**Interim Report** **R&D Project 379**

# The application and validation of *in situ* ecotoxicological assays at biologically and chemically incongruous sites

**WRc plc**

**August 1992**

**R&D 379/2/T**

HD

379/2/T

# The application and validation of *in situ* ecotoxicological assays at biologically and chemically incongruous sites

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Dissemination status
Internal: Restricted
External: Restricted

Research contractor
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WRc Report Nº NR 3135/4279

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## EXECUTIVE SUMMARY

This interim report describes work undertaken in the first six months of project 379. The principal aim of this project is to apply suitable toxicological test systems to field-based problems in order to assess the usefulness of these systems to the NRA. The results of the 1990 River Quality Survey undertaken by the NRA have been used to identify possible study sites where the provisional chemical class differs considerably from the provisional biological class. Eight of the ten NRA regions have been visited and potential sites discussed with relevant personnel. Three secure and accessible sites have been selected for the deployment of *in situ* assays: the River Tame above and below Saddleworth Yarn Dyers in NRA North-West Region, the River Calder above and below Huddersfield Sewage Treatment Works in NRA Yorkshire Region, and Brenchley Stream in Southern Region.

A suitable combination of *in situ* ecotoxicological tests in both the Tame and the Calder will include *G. pulex* feeding rate, *Dreissena* valve monitor and induction of glutathione-s-transferase (GST) or metallothionein in *Anodonta* all developed or evaluated in NRA R&D Project 061. The laboratory sediment toxicity test, involving *Chironomus* emergence, developed in NRA R&D Project 024 will also be used to provide information on sediment quality. Finally an *in situ* fish growth test and artificial substrata for macroinvertebrate colonisation will be used at least on the first deployment in an attempt to maximise the information available for final evaluation of the NRA selected methods. A limited number of *in situ* assays over a wide area will be deployed in the Brenchley Stream system in order to trace the source and type of any contamination. Assays in this category will include the induction of GST and metallothioneins in *Anodonta* and *G. pulex* feeding rate. A timetable for remaining work in the project is provided in Section 2.4 of this interim report.

Considerable effort has also been expended upon evaluating the use in freshwater systems of the mussel monitor apparatus developed by the Netherlands Organisation of Applied Scientific Research (TNO). The objectives of this work were to identify the most appropriate freshwater bivalve species for use in freshwater field deployments of the mussel valve monitor, to prepare the system for use in the field deployments described above, and to determine the response of the most appropriate species to ammonia, lindane, copper, zinc and endosulphan.

Only *Dreissena polymorpha* proved suitable for use with the monitor. Of the four test substances present at measurable concentrations only un-ionized ammonia showed responses at all concentrations based on activation of the activity alarm. This resulted in alarm responses at 0.3, 0.6 and 3.4 mg $l^{-1}$ un-ionized ammonia.

## KEY WORDS

Ecotoxicology, *Gammarus pulex* feeding rate, *Dreissena* valve movement, *Anodonta* glutathione-s-transferase and metallothionein induction, *Chironomus* emergence, Fish growth, Macroinvertebrate richness

25 AUG 1992

cc: Dr Forshaw
R+D Co-ordinator
NRA Thames Region

WORLD HEALTH ORGANIZATION
COLLABORATING CENTRE FOW
DRINKING WATER AND
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21 August 1992

Dear Derek

NR 3135. THE APPLICATION AND VALIDATION OF IN SITU ECOTOXICOLOGICAL ASSAYS

Please find enclosed 12 copies of this report, which I hope you find satisfactory. If you have any questions or comments, please do not hesitate to contact me.

Yours sincerely

pp T J Shipp

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MEDMENHAM

# 1. INTRODUCTION

This interim report describes work undertaken in the first six months of project 379. The principal aim of this project is to apply suitable toxicological test systems to field-based problems in order to assess the usefulness of these systems to the NRA. The results of the 1990 River Quality Survey undertaken by the NRA have been used to identify possible study sites where the provisional chemical class differs considerably from the provisional biological class. This type of site was considered to be a useful testing ground for these techniques, because it was hypothesised that toxic pollution was the most likely cause of 'poor' biological class and 'good' chemical class.

*In situ* assays were chosen as the primary tools in this study because, like indigenous macroinvertebrate communities, they can be used to integrate site-specific effects over different periods of time. However, unlike standard macroinvertebrate survey techniques, they can be used in controlled experiments; they can be placed in different physical compartments of the river and are not constrained by habitat differences; a range of endpoints can be measured, some of them specific to particular classes of pollutants; and certain techniques are able to identify the precise timing of a pollution event.

Eight of the ten NRA regions have been visited so far, and potential sites discussed with relevant personnel. The emphasis has been on identifying three sites nationally at which selected ecotoxicological techniques can be deployed on several occasions during a year-long exercise. The relative efficacy of the *in situ* techniques can then be assessed. Considerable effort has also been expended upon evaluating the use in freshwater systems of the mussel monitor apparatus developed by the Netherlands Organisation of Applied Scientific Research (TNO), described in Johnson *et al* (1991).

## 2. SELECTION OF STUDY SITES

### 2.1 Method of site selection for ecotoxicological deployments

Chemical and biological data from the 1990 Water Quality Survey were made available to WRc by the NRA.

The selection of potential study sites was made using the following three main criteria:

1. Biological class should be worse than chemical class. It is possible that unmeasured pollutants at such sites are affecting the biota and therefore contributing to the difference in class i.e. these are sites where ecotoxicological investigations could prove most useful.

2. The difference in biological and chemical class should be as great as possible, to avoid problems of misclassification producing potential incongruities where, in fact, there were none.

3. We should be guided by the experience and expertise of regional NRA pollution officers, biologists and chemists, who are the individuals best placed to decide upon appropriate study sites.

The Biological Liaison Officer in all ten NRA regions was contacted by telephone and asked for assistance. He or she was then sent a copy of the Project Investment Appraisal (Appendix B), a short list of ideal site conditions (Appendix C) and a list of sites in their region that appeared to have incongruous biological and chemical classes. Arrangements were then made to visit the Biological Liaison Officers and, if possible, their chemistry counterparts, to discuss potential sites in more detail. Eight of the ten regions have been visited to date, and the remaining two, Wessex and Anglian, will be visited shortly.

The data set used to derive potentially anomalous sites was provided to WRc by Dr Tony Warn of NRA Anglian Region as part of another R & D project in NRA Topic A13 (The use of biological information in a river quality classification). These data comprised chemical (dissolved oxygen, biochemical oxygen demand and ammonia) and biological (actual and RIVPACS predicted BMWP and ASPT scores). These data were used to calculate provisional chemical and biological classes for river stretches based upon the criteria in 'Proposals for Statutory Water Quality Objectives' (NRA 1991b). Matrices of provisional biological against provisional chemical class were then produced for each region, from which subsets of data could be selected (Figure 2.1).

The Biological Liaison Officers in each region, the individuals responsible for collating the 1990 biological data for their region, were asked to provide detailed comments upon those few sites where the data suggested a chemical class of A or B and a biological class D. These sites were:

- Anglian Region. Prittle Brook (entire length);

- Northumbrian Region. None identified;
- North-West Region. Cowpe Beck (Higher Boarsgreave to Irwell);
- Severn-Trent Region. Sow Brook (Overslade Lane to River Avon); Cow Honeybourne Brook (downstream from Cow Honeybourne Road to Bretforton Brook); Bretforton Brook (Cow Honeybourne Brook to Badsey Brook); Hooton Dyke (Thurcroft to Slade Hooton Sewage Treatment Works); River Tame (Minworth Sewage Treatment Works to River Blythe);
- Southern Region. Brenchley Stream (River Teise Confluence to Palmers Green);
- South-West Region. None identified;
- Thames Region. Latchford Brook (Tetsworth Common to Haseley Brook); Padworth Stream (Old Warren to Kennet);
- Welsh Region. River Piliau (confluence Teifi above Cardigan to Rhiwlas); Brandy Brook (Newgale to Roch Bridge); the Nant-y-Fendrod (Tidal limit at Landore to the confluence with a tributary at Llansamlet).;
- Wessex Region. None identified;
- Yorkshire Region. Thick Hollins Dike (Magdalen Springs to Hall Dike); River Colne (Merrydale Clough to Crimble Brook).

**Figure 2.1 Matrices of chemical class against biological class**

1. Anglian Region

| | | Provisional Chemical Class | | | | | |
|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | Total |
| | A | 1 | 141 | 214 | 52 | 0 | 408 |
| Provisional | B | 0 | 29 | 90 | 70 | 1 | 190 |
| Biological | C | 0 | 10 | 36 | 42 | 0 | 88 |
| Class | D | 0 | 1 | 5 | 15 | 0 | 21 |
| | Total | 1 | 181 | 345 | 179 | 1 | 707 |

2. Northumbrian Region

| | | Provisional Chemical Class | | | | | |
|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | Total |
| | A | 15 | 98 | 42 | 3 | 0 | 158 |
| Provisional | B | 0 | 30 | 22 | 8 | 0 | 60 |
| Biological | C | 0 | 8 | 24 | 14 | 1 | 47 |
| Class | D | 0 | 0 | 9 | 21 | 9 | 39 |
| | Total | 15 | 136 | 97 | 46 | 10 | 304 |

3. North-west Region

| | | Provisional Chemical Class | | | | | |
|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | Total |
| | A | 41 | 44 | 4 | 0 | 0 | 89 |
| Provisional | B | 9 | 12 | 12 | 6 | 0 | 39 |
| Biological | C | 0 | 5 | 22 | 20 | 10 | 57 |
| Class | D | 0 | 1 | 20 | 54 | 37 | 112 |
| | Total | 50 | 62 | 58 | 80 | 47 | 297 |

**Figure 2.1 continued**

4. Severn-Trent Region

| | | Provisional Chemical Class | | | | | |
|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | Total |
| | A | 15 | 103 | 76 | 21 | 3 | 218 |
| Provisional | B | 17 | 60 | 130 | 43 | 10 | 260 |
| Biological | C | 4 | 35 | 104 | 81 | 20 | 244 |
| Class | D | 1 | 5 | 14 | 54 | 25 | 99 |
| | Total | 37 | 203 | 324 | 199 | 58 | 821 |

5. Southern Region

| | | Provisional Chemical Class | | | | | |
|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | Total |
| | A | 19 | 83 | 87 | 35 | 10 | 234 |
| Provisional | B | 0 | 10 | 23 | 25 | 12 | 70 |
| Biological | C | 0 | 6 | 17 | 10 | 11 | 44 |
| Class | D | 0 | 1 | 1 | 1 | 1 | 4 |
| | Total | 19 | 100 | 128 | 71 | 34 | 352 |

6. South-West Region

| | | Provisional Chemical Class | | | | | |
|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | Total |
| | A | 70 | 184 | 132 | 15 | 4 | 405 |
| Provisional | B | 5 | 20 | 12 | 7 | 0 | 44 |
| Biological | C | 4 | 6 | 5 | 9 | 1 | 25 |
| Class | D | 0 | 2 | 2 | 2 | 1 | 7 |
| | Total | 79 | 212 | 151 | 33 | 6 | 481 |

**Figure 2.1 continued**

7. Thames Region

| | | Provisional Chemical Class | | | | | |
|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | Total |
| | A | 16 | 111 | 102 | 44 | 3 | 276 |
| Provisional | B | 1 | 14 | 39 | 26 | 1 | 81 |
| Biological | C | 0 | 7 | 34 | 44 | 10 | 95 |
| Class | D | 0 | 2 | 9 | 14 | 5 | 30 |
| | Total | 17 | 134 | 184 | 128 | 19 | 482 |

8. Welsh Region

| | | Provisional Chemical Class | | | | | |
|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | Total |
| | A | 292 | 397 | 125 | 25 | 0 | 839 |
| Provisional | B | 48 | 105 | 98 | 16 | 8 | 275 |
| Biological | C | 12 | 39 | 35 | 23 | 12 | 121 |
| Class | D | 0 | 3 | 7 | 7 | 8 | 25 |
| | Total | 352 | 544 | 265 | 71 | 28 | 1260 |

9. Wessex Region

| | | Provisional Chemical Class | | | | | |
|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | Total |
| | A | 47 | 208 | 193 | 57 | 11 | 516 |
| Provisional | B | 5 | 39 | 85 | 63 | 6 | 198 |
| Biological | C | 0 | 9 | 20 | 24 | 4 | 57 |
| Class | D | 0 | 0 | 2 | 2 | 0 | 4 |
| | Total | 52 | 256 | 300 | 146 | 21 | 775 |

10. Yorkshire Region

| | | Provisional Chemical Class | | | | | |
|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | Total |
| | A | 57 | 82 | 35 | 6 | 0 | 180 |
| Provisional | B | 2 | 29 | 13 | 16 | 3 | 63 |
| Biological | C | 1 | 5 | 48 | 20 | 4 | 78 |
| Class | D | 0 | 2 | 18 | 64 | 10 | 94 |
| | Total | 60 | 118 | 114 | 106 | 17 | 415 |

They were also asked to comment briefly upon, what was in some regions, the large number of sites where the biological class was very good and the chemical class was very poor. Most importantly, they were asked to identify any further sites that could usefully be assessed in this project. These sites could, for example, have been amongst the 87 where the chemical class was C and the biological class was D in 1990. The responses to our enquiries are summarised in the next section.

## 2.2 Summary of interviews with regional personnel

### 2.2.1 Anglian Region

Not yet visited.

### 2.2.2 Northumbrian Region

Anne Lewis (NRA), Malcolm Helm (NRA), Craig Turner (NRA), Neil Adams (WRc) and Chris Mainstone (WRc) met on 19/3/92. No sites of chemical class A/B and biological class D were found by WRc. However, several potential sites were identified by Northumbrian NRA:

1. The River Leven between Broughton Bridge Beck and Great Ayton Sewage Treatment Works (provisional chemistry B, biology C). The chemical site is located upstream of the town, whereas the biological site lies in the middle of town. A number of storm sewer overflows discharging between the two sites are likely to be responsible for the observed discrepancy.

2. The River Leven upstream of Great Ayton Sewage Treatment Works (provisional chemistry and biology D). This reach would be expected to support a good fauna but it scores very badly. Influences are minewater drainage (mainly iron), sewage and livestock farming. Minewater is unlikely to be causing a serious impact; the most likely cause of the problem is farming. The impact appears to extend up to the source so there may be no clean site available for monitoring purposes. Bankside trees would provide cover for equipment.

3. Other sites identified by Northumbrian NRA personnel. All of the sites identified have similar problems in that they drain ore-mining areas and consequently exhibited high metals levels, particularly lead and zinc. The biological classification was not felt to be identifying the biological problems known to exist, which are manifested by reduced invertebrate abundances rather than reduced BMWP scores or ASPT. ASPT is even worse than BMWP score in that it makes the sites appear to be of excellent quality.

Water quality in the Nent catchment is chemical class A/B and biological class A, but Northumbrian biologists believe that it should really be classed as biology C because of the low observed abundances.

Rivers with similar problems are the Rookhall Burn and the East and West Allens, all draining the same ore field. Further north, Tipalt Burn and Elsdon Burn are affected by drainage from abandoned coal mines.

The main problem with all of these watercourses is that because the pollution occurs in the headwaters, there are often no 'clean' sites for reference purposes. The Tipalt Burn is perhaps the exception to this, since mine drainage enters as a discrete discharge further downstream; however, this site was visited and it was found that a by-pass is likely to be constructed in the near future that might affect the siting of equipment.

The Nent was visited at Nent Head and Nent Hall. It is generally exposed, but there are some sites that are reasonably inconspicuous. The substrate is frequently of sheet rock, but there are also some gravelly areas. The river is quite flashy.

The East and West Allens are very flashy with a great deal of bed movement. The NRA has lost a number of colonisation chambers. British Steel land could be used to site gear on Rookhall Burn. There are secure sites on Elsdon Burn but access to these sites would involve a relatively long walk.

### 2.2.3 North-West Region

Dick Chambers (NRA), Chris Mainstone (WRc) and Mark Crane (WRc) met on 8.4.92. WRc had identified one site, Cowpe Beck, with a chemical class B and a biological class D. Dick Chambers identified another three sites where the biological class was lower than the chemical class for no apparent reason.

1. Cowpe Beck. A textile mill (Kearns of Waterfoot Ltd) discharges into the beck, which is narrow (1.5 m) and shallow (10 cm) both above and below the mill. The stream bed is stony, with ochre deposits upon the pebbles; water flow is moderate. Poor biological quality is probably caused by intermittent discharges of pesticides, mainly synthetic pyrethroids, from the mill just below the headwater reservoir. The impact is observable for a considerable distance downstream of the confluence with the River Irwell, eliminating pesticide-sensitive species such as *Asellus* and *Baetis rhodani*. The stretch in question runs through a village, so access is good but vandalism might be a problem.

2. River Tame below Saddleworth Yarn Dyers. The river is 6-7 m wide and approximately 30 cm deep. The river bed consists of rocks, boulders and some finer sediments and the flow is moderate. Access both above and below the yarn dyers is good and security is reasonable. It would probably be possible to use a private garden below the dyers, and the upstream site is not obvious from nearby footpaths. A kick-sample at the upstream site revealed *Gammarus pulex*, *Asellus aquaticus*, *Hydropsyche*, *Baetis*, *Glossiphonia* and *Ancylus*. Only one oligochaete and one

*Ancylus* were found below the dyers. Intermittent discharges of synthetic pyrethroids are thought to cause the poor biological quality downstream of the yarn-dyers. The company is authorised to discharge to sewer but contamination seems to gain access to the river directly.

3. River Bollin below Macclesfield Sewage Treatment Works (provisional chemistry and biology D). The river is 15-20 m wide, with meanders and a range of flow rates and depths. The river bed consists of sand and pebbles. The site above the Works is reasonably secure, with good access. There was evidence of sewage fungus and a kick sample revealed only *Baetis*, *Asellus* and oligochaetes. *Glossiphonia* was abundant at the downstream site, and *Asellus*, *Simulium* and oligochaetes were also found. The downstream site was more exposed to walkers. The biological effect of the STW discharge is considered by the NRA to be greater than would be expected from organic pollution alone. A major chemicals manufacturer (ICI) discharges to sewer and low levels of lindane have been detected.

4. River Dane below a Sewage Treatment Works taking complex effluent from Fisons Ltd (provisional chemistry C, biology D). The river is 25-30 m wide. There are a range of depths both above and below the works, although few riffles, especially upstream; the flow rate varies from moderate to rapid and the water was turbid when we visited the site. Both access and security are reasonably good. A kick sample taken above the STW revealed *Hydropsyche* and *Sphaerium*, but sampling was difficult. A sample taken below the STW revealed *Agrion*, *Hydropsyche*, *Asellus* and *Glossiphonia*. The NRA believe that the catchment suffers from farm pollution problems generally, so identifying an adequate reference site might be difficult.

### 2.2.4 Severn-Trent Region

Shelley Howard (NRA) and Mark Crane (WRc) met on 10/4/92. The River Tame site selected by WRc could be dismissed immediately because of errors in the NRA database which have since been resolved. There were also problems with the data for Sow Brook and Hooton Dyke.

1. Cow Honeybourne Brook and Bretforton Brook (provisional chemistry B, biology D) . These are in the Avon catchment in the Vale of Evesham, and there is a suspicion that they may be affected by agricultural pesticides. The streams are 3 m wide and about 30 cm deep, with a moderate flow and a clay and pebble bed. An upstream site emerging from the Cotswolds was heavily populated with *G. pulex*. Security was reasonably good along the streams, but access could be a problem without permission to drive onto farmland.

2. Bentley Brook below a dye works (provisional chemistry C, biology D). The brook is in the Derwent catchment and is 6 m wide with a variable depth, moderate flow and a bed of boulders and pebbles. Access is reasonably good, but security is poor. The stream has recently been downgraded to chemical class 3.

3. Other potential sites identified by Shelley Howard. The River Amber in the Derwent catchment may also be affected by a large dye works; Cannop Brook in the Forest of Dean may be affected by a pulp mill discharge, acidification and metals; the Cam may be impacted by urban runoff from Cheltenham; there is a suspicion that pesticides used in a Sewage Treatment Works have an effect on the River Leadon. The River Erewash at Shipley was also visited, although there is no incongruity between chemical and biological class: both are bad. However, this was thought to be a potentially useful site for future deployment of the mussel monitor in conjunction with a continuous chemical monitor that is already in place. The river is affected by many discharges, and although the sanitary determinands are improving, permethrin and other toxic compounds are suspected to still be causing problems.

### 2.2.5 Southern Region

Bob Dines (NRA), Mark Crane (WRc) and Paul Delaney (WRc) met on 15.4.92. WRc had identified one site, Brenchley Stream, with a chemical class B and biological class D. Bob Dines identified two more streams in the same catchment that were in a similar condition. Two other watercourses near Havant in Hampshire were also identified by Bob Dines as incongruous.

1. Brenchley Stream, Curtisden Green Stream and Curtisden Green Stream Tributary. These streams are tributaries of the River Teise near Paddock Wood in Kent. They are all class A or B chemically, but with a very poor invertebrate fauna (class D). The Curtisden Green Stream Tributary is only 50 cm wide and 10 to 30 cm deep; the other two streams are 2-3 m wide and 20 to 30 cm deep. The bed of all three comprises cobbles, pebbles and silt, the flow rate in each is moderate and access and security are good. NRA suspect that pesticides used on the many orchards and hopfields in the area may be responsible for the paucity of the fauna. However, a local farmer showed us one of the springs that fed Brenchley Stream; ochre deposits were in great evidence, suggesting that natural contamination may influence the invertebrate communities.

2. River Lavant and Southleigh Stream, Hampshire. Both of these streams are class B chemically and class C biologically. The Lavant is incongruous below an IBM plant where oil and plastic debris have been observed on occasion by NRA field biologists. The river is 4 m wide and 30-50 cm deep with a moderate flow; access is good, but security poor. The NRA believe that Southleigh Stream may be affected by diffuse urban inputs. The stream is 3-4 m wide and 20-60 cm deep, with a slow flow, good access and poor security.

### 2.2.6 South-West Region

Roseanne Broome (NRA), John Murray-Bligh (NRA), Neil Adams (WRc) and Mark Crane (WRc) met on 27.3.92. WRc identified two sites on the Red River where the chemistry was class B and the biology class D. However, the cause of this incongruity, metal contamination, was well known. The NRA identified several other sites, including Alphin

Brook and the River Dart below Buckfastleigh, at which they encountered suspected sediment contamination problems, or logistical difficulties in sampling macroinvertebrates (e.g. large boulders), although none of these sites could be described as 'incongruous' in the 1990 survey. Both Alphin Brook and the Dart were visited and would be suitable for an ecotoxicological assessment.

### 2.2.7 Thames Region

John Steele (NRA) and Mark Crane (WRc) met on 13.4.92. WRc had identified two sites, Latchford Brook and Padworth Stream, with chemistry class B and biology class D.

1. Latchford Brook (from Tetsworth Common to Haseley Brook). This stream is 2-3 m wide and 10 cm deep with a moderate flow and variable BMWP score. The NRA believe that a sewage treatment works above the sampling point occasionally fails on sanitary determinands, but that this has not yet been detected by chemical sampling methods. Access and security are good.

2. Padworth Stream (from Old Warren to the Kennet). It is the NRA's view that the variation in the fauna sampled from this site is due to low river flows.

### 2.3.8 Welsh Region

Frank Jones (NRA), Neil Reynolds (NRA), Mark Crane (WRc) and Paul Delaney (WRc) met on 14.4.92. The three incongruous sites identified by WRc were well understood by the NRA. Brandy Brook is affected by a STW, the Pilau by tidal influences and the Nant-y-Fendrod by metals. Frank Jones and Neil Reynolds identified three catchments, the Dee, the Llynfi and the Frome, in which traditional survey techniques were inadequate for ascribing cause and effect to water quality problems. The two latter catchments were visited and would be suitable for an ecotoxicological study. The Llynfi is affected by several STW and Storm Sewer Overflows, and a coal washery; fish removed from the main river were found by the NRA to contain several complex organic compounds. The Frome is probably affected by agricultural pesticides from hop growing and horticulture. The Dee has been affected in the past by sublethal episodes of phenol and formaldehyde from a Monsanto chemical works.

### 2.2.9 Wessex Region

Not yet visited.

### 2.3.10 Yorkshire Region

Colin Urquhart (NRA), Brian Hemsley-Flint (NRA), Richard Brook (NRA), Richard Armitage (NRA), John Housham (NRA), Neil Adams (WRc) and Mark Crane (WRc) met on 1.4.92. WRc had identified two sites, Thick Hollins Dike and a stretch of the River Colne, with class B chemistry and class D biology. The NRA identified several more sites where incongruities between chemical and biological class could not easily be explained, of which two were a priority.

