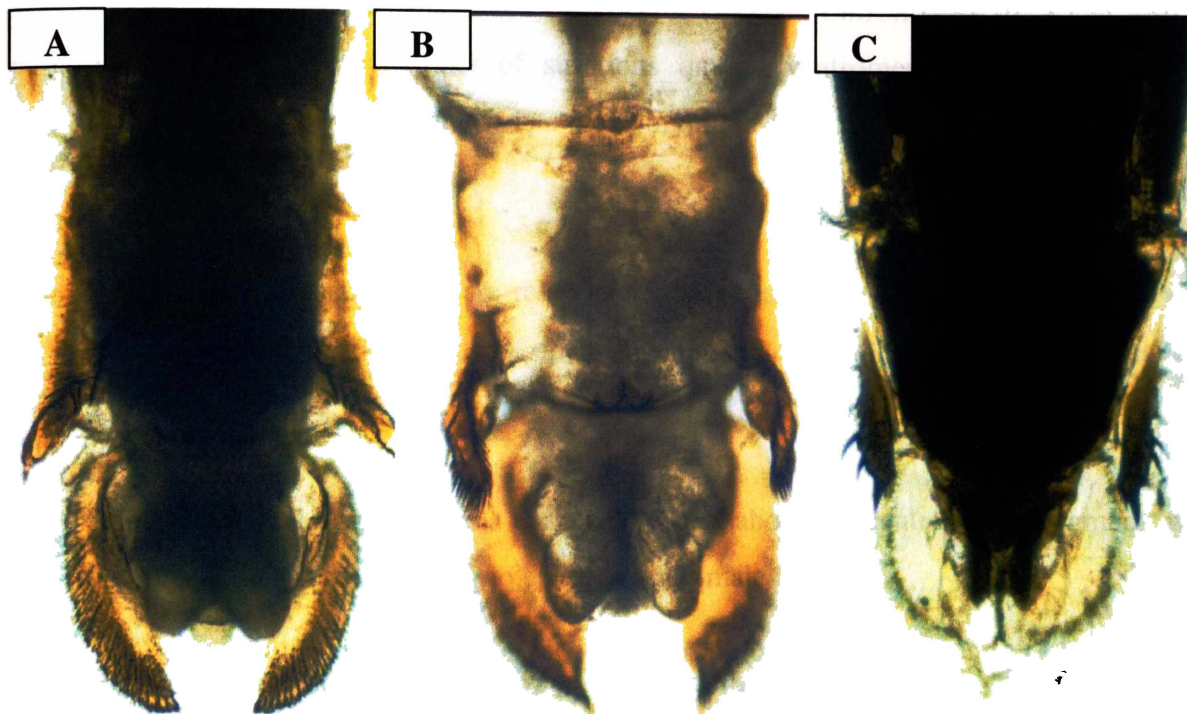


Fig. 3.3. Pupal skins of test chironomid species showing pupal spur. A, B and C are posterior ends in pupal skins of *C. zealandicus*, *C.* sp. a and *P. pavidus* respectively.

Larvae belong to the salinarius type (Fig. 3.2 B) with anal gills which are generally shorter and rounded as illustrated by Forsyth (1971). *Chironomus* sp. a can also be distinguished by the number of striations on the ventromental plates. About 80 striations on each ventromental plate are common in *C.* sp. *a* while there are about 40 in *C. zealandicus*.

Pupae and 4th instar larvae are relatively longer than those of *C. zealandicus*. Pupal exuviae contain 16 to 25 splayed, open-fingered spines on the postero-lateral spur (Fig. 3. 3 B).

Adult abdominal tergites have a dark saddle spot about the centre of the segment, with the surrounding region tending to green in adult males.

Taxonomy and life stages of *Polypedilum pavidus* (Hutton, 1902)

Genus: *Polypedilum*
Species: *pavidus*

The ovoid eggs are dark brown. An average of 250 to 300 eggs are encapsulated in a crescent-shaped gelatinous envelope deposited in a loose spiral with a holdfast at one end. Average length of an egg mass varies from 6 to 7 mm (Fig. 3.1 A3).

Larvae are relatively shorter than those of the other two species (Fig. 3.1 B3). The mentum consists of 16 teeth of which the central pair are the longest. Paralabial plates are separated and radially striated. Larval instars have no ventral tubules. Anal tubuli are short and nearly as wide as they are long. The average length of 4th instar larvae is about 10 mm and they are deep red.

There are a pair of sclerotised compound spurs on the lateral margins of the eighth abdominal segment of the pupa. Each spur is composed of 10 - 12 spines (Fig. 3. 3 C).

3.3. LABORATORY CULTURE OF TEST SPECIES

Methods for rearing and maintaining cultures of several genera and species of Chironomidae exist in the literature (Biever 1965; Credland, 1973; Downe and Caspray 1973; Gallepp 1979; Batac-Catalan and White 1982). Difficulties in developing methods for successful culture have been both biological (such as the short life span of adults), and physical (such as availability of space for mating). A variation in optimal physical and biological parameters between chironomid species may be a reason why a common culture method does not suit all species (Maier 1990). Therefore, a method described for the culture of a particular chironomid species may require considerable alteration for another species and that may take a considerable time of trial and error to succeed. The methods and materials used in this study to create and maintain cultures in the laboratory provided all life stages for use in toxic bioassays.

3.3.1. METHODS

3.3.1.a. Assembling aquaria

A stocking aquarium for maintaining wild-collected larvae in the laboratory in order to obtain adults, and a rearing aquarium for rearing eggs to adult stage were set up separately. Two different sizes of aquaria were used to create and maintain cultures, depending on the number of larvae stocked or reared. A 20 L (210 x 360 x 260 mm) clear plastic aquarium was used to stock up to 500 fourth instar larve or a 220 L (920 x 380 x 380 mm) glass aquarium was used to stock more than this number. A white nylon net of 250 µm mesh was used to cover these aquaria.

3.3.1.b. Substrate

Specimens of chironomid larvae were collected from study sites and stocked in aquaria in the laboratory in order to obtain adults for each new laboratory culture. The particular lake sediment with corresponding lake water above it in an aquarium was used to maintain each new stock of 4th instar larvae collected from each site. Sediment and

water from the site was collected and brought to the laboratory before the collection of larvae. Each aquarium was set up containing 50 mm depth of respective substrate with its appropriate lake water overlying the sediment to a depth of 80-100 mm and aerated gently and continuously. A water volume of 6-8 L in the 20 L aquarium and 30-34 L in the 220 L aquarium was maintained during the period of culture.

In starting a new culture from an egg mass, sterilized paper towel was used as the substrate. The paper towel was sterilized either by boiling in water followed by autoclaving or by submerging in acetone, and boiling and washing in water. In order to sterilize by the first method, white Double Quilted Handee Towel ™ (228 x 272 mm two-ply) was boiled in MILLI-Q water for 5 hours to remove any possible contaminants and to make the paper towels easy to cut or tear into small pieces. Small pieces of boiled paper towel were sterilized by autoclaving. The sterilized paper towel was kept frozen until used. Sterilized paper towel stored for more than 3 months was discarded and not used as substrate.

In order to sterilize by the second method, paper towels were cut into strips. These were loosely packed into a glass beaker or an aquarium depending on the quantity, submerged in acetone, covered and placed in a fume hood and soaked overnight to solubilize organic contaminants. On the following day, MILLI-Q water was added and brought to the boil in a water bath. The paper towel/acetone/water mixture was stirred from time to time to drive off acetone vapour. This process was repeated up to five times. Finally, the towelling was rinsed five times with cold MILLI-Q water. Small batches of sterilized toweling were placed in a blender with sufficient MILLI-Q water in it and blended until the strips were broken apart to form a pulp. Excess substrate was frozen for later use.

3.3.1.c. Soft synthetic water

Soft synthetic water was used as an overlying medium in culture tanks with paper towel substrate. Soft synthetic water was prepared according to USEPA (1991). Reagents used to prepare soft synthetic water and the final quality of it are given in Table 3.1. Sodium bicarbonate ($NaHCO_3$), magnesium sulphate ($MgSO_4$) and potassium chloride

(KCl) were dissolved in an appropriate volume of MILLI-Q water and the solution was aerated overnight. On the following day, the appropriate amount of calcium sulphate ($CaSO_4.2H_2O$) was added to the solution and aerated for another hour. Soft synthetic water was prepared in 50 L plastic containers.

Table 3.1. Chemical reagents used to prepare soft synthetic water and the final quality of soft synthetic water.

Reagent Added (mg/L)				Final Water Quality		
$NaHCO_3$	$CaSO_4.2H_2O$	$MgSO_4$	KCl	pH	Hardness	Alkalinity
48.0	30.0	30.0	2.0	7.2 - 7.6	40 - 48	30 - 35

3.3.1.d. Food and Feeding

Finely ground TetraMin fish flake was used to feed larvae. Feeding started on the third day after hatching at a rate of 0.5 g per 3 days during the first week and 1.0 g per 3 days from the second week till the emergence of adults in 20 L aquaria and this was 4.0 g and 8.0 g per 3 days respectively in 220 L aquaria.

3.3.1.e. Photoperiod and Temperature

A photoperiod of 12:12 (L:D) and temperature of 23°C were maintained for all cultures in this study. Ordinary aquarium heaters were used to maintain the temperature.

3.3.1.f. Aeration

A diaphragm air pump and air stones were used to provide continuous gentle aeration. A strong supply of air from the bottom, particularly of the rearing aquarium, resuspends settling substrate. This disturbs settling and, as a result, tube building of larvae. Strong

aeration, like unimpeded flow of water, may result in unsuccessful emergence of adult midges (Credland 1973), since pupae are unable to stabilize themselves at the water surface. The emerging imagines are consequently wetted, are unable to fly, and ultimately killed. Therefore, the air supply to the aquarium was provided at a rate that did not resuspend the substrate. This was done by suspending air stones just below the surface of the water (Batac-Catalan and White 1982). Air stones do not function properly for long periods in rearing aquaria, since food and other suspended particles block the pores. It is therefore essential to regularly replace the air stones to ensure proper aeration.

3.3.1.g. Collection of adults

Emerging adults were collected from stocking aquaria daily using an aspirator connected to a small vacuum pump, and introduced into a mating cage. Adults' wings, antennae and legs may be damaged when the power of the suction is high or too many insects are aspirated at a time. This may affect normal dispersal and mating performance in mating cages. The suction power of the vacuum pump and the number of adults collected at a time were adjusted to a level that did not cause damage to adults in the aspirator.

Collected adults were released into a mating cage. The mating cage was a wooden cage of 350 x 350 x 350 mm covered on all sides with nylon net of 250 μm mesh, except for the base which was not covered. Either a white plastic tray or a set of twenty 100 mL crystallizing dishes with soft synthetic water up to 30 mm depth was placed at the bottom of the cage. A piece of 100 x 100 mm nylon net was removed from the roof of the cage. This small area was covered by a piece of over-head projector transparency sheet, by cello-taping an edge of it to the cage. Adults were released into the cage by lifting the transparency door. A light source was focussed from above at the opposite corner when new adults were released. Midges already in the cage and newly released adults were attracted towards the light and did not escape through the door. In addition, this set-up enables most of the released adults not to be drowned in the water as soon as they are released.

Mating takes place in the air during swarming in most of the chironomid species (Credland 1973). Swarming and mating are highly determined by the intensity of light. Ordinary electric bulbs with different power consumption were positioned over the mating cage at different heights in order to achieve the best performance of adults in mating as well as in producing fertilized eggs. A 100 W bulb positioned 600 mm above the mating cage was selected as the best combination to induce swarming as well as mating of adults. A majority of female adult midges laid eggs at dusk, or shortly after dusk. Swarming space mainly depends on the species and number of adults in the mating or swarming container at a time. A maximum of about 500 to 600 adults were allowed in the mating cage at a time in this study. Additional mating cages were used when more adults were emerged from the stocking or rearing aquarium.

3.3.1.h. Oviposition

A set of 100 mL crystallizing dishes or a plastic tray with water in it was used for adults to lay eggs. Eggs developing vacuoles inside by the fifth day were identified as unfertilized. Hatching egg masses were kept separately in glass beakers for up to 10 days and then added to the rearing aquaria. This helped to avoid or reduce the risk of possible cannibalism in the rearing aquaria.

3.4. RESULTS AND DISCUSSION

Shredded paper towel pulp of about 150 mL gives enough substrate for one 20 L aquarium and about 2 L were needed for a 220 L aquarium. All test species were successfully grown in paper towel sterilized by either of the methods described above.

Various workers (Strenzke and Neumann 1960; Engleman and Shappirio 1965; Syrjamaki 1965; Credland 1973; Batac-Catalan and White 1982) have used various substrates in cultures of different species of Chironomidae. However, Maier et al. (1990) found that the culture diet alone provided both nutrition and the substrate necessary for *Chironomus decorus* larvae. A substrate was observed to be essential for chironomid species used in this study in addition to the food as larvae build nests and

spend most of their life time (from 1st larval instar to early pupal stage) within the nest. Fourth instar larvae of *C. zealandicus* and *C.* sp a are unlikely to survive more than 5 days without a substrate (pers. observation). McKinney (1995) reported that the death of 4th instar larvae during an acclimatization period before the toxicity tests was a serious problem when a substrate was not provided. Providing a sterilized sand substrate considerably reduced this mortality.

Sterilized double quilted Handee™ towels were selected as the control substrate in this study for the following reasons:

1. easy to sterilize and preserve the sterilized paper towel.
2. large batches of paper can be sterilized at one time (Batac-Catalan and White 1982).
3. the double quilted nature of paper towels appears to provide better area, and shelter for tube building larval instars (pers. observation).
4. no damage is caused by paper to the morphology of the animal (sand may damage mouthparts of larvae, particularly during feeding [Maier et al. 1990]).

Credland (1973) found that the survival of the 4th instar of *Chironomus riparius* was 82% in mud, 10% in sand and, 7.5% in gravel in a 5-day substrate preference test. He also found that a boiled mud substratum resulted in a large yield of imagines and many egg ropes. In contrast, gravel yielded small populations of adults and consequently very small numbers of eggs were laid. However, paper towel was a reliable substrate for the test species of this study. There is a possibility for adverse effects due to the bleach material that may be found in the paper towel. However, paper towels used in this study had no such obvious effect on test organisms. Survival of all species on the paper towel substrate was more than 87% (see chapter 6). In addition, the paper towel substrate reduced the incidence of head capsule deformities and physical wear in successive generations in a laboratory test with *C. zealandicus* (Chapter 4).

Various diets such as alder leaves, cellulose wool, paper pulp, dried nettle, yeast (Strenzke and Neumann 1960); cellulose, nettle (Kroeger 1964); chitin, milk powder, yeast (Laufer and Nakase 1965; Doyle and Loafer 1969; Laufer and Schin 1971); filter paper (Engleman and Shappirio 1965); soil (Syrjamaki 1965); and TetraMin fish food (Credland 1973; Batac-Catalan and White 1982; Maier et al. 1990) have been used to

culture various species of Chironomidae. Credland (1973) found that TetraMin, a proprietary fish food, given in small quantities proved much more satisfactory compared with dried nettle powder and ground alder leaf. All species used in this study grew successfully on TetraMin.

Excess food decomposed and encouraged fungal growth. This can decrease dissolved oxygen concentration in the water to levels that result in midge larval mortality (Maier et al. 1990). Overfeeding also turns water cloudy by the following day. Water in the aquarium was changed if this happened. Underfeeding and insufficient aeration yielded free swimming larvae in large numbers in culture tanks, particularly near the surface of the water. The feeding rate used in this study that had been determined through preliminary experimentation was optimal for the test species and did not cause any over or under-feeding problems as described above.

Strenzke (1959) listed natural sites at which he found eggs, and females in captivity oviposited in a similarly wide variety of locations. Although glass, wood, cork, non-toxic plastics, filter paper, and the leaves of many plants would serve, Credland (1973) found that the most convenient site for oviposition of *C. riparius* was suspending a strip of filter paper so that one end dipped in water. In the case of *C. zealandicus* and *C.* sp. a, adult females were observed to prefer walls of the aquaria (plastic or glass) rather than a strip of filter paper for ovipositon. More egg masses were observed along the walls of the aquarium just below the water surface than on the filter paper. Only a very few egg masses were observed on suspended filter papers. Providing water in small dishes resulted in more fertilized eggs than water in a single container at the bottom of the mating cage.

Credland (1973) reported that eggs of *C. riparius* hatched in about 36 hours and adults began to emerge after a further 28 to 30 days at 24 °C. They continued to emerge for a period of about 10 days. Batac-Catalan and White (1982) found that eggs of *C. tentans* hatched in 2 - 3 days after deposition at 21°C and adults began to emerge after 4 to 5 weeks. Maier et al. (1990) found that two days were needed for eggs to hatch and a period of 33 days to adult emergence at 20°C which was recommended as the optimum temperature for *C. decorus*.

In this study, eggs of *C. zealandicus* hatched about 3-4 days after they were laid and adults began to emerge after about 25 to 30 days and continued to emerge until about 35 to 40 days at 23°C. Eggs of *C.* sp. a and *P. pavidus* start hatching about a day earlier than those of *C. zealandicus.* Adults of *P. pavidus* start emerging from about the 19th day while those of *C.* sp. a from the 25th to 30th day at 23°C. However, substrate influenced both the time taken to the emergence of the first adult as well as the average emergence time (Chapter 5).

Adults were over-crowded towards the light when more powerful (150 and 200 W) bulbs were positioned above the aquarium, while they did not respond well when a less powerful (40 W) bulb was in place or under normal daylight. Maximum numbers of egg masses were found to hatch when a 100 W bulb was positioned 600 mm above the cage. A minimum number hatched with no artificial light provided. The number of eggs that developed vacuoles (unfertilized egg) was also higher when no artificial light was provided in the mating aquarium.

Credland (1973) reported that varying light intensity from 8 to 16 hours per day had no effect on cultures of *C. riparius* over three generations and used 13:11 (L:D) for his culture. However, Maier et al. (1990) found that photoperiod was important for midge culturing and maintained 16:8 (L:D) throughout their study for the best yield of *C. decorus*. These workers found that when the amount of light was decreased for a period greater than 2 days adult emergence was delayed. In this study, no major difference was found in adult emergence of the test species within a range of 8 to 16 hours of light per day during preliminary trials. However, 12:12 (L:D) maintained a satisfactory culture in this study.

Many workers prefer temperature ranges of between 18 to 24°C to culture different species of Chironomidae. Credland (1973) found the higher part of a temperature range of 15 to 25°C was satisfactory for *C. riparius* culture. *C. tentans* was successfully cultured at 21 $\pm$ 1°C (Batac-Catalan and White 1982). Maier et al (1990) found that the time to egg hatching and time to emergence are negatively correlated with temperature. These workers found an upper and lower thermal limit for *C. decorus* of 28 to 30°C and

8 to 10°C respectively. They recommended 20°C as the optimum temperature that would maximize larval growth. In this study, a maximum number of fertilized eggs was obtained from adults emerged at 23°C.

Methods for culturing chironomids rely on regular attention, availability of suitable food, and the renewal of water and media at short intervals (Credland 1973). In this study, water in culture aquaria was replaced once every two or three weeks. This varied depending on other factors, such as aeration and accidental excess feeding. Water in the mating cage was replaced every three days and egg masses and dead adults were removed daily from the mating cage in order to avoid any possible infection of hatching egg masses by the dead adults. In addition, numbers of larvae that can be maintained in a vessel are limited by the area of the tank bottom available to each larva (Credland 1973). Larvae of *C. tentans* each required about 50 mm^2 (Beermann 1952) and *Chironomus thummi* needed 40 mm^2 (Strenzke and Neumann 1960). However, Credland (1973) reduced the required area for the culture of *C. tentans* to 20 mm^2 from 40 mm^2 by providing a three dimensional medium of filamentous algae in his culture. Double quilted paper towel used either in multi-layers or as a pulp in this study provided an excellent medium for chironomid species.

Sterilized paper towel was observed to be shredded into very small particles by growing larvae and fresh paper towel substrate was regularly added to the bottom of aquaria to ensure sufficient substrate for the larval population. Excess and older substrate together with excess food enhanced the growth of rotifers and copepods. The substrate was completely replaced with fresh paper towel after 10 to 12 months. Pupal exuviae were removed once a week to prevent trapping TetraMin food on the water surface.

The set of biological and non-biological parameters adopted for culturing test species resulted in satisfactory provision of all desired life stages for laboratory bioassays throughout this study. These conditions may be used to create and maintain cultures of these three species for bioassay purposes in the future

Influence of site, season, and substrate on head capsule deformities in *Chironomus zealandicus*.

4.1. INTRODUCTION

Chironomids possess many advantages as indicator organisms (Warwick 1990b). The family is very large and its members inhabit virtually every type of aquatic habitat. This widespread distribution permits a very broad interpretation of temporal and spatial changes in aquatic environments. In addition, the larvae of most chironomid species dwell in sediment and feed on organic matter and mineral particles with their associated micro-fauna and flora. Because of their benthic feeding habits, these larvae may be exposed to contaminated sediments throughout their entire larval stage.

Two important characters of chironomids that are used as indicators in biomonitoring are fluctuating asymmetry and deformity. Deformity is considered as relatively a more general class of abnormalities compared with fluctuating asymmetry and animals are commonly defined as phenodeviants in the developmental stability literature. There may be a relationship between fluctuating asymmetry and the frequency of phenodeviants (Clarke et al. 1995). However, the relative sensitivities of the two types of measurement may be dependent on the structure of the test species under investigation. For chironomids, relatively more literature is available on head capsule deformity than on fluctuating asymmetry.

Morphological deformities in chironomid larvae offer promising biological indicators for the assessment of contaminated sediments. Such abnormalities represent sub-lethal effects and can be considered as early warning signals of environmental degradation by chemical contaminants (Warwick 1990a, 1990b). The use of head capsules offers several advantages because they are easily prepared for examination and are available as sub-fossils allowing assessment of environmental health through time. Examination of whole, prepared head capsules allows simultaneous assessment of deformities in a

number of structures. Indices of effect can be constructed based on the presence of single or multiple deformities and bilateral symmetry as well as the severity of deformations in a single structure (Warwick 1985).

Various workers have examined deformities in the head capsules of chironomid larvae in response to contaminant exposure. Results from these studies indicate a relationship between increased incidence of deformation and toxic sediment stress (Hamilton and Saether 1971; Kohn and Frank 1980; Warwick 1980a, 1980b, 1980c; Wiederholm 1984; Warwick 1985; Dickman et al. 1990; Dermott 1991). Hamilton and Saether (1971) proposed that deformities in chironomid larvae were caused by chemical contaminants because deformed larvae were commonly found in areas receiving industrial and/or agricultural contaminants, but not in areas polluted by domestic sewage. The frequency of deformities has been found to be generally higher at localities in which high concentrations of contaminants are present or might be assumed to be present in the environment because of the proximity of waste water discharges (Wiederholm 1984; Warwick 1988). However, the direct linkage between deformities and chemical contaminants still remains to be confirmed. Although most of the data in these studies are semi-quantitative and remain largely circumstantial, more substantial evidence was offered in recent field investigations concentrating on the relationship between deformities and contaminant concentration in sediments and larvae (e.g. heavy metals: Janssens de Bisthoven et al. 1995 and polyaromatic hydrocarbons (PAHs): Dickman et al. 1992).

There is experimental evidence that heavy metals cause deformities in the epipharyngeal pecten of *Chironomus* species (Kosalwatt and Knight 1987; van de Guchte and van Urk 1989). Furthermore, in laboratory experiments, chironomid deformities have been induced by organochlorine pesticides (Hamilton and Saether 1971; Warwick 1985), coal-derived oil (Cushman 1984), and xylene (Janssens de Bisthoven 1995). These studies present promising evidence for some specificity of response but so far, no single contaminant or class of contaminants has been found to which induction of deformities can be directly attributed (van Urk et al. 1992).

As Clarke et al. (1995) mentioned, despite several advantages in the head capsule deformity approach and its popularity, there are inherent problems in it. For example, identification and classification of deformities is very subjective and it is often difficult to distinguish between developmental deformities and physical changes resulting from wear and tear. It is also essential to know the underlying normal variation in character expression in unstressed environments. Interpretation of data is often difficult due to the lack of baseline data on the frequency of deformities in natural, unstressed populations (van Urk et al. 1992) and due to the degree of spatial and temporal variation in these parameters. However, despite such problems, for reasons of sensitivity, relative simplicity, and reduced expense, biomonitoring of aquatic ecosystems using the incidence of chironomid deformities has gained wide acceptance among environmental agencies throughout the world and has often been used in environmental assessment programmes (Clarke et al. 1995).

Objectives of this chapter were to investigate the effect of 1) site; 2) substrate; and 3) seasonal variation on the incidence of head capsule deformity in *C. zealandicus.* Jeyasingham and Ling (1997) investigated the natural incidence of deformities in the head capsules of *C. zealandicus* at four lake sites in the central North Island in the summer and winter of 1995. This chapter extends those data to include seasonal sampling from summer 1994/95 to summer 1996/1997. Furthermore, larvae from two different locations were cultured through their entire life cycle in three different substrates in order to assess the influence of substrate type in inducing head capsule deformity.

4.2. METHODS

Specimen samples were collected from four study sites. Details of these sites are given in Chapter 2.

4.2.1. Collection of larvae and sediment

Sediment samples were collected as described in Chapter 2 from each site in summer and winter of 1994/95 to 1996/97. At least 200 4th instar larvae of *C. zealandicus* were separated from the sediment and returned to the laboratory in 20 L plastic buckets with some lake water. Samples of the sediment were also collected from each site for laboratory culture of larvae

4.2.2. Preparation of head capsules

Head capsules were removed from 50 randomly selected 4th instar larvae using a sharpened probe. They were cleared in warm 10% KOH, followed by rinsing in distilled water and then 70% ethanol (Simpson and Bode 1980). Head capsules were mounted in Surgipath Clearium mounting medium with the ventral side upward on a slide. A small paintbrush was used to apply pressure without breaking the coverslip to fully flatten the head capsule.

4.2.3. Categorization of deformities

Any morphological feature departing from the normal configuration, excluding effects from mechanical wear, was considered a deformity.

Deformities of the antennae, mentum, mandible, premandibles and epipharyngeal pecten were identified and categorized according to either one or a combination of Warwick (1985), Warwick and Tisdale (1988) and Janssens de Bisthoven et al. (1995).

Each antenna is a five segmented, complex sensory organ with a conjoined blade and an accessory blade arises from the top of the basal segment (Fig. 4.1A). A style lies on the articulating membrane linking the second and third antennal segments. A ring organ responsible for chemoreception is situated in the lower third of the basal segment (Warwick 1985). Obvious deformities involve the loss of individual segments, fusion of one or more adjacent segments, segments of questionable equivalence, or the presence of

an additional segment. In some cases, the length of the individual segments is reduced or the ring organ may be displaced or entirely lacking. Other sensory organs such as the blade/accessory blade complex or the style may be displaced from their normal positions at the apices of either the first or second segment respectively (Fig. 4.2B-I).

The mentum has 15 darkly pigmented teeth comprising a tripartite median arrangement of one large tooth flanked on either side by single smaller teeth and two larger and four smaller outer teeth on either side (Fig. 4.1B). Deformities could arise due to absence or asymmetry of any of these structures (Fig. 4.3B-H). Preliminary findings indicated that there appeared to be some physical wearing of the teeth of menta from specimens collected at the Hamurana Stream site presumably associated with the coarse sand substrate at this site. In order to quantify this, the height of the first inner lateral tooth and the width of the tripartite median tooth of the mentum were measured in fifty randomly selected larvae from each of the four sites.

The mandible has a single strong apical tooth, three inner subapical teeth, and a single outer apicodorsal tooth (Fig. 4.1C). The mandible is equipped with a subapical spur and a row of pecten mandibularis apically. The setae interna, a tuft of three branched setae (the number of branches may vary with species), is located on the inner proximal margin. The outer lateral margin is equipped with a lateral seta, and an external seta. Only deformities arising in the teeth of the mandible were considered in the present study (Fig. 4.4B-H).

Premandibles are movable, inwardly curved appendages located on either side of the ventral surface of the labrum attached to the tormal bar (Fig. 4.1D). Each has two apical teeth or rami and a brush of microtrichia, the premandibular brush (Saether 1971), on the mesal margin. It is believed that premandibles probably function in food manipulation. Premandibular deformities involve the number of apical teeth, the configuration of the premandible as a unit, as well as the configuration of individual teeth (Fig. 4.5B-G). A premandible with a single tooth is considered to be the most common type of deformity in the premandible. However, examples with up to five teeth have been recorded (Warwick and Tisdale 1988). Relative length and thickness of teeth are also considered indications of deformation.

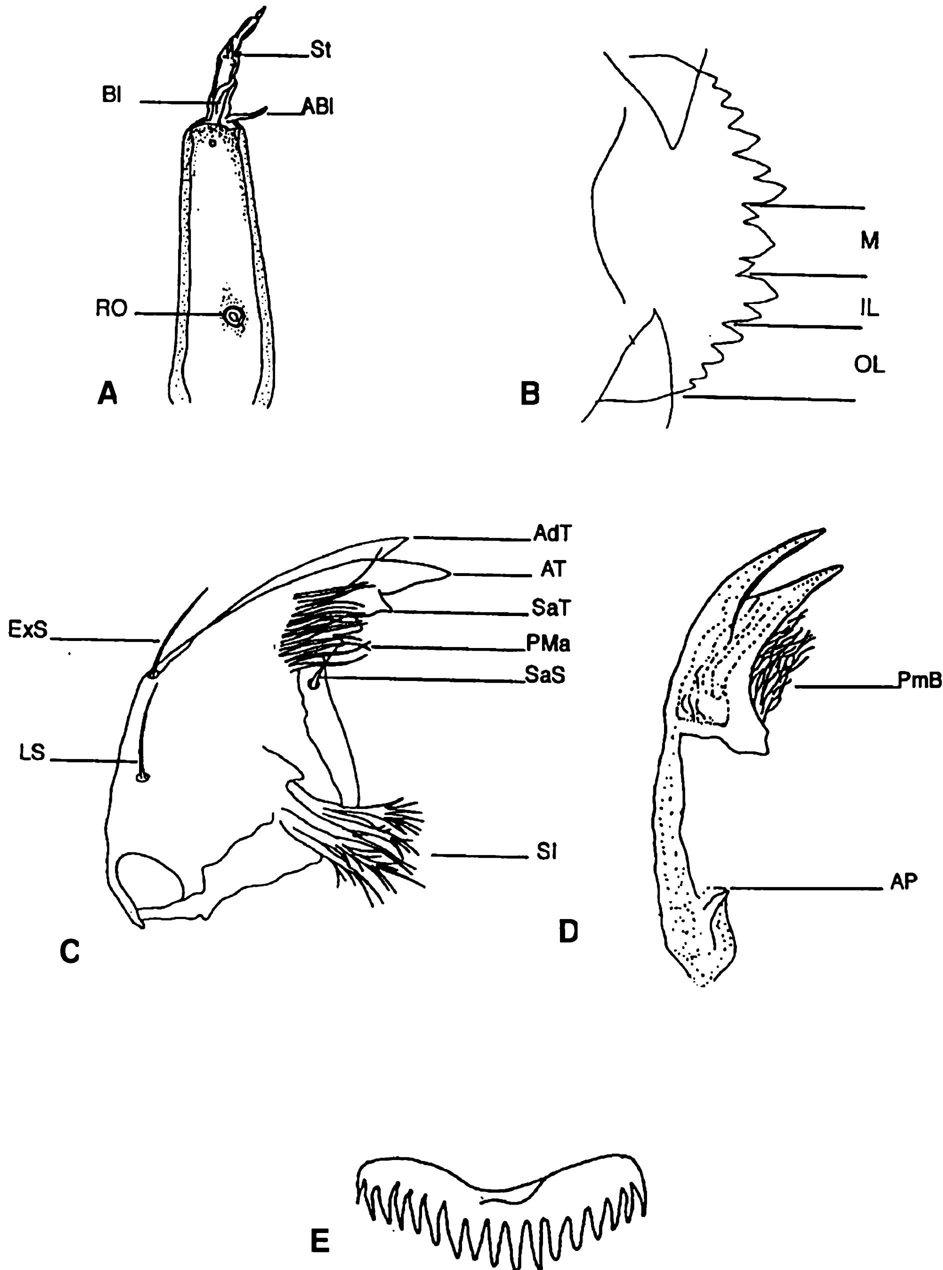


Fig. 4.1. Schematic drawings of normal A, antenna; B, mentum; C, mandible; D, premandible; and E, epipharyngeal pecten of *Chironomus zealandicus*. ABl, acessory blade; AdT, apico-dorsal tooth; AP, articulating process; AT, apical tooth; Bl, blade; ExS, external seta; IL, inner lateral teeth; LS, lateral seta; M, median tooth; OL, outer lateral teeth; PMa, pecten mandibularis; PmB, premandibular brush; RO, ring organ; St, style; SI, setae interna; SaS, sub-apical spur; SaT, sub-apical teeth.

The epipharyngeal pecten is a median shallow "U" shaped, transverse toothbar located directly behind the labral margin between the tormal bar and ungula (Fig. 4.1E). In the normal pecten, teeth generally increase in size from the outer margins inwards towards the centre. The function of the pecten is not clearly known, although it is believed to be involved in food manipulation. Deformities in the pecten range from fusing of the teeth to overlapping, asymmetry, stunting, missing teeth, and disjunction of the epipharyngeal structure (Fig. 4.6B-J).

4.2.4. Examination of deformities

Each of the paired antennae, mandibles and premandibles and the single median mentum and epipharyngeal pecten was examined for abnormal appearance using a binocular dissecting microscope (Citoval, Carl Zeiss Jena) at magnifications up to 100x. When any one of these mouthpart structures was found to be deformed it was considered as a "single" deformity whilst if more than one was deformed, it was considered to be a "multiple deformity" (up to five structures deformed). Bilaterally symmetrical abnormality of paired structures was also regarded as a single deformity. The occurrence of multiple deformities was assumed to represent an increased severity of response compared with any single abnormality.

