Chironomids as Indicators of Biological Quality: Present Use and Future Potential

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Thames Region Operational Investigation

Chironomids as Indicators of Biological Quality: Present Use and Future Potential

A Report of a Workshop Held at the Environment Agency - Thames Region on 18 July 1995

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Statement of use
This document is to provide Agency biologists with information about methods for assessing sediment quality using chironomids, to help the Agency identify those which are potentially of greatest use to it, and to identify where further development is needed to make the methods suitable for operational use.

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EXECUTIVE SUMMARY

This report describes research on chironomid ecology in relation to environmental quality assessment that was being undertaken by most of the principal workers in this field in Britain. It is the result of a workshop held on the 18 July 1995 at the Environment Agency, Thames Region. The areas covered in the workshop included:

acute lethal and chronic sub-lethal toxicity test methods;

the use of morphological deformities as a sub-lethal indicator of sediment quality;

the use of chironomid pupal exuviae as a means of using chironomid assemblages for assessing water and sediment quality.

As well as describing individual research projects that were being undertaken, the report includes a summary of the discussion held at the workshop. This was on the ways in which the various approaches could be linked to provide an assessment of sediment quality based on all levels of organisation, from community to individual organism.

The information presented in the workshop has been used to map a programme of work to develop a biological index of sediment contamination as part of an integrated approach to freshwater sediment quality assessment.

KEY WORDS

Chironomids, toxicity tests, development, growth, lethality, morphological deformities, pupal exuviae.

1. INTRODUCTION

This report has been produced as a result of a meeting of people actively working on the use of chironomids for environmental assessment. The participants are listed in Appendix A. The purpose of this document is to provide biologists and water quality managers, within the Environment Agency and outside, with a 'state of the art' overview of the use of chironomids for environmental evaluation. It includes a brief summary of current research, present uses and an indication of where future work is needed in order to develop methods for operational use.

The British chironomid fauna comprises more than 450 species in 120 genera (Smith 1989). This abundance, their ubiquity in freshwaters of all types and qualities and their commensurate breadth of ecological niches means that chironomids are potentially a good taxonomic group for monitoring water quality, both in terms of its pollution and trophic status. In particular chironomids are likely to be good indicators of sediment quality, because the aquatic larvae of most species live in the sediments and many ingest silt. Sediment toxicity is of increasing concern to regulators as it has now been recognised as a major contributor to the degradation of water quality and decline in biota (Adams *et al.* 1992). It is a limiting factor to the restoration of environmental quality in rivers affected by heavy industry, such as the River Mersey (NRA 1995) and the Rhine (van de Guchte 1992).

Methods of environmental assessment using chironomids cover a spectrum of techniques from sub-lethal and lethal effects on individuals, through evaluations of sub-lethal toxicity (for example morphological deformities) on populations, to evaluations of effects on benthic communities.

At the individual level, sub-lethal and lethal toxicity test methods have been developed using larval *Chironomus riparius* as part of the Environment Agency's Method Development programme. Section 2.1 describes the development of an acute lethal test of 10 days duration and a chronic sub-lethal test measuring growth and lethality over a 28 day exposure. The application of sediment toxicity tests in water quality management is also discussed.

Long-term chronic toxicity tests for detecting sub-lethal effects may be supplemented by measurements of developmental abnormalities within benthic populations. Deformities in the antennae and mouthparts, such as the mentum, ligula, mandibles, premandibles and the epipharyngeal comb, have been recorded in larvae of the genera *Procladius*, *Chironomus* and *Cryptochironomus* (Warwick 1985, 1988, 1989, 1990). Deformities can range from mild abnormality, such as the addition or deletion of a single tooth, to massive thickening and fusing of structures (see Figure 1.1). However, pollutant-induced deformities have to be distinguished from the normal wear or breakage of mouthparts, which occur due to feeding activities in coarse sediments. Deformities alter the symmetry of the mouthparts, while normal wear can be recognised by abrupt breaks within the overall symmetry of the head capsule.

Positive correlations between chironomid deformities and the degree of sediment contamination have been established (for example Wiederholm 1984; Warwick 1985; Klink

1989; Pettigrove 1989), though these relationships are only qualitative. The Murray-Darling Basin Commission in Australia (Bennison *et al.* 1989) has investigated mouthpart deformities as indicators of chronic sub-lethal toxicity. Their application in this role is considered further in Section 2.2.

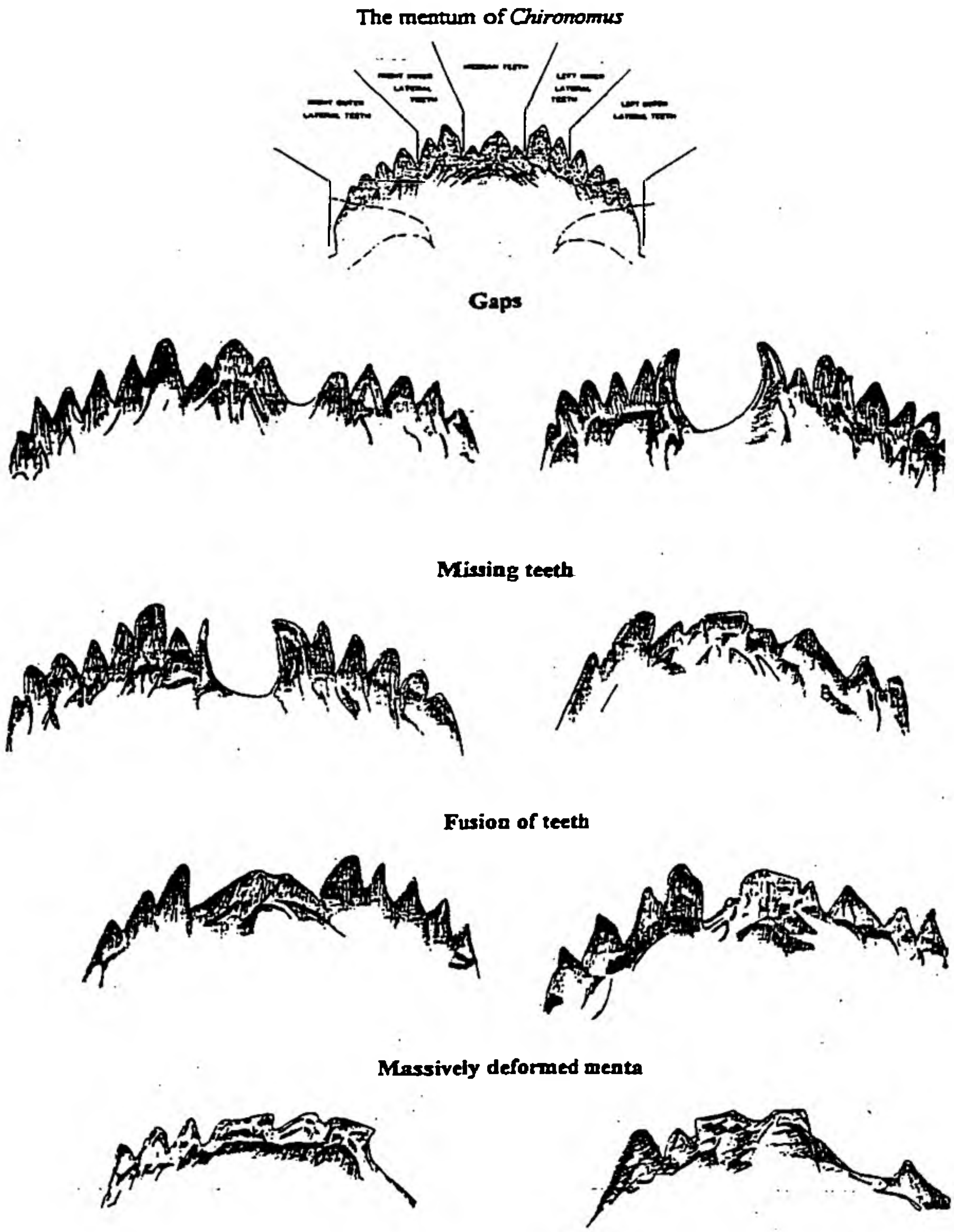

Figure 1.1 Types of mouthpart deformities observed in chironomids

Despite their abundance, diversity, and ubiquity, chironomid communities have not been used widely for monitoring water quality. Red chironomid larvae (possessing haemoglobin) are sometimes differentiated from other chironomid larvae because, when abundant, they indicate low oxygen concentrations. However, biologists rarely identify larval chironomids even to sub-family. Since they are not differentiated, the ability of different chironomid taxa to indicate environmental quality is not exploited. Identification beyond sub-family or tribe is perceived to be relatively difficult. Advances have been made in the taxonomy of chironomid larvae and Wiederholm (1983) has published a key to sub-families and genera. However, the need to mount specimens on slides makes identification comparatively time-consuming, and the keys in Wiederholm (1983) are not user friendly.