1. Thick Hollins Dike (from Magdalen Springs to Hall Dike). This is a small stream that rises in peat moorland and receives effluent from a sewage treatment works. A textile mill releases permethrin and dieldrin to sewer and has also been responsible for spillages directly into the dike.

2. River Colne (from Merrydale Clough to Crimble Brook). There are approximately 30 Storm Sewer Overflows in this catchment, one of which carries effluent containing mothproofers from a raw wool scourer and finisher-dyer. A chemical works (Pennine Chemicals) has, in the past, been implicated in pollution incidents, but new owners appear to have considerably improved the integrity of the site.

3. River Calder above and below Huddersfield STW. The river is approximately 20 m wide with variable depths and flow rates. The chemical and biological class is poor (class D) below the STW and has led Yorkshire Water plc to invest several tens of millions of pounds in upgrading the facility. However, although chemical class is relatively higher at sampling sites above the STW (class C), biological class is similar to that found below the STW (class D). Sites immediately above and below the STW have good access and reasonable security. Sites further up the Calder at Elland and Brighouse have easy access, but very low security.

4. Holme Brook in the Rother catchment is chemical class B and biological class C-D. The stream is 3-4 m wide and 20-50 cm deep with moderate flow rates. Access is good in parts, but security is poor. The NRA are unaware of any reason for the low numbers of macroinvertebrates; however, considerable earth-moving activity was apparent on our site visit and field drains expelling turbid effluent were observed.

## 2.3 Recommendations for *in situ* investigation sites in 1992/93

All of the sites described above could have been assessed using ecotoxicological techniques. However, only three have been selected from those described in Section 2.3 above, and are recommended for use in the field-work phase of this programme. These sites were selected because they are the most secure and accessible and are amongst those of greatest operational importance to NRA regions. Historical biological and chemical data received from the relevant NRA regions also support their choice.

The River Tame in NRA North-West Region is secure and has good site access. There is clearly a persistent problem with macroinvertebrate diversity below the yarn-dying works,

although it is unclear whether this is due to episodic water column pollution or the presence of persistent contaminants in the sediment.

The River Calder in NRA Yorkshire Region is also reasonably secure and has good site access. Work undertaken by Alan Cansdale, the regional toxicologist, suggests that samples of the receiving water below Huddersfield STW are only occasionally toxic to *Daphnia magna* in laboratory tests; samples of river water taken from above the STW are not toxic. However, it is difficult to ascribe any biological effects to the STW because the macroinvertebrate biology is poor both above and below the STW outfall.

A suitable combination of *in situ* ecotoxicological tests in both the Tame and the Calder could include *G. pulex* feeding rate, *Dreissena* valve movement, induction of GST or metallothionein in *Anodonta, Chironomus* emergence, fish growth and macroinvertebrate colonisation of artificial substrata. This combination of continuous *in situ* experimental systems includes fish, insects, crustaceans and molluscs; toxicological endpoints include analyses of effects at the biochemical, physiological, behavioural, individual and community level; and both the water column and sediment compartments would be assessed.

A study in Brenchley Stream and Curtisden Green Stream would provide a contrast with the two industrial sites selected above. Potential pollutants in this catchment include agricultural wastes and pesticides, and naturally-occurring iron deposits. Pollution may be both intermittent and dispersed in these streams. Our strategy at these sites would be to deploy a limited number of *in situ* assays over a wide area in order to trace the source and type of any contamination. Assays in this category could include the induction of GST and metallothioneins in *Anodonta* and *G. pulex* feeding rate.

The indigenous macroinvertebrate fauna at each deployment site would be regularly assessed using standard kick and Surber sampling techniques.

## 2.4 Timetable of work

| | |
|---|---|
| 1992 | |
| June 30 - July 28 | River Calder deployment 1 |
| July 22 - August 19 | Curtisden Green Stream deployment 1 |
| August 4 - September 1 | River Tame deployment 1 |
| | |
| September - October | River Calder deployment 2 |
| September - October | Curtisden Green Stream deployment 2 |
| October - November | River Tame deployment 2 |
| | |
| 1993 | |
| March - April | River Calder deployment 3 |
| March - April | Curtisden Green Stream deployment 2 |
| April - May | River Tame deployment 3 |
| | |
| July | Final report |

# 3. THE USE OF THE MUSSEL VALVE MOVEMENT MONITOR IN FRESHWATERS

## 3.1 Introduction

Control of discharges to natural waters has traditionally been achieved by establishing maximum permissible concentrations of substances in an effluent, and ensuring that these concentrations are not exceeded, by undertaking appropriate sampling and chemical analysis of the effluent. In line with this approach the NRA has made a policy statement to the effect that it "should promote continuous monitoring of environmentally significant discharges where technology and circumstances make that possible with adequate reliability." (National Rivers Authority 1990). This was stated in the context of chemical monitoring; however, a physico-chemical specific approach does have drawbacks.

Many effluents contain organic chemicals which are not accurately identifiable, are costly to measure accurately and may have interactive toxicological effects; difficulties may be further compounded by the variable composition of complex effluents (Hunt 1989). Further, when using a physico-chemical monitoring programme it is not possible to detect chemicals that are not specifically monitored for or those that are toxic at a level below detection limits; nor is it possible to measure the effects of water quality conditions or interactions of pollutants on toxicity (Van de Schalie 1991).

These problems may be approached by using organisms to provide a direct measurement of the toxicity of pollutants in water systems. This can be done using biological early warning systems (BEWSs) that are designed to monitor automatically the physiological or behavioural responses of aquatic organisms to changes in water quality. The choice of suitable test organism should be based on a variety of factors that are partly of logistical importance. They should:

- be easily obtained or grown in the laboratory;
- live long enough without major changes in appearance;
- possess a measurable parameter that does not involve disturbing the animal;
- require a minimum of maintenance and;
- have available ample ecotoxicological literature (Kramer and Botterweg 1991).

In most systems the test organism is in a continuous, flow-through system and the responses detected as, or converted to, electrical signals. A computer or monitor can then determine if the signal is indicative of toxic conditions and provide an appropriate alarm signal.

To date a variety of organisms have been used in BEWS studies (Van de Schalie 1986, Kramer and Botterweg 1991):

| Organism | Number of systems | Parameter measured |
|---|---|---|
| Fish | 5 | Ventilation rates |
| | 4 | Rheotaxis |
| | 2 | Activity |
| | 1 | Electric pulse discharge |
| Daphnia | 1 | Activity |
| Bivalves | 1 | Valve movement |
| Bacteria | 3 | Oxygen consumption |
| | 1 | Nitrification |

While fish are the most commonly used organisms, a number of studies have investigated the use of the valve movement of freshwater and marine bivalve molluscs as a stress response to aquatic contaminants (Salanki *et al* 1991, Sloof *et al* 1983, Kramer 1989). This method is based on the fact that most mussels have their shells open for the majority of the time for feeding and respiration. Under stress however, they will respond by rapidly opening and closing, or even complete closing, their shells for extended time periods. This valve movement has been used as an indicator of contaminants in the surrounding water.

As valve movement is a clearly defined and readily measurable response and bivalves are easily maintained and deployed in receiving waters bivalve mussels have scope for use as a monitor organism. Previous work at WRc (Johnson *et al* 1991) using a bivalve shell activity monitor supplied by the Netherlands Organisation of Applied Scientific Research (TNO) concluded that further work with the system was merited, particularly with regard to suitable freshwater species. As a result of this a further evaluation of the shell valve activity monitor has been carried out as part of this project to ensure that the equipment is ready for field deployment. The aims of the evaluation were:

1. To identify the most appropriate freshwater bivalve species for use in freshwater field deployments of the mussel valve monitor. The choice of test species was based on the valve opening and closing regimes of *Dreissena polymorpha, Anodonta* and *Unio* species under non-polluted conditions.

2. To determine the response of the most appropriate species to a variety of potential pollutant chemicals. Ammonia, lindane, copper, zinc and endosulphan were chosen to enable comparison with past work, and provide data for untested chemicals.

## 3.2 Materials and methods

### 3.2.1 Monitoring apparatus

The *in situ* version of the shell activity monitor evaluated in this study can simultaneously detect pollution induced changes in the valve movement of eight bivalves. The monitoring system is based on an electromagnetic induction system that has been extensively described in Kramer *et al* (1989) and is summarised in this report.

The system consists of a high frequency oscillator, two tiny coils and an amplifier, with a power supply. One of the coils located in each attached plate acts as a transmitter of a magnetic field generated by a 500 kHz high-frequency oscillating current. A mussel is glued to each plate and a second coil is attached to the opposite valve of the animal. The current induced by the intercepting magnetic fields of the two coils is proportional to the distance between the shell valves. The signal received from each mussel is continuously monitored and normalised to maximum and minimum valve opening, to provide an indication of the status of each animal.

### 3.2.2 Operation

The mussel monitor is controlled through the software program called 'Procomm', which provides menu driven functions for changing data and terminal settings, alarm parameters, installation of mussels and downloading of data. Interaction with the electronic system of the mussel monitor can be achieved with any appropriate portable computer, and in the evaluation a Toshiba TX1000E and T1600 were used. The means of communication between the mussel monitor and the portable computer, in terms of baud rate and access ports used is defined by changing the terminal settings within 'Procomm'.

### 3.2.3 Installation

Each of the eight mussels in an experiment was attached to a PVC plate, containing a transmitting coil, using a fast setting non-toxic two component acrylic resin (Trim, Bosworth Company). The second receiving coil was then attached to the other valve of each mussel so that the distance between each coil was approximately 20 mm. In order to check, during gluing, that the coils were fitted at the correct distance from each other, the distance was estimated using the installation option in the main menu of 'Procomm'.

### 3.2.4 Initialisation

There is a range of responses available with the mussel monitor which can provide evidence of changes in water quality at different levels. Changes in water quality may be apparent as:

- closure of a number of mussels for a certain time;
- changes in the activity of a number of mussels over a preset time period;
- a decrease in the average valve position of a number of mussels;
- gaping, i.e. full opening of the mussel valve following death, of a number of mussels.

These criteria may be changed using the changing parameters option within Procomm. Sensitivity of different alarm criteria can be varied by altering these parameters. For example, the higher the closed percentage is set and the longer the period set for activity observations the more sensitive these individual alarms will become. This enables considerable scope for the adjustment of the pollution detection capability of the valve activity monitor. The parameters used during this evaluation are given in Appendix A.

### 3.2.5 Experimental studies

The algal dose studies with the mussel monitor were carried out in a flow-through 70 l circular black PVC container to restrict the impact of visual stimuli. The flow of groundwater to the tank was controlled through a rotameter, which permitted inflows of 50-500 ml $min^{-1}$. Initially the container received a groundwater flow of 150 ml $min^{-1}$ to remove potentially toxic leachates prior to use.

After this period the opening/closing regime under different feeding conditions was determined using a range of algal densities. Laboratory cultured *Chlorella vulgaris* was used. A density of 100 000 cells $ml^{-1}$ was used as the highest concentration because it is a typical value for phytoplankton densities in moderately flowing, nutrient rich rivers during the growing season (Bill Parr, pers. comm.). This is also within the range of maximum filtration shown by *Mytilus edulis* (Foster-Smith 1975). *Dreissena polymorpha* is considered to have a lower filtration rate than *Mytilus* (Foster-Smith 1975) and so tests were also carried out at algal densities of 50 000 and 25 000 cells $ml^{-1}$ . These values were considered to be more typical of less nutrient-rich rivers and conditions outside the growing season.

The duration of active and rest periods can be different in animals under the same conditions (Salanki 1979). Because of this variability the same animals were used to test the range of algal concentrations. The algal dose was increased over a period of 12 hours with each nominal concentration (25 000, 50 000 and 100 000 cells $ml^{-1}$ ) kept constant for a period of four hours. Cell densities were derived from absorbance values using the regression equation, $y=0.04803+12.5955x$ (where y is the cell density (cells $l^{-1}$ ) and x the absorbance

value) derived from *Chlorella* culture data (I Sims, personal communication). Water samples were taken at 15, 30, 60, 120, and 240 minutes after the addition of each algal dose and the absorbance of the sample determined. This was converted to cell density using the regression equation. Algae were then added to the vessel when required.

The mussels were acclimated in the vessel for 14 hours before feeding commenced under conditions of aerated groundwater at a flow rate of 100 (±50) ml $min^{-1}$ and a temperature of 14.5±1 °C. The water was continually recirculated using an EHEIM pump. It was not possible to produce the volume of algae required to maintain nominal algal concentrations in a flow-through system so static conditions were used for the 4-hour feeding periods. Flow was terminated with the addition of algae and the medium was recirculated using the EHEIM pump alone.

Valve activity patterns shown in these experiments were used to determine:

- which species showed the most reliable opening/closing regime with which to compare pollutant-induced effects;
- which level of algal dose should be used in the pollutant addition tests.

Once the species with the most reliable opening/closing regime were determined, the effects of episodes of ammonia, lindane, copper, zinc and endosulphan on the chosen species were assessed.

The nominal exposure ranges for the the pollutants tested were:

| | |
|---|---|
| Ammonia: | 8.3, 30.0 and 96.1 mg $l^{-1}$ - Total |
| | 0.1, 0.32 and 1.0 mg $l^{-1}$ - Un-ionized |
| Lindane: | 32, 100 and 320 µg $l^{-1}$ |
| Copper: | 12.5, 40, 125 µg $l^{-1}$ |
| Zinc: | 250, 500 and 1000 µg $l^{-1}$ |
| Endosulphan: | 2, 10 and 20 µg $l^{-1}$ |

These concentrations were obtained by the addition of appropriate amounts of stock solution (10 g $l^{-1}$ total ammonia, 2 mg $l^{-1}$ , lindane, 1 g $l^{-1}$ , copper, 0.5 g $l^{-1}$ , zinc and three endosulphan solutions of 120, 600 and 1200 µg $l^{-1}$ ). Ammonia exists in water in ionized ($NH_4^+$) and un-ionized ($NH_3$) forms with toxicity being due almost entirely to the un-ionized form within the normal pH range. The conversion from total ammonia to un-ionized ammonia was carried out using the 'UNION' model (Emerson *et al* 1975) assuming the following water quality parameters: temperature 16 °C, pH 7.7.

In each toxicant exposure experiment the newly attached mussels on the monitor were acclimated for 14 hours at 15.5 (±1) °C in aerated non-polluted test medium with a flow rate to the container of 150 (± 50) ml $min^{-1}$. The water in the tank was constantly recirculated by an EHEIM pump. The mussels were fed with *Chlorella* at the optimum feed rate, determined during the algal dosing study, for two hours before pollutant addition. This ensured that each mussel exhibited normal valve opening for feeding prior to toxicant exposure. Flow was stopped at this point to maintain algal density within the test vessel.

The pollutant was added as a spike diluted in one litre of groundwater to facilitate mixing in the test vessel. The EHEIM pump was kept on throughout the test with all of the pollutants except lindane to ensure an even distribution of the pollutant within the vessel. It was assumed that losses of lindane within the test vessel would be minimal because a large volume to surface area ratio was present in the test vessel, therefore minimising loss of lindane onto the vessel sides and mussel monitor. This assumption was not made with regard to the EHEIM pump, and so it was switched off when using lindane to eliminate it as a source of loss of lindane onto its internal components.

Water samples were taken immediately before the addition of the test substance (Time 0) and then 15, 30, 60, 120 and 240 minutes after addition. The water temperature (°C), pH and dissolved oxygen level (% ASV) were measured at the beginning (Time 0) and end (Time 240) of the test.

The valve opening of each mussel was recorded every 30 secs and stored on 3.5" floppy discs for subsequent evaluation at the end of each experiment. In the evaluation the large data files were split into small blocks using the 'Splits' program supplied by TNO. These smaller blocks were then manipulated on Lotus 1-2-3 spreadsheets for graphical analysis. Any alarm records were also separated from the data by the 'Splits' program.

## 3.3 Results

In each experiment only mussels showing valve openings of >25% before addition of algae or test substance were considered for graphical representation. Control levels for each mussel were based on readings taken over the hour prior to addition of the test substance spike.

### 3.3.1 Algal Dosing - *Dreissena*

The patterns of valve opening in a group of *Dreissena* exposed to a stepped increase in algal concentration from 0 to 100 000 cells $ml^{-1}$ are shown in Figure 3.1. Measured algal concentrations are shown in Figure 3.4a. Algal concentrations at each dose level remained fairly constant and were generally close to the nominal level (±5000 cells $ml^{-1}$). Physico-chemical parameters recorded during the experiment are given in Table 3.1. Changes occurred in temperature, by 0.9 °C, and pH, by 0.16 units across the dosing period.

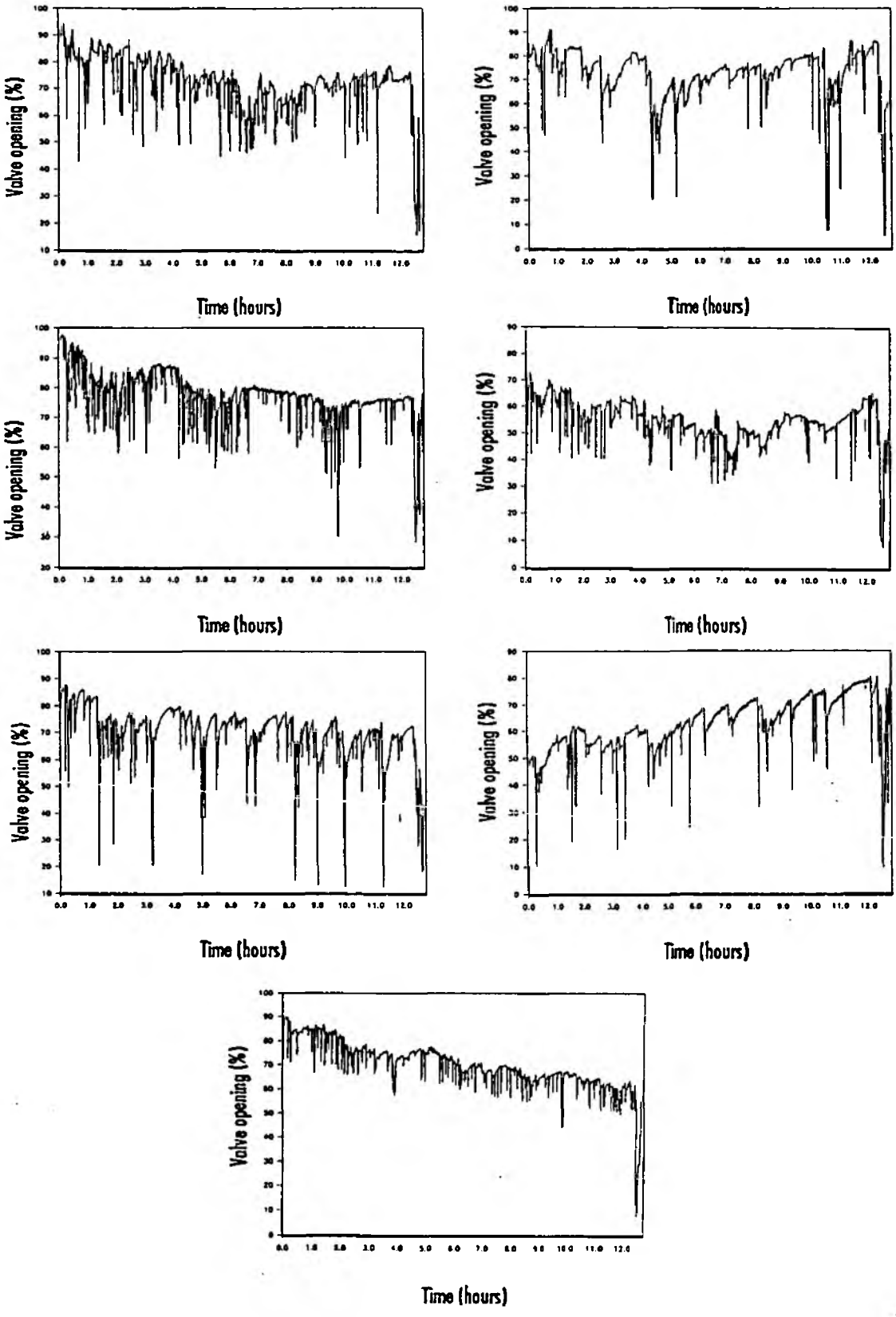


**Figure 3.1** Valve opening (%) of *Dressiena* exposed to 3 algal concentrations at 0 hours (25,000 cells/ml), at 4 hours (50,000 cells/ml) and at 8 hours (100,000 cells/ml). Groundwater flow restored at 12 hours.

**Table 3.1 Physico-chemical parameters (pH, temperature and dissolved oxygen) measured during the testing of the shell valve activity monitor with *Dreissena polymorpha* and algae**

| | 25 000 cells $ml^{-1}$ | | | 50 000 cells $ml^{-1}$ | | | 100 000 cells $ml^{-1}$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | pH | Temp. (°C) | DO (%) | pH | Temp. (°C) | DO (%) | pH | Temp. (°C) | DO (%) |
| Time 0 | 8.13 | 12.8 | 99 | 8.21 | 14.8 | 99 | 8.30 | 16.4 | 95 |
| Time 240 | 8.21 | 14.8 | 99 | 8.28 | 16.3 | 95 | 8.35 | 16.7 | 95 |

It was decided to include only seven mussels for evaluation of valve opening because one of the coil sets produced an abnormal signal leading to percentage values that fluctuated rapidly between 100 and 0%. This position on the monitor was excluded from all subsequent analyses using the change parameters menu in Procomm.

Throughout the exposure to algal concentrations there were no marked changes in average valve opening (Figure 3.5). Even though reductions in valve opening in individual mussels were evident all individuals remained open throughout the dosing period. Irregular changes in activity occurred in individuals but were not of sufficient frequency to activate the activity alarm. A decrease in average valve opening occurred across the 12-hour dosing period (Figure 3.5); however decreases in the opening of individual mussels were not of sufficient magnitude to activate the decreased average valve opening alarm. No valve closures occurred across the test period and all mussels maintained an average of between 60 and 90% valve opening throughout the algal dosing.

### 3.3.2 Algal Dosing - *Unio*

The patterns of valve opening in a group of *Unio* exposed to a stepped increase in algal concentration from 0 to 100 000 cells $ml^{-1}$ are shown in Figure 3.2. Measured algal concentrations are shown in Figure 3.4c. Algal concentrations at each dose level remained fairly constant and were generally close to the nominal cell count. Physico-chemical parameters recorded during the experiment are given in Table 3.2. Changes occurred in temperature, by 3.6 °C, and pH, by 0.41 units, across the dosing period.