Although each departure from the normal pattern in the head capsule was considered as a deformity, abnormalities that appeared to have resulted from mechanical wearing (probably by feeding activity of larvae) and/or mounting artifacts were carefully examined and excluded. Physical wear was reported separately in this experiment. As Janssens de Bisthoven et al. (1995) discussed, physical wear was easily distinguished from any real deformity by its structural characteristics.

Fifty head capsules prepared for the deformity analysis from each site were randomly selected and the height of the first inner lateral tooth of the mentum and the width of the tripartite median tooth were measured.

Based on the initial examination of head capsules of larvae collected from all four sites, Lake Ngaroto and Sulphur Point were selected as sites having high frequencies of deformity for the laboratory experiments outlined below.

4.2.5. Culture and rearing of larvae and adults

Clear plastic 20 L aquaria were set up containing either Lake Ngaroto or Sulphur Point sediment (50 mm depth of substrate with 100 mm of overlying lake water). About two-hundred 4th instar larvae from the appropriate site were introduced to aquaria containing water and substrate from the same site. Larvae were fed on TetraMin fish food (0.5 g per aquarium twice weekly). All aquaria received gentle aeration and were maintained at room temperature (approximately 20°C). Emerging adults were collected daily using an aspirator and introduced into a mating aquarium. The mating aquarium was a clear glass tank of 220 L capacity filled to a depth of 50 mm with soft synthetic water. A 100 W tungsten lamp was positioned approximately 600 mm above the tank to induce swarming activity in the adults. Egg masses were collected from the water in the bottom of the tank for rearing of F1 generation larvae.

Aquaria were set up containing sediment from either Lake Ngaroto or Sulphur Point or a control substrate consisting of shredded paper towels (washed and sterilized by autoclaving). Each aquarium received a single egg mass collected from adults reared from each site (either Ngaroto or Sulphur Point) and these treatments were performed in triplicate. Upon hatching, the larvae were fed as above until the 4th instar developmental stage (approximately 4 weeks). At this time all larvae collected from the three replicates of each sediment type for each larval stock (Ngaroto or Sulphur Point), were pooled and 50 larvae selected at random for head capsule analysis.

In order to separate the effects of culture in the laboratory from sediment effects a further experiment was performed using F2 generation larvae. This experiment used an identical design to that above for F1 larvae. However, the F1 larvae from which F2 stocks were obtained had been reared in the laboratory on the control paper towel substrate with conditions and feeding as above. Adults raised on the control substrate were mated as above to provide egg masses for the rearing of the F2 larvae. The larvae

were raised on three different substrate types in triplicate and examined for abnormalities as for the F1 generation above.

4.2.6. Statistical analyses

Head capsule deformities have been analysed using a variety of methods in the past. Numerical indexing (Warwick 1985; Dermott 1991); calculation of proportion (Hudson and Ciborowski 1996) or percentage (Bird et al. 1995) deformed with standard error; correlation analysis (Janssens de Bisthoven et al 1995); transformation of frequencies of deformities with calculation of 95% confidence intervals (van Urk et al. 1992); and the Chi-squared test (Janssens de Bisthoven et al. 1998), have all been used depending on the objective of the experiment. In this study, statistical comparisons of deformities and physical wear were made separately using the Chi-squared test between sites and seasons. Lakes Ngaroto and Hamilton were treated as a pair and the other two sites as another pair on the basis of the geomorphological similarity of the sites. Comparisons of all four sites were also made. Differences in the frequency of deformities in relation to the effects of sediment type on F1 and F2 generation larvae were also made using the Chi-squared test. One-way ANOVA was used to test variation in the height of the inner lateral teeth and the width of the median teeth of the mentum of wild caught larvae from all four sites.

4.3. RESULTS

Representative examples of normal and deformed head capsule structures examined in this experiment are given in Figs 4.1 to 4.6.

4.3.1. Frequency of abnormalities

The number of head capsules showing deformities during each sampling period are given in Table 4.1 with statistical comparisons of each site within a season. Comparisons between seasons for each site are given seperately in Table 4.2 for clarity.

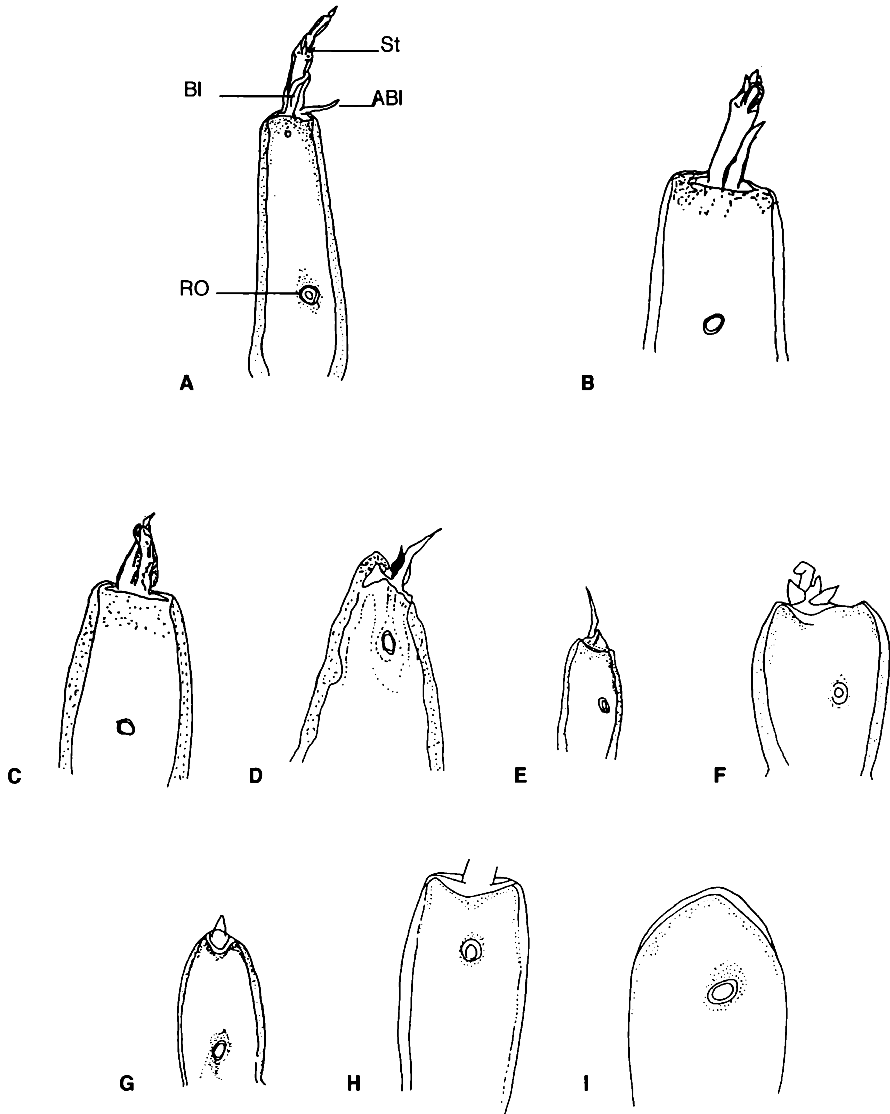


Fig. 4.2. Representative examples of normal and deformed antenna drawn from preserved specimens. A, normal antenna; B-I, deformed antennae; B & C, reduced number of segments; D-F, reduced number of segments with ring organ located above the lower third of the basal segment. H: ring organ displaced distally; G & I, lack of segments. ABl, acessory blade; Bl, blade; RO, ring organ; St, style.

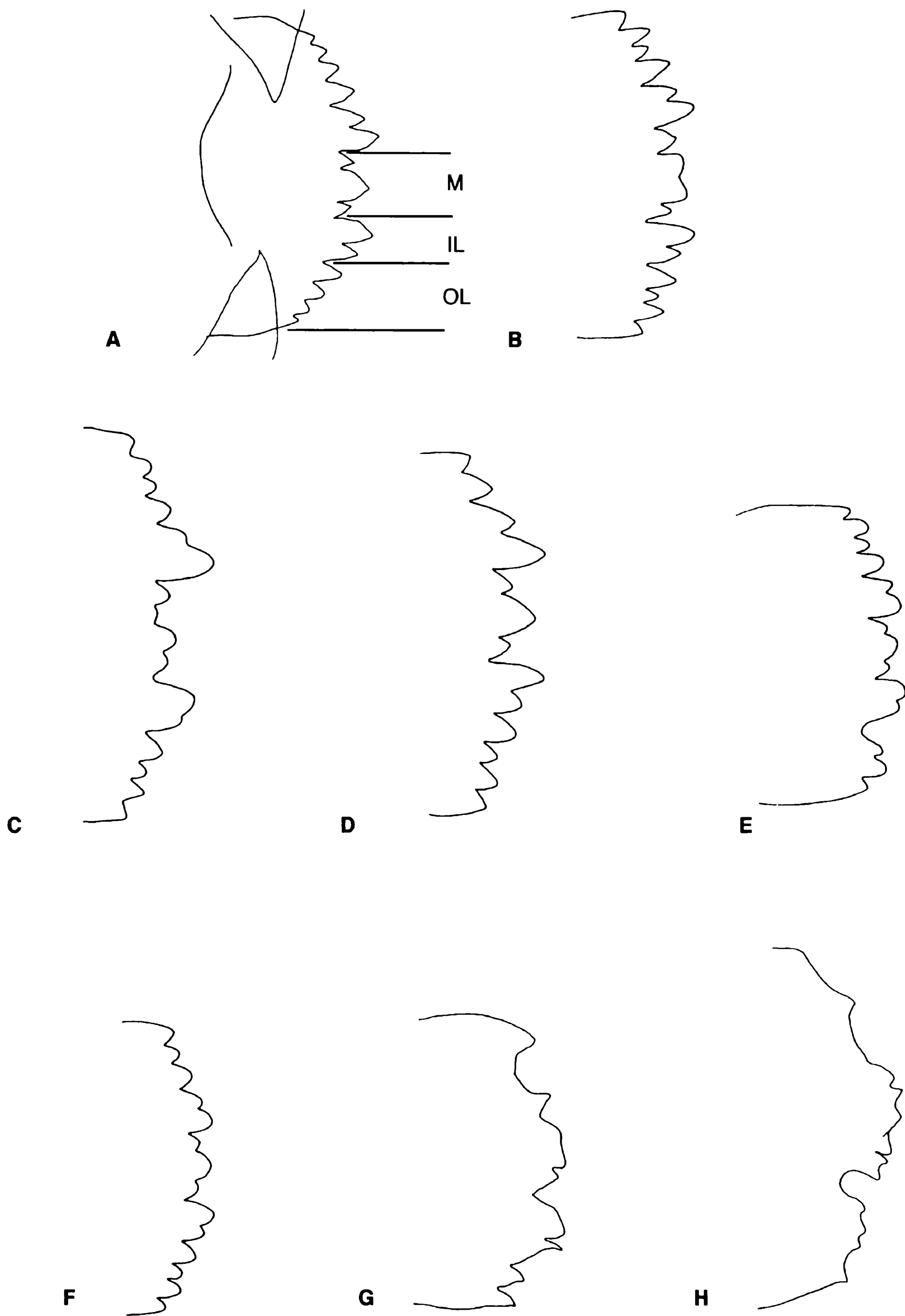


Fig. 4.3. Representative examples of normal and deformed menta drawn from preserved specimens. A, normal mentum; B-H, Deformed menta; B & C, deformed median tooth; D-F, deformed outer lateral teeth. M, median tooth; IL, inner lateral teeth; OL, outer lateral teeth.

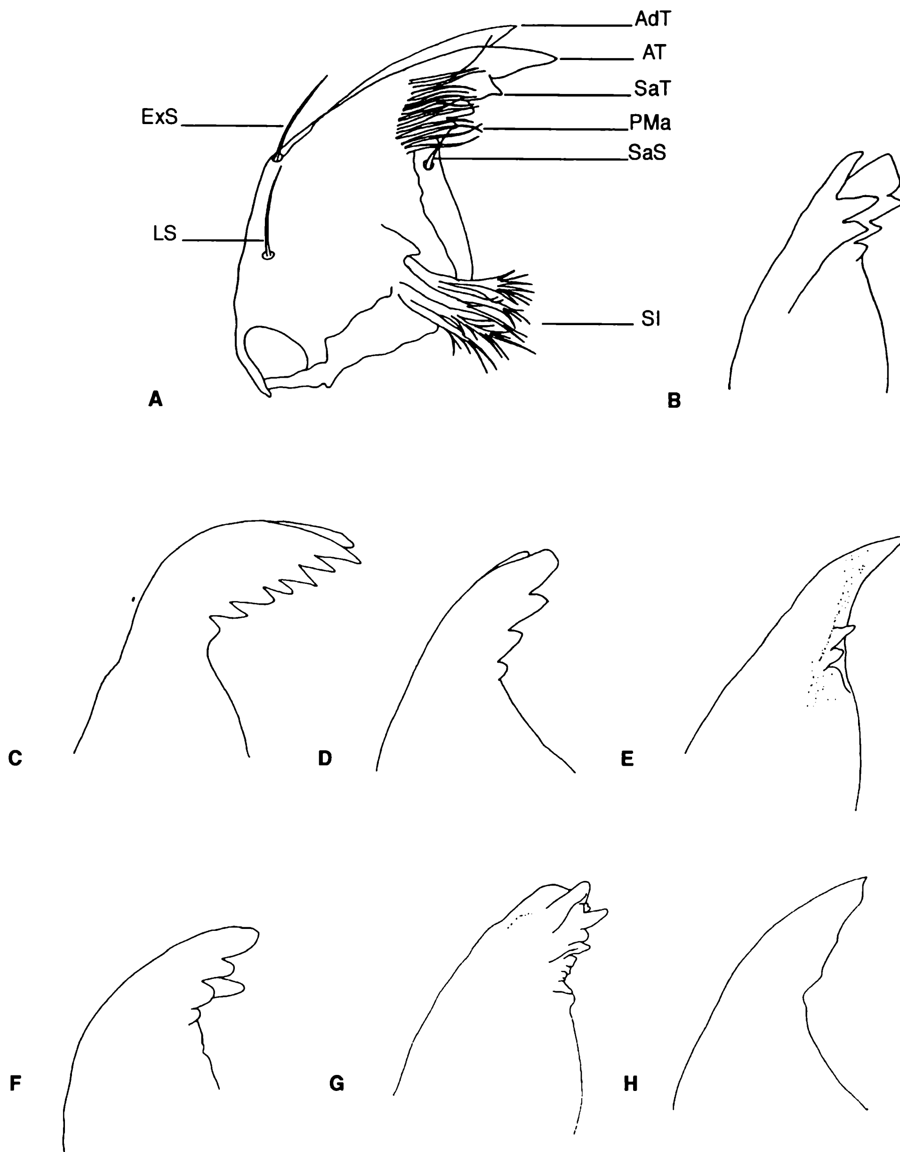


Fig. 4.4. Representative examples of normal and deformed mandibles drawn from preserved specimens. A. normal mandible; B-H, Deformed mandibles; B, two rows of teeth; C, additional teeth; D, blunt apical and apico-dorsal teeth; E, loss of teeth; F & G, irregular teeth; H, lack of teeth. AdT, apico-dorsal tooth; AT, apical tooth; SaT, sub-apical teeth; PMa, pecten mandibularis; SaS, sub-apical spur; SI, setae interna; LS, lateral seta; ExS, external seta.

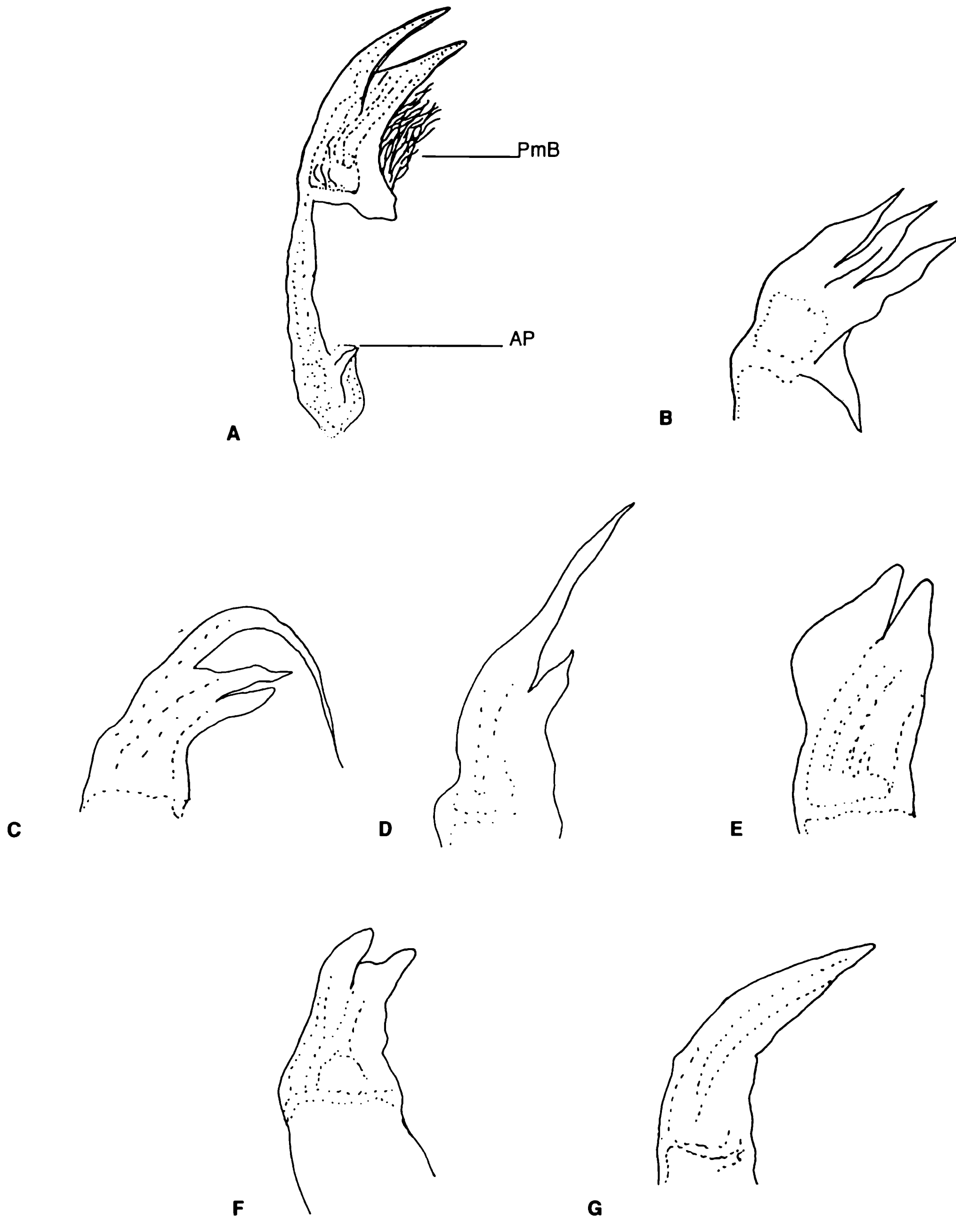


Fig. 4.5. Representative examples of normal and deformed premandibles drawn from preserved specimens. A, normal premandible; B-G, deformed premandibles; B & C, additional (3rd) tooth; D, one elongated tooth; E & F, shortened teeth; G, fusion of teeth or lack of one tooth. PmB, premandibular brush; AP, articulating process.

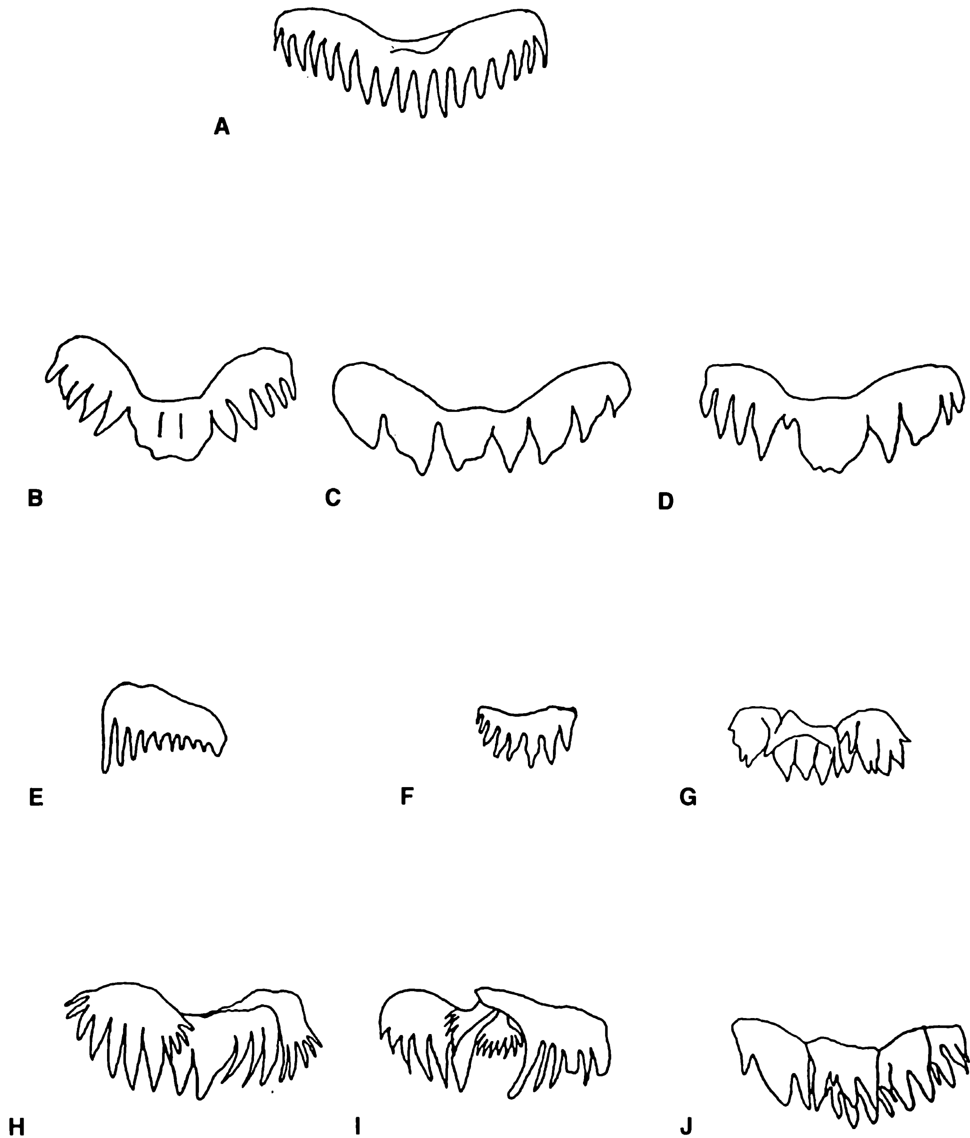


Fig. 4.6. Representative examples of normal and deformed epipharyngeal pecten drawn from preserved specimens. A, normal pecten; B-K, deformed pecten; B-F, fusion or lack of teeth; G-J, complex of deformities.

Table 4.1. The number of head capsules deformed in *Chironomus zealandicus* at each of four sites. Larvae were collected in summer (December) of 1994, 1995, and 1996 and winter (June) of 1995 and 1996. 50 individuals were examined from each site, χ^2 comparisons for data within each season ($P<0.05$): $\uparrow$ indicates an increased and $\downarrow$ indicates a decreased incidence of deformities compared to a, Hamilton Lake, b, Hamurana Stream, c, all other sites.

	Hamilton	Ngaroto	Hamurana	Sulphur Point	Hamilton	Ngaroto	Hamurana	Sulphur Point
	Summer				Winter			
Deformity								
	1994				1995			
Antennae	11	$29^{a\uparrow c\uparrow}$	2	$10^{b\uparrow}$	2	$18^{a\uparrow c\uparrow}$	3	3
Menta	10	$28^{a\uparrow c\uparrow}$	12	13	10	14	11	17
Mandibles	9	6	5	3	7	$0^{a\downarrow c\downarrow}$	10	3
Premandibles	14	8	8	$19^{b\uparrow}$	4	7	6	4
Pecten	$22^{c\uparrow}$	$12^{a\downarrow}$	8	7	4	11	6	5
Single deformities	21	15	$7^{c\downarrow}$	$19^{b\uparrow}$	15	17	10	17
Multiple deformities	18	24	12	13	3	14	10	7
Total deformities	39	39	$19^{c\uparrow}$	$32^{b\uparrow}$	21	31	20	24
	1995				1996			
Antennae	5	11	$1^{c\downarrow}$	$11^{b\uparrow}$	6	4	5	$13^{c\uparrow}$
Menta	10	16	7	$18^{b\uparrow}$	10	17	11	11
Mandibles	12	12	13	12	4	4	6	5
Premandibles	14	6	8	14	5	4	3	8
Pecten	$24^{c\uparrow}$	$8^{a\downarrow}$	7	10	14	8	9	9
Single deformities	15	21	$5^{c\downarrow}$	$19^{b\uparrow}$	10	14	6	8
Multiple deformities	17	12	13	15	11	10	9	15
Total deformities	32	33	$18^{c\downarrow}$	$34^{b\uparrow}$	21	24	16	23
	1996							
Antennae	10	8	$1^{c\downarrow}$	$11^{b\uparrow}$				
Menta	11	13	4	$13^{b\uparrow}$				
Mandibles	5	8	4	7				
Premandibles	8	8	7	16				
Pecten	18	14	13	10				
Single deformities	17	16	14	18				
Multiple deformities	15	17	6	$16^{b\uparrow}$				
Total deformities	32	33	$20^{c\downarrow}$	$34^{b\uparrow}$				

Table 4.2. Comparison of the number of deformed head capsules in *Chironomus zealandicus* between seasons. Larvae collected from four sites in summer (December) of 1994, 1995 and 1996 and winter (June) of 1995 and 1996. 50 individuals were examined from each site, χ^2 comparisons ($P<0.05$): $\uparrow$ indicates an increased and $\downarrow$ indicates a decreased incidence of deformities compared to: a, the same sites between summers or between winters of consecutive years; b, the previous summer; c, the previous winter.

	Hamilton		Ngaroto		Hamurana		Sulphur Point	
	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
Deformity	1994	1995	1994	1995	1994	1995	1994	1995
Antennae	11	$2^{b\downarrow}$	$29^{a\uparrow}$	$18^{b\downarrow}$	2	3	10	3
Menta	10	10	29	$14^{b\downarrow}$	12	11	13	17
Mandibles	9	7	$6^{\downarrow}$	$0^{b\downarrow}$	5	10	$3^{a\downarrow}$	3
Premandibles	14	$4^{b\downarrow}$	8	7	8	6	19	$4^{b\downarrow}$
Pecten	22	$4^{a\downarrow\, b\downarrow}$	12	11	8	6	7	5
Single deformities	21	15	15	17	7	10	19	17
Multiple deformities	18	$6^{b\downarrow}$	24	14	12	10	13	7
Total deformities	39	$21^{b\downarrow}$	39	31	19	20	32	24
	1995	1996	1995	1996	1995	1996	1995	1996
Antennae	5	6	11	4	1	5	11	13
Menta	10	10	16	17	7	11	18	11
Mandibles	12	4	$12^{c\uparrow}$	4	13	6	$12^{c\uparrow}$	5
Premandibles	$14^{c\uparrow}$	$5^{b\downarrow}$	6	4	8	3	$14^{c\uparrow}$	8
Pecten	$24^{c\uparrow}$	14	8	8	7	9	10	9
Single deformities	15	10	21	14	$5^{a\uparrow}$	6	19	$8^{b\downarrow}$
Multiple deformities	$17^{c\uparrow}$	11	12	10	13	9	15	15
Total deformities	$32^{c\uparrow}$	$21^{b\downarrow}$	33	24	18	16	34	$23^{b\downarrow}$
	1996		1996		1996		1996	
Antennae	10		8		1		11	
Menta	11		13		4		13	
Mandibles	5		8		4		7	
Premandibles	8		8		7		6	
Pecten	18		14		13		5	
Single deformities	17		16		14		$18^{c\uparrow}$	
Multiple deformities	15		17		6		16	
Total deformities	$32^{c\uparrow}$		33		20		$34^{c\uparrow}$	

Although the incidence of total head capsule deformity was always relatively high for all sites, statistically significant differences were observed. In any one seasonal sample, the frequency of total deformities in larvae from Hamurana Stream was always lower than other sites and significantly so in all summer samples ($P<0.05$). There were no significant differences between any of the other sites within a season.

The frequency of head capsule deformities varied between summer and winter of a year and between consecutive summers and winters. Total deformities were always higher in summer than in winter for all sites other than Hamurana Stream where this seasonal trend was not apparent.

Analyses of individual classes of deformity are less straightforward although similar trends between sites and seasons can be seen. Between sites in any one season, significant differences are more commonly observed in deformities of the antennae followed by the menta, pecten, and mandibles and premandibles. However, seasonal differences in deformity at each site are more commonly associated with premandibles, followed by mandibles, antennae and pecten, and the menta.

4.3.2. Physical effects of substrate type on mouthparts

The menta and mandibles of individuals from Hamurana Stream showed considerable flattening of teeth, which is associated with physical wearing of these structures during feeding in the coarse sand substrate at this location. No significant differences were observed in the width of the median tooth indicating that the overall size of the head capsule was relatively constant for larvae from all sites. However, the inner lateral tooth of larvae from Hamurana Stream is significantly shorter ($P<0.05$) when compared with individuals from the other sites (Table 4.3).

The frequency of physical wear was higher ($P<0.05$) in larvae collected from Hamurana both in summer and winter compared with all other sites (Table 4.4). Antennae did not show any physical damage during the entire survey, while menta showed a relatively

Table 4.3. Height of the first left inner lateral tooth and width of the tripartite median tooth of the mentum (in mm) of *Chironomus zealandicus* from four lake sites (Means ± standard error, n=50 larvae from each site, * denotes significantly different (P<0.05) from all other sites.)

	Height of inner lateral tooth	Width of median tooth
Hamilton	0.0361 (0.0007)	0.0607 (0.0006)
Ngaroto	0.0360 (0.0006)	0.0676 (0.0098)
Hamurana	0.021* (0.0009)	0.0605 (0.0006)
Sulphur Point	0.0351 (0.0007)	0.0610 (0.0008)

high incidence of physical wear. Mandibles and menta of Hamurana stream individuals showed greater incidence of physical wear in summer than in winter while the premandibles and pecten showed no such difference between seasons.

4.3.3. Effects of substrate type on the F1 and F2 generation

The effects of differing substrate types on the frequency of head capsule deformity are shown in Table 4.5 along with frequencies for the respective parent stock. There are indications that substrate may influence the development of mouthparts although clear trends are not immediately apparent. However, there is a clear decrease in antennal deformities in response to a change in substrate type for both larval stocks but this does not appear to be associated with any one particular substrate. The number of deformed menta in Ngaroto larvae was lower (P<0.05) when raised on Sulphur Point sediment and the frequency of this deformity was greater when Sulphur Point individuals were raised on Ngaroto sediment indicating that the latter substrate type may indeed increase

Table 4.4. Number of individuals showing physical wear of the head capsule in *Chironomus zealandicus* from four sites in summer (December) 1994, 1995 and 1996 and winter (June) 1995 and 1996. n=50 individuals from each site, χ^2 comparisons ($P<0.05$): a indicates an increased incidence of wear compared to all other sites in the same season; b, increased incidence of wear compared to the following winter.

	Hamilton		Ngaroto		Hamurana		Sulphur Point	
Deformity	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
	1994	1995	1994	1995	1994	1995	1994	1995
Worn antennae	0	0	0	0	0	1	0	0
Worn menta	5	4	2	1	30[a,b]	10[a]	8	6
Worn mandibles	4	2	0	1	26[a,b]	7[a]	6	3
Worn premandibles	0	2	0	1	10[a]	5	1	2
Worn pecten	0	2	0	0	8[a]	2	0	3
Single wear	5	1	2	3	7	13[a]	10	5
Multiple wear	3	4	0	0	27[a,b]	5	2	4
Total wear	8	5	2	3	34[a, b]	18[a]	12	9
	1995	1996	1995	1996	1995	1996	1995	1996
Worn antennae	0	0	0	0	0	0	0	0
Worn menta	5	3	2	1	29[a]	19[a]	8	6
Worn mandibles	3	2	0	0	24[a,b]	10[a]	4	4
Worn premandibles	0	0	0	0	6[a]	4[a]	1	0
Worn pecten	0	0	0	0	11[a]	8[a]	0	0
Single wear	4	3	2	1	8	1	8	6
Multiple wear	2	1	0	0	24[a,b]	18[a]	2	2
Total wear	6	4	2	1	32[a,b]	19[a]	10	8
	1996		1996		1996		1996	
Worn antennae	0		0		0		0	
Worn menta	8		3		20[a]		8	
Worn mandibles	2		1		18[a]		5	
Worn premandibles	0		0		4[a]		0	
Worn pecten	0		0		6[a]		0	
Single wear	6		4		16[a]		11	
Multiple wear	2		0		12[a]		1	
Total wear	9		4		28[a]		12	

Table 4.5. Number of individuals showing head capsule deformities in *Chironomus zealandicus* first (F_1) and second (F_2) generation cultured larvae from two sites, raised in three different substrates. F_2 larvae were cultured from animals raised on control substrates for the first generation. (n=50 larvae from each treatment, χ^2 comparisons ($P<0.05$): ↑ indicates an increased, and ↓ indicates a decreased incidence of deformities compared to a, Ngaroto parent stock, b, Ngaroto larvae raised in Ngaroto sediment, c, Sulphur Point parent stock, d, Sulphur Point larvae raised in Sulphur point sediment, e, F_1 generation larvae raised in the same sediment.)