Adult chironomids are not used for assessing environmental quality, even though their taxonomy is relatively well known and keys have been published for British species (Pinder 1978). The same is true for most other aquatic insect taxa, though the use of caddis to assess water quality in large rivers is an exception (Malicky 1981; Chantaramongkol 1983). This is a reflection of the ease of sampling the aquatic stages in most environments and the success of assessment methods based on the aquatic stages. Furthermore, few biologists in the water industry are familiar with adult insect morphology and terminology.

Consequently, most of the work relating chironomid communities to water quality has been based on the use of chironomid pupal exuviae (cast pupal skins), because the pupae are much more easily identified than larvae and the exuviae are easy to collect. Pioneering research was undertaken in the 1970s by a group at Bristol University led by Ron Wilson, with funding from the Department of the Environment (DoE) (see Wilson and McGill 1979, Wilson 1980). This work resulted in considerable progress into the association between chironomids and water quality, and advances in the identification of chironomid pupae (Langton 1984, 1991). The culmination was the production of a simple identification guide to the British genera of chironomids as pupae with an index of pollution tolerance of each genus aimed at environmental biologists in the water industry (Wilson and McGill 1982). This has been revised recently (Wilson, 1996). Practical methodologies were also developed for the collection and examination of chironomid pupae (Wilson, 1996) The application of these techniques for assessing water quality is discussed in Sections 2.3.1-2.3.3.

In addition to evaluating pollution, chironomids can be used to evaluate the trophic status of waterbodies, particularly lakes, where the nature of the sediments is closely related to the trophic status of the water. Both the current status (using pupal exuviae, adults or living larvae) and historical status (using sub-fossils in the sediment, e.g. Warwick 1980) can be evaluated.

Because of the abundance of chironomids in many aquatic ecosystems, they are often an important component of food webs. Section 2.3.4 describes an investigation into the trophic relationship between chironomids, eels and otters in Scottish lochs. The Environment Agency has also begun investigations into the trophic interactions in the River Thames, the aim of which is to provide information on the biological effects of possible water resource developments in the Thames catchment. This work may lead to the production of a predictive model.

Chironomids are a major source of food for fish in the River Thames (Mann 1972; Mann *et al.* 1972, Berrie 1993) and are, therefore, important to the Agency's investigation. The origin of detritus on which most chironomids feed in the River Thames was also the subject of an investigation using stable radioisotope ratios (Dennis 1996).

2. INDIVIDUAL CONTRIBUTIONS

2.1 Assessment of sediment toxicity to individual chironomids

2.1.1 The use of chironomids for evaluating sediment toxicity (R Fleming and I Johnson, WRc plc)

Introduction

In Europe, the need for standardised sediment toxicity test methods is increasing as regulators are incorporating measures of effects in the sediment compartment into their environmental quality assessment schemes and in risk assessment procedures for new and existing chemicals. Methods are required for both the toxicity testing of chemicals spiked onto sediment and for the controlled assaying of field sediments. Standard methods exist in North America and Canada either in draft or final form (ASTM 1995a, b: Environment Canada 1995: Burton *et al.* 1994) but these are largely for species that are not indigenous to European waters. For several years, researchers within Europe have been independently developing test methods for indigenous freshwater and estuarine/marine species, often derived from existing guidelines, but no effort had been directed towards producing standard European guidelines which have the agreement of member states.

Objectives

In 1992 a four year programme of work funded by the European Commission and regulators in the United Kingdom, the Netherlands, Germany and Portugal was initiated to promote harmonisation among key research organisations in the field of toxicity test method development and standardisation (Fleming *et al.* 1996). The contributing laboratories comprised WRc, who co-ordinated the programme, the Dutch Institutes of Inland and Marine/Coastal Water Management (RIZA and RIKZ respectively), the Universities of Utrecht and Hamburg and the Portuguese Institute of Marine Research. The programme of work had the objectives of:

1. optimising and standardising a method for spiking chemicals onto sediments;
2. standardising methods for assessing the acute lethal toxicity of sediment bound contaminants;
3. investigating methods for assessing chronic sub-lethal sediment toxicity.

Results

From the start of the programme in 1992, *Chironomus riparius* was the primary freshwater test organism used because of its ubiquity in freshwaters and its ease of culture. Acute lethal and chronic sub-lethal toxicity tests were developed and ring-tested. Table 2.1 summarises the procedures recommended for each test including the test duration, the age of the organisms at the start of the test, test temperature and feeding regime and the test endpoints.

The life stage used in the tests reflected the most sensitive that could be practically adopted to allow the test endpoints to be measured over the required test durations.

Table 2.1 Summary information on the acute and chronic toxicity tests using *Chironomus riparius*

Type of test	Test duration (days)	Age of organisms at start (days)	Test temp (°C)	Feeding	Test endpoints
Acute	10	2nd instars (10-12)	20	N	Lethality
Chronic	28	Egg packets	20	Y	Growth (dry weight) Development Lethality

Comparative data are available on the tests for lindane and permethrin (see Table 2.2) and indicate that the chronic sub-lethal test is more sensitive than the acute lethal test. In the chronic sub-lethal test development (as the number of 4th instar or above) was a more sensitive endpoint than lethality and more reliable than growth (as dry weight) as it is not influenced by the number of survivors.

Table 2.2 Comparative toxicity data for the acute and chronic toxicity tests using *Chironomus riparius*

Test substance	Toxicity test and endpoints (μg g dw^{-1})		
	Acute lethal	Chronic sub-lethal	
	10 d LC_{50}	28 d LC_{50}	28 d NOEC (development)
Lindane	0.6-0.72	0.23-0.27	<0.14-0.14
Permethrin	7.73	2.51	2.0

LC_{50} - concentration of test substance causing 50% lethality of the organisms
NOEC- no observed effect concentration (that is the highest test substance concentration at which responses in organisms are not significantly different from those in control organisms)

Both the acute and chronic sub-lethal *C. riparius* tests have been used in the Environment Agency Toxicity-Based Criteria for Receiving Waters study (R&D Project 703). Tests were carried out on sediments collected from six sites on the River Aire with water quality ranging (in biological quality) from good to poor/bad. Both tests discriminated between the stations and lower levels of effects were associated with the good quality rather than the bad sites. In addition to laboratory tests, *in situ* tests were developed in which animals were exposed in cages in the field and lethality was measured after 10 days exposure. These were designed to mimic the laboratory tests as closely as possible and to compare the effects of the different exposure regimes in the two tests. The *in situ* tests were also shown to discriminate between stations, and lethality was higher at the poor/bad sites than those of good biological quality.

In 1995 the OECD instigated an initiative to standardise benthic toxicity test protocols. Chironomidae were identified as the highest priority for standardised test guidelines for risk assessment (OECD 1995). The guidelines developed in the European Commission programme were considered in conjunction with existing North American guidelines. WRc are responsible for co-writing the OECD guidelines in conjunction with German regulators and have recently produced a final version for acceptance by the OECD.

Application of sediment toxicity tests

In addition to toxicity test method development, WRc have drafted strategies for the assessment of *in situ* sediments on a national and international scale. This has involved drafting recommendations for the sediment component of the proposed European Commission Water Framework Directive, for the sediment chemistry component of the Environment Agency's estuarine General Quality Assessment scheme (Nixon *et al.* 1995), and for the assessment of freshwater sediments in Scotland and Northern Ireland (Fleming *et al.* 1995). The overall recommendation is that in order to make reliable judgements of sediment quality, an integrated approach is required which takes into account measurements of sediment toxicity, biology and chemistry. Toxicity tests can give an indication of direct cause and effect but biological measures are needed to show that contaminated sediments have an impact in the field. Although River Invertebrate Prediction and Classification System (RIVPACS) data are available, an index more easily related to sediment contamination is desirable.