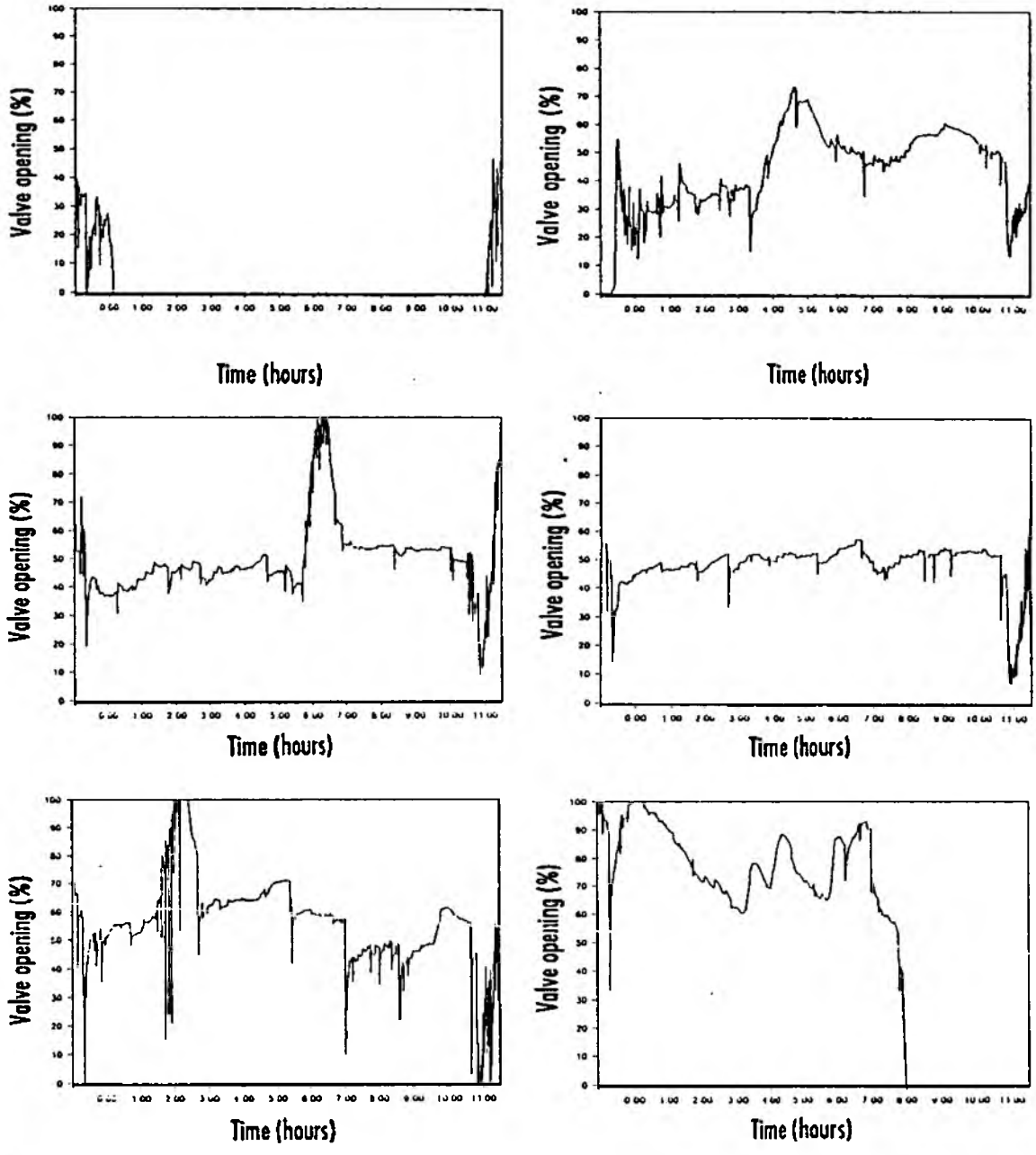


**Figure 3.2** Valve opening (%) of *Unio* exposed to 3 algal concentrations at 0 hours (25,000 cells/ml), 3 hours (50,000 cells/ml) and 7 hours (100,000 cells/ml). Groundwater flow restored at 11 hours

**Table 3.2 Physico-chemical parameters (pH, temperature and dissolved oxygen) measured during the testing of the shell valve activity with *Unio* and algae**

| | 25 000 cells $ml^{-1}$ | | | 50 000 cells $ml^{-1}$ | | | 100 000 cells $ml^{-1}$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | pH | Temp. (°C) | DO (%) | pH | Temp. (°C) | DO (%) | pH | Temp. (°C) | DO (%) |
| Time0 | 8.19 | 13.1 | 94 | 8.41 | 15.6 | 93 | 8.48 | 16.5 | 97 |
| Time 240 | 8.41 | 15.6 | 93 | 8.48 | 16.5 | 97 | 8.50 | 16.7 | 97 |

The change in average valve opening at all algal concentrations was far greater than that exhibited by *Dreissena* (Figure 3.2), but did not appear to be consistent with any change in algal concentration. Valve opening during the test period was on average lower than pre- and post-test opening. This was also observed in individual mussel valve movement patterns. Of the individual mussels only one exhibited a consistent opening regime across the algal exposure range, one closed throughout, three opened erratically and one opened erratically and closed at the start of the 100 000 cells $ml^{-1}$ dose. While increased activity and decreased average opening occurred in some individuals, no relevant alarms were activated. The closure alarm was activated at 10 hours, 50 minutes and remained on for a further six minutes.

### 3.3.3 Algal Dosing - *Anodonta*

The patterns of valve opening in a group of *Anodonta* exposed to a stepped increase in algal concentration from 0 to 100 000 cells $ml^{-1}$ are shown in Figure 3.3. Measured algal concentrations are shown in Figure 3.4b. Algal concentrations at each dose level remained fairly constant and were close to the nominal cell count. Physico-chemical parameters recorded during the experiment are given in Table 3.3. Changes occurred in temperature, by 2.3 °C, and pH, by 0.31 units, across the dosing period.

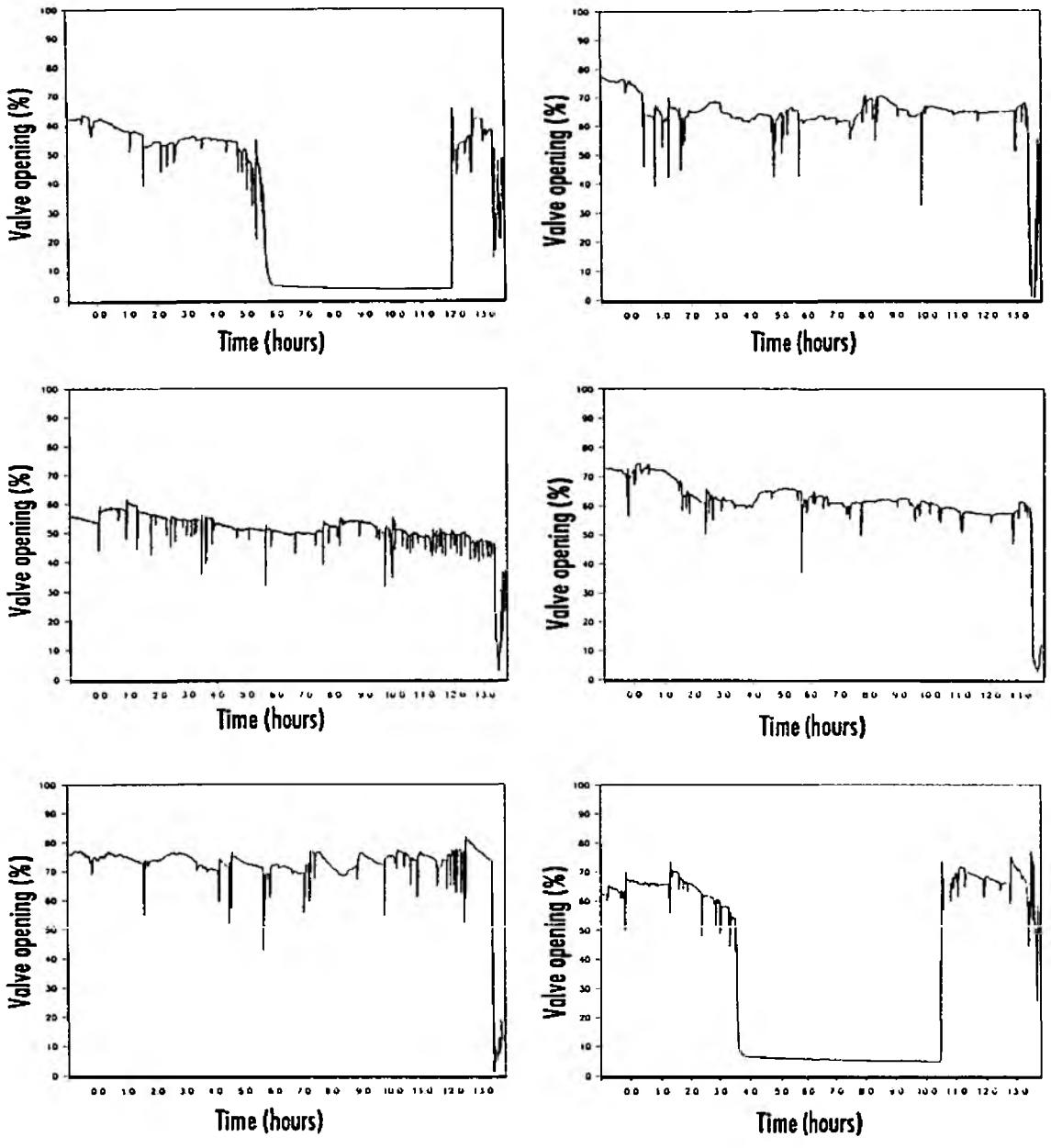


**Figure 3.3** Valve opening (%) of *Anodonta* exposed to 3 algal concentrations at 0 hours (25,000 cells/ml), 5 hours (50,000 cells/ml) and 9 hours (100,000 cells/ml). Groundwater flow restored at 13 hours

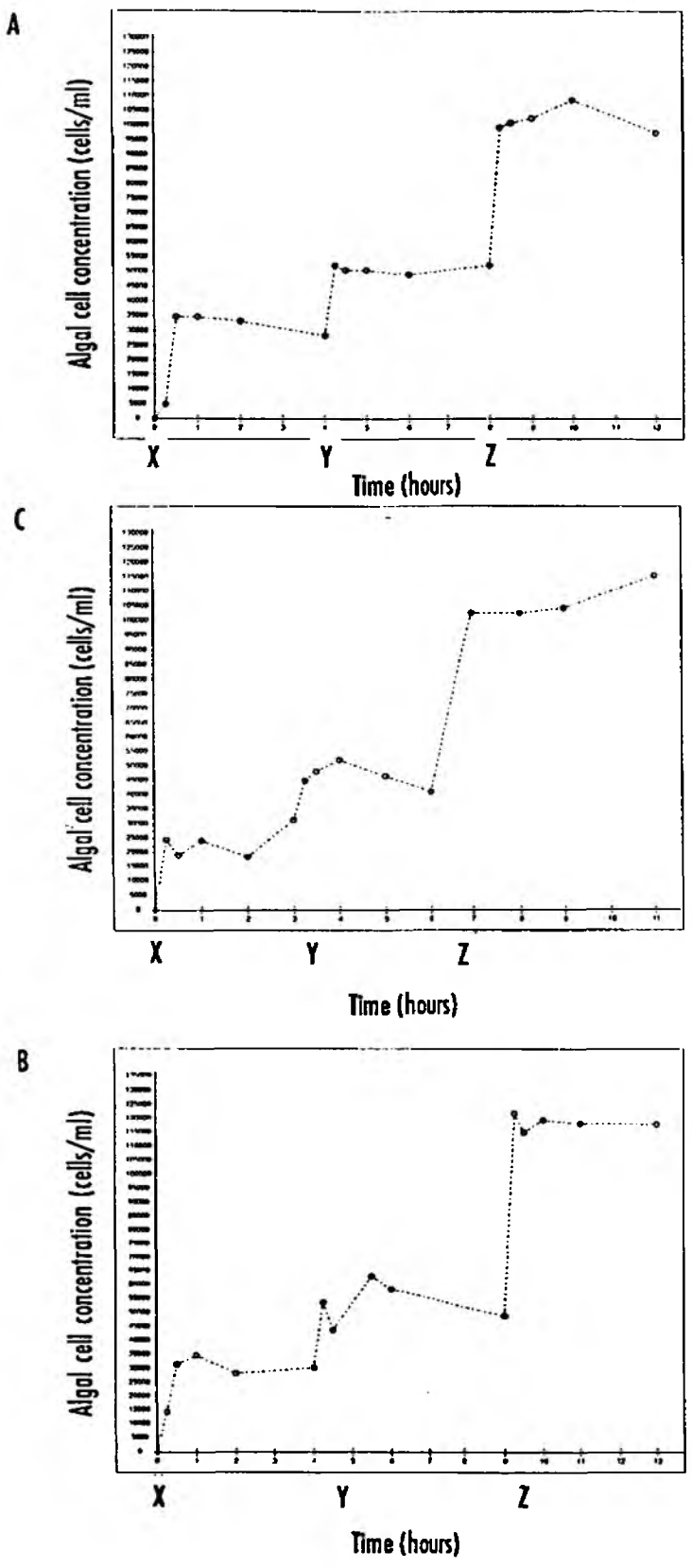


**Figure 3.4** Algal levels measured in the test vessel during each experimental study, for (A) *Dreissena* (B) *Anodonta* and (C) *Unio*. Doses introduced at X (25,000 cells/ml), Y (50,000 cells/ml) and Z (100,000 cells/ml)

**Table 3.3 Physico-chemical parameters (pH, temperature and dissolved oxygen) measured during the testing of the shell valve activity with *Anodonta* and algae**

| | 25 000 cells $ml^{-1}$ | | | 50 000 cells $ml^{-1}$ | | | 100 000 cells $ml^{-1}$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | pH | Temp. (°C) | DO (%) | pH | Temp. (°C) | DO (%) | pH | Temp. (°C) | DO (%) |
| Time 0 | 8.33 | 13.9 | 96 | 8.59 | 14.8 | 96 | 8.61 | 15.6 | 93 |
| Time 240 | 8.59 | 14.8 | 96 | 8.61 | 15.6 | 93 | 8.62 | 16.2 | 98 |

Average valve opening decreased across the first two concentrations and regained the initial level of 65-70% at the end of the final concentration (Figure 3.5c). The valve opening behaviour of four of the individual mussels remained reasonably constant across the dose period, however two closed between 3.5 and 10.5, and 5.5 and 12 hours respectively (Figure 3.3). Each closure coincided with a step in the average opening. It can be inferred from this that the large stepped closures in the average valve opening were produced by the staggered closing of the two closed individuals. The closure alarm was not activated at the preset sensitivity by these closures because closure occurred in only two mussels.

### 3.3.4 Choice of species

Of the three species, *Dreissena* produced the most constant average and individual valve opening across the three algal doses (Figure 3.5). While closures and marked activity changes occurred in individual *Dreissena* these could be attributed to disturbance from external stimuli. If this is the case, *Dreissena* is more susceptible to such disturbance than both *Unio* and *Anodonta*.

However the response of both *Unio* and *Anodonta* was more variable than that of *Dreissena*. Only six of the *Unio* and *Anodonta* mussels produced responses that exhibited >25% opening in the 1-hour control period. Of the *Dreissena* all individuals were open >25% during the control period. While closures of long duration occurred in individual valve activity for both *Unio* and *Anodonta*, closure by *Dreissena*, when it occurred, was limited to periods of only short duration.

To be suitable for reliable detection of pollutants over any time scale a group of mussels must:

- maintain an open state the majority of the time;

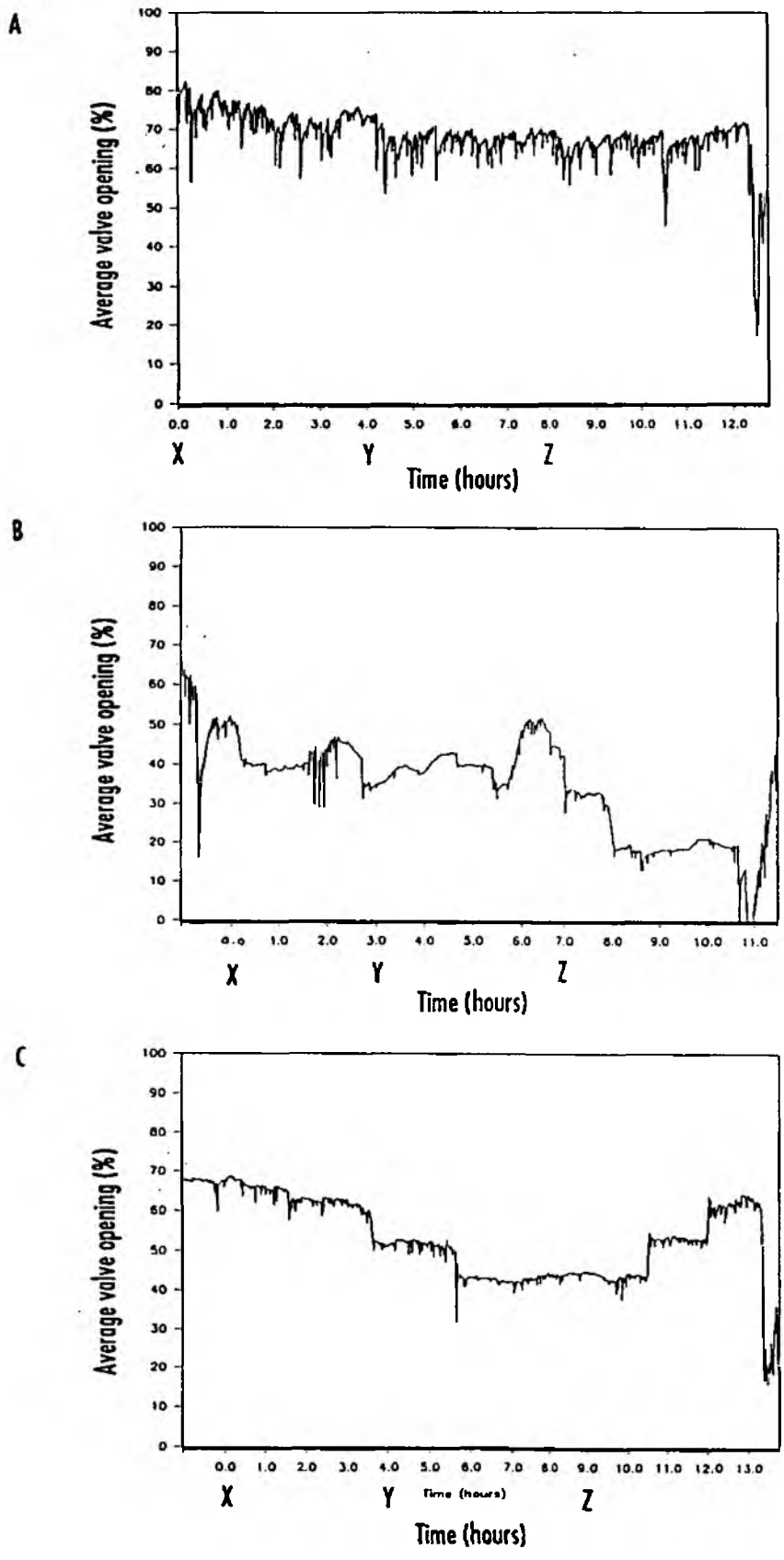


**Figure 3.5** Average valve opening (%) of (A) *Dreissena*, (B) *Unio* and (C) *Anodonta* in response to algal concentrations of (X) 25,000, (Y) 50,000 and (Z) 100,000 cells/ml

- when open maintain a relatively constant percentage valve opening over time;
- exhibit little variation between individuals.

Of the three species tested in this study *Dreissena* proved the most reliable using these criteria and so was selected to determine the effects of episodes of the chosen test substances.

### 3.3.5 Ammonia

The valve opening responses of a group of *Dreissena* exposed to total ammonia concentrations of 8.3, 30.0 and 96.1 mg $l^{-1}$ are shown in Figures 3.6 to 3.8. The measured un-ionized ammonia levels responsible for toxic impact (calculated using the 'UNION' model) are shown in Figure 3.9. Measured un-ionized ammonia levels differed markedly from expected values. The pH values assumed in calculating the required ammonia levels using the UNION model differed by up to 0.6 units from those measured in the test vessel. This resulted in a greater proportion of ammonia remaining un-ionized, and hence much higher measured un-ionized ammonia levels. Physico-chemical parameter recorded during the experiment are given in Table 3.4. Increases occurred in temperature, by 0.9 to 1.4 °C, and pH, by 0.05, 0.09 and 0.06 units respectively, across the dosing period.

**Table 3.4 Physico-chemical parameters (pH, temperature and dissolved oxygen) measured during the testing of the shell valve activity monitor with *Dreissena polymorpha* and ammonia**

| | 100 µg $l^{-1}$ ammonia | | | 320 µg $l^{-1}$ ammonia | | | 1000 µg $l^{-1}$ ammonia | | |
|---|---|---|---|---|---|---|---|---|---|
| | pH | Temp. (°C) | DO (%) | pH | Temp. (°C) | DO (%) | pH | Temp. (°C) | DO (%) |
| Algae added | 8.16 | 14.2 | 97 | 7.69 | 14.3 | 96 | 7.52 | 15.1 | 97 |
| Time 0 | 8.26 | 14.8 | 96 | 8.04 | 15.1 | 94 | 8.03 | 15.1 | 96 |
| Time 240 | 8.21 | 15.9 | 98 | 7.95 | 16.0 | 98 | 8.06 | 16.7 | 95 |

During exposure to 0.3 mg $l^{-1}$ un-ionized ammonia (8.3 mg $l^{-1}$ total ammonia) visible changes occurred in average valve opening (Figure 3.10); a reduction in average valve opening occurred from the point of pollutant addition until approximately 2.5 to three hours

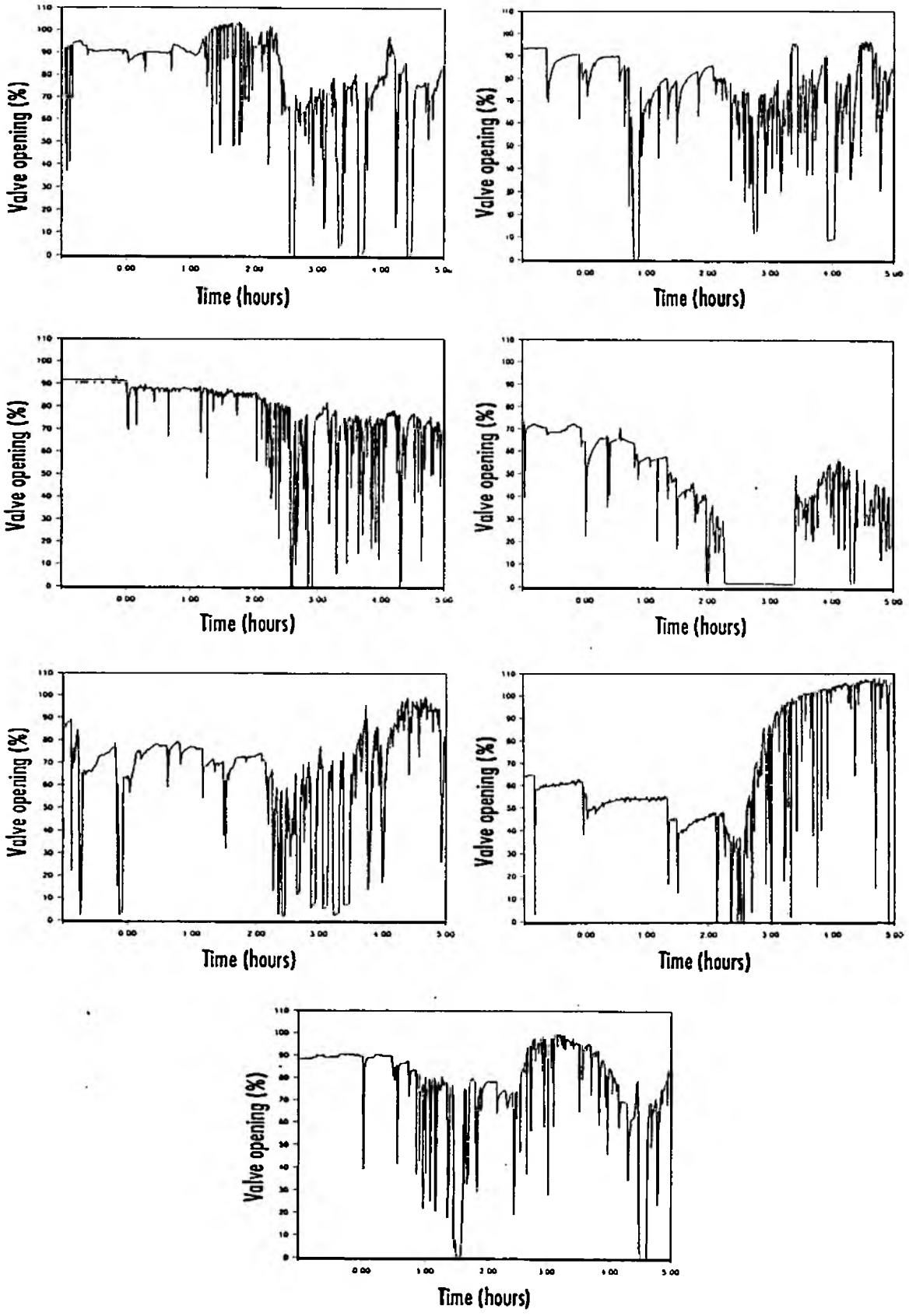


**Figure 3.6** **Valve opening (%) responses of *Dreissena* to 100 µg/l unionised ammonia. Pollutant added at 0 hours and groundwater flow restored at 4 hours**