	Ngaroto larvae raised in				Sulphur Point larvae raised in			
	Ngaroto parent stock	Ngaroto sediment	Sulphur Point sediment	Control substrate	Sulphur Point parent stock	Sulphur Point sediment	Ngaroto sediment	Control substrate
F1 generation								
Deformed antennae	29	27	$4^{\downarrow a \downarrow b}$	$4^{\downarrow a \downarrow b}$	10	10	$0^{\downarrow c \downarrow d}$	$0^{\downarrow b \downarrow c}$
Deformed menta	28	29	$6^{\downarrow a \downarrow b}$	20	16	14	22	10
Deformed mandibles	6	5	$24^{\uparrow a \uparrow b}$	4	3	4	8	$12^{\uparrow c}$
Single deformities	17	17	26	20	14	14	18	18
Multiple deformities	22	21	$4^{\downarrow a \downarrow b}$	$4^{\downarrow a \downarrow b}$	6	8	6	2
Deformed (total)	39	38	30	24	20	22	24	20
F2 generation								
Deformed antennae	29	$4^{\downarrow a \downarrow e}$	$3^{\downarrow a}$	$7^{\downarrow a}$	10	6	4	$6^{\downarrow e}$
Deformed menta	28	$16^{\downarrow e}$	14	$11^{\downarrow a}$	16	14	$11^{\downarrow e}$	9
Deformed mandibles	6	5	$7^{\downarrow e}$	$0^{\downarrow a \downarrow b}$	3	5	3	$0^{\downarrow e}$
Single deformities	17	17	18	16	14	13	14	15
Multiple deformities	22	$4^{\downarrow a \downarrow e}$	$3^{\downarrow a}$	$1^{\downarrow a}$	6	5	2	$0^{\downarrow b \downarrow c}$
Deformed (total)	39	$21^{\downarrow a \downarrow e}$	$21^{\downarrow a}$	$17^{\downarrow a}$	20	18	16	15

Table 4.6. Number of individuals showing physical wear of head capsule deformities in *Chironomus zealandicus* first (F_1) and second (F_2) generation cultured larvae from two sites, raised in three different substrates. F_2 larvae were cultured from animals raised on control substrates for the first generation. (n=50 larvae from each treatment, χ^2 comparisons (P<0.05): a indicates a decreased incidence of physical wear compared to Sulphur Point parent stock)

	Ngaroto larvae raised in				Sulphur Point larvae raised in			
	Ngaroto parent stock	Ngaroto sediment	Sulphur Point sediment	Control substrate	Sulphur Point parent stock	Sulphur Point sediment	Ngaroto sediment	Control Substrate
F1								
Worn mentum	2	3	4	1	8	2	4	1[a]
Worn Mandible	0	1	3	0	6	2	2	1
Total Wear	2	4	7	1	12	4	6	2[a]
F2								
Worn mentum	2	1	3	1	13	3	3	0[a]
Worn Mandible	0	2	5	0	6	3	3	1
Total Wear	2	3	8	1	12	6	6	1[a]

deformity of this structure. The frequency of mandible deformity was greater (P<0.05) in Ngaroto larvae raised on Sulphur Point sediment. There were no significant dfferences in any parameter between the first generation offspring raised on their own sediment type and the parent stock.

There were no clear effects of differing substrate type on the incidence of mouthpart deformity in F2 generation larvae other than a decrease in the number of deformed mandibles in both larval stocks raised on the control substrate. There was, however, an overall reduction in the total incidence of deformities in both larval groups when compared with their respective parent stocks.

There were no significant differences in the overall frequency of single deformities in any offspring when compared with their parent stock. However, there was a clear

decrease in the incidence of multiple deformities, particularly on the control paper substrate.

This indicates that this latter parameter may indeed represent a good indicator of some overall effect of environment on mouthpart abnormality. These results indicate an effect associated with laboratory culture on the control substrate, which has reduced the overall incidence of abnormality through successive generations and also implies that some factor associated with the sediment is responsible for inducing mouthpart abnormalities in the larvae.

4.3.4. Physical effects of substrate type on the F1 and F2 generation

There was no difference in the incidence of physical wear observed in larvae raised in Lake Ngaroto sediment. However, total wear and mentum wear were less ($P<0.05$) when compared with the parent stock in Sulphur Point larvae raised in control paper towel substrate both in F_1 and F_2 generations (Table 4.6).

4.4. DISCUSSION

Any morphological feature that departs from the normal configuration is defined as a deformity (Warwick 1988). The deformities encountered in the antennae, menta, mandibles, premandibles, and epipharyngeal pectens of *C. zealandicus* were similar to some of those described for Chironomidae in polluted waters by Kohn and Frank (1980), Warwick (1988), and Warwick and Tisdale (1988). Numerous pollutants have been implicated as possible causal agents but many field studies of deformities in chironomids have been poorly controlled so the attribution of deformities to contaminants remains uncertain (Rosenberg 1992).

The link between the occurrence of structural abnormalities in chironomid larvae and exposure to toxic stress has been substantiated in relation to copper (Kosalwatt and Knight 1987; Van De Gutche and Van Urk 1989; Janssens de Bisthoven et al, 1992), lead (Percy et al. 1986), the organochlorines DDE and DDT (Warwick 1985), the

herbicide Dacthal (Madden et al. 1992), and has been suggested in relation to a pesticide mixture (Hamilton and Saether 1971) and industrial pollution (Dermott 1991).

Diggins and Stewart (1992) reported elevated frequencies of abnormalities among several species of Chironomidae including *Procladius*, *Polypedilum*, *Cryptochironomus* and *Dicrotendipes* under the influence of industrial effluents. Hamilton and Saether (1971) reported 100% abnormality of the mentum in *Chironomus* sp. near the inlet of the Maumee River to Lake Erie, which implicated the effect of contaminant inflows since the frequency of abnormality reduced to near zero when the entire lake was considered.

Janssens de Bisthoven et al. (1992, 1995) determined the concentrations of cadmium, lead, copper and zinc in *Chiromonus* larvae from several locations in the polluted Dyle Basin and Dommel River in Belgium, and compared levels in normal larvae with those in deformed larvae. In most cases, metal levels were significantly higher ($P<0.05$) in deformed larvae than in normal larvae. The authors explained that the populations without significant differences between deformed and normal larvae for their trace metal contents might have been exposed to the action of other organic xenobiotics. A similar investigation was carried out by Dickman et al. (1992) in which levels of polyaromatic hydrocarbons (PAHs) in the body tissue of *Chironomus* larvae from a site in the Niagara River watershed in Canada, were measured. Higher levels of PAHs were found in deformed larvae compared with normal larvae and it was suggested that PAHs may act synergistically to cause the observed deformities. In both of these studies the authors concluded that deformed larvae represented individuals more often exposed to higher local contaminant concentrations than their apparently normal counterparts.

Hamilton and Saether (1971) suggested that different types of deformities might be caused by different contaminants. The first systematic attempt to study the contaminant specificity of deformities was a study in the polluted Dyle River in Belgium by Janssens de Bisthoven (1990). More recently, 20 more Belgian lowland rivers have been included in the study and deformities were correlated with contaminant concentrations in the sediment (Janssens de Bisthoven et al. 1995). Although many deformity types were rather pollutant-specific, some deformity types showed significant correlations

with contaminant concentrations and may prove useful in identifying the presence of specific compounds in contaminated sediments (Vermulen 1995). Vermulen (1995) also indicated that, in a study carried out in Denmark in which the effect of a paper mill effluent on chironomid deformation was investigated, diffuse domestic and agricultural pollutants could be distinguished from industrial point source pollutants (heavy metals) using multivariate deformity patterns. The proportional occurrence of the different deformity types seemed to shift consistently according to the type of pollution. A mentum deformity, the Kohn gap, had a high discriminative power. This was encountered at all sites receiving heavy metal inputs, but only in a few sites receiving domestic sewage and agricultural run-off. This might imply that mentum gaps are specifically induced by heavy metals, as suggested in some previous studies (Kohn and Frank 1980; Janssens de Bisthoven 1995). Other studies also showed that some specific deformities were induced by particular types of pollutant. For example, a split median mentum tooth could be experimentally induced by organochlorine pesticides (Madden et al. 1992) as well as heavy metals (Janssens de Bisthoven 1995). Therefore, contaminant specificity of deformities may prove advantageous for its analytical capacities. However, for a clear cut validation of the hypotheses derived from these field studies, systematic laboratory research investigating and characterizing causal compounds of all deformity responses is essential.

Profundal Lake Erie (Hamilton and Saether 1971), uncontaminated Parry Sound, Lake Huron (Hare and Carter 1976), a non-industrial section of the Wetland River, Ontario (Dickman et al. 1991) and subfossil chironomid remains from Lake Malaren, Sweden (Wiederholm 1984), and the Bay of Quinte, Lake Ontario (Warwick 1980b) all yielded *Chironomus* specimens with abnormal menta albeit at low frequencies. These very low frequencies of abnormal menta observed in uncontaminated and pre-industrial subfossil *Chironomus* suggest that the incidence of aberrant specimens in unstressed populations is small and typically less than 1% (Warwick 1980a; Wiederholm 1984).

Results of this study revealed considerable morphological abnormality in mouthparts of *C. zealandicus* from all four sites examined, but no clear trend associated with the apparent or presumed level of site contamination. Although the presumably clean site at the Hamurana Stream showed a lower incidence of deformity than all other sites, there

were still significant numbers of abnormal larvae. Results indicated an effect related to both site and season with lower frequency of deformities during winter at sites other than Hamurana Stream, possibly mediated by changes in the seasonal physico-chemical characteristics of sediments at each site. There was no clear relationship between the incidence of deformity and the measured heavy metals, arsenic, copper, and chromium in sediments from the sampling sites during the experimental period (Chapter 2). There may be several possible reasons for a lack of relationship between metal concentration and incidence of deformity. Measured values for sediment metal concentration are instantaneous and may not be realistic because sediment samples were collected only once per season; as a result, the sediment samples may not have accurately represented the lake sediments that chironomid larvae inhabit. Nevertheless, the relative concentrations of As, Cu, and Cr in sediment samples did not vary to a great extent over the experimental period, except in December 1994. It is possible that the bioavailability of metals varies between sites depending on the nature of the substrate. The sediment at the Hamurana Stream site contained a greater proportion of coarse sand than the other sites and the lowest percentage of organic matter. Another reason for a lack of relationship between measured heavy metals and the incidence of deformity may again be a synergistic and/or antagonistic effect of the mixture of chemicals present in the sediments.

Seasonal influences on chironomid deformities have been observed before. In a continuous survey, van Urk et al. (1992) found that the frequency of deformity was lower from mid-summer to autumn than from late winter to spring in three of six sites examined. At another site, the frequency of deformities was higher in early summer than in the previous spring. These workers attributed their results to factors such as a lower mortality rate for deformed larvae, perhaps because they are less active and consequently less susceptible to predation, differential emigration of normal larvae from possibly contaminated areas, or deformed larvae being more easily sampled, perhaps because they live nearer the sediment surface. Vermulen et al. (1995) found that larvae of *C. riparius* and *C. luridus* appeared to exhibit higher frequencies of deformities in spring than in summer in two contaminated lowland rivers in Flanders (Belgium). These authors attributed this to spring overwintering larvae that had been developing at a slow rate under low temperature conditions during autumn and winter. Hence they were

exposed much longer to contaminants than the summer larvae. However, this situation is likely to be more complex because, in addition to development rate, metabolism and toxicity also change with temperature, while oxygen and flow rate of water may be other factors controlling toxicity and the bioavailability of sediment-associated contaminants. In the present study, there were obvious differences in the abundance of larvae at particular sites between seasons (Chapter 2) and this may also be a factor affecting the incidence of abnormality. Temperature-dependent effects such as substrate hypoxia during summer may also be a factor in physiological induction of structural abnormalities. A one-year monthly survey of physico-chemical parameters at sampling sites showed temperature and dissolved oxygen at Hamurana were relatively lower and higher respectively than at the other three sites (Table 2.1). These two factors may have been involved in the reduced incidence of deformity of head capsules in larvae collected from Hamurana and the marked seasonal effect at the other sites. Additionally, *Chironomus zealandicus* shows clear seasonal trends in abundance at sites other than Lake Ngaroto and Hamurana Stream where it is always rare. Lower selection pressures during summer months may simply allow greater numbers of deformed larvae to survive.

The importance of food and temperature conditions for the induction of deformities was demonstrated in a study by Parren et al. (1993). Deformities were induced with contaminated sediments from the Laan River in Belgium using two temperatures (13°C and 18°C) and two food conditions (5 and 13 mg TetraMin per larva). Only at the higher temperature condition was the frequency of deformity higher than in the diatomaceous earth control conditions. In the high food regime, the incidence was comparable with field data (10%), whereas in the low food condition the incidence was lower. The induction of deformities was enhanced by a temperature-dependent increase in metabolic rate in conjunction with higher food availability. The authors suggested that deformities were induced after ingestion of contaminants associated with food. Of the four sites examined in this study, known contaminant inputs are greatest at Hamilton Lake and Sulphur Point, which did not show the highest incidence of mouthpart deformities. There is other evidence to suggest that increased exposure to contaminants does not always result in a higher incidence of deformity. Warwick (1985) reported that both the incidence of deformities and the severity of response in *Chironomus* was

inversely related to exposure to the insecticide DDE. Therefore a lower frequency of deformity in individuals from highly contaminated sites may result from Warwick's hypothesis of a graded response at lower concentrations, but increased resistance or mortality occurring at higher exposures. Severe deformities could be expected to reduce survival, especially considering the reorganization of tissues and the sequence of events necessary for the larvae to moult through four instars (Dermott 1991). The influence of severe selection pressures should not be discounted in considering the expected condition of larvae at contaminated sites. In addition, although Sulphur Point exhibits geothermal activity, thermal discharges may not necessarily influence the frequency of morphological abnormalities (Kohn and Frank 1980).

The Hamurana Stream is considered a clean site due to its proximity to a large spring-fed inflow. The larval mouthparts, menta and mandibles showed structural wear apparently mediated by the coarse nature of the sandy substrate. Blunt and shortened teeth of menta and mandibles are evidence of abrasion occurring during feeding activity. This may cause confusion with developmental abnormalities resulting from physico-chemical influences. Abnormalities of head capsule structures that reflect the effects of physical damage related to abrasion by coarse substrate at Hamurana Stream are sometimes difficult to separate from any real deformity, although care was taken to exclude these from the analysis. As Dermott (1991) pointed out, two inherent problems in making accurate identification of deformities are wear or damage to the structure and natural variability in morphology. Care must be taken to correctly identify worn mouthparts, which occur due to feeding activities in coarse sediment. However, wear does not normally cause bilateral asymmetry of the mouthparts, which is often evident in truly abnormal specimens. Damaged antennae, mandibles, and blunt toothed menta resulting from the screening and feeding process usually have abrupt breaks that are readily visible and easily differentiated from developmentally deformed structures. In addition, the edges of deformed structures resulting from mounting damage or physical wear are irregular, so that the double chitinous wall of such structures becomes apparent. Moreover, physical wear is not usually localized. Real deformities are usually localized and asymmetrical. They exhibit a smooth edge, the double wall is invisible and there is often a lack of pigmentation at the base of a gap (Janssens de Bisthoven 1995).

Results of the substrate rearing experiment indicate that the substrate type does influence deformity in the structures examined although there is a continual decline in the incidence of mouthpart deformity with continued laboratory culture through successive generations.

Although the frequency of deformity tends to decrease in the cultured F1 and F2 generations, the general pattern resembles the results of the environmental surveys. The results presented in Chapter 7 demonstrate the laboratory induction of mouthpart deformities in this species by arsenic. Nevertheless, it is not clear from this study whether the natural incidence of mouthpart deformity is influenced primarily by substrate type, chemistry, or parent stock. It is likely that these, as well as other abiotic factors such as temperature and dissolved oxygen, are involved given the greater incidence of deformity in summer. Janssens de Bisthoven (1995) suggested that experimentally induced deformities are generally weaker and the number of deformed larvae is generally lower than in natural populations from contaminated sites. A combination of physical, chemical, and biological environmental parameters is probably responsible for the observed higher frequencies in field studies. In this context, mixtures of chemicals may play an important role in deformity induction (Hamilton and Saether 1971; Janssens de Bisthoven et al. 1992; Dickman et al. 1992). The decline in abnormalities through successive laboratory generations of *C. zealandicus* may reflect the influence of culturing animals on the paper towel control substrate or some form of selection pressure for normal individuals. This may relate to selective mortality of deformed individuals in the culture stocks. This study did not investigate whether deformed larvae are able to pupate and mate successfully. Such selective pressures and the influence of genetic factors in contributing to the overall incidence of deformities and natural variation in the pattern of mouthpart structures cannot easily be dismissed.

Vermulen et al. (1994) investigated mechanical wear of the mouthparts of *C. riparius* larvae reared in two artificial substrates and found that the type of mechanical wear was substrate specific. Mouthparts of larvae reared in cellulose fibres displayed randomly occurring broken teeth with sharp fractures, and outlines of mouthparts were normally asymmetric and showed a high variability. Larvae reared in diatomite particles were

more uniform, and any teeth abraded by the diatomite particles showed a rounded top with a coarse surface. In addition, these authors noted that the occurrence of mechanical wear in experimental conditions was relatively high when compared with field conditions and attributed this to low food concentration and high competition in culture. Results of physical wear reported in the present study contrast with the results of Vermulen et al. (1994). Physical wear of head capsules was lower in larval *C. zealandicus* cultured in the paper towel substrate compared with parent stocks collected from the wild.

In summary, significant differences were observed between sites and seasons. Individuals from Hamurana Stream had the lowest incidence of deformities, particularly during summer. The incidence of deformity declined in winter in larvae from all other sites. No seasonal differences were observed in larvae from Hamurana Stream.. The frequency of deformity was similar at all sites during the winter surveys. Overall results of the substrate tests indicated no clear pattern of substrate effect although some structures may be more sensitive indicators of substrate physico-chemistry than others. The incidence of deformity tended to decline with successive laboratory culture. The substrate type contributed to physical wear and this was more common in the coarse sand substrate of Hamurana Stream. Physical wear of larvae cultured in paper towel substrate was significantly ($P<0.05$) lower than when cultured in Sulphur Point sediment. This supports the hypothesis that coarse sand substrate has contributed to the physical wear in this study, although this contrasts with some previous findings (Vermulen et al. 1994).

Although the present study doesn't give any indication of contaminant specificity in head capsule deformities, it reveals that care must be used in interpreting such findings particularly where comparisons are made between different substrate types and seasons. There are indications that substrate type, season, and genetic factors, as well as sediment chemistry may collectively contribute to the incidence of head capsule abnormalities in *C. zealandicus*. More work is needed on the influence of specific physico-chemical aspects of the environment on larval development and the susceptibility of particular head capsule structures to such influences.

Effect of substrate on the emergence of *Chironomus zealandicus* and *Chironomus* sp. a

5.1. INTRODUCTION

Chemical contamination of aquatic sediments is a problem in many water bodies around the world. Highly industrialized areas of the Great Lakes Basin, for example, have sediments that contain a variety of inorganic and organic chemicals, frequently at concentrations that are toxic to members of the natural benthic community (Liber et al. 1996).

Traditionally, the study of aquatic toxicology has paid major attention to the potential effects of dissolved pollutants on pelagic organisms with acute toxicity data for algae, crustaceans, and fish forming the basic regulatory framework. However, these data fail to take into account effects attributable to the underlying substrate, which can greatly influence the fate of a given pollutant (Watts and Pascoe 1996). As mentioned in chapter 1, most of the organic and inorganic pollutants that enter the aquatic ecosystem may rapidly become associated with sediments which consequently act as a sink, accumulating concentrations several-fold higher than those in the water column (Ingersoll and Nelson 1990).

Laboratory tests with benthic macroinvertebrates are commonly utilized to assess the potential toxicity of contaminated sediments. The use of freshwater macroinvertebrates in pollution studies has been restricted largely to biological changes associated with chronic and episodic pollution and to acute lethal tests in the laboratory. Although the latter provide data on the relative tolerance of species and on factors influencing toxicity (Williams et al. 1984), it is generally recognized that investigations examining whole life cycles and effects other than mortality are needed in order to estimate the maximum acceptable toxicant concentration (MATC) for use in the formulation of water quality

standards, and to predict the effects of aquatic pollution. In addition, using older larvae may underestimate the test animals' sensitivity to toxicants (Williams et al. 1986).

Many aquatic invertebrates are sensitive to stress, relatively long-lived and, unlike highly mobile organisms such as fish, are unable to escape a temporary stress (Coler and Kondratief 1989). Thus they are useful as indicators of pollution. Previous long-term toxicity studies with macroinvertebrates have principally involved daphnids (Winner and Farrel 1976; Marshall 1978), molluscs (Flannagan 1974; Borgmann et al. 1978) and chironomids (Thornton and Whilm 1975; Macek et al. 1976; Wentsel et al. 1977a, 1977b). Several important factors have contributed to the widespread use of Chironomidae as test species in the evaluation of sediment toxicity. The use of life history endpoints as measures of stress is becoming common in pollutant assessment studies (Wiederholm 1984), and studies in chironomids provide excellent examples of this use (Thornton and Wilhm 1975; Wentsel et al. 1977a, 1977b; Hatakeyama and Yasuno 1981; Havas and Hutchinson 1982; Hatakeyama 1987). Tests with many species of chironomid have started with 2nd or 3rd instar larvae (10 to 14 days old) and continued for 10 to 14 days (ASTM 1993). At termination of exposure, the sediment is sieved and larvae are recovered. Endpoints, or measurements, typically include survival and dry weight or growth. In some cases, endpoints such as adult emergence and larval head capsule width have been included (ASTM 1993). It has been observed that some sediments, while not causing mortalitiy to *C. tentans*, may substantially inhibit growth of the larvae. The adverse endpoint of growth reduction or retardation commonly observed at sublethal concentrations of chemical stressors is more difficult to interpret. It is not clearly known whether a statistically significant retardation in larval growth will adversely affect future population dynamics and abundance.

Considering appropriate endpoints is essential in properly estimating the sensitivity of an animal to a toxicant in toxicity tests. There have been a number of classifications for the endpoints in chironomid life history toxicity tests. Johnson et al. (1993) categorized life history indicators or life history endpoints of freshwater stress in freshwater benthic macroinvertebrates into three groups: (1) survival or mortality, (2) growth or development, (3) reproduction or emergence. Benoit et al. (1997) categorized the endpoints of the life cycle tests into four groups: (1) survival at each life stage; (2) 20

day growth as larval and adult dry weight or ash weight; (3) emergence (time to first emergence, cumulative emergence); (4) reproduction (time to oviposit, proportion of females ovipositing, sex ratio, egg production, and percent hatch). Kosalwatt and Knight (1987) categorized chronic toxicity and the resulting indices into four groups according to the effects on the organism's: (1) physiology, (2) biochemistry/cell structure, (3) behaviour, and (4) reproduction.

Thornton and Wilhm (1975) identified two stages that are relatively weak in the life cycle of *C. attenuatus*. One of those was between 1st and 2nd instars caused by experimental conditions and the other stage was between 4th instar and adult caused by the treatment.

Emergence of aquatic insects is a critical stage in the life cycle and is useful for determining the impact of pollutants on aquatic systems (Nordlie and Arthur 1981). However, many other physical factors affect the emergence of aquatic insects. Physical factors that influence emergence include water temperature, photoperiod, and dissolved oxygen (Nebeker 1971, 1972; Danks 1978).

Larvae of *C. zealandicus* were often found in very high numbers at Sulphur Point while *C.* sp. a and *P. pavidus* were the predominant species at Lake Ngaroto and Hamurana respectively (Chapter 2). Substrate type and chemistry as well as other factors such as indigenous animals of each sediment may collectively contribute to the abundance of each species at respective sites. There were clear differences in the incidence of larval head capsule deformity between different study sites observed (Chapter 4). Furthermore, the incidence of deformity decreased with successive generations cultured in control paper towel substrate. These results suggest that test chironomid species may have a substrate preference. The objective of this chapter was to investigate the effect of substrate type from four study sites on survival, growth and emergence of *C. zealandicus* and *C.* sp. a under laboratory conditions. The first part of the experiment was designed to examine the effect of substrates on survival and emergence and the second part to investigate the survival and growth of the larvae of the two species.

5.2. METHODS

5.2.1. Emergence test

Sediments from four sampling sites and two of the test species, *C. zealandicus*, and *C.* sp a were used for this experiment. Collection of sediment samples and test species and handling were described in Chapter 2.

On the day before test initiation, 4 pairs of 20 L clear plastic aquaria were prepared with each pair containing a 50 mm layer of sediment from one of the 4 sites. The sediments were overlain by a 100 mm depth of soft synthetic water. All aquaria were aerated gently and covered by a screen with 250 μm mesh to retain emerging adults. At the initiation of a test, 100 first instar cultured larvae (less than 72 hours old post hatch) of *C. zealandicus* and *C.* sp. a were carefully transferred into each aquarium. This test was repeated three times.

Dissolved oxygen (YSI 55 meter), pH (M20 standard pH meter), conductivity and salinity (YSI 30 meter), and hardness (as mg $CaCO_3$/L) of the water in the test aquaria were monitored at the beginning, middle and end of the experiment. Temperature was measured continuously throughout the experiment using a Hobo™ temperature data logger. If the water level became lower in any aquarium, freshly prepared soft synthetic water was added without disturbing the sediment layer.

Feeding was started on the 4th day post-hatching (the day after test initiation). Finely ground TetraMin flake food was used; 0.02 g/day/aquarium for the first 10 days continued as 0.5 gram per 3 days till the end of the experiment. A photoperiod cycle of L:D = 16:8 hour was maintained throughout the experiment.

Each morning, all fully emerged adults were removed from the aquaria by aspirating into a 500 mL Erlenmeyer flask using a small vacuum pump. Emergence in all aquaria was recorded by sex of the adults. Adults found on the water surface and still partially

attached to their pupal exuviae were not considered to have successfully emerged. Adults that had apparently successfully emerged (i.e., not attached to pupal exuviae and normally developed), but found dead on the water surface, were considered to have successfully emerged. Adult insects which had successfully emerged but escaped (exuviae present but no adults), were considered to have successfully emerged. These were not included in the sex ratios, however. Males were distinguished from females by the presence of large plumose antennae, thinner and slightly upwardly curved abdomen, and visible genitalia. On the 37th day, all aquaria were drained through a sieve with 250 μm mesh and remaining larvae and pupae recovered and counted.

Average time to emergence was calculated using the equation shown below (Kosalwatt and Knight 1987):

$$T = \sum \frac{(DxN)}{(N)}$$

Where: T = the average time to emergence (days)

D = number of days from hatching to emergence (age in days)

N = number of insects emerged at that age.

5.2.2. Survival and growth test

The same sediments as in the emergence tests above were used. In addition, shredded paper towel and another treatment with no substrate were included as controls.

On the day before starting the test, fifteen 300 mL beakers were set up to each contain 100 mL of the appropriate sediment with three replicates for each substrate type. Another set of three beakers contained no substrate. Water was provided through a water renewal system as described by Zumwalt et al. (1994) with complete renewal of test water 3 times daily.

At the initiation of the test, 10 second instar larvae (8 to 10 day post-hatch) of *C. zealandicus* were carefully transferred into the test beakers which were then covered by 40 μm mesh net secured by rubber bands. The same set up was used to test *C.* sp. a

Non-biological parameters were measured at the beginning and at the end of the experiment as in the emergence test.

Finely ground TetraMin (0.02 g) was added to each beaker on the day before starting the test. Feeding was continued at 0.01g per beaker on alternate days until the end of the experiment.

On the 14th day of the test the contents of each beaker were drained through a sieve with 250 µm mesh, and the number of surviving larvae in each beaker was counted.

Analysis of sediment

Subsamples of the sediment from each site were analysed for a selection of heavy metals, total organic matter, and particle size.

Data analysis

The total number of adults emerged, time to the first adult emergence, average time to emergence, sex ratio, survival and growth were analyzed using ANOVA. Post-tests were performed by Tukey - Kramer multiple comparisons using Instat 2.01.

5.3. RESULTS

Total arsenic, copper, and chromium concentrations, particle size, and organic matter for each of the four lake site sediments used in the emergence test are given in Table 5.1. Concentrations of total As, Cu, and Cr were relatively higher in Hamilton Lake sediment than in other lake sediments. Total organic matter was higher in sediments from Lake Ngaroto and Hamilton Lake than in Lake Rotorua sites.

Table 5.1. Total arsenic, copper and chromium, particle size and organic matter of four lake site sediments used in the emergence test.

	Site			
	Hamilton	Ngaroto	Hamurana	Sulphur Point
As (mg/kg)	11.00	7.20	10.10	8.40
Cu (mg/kg)	17.80	8.40	0.20	1.90
Cr (mg/kg)	33.90	13.00	0.04	2.07
Clay (0.050 μm - 2.00 μm)	3.5	3.3	0	0
Silt (2.00 μm - 63.00 μm)	34.4	47.9	2.0	8.9
Fine Sand (63.00 μm - 250.00 μm)	45.8	32.6	13.0	24.9
Coarse sand (250 μm - 1000 μm)	16.6	15.6	65.0	61.8
Very coarse sand (1000 μm - 2000 μm)	0	0	17.6	4.5
Total Organic Matter (%)	10.6	14.9	1.9	2.55

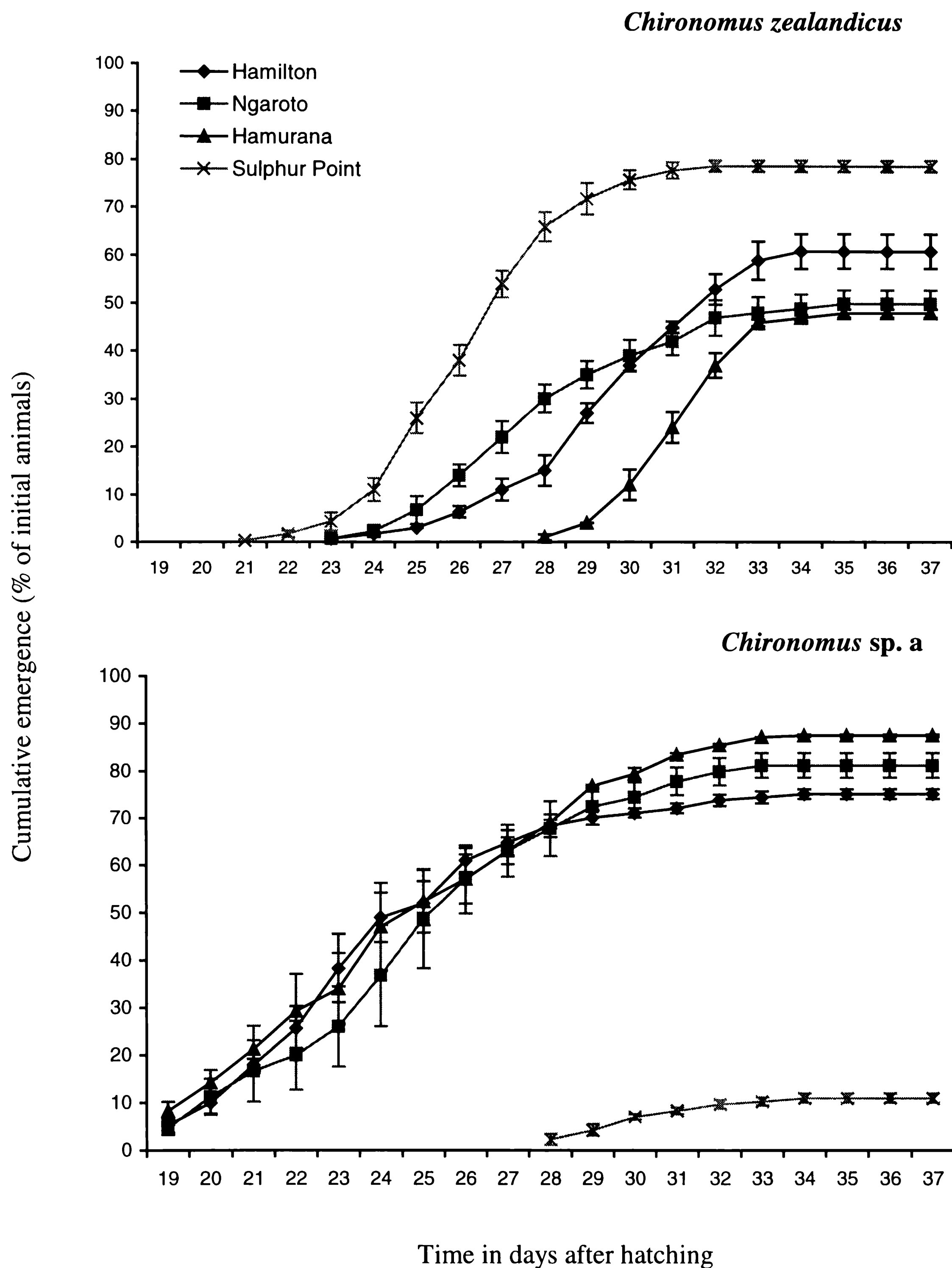


Fig 5.1. Cumulative emergence of *Chironomus zealandicus* and *Chironomus* sp. a on four different sediments given in the legend. Each point is a mean of three replicated tests ± SE.