Because toxicity tests with Chironomidae are most likely to be used in the toxicity testing component of integrated schemes, a biological index including a measurement of the effects on these animals in the field would allow clear relationships to be drawn. Such indices may involve individual organism measures such as morphological deformities in the head capsules, *in situ* measures of toxicity or population, and community measures such as chironomid diversity and the abundance of sensitive species.

2.2 Assessment of sub-lethal sediment toxicity based on deformities in populations

2.2.1 The use of chironomids in the bio-monitoring of polluted freshwater sediments (W Aston and P Mitchell, Staffordshire University)

Introduction

Increasing concern about the impact of complex industrial wastes and other forms of pollution on freshwater ecosystems has led to the search for reliable bioindicators which assess the overall level of contamination, notably within sediments (Rosenberg 1992).

Although the aquatic stages of chironomids have been used to assess organic enrichment and trophic level changes in lakes (Saether 1979; Warwick 1980), their use in running waters has been far less frequent. Morphological deformities in the mouthparts of chironomid larvae may be caused by environmental contaminants, and the frequency and/or level of deformity can be used as an effects-based index of sediment pollution (Warwick 1980, 1989).

Objective

The aim of the research carried out at Staffordshire University was to investigate evidence of contaminant-induced morphological deformities in running waters.

Results

Results from extensive surveys of three chironomid genera (*Chironomus*, *Prodiamesa* and *Stictochironomus*) in relatively unpolluted rivers in Scotland and in Yorkshire show that the normal frequency of mentum deformity is between 0 and 2%. This value is consistent with other authors' results (Warwick 1980; Madden *et al.* 1992).

The incidence of mentum deformities found in unpolluted rivers contrast with those found in surveys of urban watercourses. Three surveys have been conducted on an industrially and domestically polluted watercourse (Savick Brook) which flows through north Preston in Lancashire (see Table 2.4). Mentum deformities have been measured in three chironomid genera with incidences of mentum deformities in *Chironomus* usually being higher than in *Stictochironomus* and considerably higher than in *Prodiamesa*.

Deformities in *Chironomus* rose steadily from a relatively unpolluted site upstream to the normal tidal limit downstream (a distance of 0.8 km) and there was a significant correlation between the percentage deformities in *Chironomus* and the distance downstream ($r = 0.76$, $P < 0.001$). The rate of deformity also fluctuated over the year, ranging from 5.6 to 26.7% in July 1994, from 16.7 to 55.6% in February 1995, and from 8.8 to 36.7% in July 1995. The higher incidence of deformity at a site in the winter may reflect the longer exposure of larvae present at this time of year compared to the more rapidly developing summer generation.

Table 2.3 Incidences of mentum deformities in chironomid genera from unpolluted sites in the United Kingdom, based on the examination of 30-90 individuals

Site	Incidence of mouthpart deformities (%) in different genera		
	Chironomus	*Prodiamesa*	*Stictochironomus*
River Dee	0-1.1	0-3.3	-
River Loyne	0	-	-
River Moriston	0-2.0	0-3.3	0-3.3
River Ribble	0-2.2	0	-
River Wenning	0-1.1	0	-

Table 2.4 Incidences of mentum deformities in chironomid genera from different sites on Savick Brook

Site	Incidences of deformities (%) in different genera on three sampling occasions (22.7.94, 13.2.95, 22.6.95)		
	Chironomus	*Prodiamesa*	*Stictochironomus*
5	5.6, ND, 8.8	3.3, 6.7, 0	ND, 6.7, 0
9	8.9, 16.7, 13.3	0, 3.3, 0	0, 6.7, 0
4	14.4, 46.7, 16.7	0, 0, 0	10, 13.3, 6.7
3	14.4, 52.2, 31.1	0, ND, 0	13.3, ND, 16.7
1	26.7, ND, 41.1	0, 3.3, 10	26.7, 20, ND
10	15, ND, ND	3.3, ND, ND	16.7, ND, ND
6	26.7, 45.6, 28.9	0, 6.7, 6.7	ND, 37, ND
7	24.4, 55.6, 30	0,0,6.7	ND, 30, ND
8	21.7, 51.3, 17.8	10, 0, 3.3	ND, 27, ND
2	20, ND, ND	3.3, ND, ND	10, ND, ND

Two other genera, *Stictochironomus* and *Prodiamesa*, also show an increase in the number of deformities further downstream, and a seasonal increase from summer to winter (see also van Urk *et al.* 1992). The difference between the sensitivities of *Chironomus*, *Prodiamesa* and *Stictochironomus* may be due to a resistance in *Prodiamesa* to pollutants which have an adverse effect upon other genera.

Table 2.5 shows the mentum deformities in *Chironomus* sampled from the River Lostock (Lancashire) and the River Trent (Staffordshire). On the River Trent sites 7 and 8 were away from built up areas and could be considered analogous to cleaner upstream sites. The data for the River Lostock appear to support the conclusions found in Savick Brook with increasing incidences of deformities being associated with increasing urbanisation. The level of responses found in the River Trent were low compared to other polluted rivers and this may be the result of the type of substances being discharged to the river.

Table 2.5 Incidence of mentum deformities in *Chironomus* from different sites on the Rivers Lostock and Trent

River Lostock		River Trent	
Site	% deformities	Site	% deformities
2 (U)	9.2	2 (U)	6.7
3 (U)	30	3 (U)	11.2
4 (U)	31.1	4 (U)	5.6
5 (U)	22.2	5 (U)	5.6
6 (U)	20.5	6 (U)	7.7
7 (U)	22	7 (NP)	2.3
-	-	8 (NP)	1.1

NP - Non-polluted site
U - Urban site

Categorising and quantifying deformities

Several methods for assessing deformities both qualitatively and quantitatively have been proposed (see Table 2.6). Quantification analysis can be used to determine certain factors (e.g. common deformities at sites, which may relate to specific contaminants). Qualitative data such as the percentage of individuals showing deformity can be used as a quick and effective way of assessing the amount of deformity in relation to an area of the river and the 'health' of a genus' populations. Quantification of deformity and the derivation of scores are far more time consuming and the serial progression of deformity and the cause of deformity are open to debate.

The two techniques give two very different results in terms of expressing the health of the population. On a practical note, simple qualitative data is quicker and easier to collect compared to the more detailed and time-consuming method of quantification.

Table 2.6 Advantages and disadvantages of qualitative and quantitative techniques

Method	Advantage	Disadvantage
% deformity (Cushman 1984, Warwick 1980, 1987, 1990)	1. Quick and easy. 2. Assessment is simply made as a break from the normal. 3. Can be used on all genera. 4. Derived score gives indication of population health.	1. Affected by seasonal variation. 2. Familiarity with mechanical wear as not to falsify results. 3. Sample size should be constant.
Categorisation (Klink 1989)	1. Can be used on all genera. 2. Categorises deformity from which degree of deformity may be assessed.	1. Problems with some types of deformity. 2. Does not take into account aberrants. 3. Can not be used to assign scores to individuals or populations.
'Deformity Classes' (Lenat 1993)	1. Can be used on all genera. 2. Derived score gives indication of population health.	1. Deformity classes are broad. 2. Mechanical wear considered as deformity. 3. Deformity types overlap classes.
Fluctuating Asymmetry (Clarke 1995)	1. Can be used on all genera. 2. Quick and easy. 3. Assessment is simply made as a break from the normal.	1. Not known whether seasonally affected. 2. Sample size should be kept constant.
***Procladius* Index** (Warwick 1990)	1. Results in a score for the individual and population. 2. Generally easy to use.	1. Can only be used for *Procladius*. 2. Assumes one deformity follows another. 3. Subjective. 4. Assumes all types of deformity have been found. 5. Time consuming.
***Chironomus* Index** (Janssens de Bisthoven 1995)	1. Results in a score for the individual and population. 2. Generally easy to use.	1. Assumption that one deformity follows another. 2. Subjective. 3. Time consuming. 4. Assumes all types of deformities have been found. 5. Some problems arise when trying to categorise certain deformities. 6. Can only be used on *Chironomus* in present form.