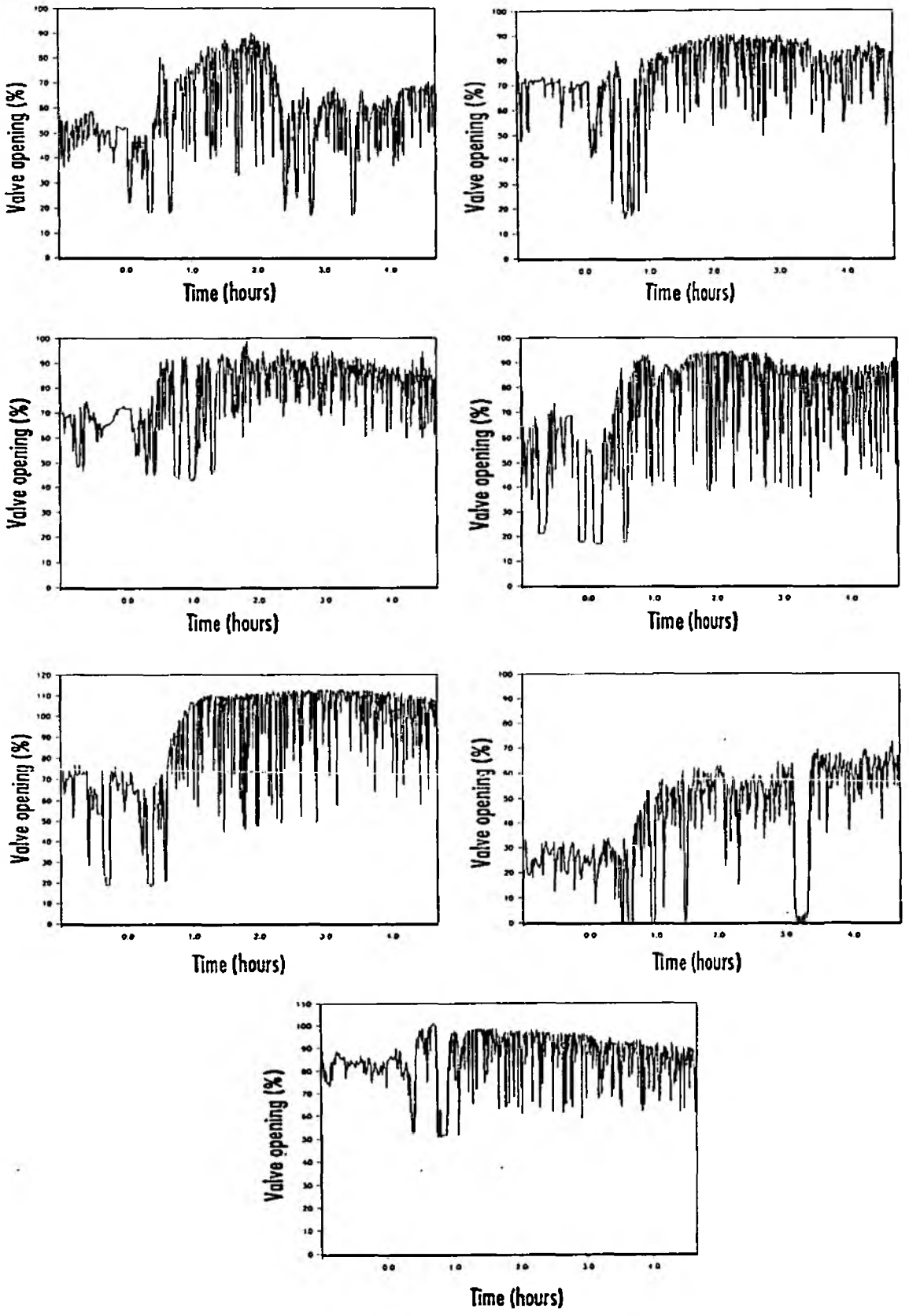


**Figure 3.7** **Valve opening (%) responses of *Dreissena* to 320 µg/l unionised ammonia. Pollutant added at 0 hours and groundwater flow restored at 4 hours**

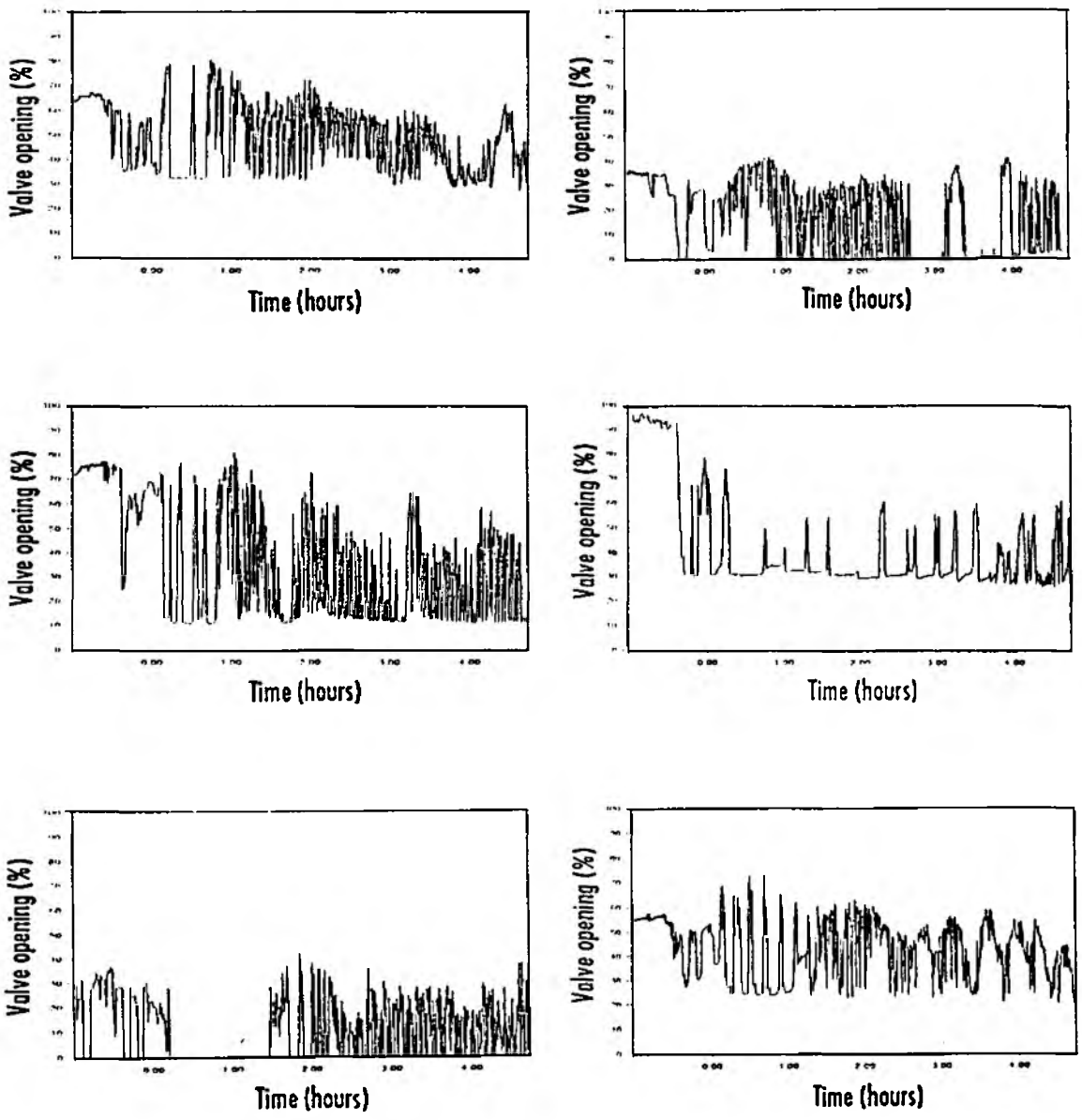


**Figure 3.8 Valve opening (%) responses of *Dreissena* to 1000 µg/l unionised ammonia. Pollutant added at 0 hours and groundwater flow restored at 4 hours**

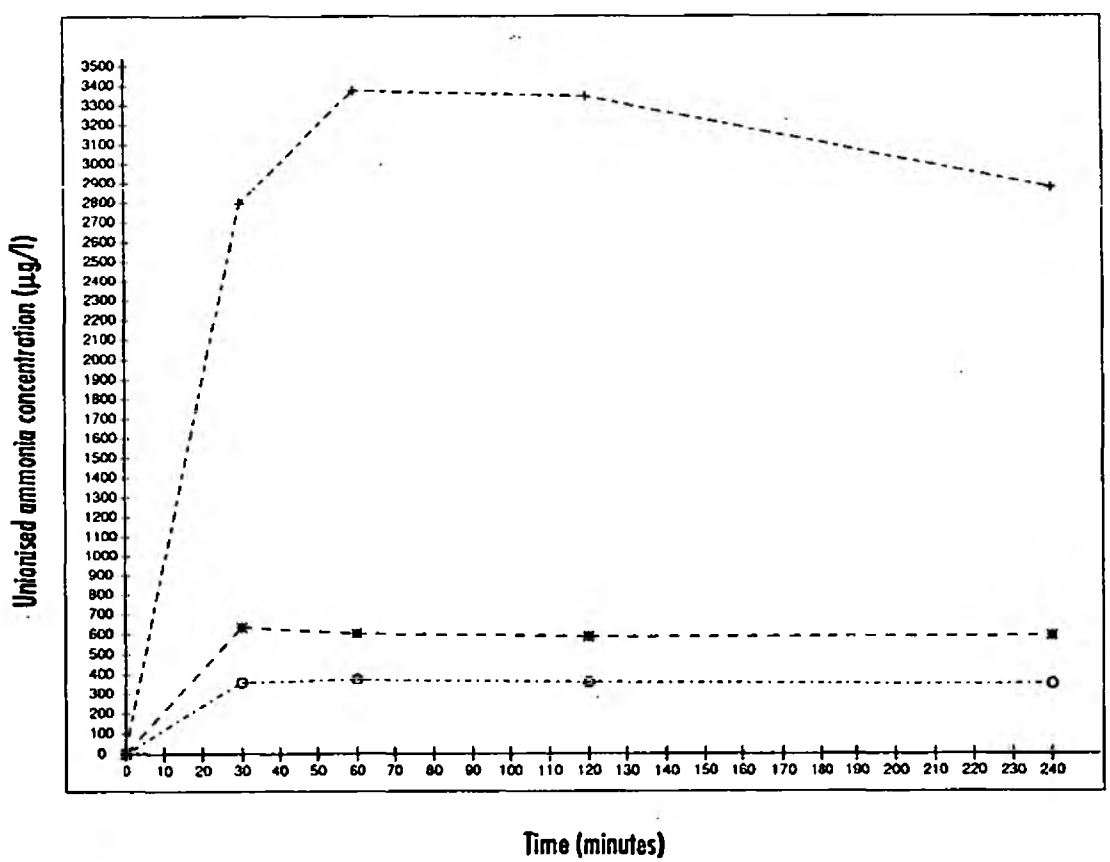


**Figure 3.9 Measured unionised ammonia concentrations at nominal total ammonia concentrations of 8.3 µg/l (○), 30 µg/l (✱) and 96.1 µg/l (✚)**

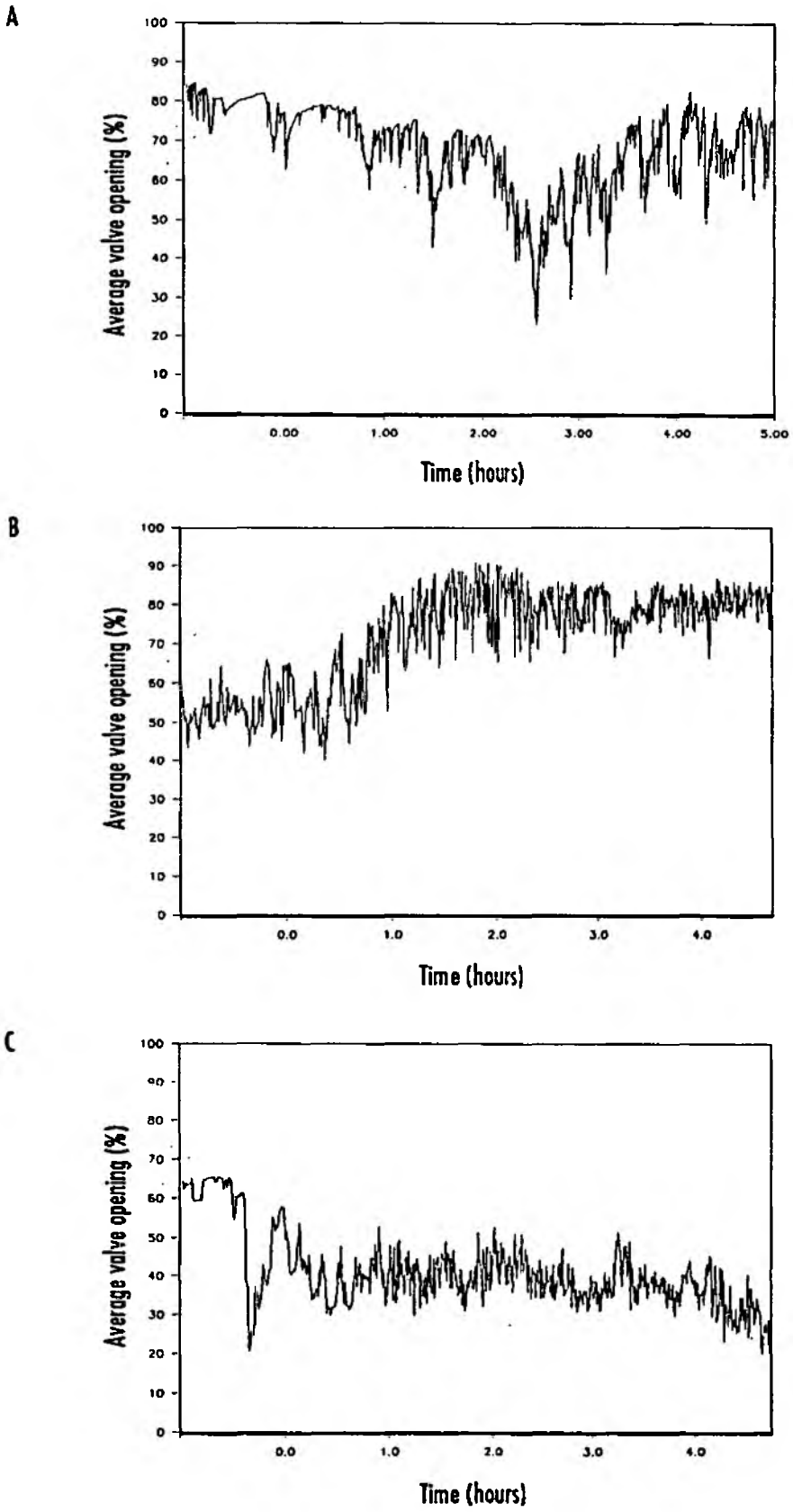


**Figure 3.10** Average valve opening (%) for the groups of *Dreissena* exposed to (A) 100 µg/l, (B) 320 µg/l and (C) 1000 µg/l unionised ammonia. Pollutant added at 0 hours and groundwater flow restored at 4 hours

after (from 80% to 40%), then increasing to a value equivalent to that before pollutant after four hours. Further, after one hour, an increase in activity occurred resulting in a visibly more variable trace. More complex responses occurred in the individual mussels (Figure 3.6), in most individuals, marked changes in valve activity occurred after two hours, with rapid opening and closing in all but one individual, which closed for one hour. Changes in activity were also associated with larger changes in valve opening values during this interval of the test period. After three hours, activation of the activity alarm occurred. This continued for a further hour. While closure did occur in some individual mussels this was, in the majority of instances, of too short a duration to overlap with other mussel closures. As a consequence, the closure alarm was not activated at any point during the test period.

At 0.6 mg $l^{-1}$ un-ionized ammonia (30 mg $l^{-1}$ total ammonia) increases in valve opening in most individuals during the exposure period (Figure 3.7) resulted in an increase in average valve opening (Figure 3.10) from approximately 50% to 80% over the first two hours; this was maintained for the remaining period. Almost immediately after test substance addition individual mussels exhibited changes in valve opening behaviour as a marked increase in activity. As a result the activity alarm was activated after one hour and continued to 4.5 hours after addition of the test substance. It should be noted that the extent of this change in activity in individual mussels was not apparent in the average valve opening data (Figure 3.10).

At 3.4 mg $l^{-1}$ un-ionized ammonia (96 mg $l^{-1}$ total ammonia) a gradual decrease occurred in average valve opening (Figure 3.10), from 55% to 30%, over the test period. Changes in valve opening activity occurred almost immediately - in most cases after 15 minutes. In some individuals there is evidence of activity changes before the addition of the test substance; this may be due to external stimuli, e.g. sound or vibration. As a result of changes in activity the activity alarm was activated after one hour and continued until 3.5 hours after test substance addition. While closures occurred in individual mussels, these were not of sufficient magnitude to activate the closure alarm at the pre-set limits (see Appendix A).

### 3.3.6 Lindane

Analysis of water samples taken from the test vessel after addition of the test substance indicated that lindane was present at all nominal exposure concentrations at or below the limit of detection ($\leq 2$ µg $l^{-1}$). Since lindane is readily soluble at 8 mg $l^{-1}$ in water without a carrier substance, it can be assumed that losses of lindane through adsorption to surfaces of the test vessel, or volatilisation were responsible for the very low concentrations of lindane that occurred. Lindane may not have been present in individual samples because of a combination of poor mixing in the vessel, after the recirculation pump had been switched off, and the low concentrations at which it was present in the vessel.

The valve opening responses of a group of *Dreissena* exposed to nominal lindane concentrations of 32, 100 and 32 µg $l^{-1}$ are shown in Figures 3.11 to 3.13. Measured changes in physico-chemical parameters recorded during the experiment are given in

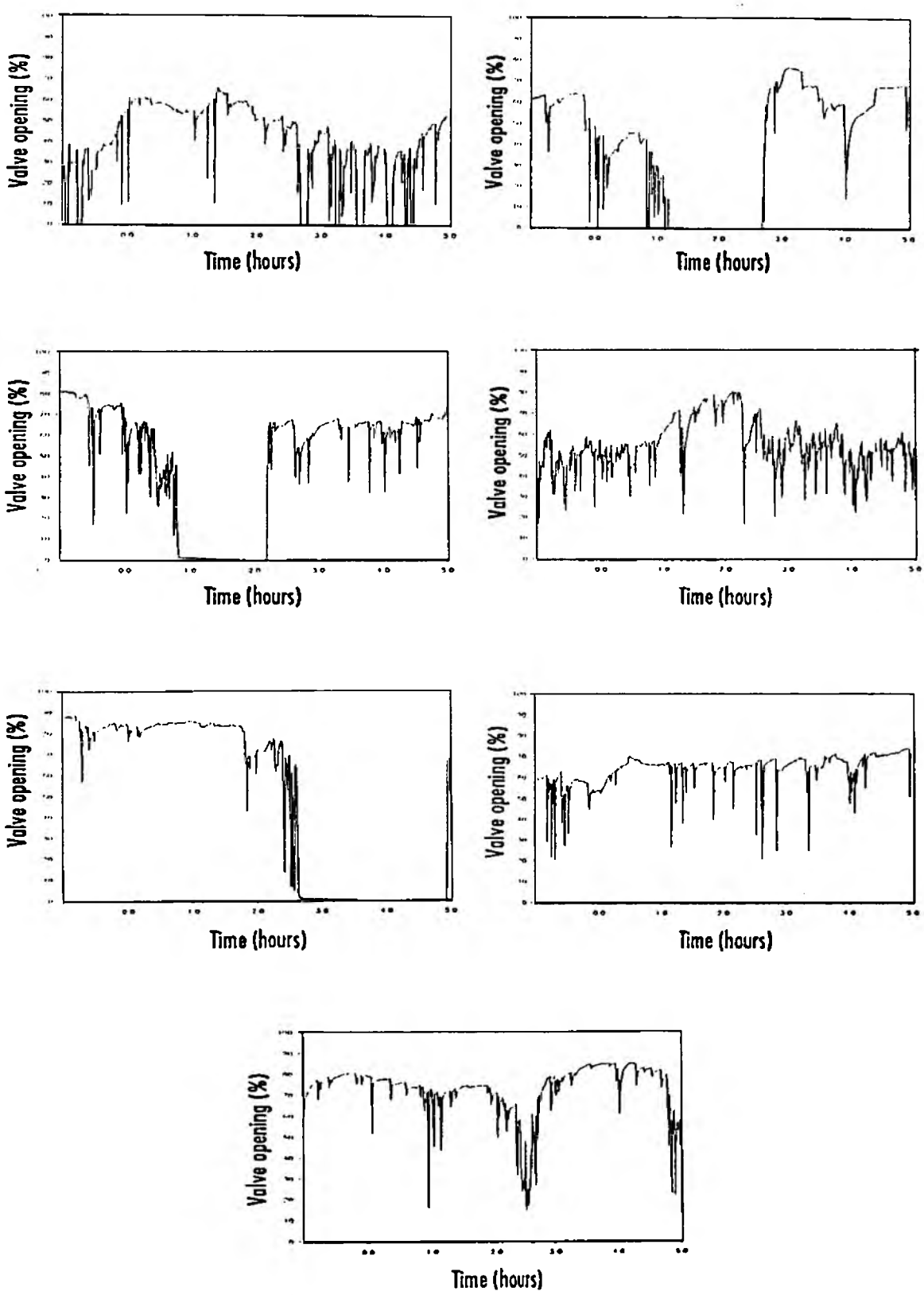


**Figure 3.11** **Valve opening (%) responses of *Dreissena* to 32 µg/l lindane. Pollutant added at 0 hours and groundwater flow restored at 4 hours**

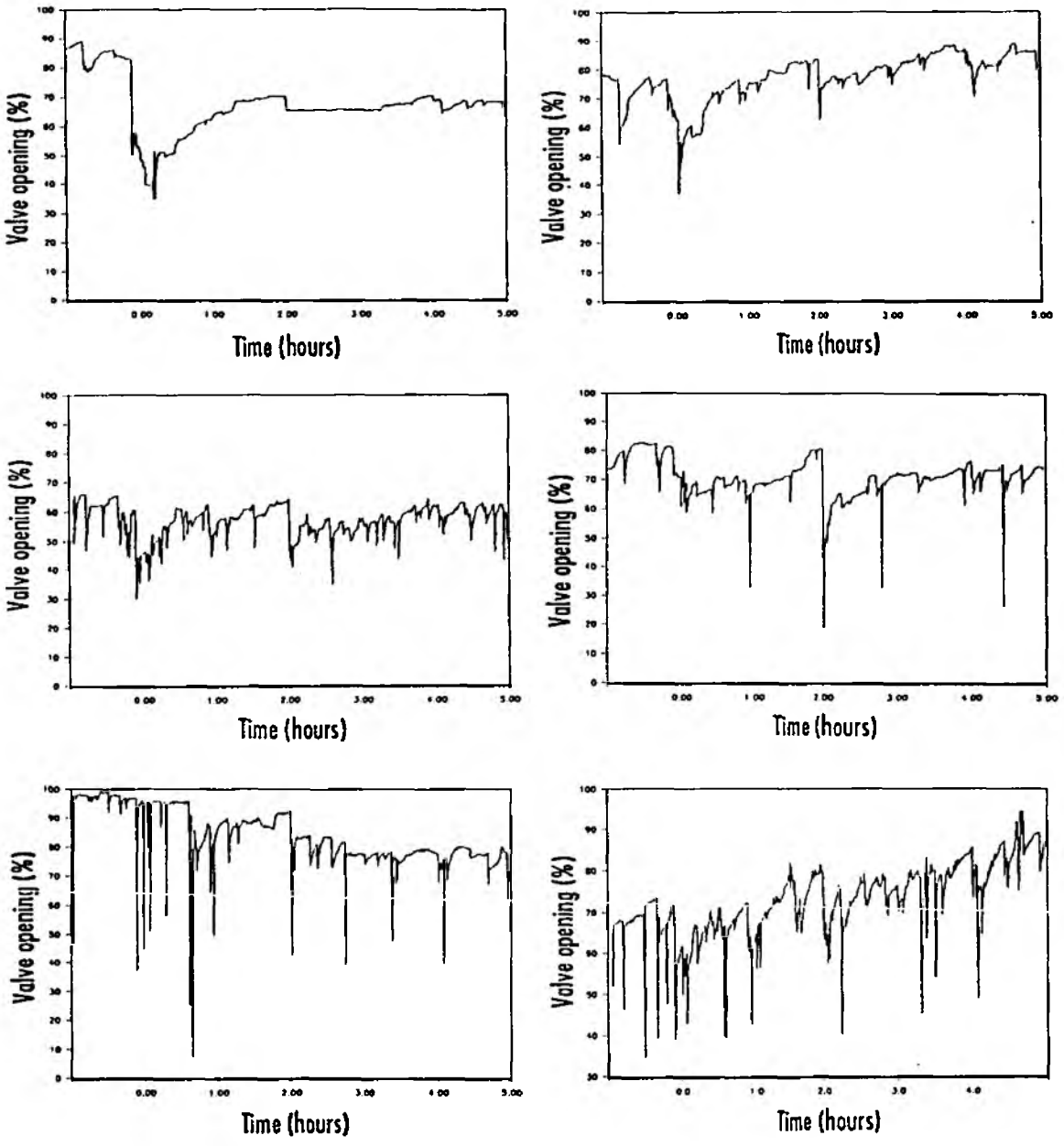


**Figure 3.12** **Valve opening (%) responses of *Dreissena* to 100 μg/l lindane. Pollutant added at 0 hours and groundwater flow restored at 4 hours**