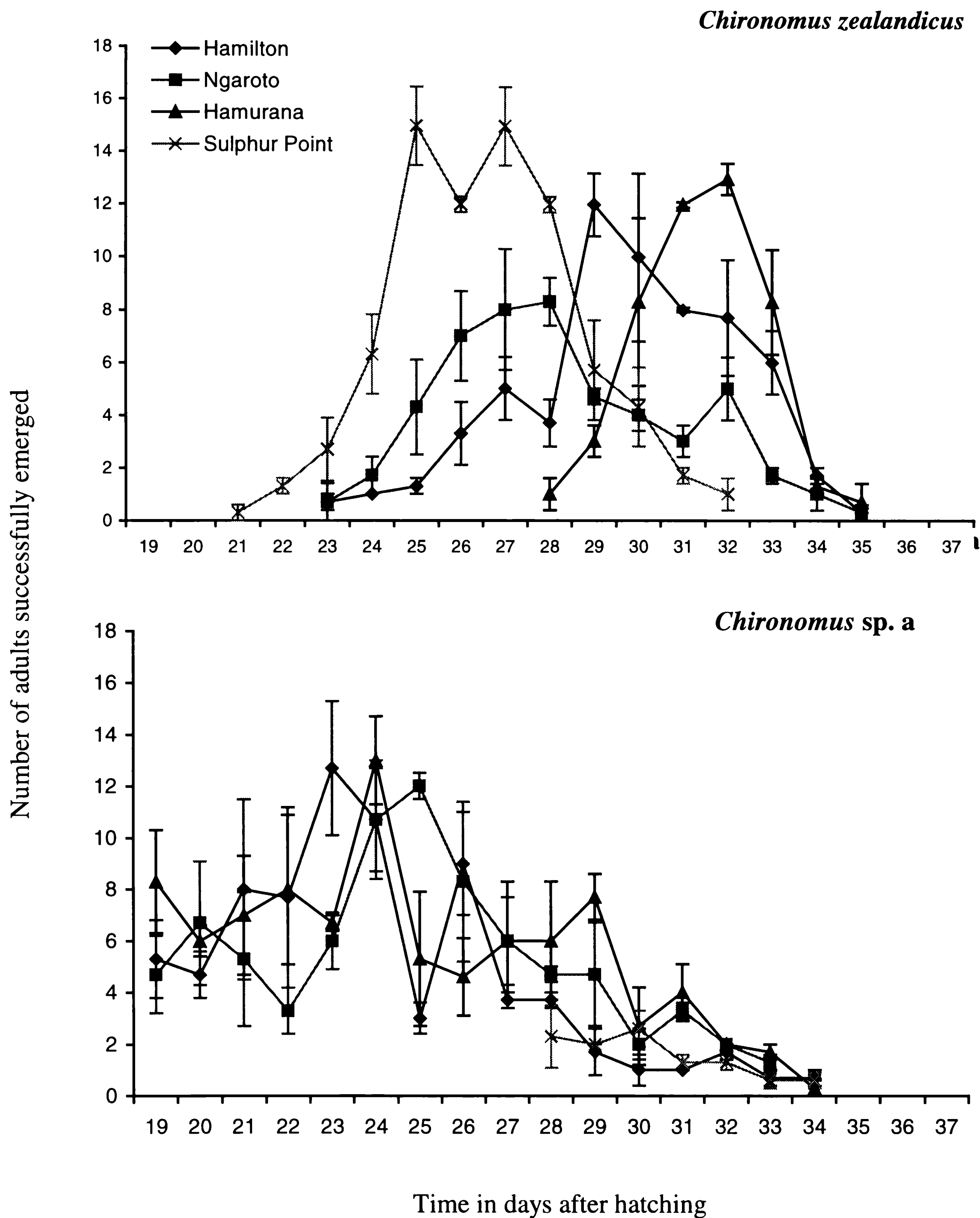


Fig 5.2. Emergence pattern of *Chironomus zealandicus* and *Chironomus* sp. a on four different sediments. Each point is a mean of three replicated tests ± SE.

Table 5.2. Physical and chemical parameters measured in the test aquaria and at the sampling sites.

	Hamilton		Ngaroto		Hamurana		Sulphur Point	
	Aquarium	Site	Aquarium	Site	Aquarium	Site	Aquarium	Site
Dissolved Oxygen (mg/L)	8.28	5.8 - 10.3 (8.2)	8.3	5.5 - 10.2 (7.3)	8.14	9.0 - 12.8 (10.7)	7.93	1.1 - 9.63 (3.8)
Conductivity (μS/cm)	277.6	99 - 115 (100)	284	123 - 160 (140)	323	108 - 189 (145)	856	202 - 573 (406)
Temperature °C)	20	9.2 - 23.3 (16.2)	20	9.4 - 24.2 (16.5)	20	10.8 - 22.9 (15.2)	20	11.7 - 24.9 (17.7)
pH	6.65	6.5 - 7.4 (6.8)	6.33	5.2 - 6.9 (6.2)	6.83	6.5 - 7.9 (7.2)	3.04	2.9 - 6.5 (4.2)
Hardness (as mg/L $CaCO_3$)	42.3	32.1	42.0	39.4	42.4	21.7	44.6	13.5

Values for the sites are ranges and mean values (in parentheses) of the monthly observations from June 1996 to May 1997. Values for the aquaria are mean values of nine observations.

Temperature, dissolved oxygen, conductivity, pH and hardness of water measured in the emergence test aquaria and at the lake sites are given in Table 5.2. Mean values of conductivity and hardness of water were relatively higher in test aquaria containing lake sediments than at the lake sites. Among the other three parameters, dissolved oxygen and temperature were optimised in the test aquaria by aeration and aquarium heaters respectively. Even though the pH was not controlled in the test aquaria, major differences were not observed between values recorded in test aquaria and the respective lake sites.

Results of the emergence experiment are summarized in Table 5.3. The type of sediment clearly affected both survival and development of both species. Survival of *Chironomus zealandicus* was significantly better on Sulphur Point sediment and worst in sediment from Lake Ngaroto. Conversely, *C.* sp.a fared poorly in Sulphur Point sediment but coped well with sediment from the other three sites, although survival was significantly lower in sediment from Hamilton Lake. This species survived best in the coarse sand substrate from the Hamurana Stream site.

Table 5.3. The emergence data for *C. zealandicus and C.* sp. a larvae reared on sediments from four lake sites. Each value is a mean of three replicates with standard error of the mean within parentheses. Mean values were compared using one way ANOVA (P<0.05).

	Chironomus zealandicus				*Chironomus* sp. a			
	Hamilton	Ngaroto	Hamurana	Sulphur Point	Hamilton	Ngaroto	Hamurana	Sulphur Point
Percent of animals successfully emerged as adults	61 [d] (4)	50 [d] (3)	48 a [d] (1)	79 (1)	75 [bc] (1)	81 (3)	87 (0)	11 [a b c] (0)
Percent recovered as pupae at the end of the experiment	3.0 (0.6)	0.0 [d] (0.0)	2.0 [d] (1.1)	6.0 (1.0)	0.0 [b] (0.0)	3.0 (0.6)	2.0 (1.2)	0.0 [b] (0.0)
Percent recovered as larvae at the end of the experiment	11.0 [c] (1.0)	9.0 [c] (0.9)	19 (0.6)	9.0 [c] (0.9)	3.0 (0.6)	9.0 (2.6)	7.0 (2.1)	1.0 (1.0)
Total percent survived at the end of the experiment (adults+pupae+larvae)	75.0 [d] (2.9)	59.0 [a d] (3.6)	69.0 [d] (1.3)	94.0 (2.5)	78.0 [bc] (1.5)	93.0 (3.5)	96.0 (1.4)	11.0 [a b c] (0.9)
Percent mortality during emergence[(1)]	3.0 (1.7)	2.0 (1.0)	3.0 (0.6)	1.0 (1.0)	1.0 [d] (0.5)	0.0 [d] (0.0)	0.0 [d] (0.0)	3.0 (0.6)
Percent mortality during larval stages[(2)]	22.0 [b] (4.0)	39.0 (4.3)	28.0 (0.8)	5.0 [a b c] (5.0)	21.0 [d] (11.1)	7.0 [a d] (3.5)	4.0 [a d] (1.4)	85.0 (1.45)
Day first adult emerged after hatching	23.0 [c] (0.6)	24.0 [c] (0.6)	28.0 (0.6)	22.0 [c] (0.6)	19.0 [d] (0.0)	19.0 [d] (0.0)	19.0 [d] (0.0)	29.0 (0.3)
Average time in days to emergence after hatching	31.0 (2.0)	30.0 (3.0)	31.0 (0.5)	27.0 (0.4)	24.0 [d] (0.3)	25.0 (0.7)	25.0 (0.6)	33.0 (3.5)
Sex ratio (Male: Female)	1.06 (0.11)	1.01 (0.02)	0.98 (0.01)	1.07 (0.03)	1.01 (0.03)	0.98 [d] (0.07)	0.99 [d] (0.01)	1.20 (0.02)

(1) - Dead adults found on the water surface attached to the pupal exuviae
(2) - Larval mortalities inferred by subtraction
a - Significantly less than on Hamilton sediment
b - Significantly less than on Ngaroto sediment
c - Significantly less than on Hamurana sediment
d - Significantly less than on Sulphur Point sediment

For both species, these effects are mostly related to differences in larval survival on the different substrates. A substrate effect is also apparent in the rate of development with *Chironomus zealandicus* developing fastest in Sulphur Point sediment and significantly slower in sediment from Hamurana Stream as seen in the time to emergence of the first adult. Those few individuals of *C.* sp. a that survived in sediment from Sulphur Point developed very slowly compared with all other substrates. There is also an indication that the mortality of *C.* sp. a larvae in Sulphur Point sediment may be sex dependent since the sex ratio of imagines is significantly skewed towards males. However, this effect is relatively small.

Table 5.4. Summary of the results of survival experiments. Each value is an average of three replicates with standard error of mean in parentheses. Mean values were compared using one way ANOVA (P<0.05).

		Substrate used					
Species		Hamilton	Ngaroto	Hamurana	Sulphur Point	Papertowel	Water
Chironomus zealandicus	Percent survival	75.6 (9.6)	64.4 (7.4)	81.1 (9.0)	86.7 (3.0)	65.6 (4.1)	54.4[d] (4.1)
	Mean dry weight (mg)	1.12 (0.32)	1.02 (0.26)	1.03 (0.18)	1.01 (0.16)	0.28[a] (0.03)	0.32 (0.02)
Chironomus sp. a	Percent survival	84.1 (5.0)	95.6 (2.4)	80.0 (4.7)	83.3 (5.8)	87.8 (3.2)	60.0[abcdc] (5.3)
	Mean dry weight (mg)	0.85[c] (0.26)	1.07 (0.14)	1.63 (0.23)	1.03 (0.45)	0.62[c] (0.23)	0.28[bcd] (0.07)

[a] - Significantly less than on Hamilton sediment
[b] - Significantly less than on Ngaroto sediment
[c] - Significantly less than on Hamurana sediment
[d] - Significantly less than on Sulphur Point sediment

Results of the ten-day survival and growth tests using second instar larvae are presented in Table 5.4. There was no significant difference in the survival of either species in any of the substrates used although both species fared poorly in the absence of any substrate. Natural sediment from any of the field sites did not affect the growth of *C. zealandicus* larvae but *C.* sp. a grew best on Hamurana Stream sediment and poorest on sediment from Hamilton Lake. Neither species grew well on the paper towel substrate or in the absence of any substrate.

5.4. DISCUSSION

The substrate type influenced emergence of the chironomids tested in this experiment. In bioassay evaluations for acute toxicity, mortality is often measured by exposing organisms to high levels of substances over a short time, usually 96 hours or less. Although the information obtained from these evaluations is useful in determining the sensitivity of an organism to a toxicant, the results are difficult to interpret and even more difficult to extrapolate to field conditions (Kosalwatt and Knight 1987). The deleterious effects of many substances may not be evident for weeks, months, or longer. They may be influenced by changes in appetite, metabolism, morphology, growth, reproduction, development of sex products, maturation, hatching, survival of different life stages, deformities and behaviour or vital functions which do not result in early death. Changes in these functions are important as precursors of chronic toxicity and eventual reduction or elimination of the species (McKee and Wolf 1963). On the other hand, emergence is an endpoint that represents almost the entire life cycle of chironomids, particularly when a test is begun with eggs. Successful emergence of adult chironomids includes completion of many of the processes mentioned above including survival, growth, and development of larvae, which are the common endpoints in standard 10- or 14-day toxicity tests. Therefore the life cycle test provides a complementary method to the 10- and/or 14-day test, and is relatively straightforward to conduct, yet provides a comprehensive basis upon which sediment toxicity can be assessed (Benoit et al. 1997). Although the chemical contaminants of sediments affect many aspects such as survival, growth, and development, all these may eventually result

in the emergence of chironomids. Therefore, studying emergence may give an overall indication of sediment toxicity to chironomids. A life cycle test can be followed by other standard toxicity tests with contaminated sediment to identify the most vulnerable life stage of the test animal and specific effects on the organism.

In the life-cycle test begun using 1st instar larvae, survival, growth, and emergence of the test species have clearly been affected by the sediments used. Survival and emergence of *C. sp.* a were adversely affected by sediment from the Sulphur Point site whereas *C. zealandicus* clearly shows a preference for this substrate. In the 10-day tests that were begun with the early 2nd instar larvae, no difference was found in survival for either species between the substrates, except in the water-only control (Table 5.4). This test shows that these substrates have no effect on survival of larvae between the 2nd and 4th instar stages. Therefore, the 1st larval instars are the most vulnerable larval stage to sediment induced stress although sediment type did affect growth of older larvae. However, the actual factor that caused the stress to the test animals in the sediment can not be identified from this experiment alone. There may be one or more physical and/or chemical factors that could have stressed the test animals in the sediments.

Although there was no difference in the survival of the species on the different substrates, growth was significantly less on the paper towel substrate. Survival was lower in the water only treatment than in all other treatments for both species. Lack of substrate in the water only control treatment and lower organic content in the paper towel substrate compared with other treatments may be the probable cause for high larval mortality (McKinney 1995) and a relatively lower growth respectively in this experiment.

Mattingly et al. (1981) found that growth of a stream chironomid, *Stictochironomus annulicrus,* on a substrate with low organic content was less than that on two other substrates with higher organic contents. In the present study, the highest percentage of organic content was measured in the sediment from the Lake Ngaroto (14.9%) and the lowest in the sediment from Hamurana (1.87%). Contrary to Mattingly et al.'s (1981) results, no significant difference was observed in the growth of the two species tested between sediments from Ngaroto and Hamurana in this experiment (Table 5.4).

Liber et al. (1996) controlled the growth of *C. tentans* by altering feeding levels (from 0.2 to 5.9 mg dry weight Tetrafin fish food per day), and studied the effects of larval growth retardation on adult emergence and ovipositing success on silica sand. Mean 10-day survival was ≥ 88% at all feeding levels, but larval growth decreased significantly with each decrease in feeding level. Cumulative successful emergence of adult *C. tentans* decreased significantly with decreasing larval growth. Mean times to emergence always increased with decreasing growth rates and effects were generally more pronounced for females than males. They also observed that at the lowest 10-day mean growth where successful emergence occurred, the time to emergence doubled relative to the times observed at the highest 10 day mean growth. Ten-day larval growth retardation was strongly correlated with reduction in adult emergence success. Growth retardation of ≥ 64% resulted in 86-100% reductions in adult emergence. Adult *Paratanitarsus parthenogeneticus* emerged 18±1 days after oviposition when 1.4 mg to 2.8 mg of food was supplied. However, emergence was delayed by one day at 0.7 mg and by two to five days with 0.35 mg of food. Development of *Paratanitarsus parthenogeneticus* stopped at the 2nd and 3rd instar larval stage with 0.18 mg food. Successful laboratory cultures of test species of the present experiment were created and established on the paper towel substrate with the feeding rate varying according to the number and age of the larval instars. The optimum rate of feeding for this experiment was determined from the preliminary laboratory culture practices. Therefore, the influence of feeding on differences in emergence between sediments may not be significant.

Chemical contaminants are known to affect the bioenergetics of aquatic animals (Maltby 1992). Organisms stressed by contaminants for extended periods are likely to have less energy available for both growth and reproduction than unstressed organisms. Continued exposure to chemical stressors could therefore lead to severe population declines and even local extinction of the species. From this perspective, growth would appear to be a logical measurement in the assessment of sublethal sediment toxicity. Heavy metals are among the most common pollutants in many contaminated aquatic ecosystems. Several studies have shown that heavy metals affect many aspects of the life cycle of chironomids. Wentsel et al. (1977a, 1977b, 1978) observed an inverse relationship between growth of *C. tentans* larvae in laboratory tests and concentrations of heavy metals (Cu, Zn and Cr) in lake sediments. Growth of *C. tentans* was reduced

by 50-55% in contaminated sediment compared with a reference (clean) sediment and emergence success was reduced by 69% and delayed by 2 days in separate laboratory studies. The authors concluded that the reason for the large decline and the delay in emergence from the test sediment was that the sediment stressed and/or killed the chironomids.

Pascoe et al. (1989) found that, in addition to causing a delay in the development of 3rd and 4th larval instars, exposure to 0.15 mg/L Cd resulted in a delay in the emergence of adult *C. riparius*, and an overall reduction in the number of adults emerged, reflecting earlier 1st instar mortalities. Kosalwatt and Knight (1987) reported that the higher the copper concentration in food substrate, the slower the growth rate of the midges. There was a very low growth rate of only < 0.003 mg/day when the substrate copper concentration was higher than 3400 g/m^3. The authors concluded that copper in food substrate retarded midge growth.

This experiment does not clearly indicate whether the effect on emergence of two species of chironomid is due to the heavy metals (arsenic, copper and chromium) measured in sediments. The highest concentrations of arsenic, copper and chromium (11.0, 17.8 and 33.9 g/m^3) were found in sediments from Hamilton Lake (Table 5.1). However, none of the endpoints measured at the end of this experiment showed significant reduction in Hamilton sediment compared with other sediments (Table 5.3). Percentages of (total) chironomids that survived and successfully emerged as adults show that Lake Ngaroto and Hamurana Stream sediments were not favourable to *C. zealandicus*, whereas Sulphur Point and Hamilton Lake sediments adversely affected *C.* sp a. Results of the habitat surveys presented in Chapter 2 show that *C. zealandicus* is rare at Lake Ngaroto and Hamurana Stream while *C.* sp. a is not common at Hamilton Lake and absent from Sulphur Point. This implies that physico-chemical factors other than heavy metal content are responsible for the effects observed. Time for the first adult emergence and average time for emergence also did not show any direct relationship with the heavy metals measured in sediments

There may be several reasons for the lack of direct relationship between the three heavy metal concentrations and the emergence of chironomids. However, a probable cause for

this may be that a combined action of other chemicals present in the sediments may have affected the overall emergence of chironomids in the laboratory test or the bioavailability of the heavy metals present in sediments may not be the same in all sediment types. As well as arsenic, Hamilton Lake is known to contain several other metals such as Cd, Hg, Ni, Pb, Zn, Mn and Fe (Rajendram 1992). Two or more chemicals delivered together may alter the actions of each other. They may have a synergistic action, where overall toxicity is increased, or an antagonistic action, where overall toxicity is less than expected. Since the total organic matter is relatively higher in Hamilton Lake than in Hamurana Stream and Sulphur Point, it may bind with metal ions and, as a result, reduce the bioavailability of metals. This may be an explanation as to why Hamilton sediment is not more toxic than other sediments.

Various studies have reported the effects that physico-chemical parameters can have on growth, survival, and emergence of chironomids. However, tests in this study were conducted under controlled laboratory conditions in order to minimize the influence of factors other than sediment type.

Water hardness is one of the important factors that affects the toxicity of heavy metals. For example, Gauss et al. (1985) found that the concentrations of copper necessary to immobilise 50% of the first larval instars of *C. tentans* after 96 hours was significantly less in soft and medium (43 and 110 mg/L as $CaCO_3$ respectively) waters than in hard (172 mg/L as $CaCO_3$) water. This 96 hour EC_{50} was significantly lower in soft water than in medium and hard water for the fourth larval instars of *C. tentans*. However, water hardness did not vary greatly in the present experiment. Water hardness in the this experiment varied between 42.05 and 44.63 as mg/L $CaCO_3$ in all the test aquaria throughout the test (Table 5. 2). These values were approximately 13 to 70% higher than at the sampling sites (Chapter 2).

The start of insect emergence has been attributed to rising water and mud temperatures and photoperiod changes (Britain 1976). Therefore, both temperature and photoperiod may have been involved in the beginning of emergence and appear not to be separable. However, the relationship between elevated water temperatures and emergence has not been clearly elucidated (Coler and Kondratief 1989). Several studies have indicated that

a mildly elevated temperature had no or little effect on the emergence pattern of chironomids and other aquatic insects (Ferguson and Fox 1978), whereas other studies (Coutant 1967) showed that higher temperatures produce variations in the normal emergence patterns. However, temperature was maintained at 20°C in all the test aquaria throughout the test duration in the present study. This was 2 to 5°C above the average water temperatures at sites (Table 5.4) and was 2 to 5°C below the maximum temperatures reported during summer months at sampling sites.

There is evidence that many aquatic insects have a high short-term tolerance to dissolved oxygen concentrations of less than 2 mg/L. For example, Curry (1965) in his review pointed out that several chironomids are able to withstand dissolved oxygen concentrations as low as 1 mg/L. Insects continued to emerge from lower channel pools despite dissolved oxygen concentrations of 1 mg/L (Nordlie and Arthur 1981). Dissolved oxygen concentrations were well above these values and varied between 7.93 and 8.28 mg/L in test aquaria throughout this experiment (Table 5.4). Therefore, this would not have affected the emergence of test animals.

It is therefore unlikely that parameters such as water hardness, food, temperature, or dissolved oxygen would have caused the observed differences in emergence of the two species of chironomid in this experiment. However, the pH was relatively lower in aquaria with Sulphur Point substrate. This value corresponds to the pH values recorded at the Sulphur Point site, where *C. zealandicus* dominates the macroinvertebrate community (Chapter 2). A tolerance to low pH may possibly be one of the reasons that *C. zealandicus* emerged successfully in the Sulphur Point substrate under laboratory conditions whereas *C.* sp. a may be intolerant of low pH. However, further study is essential to examine whether pH is the major factor influencing both survival and emergence of the test species on these sediments.

One of the aspects that may influence the results of a life cycle test is the starting life stage of the test organism. There is evidence that the younger instars of chironomids are more sensitive to toxicants than the older instars (Robinson and Scott 1995). Many authors (Gauss et al. 1985; Kosalwatt and Knight 1987) observed that the most sensitive chironomid life stage to a wide range of toxicants is the 1st larval instar. It has been

shown that the 1st instar stage of *C. tentans* is the most sensitive to cadmium (Williams et al. 1986; Pascoe et al. 1989) and copper (Nebeker et al. 1984; Gauss et al. 1985). The 1st instar of *C. zealandicus, C.* sp. a and *P. pavidus* was the most sensitive stage to arsenic (Chapter 6), while 1st instar larvae of *C. zealandicus* was the stage most sensitive to cyromazine (Robinson and Scott 1995). In the present study, 1st instar larvae were used to study the effect of sediment type on emergence and early stages of the 2nd instar larvae were used to assess the effect of sediments on survival and growth. Results of these tests clearly demonstrate that the 1st instars of both species are the most sensitive stage of the life cycle to sediment induced stress.

In summary, substrate type influences both survival, growth, and emergence of *C. zealandicus*, and *C.* sp. a. Sediments from Lake Ngaroto and Hamurana Stream appear to favour *C.* sp. a while Sulphur Point sediment favours *C. zealandicus*. This parallels the natural abundance of these species at these sites. However, the larvae used in this experiment were obtained from established laboratory cultures. The separate cultures of *C.* sp. a and *C. zealandicus* were created from parent stocks collected from Lake Ngaroto and Sulphur Point respectively. It is therefore not clear from this study whether these two species generally have a natural preference for these sediment types or whether they simply reflect genetically determined preferences of the parent stocks. A single sediment property that affects emergence could not be identified from this experiment. However, one or more of the chemical (such as contaminant, total organic carbon) or physical (such as particle size or pH) factors of the sediment could be a probable cause. Further study of these two test species under various laboratory conditions (e.g., temperature, pH) or by using mesocosms may lead to a more definitive conclusion on the effect of substrate on chironomid emergence.

Acute toxicity of arsenic and copper to *Chironomus zealandicus, Chironomus* sp. a and *Polypedilum pavidus*

6.1. INTRODUCTION

Acute toxicity tests are important in establishing appropriate water quality criteria and standards. Most toxicity studies were carried out with fish species (EIFAC 1977; Taylor 1977) until it was realised that water quality criteria established for the protection of fish may be inadequate to protect many invertebrates. Consequently, toxicity tests with invertebrates are now generally included in the hazard evaluation process (Williams et al. 1985, 1986).

It is important that the most sensitive life stage should be used in toxicity tests to properly estimate the sensitivity of the test animal and to make toxicity tests ecologically relevant (Williams et al. 1986). Although different species of chironomid have frequently been used in acute toxicity tests, these tests have evaluated the sensitivity of only one stage of the life cycle, usually the 4th instar larvae. Data available for crustaceans (Hubüschman 1967), molluscs (Wurtz 1962; Rehwoldt et al. 1973) and insects (Sanders and Cope 1968; Clubb et al. 1975; Maki et al. 1975; Gauss et al. 1985; Williams et al. 1986) however, suggest that earlier life stages are more sensitive than later ones. For example, Gauss et al. (1985) found that in acute toxicity tests, 4th instar larvae of *C. tentans* were 12 - 27 times more resistant to copper than 1st instar larvae. If this pattern is representative of sensitivity to other chemicals, then the use of 4th instar larvae in toxicity tests may underestimate the impact of the toxicant on the species (Gauss et al. 1985).

The results of Chapter 5 clearly indicated that the substrate type influenced the emergence of *Chironomus* sp. a and *Chironomus zealandicus*. Measured sediment As, Cu, and Cr concentrations were relatively higher in Hamilton Lake than in Sulphur Point sediment. The total survival and emergence of *C. zealandicus* were relatively

higher in Sulphur point sediment while those of *C.* sp. were higher in sediments of the other three sites. However, it was not known from the results of Chapter 5 whether the test species have differing sensitivities for the heavy metals measured. The present experiment determines the sensitivity of these chironomids to As and Cu in acute toxicity tests.

Arsenic enters the aquatic environment from a variety of natural and industrial sources and is generally regarded as highly toxic to all components of aquatic communities. In general, inorganic arsenicals are more toxic than organo arsenicals to aquatic biota. Trivalent (As^{3+}), and pentavalent (As^{5+}) arsenic are known to be the second and third most toxic forms of arsenic respectively, while arsine (AsH_3) is the most toxic of all the forms (Aggett 1988). Toxic and other effects of arsenicals on aquatic life are significantly modified by numerous biotic and abiotic factors (Woolson 1975; NAS 1977; NRCC 1978; Howard et al. 1984).

In New Zealand, dominant sources of As input into waterways are geothermal activity, herbicides and timber preservation. Aggett (1988) studied the arsenic cycle in Waikato lakes (central North Island, New Zealand), and noted that As^{3+} and As^{5+} were the predominant forms. Total As concentrations of 0.005 - 0.095 g/m^3 have been found for waters along the Waikato River due to the release of arsenic from hot springs high in arsenic (Aggett 1988). Another source of input is herbicides containing arsenic as an active ingredient.

Copper is a soft heavy metal and occurs naturally as sulphides and oxides and occasionally as metallic copper. Solutions of these natural copper deposits result in background levels of copper in natural surface waters. Higher concentrations of copper are usually due to anthropogenic sources. These sources include corrosion of brass and copper pipes by acidic waters; industrial effluents and fallout; sewage treatment plant effluents; and the use of copper compounds as aquatic algaecides. However, the major industrial sources include the smelting and refining industries, copper wire mills, coal burning industries, and iron and steel producing industries.

Relatively little work has been carried out on the toxicity of arsenic to freshwater macroinvertebrates including chironomids. Khangarot and Ray (1989) tested the sensitivity of *C. tentans* larvae to 10 heavy metals in relation to the effects of starvation of larvae and their possible development into the succeeding instar. They found that As was the 4th most toxic of the 10 heavy metals tested for a 48 hour EC_{50}, while Ag, Hg, and Cu were the three more toxic metals in decreasing order. McKinney (1995) studied the toxicity of two forms of arsenic (As^{3+} and As^{5+}) to *C. zealandicus* 4th instar larvae collected from two lakes in the Waikato region. The author found that the acute toxicity of As to *C. zealandicus* was dependent on the valency state of arsenic and the location from which larvae were collected. However, McKinney (1995) did not investigate the toxicity of As with differing physico-chemical parameters such as water temperature, pH, Eh, phosphate concentration and duration of exposure which may affect the LC_{50} values.

The objective of the present experiment was to study how test species' sensitivity varies with other biological and non-biological parameters such as differing age, season, temperature and population, in addition to the determination of the LC_{50} values of As and Cu. Three sets of experiments were carried out with each species to test the toxicity of arsenic. Each larval instar from the laboratory cultures and 4th larval instars collected from the wild were exposed to arsenic at 18°C in the first set of experiments. Wild-collected 4th instar larvae of three species were tested for sensitivity to arsenic at three different laboratory temperatures, 13°C, 18°C, and 23°C in the second set. The difference in sensitivity of the 4th instars of each species collected in two seasons, summer (November 1996), and winter (June 1997) was compared in the third set of experiments.

Copper toxicity tests were carried out in two sets. The toxicity of copper to *C. zealandicus* was determined at three temperatures 13°C, 18°C, and 23°C in the first set. The sensitivity of *C. zealandicus* and *C* sp. a to copper was compared at 18°C in the second set of experiments.

Specific aims of this experiment were to

1). determine the sensitivity of three species of chironomids to arsenic.

2). determine the sensitivity of different larval instars to arsenic.

3). determine whether the chemical species or valency status influenced the toxicity of arsenic.

4). determine whether the sensitivity of a test species changes with season of the year when it is collected.

5). determine whether there is any difference in sensitivity to arsenic between laboratory cultured and wild-collected larvae of the same age.

6). evaluate how temperature affects the toxicity of arsenic and copper to test species.

6.2. METHODS

First, 2nd and 3rd larval instars were obtained from the cultures established in the laboratory. Fourth larval instars were obtained from either laboratory cultures or the field. For the collection of wild 4th instar larvae from the study sites in this experiment, Lake Ngaroto was chosen for *C.* sp. a and Sulphur Point and Hamurana Stream were chosen for *C. zealandicus* and *P. pavidus* respectively, because each species was relatively more abundant at these respective sites. Methods of collection and culture of test species were described in chapters 2 and 3 respectively).

6.2.1. Handling and acclimation

Required larval stages were obtained either from culture tanks or from stocking tanks that were maintained at 18°C, 24 hours before starting the test. This was done by sieving substrates in 20 L buckets containing water. A mesh size of 40 μm was used to sieve 1st, 2nd, and 3rd larval instars, and 250μm mesh was used to sieve 4th larval instars. Test animals were carefully transferred with a paintbrush (4th instar) or a Pasteur pipette (earlier instars) into beakers containing soft synthetic water at the test temperature and acclimatised for 24 hours before the test initiation. Beakers were gently aerated during the acclimation period.

6.2.2. Test media and parameters

Stock solutions of As^{3+} and As^{5+} were prepared from reagent grade sodium arsenite ($NaAsO_2$) and sodium arsenate ($Na_2HAsO_4.7H_2O$) respectively, and Cu^{2+} stock solution was prepared from reagent grade copper sulphate ($CuSO_4.2H_2O$). A final range of concentrations for test solutions was determined by conducting preliminary range-finding tests with each instar of each species for As^{3+}, As^{5+}, and Cu^{2+} separately. Test concentrations were prepared by diluting appropriate aliquots of stock solutions with soft synthetic water before larvae were exposed. All glassware was cleaned by washing in 20% laboratory grade nitric acid and then rinsing in hot water and MILLI-Q water at least five times to remove any trace of acid.

A series of five concentrations of arsenic or copper and a soft synthetic water control were run during this study. Beakers (150 mL) with 100 mL of test solution were used for 3rd and 4th larval instars and 25 mL beakers with 20 mL of test solution were used for 1st and 2nd larval instars in each test. Five acclimated larvae were exposed to each arsenic or control solution with three replicates per test. Each test was run over 96 hours except tests with 1st instars. Tests with 1st instar larvae were terminated after 72 hours since these animals metamorphose within 4 to 5 days of hatching. Test conditions were static without replacement of the test solution. Each test was replicated 3 times. Mortality was defined as lack of movement of the body, anal papillae, or mouthparts when subjected to a rapid stream of water from a Pasteur pipette or gentle probing (Gauss et al. 1985).

Water quality parameters such as dissolved oxygen, salinity, conductivity and pH were measured at the beginning and end of each test by standard methods described in Chapter 2. Concentrations of arsenic and copper in the stock solutions and randomly selected test solutions were determined at the end of testing by either flame or graphite furnace atomic absorption spectroscopy. Each set of tests was carried out in a temperature-controlled system with a photoperiod of 16:8 (L:D). Test chambers were not aerated and no food was given during the experiments.

6.2.3. Data analysis

A test was considered to be valid only if control survival was greater than 90%. Four methods for estimating the Median Lethal Concentration were used as recommended by the USEPA (1991) depending on the number of partial mortalities.

1. Graphical Method:

The graphical method is used to determine the LC_{50} when there is no partial mortality and the observed percent mortalities bracket 50%.

2. Spearman-Karber Method:

The Spearman-Karber method is used when one or more partial mortality occurrs and the mortality is zero at the lowest concentration and 100% at the highest concentration. The Spearman-Karber method is used when the requirements for the probit method are not met.

3. The trimmed Spearman-Karber Method:

The trimmed Spearman-Karber method is used when there is one or more partial mortality and the mortality is not zero at the lowest concentration and 100% at the highest concentration. The trimmed Spearman-Karber method is used when the requirements for the probit and Spearman-Karber methods are not met.

4. Probit Method:

The Probit method is used when there are two or more partial mortalities and observed mortalities bracket 50%.

LC_{50} values in the present study were determined by one of above methods depending on the number of partial mortalities in each test. Each replicate in these experiments was treated as an independent test. The differences between mean LC_{50} values were analysed by ANOVA followed by Tukey-Kramer pairwise comparison. Significances were determined at the 95% level ($p < 0.05$).

6.3. RESULTS

Control survival in all valid tests at 18°C is given in Table 6.1. Survival did not decline appreciably during the duration of the tests. Survival was relatively higher for *C. zealandicus* than the other two species and older instars of each species survived better than younger instars.