In Table 2.6, the advantages and disadvantages of different qualitative and quantitative methods are assessed.

The data for *Chironomus* at Savick Brook and the Rivers Lostock and Trent has also been used to assess whether the qualitative percentage deformity measure is as discriminating as a more quantitative index-based measure. In this assessment, the system of quantifying mentum deformities proposed by Janssens de Bisthoven (1995) was used (see Table 2.7). The highly significant Spearman Rank correlations found between the qualitative and quantitative data indicated that either method could be used to evaluate incidences of deformed larvae. However, the qualitative measure is simpler and easier to carry out than the more specialised and time-consuming quantification method.

Table 2.7 Quantification of deformity using Janssens de Bisthoven system (1995)

Structure	Deformity type	Score allocation
Mentum	- 1 tooth	3
	- ½ tooth	2
	minus > 1 tooth in 4 external (outer) lateral teeth	10
	minus > 1 tooth in 2 inner lateral teeth	20
	minus > 1 tooth in trifid medial tooth	30
	Koehngap of size equal to 1 tooth of corresponding zonation	10
	1½	15
	2	20
	3	40
	4	80
	>5	100
	+ 1 tooth	30
	+ 2 additional structures	20
	+ 3 additional structures	40
	twisted or split tooth	10

In future, observations on the type of deformity, such as that described by Warwick 1989, may be a useful way of assessing the toxicity of sediments. However, without the results of toxicity tests to indicate what levels of pollutants cause different types of deformities, any categorisation system will remain relative and not comparable with other systems except by effect.

Preliminary laboratory experiments have been conducted in an attempt to demonstrate a causal link between contaminant levels in sediments and the frequency and severity of mouthpart deformities in larvae. In these studies, polluted sediment has been shown to be capable of inducing mouthpart deformities. Concentration-dependent increases in mentum deformities were also evident in the head capsules of larvae which had been exposed to a series of permethrin concentrations in a laboratory study at WRc (see Table 2.8). The types of deformities found (gaps, missing teeth in the left and right outer lateral areas, additional teeth and distortion) were similar to those recorded in field populations. Mandibular deformities only occurred in the higher exposure concentrations and were not found in individuals with mentum deformities. Deformities of the pecten epipharyngis were difficult to assess due to natural variations in their number and shape. The degree of effect reported was determined by comparing the number and shape of deformities in treatment samples with those from control larvae. No deformities were observed in the antennae and labral lamellae.

Table 2.8 Incidences and types of deformities recorded in *Chironomus* exposed to different permethrin concentrations

		Type and number of deformities (%)		
Treatment	No. of larvae measured	Mentum	Mandibles	Pecten epipharyngis
Control	20	0	0	0
510 ng l^{-1}	19	3 (15.8)	0	8 (42)
1090 ng l^{-1}	14	2 (14.3)	0	4 (28.6)
2000 ng l^{-1}	22	5 (22.8)	2 (9.1)	3 (13.6)
4250 ng l^{-1}	2	1 (50)	1 (50)	2 (100)

Further laboratory testing to investigate morphological deformities and toxicity test endpoints (such as growth and survival) is needed to link the two measures, using chemicals with different modes of toxic action to determine the biological significance of the endpoint for individuals and populations.

2.2.2 Chironomid deformities in the River Erewash Catchment (C Pinder, Institute of Freshwater Ecology)

Introduction

The measurement of morphological deformities in chironomids represents a potentially useful sub-lethal index of sediment quality. Ideally, however, morphological deformities should be observable before changes in the invertebrate community are apparent (and, therefore, capable of serving as an early warning of sediment contamination). In addition, specific types of deformity should be associated with specific pollutants or classes of pollutants.

Objective

The aim of the research was to investigate evidence of contaminant-induced morphological deformities in lotic systems.

Results

An investigation of chironomid head deformities in the River Erewash catchment has been carried out at the twelve sites given in Table 2.9. The biological and classical chemical classifications at many of the sites did not match and these sites were of particular interest since they may have been subject to toxic impacts. Cuttail Brook (Site 2) was classified as good chemical quality, but only fair biological quality. It was susceptible to discharges from an industrial estate and by mine drainage. Nut Brook (Sites 10 and 11) flowed through contaminated land (the old Stanton Ironworks/coke works) and was culverted to avoid contamination from ground water.

Table 2.9 Chemical and biological water quality data at twelve sampling stations on the River Erewash catchment

Site Number	Chemical quality	Biological quality
1. Upper Erewash	Fair	Poor
2. Cuttail Brook	Very Good	Fair
3. Birchwood Brook	Poor	Fair
4. Jacksdale Tributary	Poor	Poor
5. Jacksdale Erewash	Poor	Poor
6. Bagthorpe Brook	Good	Fair
7. Bailey Brook	Good	Poor
8. Gilt Brook	Fair	Poor
9. Middle Erewash	Poor	Poor
10. Upper Nut Brook	Good	Good
11. Lower Nut Brook	Good	Fair
12. Lower Erewash	Poor	Poor

Table 2.10 shows the chironomid specimens examined from each site, the most common genus/species and the percentage of all chironomids and the most common species which had deformities. Sites 6 and 8 had the highest number of chironomid genera present with nine separate genera whereas sites 9 had only 1 genus present. At most sites a single genus contributed between 43-70% of the total number, the exceptions being sites 8 and 11 which were not dominated by one genus and site 9 which only comprised one genus. The percentage of deformed larvae was as high as 40%, compared to a small percentage of deformities at clean sites.

Table 2.10 **Summary of generic richness, the most common genera and the percentage deformities in all genera and the most common genera at the different R. Erewash catchment sampling stations**

Site number	Generic richness	Most common genus	Most common genus as % of total	Total no. of larvae deformed (%)	No. of most common genus deformed (%)
1	6	*Rheocricotopus*	46	4 (13)	3 (21)
2	7	*Macropelopia*	70	8 (26)	5 (23)
3	6	*Thienemannimyia*	50	2 (7)	2 (13)
4	6	*Chironomus*	63	5 (16)	5 (26)
5	8	*Macropelopia*	43	5 (16)	2 (15)
6	9	*Chironomus*	58	12 (40)	10 (58)
7	4	*Micropsectra*	53	4 (13)	2 (13)
8	9	*Micropsectra*	26	1 (3)	0 (0)
9	1	*Micropsectra*	100	11 (37)	11 (37)
10	5	*Cricotopus*	56	5 (16)	2 (12)
11	4	*Cricotopus*	37	1 (13)	0 (0)
12	5	*Micropsectra*	60	10 (33)	8 (44)

Table 2.11 summarises the types of deformities found in the genera at the different sampling stations. In *Chironomus* most obvious deformities were observed in the mentum, but a few deformities were also seen on the mandible. It is possible that so few were seen on the mandible because they were not visible on the slide preparation. Deformities were also seen is the pecten epipharyngis and the first segment of the antenna. The deformities of the mentum varied from the degree of fusing in the central trifid teeth to missing lateral teeth.

In *Micropsectra,* deformities included the loss of apical segments of the antennae, the loss or addition of the ring organ around the antennae, or the loss or addition of plates on the pecten epipharyngis. In *Macropelopia* an additional tooth was sometimes present on the side of the labium, the configuration of teeth on the hypopharyngis varied, and there were gaps or stunted teeth on the mentum.

No relationship was apparent between morphological deformity and biological quality, though further studies are needed to clarify whether deformities are capable of serving as an early warning of sediment contamination.