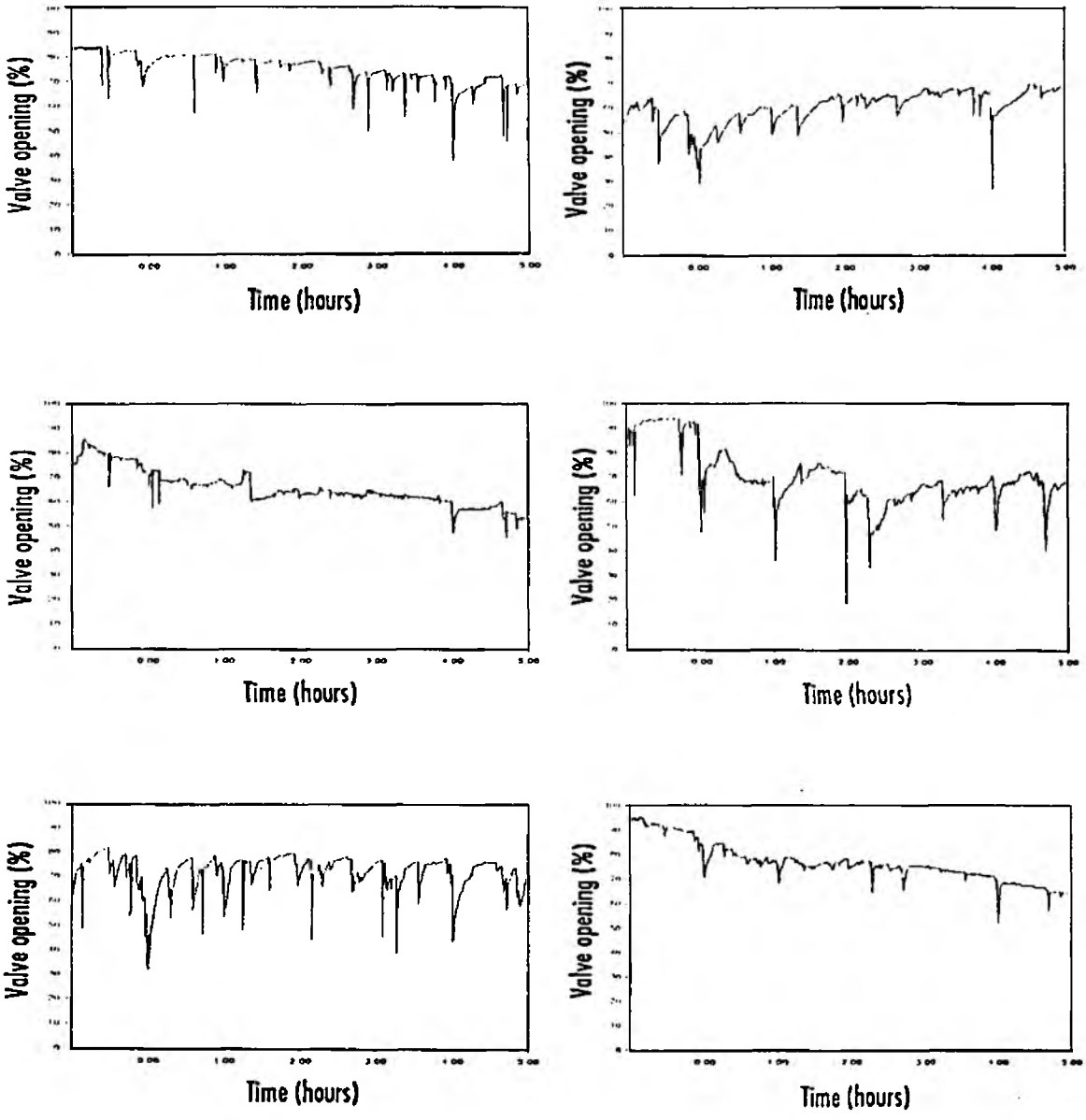


**Figure 3.13** **Valve opening (%) responses of *Dreissena* to 320 µg/l lindane. Pollutant added at 0 hours and groundwater flow restored at 4 hours**

Table 3.5. Changes occurred in temperature, by 1.1 to 1.7 °C, and pH, by 0.18, 0.11 and 0.25 units respectively, across the dosing period.

**Table 3.5 Physico-chemical parameters (pH, temperature and dissolved oxygen) measured during the testing of the shell valve activity monitor with *Dreissena polymorpha* and lindane**

| | 32 µg $l^{-1}$ lindane | | | 100 µg $l^{-1}$ lindane | | | 320 µg $l^{-1}$ lindane | | |
|---|---|---|---|---|---|---|---|---|---|
| | pH | Temp. (°C) | DO (%) | pH | Temp. (°C) | DO (%) | pH | Temp. (°C) | DO (%) |
| Algae added | 8.02 | 14.1 | 102 | 8.00 | 15.7 | 97 | 7.48 | 16.0 | 98 |
| Time 0 | 8.22 | 14.8 | 100 | 8.12 | 16.6 | 93 | 7.88 | 16.6 | 98 |
| Time 240 | 8.40 | 16.1 | 102 | 8.23 | 18.3 | 96 | 8.13 | 17.7 | 98 |

At 32 µg $l^{-1}$ lindane valve closure occurred in three individual mussels for approximately 1.5 to 2 hours each; one other mussel closed for intermittent periods between 2.5 and 4 hours after test substance addition (Figure 3.11). The remaining mussels produced no easily interpretable changes in valve opening. A decrease occurred in average valve opening (Figure 3.14) between one and three hours after test substance addition. This was due to the valve closure of two mussels at this time. No alarms were activated during the test period.

At 100 µg $l^{-1}$ lindane no direct changes occurred in the valve opening of individual mussels. Reductions in valve openings of some individual mussels occurred at the addition of the test substance and at a point two hours after (Figure 3.12). This is reflected in the small decreases in the average valve opening seen at these times (Figure 3.14). No alarms were activated during the test period.

At 320 µg $l^{-1}$ gradual reductions in valve opening occurred in three individuals over the test period. No other changes occurred in individual valve opening and no marked changes were apparent in the average valve opening (Figure 3.14).

### 3.3.7 Copper

The valve opening responses of a group of *Dreissena* exposed to copper concentrations of 12.5, 40 and 125 µg $l^{-1}$ are shown in Figures 3.15 to 3.17. Measured changes in copper concentrations and physico-chemical parameters recorded during the experiment are given in Figure 3.18 and Table 3.6. At all nominal copper concentrations decreases occurred in the

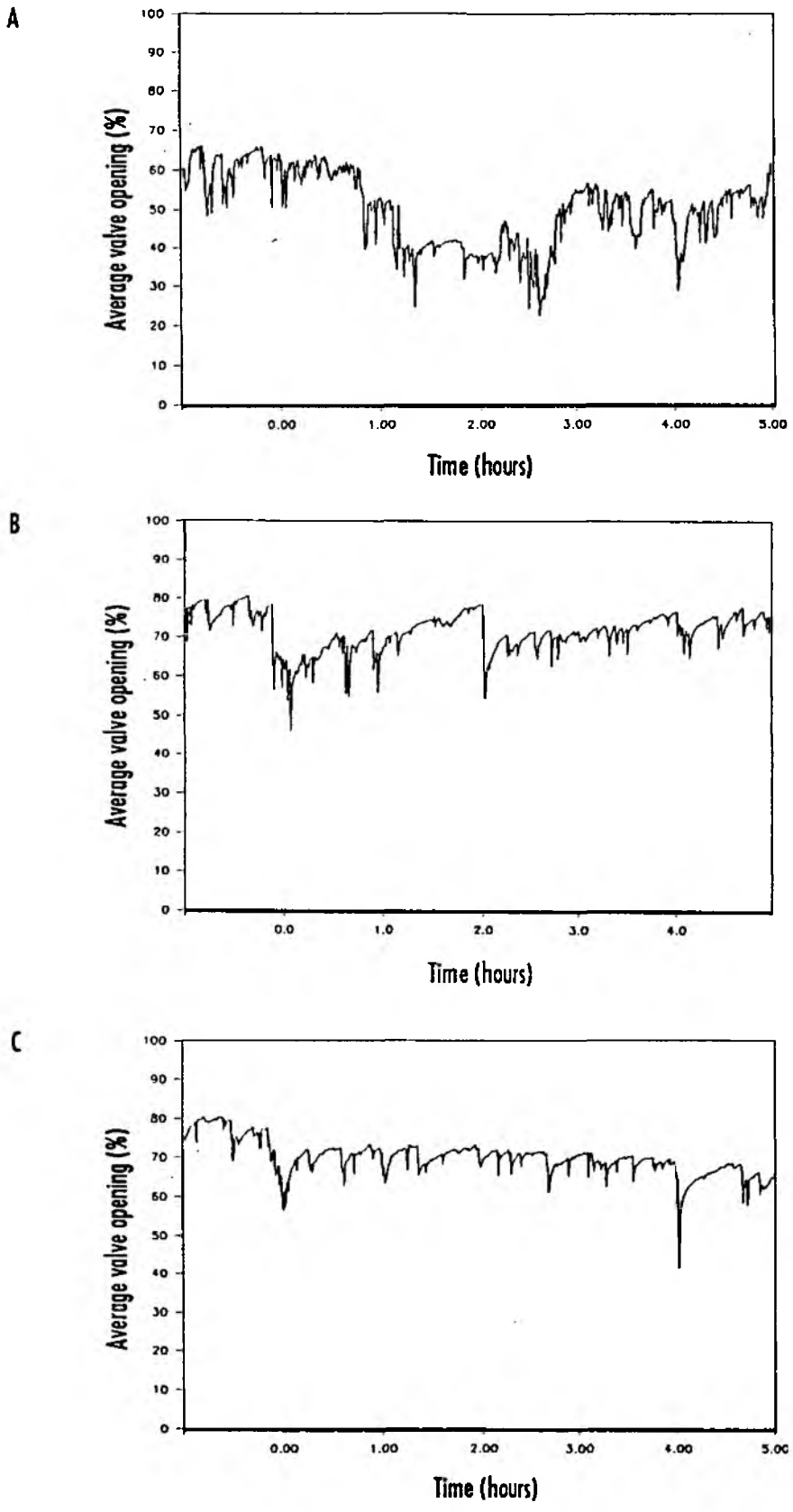


**Figure 3.14** Average valve opening (%) for the groups of *Dreissena* exposed to (A) 32 μg/l, (B) 100 μg/l and (C) 320 μg/l lindane. Pollutant added at 0 hours and groundwater flow restored at 4 hours

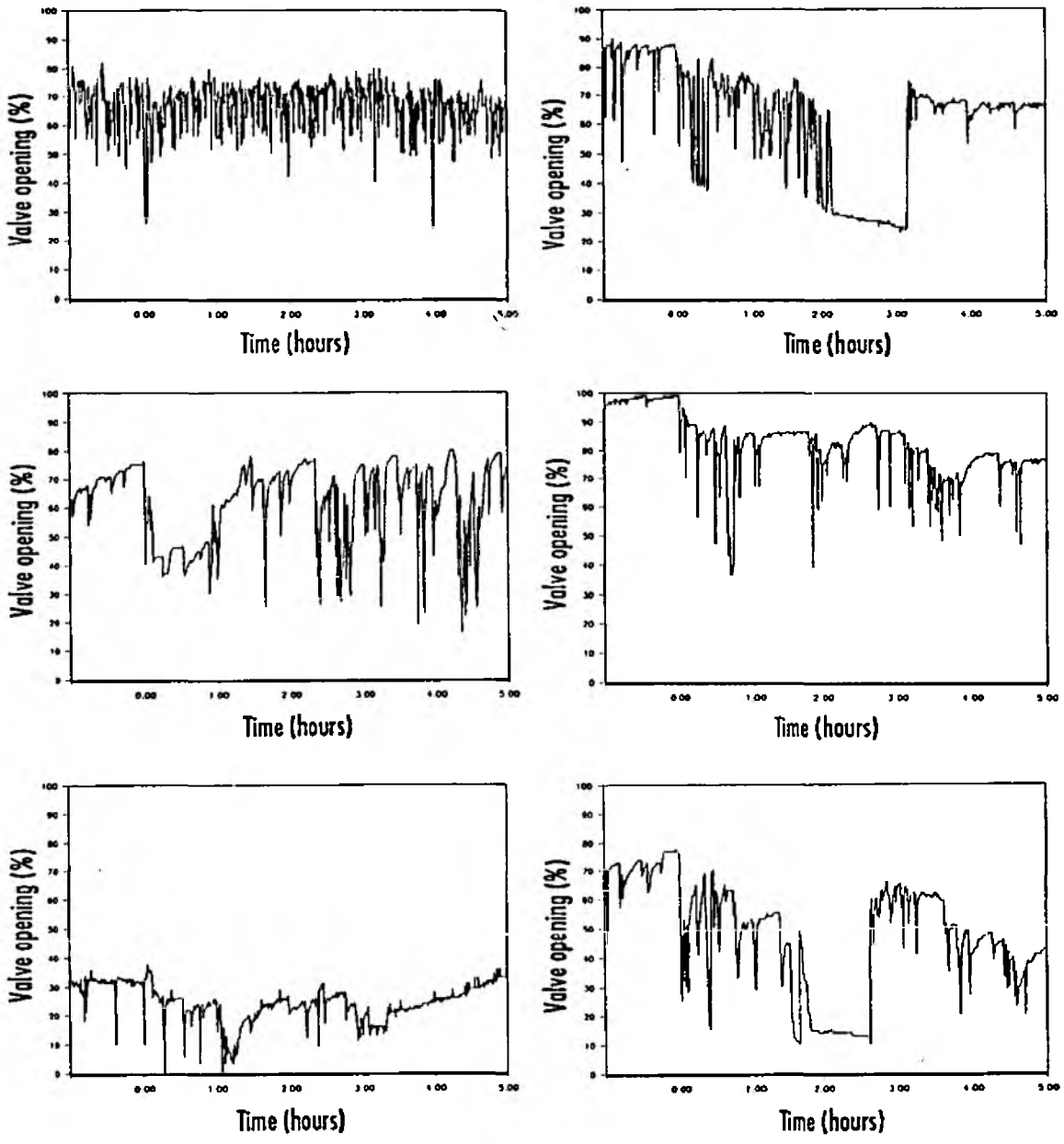


**Figure 3.15 Valve opening (%) responses of *Dreissena* to 12.5 µg/l copper. Pollutant added at 0 hours and groundwater flow restored at 4 hours**

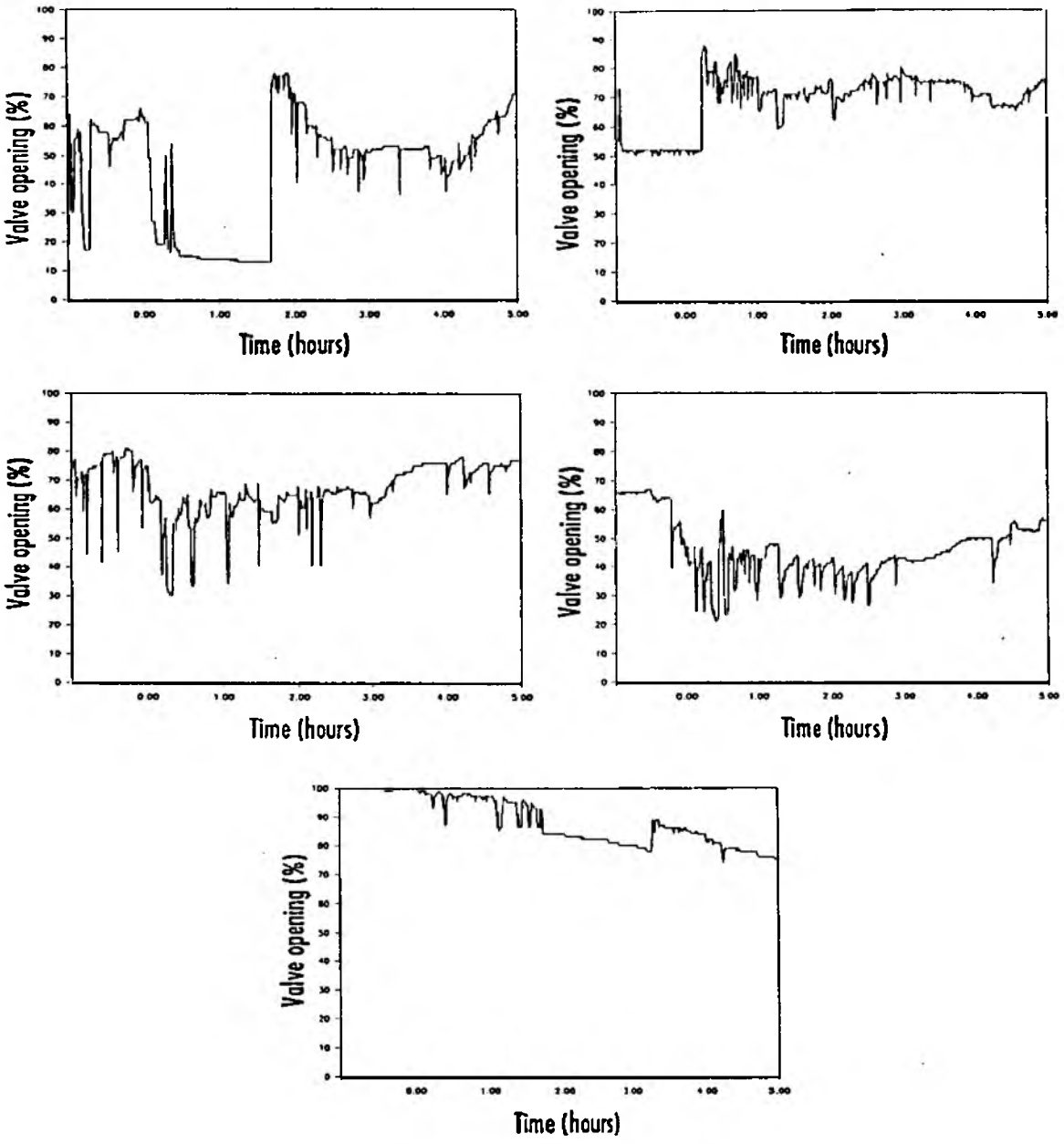


**Figure 3.16** **Valve opening (%) responses of *Dreissena* to 40 µg/l copper. Pollutant added at 0 hours and groundwater flow restored at 4 hours**

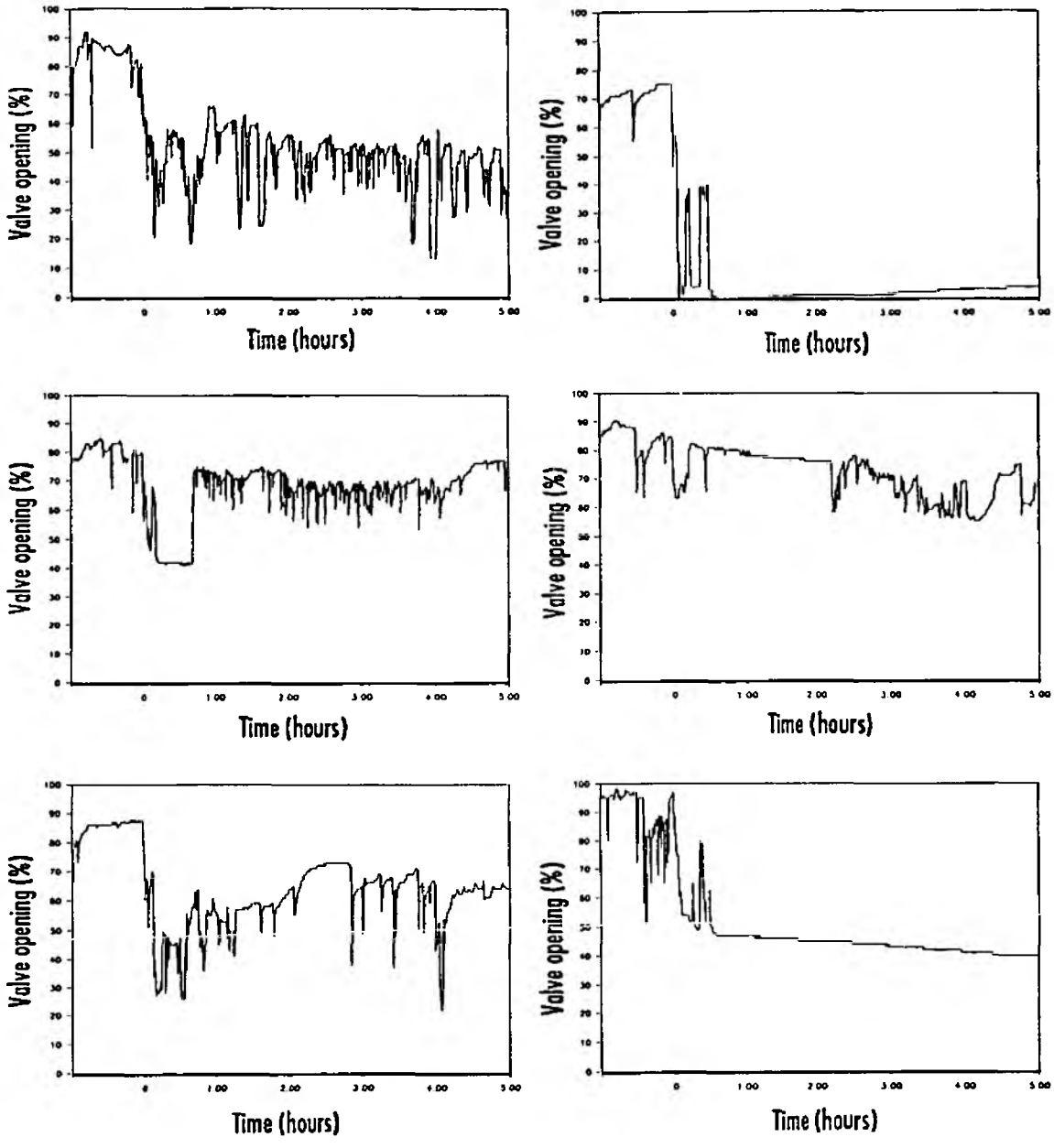


**Figure 3.17** **Valve opening (%) responses of *Dreissena* to 125 µg/l copper. Pollutant added at 0 hours and groundwater flow restored at 4 hours**

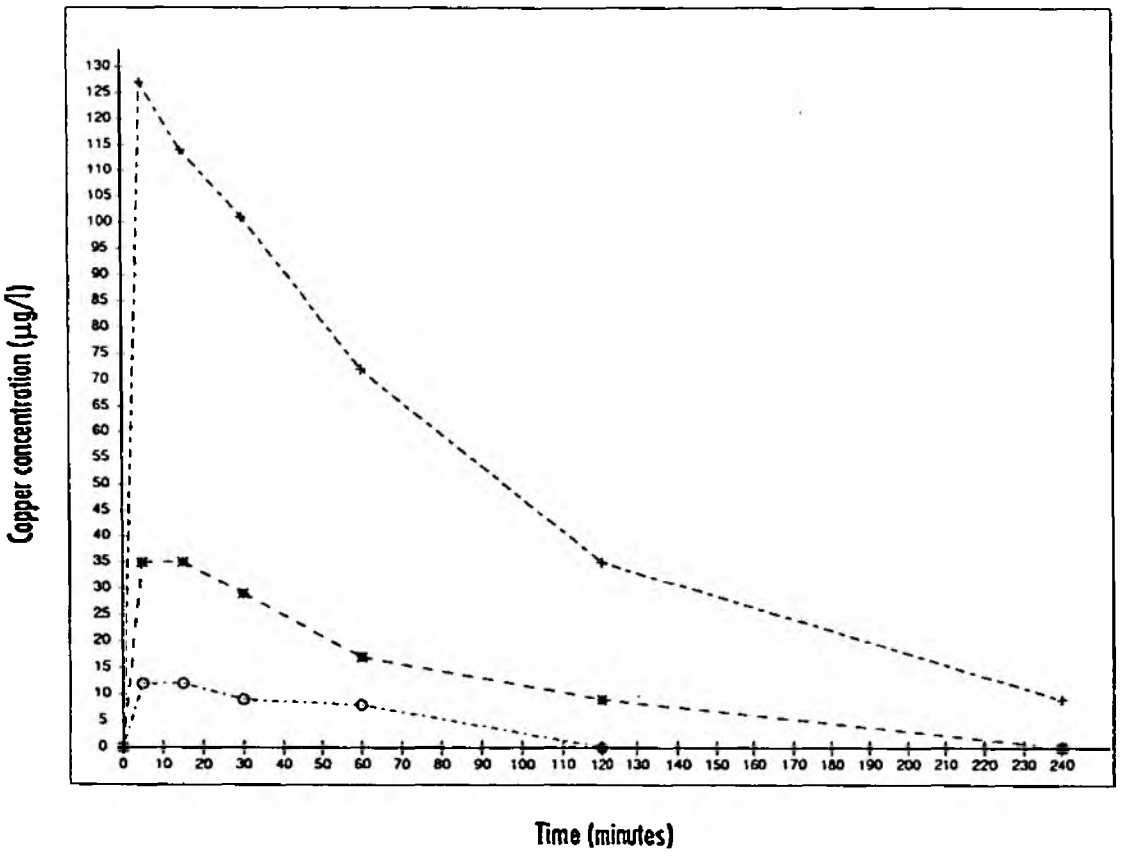


**Figure 3.18** Measured copper concentrations at nominal copper concentrations of 12.5 µg/l (○), 40 µg/l (✱) and 125 µg/l (✚)

measured copper concentration over time. This took place at a similar rate for all concentrations of copper and, since samples were filtered before analysis and copper will form insoluble carbonates in hard water such as that used in the test (Mallet *et al* 1992), it can be assumed these losses were due to the formation of insoluble copper carbonate. Changes occurred in temperature, by 1.4 to 2.8 °C, and pH, by 0.01, 0.08 and 0.03 units respectively, across the dosing period.