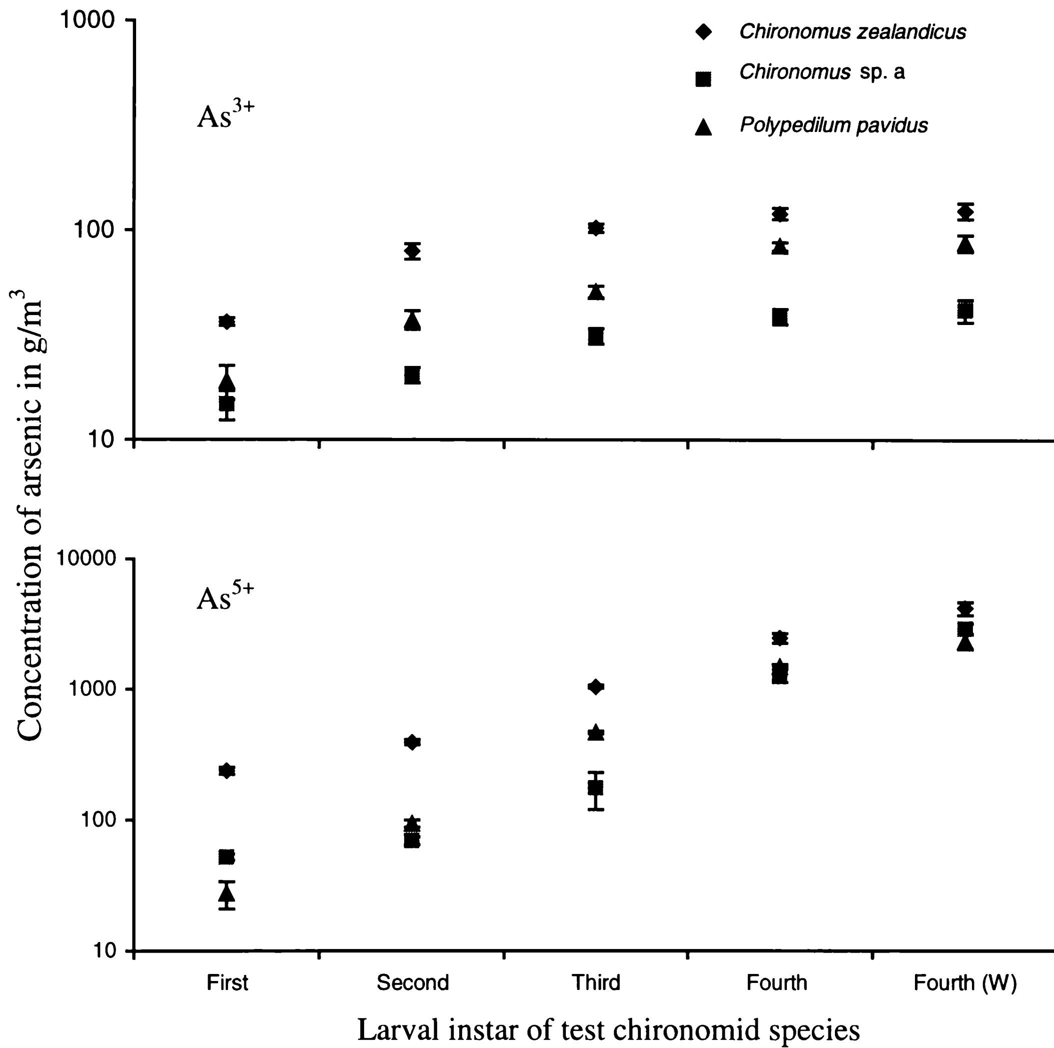
6.3.1. Acute toxicity of arsenic

Significant differences in sensitivity to arsenic were recorded between larval instars, and between chironomid species. *C. zealandicus* was more tolerant to both forms of As than the other two species (Tables 6.2, 6.3, 6.4). *C.* sp. a was the most sensitive species. The toxicity of arsenic increased with test duration. In general, 48 hour LC_{50} values for all three species increased with age. However, differences in LC_{50} values between instars were relatively less at 72 hours and were least at 96 hours. Trivalent arsenic was always more toxic than pentavalent arsenic to all three test species. The wild-collected 4th instar larvae were relatively more tolerant to As^{5+} than the laboratory cultured animals of the same age. This effect declined with test duration with the exception of *C. zealandicus*. Wild-collected 4th instar larvae were slightly more tolerant to As^{3+} after 48 hours but became more sensitive with time (Tables 6.2, 6.3, 6.4).

In general, the tolerance of larvae to both forms of arsenic increased with age with few exceptions (Fig. 6.1).

Table 6.1. Control survival (%) of larval instars of three species of chironomid used in the toxicity tests after 48, 72 and 96 hours. Each point is a mean of 18 replicates with % covariance in parentheses. (W - Collected in the wild.)

Test species	Duration (hours)	Larval instar				
		First	Second	Third	Fourth	Fourth (W)
Chironomus zealandicus	48	94.4 (9.8)	94.4 (9.8)	95.6 (9.0)	96.7 (8.0)	97.8 (6.7)
	72	93.3 (10.4)	94.4 (9.8)	95.6 (8.9)	96.7 (7.9)	97.8 (6.6)
	96	93.3 (10.4)	94.4 (9.8)	95.6 (8.9)	96.7 (7.9)	97.8 (6.6)
Chironomus sp. a	48	91.1 (11.2)	91.1 (11.2)	92.2 (10.9)	94.4 (9.8)	94.4 (9.8)
	72	91.1 (11.2)	91.1 (11.2)	92.2 (10.9)	94.4 (9.8)	94.4 (9.8)
	96	90 (11.4)	91.1 (11.2)	92.2 (10.9)	94.4 (9.8)	94.4 (9.8)
Polypedilum pavidus	48	93.3 (10.4)	95.6 (8.9)	95.6 (8.9)	96.7 (7.9)	97.8 (6.6)
	72	93.3 (10.4)	95.6 (8.9)	95.6 (8.9)	96.7 (7.9)	96.7 (7.9)
	96	93.3 (10.4)	95.6 (8.9)	95.6 (8.9)	96.7 (7.9)	95.6 (8.9)



W – collected in the wild

Fig. 6.1. Comparison of 48 hour LC_{50} values for As^{3+} and As^{5+} to four larval instars cultured in the laboratory and the fourth larval instars collected in the wild for three species of chironomid. Each point is a mean of 9 replicates ± SE.

Table 6.2. 48 hour LC_{50} values for As^{3+} and As^{3+} in g/m^3 to cultured 1st, 2nd, 3rd, 4th instars and wild-collected 4th instars of *Chironomus zealandicus, Chironomus* sp. a, and *Polypedilum pavidus* at 18°C. Each value is a mean of 9 replicates from three tests with standard errors in parentheses. (* - significantly greater than that of the previous instar (P<0.05); W - Collected in the wild)

	As^{3+}					As^{5+}				
Larval instar	First	Second	Third	Fourth	Fourth (W)	First	Second	Third	Fourth	Fourth (W)
C. zealandicus	36.7 (1.6)	79.4* (6.7)	103 (5)	122 (8)	116 (14)	239 (15)	395* (19)	1055* (27)	2490* (216)	4190* (472)
C. sp. a	14.7 (2.4)	20.4* (1.7)	31.3* (2.7)	39.1* (3.4)	42.1 (5.3)	52.1 (6.4)	69.3 (5.8)	176* (10)	1303* (72)	2925* (304)
P. pavidus	19.0 (3.5)	37.4* (3.8)	51.0* (3.3)	84.7* (3.7)	88.6 (8.1)	21.4 (4.6)	93.5* (4.6)	470* (56)	1492* (196)	2336* (266)

Table 6.3. 72 hour LC_{50} values for As^{3+} and As^{3+} in g/m^3 to cultured 1st, 2nd, 3rd, 4th instars and wild-collected 4th instars of *Chironomus zealandicus, Chironomus* sp. a, *and Polypedilum pavidus* at 18°C. Each value is a mean of 9 replicates from three tests with standard errors in parentheses. (* - significantly greater than that of the previous instar (P<0.05); W - Collected in the wild)

	As^{3+}					As^{5+}				
Larval instar	First	Second	Third	Fourth	Fourth (W)	First	Second	Third	Fourth	Fourth (W)
C. zealandicus	29.5 (2.1)	24.5 (3.8)	81.3* (5.7)	105 (10)	92.1 (5.4)	184 (17)	363* (14)	944* (23)	1707* (222)	4028* (253)
C. sp. a	12.7 (2.3)	16.6 (0.8)	19.6* (1.0)	18.0 (2.8)	18.3 (2.3)	41.7 (1.3)	49.2 (4.0)	128* (5)	735* (31)	1155* (162)
P. pavidus	6.2 (0.5)	22.2* (0.8)	30.4 (4.2)	38.7 (4.7)	30.4 (3.6)	31.1 (3.0)	73.6* (4.0)	267* (10)	1127* (133)	1191 (176)

Table 6.4. 96 hour LC_{50} values for As^{3+} and As^{3+} in g/m^3 to cultured 2nd, 3rd, 4th instars and wild-collected 4th instars of *Chironomus zealandicus, Chironomus* sp. a, *and Polypedilum pavidus* at 18°C. Each value is a mean of 9 replicates from three tests with standard errors in parentheses. (* - significantly greater than that of the previous instar ($P<0.05$); ** - significantly lower than that of the previous instar ($P<0.05$); W - Collected in the wild)

	As^{3+}				As^{5+}			
Larval instar	Second	Third	Fourth	Fourth (W)	Second	Third	Fourth	Fourth (W)
C. zealandicus	16.2 (1.2)	63.4* (2.5)	70.4 (5.4)	60.0 (10.0)	285 (10)	919* (17)	814 (63)	4176* (132)
C. sp. a	14.9 (0.8)	17.3* (0.5)	9.8** (0.5)	6.9 (0.3)	33 (1)	104* (3)	481* (28)	502 (35)
P. pavidus	19.7 (0.9)	20.9 (1.1)	26.6 (4.4)	13.6 (1.5)	47 (2)	235* (5)	700* (58)	608 (38)

Polypedilum pavidus shows greater variation in sensitivity than *C. zealandicus* and *C.* sp. a between 1st and 4th instar larvae (Table 6.5). Values of the ratios of median lethal concentrations of the laboratory cultured 4th instar larvae to the 1st instar larvae for As^{3+} at 48, 72 and 96 hours were smaller for *C. zealandicus*, and *C.* sp. a than for *P. pavidus*. These values for As^{5+} also showed the same pattern.

Effects of temperature on arsenic acute toxicity

No direct relationship between LC_{50} values and temperature was observed in the test designed to study the effect of temperature on arsenic toxicity (Tables 6.6, 6.7). Larvae of the test species were more sensitive at 23°C with few exceptions. *Chironomus zealandicus* was relatively more sensitive at 13°C, and 23°C, than at 18°C to both forms of arsenic. The same relationship was observed with *C.* sp. a and *P. pavidus* for As^{5+}. There were no significant differences in LC_{50} values for As^{3+} at 13°C and 18°C for *C.* sp. a and *P. pavidus*. However, these values were significantly lower than at 23°C.

Table 6.5. Ratio of LC_{50} values (As^{3+} and As^{5+}) of the 2nd, 3rd and 4th instar larvae to the 1st instar larvae and 4th (W) instar larvae to 4th instar larvae with 95% confidence limits within parentheses. Each point is a mean of 9 replicates. (W - Collected in the wild; CL - 95% confidence interval

Test duration		48 hours				72 hours				96 hours			
Ratio		2nd: 1st instar	3rd: 1st instar	4th: 1st instar	4th(W) :4th instar	2nd: 1st instar	3rd: 1st instar	4th: 1st instar	4th(W) :4th instar	2nd: 1st instar	3rd: 1st instar	4th: 1st instar	4th(W): 4th instar
C. zealandicus	As^{3+}	2.18 (0.40)	2.82 (0.17)	3.33 (0.19)	0.96 (0.27)	0.84 (0.30)	2.85 (0.72)	3.57 (0.33)	0.91 (0.21)	0.92 (0.18)	3.63 (0.57)	3.97 (0.21)	0.87 (0.33)
	As^{5+}	1.68 (0.27)	4.44 (0.38)	10.4 (0.5)	1.73 (0.48)	2.04 (0.42)	5.16 (0.51)	8.87 (0.56)	2.69 (0.85)	2.18 (0.43)	6.85 (0.62)	6.02 (0.21)	5.28 (0.97)
C. sp. a	As^{3+}	1.56 (0.59)	2.26 (0.63)	2.72 (0.49)	1.11 (0.35)	1.53 (0.61)	1.69 (0.51)	1.50 (0.47)	1.14 (0.47)	2.55 (0.36)	2.93 (0.12)	1.66 (0.07)	0.71 (0.09)
	As^{5+}	1.42 (0.44)	3.51 (0.75)	25.2 (2.2)	2.27 (0.52)	1.18 (0.19)	3.08 (0.26)	17.6 (0.6)	1.57 (0.38)	1.23 (0.22)	3.84 (0.58)	17.4 (1.0)	1.06 (0.20)
Polypedilum pavidus	As^{3+}	2.32 (1.02)	2.79 (0.58)	4.54 (0.68)	1.05 (0.20)	3.65 (0.64)	4.88 (0.42)	6.21 (0.38)	0.84 (0.29)	5.63 (0.98)	5.84 (0.23)	7.45 (0.46)	0.58 (0.24)
	As^{5+}	5.50 (2.56)	22.2 (2.7)	69.8 (1.7)	1.66 (0.55)	2.50 (0.67)	8.70 (0.96)	36.2 (1.4)	1.12 (0.44)	1.82 (0.37)	8.86 (0.98)	26.1 (0.7)	0.89 (0.19)

Effect of season on sensitivity to arsenic

From the third set of experiments with arsenic, clear differences were observed in 48 hour LC_{50} values for each species with differing seasons (Table 6.8). *Polypedilum pavidus* collected from the wild in summer, when the water temperature was higher (16°C), was significantly more sensitive than when collected in winter (11°C), to both forms of arsenic at the test temperature of 18°C. The reverse of this relationship was observed with *C. zealandicus,* although the difference was not significant with As^{5+}. No seasonal effect was observed for *C.* sp. a.

Table 6.6. 48, 72 and 96 hour LC_{50} values for As^{3+} in g/m^3 to *Chironomus zealandicus*, *Chironomus* sp. a, and *Polypedilum pavidus* at 13°C, 18°C, and 23°C (each value is a mean of 9 replicates with standard errors in parentheses).

Species	*Chironomus zealandicus*			*Chironomus* sp a			*Polypedilum pavidus*		
Duration	48 hour	72 hour	96 hour	48 hour	72 hour	96 hour	48 hour	72 hour	96 hour
Temperature									
13°C	42.9 (1.5)	32.9 (2.8)	27.9 (1.7)	64.6 (2.9)	44.6 (3.0)	33.7 (1.5)	142 (10)	72 (3)	42 (4)
18°C	58.6 (3.3)	34.5 (1.6)	29.2 (0.9)	54.8 (4.1)	25.1 (1.1)	20.4 (0.4)	146 (10)	125 (9)	76 (2)
23°C	40.9 (2.1)	33.4 (1.0)	26.2 (1.1)	27.1 (0.3)	21.7 (0.5)	16.0 (0.0)	90.6 (4.3)	46.6 (2.4)	24.8 (1.0)

Table 6.7. 48, 72 and 96 hour LC_{50} values for As^{5+} in g/m^3 to *Chironomus zealandicus*, *Chironomus* sp. a, *and Polypedilum pavidus* at 13°C, 18°C, and 23°C (each value is a mean of 9 replicates with standard errors in parentheses).

Species	*Chironomus zealandicus*			*Chironomus* sp. a			*Polypedilum pavidus*		
Duration	48 hour	72 hour	96 hour	48 hour	72 hour	96 hour	48 hour	72 hour	96 hour
Temperature									
13°C	3619 (170)	2262 (223)	696 (89)	1654 (177)	811 (100)	358 (28)	1904 (192)	906 (119)	394 (39)
18°C	5104 (450)	2456 (193)	1971 (265)	2241 (139)	709 (59)	454 (26)	3982 (299)	2251 (302)	1235 (172)
23°C	3519 (457)	1498 (173)	692 (102)	1069 (131)	639 (63)	436 (19)	1509 (148)	790 (78)	728 (73)

Table 6.8. 48 hour LC_{50} values of As^{3+} and As^{5+} in g/m^3 to *Chironomus zealandicus, Chironomus* sp. a, *and Polypedilum pavidus* collected from sampling sites in two seasons (each value is a mean of 9 replicates with standard errors in parentheses).

	As^{3+}		As^{5+}	
	November 1996	June 1997	November 1996	June 1997
C. zealandicus	116.0 (13.6)	58.6 (3.3)	4191 (472)	3619 (171)
C. sp. a	42.1 (5.2)	54.8 (4.1)	2925 (304)	2240 (139)
P. pavidus	88.6 (8.1)	145.6 (10.0)	2336 (266)	3981 (299)

6.3.2. Acute toxicity of copper

The sensitivity of *C.* sp. a to Cu was almost twice that of *C. zealandicus* at 18°C at 48 and 72 hours (Tables 6.9, 6.10). Sensitivities were not significantly different after 96 hours. The toxicity of copper to *C. zealandicus* increased with temperature. Copper was approximately 139 and 81 times more toxic at 23°C than at 13°C and 18°C respectively at 48 hours to *C. zealandicus* (Table 6.10).

Table 6.9. 48 hour LC_{50} values of Cu^{2+} in g/m^3 to *Chironomus zealandicus* and *Chironomus* sp. a. at 18°C (each value is a mean of 9 replicates with standard errors in parentheses).

	Test species	
Duration	*Chironomus zealandicus*	*Chironomus* sp. a
48 hour	96.3 (16.6)	45.6 (13.5)
72 hour	30.3 (12.7)	17.0 (7.9)
96 hour	3.10 (1.17)	3.30 (0.51)

Table 6.10. LC_{50} values of Cu^{2+} in g/m^3 to fourth instar larvae of *Chironomus zealandicus* (each value is a mean of 9 replicates with standard errors in parentheses).

	Test temperature		
Duration	13°C	18°C	23°C
48 hour	164 (40)	96.3 (16.6)	1.19 (0.14)
72 hour	84.3 (20.8)	30.3 (12.7)	0.63 (0.09)
96 hour	21.1 (11.0)	3.12 (1.17)	

6.4. DISCUSSION

6.4.1. Arsenic toxicity

The trivalent form of inorganic arsenic was significantly more toxic to all three species investigated with *C.* sp a being the most sensitive overall. Howard et al. (1984) pointed out that the primary mode of toxicity of inorganic trivalent arsenic (As^{3+}) is through reaction with sulfhydral groups of proteins and subsequent inhibition of enzymes such as xanthine oxydase, alpha-keto acid dehydrogenase, pyruvate oxydase and glycine reductase (Fowler, 1983). Inorganic pentavalent arsenic (As^{5+}) does not react as readily with sulfhydral groups, but may uncouple oxidative phosphorylation. Inorganic arsenite (As^{3+}) interrupts oxidative metabolic pathways and sometimes causes morphological changes in liver mitochondria in vertebrates (Belton et al. 1985). Arsenate (As^{5+}) may compete for and substitute for phosphate in some glycolytic pathways because it is isoelectric and isosteric with phosphate. The main reason that arsenate is toxic is that it forms unstable arsenical esters which cause instant hydrolysis or arsenolysis. This action uncouples the enzyme system into which arsenate has been incorporated.

One of the important factors that affects the sensitivity of a test animal to a toxicant is its age. Previous studies with *C. tentans* (Gauss et al. 1985) and *C. riparius* (Williams et al. 1985, 1986) showed that earlier larval instars were more sensitive than 4th instars to heavy metals. Fourth instars of *C. tentans* were 12 to 27 times more resistant to copper stress than were 1st instars (Gauss et al. 1985). Fourth instar larvae of *C. riparius* were 15.7 times more resistant at 10 hours and 925 times more resistant at 24 hours than 1st instar larvae to cadmium (Williams et al. 1986). Robinson and Scott (1995) observed that the acute toxicity of cyromazine, an insect growth regulator, to *C. zealandicus* was age dependent, and LC_{50} values varied from 100 – 400 g/m^3 for 2nd and 3rd instars to 1000-10000 g/m^3 for 4th instars. This relationship is supported by findings from the present study for all three species of New Zealand chironomids. Results of this study show that the laboratory cultured 4th instar larvae of *C. zealandicus, C.* sp a , and *P. pavidus* were 3.3, 2.6, and 4.4 times more resistant respectively to As^{3+} after 48 hours exposure than 1st instar larvae. Corresponding ratios for As^{5+} were 10.4, 25.0, and 54.4 respectively. This relationship did not change

appreciably with increasing test duration. This clearly indicates the importance of using early instars if possible in toxicity tests since one of the aims of such bioassays is to use the most sensitive life stages available. Although 1st instar larvae of each species were always the most sensitive stage, they were somewhat difficult to use for routine testing due to their small size and the difficulty of easily establishing their mortality. In almost all cases the difference between the sensitivities of the 1st and 2nd instars was around two-fold or less, so the larger more visible 2nd instars would be more suitable as routine test subjects.

There were differences in sensitivity between the wild-collected and laboratory-cultured populations of the test species. Wild collected 4th instars were slightly less sensitive to As^{5+} than the cultured animals for shorter test durations but became progressively more sensitive with time with the exception of *C. zealandicus*. This time dependent alteration in senstivity was also shown to As^{3+} and may reflect developing stress to laboratory conditions in the other two species. The most pronounced effect was shown by *P. pavidus* which seems to prefer lower temperature conditions and may have been stressed by acute acclimation to the laboratory test temperature. A high mortality rate of wild-collected larvae of all three species occurred due to stress by handling and the marked change in physical conditions. Larvae for this experiment had been collected from sites not more than 48 hours before the test initiation. Environmental and handling stress might have had some effects on the sensitivity of larvae to arsenic.

The differences in sensitivity between wild and cultured animals could also possibly be due to a variety of factors such as genetic variation or differences in the amount of haemoglobin that could affect toxicant sensitivity as outlined below.

Although cultures of each species were originally established from the same sites from which wild animals were collected for these tests, the cultures were established approximately 30 months before the test date. There is a possibility that the genetic structure of the laboratory population could have changed during this period. Furthermore, initial high mortality in wild-collected larvae may select for animals with altered sensitivity.

An increasing number of investigations on a variety of organisms have revealed that population level responses to environmental stressors may be genetically dependent (Woods et al. 1989; Guttman 1994; Duan et al. 1997). In those studies, certain genotypes were found to be more resistant than others to environmental stressors. The conditions of the laboratory environment may cause unanticipated alterations in the population's genetic structure such as changes in allele frequency, loss of alleles, and diminished heterozygosity. These changes could result from founder events, genetic drift, nonrandom mating, and artificial selection (Woods et al. 1989; Duan et al. 1997). Such effects will occur more quickly in animals with short life cycles and the 30 month culture period covers approximately 30 generations. Woods et al. (1989) investigated genetic variability in *C. tentans* from seven scattered laboratories and a population sampled directly from the wild, and found populations differed significantly in heterozygosity, both among laboratory strains and between laboratories and the wild strain.

Genetic differences among laboratory stocks, or between laboratory and natural populations, may be reflected in differential sensitivity to toxicant stress. Since different field populations will manifest somewhat different genetic profiles in response to local conditions, an exact match in toxicant sensitivity is unlikely and, even if possible, of limited utility. Woods et al. (1989) recommended that differing responses to toxicants under conditions of altered genetic integrity must be viewed as suspect until proven otherwise.

However, the fact that this relationship between the sensitivity of wild and cultured animals is common to all three species implies that some causative agent other than random genetic drift may be responsible. As discussed earlier, arsenic is known to bind with proteins and thereby alter the metabolism of animals. Chironomid larvae are distinctly red due to high levels of the oxygen carrying pigment haemoglobin. If there is a variation in the amount of haemoglobin between wild-collected and laboratory-cultured populations of each species, there may be a variation in available binding sites for arsenic. This could have been one reason for the lower sensitivity of wild populations to arsenic.

The characteristic red colour of the laboratory-cultured chironomid larvae was observed to be pale when compared with the wild-collected larvae in this study. This may be an indication of less haemoglobin in the laboratory-cultured animals. If this is true, it may be due to physiological adaptation to lower dissolved oxygen in lake sediments while regular aeration provides sufficient oxygen in the more open paper towel substrate of the laboratory culture tanks. Differences in the haemoglobin content of chironomid populations may either positively or negatively affect the toxicity of arsenic. When haemoglobin binds with bioavailable arsenic, it may act as a sink for arsenic and prevent it from binding with other essential enzymes or hormones. In this case, high haemoglobin content may reduce arsenic toxicity. However, this may adversely affect overall oxygen carrying capacity. The effect of arsenic on invertebrate haemoglobin oxygen binding therefore requires further study. There may also be variation in the amount of haemoglobin present in different larval instars that may affect the sensitivity of those instars to arsenic.

The present study investigated neither genetic properties nor haemoglobin content of test organisms. Future work should study the genetics of laboratory and field populations of test species to determine whether genetic differences could be a reason for the varying sensitivity to the same chemical in the toxicity tests. Likewise, the relationship between haemoglobin content and sensitivity to arsenic could also be a valuable subject of further research.

The declining LC_{50} values with increasing test duration may be due to progressive toxicant uptake or to starvation. Neither feeding nor water renewal was made over the 96 hours of the experiment. Nutritional factors are generally not regulated and often overlooked, particularly in water only toxicity tests. However, diet is well known to alter the toxicity of many chemicals (Mehrle et al. 1977; Dixon and Hilton 1985). Feeding may increase or decrease a toxicant's effects. Feeding obviously increases the levels of chemical contaminant taken up and its metabolism (Jobling 1981), therefore toxicity may be increased. However, a decrease in copper toxicity to a copepod, *Acartia tonsa*, with feeding was recorded by Sosnowski et al. (1979). Thus the effect of feeding during toxicity tests is not always known and may modify the results significantly.

However, fasting is commonly recommended in short term toxicity tests (ASTM 1990) and it was decided not to feed test animals during the present experiment.

Experimental conditions are also well known to alter the sensitivity of chironomids to heavy metals. Gauss et al. (1985) reported that the sensitivity of *C. tentans* decreased with increasing water hardness and other factors are known to affect the toxicity of arsenic. Studies with radioarsenic in mussels (*Mytilus galloprovincialis*) showed that accumulation varied with nominal arsenic concentration, tissue, age of the mussel, and temperature and salinity of the medium (Unlu and Fowler 1979). Ronald (1994) pointed out that although the response was not linear, arsenic uptake increased with increasing arsenic concentration in the medium, but the accumulation was suppressed at higher external arsenic concentrations. Smaller mussels took up more arsenic than larger ones. Uptake was higher at 1.95% salinity than at 3.8%, but the loss rate was about the same at both salinities (Ronald 1994). Williams et al. (1986) found that a flow-through system of water renewal was less stressful than the static renewal method. In the present study, the volume of the test solution in each test beaker was monitored regularly for evaporative loss and its level was topped up with soft synthetic water when required. However, no great loss of test solution was recorded from the beakers at 13 and 18°C, and only a small proportion of the original test volume had to be replaced with soft synthetic water at 23°C. In addition, no significant change in water quality parameters was recorded during the tests.

The temperature effect on metal toxicity appears contradictory (Wang 1987). Generally, temperature and toxicity are positively correlated for most chemicals (Mayer and Ellersieck 1986; Ronald 1994). It is apparent that the temperature-mediated metabolic rate has an important role in metal toxicity. Cairns et al. (1975) explained that the reason for this positive correlation was the exposure of test animals to a greater amount of toxicant as the temperature increases. These authors explained that increasing diffusion or active uptake because of increased temperature induces greater increases in rates of water and solute movement across gills or other cell membranes. It was observed that temperature was one of the physico-chemical parameters that considerably changed throughout the year at the sampling sites during this study (Chapter 2). Results of present tests carried out with As at different temperatures did not agree with this

general trend of increasing toxicity with temperature. All species examined in this study were more tolerant at 18°C than at either 13°C or 23°C. The effect of temperature was more pronounced for As^{5+} than As^{3+} to *C. zealandicus* and *P. pavidus.*

The other factor that influenced the toxicity of arsenic in this study was seasonal variation. Seasonal cycle alone has been observed to cause variation in toxicity by several other workers. For example, Storch (1977) used a natural community of Lake Erie phytoplankton to determine the effects of Fe^{3+}, and found that Fe^{3+} might either stimulate or inhibit algal photosynthesis depending on the concentration and the time of the year. In another study, Erickson (1972) found that Cu inhibition on the growth of *Thallassiosira pseudonana* in unenriched seawater samples varied with season and location of collection. The present study shows that *C. zealandicus* collected in November (1996), when the water temperature was 23°C, was more resistant than specimens collected in June (1997), when water temperature was only 14°C, particularly to As^{3+}. Conversely, *P. pavidus* collected in November (1996), when the water temperature was 16°C, was less resistant than those collected in June (1997) while water temperature was only 12°C. *Chironomus* sp. *a* did not exhibit such a great difference in sensitivity to arsenic with season. The water temperatures at the site of collection of *C.* sp. a were 18°C and 14°C in November (1996) and June (1997) respectively. A possible explanation for the response observed is that the optimum temperature might be species specific. *Chironomus zealandicus* appears to live in waters with elevated temperatures and has been reported to live in waters with temperatures as high as 34°C (Stark 1989). Temperature is relatively lower at Hamurana (Chapter 2), where *P. pavidus* dominates the benthic fauna. Therefore, the reason for differential sensitivity of test species collected in different seasons might be that *C. zealandicus* and *P. pavidus* collected when environmental temperatures were lower or higher respectively may not be as healthy. Animals collected when the temperatures were unfavourable might therefore be more vulnerable to arsenic stress. However, temperature and seasonal effects on the test species' sensitivity to arsenic have to be studied in detail under more controlled conditions before any definitive conclusions can be drawn.

6.4.2. Copper toxicity

Unlike arsenic, copper is one of the trace elements essential to the life of many plants and animals. The important function of copper is its participation in indispensable catalytic reactions (Kosalwatt and Knight 1987). This element is part of the active site of a number of enzymes (Sarkar 1981). In larger quantities, copper may be toxic and have deleterious effects.

Certain plants and animals, or certain tissues or organs accumulate abnormally high levels of copper. Such accumulation can be attributed to high levels of copper in the substrate or medium, in which the organism lives, or to unusual metabolic activity or to both. Accumulation of extremely high levels may be accompanied by pathological symptoms (Wieser 1979). Copper has been recorded to be relatively more toxic than arsenic to chironomids. Khangarot and Ray (1989) reported that As^{3+} and Cu^{2+} concentrations required to immobilise 50% percent of *C. tentans* (EC_{50}) at 48 hours were 0.68 and 0.327 g/m^3 respectively at an average temperature of 14°C. In another study, Gauss et al. (1985) found that the 96 hour EC_{50} of Cu^{2+} to *C. tentans* in soft, medium, and hard water were 0.211, 1.238, and 1.484 g/m^3 respectively at 21$\pm$1°C. Kosalwatt and Knight (1987) determined 48 and 72 hour LC_{50} values for Cu^{2+} to *C. decorus* in a water-only toxicity test, and substrate-spiked bioassay were 0.739 g/m^3 and 5830 g/m^3 respectively at 20 $\pm$ 0.02°C.

Compared with these values, LC_{50} values for *C. zealandicus* and *C.* sp. a are comparable. The 72 hour LC_{50} value for *C. decorus* (0.739 g/m^3 at 20°C) reported by Kosalwatt and Knight (1987) is similar to that for *C. zealandicus* (0.633 g/m^3 at 23°C) in the present study. As for arsenic, *Chironomus* sp. *a* was more sensitive to copper than *C. zealandicus*. A positive relationship was observed between temperature and copper toxicity for *C. zealandicus*. This agrees with the results of several previous works (Cairns et al. 1975; Mayer and Ellersieck 1986; Ronald 1994). It is important to note that most of the toxicity tests described above were carried out with laboratory-cultured larvae whereas toxicity tests with copper in this study were carried out using wild-collected larvae. The results of acute tests with arsenic showed differences in sensitivity between wild and cultured larvae.

Summary

Biological (age), chemical (valency state), and physical (season, temperature) factors affected the acute toxicity of arsenic and copper to the test chironomid species. Although two of the test species (*C. zealandicus* and *C* sp. a) in this study are very closely related, they showed considerable differences in sensitivity to arsenic and copper. It was observed that *C. zealandicus* was the most tolerant of the three species tested to both forms of arsenic and to copper at 18°C. Earlier instars were more sensitive than the later instars of each species to arsenic. All species were more tolerant to arsenic at 18°C than at higher or lower temperatures. However, the toxicity of copper to *C. zealandicus* increased with increasing temperature. There were also differences in sensitivity of the test species to arsenic with season although *Chironomus* sp. a showed relatively little variation in sensitivity with either temperature or season.

The use of 4th instar larvae in acute toxicity tests is a practice that may seriously underestimate the sensitivity of these species since it is the least sensitive life stage. Therefore this practice cannot be recommended for tests that are to be used in generating data for water quality criteria and standards. If one were to test only 4th instar larvae it could be concluded that these three species were relatively insensitive to acute arsenic stress. This study illustrates the importance of examining how toxicity of a substance varies with other biotic and abiotic factors. Investigation of as many life stages as possible rather than just the most easily available stage is essential in order to set accurate water quality standards for the protection of the aquatic environment. Routine use of 2nd instar larvae is recommended due to their greater acute sensitivity and the option of prolonging test duration to study chronic effects (Chapter 7). Differences in sensitivity between cultured and wild animals indicate that the effect of factors such as haemoglobin and genetic variation on As toxicity in chironomid species may be a valuable subject for further research.

Chronic sublethal toxicity of arsenic (As^{5+}) to *Chironomus zealandicus* and *Chironomus* sp. a

7.1. INTRODUCTION

Sediments provide habitat and act as a source of contaminants for many benthic and epibenthic organisms which are critical components of aquatic ecosystems. Sediments also act as a sink for many chemical substances that enter the aquatic environment. They often contain complex mixtures of contaminants at concentrations that are orders of magnitude greater than in the overlying water. Contaminants may be transferred from benthic invertebrates to higher trophic levels through the food chain. However, much of the information regarding effects of contaminants in aquatic systems is based on aqueous exposure alone.