Table 2.11 Summary of the types of deformities found at the different R. Erewash catchment sampling stations

Site no.	Antennae	Pecten/epipharyngis	Mentum/ligula	Other
1	*Rheocricotopus* (3)	None	*Polypedilum*	None
2	None	*Micropsectra* (1)	*Macropelopia* (5) *Polypedilum* (1)	*Apsectrotanypus* (1)
3	None	None	*Thienemannimyia* (2)	None
4	*Chironomus* (1)	*Chironomus* (1)	*Chironomus* (1)	None
5	None	None	*Macropelopia* (5) *Prodiamesa* (1)	*Cricotopus* (2)
6	*Chironomus* (7)	*Chironomus* (3) *Prodiamesa* (1)	*Chironomus* (1)	None
7	Cricotopus (1)	None	*Macropelopia* (1) *Micropsectra* (1)	*Micropsectra* (1)
8	None	None	*Brillia* (1)	None
9	*Micropsectra* (1)	None	*Micropsectra* (10)	None
10	*Cricotopus* (2)	None	*Micropsectra* (3)	None
11	None	None	*Micropsectra* (1)	None
12	*Cricotopus* (1)	None	*Micropsectra* (6) *Dicrotendipes* (1)	*Micropsectra* (2)

2.3 Assessment of water quality effects on whole community responses

2.3.1 Chironomids as indicators of water quality (L Ruse, Environment Agency - Thames Region)

Introduction

At present, existing freshwater biological community survey methods are largely based on components of the macro-invertebrate community other than chironomids and are considered to be effective tools. Practical methodologies are available for the use of chironomid pupae in monitoring water quality and considerable progress has been made in the taxonomy of pupal chironomids, such that most species can now be identified to species levels using available keys (Langton 1984, 1991; Wiederholm 1986). However, these techniques have not been adopted widely by biologists in the water industry because pupal exuviae of chironomids are not collected by the routine sampling methods that they employ for collecting invertebrates. In addition, the need for training in the identification of what was perceived to be a 'difficult' group has yet to be addressed.

However, the Environment Agency has successfully used the pupal exuvial technique in a number of studies.

Results

One application of the pupal exuvial technique has been in a long term study of chironomids in the River Thames designed to assist with the evaluation of the effects of future river regulation schemes (Ruse and Wilson 1995). Thirteen sites along the River Thames have been sampled for chironomid pupal skins in each of four surveys. Monthly samples were taken from April to September 1977 and repeated during the same months in 1978. The same sites were sampled subsequently on four occasions between September 1986 and July 1987 and on three occasions between May and August 1992. Any combination of monthly samples between May and September were expected to yield at least 80% of the available genera (Ruse and Wilson 1984). All the samples were collected with a 250 μm mesh net and pupal skins were sub-sampled randomly and identified to at least genus level. At least 600 pupal skins were collected from each site during a survey. Chemical (BOD, DO, unionised ammonia, TON and orthophosphate) and physical (flow, altitude, slope, distance downstream of river source, channel mean depth and width) data were also obtained for each site. The data were analysed in two ways:

1. the physical and chemical quality of each site was assessed from an *a priori* assumption of chironomid substratum preferences and pollution sensitivity. Each taxon was classified as preferring fine (silt, fine sand) or coarse (gravel, rocks, plants) substrata and being tolerant or intolerant of organic pollution;

2. *a posteriori* by calculating Shannon-Weaver diversity indices and applying constrained ordination, canonical correspondence analysis (CCA) using the program package CANOCO version 3.1.

Assessment of river quality from a prior knowledge of chironomid species preference showed a consistent pattern of downstream change through the last three surveys. Biological, chemical and physical data showed that October 1976 to September 1977 was distinct from subsequent survey periods. The highest flows occurred over this period following a severe drought, which ended in September 1976, and these abrupt hydrological changes may have increased the concentrations of ammonia and nitrates entering the river. Analysis of chironomid data using *a priori* classification of preferences was sensitive to these changes and would be expected to respond to future fluctuations in discharge and river quality.

In the canonical correspondence analysis, 68% of the variation in chironomid data was not explained by the six most influential environmental variables supplied. However, the analysis indicated that the variables supplied were sufficient to explain major non-random variation of chironomid data. Unexplained variation could have resulted from changes in the chemical monitoring sites chosen for each survey and in the number of chemical samples and pupal exuviae collected. Among the variables supplied, the most important predictor of the River Thames chironomid assemblage was the distance downstream of the source. Mean ammonia concentrations and annual variations in flow best explained the variations within the chironomid assemblage at particular stations along the river.

The pupal exuvial technique has also been applied to studies of the Grand Union Canal where it is difficult for biologists to sample macro-invertebrates using conventional pond net or box sampling equipment (Ruse 1993). In the Grand Union Canal study, chironomid pupal exuviae were collected from seven sites between Tring and Ealing during May, July and September 1992 (see Figure 2.1). The highest proportion of pollution-sensitive chironomids were found above Tring STW (site number TA8), while the next two sites downstream of Tring and Berkhamsted STWs respectively (CN50 and CN47) had very low proportions of sensitive individuals (Table 2.12). At Springwell Lock (CN205), downstream of the River Gade, there was a recovery in the proportion of pollution-sensitive chironomids to a level comparable with that found above Tring STW. Below Maple Lodge STW (CN186) there was another decline in sensitive chironomids which continued steadily to Lock 97 (GU21) in the Cowley Reach. The low numbers of pollution-sensitive chironomids at sites CN50 and CN47 coincided with the highest BOD values in the sampling period whilst the highest unionised ammonia values were found at sites CN47 and CN186.

Ruse (1997) has developed a simple non-expert scoring system using the chironomid pupal exuviae technique for assessing water quality in canals. Scores are attributed to widely distributed, frequently occurring and easily recognisable indicator taxa. A dichotomous key based on six of these indicators is provided for classifying canal water quality.

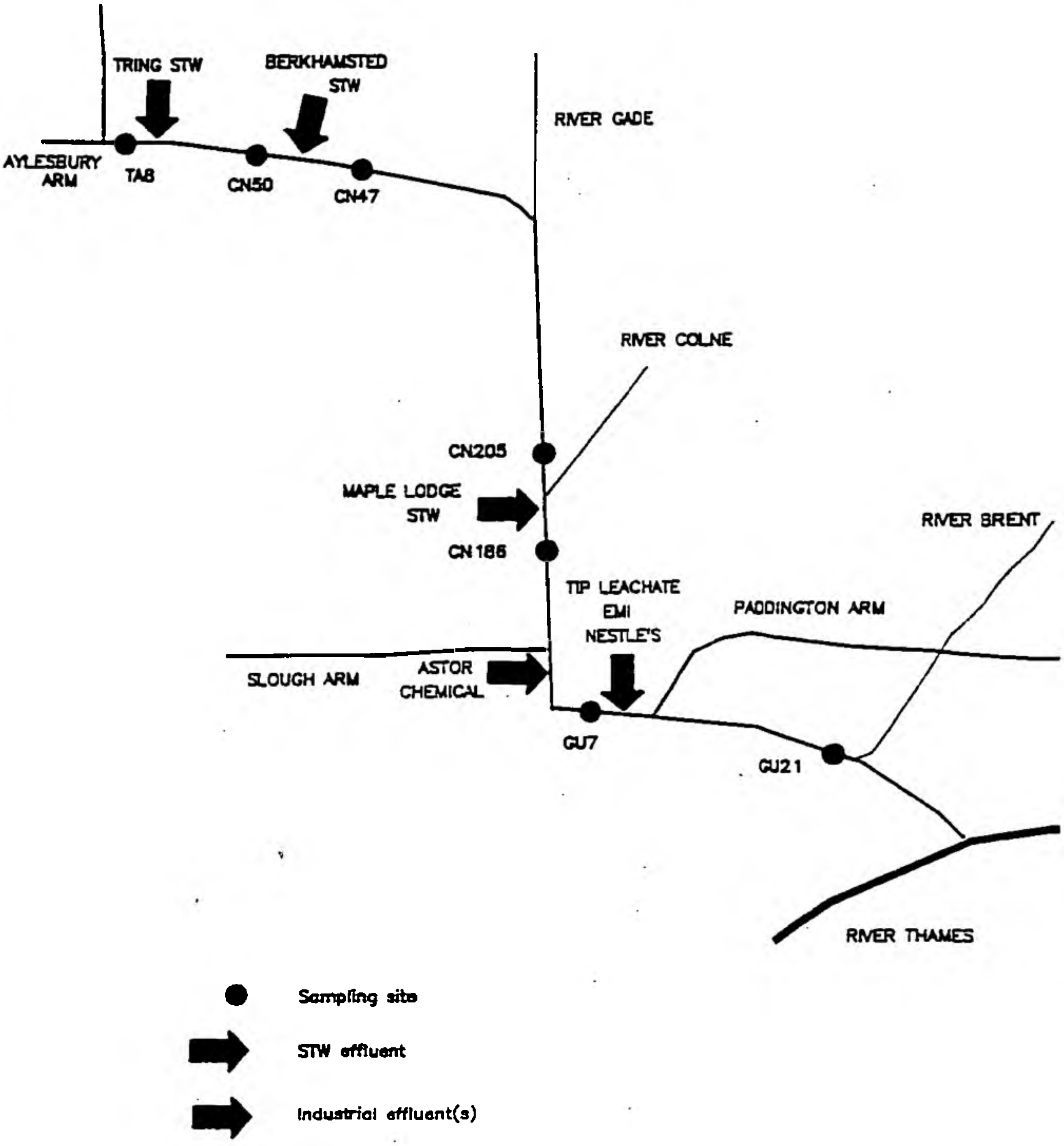

Figure 2.1 Sampling sites used in a study of the chironomid pupal exuviae technique on the Grand Union Canal

