

Methods of Biological Sampling Sampling Macro-invertebrates in Water Supply Systems 1983

Methods for the Examination of Waters and Associated Materials

Methods of Biological Sampling

Sampling of Macro-invertebrates in Water Supply Systems

1983

Methods for the Examination of Waters and Associated Materials

Contents

Warning to Users	2
About this Series	3
Performance Characteristics	4
Introduction	4
Sampling for Infestation of the Distribution Systems	5
Flushing through nets	5
Inserting traps into the mains	7
Direct observation	7
Sampling for Ingress to the Distribution System	7
Traps and filters in line	7
Filters in conjunction with pump sampling	7
Core samples and sand filters	7
Insect attractors	8
Direct Observation	8
Laboratory Examination	8
References	9
Figures	10-13
Address for correspondence	14
Members of the committee responsible for this booklet	15

The Compilers of this booklet have suggested suppliers for some of the equipment. This in no way endorses these suppliers and use of any other equipment with similar performance is acceptable.

Warning to Users

The analytical procedures given in this booklet should only be carried out by competent trained persons, with adequate supervision when necessary. Local Safety Regulations must be observed. Laboratory procedures should be carried out only in properly equipped laboratories. Field operations should be conducted with due regard to possible local hazards, and portable safety equipment should be carried. Care should be taken against creating hazards. Lone working, whether in the laboratory or field, should be discouraged. Reagents of adequate purity must be used, along with properly maintained apparatus and equipment of correct specifications. Specifications for reagents, apparatus and equipment are given in manufacturer's catalogues and various published standards. If contamination is suspected, reagent purity should be checked before use.

There are numerous handbooks on first aid and laboratory safety. Among such publications are: 'Code of Practice for Chemical Laboratories' and 'Hazards in the Chemical Laboratory' issued by the Royal Society of Chemistry, London; 'Safety in Biological Laboratories' (Editors Hartree and Booth), Biochemical Society Special Publication No 5, The Biochemical Society, London, which includes biological hazards; and 'The Prevention of Laboratory Acquired Infection', 'Public Health Laboratory Service Monograph 6, HMSO, London.

Where the Committee have considered that a special unusual hazard exists, attention has been drawn to this in the text so that additional care might be taken beyond that which should be exercised at all times when carrying out analytical procedures. It cannot be too strongly emphasized that prompt first aid, decontamination, or administration of the correct antidote can save life; but that incorrect treatment can make matters worse. It is

suggested that both supervisors and operators be familiar with emergency procedures before starting even a slightly hazardous operation, and that doctors consulted after any accident involving chemical contamination, ingestion, or inhalation, be made familiar with the chemical nature of the injury, as some chemical injuries require specialist treatment not normally encountered by most doctors. Similar warning should be given if a biological or radio-chemical injury is suspected. Some very unusual parasites, viruses and other micro-organisms are occasionally encountered in samples and when sampling in the field. In the latter case, all equipment including footwear should be disinfected by appropriate methods if contamination is suspected. Known or suspected poisoning cases are usually sent to the nearest hospital having special equipment. To ensure admission to the correct hospital at once, always state whether poisoning is likely when calling an ambulance or arranging for an admission to hospital.

The best safeguard is a thorough consideration of hazards and the consequent safety precautions and remedies well in advance. Without intending to give a complete checklist, points that experience has shown are often forgotten include: laboratory tidiness, stray radiation leaks (including ultra violet), use of correct protective clothing and goggles, removal of toxic fumes and wastes, containment in the event of breakage, access to taps, escape routes, and the accessibility of the correct and properly maintained first-aid, fire-fighting, and rescue equipment. Hazardous reagents and solutions should always be stored in plain sight and below face level. Attention should also be given to potential vapour and fire risks. If in doubt, it is safe to assume that the hazard may exist and take reasonable precautions, rather than to assume that no hazard exists until proved otherwise.

© Crown copyright 1985
First published 1985

ISBN 0 11 751789 5

About this series

This booklet is part of a series intended to provide both recommended methods for the determination of water quality, and in addition, short reviews of the more important analytical techniques of interest to the water and sewage industries. In the past, the Department of the Environment and its predecessors, in collaboration with various learned societies, have issued volumes of methods for the analysis of water and sewage culminating in 'Analysis of Raw, Potable and Waste Waters'. These volumes inevitably took some years to prepare, so that they were often partially out of date before they appeared in print. The present series will be published as individual methods, thus allowing for the replacement or addition of methods as quickly as possible without need of waiting for the next edition. The rate of publication will also be related to the urgency of requirement for that particular method, tentative methods being issued when necessary. The aim is to provide as complete and up to date a collection of methods and reviews as is practicable, which will, as far as possible, take into account the analytical facilities available in different parts of the Kingdom, and the quality criteria of interest to those responsible for the various aspects of the water cycle. Because both needs and equipment vary widely, where necessary, a selection of methods may be recommended for a single determinand. It will be the responsibility of the users — the senior analytical chemist, biologist, bacteriologist etc. to decide which of these methods to use for the determination in hand. Whilst the attention of the user is drawn to any special known hazards which may occur with the use of any particular method, responsibility for proper supervision and the provision of safe working conditions must remain with the user.