An integrated sediment assessment evaluation using toxicity testing, measures of benthic community structure, and physicochemical characteristics is necessary for accurate evaluation of the degree of sediment contamination. Identification of cause-and-effect relationships for specific chemical contaminants requires further evaluation through the use of spiked sediment toxicity tests (Lamberson and Swartz 1992) or Toxicity Identification Evaluation (TIE) procedures (Ankley and Thomas 1992). Toxicity tests on sediments spiked with known concentrations of contaminants can be used to establish cause-and-effect relationships between chemicals and responses, but the behaviour of contaminants in spiked sediments cannot necessarily be equated with that in field-contaminated sediments.

The US Environmental Protection Agency has published standard methods for determining the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates (USEPA 1994a, 1994b).

Burton (1992) provided a comprehensive review of sediment toxicity test methods, their advantages and disadvantages, and considerations related to sampling and testing of sediments. The Assesment and Remediation of Contaminated Sediments (ARCS) Program (USEPA 1994a) evaluated 20 single-species and 5 community toxicity tests comprising a total of 55 endpoints. Species used in the tests included bacteria, algae, macrophytes, rotifers, cladocerans, chironomids, amphipods, mayflies, and fish. These species represent many of the major trophic groups in aquatic ecosystems.

Two of the test organisms used most frequently to evaluate toxicity of freshwater sediments are the amphipod, *Hyalella azteca,* and the midge, *Chironomus tentans* (Burton 1991; Burton and Scott 1992; Ingersoll et al. 1993). Both species are considered sensitive to toxic substances and standardized methods for conducting toxicity tests using these two species have been developed by the American Society for Testing and Materials (ASTM 1993). In addition, Environment Canada has developed draft methods for conducting toxicity tests using the two species. Following an evaluation of sediment toxicity studies in several parts of the United States, Burton and Scott (1992) concluded that tests using *H. azteca* and *C. tentans* were among the most efficient in assessing toxicity of whole sediments.

Although an adequate database exists for several promising test species that can be used in sediment toxicity testing, each species has its specific advantages and disadvantages as a test organism (USEPA 1994a). Sensitivity to a toxicant can be species-specific even within a family or subfamily and might vary with environmental testing conditions. It is widely accepted that as many test organisms as possible, and the most sensitive life stage of each organism (Williams et al. 1986), should be used in toxicity tests to properly estimate the toxicity of a compound and to make toxicity tests ecologically relevant.

Laboratory sediment toxicity tests generally include the use of a control sediment sample. Control sediment is sediment that is essentially free of contamination and is used routinely to assess the acceptability of a test, although control sediment is not necessarily collected near the site of concern (USEPA-USACOE 1991). The control sediment may consist of formulated components, such as clay, sand, and organic matter

(USEPA 1994a). The control sediment provides a measure of test acceptability, evidence of test organism health, and is one basis for interpreting data obtained from the test sediments.

The sediment toxicity test procedures range in complexity from short-term lethality tests that measure effects of individual contaminants on single species, to long-term tests that determine the effects of chemical mixtures on the structure and function of communities. The sediment phase tested may include whole sediment, suspended sediment, elutriates, or sediment extracts (Burton 1991; Lamberson et al. 1992).

Toxicity tests of sediment interstitial water were developed for evaluating the potential *in situ* effects of contaminated sediment on aquatic organisms (Ankley et al. 1991). For many benthic invertebrates, the toxicity and bioaccumulation of sediment-associated contaminants such as metals and nonionic organic contaminants have been correlated with concentrations of these chemicals in interstitial water (Di Toro et al. 1991). Interstitial water may be an important route of exposure for many infaunal benthic invertebrates in contaminated sediments. However, interstitial water might not be the relevant route of exposure for evaluations of organisms that ingest sediment.

Testing of the elutriate (water-extractable) fraction of the sediment is a commonly used technique. The elutriate test was developed for evaluating the potential short-term effects of open-water disposal of dredged material. Tests with elutriate samples measure the potential effects of the release of water-soluble constituents from sediment to the water column during the disposal of dredged material. The advantages of testing elutriates are similar to those for interstitial water because the test method is similar to water column testing and is easy to perform. Elutriate samples are generally less toxic than either whole-sediment or interstitial water samples (Ankley et al. 1991; Sasson-Brickson and Burton 1991).

Whole-sediment toxicity tests are the most appropriate for organisms that live directly in or on the sediments and ingest sediment particles (USEPA 1994a). Use of whole sediments for toxicity tests also requires less manipulation of the original sample and does not require preparation of special sample phases for testing. Whole-sediment

toxicity tests with field-collected sediments are of limited use for establishing cause-and-effect relationships. However, spiking of clean sediments with individual chemicals can be useful for this purpose.

Sediments are semi-solid media composed of minerals, organic material, interstitial water, and a myriad of physico-chemical and biological components. The ASTM Standard E 1391-90 (ASTM 1991) provides guidance on methods for collection, storage, and manipulation of sediments for toxicity testing.

It is impossible to collect sediments in the field, transport them to the laboratory, store them, and then test for toxicity without some alteration to their original structure. Some methods of sample collection and testing are more disruptive than others. For example, the use of a sediment grab sampler (e.g., Ponar, Ekman, van Veen, Shipek, Peterson) is more disruptive than a sediment core sampler, and a standard core sampler is more disruptive than a box core sampler (USEPA 1994a).

Most protocols for conducting whole sediment toxicity tests recommend that manipulation of field-collected sediments (sieving and mixing) be limited to maintain the chemical equilibria of any potential contaminants associated with these sediments. Manipulation or storage of whole-sediment samples can alter the bioavailability of contaminants in sediment; however, the alterations that occur may not substantially affect toxicity (Stemmer et al. 1990; Burton 1991). Sediments contaminated primarily with nonionic, semivolatile organic compounds may change little during storage at 4°C because of their relative resistance to biodegradation and sorption to solids. However, metals and metalloids may be affected by changing redox, oxidation, or microbial metabolism. Metal-contaminated sediments may need to be tested relatively soon after collection with as little manipulation as possible.

It is recommended that the sediments be held in the dark at 4°C soon after collection from the field until their use in toxicity tests. Recommended sediment holding time ranges from less than 2 weeks to less than 8 weeks (ASTM 1993). If whole-sediment toxicity tests are started more than 2 weeks after collection, it is recommended that additional characterizations of the sediment also be conducted to evaluate possible

effects of storage on sediment. Freezing and long-term storage might further change sediment properties such as grain size or partitioning and should be avoided (Schuytema et al. 1989; ASTM 1990). Sediments should be stored with no air over the sealed samples (no head space) at 4°C before the start of a test (Shuba et al. 1978; ASTM 1990). Sediments should be stored in containers constructed of suitable materials. Plastic containers are usually used for collection and short term storage of sediment and water samples.

The bulk chemical concentrations alone cannot be used to evaluate bioavailability (Di Toro et al. 1991). Therefore, the characterization of sediment is important in evaluating its toxicity to test animals. Characterization includes measurement of factors such as sediment organic carbon, percent water, and grain size (e.g., percentages of sand, silt, and clay) that are known to control the availability of contaminants in sediment.

Chapter 5 indicated that *Chironomus zealandicus* and *Chironomus* sp. a had differential preference for the sediments of study sites. Between sediments from Hamilton Lake and Lake Ngaroto, measured total As, Cu, and Cr were relatively higher in Hamilton Lake than in Lake Ngaroto. Survival and emergence of *C. zealandicus* were higher in Hamilton Lake sediment than in Lake Ngaroto sediment while those of *C.* sp. a were higher in Lake Ngaroto sediment than in Hamilton Lake sediment. This indicated that *C. zealandicus* might be more resistant to heavy metal toxicity than *C.* sp. a and the results of Chapter 6 supported this fact. A set of heavy metal spiked sediment bioassays was therefore conducted to further investigate the sensitivity of these two test species and the toxicity of the two site sediments. This chapter investigates the toxicity of sediment spiked with arsenic (As^{5+}) to *C. zealandicus* and *C.* sp. a.

The experiments described in subsequent sections of this chapter were designed to:

1. compare the sensitivity of *C.* sp. a and *C. zealandicus* to arsenic (As^{5+}) in a 10-day chronic exposure;
2. study the potential of *C. zealandicus* as a test organism in sediment toxicity tests;
3. study the sublethal effects of arsenic (As^{5+}) on *C. zealandicus*;
4. to compare the effect of substrates on the toxicity of arsenic (As^{5+}) to *C. zealandicus*;

5. to investigate the laboratory test conditions such as renewal of overlying water, length of exposure to arsenic (As^{5+}) and endpoints of toxicity tests, on the toxicity of arsenic to *C. zealandicus.*

7.2. MATERIALS AND METHODS

Four sets of toxicity bioassays were carried out:

1. Second instar larvae were exposed to sediment from Hamilton Lake or Lake Ngaroto that was spiked with different concentrations of arsenic (As^{5+}), and clean overlying water was renewed throughout the test.
2. Bioassays were carried out as in the first set but under static test conditions and for two differing periods of test duration.
3. First instar larvae were exposed to different concentrations of arsenic (As^{5+}) solutions for the first 96 hours (four days) and then exposed to unspiked sediments from either Hamilton Lake or Lake Ngaroto.
4. Second instar larvae were exposed to shredded paper towel substrate that was spiked with different concentrations of arsenic (As^{5+}).

Endpoints being either one or more of survival, growth (by mean dry weight or total length of larva), developmental stage (larval instar stage, pupa, adult) and head capsule deformity (antenna, mentum, mandible, premandible, epipharyngeal pecten) were determined at the end of each bioassay.

Collection of sediment samples and preparation of paper towel substrate were done as described in Chapters 2 and 3 respectively.

Sediment and paper towel substrate samples were thoroughly mixed in the storage container before the beginning of each bioassay. Sub-samples of the whole sediment or paper towel were spiked with the required concentration of arsenic (As^{5+}) as detailed below. Overlying water was then gently poured along the side of the test chambers containing whole sediment to minimize resuspension of the sediment. Gentle aeration

was provided throughout each test (ASTM 1993). Conditions of toxicity tests carried out in this experiment are summarised in Table 7.1.

7.2.1. Ten-day spiked sediment flow-through test

On the day before the start of a test (day -1), 300 mL sub-samples of whole sediment collected from Hamilton Lake and Lake Ngaroto were mixed with appropriate volumes of 1000 g/m^3 arsenic (As^{5+}) stock solution to give final concentrations of 6.25, 12.5, 25, 50 and 100 g/m^3. Total volume of the arsenic-spiked whole sediment was made up to 500 mL by adding soft synthetic water into 1000 mL glass beakers. Beakers containing whole sediment were thoroughly mixed for 24 hours on an orbital shaker. The overlying water was carefully drained after mixing (day 0) and 75 mL of the remaining sediment sample was placed into each 250 mL glass test beaker. Sediment in these beakers was overlain with freshly prepared clean soft synthetic water, and was renewed at a rate of three times per day. A water renewal system as described by Zumwalt et al. (1994) was used to deliver water to the test beakers. On the following day (day 1), ten 2nd instar larvae of either *C. zealandicus* or *C.* sp. a that had hatched from a single egg mass were randomly collected using a Pasteur pipette and added to each test beaker. Each concentration was triplicated in a test and each test was replicated three times. The bioassay was ended on day 10.

7.2.2. Fourteen and eighteen-day spiked sediment static test

Two sets of toxicity tests were carried out in the second bioassay. One set of tests was carried out for 14 days while the other set was continued for 18 days in order to monitor the later developmental stages such as pupae and adults of the test animals. In a slight modification to the first set of bioassays, on day -1, 75 mL of whole sediment samples from Hamilton Lake or Lake Ngaroto were mixed with required volumes of 1000 g/m^3 arsenic stock solution to achieve concentrations of 6.25, 12.5, 25, 50 and 100 g/m^3 of arsenic (As^{5+}). They were then made up to a final volume of 200 mL in the 250 mL test beakers by adding soft synthetic water. Beakers were shaken for 24 hours on an orbital shaker. Test beakers with spiked sediment were allowed to stand for another 24 hours and gentle aeration was started. On the following day (day 1), ten 2nd instar larvae of *C.*

zealandicus that had hatched from a single egg mass were randomly collected using a Pasteur pipette and added to each test beaker. In this bioassay, overlying water was not renewed and evaporated water was replaced with soft synthetic water as required. Each concentration was triplicated in a test and each test was replicated three times. Bioassays were terminated on day 14 or 18.

Table 7.1. Summary of conditions of chronic toxicity tests carried out in this experiment.

Test	Duration	Species	Substrate	Replicates	Animals per replicate	Endpoints
Flow-through spiked sediment	10 day	*C. zealandicus* and *C.* sp. a	Ngaroto Hamilton	9	10	Survival, Growth (mean dry weight)
Static spiked sediment	14 day	*C. zealandicus*	Ngaroto Hamilton	9	10	Survival, Development, Growth (length), Head capsule deformity
Static spiked sediment	18 day	*C. zealandicus*	Ngaroto Hamilton	9	10	Survival, Development, Growth (length), Head capsule deformity
Pre-exposed animal static unspiked sediment	14 day	*C. zealandicus*	Ngaroto Hamilton	9	10	Survival, Development, Growth (length), Head capsule deformity
Static spiked paper towel	18 day	*C. zealandicus*	Ngaroto Hamilton	3	50	Survival, Development, Growth (length), Head capsule deformity
Static-renewal spiked paper towel	18 day	*C. zealandicus*	Ngaroto Hamilton	3	50	Survival, Development, Growth (length), Head capsule deformity

7.2.3. Fourteen-day pre-exposed unspiked sediment static tests

Approximately two hundred 1st instar larvae of *C. zealandicus* that had hatched from a single egg mass were exposed to each of a series of 30, 60, 90, 120 and 150 g/m^3 arsenic solutions and a soft synthetic water control for 96 hours (4 days), from day -3 in this experiment. Aeration and food were provided during exposure and all other conditions were as in the acute toxicity tests (Chapter 6). Ten larvae were randomly selected from each of the above concentrations after 96 hours (day 1) and placed into 250 mL test beakers that contained 75 mL of unspiked sediment from either Hamilton Lake or Lake Ngaroto with overlying soft synthetic water. Overlying water was not renewed in this bioassay and was replaced with soft synthetic water as needed. Each concentration was triplicated in a test and each test was replicated three times. Tests were ended on day 14.

7.2.4. Eighteen day arsenic (As^{5+}) spiked paper towel static and static-renewal test

Samples of 100 mL shredded paper towel substrate were spiked with the required volume of 1000 g/m^3 arsenic (As^{5+}) stock solution necessary to give final concentrations of 2, 4, 8, 16 and 32 g/m^3 in 1000 mL beakers on the day before the start of the test (day -1). The final volume was made up to 500 mL by adding soft synthetic water. This mixture of paper towel and arsenic (As^{5+}) solution was shaken on an orbital shaker for 24 hours. Test beakers with spiked paper towel substrate were allowed to stand for another 24 hours with gentle aeration. Fifty 2nd instar larvae of *C. zealandicus* that hatched from a single egg mass were randomly collected using a Pasteur pipette and added to each test beaker on the following day (day 1).

Two different bioassays were performed with arsenic-spiked shredded paper towel substrate. Overlying water was renewed using freshly prepared arsenic (As^{5+}) solution of the appropriate concentration on every other day in one bioassay. Overlying water was not renewed in the second bioassay. Each concentration was triplicated in every test, and the bioassay was terminated on day 18.

7.2.5. Food and Feeding

Test animals were fed with finely ground TetraMin fish flakes. Different doses of feeding were used in different bioassays. Feeding rates were determined through preliminary laboratory culture practices according to the number and age of test animals and the size of each test chamber.

Six mg Tetramin fish flake were added to each test chamber that contained lake sediment substrates on day 0 and continued at a rate of 6 mg/beaker daily. Fifty mg Tetramin were added to each beaker that contained shredded paper towel substrate and feeding was continued at 50 mg/beaker daily. In the pre-exposure bioassay, feeding was maintained at 4 mg/beaker during the period of pre-exposure to arsenic (As^{5+}) solutions of different concentrations and continued at 6 mg/beaker after the test animals were transferred to unspiked sediment substrates. Feeding was stopped on the day before the end of each test.

7.2.6. Physico-chemical parameters

Sediment subsamples were analyzed for moisture content, total organic matter and heavy metals as described in chapter 2.

Concentrations of As, Cu and Cr were measured in interstitial (pore) water of randomly selected sediment samples of each concentration at the beginning and end of each test. Pore water was collected by centrifuging sediment samples at 6000 rpm for 15 minutes at 4°C. Pore water samples were acidified to pH 2 as soon as collected. Either FAAS, with or without a hydride generator or GFAAS was used to measure metals in pore water.

The pH, hardness, dissolved oxygen, and conductivity were measured at the beginning and end of each bioassay as described in chapter 2. Toxicity tests were conducted at 23°C. The dark-light cycle was maintained at 8:16 (D:L) throughout bioassays using 60 watt tungsten filament electric bulbs.

Test animals hatched from a single egg mass were used in each bioassay in order to keep the biological variability of larvae constant between replicates in a bioassay. When larvae hatched from a single egg mass were not sufficient for all replicates, larvae of the same age but from another egg mass were used and randomly allotted to test beakers.

Test animals were handled as little as possible and were introduced into the overlying water below the air-water interface by Pasteur pipette. At the end of an exposure, test organisms were removed from the chambers by wet-sieving the sediment through a 250-μm sieve.

7.2.7. Endpoints

In the spiked sediment toxicity tests with flow-through conditions, survival and larval growth (mean dry weight) were determined at the end of the experiment. Recovered larvae from each test beaker were pooled, placed in pre-weighed plastic vials and dried in the oven at 60°C to a constant weight in order to obtain mean dry weight. In other bioassays, survival, developmental stage, larval growth and the head capsule deformity of 4th instar larvae were recorded for each concentration at the end of the experiment. Number of larval instars, pupae and adults were counted for each concentration of a test to determine the average developmental stage. Head capsules of 4th instar larvae were prepared on slides as described in Chapter 4. Mentum, mandible, premandible, epipharyngeal pecten and antenna were examined for deformity.

Test beakers were monitored twice every day, particularly at dawn and dusk, in order to estimate the number of adults emerged. For the measurement of the length of larvae, instars were straightened on a glass slide and the length was determined by measuring the distance from the anterior of the labrum to the posterior of the last abdominal segment. Measurements were made by using an eyepiece that was calibrated with a slide micrometer in a stereo microscope (ASTM 1995).

7.2.8. Data analyses

The kind of statistical test to be used is usually determined by the study objectives. If the objective is to compare the toxicity results between test sites within an area of concern or between each test site and a reference area, analysis of variance (ANOVA) may be used to conduct the evaluation (USEPA 1994a). If the objective is to evaluate whether a gradient of toxicity exists with distance from a potential problem area, a correlation analysis or multivariate analysis approach may be used. Details of potential statistical approaches have been discussed by Green (1979), Gilbert (1987) and USEPA (1994a, 1994b). Survival, growth, and development were analysed by ANOVA and mouthpart deformity was analysed by chi-squared test in this experiment.

7.3. RESULTS

Physico-chemical parameters measured in bioassays are given in Table A.4 (Appendix), and concentrations of copper (Cu), chromium (Cr), and arsenic (As) of the pore water extracted from the whole sediment subsample at the beginning and end of each bioassay are given in Table 7.2. Total organic matter and moisture content of the whole sediments used in the four bioassays are given in Table 7.3.

The protocol used to spike sediment successfully raised initial arsenic concentrations in both sediment types (Figs 7.1, 7.2, Table 7.2). Initial arsenic concentrations in Lake Ngaroto sediment pore water are higher than corresponding samples from Hamilton Lake sediment but are lower by the end of both the static and flow-through bioassays indicating greater absorption to the Ngaroto sediment.

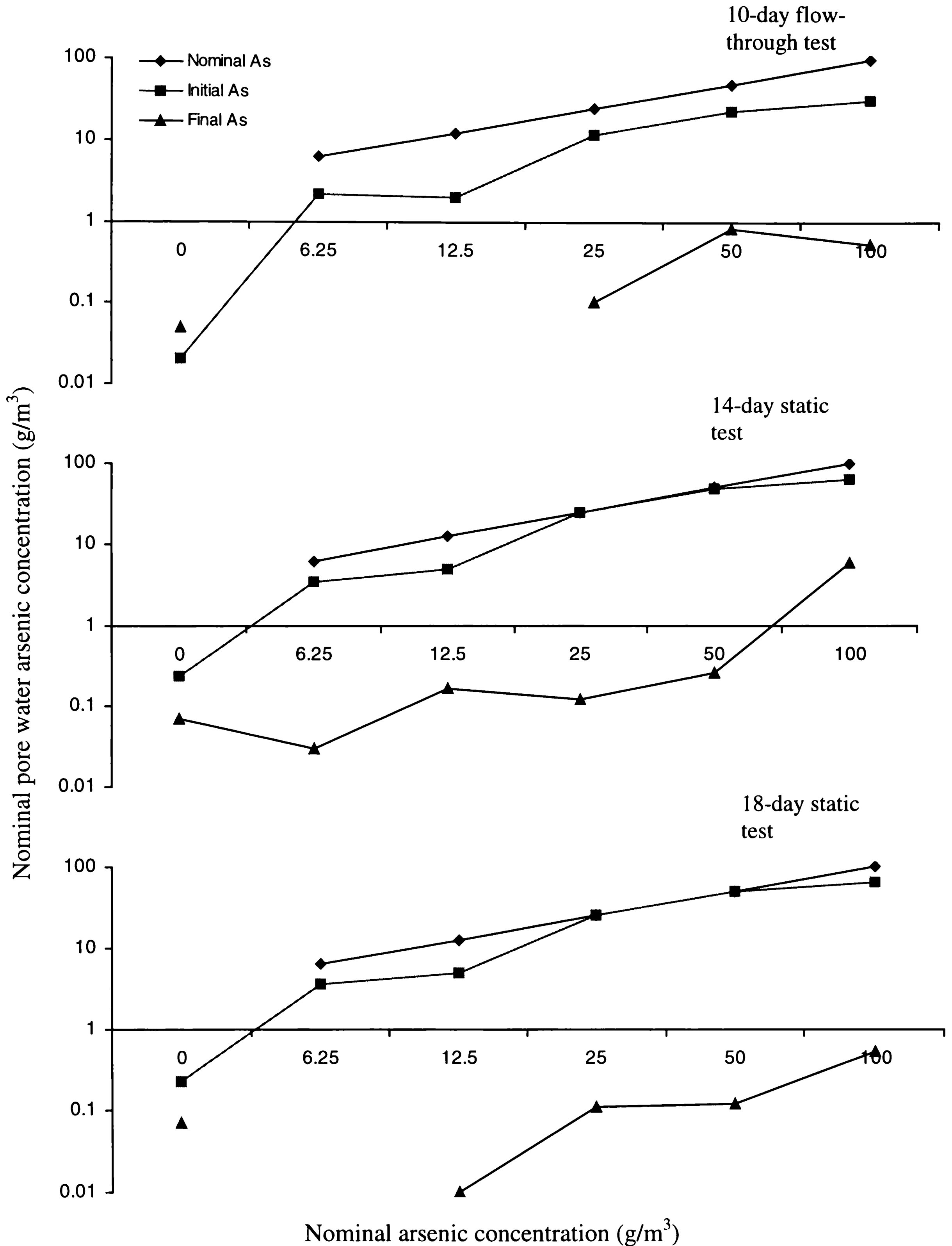


Fig 7.1. Nominal concentrations and pore water concentrations of arsenic (As^{5+}) at the beginning and end of spiked Hamilton Lake sediment chronic toxicity tests. Total pore water arsenic was determined from a randomly selected sediment replicate at each concentration.

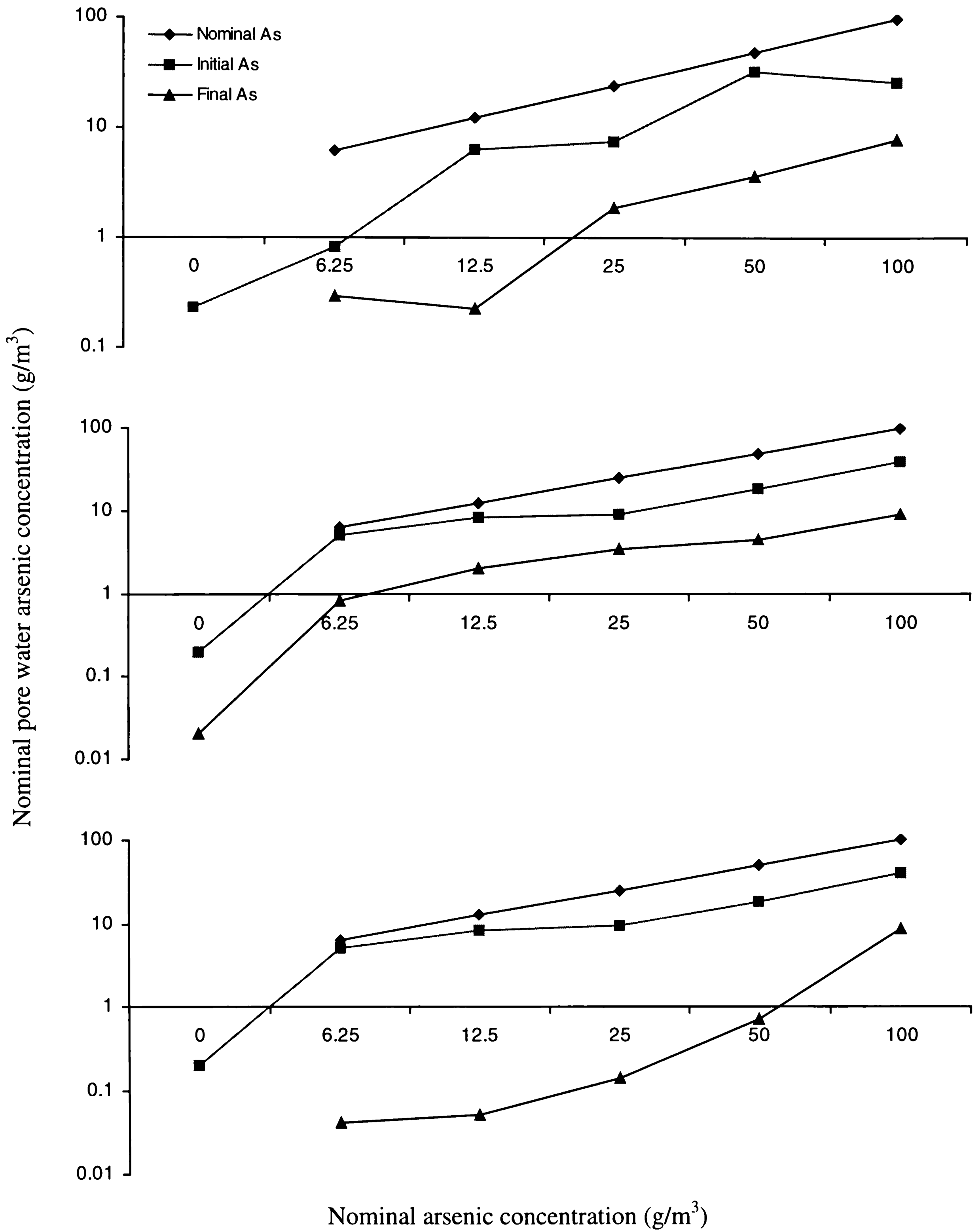


Fig 7.2. Nominal concentrations and pore water concentrations of arsenic at the beginning and end of spiked Lake Ngaroto sediment chronic toxicity tests. Total pore water arsenic was determined from a randomly selected sediment replicate at each concentration.

Table 7.2. Pore water concentration of As, Cu, Cr and Mn in mg/kg at the beginning and end of bioassays.

Nominal As	Initial As	Final As	Initial Cu	Final Cu	Initial Cr	Final Cr
- Ngaroto sediment						
10 day flow through test						
0	0.02	0.05	3.52	1.68	28.32	0.68
6.25	2.17	_	3.38	2.55	28.32	0.75
12.50	2.00	0	4.30	2.95	30.32	0.68
25.00	12.00	0.10	5.61	3.28	28.66	0.02
50.00	22.99	0.83	5.56	4.25	31.99	0.70
100.00	31.65	0.52	5.53	2.88	27.49	0.13
14 day static test						
0	0.23	0.07	5.18	4.75	16.33	0.13
6.25	3.50	0.03	4.91	3.52	16.83	0.03
12.50	4.83	0.16	6.36	5.85	15.19	0.15
25.00	25.32	0.12	6.80	4.13	8.80	0.18
50.00	48.15	0.25	6.43	4.98	14.33	0.10
100.00	63.31	5.83	6.10	4.46	13.66	0.15
18 day static test						
0		0.07		2.20		0.23
6.25		0		2.30		0.15
12.50		0.01		2.42		1.00
25.00		0.11		2.55		0
50.00		0.12		2.70		0.02
100.00		0.53		2.72		0.18
- Hamilton sediment						
10 day flow-through test						
0	0.23	_	10.60	6.32	14.75	0.36
6.25	0.83	0.29	11.18	4.34	14.00	0.24
12.50	6.50	0.22	10.15	6.00	21.50	0.45
25.00	7.75	1.86	8.98	6.25	11.25	0.10
50.00	33.00	3.70	12.25	5.30	13.50	0.07
100.00	26.00	8.02	10.65	4.65	13.00	0.15
14 day static test						
0	0.20	0.02	7.84	3.67	12.20	0.43
6.25	5.00	0.83	7.70	5.85	11.80	4.33
12.50	8.20	2.00	7.26	10.33	19.60	15.5
25.00	9.20	3.33	8.18	5.93	13.80	11.33
50.00	18.40	4.40	7.62	9.10	9.80	5.80
100.00	39.20	9.00	7.82	7.60	9.20	6.00
18 day static test						
0		0		4.50		0.36
6.25		0.04		6.32		0.26
12.50		0.05		7.52		0.12
25.00		0.14		7.94		0.08
50.00		0.69		5.36		0.08
100.00		8.40		7.22		0.06

Table 7.3. Percentages of total organic matter and moisture content of whole sediment substrates used in experiments. Each value is a mean of three replicates with SE in parentheses.

	Sediment		Flow-through test	Static, 14 day test	Static, 18 day test	Pre-exposed test
Organic matter (%)						
	Hamilton	Mean	10.9	11.6	9.6	12.0
		SE	(0.3)	(0.1)	(0. 8)	(0.7)
	Ngaroto	Mean	13.9	15.0	14.9	14.3
		SE	(0.5)	(0.5)	(0.4)	(0.6)
Moisture content (%)						
	Hamilton	Mean	36.3	30.6	39.8	36.0
		SE	(1.8)	(7.0)	(4.3)	(2. 9)
	Ngaroto	Mean	52.8	48.0	47.3	46.0
		SE	(8.7)	(4.0)	(7.9)	(2.8)

7.3.1. Ten day spiked sediment flow-through test with *C. zealandicus* and *C.* sp. a

Results of ten day spiked sediment flow-through tests are given in Tables 7.4 and 7.5. Nominal arsenic concentration significantly affected survival of *C. zealandicus*, and survival and growth of *C.* sp. a on both sediments ($P<0.05$). Survival of *C.* sp. a was also adversely affected by Hamilton Lake sediment ($P<0.001$) but sediment type had no effect on survival or growth for *C. zealandicus*. Interaction between sediment type and concentration was not significant for survival of either species but was found to have a significant effect on the growth of *C. zealandicus* ($P<0.05$).

Table 7.4. Percent survival and growth (mean dry weight in mg) of *Chironomus zealandicus* and *Chironomus* sp. a on Hamilton Lake and Lake Ngaroto sediment at the end of the 10 day bioassay. Each value is a mean of 9 replicates with SE in parentheses.

	Nominal Concentration of arsenic in g/m^3	0	6.25	12.5	25	50	100
C. zealandicus							
Hamilton sediment	Survival (%)	86.6 (8.0)	94.4 (2.4)	92.2 (3.2)	82.2 (4.6)	80 (4.1)	74.4 (5.6)
	Mean dry weight (mg)	0.78 (0.03)	0.76 (0.04)	0.52 (0.04)	0.68 (0.05)	0.64 (0.20)	0.44 (0.03)
Ngaroto sediment	Survival (%)	92.2 (2.8)	84.4 (3.8)	93.3 (3.3)	86.6 (3.0)	82.2 (4.6)	83.3 (3.7)
	Mean dry weight (mg)	0.69 (0.04)	0.53 (0.04)	0.68 (0.03)	0.63 (0.03)	0.5 (0.08)	0.6 (0.01)
C. sp. a							
Hamilton sediment	Survival (%)	91.1 (3.1)	75.6 (6.5)	70.0 (8.0)	63.3 (6.0)	60.0 (7.2)	64.4 (6.9)
	Mean dry weight (mg)	1.32 (0.09)	1.12 (0.07)	1.81 (0.91)	1.34 (0.07)	0.67 (0.08)	0.66 (0.08)
Ngaroto sediment	Survival (%)	95.6 (2.4)	80.0 (5.0)	74.4 (10.1)	85.6 (4.7)	85.6 (4.7)	83.3 (4.1)
	Mean dry weight (mg)	2.16 (0.28)	1.20 (0.05)	1.23 (0.16)	1.15 (0.05)	1.38 (0.07)	1.20 (0.13)

Table 7.5. Statistical comparisons of survival and growth of *Chironomus zealandicus* and *Chironomus* sp. a between sediment types and sediment arsenic concentrations in 10 day tests. Means of 9 replicates were compared using 2 way ANOVA. NS-Not significant (P>0.05)

		P values	
Dependent variable		Survival	Mean dry weight
Factors: Sediment type and Concentration			
Chironomus zealandicus	P(Sediment type)	NS	NS
	P(Concentration)	0.015	NS
	P(interaction)	NS	0.042
Chironomus sp. a	P(Sediment type)	<0.001	NS
	P(Concentration)	0.011	0.045
	P(interaction)	NS	NS
Factors: Test species and Concentration			
Hamilton Lake sediment	P(Species)	<0.001	<0.01
	P(Concentration)	<0.004	NS
	P(interaction)	NS	NS
Lake Ngaroto sediment	P(Species)	NS	<0.001
	P(Concentration)	NS	<0.001
	P(interaction)	NS	<0.001

7.3.2. Fourteen and eighteen day spiked sediment static test with *C. zealandicus*

Under 14 or 18 day static test conditions, both survival and development of *C. zealandicus* showed significant effects related to sediment type and the nominal concentration of arsenic. Reduced survival was observed in Hamilton Lake sediment; the effect of arsenic concentration was more significant than that of either sediment type or test duration (Tables 7.6, 7.7). Test duration had no effect on survival in either of the sediments but development showed significant effects with test duration due to growth. The interaction between test duration and sediment arsenic concentration had a significant effect on the numbers of adults, pupae, and 4th instar larvae recovered from Hamilton Lake sediment, but not from Lake Ngaroto sediment.