Table 2.12 Chironomid diversity and chemical monitoring data at different sites on the Grand Union Canal

Site number and location relative to inputs	Chironomid diversity[1]	BOD (mg l^{-1})	Unionised ammonia (μg l^{-1})
TA8 (Above Tring STW)	43	4.2	4
CN50 (Above Berkhamsted STW)	4	11.2	13
CN47 (Below Berkhamsted STW)	1	14	43
CN205 (Above Maple Lodge STW)	39	7.8	2
CN186 (Below Maple Lodge STW)	32	7	25
GU7 (Below Astor Chemicals discharge)	16	5.7	7
GU21 (Below Tip leachate discharge)	8	7	4

[1] Shannon Index

2.3.2 Assessing the effects of pollution and acidification on chironomid assemblages (M A Learner, University of Wales, Cardiff)

Introduction

The identification of pollutant-indicator species assemblages

It is known from previous work that the chironomid fauna can be used to detect and monitor the presence and impact of sewage pollution in rivers. Pupal exuviae collection provides an effective way of sampling the chironomid fauna. However, the sensitivity of this approach and its extrapolation to detect other kinds of pollution requires further study. The present investigation was initiated to learn more about the response of the chironomid fauna to a range of pollutants in South Wales. Some forty locations were selected following discussion with pollution control officers of the Environment Agency, St Mellons, Cardiff, regarding known sources of pollution.

The kinds of pollution included in the study were:

1. *Organic*: storm water overflows, sewage works effluents; farm animal wastes; waste disposal site leachate.

2. *Mining and associated activities*: mine-water discharges (ferruginous, acid and circum-neutral); coking plant effluent; quarry (limestone) discharge.

3. *Industrial*: cooling water; heavy metals.

The response of chironomids to acidification

In 1984, the Welsh Water Authority conducted a biological survey of 141 streams in its area that were sensitive to acidification. Most of the macro-invertebrates collected were identified to species, but chironomids were not included. In 1995, 81 of these streams were re-sampled along with 39 additional streams in acid-sensitive regions not surveyed in 1984.

Results

The identification of pollutant-indicator species assemblages

Pupal exuviae were collected upstream and downstream of each discharge in spring and again in summer. The upstream sites provided information about the chironomid species assemblages in streams of different character in South Wales, and the downstream faunas reflected the response of these assemblages to defined pollutants. The analysis of data from this project is currently being carried out.

The response of chironomids to acidification

Chironomid pupal exuviae were collected from many of the acid-sensitive sites during July 1995. A range of chemical and other information was also collected for each site so that relationships between water chemistry, particularly acidity, and chironomid species assemblages could be examined. The analysis of data from this project is currently being carried out.

2.3.3 Chironomids in lowland rivers in relation to water quality and physical habitat (E Hawtin, University of Birmingham)

Introduction

The objectives of the study were to:

1. monitor chironomid communities at a number of sites in the River Trent catchment;

2. examine and classify the community structure and define preferred habitat of the species in the community and to assess the importance of particular environmental parameters in determining chironomid communities by multivariate analysis of the data (namely cluster analysis and direct environmental gradient analysis);

3. construct and test the efficacy of elements of the gradient model by field and laboratory experiment.

Results

Five different underlying geologies present in the Environment Agency's Midlands Region were investigated, with two sample reaches being located on each geological type. Samples were taken 0, 5, and where possible, 10 kilometres downstream of the headwater and surveys were repeated monthly for a six month period. The chironomid communities were monitored by using pupal exuviae. This was achieved by hand-netting a surface sample at snag sites at the sides of rivers. Larval samples were taken by standard kick sampling.

The first phase of sampling started in March 1995 to capture the beginning of the year's emergence. The first full data set for all the sites was obtained in April and was analysed using CANOCO. Correspondence analysis was used to show a cluster of 'Upland' sites - that is those that are in the Peak District (the Rivers Ashop, Noe, Manifold and Dove). This clustering of 'upland' sites may be associated with a number of different variables such as altitude, temperature, etc. The scatter of the species shows a split between the groups/genera that predominately emerge in spring (and autumn), that is some Orthocladiinae, and those that are most common in summer, i.e. Tanytarsini, Tanypodinae, *Cricotopus*, *Polypedilum*, and *Chironomus*.

This pattern of seasonal emergence, which is partly temperature dependent, is also represented by the dominant species that are found in the 'upland' and 'lowland' sites. Most of the 'upland' sites are dominated by Orthocladiinae, however, the 'lowland' sites show a larger proportion of *Polypedilum*, *Cricotopus* and Tanytarsini.

The effects of temperature difference between the sites will also be examined. Some of the sites are groundwater fed, such as the Dove and the Manifold, and here temperatures may stay relatively constant through the seasons. On a macro-scale the effect of physical habitat becoming more dominant (geology accounting for the groundwater fed rivers' temperature difference) and other patterns may emerge between the species and sites.

The second phase of sampling will investigate the effect of water quality, particularly inorganic pollutants, on the chironomid community. Therefore, physical habitat will be kept as consistent as possible. Sampling will, again, be monthly, but over 18 months. An additional element of this phase of sampling may involve undertaking more intensive sampling, at weekly intervals in reach 1 for a period of 1-2 months.

The third phase may involve both field and laboratory experiments. Larvae may be taken from selected sites in order to look at the effect of drift. Also, field testing may be undertaken to establish if community structure can be predicted when known pollutants are present.

The analysis of the data from this project is currently being carried out.

2.3.4 Trophic level relationships involving chironomids (J Saddler, University of Birmingham)

Introduction

Trophic level relationships involving chironomids are being investigated in two studies:

An investigation is taking place in two Scottish lochs (both SSSIs) and includes an examination of eel guts for chironomid remains. The study is being carried out in collaboration with an otter expert, Hans Kruk, who is investigating the link between eels and otters. The project looks at a range of habitats from littoral to profundal and between lochs, which differ in terms of their trophic status.

A study is being carried out to assess the effects of eutrophication on trophic relationships in midland reservoirs. In the study the effects of the phosphorus and oxygen are also being considered.

Results

The analysis of the data from these projects is currently being carried out. Up to 50% of an eels diet in freshwater may consist of chironomids. Eels form the preferred food of otters (and herons). The usefulness of chironomid environmental quality indicators has already been referred to, but the implications of these indicators for both eel and otter success have hitherto not been recognised.

2.4 Summary

The following conclusions can be drawn from the research described in the previous sections:

Standardised acute lethal and chronic sub-lethal toxicity tests using *Chironomus riparius* are available to measure the toxicity of laboratory spiked and field collected sediments as part of risk assessment procedures, general quality assessment schemes or environment impact assessments.

Increasing incidences of morphological deformities in the head capsules of chironomid larvae have been found to be associated with declining water quality. Deformities in *Chironomus* have been induced in the laboratory by sediments spiked with permethrin and by contaminated field sediments.

Pupal exuviae collection provides an effective way of sampling the chironomid fauna. Changes in the composition of the chironomid assemblage at a site can be related to water quality with a shift from pollution sensitive to pollution tolerant species being apparent as there is an increase in sediment contamination.