**Table 3.6 Physico-chemical parameters (pH, temperature and dissolved oxygen) measured during the testing of the shell valve activity monitor with *Dreissena polymorpha* and copper**

| | 12.5 µg $l^{-1}$ copper | | | 40 µg $l^{-1}$ copper | | | 125 µg $l^{-1}$ copper | | |
|---|---|---|---|---|---|---|---|---|---|
| | pH | Temp. (°C) | DO (%) | pH | Temp. (°C) | DO (%) | pH | Temp. (°C) | DO (%) |
| Algae added | 7.86 | 14.3 | 100 | - | - | - | 7.90 | 14.5 | 95 |
| Time 0 | 7.99 | 15.6 | 99 | 7.87 | 14.3 | 100 | 7.94 | 14.7 | 95 |
| Time 240 | 7.98 | 18.4 | 99 | 7.95 | 16.7 | 95 | 7.97 | 16.1 | 95 |

At 12.5 µg $l^{-1}$ copper average valve opening fell markedly on addition of the test substance from 75% to 45% within one minute (Figure 3.15). Average valve opening values then exhibited regular changes in activity for the next 1.5 hours after which a reduction in valve opening occurs. This continued until just after two hours at which point recovery to a valve opening of 55-60% took place, which was maintained for the remaining time with some changes in activity at four hours. Closure to an alarm level occurred in only one individual, and a decrease in average opening below the preset alarm occurred within one hour of pollutant addition in three individuals. Consequently no alarms were triggered at the preset alarm levels (see Appendix A).

At 40 µg $l^{-1}$ copper less marked changes occurred in the valve opening of individual mussels (Figure 3.16). Closure at the set alarm level occurred in only one mussel within two hours of the addition of the test substance. Two individuals exhibited an initial change in activity that can be described as intermittent activity changes with periods of reduced opening. One individual increased valve opening from 50% to 75% 15 minutes after test substance addition. Average valve opening decreased initially, returning to a level comparable to control valve opening after 1.5 hours.

At 125 µg $l^{-1}$ copper marked decreases occurred in valve opening of individual mussels (Figure 3.15). These varied in effect and duration amongst individuals: immediate closure from 80% to 40% for approximately 30 minutes; immediate closure from 70% to 30% with a

gradual opening to 70% over 2.5 hours; immediate closure from 80% to 50% with a continued drop to 40% after four hours; and almost immediate full closure from 70% to 0% with a gradual increase to less than 5% after four hours. This resulted in a marked initial decrease in average valve opening from 70% to 50% with no change over the 4-hour test period. Activation of the decreased average alarm occurred 39 minutes after test substance addition.

### 3.3.8 Zinc

The valve opening responses of a group of *Dreissena* exposed to zinc concentrations of 250, 500 and 1000 µg $l^{-1}$ are shown in Figures 3.20 to 3.22. Measured changes in zinc concentrations and physico-chemical parameters recorded during the experiment are given in Figure 3.23 and Table 3.7. Changes occurred in temperature, by 1.3 to 2.8 °C, and pH, by 0.18, 0.17 and 0.20 units respectively, across the dosing period.

**Table 3.7 Physico-chemical parameters (pH, temperature and dissolved oxygen) measured during the testing of the shell valve activity monitor with *Dreissena polymorpha* and zinc**

| | 250 µg $l^{-1}$ zinc | | | 500 µg $l^{-1}$ zinc | | | 1000 µg $l^{-1}$ zinc | | |
|---|---|---|---|---|---|---|---|---|---|
| | pH | Temp. (°C) | DO (%) | pH | Temp. (°C) | DO (%) | pH | Temp. (°C) | DO (%) |
| Algae added | 7.81 | 13.7 | 99 | 7.89 | 14.6 | 94 | 8.00 | 15.5 | 96 |
| Time 0 | 8.12 | 14.4 | 95 | 8.15 | 15.7 | 95 | 8.20 | 15.8 | 95 |
| Time 240 | 8.30 | 17.1 | 96 | 8.32 | 17.5 | 93 | 8.40 | 17.1 | 94 |

At 250 µg Zn $l^{-1}$ average valve opening (Figure 3.24a) remained stable across the test period, rising by approximately 10% over four hours. Concurrently individual mussels valve opening behaviour shows little change over the test period and no alarms were activated at their preset levels.

At 500 µg Zn $l^{-1}$ zinc, average valve opening (Figure 3.24b) decreased from 85% to 60% over the first hour after test substance addition and remained at this level throughout the test period. This pattern of response was also apparent in individual mussel behaviour. No alarms were activated at their preset level.

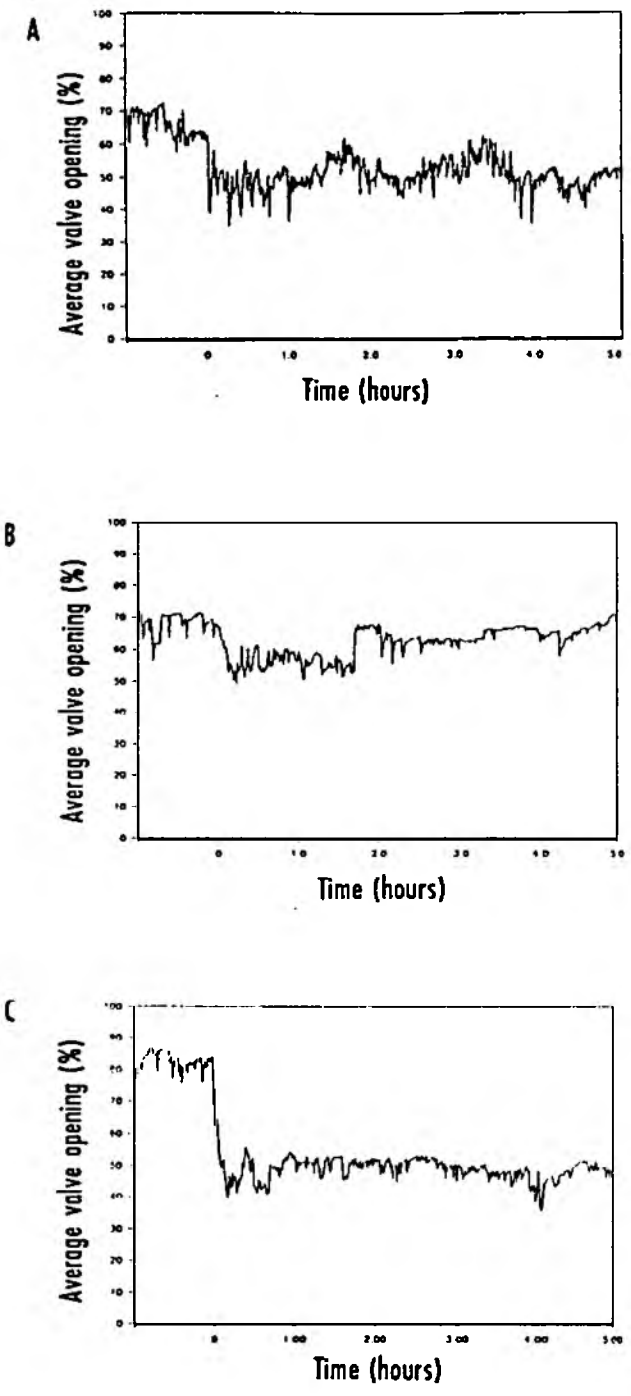


**Figure 3.19** Average valve opening (%) for the groups of *Dreissena* exposed to (A) 12.5 μg/l, (B) 40 μg/l and (C) 125 μg/l copper. Pollutant added at 0 hours and groundwater flow restored at 4 hours

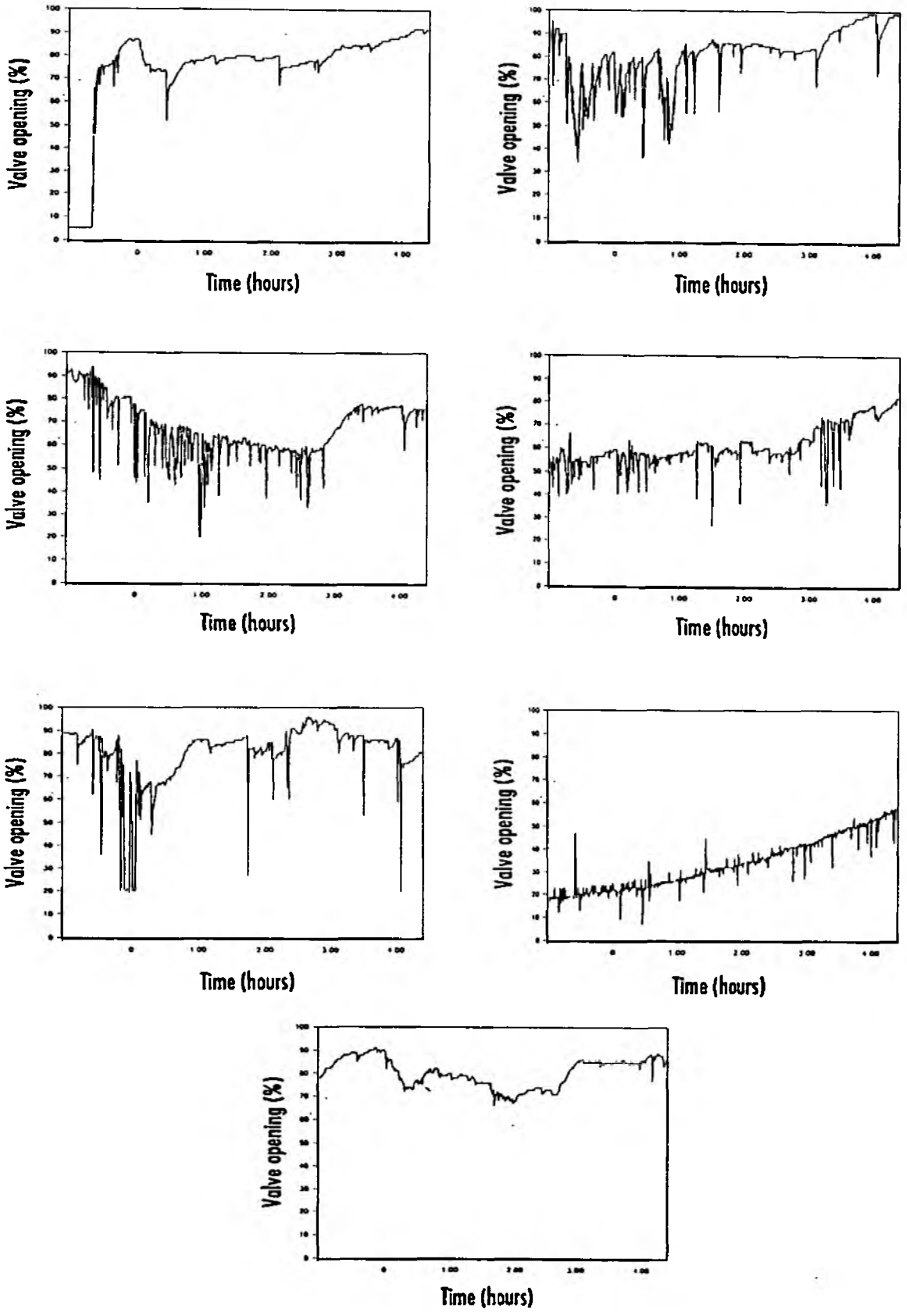


**Figure 3.20** **Valve opening (%) responses of *Dreissena* to 250 µg/l zinc. Pollutant added at 0 hours and groundwater flow restored at 4 hours**

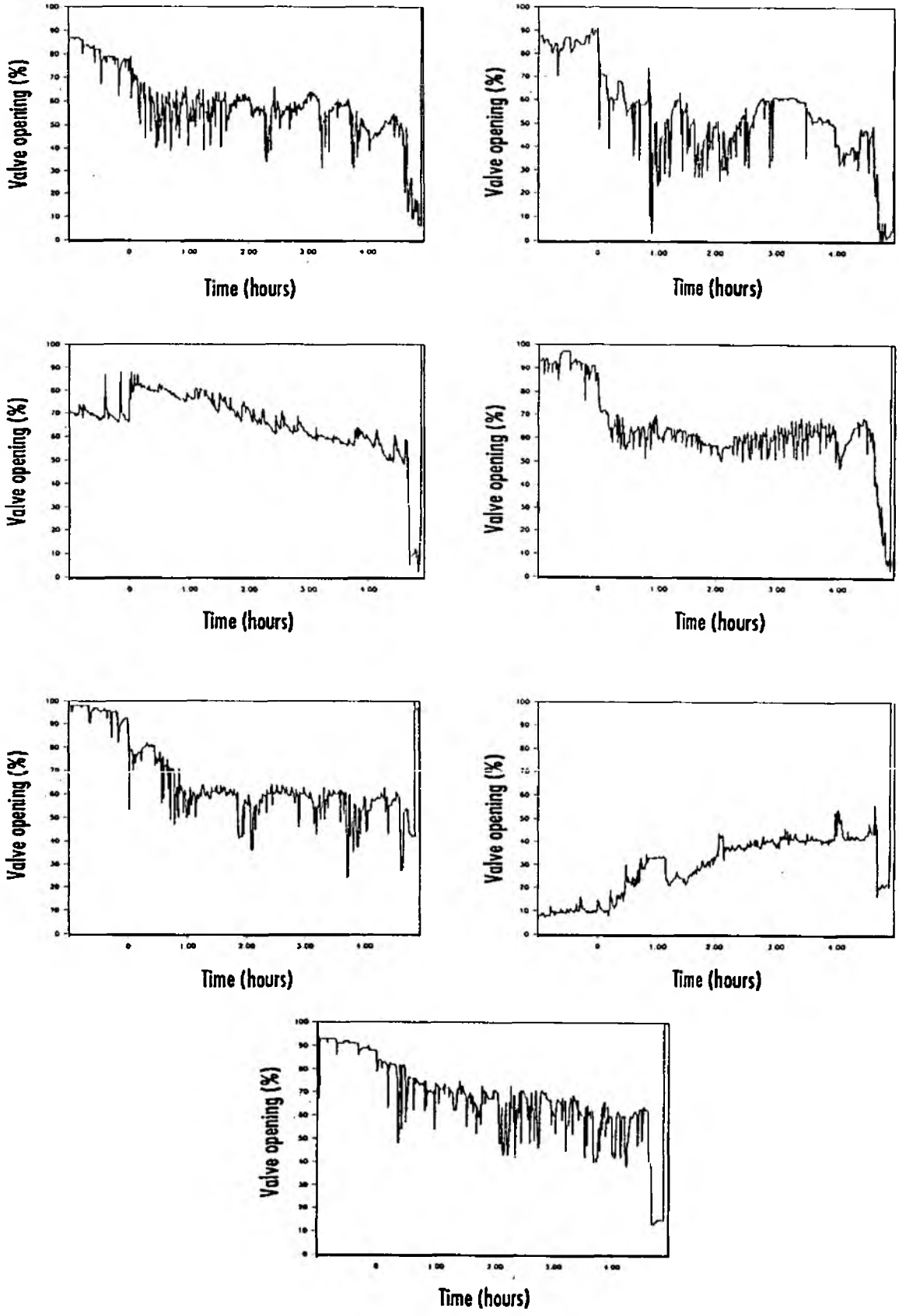


**Figure 3.21** **Valve opening (%) responses of *Dreissena* to 500 µg/l zinc. Pollutant added at 0 hours and groundwater flow restored at 4 hours**

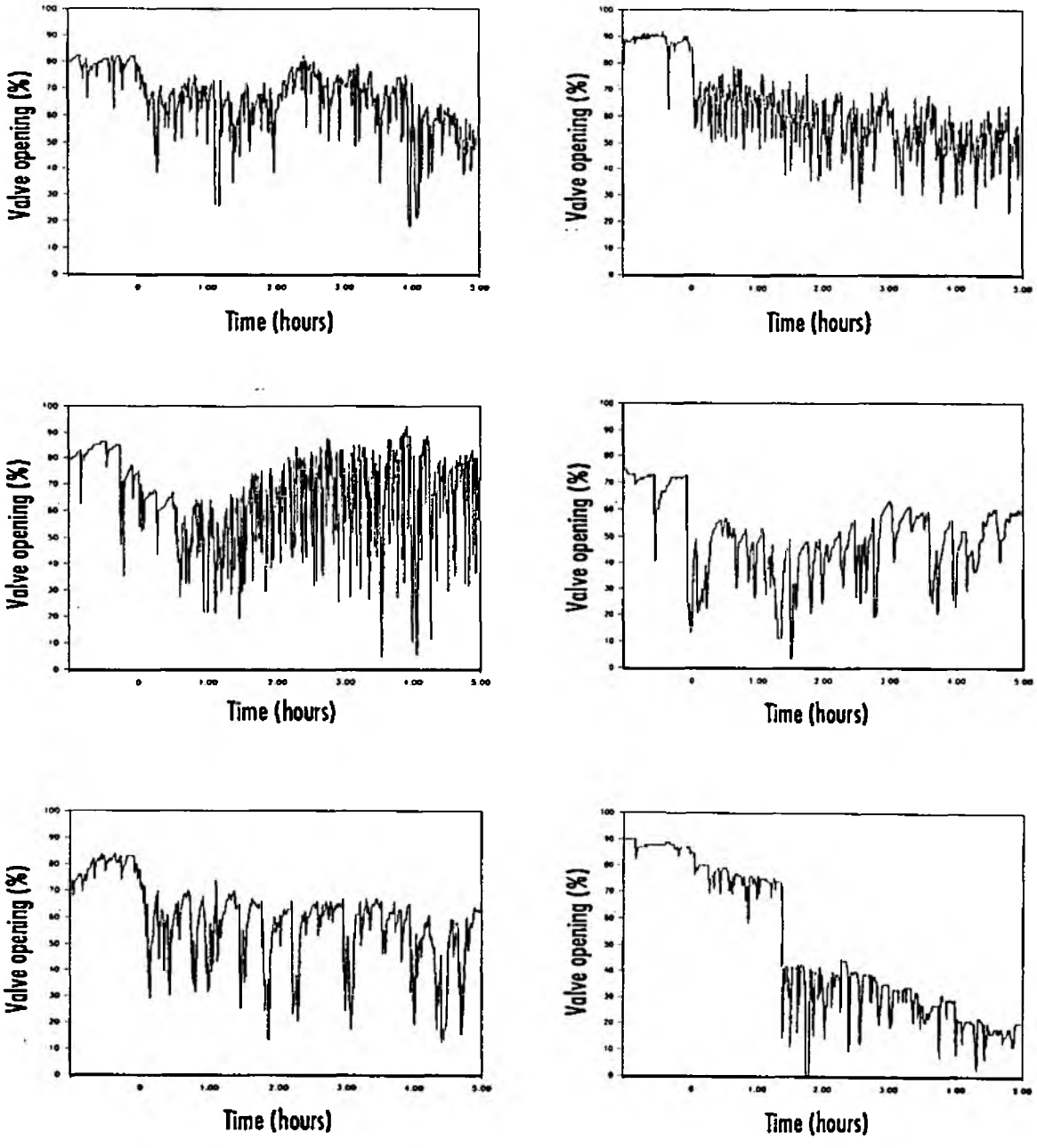


**Figure 3.22 Valve opening (%) responses of *Dreissena* to 1000 µg/l zinc. Pollutant added at 0 hours and groundwater flow restored at 4 hours**

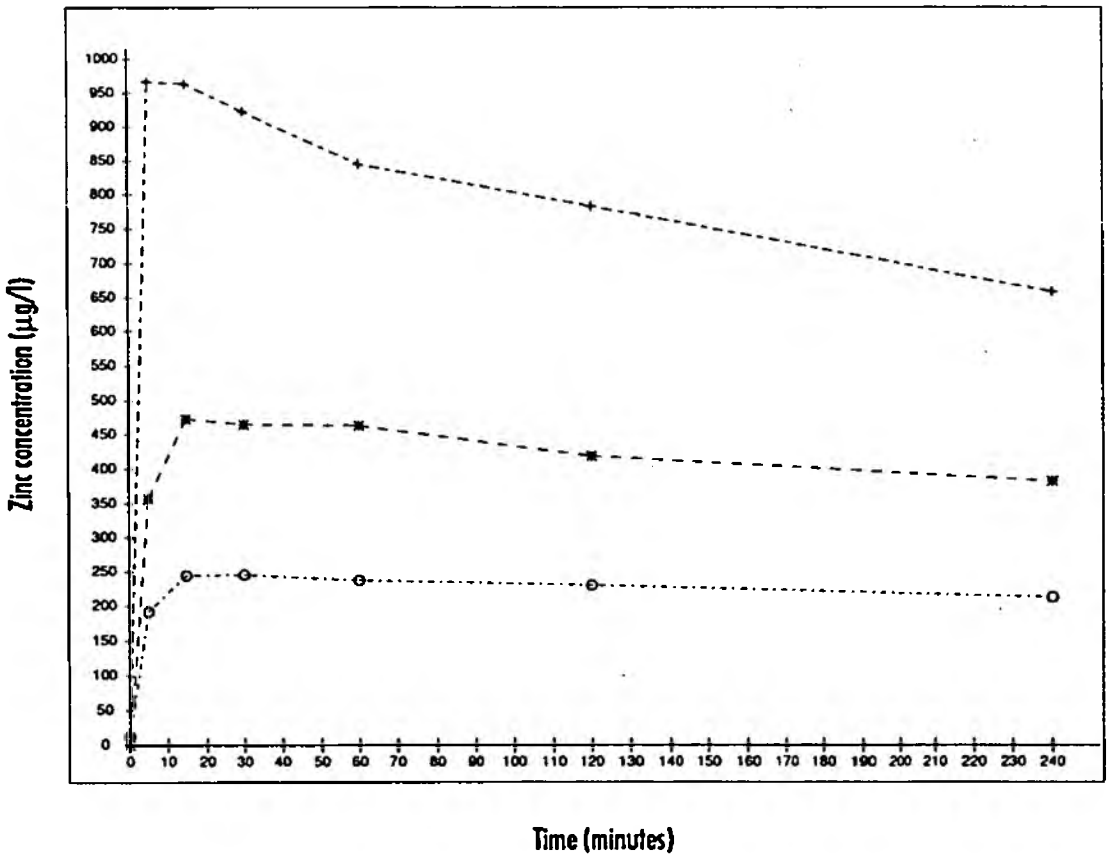


**Figure 3.23 Measured zinc concentrations at nominal zinc concentrations of 250 µg/l (○), 500 µg/l (✳) and 1000 µg/l (✚)**