The body length frequency distribution for larvae recovered at the end of experiments is shown in Table 7.8. Growth of larvae is considerably retarded when sediments are spiked with high concentrations of arsenic. This effect is greater in Hamilton Lake sediment and may relate to the fact that larvae grew faster on this substrate.

The incidence of head capsule deformity in 4th instar larvae recovered at the end of the static bioassays is presented in Tables 7.9 and 7.10. Total deformities showed a significant effect of arsenic concentration after 18 days in both sediments. The greater incidence of deformity in larvae raised on Hamilton Lake sediment at high concentrations of arsenic is mostly due to abnormalities in the antennae and the mentum, although the effect of sediment type was not significant overall. A significant relationship was observed between test duration and head capsule deformity for some combinations of arsenic concentration and sediment type.

Table 7.6. Percentage of survival and each developmental stage of *Chironomus zealandicus* recovered at the end of 14 and 18 day static bioassay on arsenic-spiked Hamilton Lake and Lake Ngaroto sediment substrates. Each value is a mean of 9 replicates with SE in parentheses.

Nominal concentration of arsenic in g/m^3	0	6.25	12.5	25	50	100
14 day bioassay						
<u>Hamilton sediment</u>						
Survival (%)	98.9	95.6	97.8	86.7	67.8	50.0
	(1.1)	(2.9)	(1.5)	(4.4)	(5.2)	(3.7)
Recovered as younger instars (%)	8.9	17.8	14.4	24.4	33.3	40.0
	(3.5)	(3.2)	(1.8)	(3.4)	(7.3)	(5.0)
Recovered as 4th instars (%)	55.6	55.6	44.4	51.1	32.2	10.0
	(2.4)	(4.1)	(4.1)	(4.2)	(9.7)	(3.3)
Recovered as pupae (%)	22.2	16.7	35.6	10.0	2.2	0.0
	(2.8)	(3.7)	(4.7)	(2.4)	(1.5)	
Recovered as adults (%)	12.2	5.6	3.3	3.3	0.0	0.0
	(1.5)	(1.8)	(1.7)	(1.7)		
<u>Ngaroto sediment</u>						
survival (%)	98.9	93.3	92.2	87.8	93.3	75.6
	(1.1)	(3.3)	(3.2)	(4.9)	(2.9)	(6.0)
Recovered as younger instars (%)	10.0	14.4	23.3	17.8	23.3	14.4
	(3.7)	(3.4)	(5.0)	(3.6)	(5.3)	(2.9)
Recovered as 4th instars (%)	53.3	57.8	50.0	63.3	67.8	61.1
	(3.3)	(3.2)	(4.4)	(5.0)	(8.1)	(6.5)
Recovered as pupae (%)	23.3	15.6	14.4	6.7	2.2	0.0
	(2.4)	(3.4)	(3.4)	(2.4)	(1.5)	
Recovered as adults (%)	12.2	6.7	4.4	0.0	0.0	0.0
	(2.2)	(1.7)	(1.8)			
18 day bioassay						
<u>Hamilton sediment</u>						
Survival (%)	97.5	92.5	97.5	72.5	65.0	53.8
	(1.5)	(4.6)	(1.5)	(4.6)	(4.7)	(4.0)
Recovered as younger instars (%)	0.0	0.0	0.0	3.8	33.8	23.8
				(1.7)	(7.8)	(7.8)
Recovered as 4th instar (%)	8.8	58.8	46.3	24.5	27.5	26.3
	(4.5)	(4.2)	(4.0)	(7.3)	(10.0)	(6.7)
Recovered as pupa (%)	53.8	21.3	33.8	26.3	3.8	3.8
	(2.5)	(4.2)	(3.5)	(1.7)	(1.7)	(1.7)
Recovered as adults(%)	33.8	13.8	17.5	15.0	0.0	0.0
	(2.5)	(2.5)	(4.6)	(3.1)		
<u>Ngaroto sediment</u>						
Survival (%)	100	95.0	91.3	75.0	92.5	81.3
	(0)	(4.4)	(3.3)	(4.4)	(3.0)	(7.9)
Recovered as younger instars (%)	0.0	0.0	1.3	2.5	15.0	3.8
			(1.2)	(1.5)	(6.4)	(1.7)
Recovered as 4th instars (%)	45.0	63.1	55.0	63.8	75.0	73.8
	(4.4)	(4.2)	(5.6)	(6.4)	(8.5)	(9.4)
Recovered as pupae (%)	37.5	25.0	30.0	8.8	2.5	3.8
	(2.4)	(6.9)	(7.6)	(2.8)	(1.5)	(1.7)
Recovered as adults (%)	17.5	8.8	5.0	0.0	0.0	0.0
	(2.4)	(2.1)	(1.8)			

Table 7.7. Statistical comparisons of survival and development of *Chironomus zealandicus* between sediment type and test duration and sediment arsenic concentrations in 14 and 18 day bioassays. Means of 9 replicates were compared using 2 way ANOVA. NS-Not significant (P>0.05)

		P values			
Dependent variable	Survival	Adults emerged	Pupa recovered	Fourth instar recovered	Younger instar recovered
Factors: Sediment type and Concentration					
14 day Static test					
P(sediment)	0.001	NS	0.011	<0.001	0.017
P(concentration)	<0.001	<0.001	<0.001	0.001	<0.001
P(interaction)	<0.001	NS	<0.001	<0.001	<0.001
18 day test					
P(sediment)	0.011	<0.001	0.001	<0.001	<0.001
P(concentration)	<0.001	<0.001	<0.001	0.001	<0.001
P(interaction)	0.009	0.003	0.035	0.020	<0.001
Factors: Test duration and Concentration					
Hamilton Lake sediment substrate					
P(test duration)	NS	<0.001	<0.001	0.012	<0.001
P(concentration)	<0.001	<0.001	<0.001	<0.001	<0.001
P(interaction)	NS	<0.001	<0.001	<0.001	NS
Lake Ngaroto sediment substrate					
P(test duration)	NS	0.049	0.001	NS	<0.001
P(concentration)	<0.001	<0.001	<0.001	0.011	0.025
P(interaction)	NS	NS	NS	NS	NS

Table 7.8. Length frequency distribution of *Chironomus zealandicus* larvae recovered from the sediment substrates at the end of 14 and 18 day static bioassays. Median size class at each concentration is indicated by bold face type within a box. (Larvae recovered from 9 replicates of each concentration were pooled for the length measurement)

Nominal Concentration of As (g/m^3)	0	6.25	12.5	25	50	100	0	6.25	12.5	25	50	100
Static 14day test												
	Hamilton sediment						Ngaroto sediment					
Size Class (mm)												
3.6-4.5	1	1	2	8	11	11	1	3	8	7	6	6
4.6-5.5	3	8	8	10	8	**18**	4	7	7	6	13	6
5.6-6.5	4	7	3	4	**11**	7	4	3	6	3	2	1
6.6-7.5	0	2	2	2	1	3	0	1	2	2	6	1
7.6-8.5	2	4	1	5	6	1	1	3	1	5	5	3
8.6-9.5	4	8	2	**7**	2	2	1	6	4	3	6	8
9.6-10.5	4	**6**	**12**	6	3	1	2	4	3	8	**7**	**13**
10.6-11.5	10	7	13	14	6	1	6	1	**7**	**12**	7	15
11.6-12.5	**9**	8	5	6	5	1	3	**5**	10	12	12	15
12.6-13.5	9	7	3	6	6	0	**12**	8	9	9	15	0
13.6-14.5	6	4	2	0	0	0	5	15	7	4	3	0
14.6-15.5	6	4	0	0	0	0	15	9	2	2	0	0
15.6-16.5	0	0	0	0	0	0	3	0	0	0	0	0
Total	58	66	53	68	59	45	57	65	66	73	82	68
Static 18 day test												
	Hamilton sediment						Ngaroto sediment					
Size class (mm)												
4.6-5.5	0	0	0	0	6	6	0	0	0	0	5	3
5.6-6.5	0	0	0	0	8	12	0	0	0	0	6	6
6.6-7.5	0	0	0	0	8	**8**	0	0	0	0	6	7
7.6-8.5	0	0	0	0	6	4	0	0	0	3	6	**20**
8.6-9.5	0	0	0	4	**12**	8	0	0	1	2	**12**	6
9.6-10.5	0	0	0	10	6	2	0	3	3	6	10	10
10.6-11.5	0	15	11	**10**	7	2	0	3	3	18	8	10
11.6-12.5	0	7	**9**	3	2	2	3	10	**22**	**12**	8	5
12.6-13.5	0	**8**	13	3	3	1	13	**15**	15	15	4	1
13.6-14.5	3	12	6	0	0	0	**7**	15	6	2	3	0
14.6-15.5	**8**	8	1	0	0	0	15	10	1	1	0	0
15.6-16.5	0	0	0	0	0	0	3	0	0	0	0	0
Total	11	50	40	30	58	45	41	56	51	59	68	68

Table 7.9. Head capsule deformity (percentage) observed from the fourth instar larvae of *Chironomus zealandicus* that were recovered at the end of 14 and 18 day static bioassays on Hamilton Lake and Lake Ngaroto sediment.

Nominal concentration of arsenic in g/m^3	0	6.25	12.5	25	50	100
14 day static test						
<u>Hamilton Lake sediment</u>						
Number of fourth instars recovered	50	50	40	46	29	9
Deformed head capsules (%)	12.0	14.0	25.0	30.0	34.5	33.3
Single structure deformed (%)	6.0	10.0	20.0	23.9	20.7	11.1
Multiple structure deformed (%)	6.0	4.0	5.0	6.5	13.8	22.2
Antenna deformed (%)	4.0	4.0	7.5	4.3	6.9	22.2
Mentum deformed (%)	8.0	8.0	10.0	15.2	20.7	33.3
Mandible deformed (%)	2.0	0.0	2.5	2.2	3.4	11.1
Premandible deformed (%)	2.0	6.0	7.5	6.5	10.3	11.1
Epipharyngeal pecten deformed (%)	2.0	2.0	5.0	8.7	10.3	11.1
<u>Lake Ngaroto sediment</u>						
Number of fourth instars recovered	48	52	45	57	61	55
Deformed head capsules (%)	8.3	30.8	35.6	29.8	21.3	27.3
Single structure deformed (%)	8.3	19.2	22.2	21.1	16.4	23.7
Multiple structure deformed (%)	0.0	11.5	13.3	8.8	4.9	3.6
Antenna deformed (%)	4.2	11.5	11.5	8.8	4.9	9.1
Mentum deformed (%)	4.2	13.5	17.8	10.5	8.2	9.1
Mandible deformed (%)	0.0	1.9	0.0	1.7	1.6	1.8
Premandible deformed (%)	0.0	11.5	8.9	8.8	6.6	3.6
Epipharyngeal pecten deformed (%)	0.0	7.7	11.1	8.8	6.6	5.5
18 day static test						
<u>Hamilton Lake sediment</u>						
Number of fourth instars recovered	11	50	40	27	28	23
Deformed head capsules (%)	18.2	30.0	55.0	63.0	42.9	65.2
Single structure deformed (%)	9.1	18.0	40.0	22.2	17.9	30.4
Multiple structure deformed (%)	9.1	12.0	15.0	40.7	25.0	34.8
Antenna deformed (%)	0.0	3.0	12.5	18.5	10.7	21.7
Mentum deformed (%)	9.1	12.0	27.5	40.7	21.4	30.4
Mandible deformed (%)	0.0	2.0	2.5	7.4	0.0	8.7
Premandible deformed (%)	9.1	10.0	15.0	22.2	28.6	26.1
Epipharyngeal pecten deformed (%)	9.1	16.0	22.5	25.9	21.4	39.1
<u>Lake Ngaroto sediment</u>						
Number of fourth instars recovered	41	56	50	56	63	67
Deformed head capsules (%)	12.1	41.0	42.0	39.2	42.8	47.7
Single structure deformed (%)	2.4	32.1	24.0	28.5	37.5	35.8
Multiple structure deformed (%)	9.7	8.9	18.0	10.7	9.5	11.9
Antenna deformed (%)	7.3	10.7	10.0	14.2	9.5	13.4
Mentum deformed (%)	4.8	14.2	18.0	12.5	14.2	16.4
Mandible deformed (%)	0.0	1.7	4.0	1.7	3.2	5.97
Premandible deformed (%)	2.4	8.9	16.0	16.0	14.2	13.4
Epipharyngeal pecten deformed (%)	4.8	16.0	16.0	12.5	12.6	14.9

Table 7.10. Statistical comparisons of head capsule deformity reported for the fourth larval instars of *Chironomus zealandicus* between test concentrations, sediment types and test durations in 14 and 18 day static bioassays. The deformities were compared with expected values in a Chi-squared test. NS-Not significant (P>0.05).

				P value		
				Total number of deformed head capsules	Single structure deformed	Multiple structure deformed
Factor						
Concentration	Hamilton Lake sediment					
		14 day static test		NS	NS	NS
		18 day static test		0.006	NS	0.042
	Lake Ngaroto sediment					
		14 day static test		0.037	NS	NS
		18 day static test		0.009	0.004	NS
Sediment type		14 day static test				
			Control	NS	NS	NS
			6.25g/m³	NS	NS	NS
			12.5 g/m³	NS	NS	NS
			25 g/m³	NS	NS	NS
			50 g/m³	NS	NS	NS
			100 g/m³	NS	NS	NS
		18 day static test	Control	NS	NS	NS
			6.25 g/m³	NS	NS	NS
			12.5 g/m³	NS	NS	NS
			25 g/m³	NS	NS	0.004
			50 g/m³	NS	NS	NS
			100 g/m³	NS	NS	0.031
Test duration						
	Hamilton Lake sediment		Control	NS	NS	NS
			6.25 g/m³	0.012	NS	NS
			12.5 g/m³	0.014	NS	0.001
			25 g/m³	NS	NS	NS
			50 g/m³	NS	NS	NS
			100 g/m³	NS	NS	NS
	Lake Ngaroto sediment		Control	NS	NS	NS
			6.25 g/m³	NS	NS	NS
			12.5 g/m³	NS	NS	NS
			25 g/m³	NS	NS	NS
			50 g/m³	0.010	NS	NS
			100 g/m³	0.033	NS	NS

7.3.3. Fourteen day pre-exposed unspiked sediment static tests

Both survival and development of *C. zealandicus* larvae previously exposed to arsenic were significantly affected by the sediment type on which they were grown and the pre-exposed arsenic concentration (Tables 7.11, 7.12, 7.13). Mortality of pre-exposed larvae was higher on clean sediment from Hamilton Lake than from Lake Ngaroto. The concentration of arsenic to which larvae were exposed before they were exposed to the sediment significantly affected subsequent growth and development. As in the tests with arsenic-spiked sediment, length of larvae recovered at test conclusion decreased with arsenic concentration. Once again, larvae grew much faster on Hamilton Lake sediment. The relationship between head capsule deformity and pre-exposure to arsenic was significant for all concentrations on both sediments (Tables 7.14, 7.15).

7.3.4. Eighteen day arsenic spiked paper towel static and static-renewal tests

Renewal of overlying water in the test beakers had a positive effect on the survival of test animals in an 18 day bioassay using shredded paper towel substrate (Tables 7.16 and 7.17). More animals survived in the static renewal test for any particular nominal arsenic concentration. However, renewal of test water decreased development rate with a greater number of 4th instar larvae recovered from the static renewal test. Survival and development of test animals were significantly affected by arsenic concentration under both static and renewal conditions in this bioassay. There were differences in the occurrence of head capsule deformity in 4th instar larvae between tests. Arsenic exposure increased deformity under both static and static renewal test conditions (Tables 7. 18 and 7.19) but the incidence of deformity was also high in control substrate under static conditions.

Table 7.11. Percentage of survival and each developmental stage of *Chironomus zealandicus* recovered at the end of a 14 day static bioassay on Hamilton Lake and Lake Ngaroto sediment substrates with pre-exposure to arsenic (see text for details). Each value is a mean of 9 replicates with SE in parentheses.

Nominal concentration of arsenic (g/m^3)	0	30	60	90	120	150
Hamilton sediment						
Survival (%)	93.0 (0.2)	86.0 (3.0)	77.0 (6.0)	54.0 (6.0)	22.0 (6.0)	20.0 (5.0)
Recovered as younger instars (%)	0	0	0	3.0 (2.0)	2.0 (2.0)	6.0 (4.0)
Recovered as 4th instars (%)	3.0 (2.0)	21.0 (3.0)	23.0 (3.0)	13.0 (5.0)	10.0 (3.0)	9.0 (0.1)
Recovered as pupae (%)	13.0 (2.0)	37.0 (0.8)	33.0 (5.0)	19.0 (5.0)	2.0 (1.0)	6.0 (2.0)
Recovered as adults (%)	77.0 (2.0)	28.0 (11.0)	20.0 (4.0)	19.0 (0.5)	8.0 (0.3)	0
Ngaroto sediment						
Survival (%)	100.0 (0)	83.0 (8.0)	78.0 (6.0)	86.0 (6.0)	71.0 (0.7)	33.0 (3.0)
Recovered as younger instars (%)	0	4.0 (3.0)	22.0 (0.5)	1.0 (0.1)	10.0 (4.0)	23.0 (2.0)
Recovered as 4th instars (%)	40.0 (6.0)	46.0 (4.0)	29.0 (4.0)	57.0 (9.0)	39.0 (0.5)	10.0 (3.0)
Recovered as pupae (%)	27.0 (3.0)	27.0 (6.0)	10.0 (2.0)	21.0 (4.0)	4.0 (2.0)	1.0 (1.0)
Recovered as adults (%)	33.0 (5.0)	7.0 (2.0)	17.0 (7.0)	7.0 (3.0)	18.0 (6.0)	0

Table 7.12. Statistical comparisons of survival and development between sediment types and sediment arsenic concentrations for *Chironomus zealandicus* that were cultured on clean sediment substrates of Hamilton Lake and Lake Ngaroto for 14 days in the laboratory after exposing to arsenic solution for 96 hours. Means of 9 replicates were compared using 2 way ANOVA. NS-Not significant (P>0.05)

	P values				
Dependent variable	Survival	Adults emerged	Pupae recovered	Fourth instar recovered	Younger instar recovered
Factors:Sediment type and Concentration					
P(Sediment type)	<0.001	<0.001	0.006	<0.001	<0.001
P(Concentration)	<0.001	<0.001	<0.001	<0.001	<0.001
P(interaction)	<0.001	<0.001	<0.001	<0.001	<0.001

Table 7.13. Length frequency distribution of *Chironomus zealandicus* larvae recovered from lake sediment substrates at the end of 14 day static bioassays. Larvae were exposed to arsenic solutions of different concentrations for 96 hours prior to the beginning of the sediment bioassay. Median size class at each concentration is indicated by bold face type within a box. (Larvae recovered from 9 replicates of each concentration were pooled for the length measurement.)

Concentration of arsenic solution in g/m^3	0	30	60	90	120	150	0	30	60	90	120	150
	Hamilton Lake sediment						Lake Ngaroto sediment					
Size class (mm)												
4.6-5.5	0	0	0	0	1	1	0	0	0	0	2	8
5.6-6.5	0	0	0	0	1	4	0	0	0	6	6	**12**
6.6-7.5	0	0	0	2	3	**3**	0	0	8	6	5	5
7.6-8.5	0	0	4	3	**4**	1	0	4	8	12	**10**	2
8.6-9.5	0	1	1	1	1	2	0	4	**10**	**8**	5	1
9.6-10.5	0	1	4	**3**	1	2	0	5	12	9	8	1
10.6-11.5	0	2	5	0	0	0	3	**3**	3	7	7	1
11.6-12.5	0	7	**3**	3	0	0	4	12	1	4	1	0
12.6-13.5	0	**5**	1	3	0	0	**12**	8	2	0	0	0
13.6-14.5	**2**	2	2	0	0	0	6	6	1	0	0	0
14.6-15.5	1	1	1	0	0	0	8	1	1	0	0	0
15.6-16.5	0	0	0	0	0	0	3	2	0		0	0
Total	3	19	21	15	11	13	36	45	46	52	44	30

Table 7.14. Head capsule deformity of fourth instar larvae of *Chironomus zealandicus* that were cultured on clean sediment substrates of Hamilton Lake and Lake Ngaroto for 14 days after exposing to arsenic (As^{5+}) solution for 96 hours as 1st instars.

Nominal concentration of arsenic in g/m^3	0	30	60	90	120	150
Pre exposed test						
Hamilton sediment						
Number of fourth instar recovered	3	19	21	12	9	8
Deformed head capsules (%)	0	52.6	57.1	50.0	66.6	50.0
Single structure deformed (%)	0	26.3	28.5	16.6	55.5	25.0
Multiple structure deformed (%)	0	26.3	28.5	33.3	11.1	37.5
Antenna deformed (%)	0	10.5	9.5	8.3	0.0	0.0
Mentum deformed (%)	0	47.3	19.0	41.6	55.5	37.5
Mandible deformed (%)	0	0.0	4.7	16.6	0.0	12.5
Premandible deformed (%)	0	21.0	14.2	16.6	22.2	25.0
Epipharyngeal pecten deformed (%)	0	5.2	14.2	25.0	0.0	12.5
Ngaroto sediment						
Number of fourth instar recovered	36	41	26	51	35	9
Deformed head capsules (%)	5.5	39.0	57.6	50.9	40.0	66.6
Single structure deformed (%)	5.5	24.1	34.6	37.2	17.1	33.3
Multiple structure deformed (%)	0.0	4.8	23.0	13.7	11.4	33.3
Antenna deformed (%)	0.0	2.4	7.6	13.7	11.4	33.3
Mentum deformed (%)	2.7	17.0	30.7	24.5	22.8	55.5
Mandible deformed (%)	0.0	2.4	7.6	7.1	5.7	11.1
Premandible deformed (%)	0.0	9.7	23.0	19.6	17.1	33.3
Epipharyngeal pecten deformed (%)	2.7	12.1	23.0	13.7	25.7	11.1

Table 7.15. Comparisons of the head capsule deformity reported for the fourth larval instars of *Chironomus zealandicus* between test concentrations in a static pre-exposed bioassay on unspiked Lake sediment substrates. The deformities were compared with expected values in a Chi-squared test. NS-Not significant (P>0.05)

		P value		
		Total number of deformed head capsules	Single structure deformed	Multiple structure deformed
Factor	Sediment/test			
Concentration	Static test with pre-exposed larvae			
	Hamilton Lake sediment	NS	NS	NS
	Lake Ngaroto sediment	<0.001	0.011	<0.001

Table 7.16. Percentage of survival and each developmental stage of *Chironomus zealandicus* recovered at the end of 18 day static and static-renewal bioassay on arsenic-spiked shredded paper towel substrate. Each value is a mean of 9 replicates with SE in parentheses.

Nominal concentration of arsenic in g/m^3	0	2	4	8	16	32
Static test						
Survival (%)	90.0 (2.3)	68.0 (2.4)	64.0 (2.4)	52.0 (2.9)	52.0 (5.2)	42.0 (7.0)
Recovered as younger instars (%)	1.3 (0.7)	1.3 (0.7)	14.7 (4.8)	14.0 (2.0)	16.0 (4.6)	10.0 (8.1)
Recovered as 4th instars (%)	23.4 (5.5)	16.7 (7.7)	14.7 (4.8)	12.7 (2.4)	14.7 (4.4)	16.7 (0.7)
Recovered as pupae (%)	24.6 (4.1)	18.0 (2.0)	28.0 (5.3)	20.0 (2.3)	18.0 (2.3)	14.7 (1.7)
Recovered as adults (%)	40.6 (1.8)	31.4 (12.7)	7.34 (1.8)	6.0 (3.5)	4.0 (2.0)	0.7 (0.7)
Static renewal test						
Survival (%)	87.4 (0.7)	82.7 (4.0)	94.7 (0.7)	88.0 (3.1)	80.0 (1.2)	81.4 (0.7)
Recovered as younger instars (%)	0.7 (0.7)	4.7 (0.7)	10.0 (0.0)	14.7 (0.7)	11.3 (0.7)	28.0 (0.6)
Recovered as 4th instar (%)	20.6 (0.7)	30.0 (1.1)	40.0 (0.0)	52.6 (0.7)	52.0 (1.2)	48.6 (1.3)
Recovered as pupae (%)	33.4 (1.8)	25.3 (3.0)	32.0 (1.1)	14.7 (0.7)	12.7 (0.7)	4.0 (0.0)
Recovered as adults (%)	32.6 (1.8)	22.7 (0.7)	12.7 (1.8)	6.0 (1.1)	4.0 (0.0)	0.7 (0.7)

Table 7.17. Comparisons of survival and development between test types (static and static renewal) and sediment arsenic concentrations for *Chironomus zealandicus* in 18 day tests on arsenic-spiked shredded paper towel.
NS-Not significant (P>0.05)

	P values				
Dependent variable	Survival	Adults emerged	Pupae recovered	Fourth instar recovered	Younger instar recovered
Factors: Test type and Concentration					
P(Test type)	<0.001	NS	NS	<0.001	NS
P(Concentration)	<0.001	<0.001	<0.001	0.006	<0.001
P(interaction)	<0.001	NS	0.003	<0.001	0.014

Table 7.18. Head capsule deformity of fourth instar larvae of *Chironomus zealandicus* that were recovered at the end of 18 day static and static-renewal bioassays carried out on arsenic-spiked shredded paper towel substrate.

Concentration of arsenic in g/m^3	0	2	4	8	16	32
Static test						
Number of fourth instar recovered	35	25	22	19	22	25
Deformed head capsules (%)	34.1	44.0	45.0	31.5	40.9	60.0
Single structure deformed (%)	25.7	40.0	36.3	36.3	31.8	56.0
Multiple strucure deformed (%)	5.7	4.0	9.0	5.2	9.0	4.0
Antenna deformed (%)	0.0	8.0	18.1	5.2	9.0	8.0
Mentum deformed (%)	28.5	32.0	9.0	26.3	22.7	4.0
Mandible deformed (%)	5.7	0.0	13.6	5.2	0.0	4.0
Premandible deformed (%)	5.7	4.0	4.5	0.0	13.6	4.0
Epipharyngeal pecten deformed (%)	5.7	8.0	9.0	5.2	9.0	4.0
Static-renewal test						
Number of fourth instar recovered	31	45	60	79	78	76
Deformed head capsules (%)	9.6	48.8	55.0	48.1	47.4	51.3
Single structure deformed (%)	9.6	46.6	48.3	39.2	34.6	36.8
Multiple strucure deformed (%)	0.0	2.2	6.6	8.8	12.8	14.4
Antenna deformed (%)	3.2	8.8	10.0	11.4	10.2	13.1
Mentum deformed (%)	3.2	20.0	20.0	15.2	21.7	19.7
Mandible deformed (%)	0.0	6.6	5.0	3.7	6.4	5.2
Premandible deformed (%)	0.0	6.6	11.6	11.3	11.5	14.4
Epipharyngeal pecten deformed (%)	3.2	11.1	16.6	16.4	12.8	15.7

Table 7.19. Statistical comparisons of head capsule deformity reported for the fourth larval instars of *Chironomus zealandicus* between test concentrations in a static and static-renewal bioassay on arsenic-spiked shredded paper towel substrate. The deformities were compared with expected values in a Chi-squared test. NS-Not significant (P>0.05)

	P value		
	Total number of deformed head capsules	Single structure deformed	Multiple structure deformed
18 day static test	0.031	NS	NS
18 day static-renewal test	0.001	0.011	NS

Summary

Overall, results of the different bioassays indicate that *C. zealandicus* is again more resistant than *C.* sp. a to As. Arsenic is relatively more toxic in Hamilton Lake sediment than in Lake Ngaroto sediment in the spiked sediment bioassays. This suggests that As may be more bioavailable in Hamilton sediment which agrees with the higher measured pore water concentrations. However, Hamilton Lake sediment not spiked with As is also more stressful to *C. zealandicus* than Lake Ngaroto sediment in pre-exposed bioassays. The test conditions, prolonged exposure, extended endpoints, and renewal of the test medium all had considerable effects on the results of the toxicity tests.

In general, survival and growth and/or development of test animals varied more between nominal concentrations of arsenic than between other factors such as sediment type (Hamilton Lake, Lake Ngaroto), test species (*C. zealandicus*, *C.* sp. a), test duration (14 and 18 day) or test type (static and static renewal). These other factors affected test animals under some test conditions but not all the time. The occurrence of head capsule deformities was also observed to be significantly associated with arsenic concentrations in the substrate in most of the bioassays while other factors did not always show such a clear relationship.

7.4. DISCUSSION

Several physical and chemical parameters influence metal bioavailability. Ankley et al (1993) examined the effect of natural sediment physico-chemical properties on the results of laboratory tests with the amphipod *H. azteca*, the midge *C. tentans*, and the oligochaete *Lumbriculus variegatus*. These authors found that 10 day exposure on uncontaminated sediment with these three species differed markedly with regard to characteristics such as grain-size distribution, organic carbon content, and mineralogical composition. Food and feeding is one of the most important critical factors affecting the bioavailability of toxicants to the test organism. Survival of *H. azteca*, survival and growth of *C. tentans*, and survival, reproduction and growth of *L. variegatus* were significantly greater in tests in which the animals were fed than those in which they were not. A relatively high percentage of the tests in which *C. tentans* was not fed would have failed a control survival criterion of 70%. Hence, the authors concluded that there was significant potential for false positive results if *H. azteca* or *C. tentans* are not fed during sediment tests. In addition they found that growth of fed as well as unfed *C. tentans* may have been influenced by grain size distribution of the sediment.

Sibley et al. (1997) found that both larval and adult dry weight declined significantly with a reduction in food supply. Total emergence was reduced only at the lowest feeding level of 0.29 mg/individual/day whereas the rate of emergence declined with food supplies below 0.42 mg/ individual/day. The number of eggs per female declined significantly with a decrease in food supply below 0.42 mg/individual/day. Fecundity and expected number of progeny declined linearly with reduced food supply. The authors also found that the application of their data in a demographic model showed that the growth and predicted size of a population would decline significantly with a decline in larval growth and reproductive output.

In order to avoid or minimize all these complications, animals were fed in the present experiment throughout each bioassay at regular intervals at an optimum feeding rate that was determined through a series of preliminary laboratory culture practices.

At the beginning of the present experiment, the sensitivity of *C.* sp. a and *C. zealandicus* was assessed using a standard 10 day toxicity test under flow-through water renewal conditions, and survival and growth (by mean dry weight) were determined on arsenic-spiked sediment substrates from Hamilton Lake and Lake Ngaroto. *C. zealandicus* showed differences in survival with concentrations of arsenic, but did not show such differences with sediment type, nor was growth affected by either sediment type or arsenic concentration (Table 7.4). *Chironomus* sp. a showed a significant relationship between survival and growth with arsenic concentration and survival was also affected by the type of sediment. From this bioassay and the results of acute toxicity tests (Chapter 6), it appears that *C.* sp. a is generally more sensitive than *C. zealandicus* to arsenic. However, *C. zealandicus* was used as the test organism for further toxicity bioassays for several reasons, such as ease of culture and handling, and continuous availability in the wild throughout the year.

The length of exposure to the test substance is a critical consideration in sediment tests, for extended exposure is generally associated with greater sensitivity (Ingersoll and Nelson 1990). The effects of small toxicant doses are sometimes revealed in the form of sublethal effects after prolonged exposure. In addition, acute lethality tests are useful in identifying "hot spots" of sediment contamination, but these tests cannot be used to evaluate moderately contaminated areas where only chronic effects may occur. Concentrations of contaminants in sediments may not be lethal, but may interfere with the ability of an animal to develop, grow, or reproduce. A better understanding of the sublethal effects of chemicals in sediment is essential to identify areas with moderate contamination and evaluate chemicals that do not elicit acutely lethal responses. Furthermore, short exposure tests may be more appropriate in cases requiring a less sensitive and resource-intensive approach, which allows for a greater number of tests overall. Although there are rigorous methodologies to extrapolate the effect of acute short-term exposures to effect of long-term exposures on survival, growth and reproduction of aquatic organisms (Giesy and Hoke 1989), the value of long-term exposures in bioassays is obvious, despite possible complications arising from feeding to keep the test organisms alive (Wiederholm et al. 1987). The chronic, sublethal sediment bioassays are essential because bioaccumulation is a slow process which can affect reproduction, and benthic organisms generally experience such prolonged exposures to low contamination levels (Dillon 1993).

The duration of the exposure may have a profound influence on the response of organisms in sediment toxicity tests. The toxicity to *H. azteca* of sediment contaminated with PAHs and PCBs was evaluated after exposures of 2, 10 and 29 days in static and water-renewal exposures (Ingersoll and Nelson 1990). Survival of these amphipods was not reduced after a 2 day exposure, but was reduced by about 50 percent after a 10 day exposure, and was reduced by about 70 to 90 percent after a 29 day exposure. Body length of amphipods was reduced only in the 29 day exposure. In another experiment, the toxicity of contaminated Great Lakes sediment on *H. azteca* was also evaluated after 7, 14 or 28 day exposures (Nelson et al. 1993; Burton 1994). Survival and length endpoints were more discriminatory compared with sexual maturation. Effects after 28 days of exposure were much more severe than effects after 7 or 14 days of exposure.