Because of the major importance of chironomids in aquatic trophic webs their importance as environmental quality indicators may be much greater than biologists previously recognised.

3. DEVELOPMENT OF TOOLS FOR USE IN FRESHWATER SEDIMENT QUALITY ASSESSMENT

3.1 Objective

The workshop was held with the long term intention of identifying the potential use of chironomids by the Environment agency, and to identify where further R&D is needed to develop the methods to a stage where they could be used as operational tools for environmental management. Particular attention was paid to systems for indicating sediment condition that could be used, in conjunction with other monitoring results, as part of an integrated approach to freshwater environmental quality assessment.

3.2 Conclusions

The most scientifically justifiable and cost-effective approach to sediment quality assessment is the use of a tiered, integrated approach, measuring sediment toxicity, chemistry, and *in situ* biology. This approach is known as the sediment quality triad approach for freshwater sediments (van de Guchte 1992) and has been previously recommended to SNIFFER and the Environment Agency as the most cost-effective strategy for risk assessment of contaminated sediments (Fleming *et al.* 1995, 1996).

For the toxicity component of the freshwater sediment quality triad, most work to date has been carried out with the freshwater dipteran *Chironomus riparius*. Laboratory-based acute lethal and chronic sub-lethal toxicity test protocols for the larvae of this animal have been developed and standardised, and are now essentially ready for use.

For the *in situ* biology component, procedures such as the generation of BMWP-scores, are of relevance. However, these tend not to be indicative of sediment contamination, having been developed primarily for assessing organic water pollution. A sensitive biological index of sediment contamination is required that includes measures of sediment-dwelling animals that are known to be sensitive to various types of contaminants.

The index could include species abundance and diversity estimates for one or more relevant taxa, and also morphological measures such as head capsule deformities, which have been shown to occur in several species at contaminated sites. As the toxicity testing component will include *C. riparius*, it seems sensible at this stage to include the same species in the biological component so that direct comparisons can be made. Head capsule deformities have also been investigated with several species of this genus.

An additional consideration in the assessment of sediment quality is how to link the effects measured under controlled laboratory conditions with those measured in the field. As the sediment integrity is usually disrupted during sampling, results of laboratory tests are less likely to correlate with biological measures than water column laboratory tests. In order to compare exposure in the laboratory with that experienced in the field *in situ* bioassays can be used which are essentially the same test systems transplanted to the site of concern.

Methods for freshwater using *C. riparius* have undergone preliminary development for the Environment Agency as part of the Toxicity-Based Criteria for Receiving Waters Project.

4. RECOMMENDATIONS

To develop and calibrate a biological index as described above requires additional research and development work. As the disciplines involved are diverse, the programme could best be carried out on as a collaboration between UK experts in the different fields.

The programme could be divided into four separate research components, followed by a validation exercise which would bring together the separate research programmes. All components would be carried out using the same model test compounds (with different modes of toxic action) and the same species of Chironomidae. Where possible, single sources of the test animals would be used to minimise variability caused by differences in inter-population sensitivity.

Component 1 - Toxicity testing: Laboratory and in situ tests

Research questions to be addressed:

Which is the most sensitive and discriminatory sub-lethal endpoint for chronic laboratory toxicity tests with *C. riparius*?

What is the most suitable test design for *C. riparius in situ* tests?

How do laboratory and *in situ* sediment tests compare with head deformities and the structure of chironomid communities, and pollution indices based on them?

Component 2 - Morphological deformities

Research questions to be addressed:

Does a high incidence of morphological deformity affect population numbers and can it be linked to a decrease in growth or reproduction or an increase in mortality?

Are morphological deformities observable before changes in the invertebrate community are apparent?

How are morphological deformities induced, which contaminants are responsible and is habitat type a confounding factor?

Are specific types of deformities associated with specific pollutants or classes of pollutants?

At what life stage do deformities occur and does the condition have a genetic basis or does it occur at some particular stage of the developmental of the larvae?

Are deformities reversible

Is there a dose/response effect.

Component 3 - Chironomid diversity and abundance

Research questions to be addressed:

> How does the measurement of chironomid larvae versus chironomid pupal exuviae compare in terms of sensitivity, reliability, practicality and cost effectiveness?
>
> Can or should the sampling method be improved?
>
> Can the measurement be limited to species known to be sensitive to certain types of contaminant?
>
> How well does CPET link to RIVPACS' predictions of chironomid community composition?

Component 4 - Other benthic invertebrates

Research questions to be addressed:

> Are there any other sensitive species of benthic invertebrates that could be included in the benthic index?
>
> How does the chironomid-based index and the chironomid community predicted by RIVPACS compare with those of other invertebrates and especially the GQA biology classes?

Laboratory study

Laboratory studies could be performed, exposing *C. riparius* at different life stages (egg, larval instars) and over partial life-cycles (one generation or several generations) to concentration ranges of several contaminants with different modes of toxic action. Endpoints such as mortality, reproduction, growth, and morphological deformities of the head capsule could be measured and calibrated against each other for each contaminant.

Animals known to have morphological deformities can be placed in clean conditions and mortality, growth and reproduction measured over a prolonged time period. These could be compared with the same endpoints in healthy animals under the same conditions to assess the long term impact on populations of morphological deformities.

The assessment of which other benthic invertebrates could be used in a biological index alongside chironomids would consist primarily of a desk study. A literature search could be carried out to identify species used in similar indices in other countries (primarily the Netherlands and Canada) and data could be collated on the sensitivity of candidate species to specific contaminants.

Validation study

A 'semi-field' study could be carried out in pond systems or artificial stream tanks. These could be dosed with selected model test substances shown in the laboratory studies to cause effects on chironomids. Over a certain time period, samples of sediment and animals can be taken for measures of toxicity, morphological deformities and larval diversity and abundance. At the same time, chironomid exuviae can be removed using emergence traps, skimming or any other method recommended in Component 3. The correlation between these measures could provide considerable context to the Environment Agency.

Project management

The project should be a collaborative programme with an overall co-ordinator to draw together the research activities of the other participants. The co-ordination could be undertaken in-house by the Agency, using existing project support systems or the help of external experts who could act as referees, or it could be contracted out.

REFERENCES

Adams, W.J., Kimerle, R.A. and Barnett, J.W. (1992) Sediment quality and aquatic life. *Environmental Science and Technology*, **26,** 1865-1875.

ASTM (1995a) Standard guide for conducting 10 day static toxicity tests with marine and estuarine amphipods, E1367-92. In: *Annual Book of ASTM Standards, Vol 11.05 Biological effects and environmental fate; biotechnology; pesticides*. pp 767-792.

ASTM (1995b) Standard guide for conducting sediment toxicity tests with freshwater invertebrates, E1383-94a. In: *Annual Book of ASTM Standards, Vol 11.05 Biological effects and environmental fate; biotechnology; pesticides*. pp 802-834.

Bennison, G.L., Hillman, T.J. and Suter, P.J. (1989) Macroinvertebrates of the River Murray (Survey and Monitoring: 1980 - 1985*). Murray-Darling Basin Commission Water Quality Report No. 3.*

Berrie, A.D. (1993) *Trophic interactions in the River Thames: Scoping Report.* Report to NRA Thames Region.

Burton, G.A. Jr, Ankley, G., Ingersoll, C. and Norberg-King, T. (1994*) Progress in standardisation of sediment toxicity tests in the USA*. Presentation to the First International Symposium on Sediment Quality Assessment, Rationale, Challenges and Strategies, 22-25 August, Goteborg, Sweden.

Chantaramongkol, P. (1983) Light-trapped caddisflies (Trichoptera) as water quality indicators in large rivers: results from the Danube at Veroce, Hungary. *Aquatic Insects*, **5,** 33-37.

Clarke, G.M. (1995) Fluctuating asymmetry of chironomid larvae as an indicator of pesticide contamination in freshwater environments. In: *Chironomids from genes to ecosystems* (ed. Cranston, P.), pp101-109.

Cushman, R.M. (1984) Chironomid deformities as indicators of pollution from a synthetic, coal-derived oil. *Freshwater Biology*, **14,** 179-182.