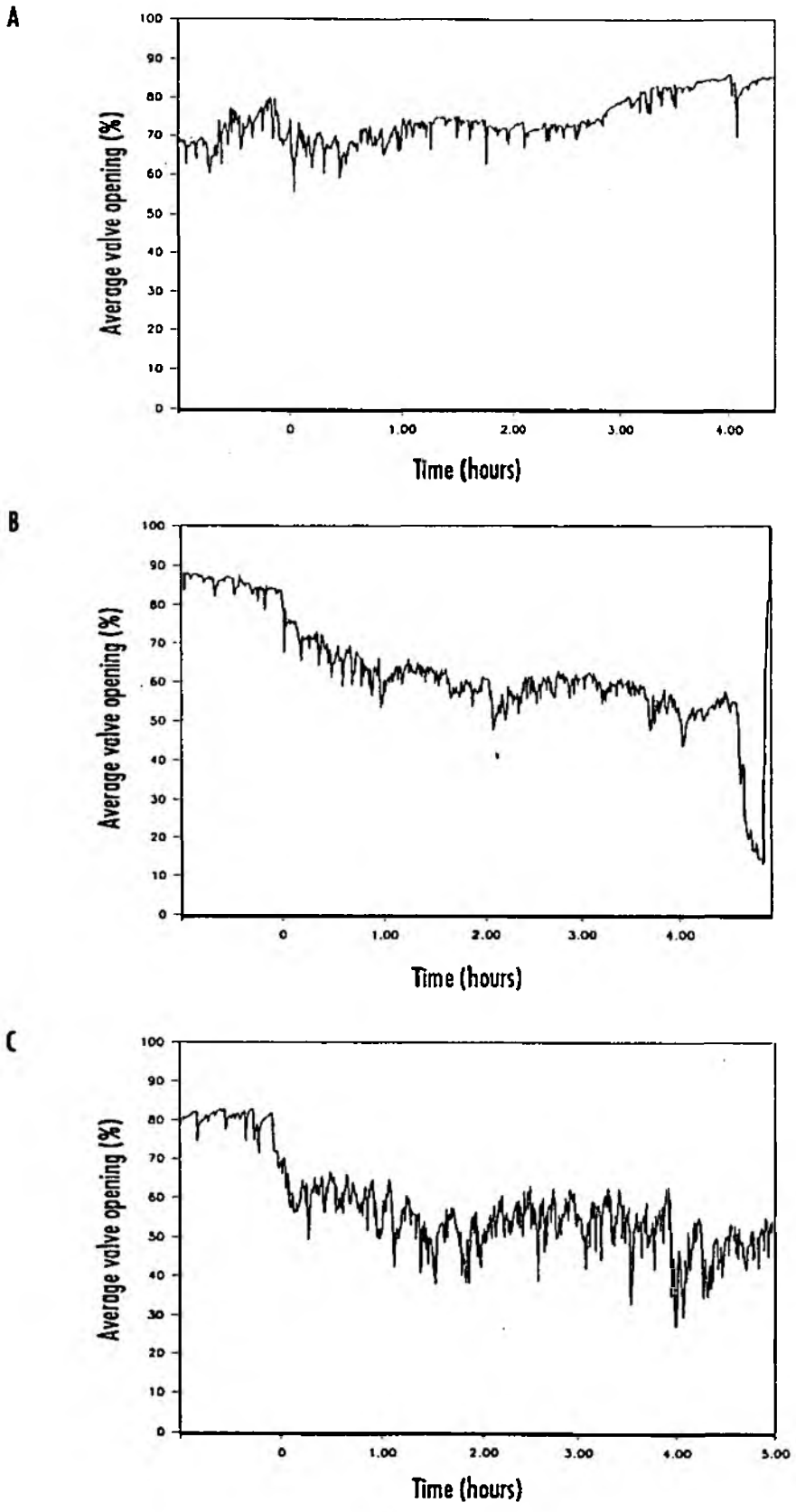


**Figure 3.24** Average valve opening (%) for the groups of *Dreissena* exposed to (A) 250 µg/l, (B) 500 µg/l and (C) 1000 µg/l zinc. Pollutant added at 0 hours and groundwater flow restored at 4 hours

At 1000 µg Zn $l^{-1}$ , average valve opening followed the same pattern as above (Figure 3.24c). A decrease from 70% to 50% occurred over the first 90 minutes and maintained this level for the remainder of the test period. Individual behaviour varied to a greater extent than at 500 µg $l^{-1}$; three individuals adopted a pattern of relative increased closure with intermittent periods of activity; another two exhibited a pattern of rapid changes in activity displayed by mussels dosed with ammonia (Figure 3.6 to 3.8). However no alarms were activated at their preset sensitivities.

### 3.3.9 Endosulphan

The valve opening responses of a group of *Dreissena* exposed to endosulphan concentrations of 2, 10 and 20 µg $l^{-1}$ are shown in Figures 3.25 to 3.27. Endosulphan is composed of an α and β isomer in a mixture of 75% α and 25% β and since the main cause of toxicity has been attributed to the α isomer of endosulphan (Crane and Jones 1991) only this component has been consider in terms of its effect upon *Dreissena*. Measured changes in α-endosulphan concentrations and physico-chemical parameters recorded during the experiment are given in Figure 3.28 and Table 3.8. Measured values of α-endosulphan were below those of the nominal values given above and at the higher concentration exhibited a fluctuating level over time. This may be due to the reasons given above in Section 3.7. for lindane, i.e. losses of endosulphan by adsorption or volatilisation processes, or poor solubility of endosulphan in the stock solution. Changes occurred in temperature, by 1.9 to 2.2 °C and pH, by 0.09, 0.22 and 0.27 units respectively, across the dosing period.

**Table 3.8 Physico-chemical parameters (pH, temperature and dissolved oxygen) measured during the testing of the shell valve activity monitor with *Dreissena polymorpha* and endosulphan**

| | 2 µg $l^{-1}$ endosulphan | | | 10 µg $l^{-1}$ endosulphan | | | 20 µg $l^{-1}$ endosulphan | | |
|---|---|---|---|---|---|---|---|---|---|
| | pH | Temp. (°C) | DO (%) | pH | Temp. (°C) | DO (%) | pH | Temp. (°C) | DO (%) |
| Algae added | 7.97 | 16.7 | 98 | 7.94 | 16.6 | 100 | 7.98 | 17.1 | 97 |
| Time 0 | 8.31 | 19.4 | 97 | 8.11 | 17.3 | 97 | 7.96 | 17.4 | 97 |
| Time 240 | 8.39 | 21.3 | - | 8.33 | 19.5 | 96 | 8.23 | 19.4 | - |

At 2 µg $l^{-1}$ endosulphan no marked changes occurs in average valve opening (Figure 3.25). A decrease of approximately 10% was apparent between one and three hours after test

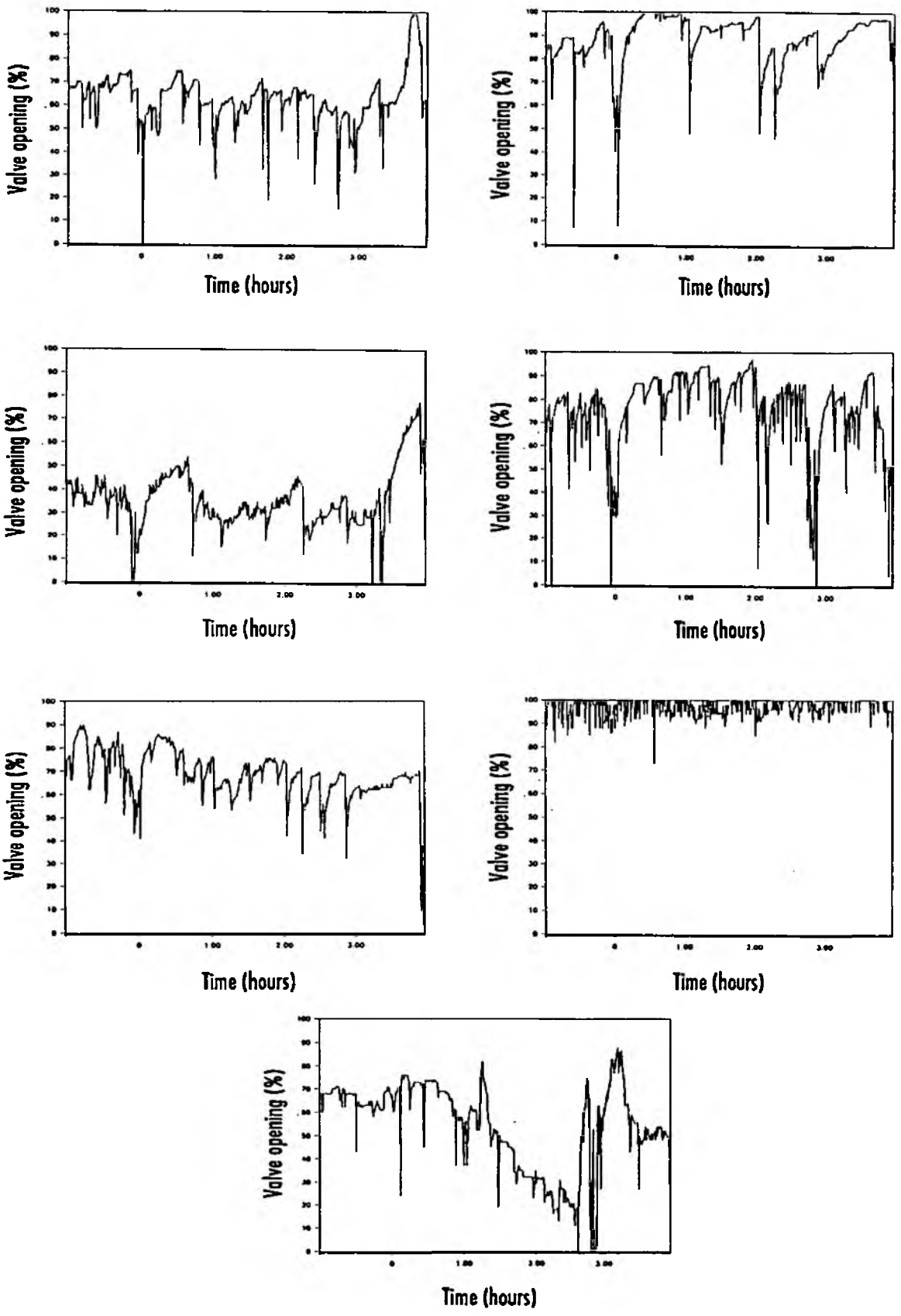


**Figure 3.25** **Valve opening (%) responses of *Dreissena* to 2 µg/l endosulphan. Pollutant added at 0 hours and groundwater flow restored at 4 hours**

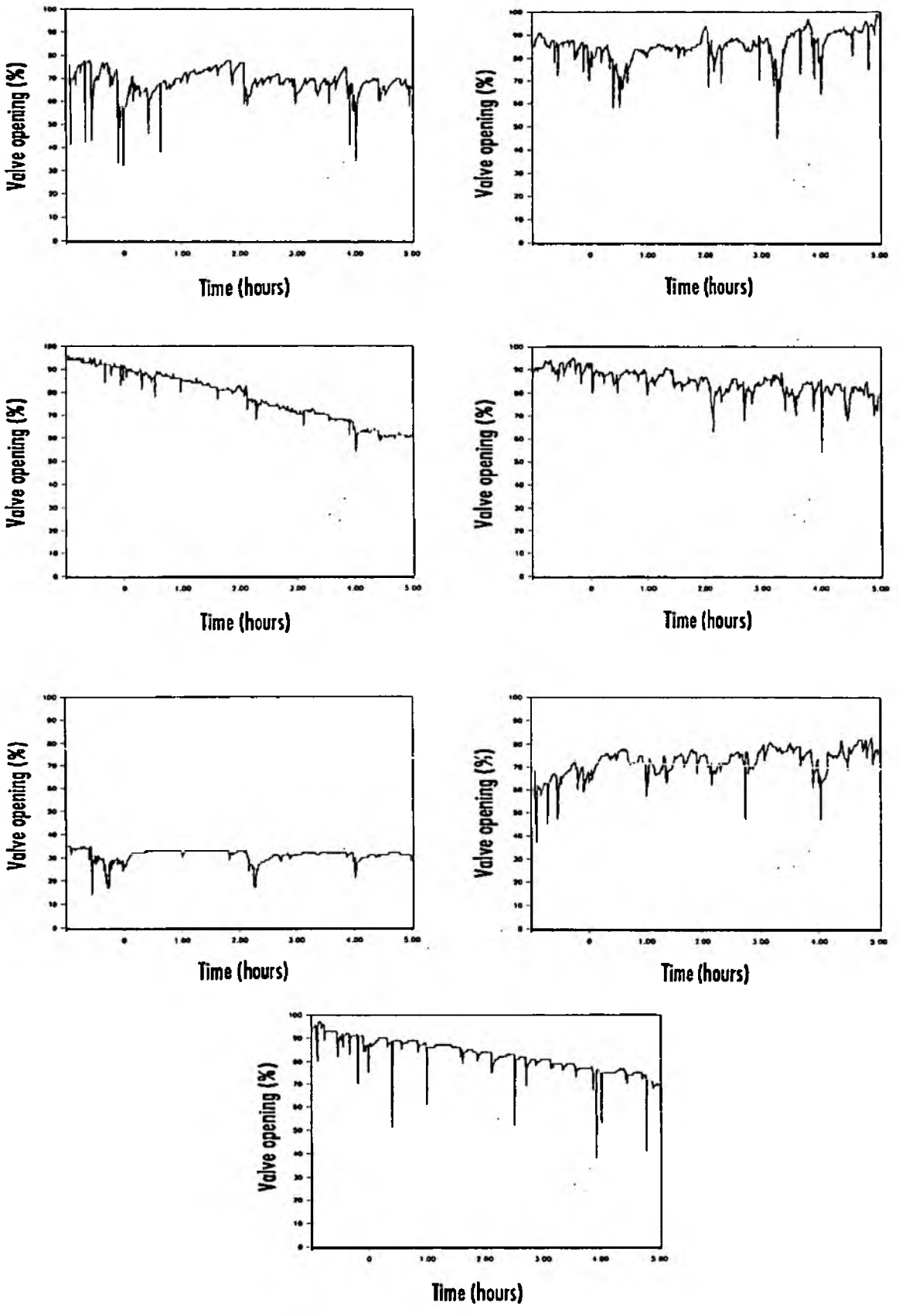


**Figure 3.26** **Valve opening (%) responses of *Dreissena* to 10 µg/l endosulphan. Pollutant added at 0 hours and groundwater flow restored at 4 hours**

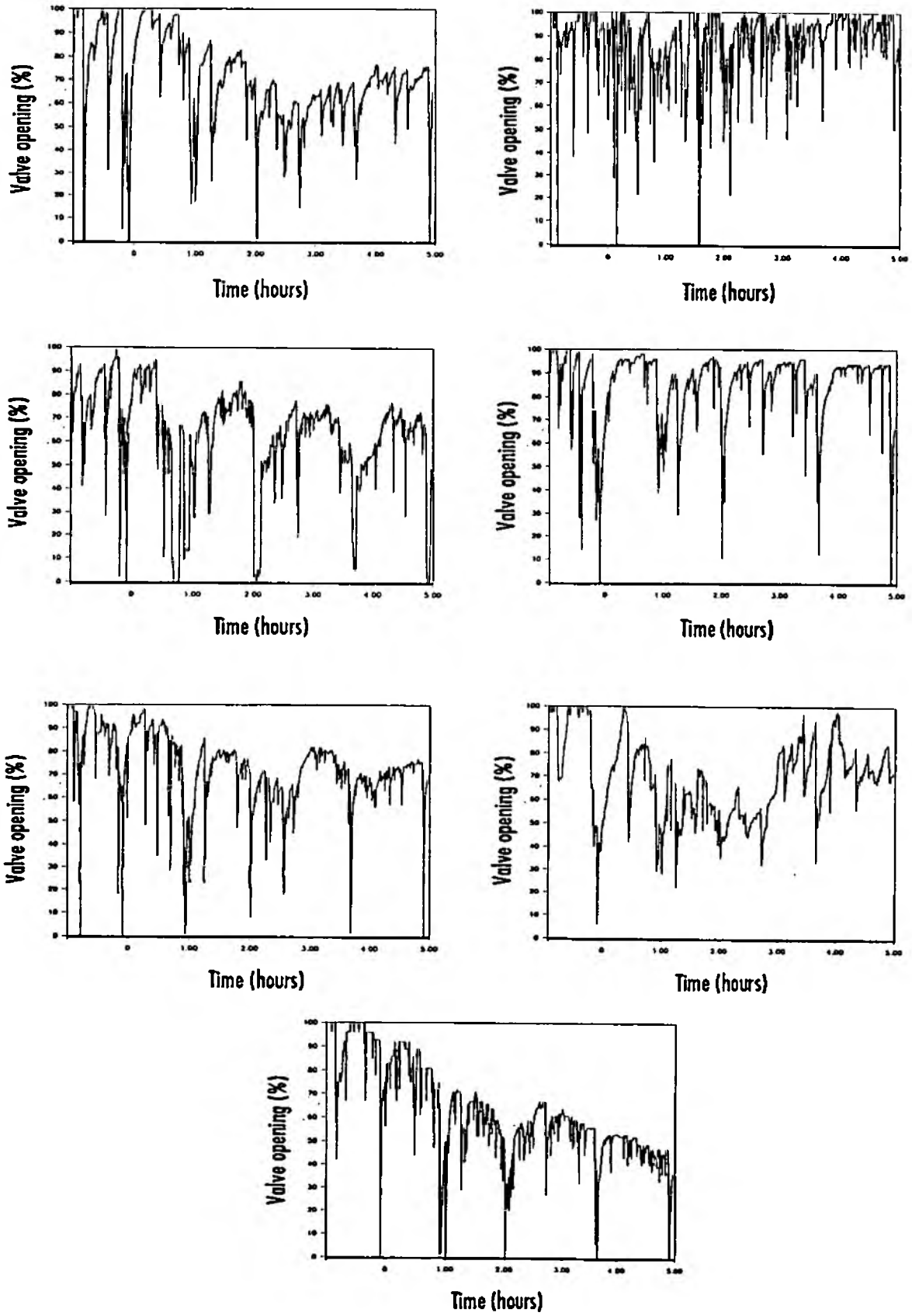


**Figure 3.27** **Valve opening (%) responses of *Dreissena* to 20 µg/l endosulphan. Pollutant added at 0 hours and groundwater flow restored at 4 hours**

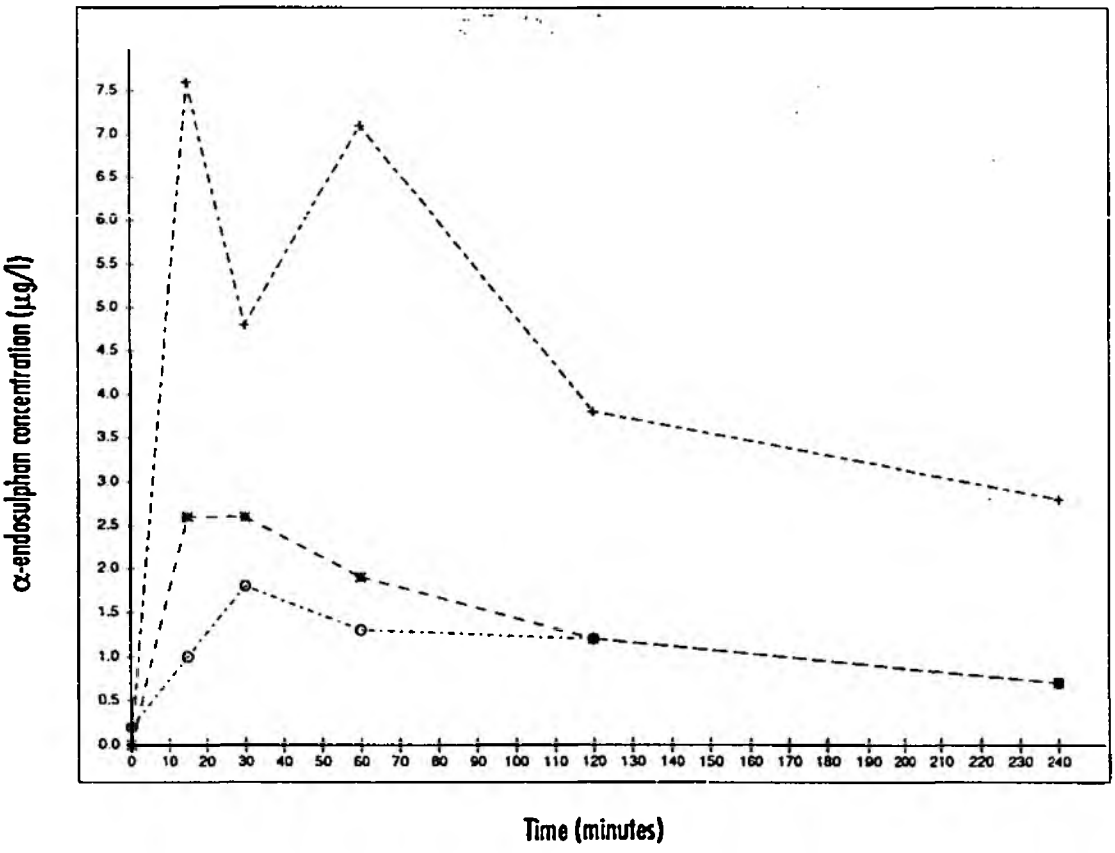


**Figure 3.28** Measured α-endosulphan concentrations at nominal endosulphan concentrations of 2 µg/l (○), 10 µg/l (✱) and 20 µg/l (✚)

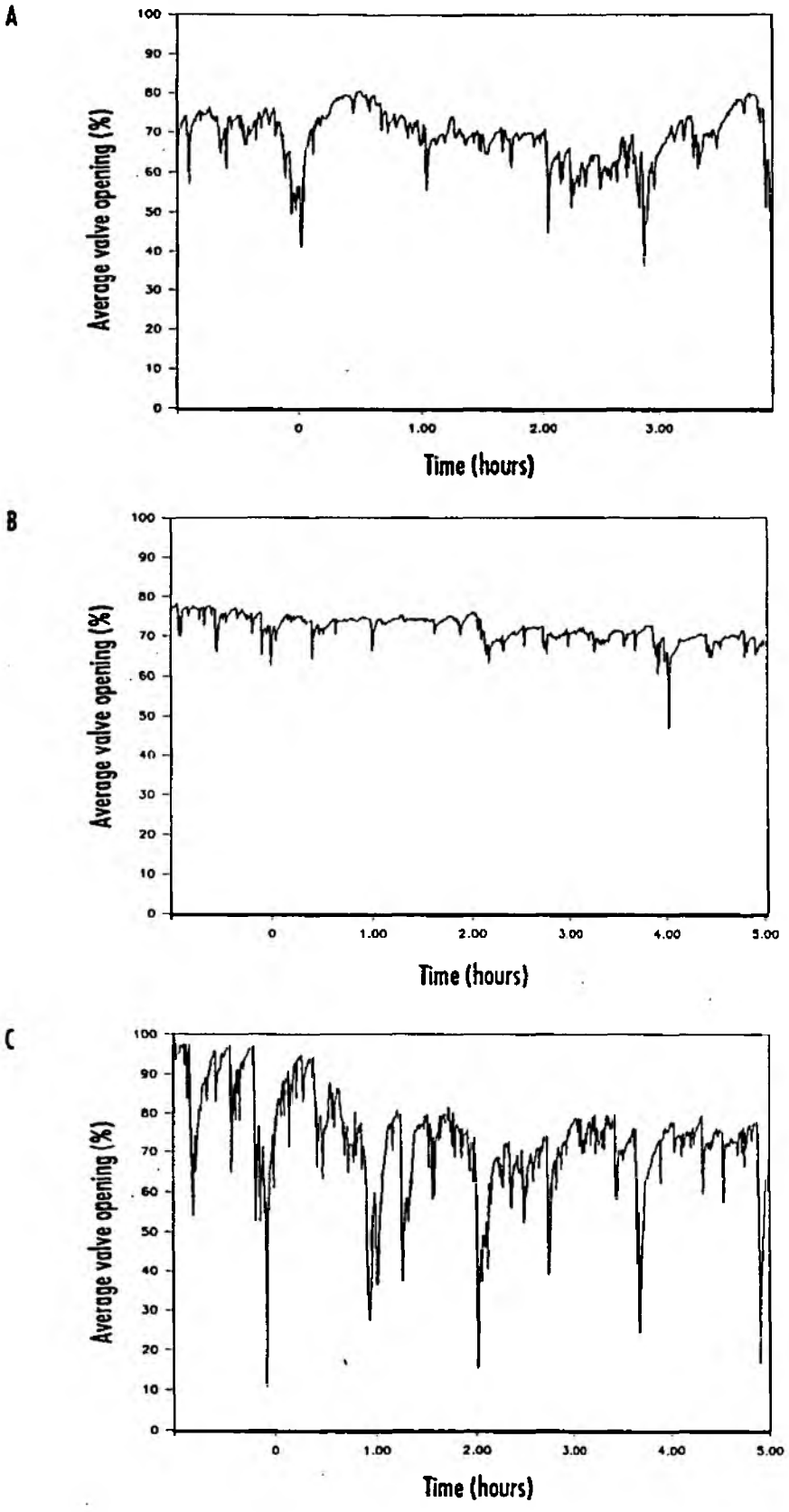


**Figure 3.29** Average valve opening (%) for the groups of *Dreissena* exposed to (A) 2 μg/l, (B) 10 μg/l and (C) 20 μg/l endosulphan. Pollutant added at 0 hours and groundwater flow restored at 4 hours

substance addition. This returned to control levels over the next hour. Most individual mussels exhibited no marked changes in valve opening behaviour. Although some closure and changes in activity occurred in individuals over the test period they were not of sufficient magnitude or duration to activate alarms at their preset levels.