Results of the present experiments agree with these findings. The sensitivity of *C. zealandicus* showed differences with test duration on two lake sediment substrates. Comparing the total survival between sediment types, there were significant differences for 14- and 18 day tests, but not in the 10 day test (Tables 7.5, 7.7). Although the comparisons between concentrations differ for all three test exposures, they were more significant after 14 and 18 day exposures ($P<0.01$) than after a 10 day exposure ($P<0.05$). Test conditions may have influenced the sensitivity as the 10 day tests were conducted under flow-through water renewal conditions whereas 14- and 18 day tests were carried out under static test conditions. Comparisons between 14 and 18 day tests show that differences in the number of adults emerged, and pupae and younger instars recovered, were more significant between the two sediments after an 18 day exposure than after 14 days. Clearly, however, test duration will affect recovery of later developmental stages.

Potential depletion of contaminants or changes in sediment during exposures may be a problem when conducting long-term tests. Although organisms in direct contact with sediment generally receive a substantial proportion of a contaminant dose directly from either the whole sediment or from the interstitial water, contaminant concentrations are reduced in the overlying water in water-renewal tests and depuration of sediment may occur. In static tests, although the contaminant concentration does not change

markedly, water quality may change profoundly during the exposure (Ingersoll and Nelson 1990). However, measured physico-chemical parameters did not change considerably between 14- and 18 day tests (Table A.4). Other characteristics of sediment, such as grain size, or the presence of other animals, e.g., predators, may affect the results of chronic exposures but this is considered unlikely in this case.

Measured total arsenic concentrations in pore water samples were relatively higher in Lake Ngaroto sediment than in Hamilton Lake sediment at the beginning of the spiked sediment toxicity tests. However, concentrations at the end of tests were higher in sediment from Hamilton Lake (Table 7.3). This may be due to the lower total organic matter (TOM) in this sediment. Therefore, the proportion of arsenic bound to sediment organic matter may be higher in Lake Ngaroto sediment and as a result, final arsenic concentrations in pore water were lower. When comparing the spiked sediments of the two lake sites, arsenic was more toxic in Hamilton Lake sediment than in Lake Ngaroto sediment. Percent survival was considerably less on Hamilton Lake sediment than on Lake Ngaroto sediment, in particular, at nominal concentrations of above 50 g/m^3. This difference in survival between sediment substrate types increased with arsenic concentration. This suggests that the bioavailability of arsenic is higher in Hamilton sediment than in Ngaroto sediment. Studies comparing the toxicity of metals in different exposure phases show that ions in overlying and pore-water are the main path of contamination for most benthic organisms, while the toxicity of sediment-bound metal is negligible (Cairns et al. 1984). Therefore, it is likely that the greater bioavailablility of pore water arsenic was the reason for the lower survival of *C. zealandicus* in Hamilton sediment.

The results of the pre-exposed test, in which *C. zealandicus* was raised in unspiked sediment show that Hamilton Lake sediment is more stressful than Lake Ngaroto sediment. Reduction of survival for a nominal arsenic concentration was comparatively higher on sediment from Hamilton Lake than from Lake Ngaroto (Tables 7.11, 7.12). This indicates that in addition to the spiked arsenic, some other factor that is already present in Hamilton Lake sediment contributes to its toxicity to *C. zealandicus*.

The choice of the appropriate endpoint (response) to measure is another important aspect in the assessment process. All toxicants do not affect the same metabolic

processes or result in the same effects, because they have varying modes of action and target receptors. Some toxicants may interfere with processes essential for reproduction or growth. Relative species sensitivity frequently varies among contaminants. Reish (1988) studied the relative toxicity of six metals (arsenic, cadmium, chromium, copper, mercury, and zinc) to marine crustaceans, polychaetes, and fishes, and found that no one species or group of organisms was the most sensitive to all of the metals. Mortality or survival is the most common endpoint used in standardized bioassays for the assessment of sediment toxicity (Traunspurger and Drews 1996) because it is an easily measurable and readily understandable response criterion, allowing comparisons among species and among chemicals or other variables within a species. Although mortality is a very amenable toxicological response, endpoints such as behaviour, reproduction and larval settlement are more sensitive than mortality for whole sediment single species assays (Luoma and Ho 1993).

Developmental criteria for benthic species such as growth, adult emergence and maturation rates are important toxicity variables in that they cover various life stages of the test organisms. However, the results can be highly variable and the bioassay may take too long for large-scale screening or monitoring work. Growth and development are used as endpoints in several bioassays for long term exposure to sediment samples. Kemble et al. (1993) found a correlation between reduction in length of amphipods and metal concentration in whole sediment and interstitial water tests. Amphipod length and benthic community evaluations both provided complementary evidence of metal-induced degradation in aquatic communities at study sites in the Milltown Reservoir and Clark Fork River in Montana. In an assessment of a metal contaminated sediment to *H. azteca,* Nelson et al. (1993) found that only a 7 percent of the samples showed reduced survival and 23 percent of the samples had reduced sexual maturation. However, 62 percent of the samples showed reduced length of the amphipods after 28 days of exposure. The authors concluded that the length of amphipods was a more sensitive endpoint than survival or sexual maturation.

Generally a reduction in growth is considered an adverse effect. In this experiment, development and growth (length of larvae recovered) showed a negative trend with nominal concentration of arsenic in 14- and 18 day sediment static tests. This effect was more significant on Hamilton Lake sediment. However, larvae clearly grew much

faster on this substrate, which accentuated this effect. This also illustrates that the major route of exposure to arsenic is likely to be via pore water rather than ingestion of organic matter. Although the percentage of total organic matter is higher in sediment from Lake Ngaroto than from Hamilton Lake, the effect of intra-sediment nutritional difference may be minimal in these bioassays since animals were fed regularly.

These examples suggest that additional endpoints have to be considered along with gross mortality or survival in correctly evaluating the sensitivity of a test organism to a toxicant. The results of the present experiment support the above statement. Total survival (or mortality) showed no effect due to test duration (length of exposure) either on Hamilton Lake sediment or on Lake Ngaroto sediment. However, an effect related to test duration was observed when the surviving developmental stage of the animal (adults emerged and pupae, 4th instars, and younger instars recovered) was considered separately.

The relationship between environmental contamination and chironomid head capsule deformity is relatively well established. Van Urk et al. (1992) found that *C. plumosus* larvae occurred at lower population densities and with higher frequencies of deformities as environmental contamination levels increased. Dickman et al. (1992) found a significantly higher frequency of mentum deformity in coal tar-contaminated sediments than at reference sites. However, although chironomids have been used in sediment toxicity tests for a long time, only very few studies have examined the effect of contaminants on head capsule deformity in the laboratory under controlled conditions. Hudson and Cyborowski (1996) found that the incidence of mentum deformities increased with increasing proportions of contaminated Trenton channel sediment. Michailova and Belcheva (1990) exposed *Glyptotendipes barbipes* to sediments spiked with two concentrations of lead and found that there was an increase in malformations of the teeth of the mandibles and sub-mentum, and in the shape of genitalia and wings of emergent adults. In another laboratory study, Kosalwatt and Knight (1987) reported that sediments heavily spiked with copper caused deformities in the epipharyngeal plate of larval mouthparts.

The greater occurrence of head capsule deformities with exposure to arsenic reported in this experiment is comparable with the results of the above studies. The incidence of

deformity was significantly different between concentrations of arsenic in the 18 day test, but not in the 14 day test, on Hamilton Lake sediment (Tables 7.9, 7.10). Although the difference in proportion of head capsules deformed between concentrations was significant in both 14 and 18 day exposures on Lake Ngaroto sediment, the difference was more significant ($P<0.01$) in the 18 day test than in 14 day ($P<0.05$) test. This may be due to the slightly higher arsenic concentrations measured in the Lake Ngaroto sediment at the start of the bioassay. Results of exposure to arsenic for a brief period during early life followed by culture on clean sediment indicate that even quite low arsenic concentrations can induce significant abnormality in later larval stages. The effect of sediment type seems minimal since head capsule deformity did not show any significant differences between sediments in spiked sediment bioassays or in unspiked sediments with pre-exposed test animals (Tables 7.14, 7.15).

From the results of the above bioassays with *C. zealandicus,* it is obvious that length of larval instars, developmental stage, and the occurrence of head capsule deformity of 4th larval instars are more sensitive endpoints than survival and growth (by mean dry weight).

Metals may be more bioavailable in synthetic sediments than in natural sediment substrates. This is a potential disadvantage associated with the use of synthetic sediments, limiting comparisons of laboratory and field observations particularly in metal toxicity testing (Harrahy and Clements 1996). However, depending on the objective of a bioassay, synthetic sediments may be used to evaluate the toxicity of a chemical under controlled conditions. Shredded paper towel was used successfully in establishing laboratory cultures of test chironomid species for more than three years. The paper towel substrate was prepared for As spiked tests as it was prepared for the laboratory culture (Chapter 3). Bioavailability of As in this bioassay may not be the same as in tests with natural lake sediment. However, the effects of other biological (other organisms in sediment), physical (grain size, composition) and chemical (other compounds, varying organic carbon) factors were minimized in determining the effect of arsenic on *C. zealandicus*. The percentages of survival, each developmental stage and occurrence of head capsule deformity all showed a significant relationship with concentration of spiked substrate arsenic in both static and static-renewal toxicity tests (Tables 7.16, 7.17, 7.18, 7.19).

Water renewal affected the toxicity of arsenic while other factors such as grain-size distribution, organic carbon content, and mineralogical composition remained typically unchanged during toxicity tests with *C. zealandicus* on arsenic-spiked shredded paper towel substrate. Although the percentages of developmental stages, except the 4th instar larvae, showed no difference between static and static renewal conditions, total survival showed a significant difference. Survival was considerably reduced in static tests with differing arsenic concentration. Only 50% of animals survived at the highest spiked arsenic concentration under static conditions compared with static renewal conditions. This is comparable with results of previous studies. For example, Williams et al. (1986) found that a continuous-flow testing procedure provided less "stressful" conditions for the 4th instar larvae of *C. riparius* than a static-replacement procedure in acute toxicity tests with Cd. In the present study with paper towel substrate under static renewal conditions, the overlying test solution was replaced with a freshly prepared solution of the appropriate arsenic concentration. Furthermore, measured physico-chemical parameters did not vary appreciably between static and static-renewal tests with paper towel substrate. Therefore, the differential survival of test chironomids in both tests may not necessarily be due to the bioavailability of the As. One of the possible reasons for the difference in toxic effect may be the accumulation of wastes in test chambers under static conditions. Water renewal conditions were also observed to affect the relationship between spiked-arsenic concentration and head capsule deformity. The head capsule deformity and arsenic concentration relationship was more significant in static renewal exposure ($P<0.005$) than in static exposure ($P<0.05$). This is mostly due to high levels of deformity in control animals under the latter conditions that may be due to waste accumulation during the test. No difference was observed in head capsule percentage deformity between static and static-renewal conditions for a particular concentration of arsenic.

There are a few limitations associated with laboratory bioassays used for sediment toxicity. These fall into four categories (Lamberson et al. 1992): (1) alterations of the toxicological properties of the sediment during sampling and handling; (2) sensitivity of test organisms to natural sedimentary features and laboratory conditions; (3) toxicological uncertainities arising from the narrow range of contaminants as yet tested and from the difficulties in measuring exposure concentrations; and (4) poorly

understood ecological interactions and relevance. Minimizing these problems and the potential for over and underestimation of toxicity (Luoma and Carter 1993) have been, and still remain, challenges in the development of standardized protocols for sediment bioassays. The results of toxicity tests on the same organism can vary depending on the exposure phase used (Chapman and Fink 1984; McCauley et al. 1992; Green et al. 1993; West et al. 1993). Different strengths and weaknesses are associated with these procedures and some exposure modes may be appropriate in some cases but not in others (Burton 1991).

Many authors have found that tests with benthic species involving direct exposure to a sediment are more adequate and ecologically relevant for an assessment of sediment toxicity than tests in isolated liquid phases (Lamberson and Swartz 1988; Ankley et al. 1990). Static bioassays have long been used to test experimentally spiked sediments (Cairns et al. 1984) and field-contaminated samples (Wentsel et al. 1977a, 1977b and 1988). Many authors agreed that the diagnosis of sediment quality should be centered around whole sediment analyses, with pore-water and elutriate tests only as complementary sources of information (Chapman and Fink 1984; Lamberson and Swartz 1988; Traunspurger and Drews 1996).

In conclusion, the toxicity of a chemical to a test organism depends on several biological, physical and chemical factors. The sensitivity of a test organism to a particular toxicant may vary depending on the length of exposure to the toxicant, endpoint concerned, test media used, and laboratory testing conditions. This experiment further strengthens the results of Chapter 5 and 6 to show that *C. zealandicus* is more resistant than *C.* sp. a to As. Arsenic adversely affected survival, growth, and development of *C. zealandicus* and *C.* sp. a. Arsenic also induced head capsule deformities in *C. zealandicus*. However, the specific type (e.g., missing tooth, split in tooth, reduced segment) and severity (weak and strong deformation) of the deformity induced by As was not considered in the present study. These aspects will be useful topics for future research.

Sediment from Hamilton Lake was more stressful with or without spiking of As than that from Lake Ngaroto. This may be due to differential bioavailability of contaminants. The higher concentrations of pore water As at the end of the spiked

sediment bioassays may be related to the reduced survival of *C. zealandicus* in Hamilton Lake sediment while the slightly higher initial concentrations in Lake Ngaroto sediment may be responsible for greater head capsule deformity. Although this implicates pore water arsenic as the most logical route of exposure, this study did not unequivocally indicate whether the pore water or sediment bound As fraction is more bioavailable to *C. zealandicus*.

The sensitivity of *C. zealandicus* to arsenic was affected by the renewal of overlying water in the laboratory toxicity tests. Furthermore, *C. zealandicus* became more sensitive to arsenic when exposed for an extended period.

Eighteen day tests measuring mortality, growth, developmental life stage, and head capsule deformity as endpoints may be used under static or static renewal conditions to test the toxicity of contaminated sediments to *C. zealandicus*. This combination of test conditions appears to be more effective than the standard 10 day chronic exposure tests described for *Chironomus* in the past.

Conclusion and Summary

Three species of chironomid, *Chironomus zealandicus, Chironomus* sp. a, and *Polypedilum pavidus* were studied for their potential as biological indicators of aquatic environmental pollution in New Zealand. This included investigation of both wild-collected and laboratory reared animals.

Although chironomids are one of the major components of the benthos at the four study sites, the species of interest were not equally abundant throughout the study period at the study sites. *Chironomus zealandicus* was present at very high densities while no other invertebrate was observed at Sulphur Point in Lake Rotorua. Physico-chemical parameters such as relatively elevated temperature due to the geothermal activity, high conductivity, and low dissolved oxygen and pH that prevail at this site may be the probable cause for this. Although all three species were observed at the other study sites, *C* sp. a and *P. pavidus* dominated the benthic macroinvertebrate community of Lake Ngaroto and Hamurana Stream respectively. Chironomid density was relatively lower at Hamilton Lake compared with the other three sites. This may be because of a higher level of pollution of this lake site. Cultures of these chironomids were established in the laboratory in order to obtain all larval stages of test species for laboratory bioassays throughout this study.

Advantages and disadvantages of using Chironomidae as indicator organisms have been described in previous studies (Rosenberg 1992; Clarke et al. 1995; Warwick 1985; USEPA 1994b). Chironomids were easily collected using relatively simple methods in this study and observed to be considerably robust for handling. Among the three species, *C. zealandicus* appeared to be more tolerant of physical handling during bioassays.

A common method was employed to create and maintain cultures of all three species of chironomid. Methods for culturing chironomids relied on regular attention, availability of suitable food, and the maintenance of culture media throughout (Credland 1973). An appropriate combination of biological and non-biological parameters was determined

after a period of 4-months of trial and error to establish reliable cultures in the laboratory. Parameters include food type (TetraMin flakes), feeding, aeration, photoperiod, substrate, stocking density of larvae and adults, and the maintenance of aquaria and mating cages.

An attention of $2^1/_2$ - 3 hours per day was needed to maintain about 4 stocking tanks, 8 rearing aquaria and 4 mating cages in the laboratory. An average of 15 to 20 egg masses was collected daily from each mating cage. These are important considerations for potential establishment of cultures for standard ecotoxicological testing.

One of the advantages of using chironomids as bioindicators is that each specimen allows examination of additional sublethal endpoints such as deformity in head capsule structures and thickness of cuticle, in addition to the standard endpoints (survival, growth, reproduction). Head capsule deformity and physical wear were examined for a wild population of *C. zealandicus* collected from the study sites in 3 summers and 2 winters during this study.

Significant differences were observed in the head capsule percentage deformity between sites and seasons. Individuals from the pristine Hamurana Stream site had the lowest incidence of deformities while deformities were uniformly common at the other sites. Generally, the percentage deformity declined in winter. Results indicated that the substrate type contributed to physical wear of mouthparts and this was more common in the coarse sand substrate at Hamurana Stream. Deformity and physical wear of larvae in laboratory cultures on a paper towel substrate indicated no clear pattern of substrate effect on the incidence of deformity, although some structures may be more sensitive indicators of substrate physicochemistry than others. However, percentage deformity declined with successive laboratory generations. Physical wear of larvae cultured on paper towel substrate was significantly lower than when animals were cultured in sediment from Sulphur Point. This supports the hypothesis that coarse sand substrate contributed to the physical wear observed.

Arsenic increased the incidence of head capsule deformities in the laboratory toxicity tests. Although the present study doesn't give any indication of contaminant specificity in the percentage head capsule deformities of larvae collected from the wild, it reveals

that care must be used in interpreting such findings particularly where comparisons are made between different substrate types and seasons. There are indications that substrate type, season, and genetic factors, as well as sediment chemistry may collectively contribute to the incidence of head capsule abnormalities in *C. zealandicus*. The influence of specific physicochemical aspects of the environment on larval development and the susceptibility of particular headcapsule structures to such influences need further study.

The acute toxicity of arsenic (As^{3+} and As^{5+}) and copper (Cu^{2+}) to chironomid species under differing conditions was investigated in this study. A number of biological (age), chemical (valency state), and physical (season, temperature) factors were observed to affect the toxicity of arsenic and copper to the tested chironomid species. Although *C. zealandicus* and *C.* sp. a are taxonomically closely related, they showed considerable difference in sensitivity to arsenic and copper. As^{3+} was more toxic than As^{5+} to all three species. *Chironomus zealandicus* was the most tolerant species to both forms of arsenic and to copper of the three species tested. Earlier instars were more sensitive than the later instars of each species to arsenic. Furthermore, wild-collected 4th instar larvae of all three species were more resistant than the laboratory-cultured population of the same age to both forms of arsenic. No direct relationship was observed between temperature and the toxicity of arsenic with these test animals. It was found that there was a difference in sensitivity of the test species to arsenic depending on the season when they were collected from the wild. *Chironomus* sp. a showed relatively little variation in sensitivity compared with the other two species to both forms of arsenic with either temperature or season.

This study illustrates the importance of examining the toxicity of a substance under different biotic and abiotic conditions. Investigation with as many life stages as possible is essential in order to set accurate water quality standards for the protection of the aquatic environment. This study has shown that the use of the fourth instar larvae in acute toxicity tests is a practice that may seriously underestimate the contaminant sensitivity of these species, and this should not be appreciated in tests that are used in generating data for water quality criteria and standards. Factors such as the effect of season (summer and winter) and the differing origin of a particular species (wild-

collected vs laboratory-cultured) may also be considered as important factors in evaluating the toxicity of substances.

The chronic tests with sediments from Hamilton Lake and Lake Ngaroto and paper towel substrate on *C. zealandicus* and *C.* sp. a included the study of both arsenic spiked and unspiked substrates. This experiment indicated that *C.* sp. a was more sensitive to arsenic spiked sediment than *C. zealandicus*. Sediment and paper towel substrates that were spiked with arsenic (As^{5+}) influenced the survival, growth, and/or development of *C. zealandicus.* Arsenic was observed to induce head capsule deformities in the larval instars of *C. zealandicus*. However, this study did not establish a dose-response relationship between arsenic concentration and head capsule deformity. Whether the arsenic induces any specific type of deformity in head capsules may be a valuable subject for a future research.

The sensitivity of *C. zealandicus* to arsenic was affected by the renewal of overlying water in the laboratory toxicity tests. In addition, *C. zealandicus* became more sensitive to arsenic-spiked sediments when exposed for an extended period and when more test endpoints were considered. The effect of arsenic appeared to be relatively higher in sediment from Hamilton Lake than from Lake Ngaroto.

The 18 day tests using mortality, growth, developmental life stage and head capsule deformity as endpoints can be used under static or static renewal conditions to test the toxicity of contaminated sediments to *C. zealandicus*. This combination of test conditions appears to be more effective than the standard 10 day chronic exposure tests previously described for other *Chironomus* species. The sensitivity of the test organisms therefore varied depending on the length of exposure to the spiked sediment, the number of endpoints examined, and other laboratory testing conditions such as the renewal of dilution water.

Unspiked sediment tests studied the influence of lake sediments on the survival and emergence of *C. zealandicus* and *C.* sp. a. Results showed that the sediment substrates from Hamilton Lake, Lake Ngaroto, Hamurana Stream, and Sulphur Point of Lake Rotorua influenced survival or emergence or both of *C. zealandicus* and *C* sp. a. No difference was observed in survival or growth of these two test species between

sediment type in 10 day tests. However, results of 37 day emergence tests showed that survival and the percentage of larvae successfully emerged as adults were higher for *C. zealandicus* on the Sulphur Point sediment, and for *C.* sp. a on Lake Ngaroto and Hamurana Stream sediments. Emergence of *C. zealandicus* was delayed on Hamurana Stream sediment by 6 days while that of *C.* sp. a was delayed on Sulphur Point sediment by 10 days.

Sediment substrate appears to also influence the species of chironomid that inhabit it. However, a unique sediment property that affects chironomids could not be identified from this study. One or a combination of chemical (e.g., contaminants and organic carbon content) and physical (e.g., particle size distribution) factors of the sediments could possibly be implicated. In addition, it is not known how the results of the emergence experiment compare with the natural emergence pattern of test species at each site. The laboratory conditions may have altered the natural emergence pattern of the test species. An emergence study using mesocosms may be a useful topic for future study.

This work shows that either one or more of the chironomid species used in this study may be used as bioindicators in monitoring freshwater lakes. Although *C.* sp. a was more sensitive than the other two species to arsenic and copper, the other two species have the advantage of relatively higher tolerance to physical handling during bioassays. Between the other two species, *P. pavidus* is comparatively smaller than *C. zealandicus* and early larval instars are relatively harder to distinguish from each other. However, *P. pavidus* is relatively easy to culture in the laboratory and the hatchability of egg masses of *P. pavidus* was higher than that of *C. zealandicus*. *Chironomus zealandicus* is the most convenient species to use in laboratory tests, due to its size and tolerance to physical handling. The possession of two pairs of ventral tubules at the eighth abdominal segment allows for easy identification, particularly at older larval stages. However, it is relatively harder to culture in the laboratory and the hatchability of egg masses is lower than that of the other two species. Regular re-stocking with wild-collected larvae of *C. zealandicus* is essential to maintain a reliable culture in the laboratory and, in particular, if a large number of larvae is needed for laboratory studies. In addition, since there was a differential abundance of chironomid species at the different lake sites observed, the suitability of a particular species for bioassay use

should be assessed taking into account its occurrence in suitable sediments or sites in the wild. Depending on the type of bioassay required, an appropriate species can be selected from these three chironomids.

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Appendix one: System parameters of atomic absorption spectrometers and particle size analyser

Table A.1 System details of the laser mastersizer used to determine particle size distribution of site sediments.

	Range lens (mm)	Beam length (mm)	Sampler	Obstruction (%)	Residual (%)	Analysis mode	Modifications
Hamilton	300 RF	2.40	MS 17	26.3	0.49	Poly disperse	None
Ngaroto	300 RF	2.40	MS 17	27.1	0.43	Poly disperse	None
Hamurana	1000	2.40	MS 17	13.6	0.81	Poly disperse	None
Sulphur Point	1000	2.40	MS 17	17.5	0.79	Poly disperse	None

Table A.2. Instrument parameters for flame AAS

Analysis parameter	Element		
	As	Cu	Cr
System type	Flame	Flame	Flame
Matrix	HNO_3/H_2O_2	HNO_3/H_2O_2	HNO_3/H_2O_2
Lamp current (mA)	10.0	4.0	6.0
Wavelength (nm)	193.5	324.7	357.9
Slit width (nm)	1.0	0.5	0.2
Read time (sec)	3	3	3
Replicates	3	3	3

Table A.3. Instrument parameters for Graphite Furnace AAS

Analysis parameter	Element		
	As	Cu	Cr
System type	Furnace	Furnace	Furnace
Matrix	HNO_3/H_2O_2	HNO_3/H_2O_2	HNO_3/H_2O_2
Lamp current (mA)	8	4	6
Wavelength (nm)	193.7	324.7	357.9
Slit width (nm)	1.0	0.5	0.2
Number of injections	1	1	1
Injection speed (µl/s)	9	9	9
Sampling mode	Auto sampling	Auto sampling	Auto sampling
Read time (s)	1.5	1.5	1.5
Replicates	3	3	3

Appendix two: Physico-chemical parameters measured during bioassays with arsenic spiked substrates (Chapter 7)

Table A.4. Dissolved oxygen (DO), temperature, conductivity and pH recorded at the beginning and end of bioassays of Chapter 7 (value at the beginning / value at the end). Each value is an average of three experiments with SE in parentheses.

DO (mg / l)	Temperature (°C)	Conductivity (μs/cm²)	PH
Flow-through test with *Chironomus zealandicus* on Hamilton sediment			
8.12 / 8.24	23.1 / 23.2	154 / 155	7.23 / 7.03
(0.06 / 0.07)	(0.46 / 0.15)	(5.61 / 6.33)	(0.02 / 0.09)
Flow-through test with *Chironomus zealandicus* on Ngaroto sediment			
8.17 / 8.16	22.9 / 23.2	162 / 151	7.16 / 7.04
(0.04 / 0.04)	(0.09 / 0.28)	(10.6 / 3.76)	(0.02 / 0.02)
Flow-through test with *Chironomus* sp. a on Hamilton sediment			
8.12 / 8.24	23.3 / 23.6	154 / 147	7.21 / 7.29
(0.11 / 0.06)	(0.46 / 0.22)	(1.45 / 2.65)	(0.06 / 0.12)
Flow-through test with *Chironomus* sp. a on Ngaroto sediment			
8.21 / 8.27	23.1 / 23.4	154 / 161	7.31 / 7.1
(0.09 / 0.09)	(0.25 / 0.18)	(3.48 / 11.9)	(0.05 / 0.04)
14 day static test with *Chironomus zealandicus* on Hamilton sediment			
8.19 / 8.13	23.2 / 23.7	155 / 201	7.21 / 7.17
(0.04 / 0.01)	(0.56 / 0.07)	(2.89 / 6.33)	(0.08 / 0.02)
14 day static test with *Chironomus zealandicus* on Ngaroto sediment			
8.12 / 8.02	22.8 / 22.8	151 / 168	7.18 / 7.17
(0 / 0.15)	(0.42 / 0.19)	(3.48 / 4.1)	(0.01 / 0.02)
18 day static test with *Chironomus zealandicus* on Hamilton sediment			
8.08 / 8.17	23 / 22.2	152 / 153	7.12 / 7.23
(0.01 / 0.02)	(0.2 / 1.11)	(1.67 / 2.33)	(0.03 / 0.02)
18 day static test with *Chironomus zealandicus* on Ngaroto sediment			
8.16 / 8.28	23.1 / 23.3	159 / 149	7.16 / 6.94
(0.04 / 0.11)	(0.23 / 0.26)	(1.2 / 2.52)	(0.13 / 0.11)
Pre-exposed test with*Chironomus zealandicus* on Hamilton sediment			
8.41 / 8.22	23.1 / 23.2	156 / 145	7.07 / 7.25
(0.04 / 0.05)	(0.43 / 0.38)	(4.7 / 2.52)	(0.05 / 0.03)
Pre-exposed test with *Chironomus zealandicus* on Ngaroto sediment			
8.2 / 8.42	23.3 / 23.2	157 / 152	7.137.13
(0.17 / 0.12)	(0.46 / 0.35)	(1.45 / 2.03)	(0.07 / 0.08)
18 day static test with *Chironomus zealandicus* on paper towel substrate			
7.75 / 8.45	22.6 / 23.3	153 / 152	7.15 / 6.99
(0.42 / 0.1)	(0.15 / 0.37)	(2.08 / 6.03)	(0.14 / 0.1)
18 day static-renewal test with *Chironomus zealandicus* on paper towel substrate			
8.1 / 8.19	23.5 / 23.4	153 / 172	7.14 / 7.18
(0.05 / 0.03)	(0.33 / 0.45)	(3.21 / 9.84)	(0.02 / 0.06)

Appendix three: Abstracts of articles and conference presentations arising from this study

Abstract 1:

New Zealand Journal of Marine and Freshwater Research. Volume 31: 175-184 (1997).

Head capsule deformities in *Chironomus zealandicus* (Diptera: Chironomidae); influence of site and substrate

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The natural incidence of deformities in the larval head capsules of *Chironomus zealandicus* was investigated at four lake sites in the central North Island in the summer (December 1994) and winter of 1995 (June 1995). Significant differences were observed between sites and seasons. Individuals from the peat lakes Ngaroto and Hamilton (Rotoroa) had the highest incidence of deformities during summer (78% and 48% respectively) and these declined significantly in winter (40% and 26%). No seasonal differences were observed in larvae from the two sites chosen in Lake Rotorua. Individuals close to the Hamurana Stream in Lake Rotorua showed the lowest number of malformed head capsules during summer (32%). In order to assess the influence of substrate type, larvae from two different locations were cultured through their entire life cycle in three different substrates. Results overall indicated no clear pattern of substrate effect although some structures may be more sensitive indicators of substrate physicochemistry than others. The incidence of deformity tended to decline with successive laboratory culture.

Abstract 2:

Annual Conference of the New Zealand Limnological Society, Nelson, New Zealand: 30th June to 4th July 1997

Effect of Substrate on the Emergence of Two Species of Chironomid

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We have been examining the effect of site and substrate on developmental anomalies in lake chironomids from sites in the Waikato regoin. We studied the effect of substrate on the emergence of two chironomid species. Laboratory cultured specimens of *Chironomus zealandicus* and *Chironomus* species *a* were allowed to grow on sediments of four lake sites namely, Hamilton Lake, Lake Ngaroto, and Sulphur Point and Hamurana stream sediment from Lake Rotorua, with over lying soft synthetic water. Each experiment was conducted using 100 first instar larvae collected from a single egg mass. Experiments were performed in triplicate. We found that the emergence of *Chironomus zealandicus* on Hamilton Lake and Lake Ngaroto sediment was delayed and reduced in number. Emergence of *Chironomus* sp. *a* was delayed and reduced on the Sulphur Point sediment from Lake Rotorua. The maximum number of adults emerged from aquaria containing Hamurana stream sediment. Although the male adults emerged first in all aquaria, there was no effect of substrate on emerged sex ratios.

Abstract 3:

Fourth Annual Conference of the Australian Society for Ecotoxicology, Brisbane, Australia: 17th to 19th July 1997

Sensitivity of Three Species of New Zealand Chironomids (Insecta: Chironomidae) to Arsenic

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Chironomids are important benthic macro invertebrates in biomonitoring fresh water habitats. *Chironomus zealandicus, Chironomus sp. a* and *Polypedilum pavidus* are three common chironomid species in soft sediments of lakes in the central North Island of New Zealand. These chironomid species were cultured in the laboratory to obtain different larval instars for this study. Four larval instars of each species were tested for their sensitivity to two forms of arsenic (As^{3+} and As^{5+}).

Median lethal concentrations indicated differences in sensitivity to As^{3+} and As^{5+} between larval instars and between different species. As^{3+} was found to be approximately five times more toxic than As^{5+} to all four larval instars of all chironomid species. Fourth instar larvae collected from the wild were more tolerant to both As3+ and As^{5+} than laboratory cultured animals of the same age.

Sensitivity of fourth instar larvae to arsenic at three temperatures (13°C, 18°C, 23°C) was also tested. No clear relationship was observed between median lethal concentrations and temperature. However, temperature shock was observed to have an impact on the sensitivity of chironomids to arsenic.

Abstract 4:

8th Annual Meeting, Society of Environmental Toxicology and Chemistry, Bordeaux, France: 14th to 18th April 1998

Toxicity of Arsenic to *Chironomus zealandicus* (Insecta: Chironomidae) under the Laboratory Test Conditions

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Chironomids are important benthic macro invertebrates in biomonitoring fresh water habitats. *Chironomus zealandicus* is one of the common chironomid species in sediments of lakes in the Central North Island of New Zealand. Ten and fourteen-day laboratory tests were conducted either with or without renewal of water to assess the toxicity of arsenic (As^{5+}) to *C. zealandicus* on sediments from Hamilton Lake and Lake Ngaroto. Tests were started with 10 second instar larvae and replicated nine times. We found that laboratory test conditions affect the toxicity of arsenic to *C. zealansdicus*. *C. zealandicus* showed difference ($P<0.05$) in survival but did not show difference in growth between concentrations of arsenic on sediments from lakes in the water renewal test. *C. zealandicus* showed difference ($P<0.05$) in survival, growth and incidence of head capsule deformity between arsenic concentrations on both sediments, in a 14-d static toxicity test. Survival, growth and incidence of head capsule deformity showed difference ($P<0.05$) between the concentrations of arsenic when the test animals were exposed to different concentrations of arsenic for the first 96 hours and then allowed to grow on lake sediments under the static condition.