Dennis, P.F. (1996*) Detritus and detritivore interactions in the River Thames: A pilot isotope study*. Report to NRA Thames Region.

Environment Canada (1995) Test for growth and survival in sediment using larvae of the freshwater midges (*Chironomus tentans* or *Chironomus riparius*). Environment Protection Series Report 1/RM/XX Conservation and Protection, Environment Canada, Ottawa, ONT.

Fleming, R., Johnson, I., Delaney, P. and Reynolds, P. (1995) Freshwater sediment assessment - Scoping study. *Report No SR 3931*. Medmenham, Water Research Centre.

Fleming, R.J., van de Guchte, C., Grootelaar, L., Ciarelli, S., Borchert, J., Looise, B. and Guerra, M.T. (1996) Sediment tests for poorly water soluble substances: European intra- and inter-laboratory comparisons. In: M Munawar and G Dave. *Development and Progress in Sediment Quality Assessment: Rationale, Challenges, Techniques and Strategies*. pp 203-211.

Janssens de Bisthoven, L. (1995) The *in situ* relationship between sediment concentrations of micropollutants and morphological deformities in *Chironomus gr Thummi* larvae from lowland rivers (Belgium): A spatial comparison. In: P Cranston. *Chironomids from genes to ecosystems*. pp 63-80.

Klink, A. (1989) The lower Rhine: Palaeoecological analysis. In: G E Petts. *Historical changes of large alluvial rivers: Western Europe*. pp 183-201.

Langton, P.H. (1984) *A key to pupal exuviae of British Chironomidae*. Published by P.H. Langton.

Langton, P.H. (1991) *A key to pupal exuviae of West Palaearctic Chironomidae*. Published by P.H. Langton.

Lenat, D.R. (1993) Using mentum deformities of *Chironomus* larvae to evaluate the effects of toxicity and organic loading on streams. *Journal of the North American Benthological Society*, **12** (3), 265-269.

Madden, C.P., Stuter, P.J., Nicholson, B.C. and Austin, A.D. (1992) Deformities in chironomid larvae as indicators of pollution (pesticide) stress. *Netherlands Journal of Aquatic Ecology*, **26** (2-4), 551-557.

Malicky, H. (1981) Der Indikatorwert von Kocherfliegen (Trichoptera) in grossen Flussen. *Mitteilungen der deutschen Gesellschaft für allgemeine und angewandte Entomologie*, **3**, 135-137.

Mann, K.H. (1972) Case History: the River Thames. In: *River Ecology and Man*. New York and London, Academic Press: pp. 215-232.

Mann, K.K., Britton, R.H., Kowalczewski, A., Lack, T.J., Mathews, C.P. and McDonald, I. (1972) Productivity and energy flow at all trophic levels in the River Thames, England. In: Kajak, Z. and Hillbricht-Ilkowska, A. (Eds). Productivity problems of freshwaters. *Proceedings of the IBP-UNESCO Symposium on Productivity Problems of Freshwaters, Kazimirez Dolny, Poland, May 6-12, 1970. Krakow, Warszawa*: pp. 579-596.

National Rivers Authority (1995) The Mersey Estuary. *NRA Water Quality Series 23*.

Nixon, S., Fleming, R., Gunby, A. and Clarke, S. (1995) *Development and Testing of General Quality Assessment Schemes: Sediment Quality in Estuaries and Coastal Waters*. NRA Project Record 469/21/HO.

OECD (1995) Draft report of the OECD Working Group meeting on Aquatic Toxicity Testing, Copenhagen, Denmark, 29-30 June 1995.

Pettigrove, V. (1989) Larval mouthpart deformities in *Procladius papudicola* Skuse from the Murray and Darling rivers, Australia. *Hydrobiologia*, **31**, 1-23.

Pinder, L.C.V. (1978) A key to adult males of British Chironomidae. *Scientific Publications of the Freshwater Biological Association*, **37**.

Rosenberg, D.M. (1992) Freshwater bio-monitoring and Chironomidae. *Netherlands Journal of Aquatic Ecology*, **26** (2-4), 101-122.

Ruse, L.P. (1993) *Biological assessment of the Grand Union Canal.* Report to NRA Thames Region.

Ruse, L.P. (1997) *A simple key to canal water quality using a biological technique*. Report to the Environment Agency.

Ruse, L.P. and Wilson, R.S. (1995) Long-term assessment of water and sediment quality of the River Thames using chironomid pupal skins. In: Cranston, P. (Ed). *Chironomids - from genus to ecosystems*. Canberra, C.S.I.R.O., pp. 113-123.

Saether, O.A. (1979) Chironomid communities as water quality indicators. *Holarctic Ecology*, **2,** 65-74.

Smith, K.V.G. (1989) An introduction to the immature stages of British flies; Diptera larvae, with notes on eggs, puparia and pupae. *Handbooks for the identification of British Insects*, **10** (14).

van de Guchte, C. (1992) The sediment quality triad: an integrated approach to assess contaminated sediment. In Newman, P.J., Piavaux, M.A. and Sweeting, R.A. *River water quality: ecological assessment and control.* EUR 14606 EN-FR. Brussels, Commission of the European Communities: pp. 417-423 and 424-431.

van Urk, G., Kerkum, F.C.M. and Smit, H. (1992) Life cycle patterns, density, and frequency of deformities in *Chironomus* larvae over a contaminated sediment gradient. *Canadian Journal of Fisheries and Aquatic Sciences*, **49,** 2291-2299.

Warwick, W.F. (1980) Chironomidae responses to 2800 years of cultural influence: a palaeolimnological study with special reference to sedimentation, eutrophication, and contamination processes. *The Canadian Entomologist*, **112,** 1193-1238.

Warwick, W.F. (1985) Morphological abnormalities in Chironomidae larvae as measures of toxic stress in freshwater ecosystems: Indexing antennal deformities in *Chironomus* Meigen. *Canadian Journal of Aquatic Sciences*, **42,** 1881-1914.

Warwick, W.F. (1987) The incidence of deformities in *Chironomus* spp from Port Hope Harbour, Lake Ontario. *J. Great Lakes Res.*, **13** (1), 88-92.

Warwick, W.F. (1988) Morphological deformities in Chironomidae larvae as biological indicators of toxic stress. In: *Toxic contaminants and ecosystem health: a Great Lakes focus.* (ed. Evans, M.B.) pp 281-320, New York, John Wiley & Sons.

Warwick, W.F. (1989) Morphological deformities in larvae of *Procladius* Skuse and their bio-monitoring potential. *Canadian Journal of Fisheries and Aquatic Sciences*, **46,** 1255-1270.

Warwick, W.F. (1990) Morphological deformities in Chironomidae larvae from the Lac St Louis and Laprarie basins of the St Lawrence river. *Journal of Great Lakes Research*, **16** (2), 185-208.

Wiederholm, T. (ed) (1983) Chironomidae of the Holarctic region - Keys and diagnoses. Part 1. Larvae. *Entomologica scandinavica Supplement*, **17**.

Wiederholm, T. (1984) Incidence of deformed chironomid larvae in Swedish lakes. *Hydrobiologia*, **109**, 243-249.

Wiederholm, T. (ed) (1986) Chironomidae of the Holarctic region - Keys and diagnoses. Part 2. Pupae. *Entomologica scandinavica Supplement*, **28**.

Wilson, R.S. (1980) Classifying rivers using Chironomid Pupal Exuviae. In: D.A. Murray. *Chironomidae, Ecology, Systematic, Cytology and Physiology*. pp 209-216.

Wilson, R.S. (1996) *A practical key to the genera of pupal exuviae of the British Chironomidae (Diptera: Chironomidae) with an account of the CPET method of water quality monitoring*. Published by R.S. Wilson, 98pp.

Wilson, R.S. and McGill, J.D. (1979) The Use of Chironomid Pupal Exuviae for Biological Surveillance of Water Quality. *Technical Memo*, **18,** Department of the Environmental Water Data Unit, 20pp.

Wilson, R.S. and McGill, J.D. (1982) *A practical key to the genera of pupal exuviae of the British Chironomidae*. University of Bristol Printing Office. 62pp.

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