At 10 µg $l^{-1}$ endosulphan no marked changes occurred in average or individual valve opening after addition of the test substance and no alarms were activated at the preset levels (Figure 3.26).

At 20 µg $l^{-1}$ endosulphan any changes in average and individual valve opening is confused by the erratic behaviour observed in the control period prior to addition of the test substance (Figure 3.27). In most individuals, rapid changes in valve opening occurred over one to five minutes both before and after time 0. The mussels used may have been under stress, or subject to external stimulus, before and after addition of the test substance to the extent that any effect of the test substance was masked by their response to this stimulus. No alarms were activated over the test period.

### 3.3.10 Changes in, and variability of, pH and temperature

In all the tests run both temperature and pH increased from the point at which groundwater flow to the test vessel was stopped, on addition of algae two hours before test substance addition. Initial pH values also differed from each other. The test vessel was located in a non temperature-controlled room located in an area under glass. It can be expected that some variation in temperature should occur when it is taken into account that the tests occurred over midday. Increases in pH values are assumed to occur after addition of algae as a result of removal of $CO_2$ from solution for use in photosynthetic metabolism.

### 3.3.11 Operational difficulties

In the use of the mussel monitor over the period of the study some difficulties occurred when communicating with and operating the monitor. The main difficulty occurred when establishing communication between the software program 'Procomm' and the monitor. This inconvenience ranged from between a few minutes to two hours. In one case, when setting up a second monitor, it was found that intermittent communication of data from six mussels could only be enabled at a low baud rate. This stage was only reached after consulting an electronics specialist within WRc, and it was decided not to continue work with the second monitor. Some problems also occurred when downloading data onto the 3.5" discs. Two test runs had to be re-run when it was discovered that data had not been downloaded onto file space on the disc. It was later discovered that this was due to an omission in the software such that when data was re-downloaded to a named data file without changing the name the original file was maintained and not overwritten. This was easily rectified by ensuring that data from a test that was restarted was sent to a file with a different name.

## 3.4 Discussion

Previous studies using *Dreissena polymorpha* and the mussel monitor have reported responses to a range of organic and inorganic chemicals (Sloof *et al* 1983, Kramer *et al* 1989, Kramer and Botterweg 1991) and the estimated detection limits are given in Table 3.9.

**Table 3.9 Estimated detection ranges for toxicants detected by the valve closure responses of *Dreissena polymorpha***

| Substance | Estimated detection limit ($\mu g\ l^{-1}$) |
|---|---|
| Cadmium | <100-370 |
| Chlorine | 5 |
| Copper | 5-30 |
| Cyanide | 530 |
| Lead | 250 |
| Selenium | <100 |
| Zinc | <500 |
| Tributyltin oxide | 5 |
| Chloroform | 106 000 |
| 1,3-Dichlorobenzene | 2 400 |
| Hexachlorobutadiene | 260 |
| Lindane | 110 |
| Pentachlorophenol | 340 |
| Phenol | 26 400 |
| Toluene | 9 400 |
| Trichloroethylene | 9 700 |
| Xylene | 16 200 |

The monitor has shown low detection levels with many of the compounds tested previously. However, this was not apparent when the monitor was used in this study.

Of the four test substances present at measurable concentrations only un-ionized ammonia showed responses at all concentrations based on activation of the activity alarm. This resulted in alarm responses at 0.3, 0.6 and 3.4 mg $l^{-1}$ un-ionized ammonia. The alarm response at 0.3 mg $l^{-1}$ indicates that a response may occur at concentrations below this, and compares favourably with the value of 0.11 mg $l^{-1}$ for the WRc Mk(III) Fish Monitor and 1.2 mg $l^{-1}$ for the Arena Basin fish rheotaxis system (Kramer and Botterweg 1991). The response time of three hours at 0.3 mg $l^{-1}$ is lower than the 11 minutes exhibited at 0.59 mg $l^{-1}$ with *Mytilus edulis* (Johnson *et al* 1991). Even at the higher concentrations of

0.6 and 3.4 mg $l^{-1}$ no response time of less than one hour was obtained. The difference can be explained by the difference in alarms triggered. With *Mytilus* a closure alarm was triggered. This alarm has an evaluation period of three minutes whereas the activity and decreased average alarm has an evaluation period of 30 minutes. No activity alarm events occurred before the addition of ammonia concentrations, therefore it may be assumed that the difference in percentage of 15% set to activate the alarm may be lowered to achieve an increase in future sensitivity. *In situ* field work at a known clean site would be useful to determine the extent to which alarm parameters can be altered to increase the monitors sensitivity.

Copper was found to induce an effect on valve opening in this study at a concentration of 125 µg $l^{-1}$. This is an order of magnitude higher than previous effect concentrations of between 5 and 30 µg $l^{-1}$ that have been reported with *Dreissena polymorpha* (Table 3.6). This may reflect variations in the inherent sensitivity of populations of *Dreissena* or possible acclimation of *Dreissena* to copper at the collection site. Copper has been shown to be more toxic to fish in soft than in hard water because complexes formed in hard water have a lower toxicity compared with the free ionic form (Mallet *et al* 1992). This may be the cause of the difference between the detection limits of Kramer (1989) and this study for *Dreissena* polymorpha.

No zinc induced effects on valve opening were found in this study at concentrations up to 1000 µg $l^{-1}$. Individuals of *Ancylus fluviatilus* collected from an unpolluted site have been found to be capable of surviving at concentrations up to 320 µg $l^{-1}$ with reduced reproductive capacity (Willis 1988). Studies on the toxicity of zinc to freshwater invertebrates have found a decrease in the acute toxicity of zinc with increasing pH and hardness (Mallet *et al* 1992). Water pH and hardness were high in this study. In view of this and the difference in response level of *Dreissena* to copper in this study compared to previous work, it is not surprising that an alarm response did not occur at 1000 µg $l^{-1}$. However some changes in valve opening did occur which may have resulted in alarm responses if alarm parameters had been made more sensitive.

For both lindane and α-endosulphan measured levels were much lower than nominal levels. This study has been the first occasion that organic compounds have been investigated using this test system. The system is freely open to the air and is not temperature controlled. Calcium carbonate precipitation on the vessel and monitor, and algae added to the vessel as part of the test procedure, provided particulate matter that may act as a site for sorption, which is an important fate for endosulphan and lindane in water systems (Crane and Jones 1991, Farida *et al* 1983). The decrease in α-endosulphan concentration over the test period suggests that losses of organics subject to volatilisation and sorption cannot be avoided. As a consequence it is advisable that a more suitable system is designed for future work. Previous work by Kramer *et al* (1989) used a continuous flow-through system with test substances added by peristaltic pump and Baldwin (1990) suggested that a ramp-dosing system would result in a two or three times lower sensitivity threshold.

Of the three freshwater bivalves chosen for testing *Dreissena polymorpha* proved the most reliable test organism in terms of consistency of valve opening behaviour (Section 3.3.4). However *Dreissena* is not native to the British Isles and as with all introduced species concern exists about its impact upon native species. *Dreissena* is a sessile organism, bysally

attaching to many kinds of solid substrate from rocks and debris to large living invertebrates embedded in a muddy matrix such as the shells of *Unio* and *Anodonta*. Mackie (1991) found that unionid shells provided an ideal substrate for *Dreissena* to colonise; he observed an average of 300 per shell throughout Lake St Clair in 1989. As a result of this the impact of *Dreissena* on other species of bivalve can be detrimental. Growths of *Dreissena* on unionids can be so great or invasive that:

- normal locomotion and burrowing activities of a mussel are impaired:
- prevention of valve closure exposes the mussel to predation, disease and pollutants:
- shell deformities occur;
- valve opening is limited to the extent that normal functions are impaired (Mackie 1991).

In lakes *Dreissena* occupies a belt around the littoral zones that Stanczykowska (1977) has shown in the Masurian lakes occupied a depth between 1 and 12 metres with the largest numbers at four metres depth. In flowing waters they may cover the total area of the river bed. In lakes *Dreissena* will reach densities of 100-1000 individuals per $m^2$, but river densities are much lower with a range of 20-300 individuals per $m^2$ (Stanczykowska 1977).

*Dreissena* are dioecious and among freshwater molluscs are characterised by a high fecundity. Egg production is estimated to vary between 30 000 and 40 000 eggs per female each year with a reproductive season of 2-8 months at an optimum water temperature of 15-17 °C (Stanczykowska 1977, Mackie 1991). Sprung (1987) found that males were the first to release gametes some one to two hours before females under ideal conditions; at 16 °C 50% of female eggs could still be fertilized after 4.5 hours (this decreased with increasing temperature). After fertilization the rearing success of the eggs is strongly dependent on pH. Sprung (1987) found that only a narrow range of pH was tolerated: below pH 7.4 and above pH 9.4 no rearing success was possible. However *Dreissena* spawns in spring when pH tend to rise as a result of phytoplankton blooms (Sprung 1987). Even so this requirement would probably exclude *Dreissena* from acidic upland water bodies. The eggs hatch into free swimming veligers which exist as plankton for 8-10 days, after which they settle to the bottom and enter a mobile post-veliger stage. It is at this stage that the presence of suitable substrate conditions are vital to the survival of the post-veligers for they must find a hard surface for attachment while they are still mobile. After determining that mortality during this period reached 99%, Stanczykowska (1977) concluded that this was the most important stage of development. Dispersal occurs passively both in the larval planktonic stage and the adult benthic stage. Mackie (1991) concluded that *Dreissena* first reached Lake St Clair as veligers in the freshwater ballast of a transoceanic ship. In the veliger and post-veliger stages transport by water currents is the main means of dispersal. Mackie (1991) observed that currents exceeding 1 m $s^{-1}$ prevented veligers from invading a river upstream of a lake colony. Adult dispersal is enabled mainly through byssal attachment to the hulls of pleasure and fishing boats.

Placement of *Dreissena* in a water system in the numbers used by the monitor should not readily result in the establishment of a population of the mussel within that system. It is

likely that gamete release will occur if the system is left *in situ* for any length of time. Sprung (1987) observed that the release of gametes occurred within a few hours under favourable conditions. This is adequate for such a species as *Dreissena* which forms dense aggregations of individuals. However, assuming that both males and females were present on the monitor, dilution of the males' gametes may occur on release to the extent that fertilization, once females release their eggs, would be low. As mentioned above fertilization would be minimal at and below a pH of 7.4. After fertilization, in fast flowing rivers (>1 $m^{-1}$) veligers would be carried downstream. Post-veligers would not be limited by water depth or substrate availability in most British rivers, and if sufficient numbers survived may become problematic.

In past studies, and to some extent in this one, *Dreissena* had been shown to give reasonable detection limits with the valve monitor to a range of pollutants under laboratory conditions. However in a field situation the monitor may be exposed to more complex pollutant conditions in the form of multi-pollutant effluents. In this case the sensitivity of the monitor may be increased due to the cumulative or interactive toxicological effects of individual pollutants on individual *Dreissena*. The effect of bioaccumulative pollutants may vary across the year. Kraak *et al* (1991) investigated biomonitoring of heavy metals in the Rhine using *Dreissena* and found that metal concentrations (expressed as mg $kg^{-1}$ dry weight) changed significantly during the year. One of the factors affecting this may be seasonal changes in soft tissue weight caused by spawning. An annual cycle in soft tissue weight with a factor of difference of two between the lowest and highest value was reported. As a result the total pollutant body burden can be diluted or concentrated; this has been shown for *Mytilus edulis* (Philips 1976). As a consequence this may in turn result in seasonal fluctuations in alarm levels. The suitability of the monitor for *in situ* monitoring of effluent effect within a river system has been shown by Bengtsson *et al* (1987) who concluded that the system was easier to operate and less susceptible to noise compared to other early warning systems, even when severe hydrological fluctuations occurred. However, they also found that the alarm criteria used (a reaction by six out of eight mussels) was not sensitive enough to evaluate the toxic conditions at the test site.

The setting of alarm criteria is an important consideration when determining the sensitivity of the monitor. The criteria used in this study were based on those used by Johnson *et al* (1991) for use with *Mytilus edulis* with some minor modifications to individual alarm parameters suggested for use with freshwater mussels (Kramer, pers. comm.). This is a valid approach for laboratory validation of responses to specific test substances. For the monitor to act as an early warning system it would be more suitable to determine alarm parameters for individual species derived from valve opening behaviour in the laboratory or at known clean field sites. This would establish alarm parameters at levels approaching maximum sensitivity. At present the alarm parameters used are based upon the reactions of a proportion of, rather than the whole of, the group of mussels used, and alarm criteria are based upon the findings of previous studies findings rather than a defined statistical procedure. Johnson *et al* (1991) suggested that the cumulative sum method (Evans *et al* 1986) would be a useful means of analysing the data to provide a means of determining statistically significant changes in valve opening. As a result the development of further alarm criteria is taking place at the Netherlands Organisation of Applied Scientific Research. Once validated these will be applied in future studies.

## 3.5 Recommendations

1. Sufficient laboratory work has been carried out in this and past studies (Johnson *et al* 1991, Kramer *et al* 1989, Sloof *et al* 1983) to justify field deployment of the monitor using *Dreissena polymorpha*, the species chosen. Bengtsson *et al* (1987) found use of the monitor in field situations to be reasonably trouble free. However, as a result of visible and sustained changes in valve opening upon test substance addition with no resulting alarm responses, it is advisable that alarm sensitivities are increased for field deployment. Deployment in this project will involve placement of two monitors above and below point incongruities at two of the sites chosen; this will involve the development of statistical methods for comparison of upstream and downstream monitor data, a process already underway at WRc. Use of statistical methods under development by TNO may occur as these become available.

2. Further laboratory work should involve repeat experimentation with test substances to assess the degree of variability in response between different groups of *Dreissena* and an assessment of the extent of inter-operator variability. Sloof *et al* (1983) reported that pre-exposure of mussels to low doses of test substances resulted in a decrease in sensitivity. The extent to which continual exposure to low levels of pollutants will result in adaptation needs to be assessed since this is of particular relevance to long term field deployments. Any further laboratory work, particularly with organic chemicals should not incorporate the system used in this study due to problems mentioned in Section 3.4. Incorporation of a ramp dosing system, and continuous flow through, is recommended to produce experimental conditions that more realistically represent environmental processes.

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## APPENDIX A - ALARM PARAMETERS USED WITH THE MUSSEL MONITOR

These parameters were used from the point that station 4 on the monitor malfunctioned (see Section 2.3.1).

Closed Evaluation:

| | |
|---|---|
| Percentage considered closed | 15 |
| Time closed in minutes | 3 |
| Number of mussels closed | 4 |

Activity Evaluation:

| | |
|---|---|
| Number of measurements | 60 |
| Difference in percentage | 15 |
| Number of detected activity | 10 |
| Number of mussels | 4 |

Decreased Average Evaluation:

| | |
|---|---|
| Interval in minutes | 60 |
| Decrease in percentage | 20 |
| Number of mussels | 4 |

Gaping Evaluation:

| | |
|---|---|
| Increase in percentage (>100%) | 100 |

## APPENDIX B - PROJECT INVESTMENT APPRAISAL

## APPENDIX B. - PROJECT INVESTMENT APPRAISAL

1. R & D Commission A - Water Quality

   Topic A12 - Ecotoxicology

   Project Title - 1990 River Water Quality Survey : Biological and Chemical Incongruities

   Proposal No. A12(91)03 Project No. 379

   Classification of R&D - Applied Research with Strategic Aims

   Primary Purpose - Operational Effectiveness

2. Project Leader - D Tinsley Tel : 0992 35566

   Environmental Quality
   Aspen House
   Crossbrook Street
   Waltham Cross
   Herts
   EN8 8HE

3. Research Contractor - WRc Medmenham
   PO Box 16 Marlow
   Buckinghamshire
   SL7 2HD

   Contract Signatory - Dr A J Dobbs

   Project Manager - M Crane

4. Contract Details

   Start date : 1/11/91
   End date : 30/3/93

   Contract type : Single Source Tender (Sole Source)

5. Objectives

   Overall Project Objective

   To evaluate the performance of selected ecotoxicological methods in assessing water quality in rivers.

Specific Objectives

(a) To evaluate the performance of selected ecotoxicological methods in assessing water quality by means of a number of case studies at selected sites on rivers at which incongruities exist between chemical and biological data collected during the 1990 national water quality survey.

(b) To provide explanations for the incongruities in water quality data at the selected sites.

## 6. BACKGROUND

A number of ecotoxicological methods have been selected by the Ecotoxicology R&D group as being of potential value to the NRA in its job of maintaining and improving water quality. Further development of these methods has taken place as part of the programme of work identified within the topic. The final phase of method development involves evaluating the performance of tests under realistic working conditions. Tests that perform well can then be brought into working practices. Those that do not perform well will need to be modified or rejected.

Ecotoxicological methods can be of value to the NRA in setting discharge consents particularly where complex effluents are involved, in providing data to help in deriving environmental quality standards, or in approving the use of substances in or near water, and in demonstrating either acute (often pollution incidents) or chronic deteriorations in water quality. To help with the latter job it is desirable to develop in situ assessments in order to reduce the error in extrapolation from laboratory generated data. Amongst the in situ methods being developed in the Ecotoxicology R&D Topic are the Gammarus feeding test, the TNO Valve monitor and the GST (glutathione-s-transferase) method.

Development of the Gammarus feeding test by WRc is due for completion at the end of March 1992 and considerable time and effort has also been spent on the development of valve monitor by TNO (the Netherlands Organisation of Applied Scientific Research) in Holland. The aim of this project is therefore to carry out a full evaluation of these methods at sites selected from the results of the 1990 survey.

The in-situ methods to be undertaken within the project are very specialised and, to date, all the NRA funded development work has been carried out by WRc. No other organisation would therefore be able to perform this project within the timescale required. WRc are a Primary Contractor in this area of work because of their unique experience and their international position as a centre of excellence in ecotoxicological research. They have carried out all the NRA ecotoxicology research to date in addition to studies conducted in this field on behalf of the DoE and EC. The study builds on WRc's current programme of work for the NRA.

Context

The work can be classified as an ecotoxicology methods / development and application project. The project is linked to study A12(89)061 as much of the development to date of in-situ methods has taken place within this project. It is also linked to project A08(91)04.

## 7. STRATEGY

Method

Desk study, field and laboratory investigations by external contractor, to be supervised by Project Leader. The programme of work will consist of the following:

(a) Provision by the NRA of 1990 survey data, which will be used by WRc to identify incongruous sites utilising robust, statistically-based criteria.

(b) Contact with NRA regions to obtain historical chemical and biological data and other relevant information (eg view of local pollution control staff on reasons for incongruities; ease of access to site etc) for sites identified in (a)).

(c) Visit to sites which look most promising from initial enquiry, and, in collaboration with project leader, selection of 3 or 4 sites for study as a part of this project.

(d) Production of detailed plan for work at each selected site in collaboration with NRA Project Leader - to include selection of in-situ methods, chemical analysis, macroinvertebrate surveys, sediment toxicity assessment etc. Consideration should be given to three season deployment and supporting analysis/surveys. Each plan will include a cost for the proposed work.

(e) Implementation of the agreed work plans at appropriate times of the year during the contract and reporting on the performance of the in-situ methods and of the likely cause of the incongruity as separate interim reports for each site.

(f) Further development of TNO in-situ valve monitor to be carried out as necessary so as to ensure that it is ready for the final phase of method development as a part of this project. Plan for further development to be agreed with project leader.

(g) Completion of draft project report.

(h) Final project report to be produced following review by NRA.

Monitoring

Project Leader is responsible for monitoring progress by means of progress meetings, progress reports and interim reports after 5 months and thereafter at suitable intervals in accordance with the site work plans. Project Leader/Topic Leader to review the draft report. Project progress to be reviewed and reported to Function Managers through the Ecotoxicology group.

Dissemination Status and Customer Acceptance Level

Approach to dissemination will be agreed with Topic Leader as the project progresses, but is likely to be as follows:

Internal - Release to Regions
External - Restricted

Customer Acceptance is likely to be at Topic Leader Level.

## 8. TARGETS AND TIMESCALES

| Work item | Date Completed | Month |
|---|---|---|
| Complete points (a) to (d) and point (f) in section 7 | end March 1992 | 5 |
| 1st Interim Report - site plans and results of further development of *TNO* Valve Monitor | end March 1992 | 5 |
| Draft R&D Note and key sections of Project Report | end of Jan 1993 | 15 |
| Final Documents - Project Report<br>- R&D Digest<br>- R&D Report | end of March 1993 | 17 |

Targets relating to implementation of site work plans and Interim Reporting associated with this to be agreed between Project Leader and WRc.

9. OUTPUTS

| Type of Report | Number Required |
|---|---|
| * Quarterly Progress Reports | 5 |
| * Interim Reports - after 5 months and following completion of each work plan, until a maximum of three Interim reports are produced. | 20 |
| * Draft R&D Report and Relevant Sections of Project Report | 20 |
| * Final Documents - Project Report | 5 |
| - R&D Report | 50 |
| - R&D Digest | 200 |

10. COST (£) excluding VAT

| | NRA | |
|---|---|---|
| Item | 1991/2 External | 1992/3 External |
| Staff (451 man days) | 41,353 | 60,366 |
| Facilities | 4,403 | 8,554 |
| Travel and Subsistence | 1,800 | 5,000 |
| Capital Items | 24,000 | - |
| Consumables | 6,660 | 4,500 |
| Production of reports | 1,065 | 1,050 |
| Computing | 725 | 500 |
| Total | 80,006 | 79,970 |

NB * It is understood that the work programme proposed represents the maximum output achievable within the project budget of £80k per annum for two years.

* WRc's charging rates for1991/92 have been subject to central negotiation by NRA Head Office.

R&D Budget Provision (£K)

| | 1991/92 External | 1992/93 External |
|---|---|---|
| NRA | 80 | 80 |

11. BENEFITS

Direct benefit will be gained from the successful final development of in-situ ecotoxicological methods which can be used by the NRA to investigate chronic deteriorations in water quality in terms of effectiveness - ie better indication of problems and efficiency - ie cost savings over presently available methods.

12. ASSUMPTIONS AND RISKS

No assumptions. Risks limited to the degree of co-operation from NRA regions in the process of site selection and in the adequacy of the contractor's performance.

13. OVERALL APPRAISAL

This work is needed as the final step in the development of in-situ ecotoxicological methods for use by the NRA. The risks are limited to the degree of co-operation from NRA regions and in the adequacy of the contractors performance.

DT
24.9.91

Updated by TGF
14 February 1992

## APPENDIX C - SITE SELECTION CRITERIA (IDEAL CONDITIONS)

APPENDIX C. - SITE SELECTION CRITERIA (IDEAL CONDITIONS)

1. Accessibility

a) To site - agreeable landowner
- suitable roads/tracks to site
- adjacent vehicle parking

b) Bankside - suitable for easy positioning of autosamplers

c) To river channel - easy access from bank
- possible to wade

2. Security

a) Guarded or unvisited location (ie. no vandals)

b) Cover from roads, footpaths, etc.

c) Presence of trees so equipment can be secured

3. Technical

a) Presence of riffles for community sampling

b) Clarity of impact supported by historical biological and chemical data

c) Lack of confounding factors (eg. downstream confluences)

d) River bed suitable for securing caged organisms

e) Prior, testable hypotheses about reason for discrepancy (but not overly obvious reasons, eg. acute levels of metals).