The preparation of this series and its continuous revision is the responsibility of the Standing Committee of Analysts (to review Standard Methods for Quality Control of the Water Cycle). The Standing Committee of Analysts is a committee of the Department of the Environment set up in 1972. Currently it has seven Working Groups, each responsible for one section or aspect of water cycle quality analysis. They are as follows:

- 1.0 General principles of sampling and accuracy of results
- 3.0 Empirical and physical methods
- 4.0 Metals and metalloids
- 5.0 General nonmetallic substances
- 6.0 Organic impurities
- 7.0 Biological methods
- 9.0 Radiochemical methods.

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, under the overall supervision of the appropriate working group and the main committee. The names of those associated with this method are listed inside the back cover. Publication of new or revised methods will be notified to the technical press, whilst a list of Methods in Print is given in the current HMSO Sectional Publication List No. 5.

Whilst an effort is made to prevent errors from occurring in the published text, a few errors have been found in booklets in this series. Correction notes and minor additions to published booklets not warranting a new booklet in this series will be issued periodically as the need arises. Should an error be found affecting the operation of a method, the true sense not being obvious, or an error in the printed text be discovered prior to sale, a separate correction note will be issued for inclusion in that booklet.

L R PITTWELL

Secretary

31 October 1983

Methods of Biological Sampling

Sampling Macroinvertebrates in Water Supply Systems

Performance Characteristics

Biota Sampled	—	macroinvertebrates which occur in water supply systems.
Habitats Sampled	—	water mains, service reservoirs, water treatment works.
Types of Sampler	—	nets, traps, filters, core samplers, insect attractors.
Basis of Operation	—	flushing and netting, filtering, trapping and direct observation.
Form of Data	—	qualitative and relative abundance.
Limitations of Method	—	flushing ability, isolation and condition of mains which may require swabbing or use of chemicals.
Logistics of Sampling	—	a small team may be required including a waterman with knowledge of the mains system. Care is needed to avoid contamination of the water supply. Staff engaged in this work may require medical clearance. Samples require laboratory examination.
Safety and Hygiene	—	When carrying out sampling procedures described in this publication, safety and hygiene precautions laid down or recommended in (a) 'Working on or near water' National Water Council Safety Advisory Broadsheet and (b) 'Working in confined spaces' Health and Safety Executive and (c) 'Water supply hygiene' National Water Council Occasional Technical Paper No 2 (1970), respectively, should be strictly followed.

Introduction

The methods described are intended to facilitate the collection and examination of the faunas of distribution systems. It is not the purpose of this document to offer advice on the treatment of infested systems, which is covered elsewhere (Sands, 1969).

Samples may be obtained in connection with infestation of water supply systems in order to achieve one or more of the following objectives.

- 1 To investigate the incidence of organisms in different areas of the distribution system from a regular sampling programme.
- 2 To confirm the nature of complaints, often the first indication of an infestation, and to establish the extent of an infestation in the region of complaints.
- 3 To establish the point and intensity of ingress of infesting organisms.
- 4 To monitor the effectiveness of mechanical and chemical infestation control measures.

The results should enable guidance to be given concerning appropriate action to be taken in order to control infestations, for example by identifying the extent of priority areas for treatment, effective methods of treatment and the formulation of a longer term policy to limit infestation and prevent complaints recurring.

The subsequent sections of this document are concerned with:

- I Infestation of the distribution system.
- II Ingress to the distribution system.

I Sampling for Infestation of the Distribution System

This includes the investigation of infestation of trunk mains, service reservoirs, tanks and water towers, and smaller diameter distribution mains. The object is to obtain samples by standardized methods to allow comparison of results. The principal means of sampling are:

- 1 flushing through nets,
- 2 inserting traps into the main and
- 3 direct observation, where access is possible.

1.1 Flushing through nets

All flushing should be through fire hydrants with an outlet diameter of 64mm (2½ inches) into Water Research Centre (WRC) sampling nets (Sands, 1969). These nets are of nylon with an aperture size of 142µm and are available commercially¹.

It is important to achieve sufficient flow to remove debris from the mains. Table 1 (abridged from Sands (1969), based on the work of Durand and Condolios (1952) gives estimates for minimum flow rates to remove particles of specific gravity 1.5 and equivalent diameter of 0.2mm (Table 1). Many animals, their faeces and decomposition products have a specific gravity of about 1.0 and so may relatively easily be transported by flowing water. However, many animals live within sediments of higher specific gravity (2.5–3.0) and these must be disturbed before the animals can be moved.

Table 1 Minimum flow necessary to remove particles of 0.2mm diameter and specific gravity 1.5, from a main of given diameter

Diameter of Main		Minimum Effective Flow	
mm	inches	l/s	gals/min
50	2	1.5	20
75	3	3.8	50
100	4	7.5	100
125	5	12.8	170
150	6	20.2	270
200	8	41.3	550

In older mains, the effective internal diameter is often reduced and high rates of flow are generally not possible because of inadequate water pressure. Thus, although flows of 7.5 l/s (100 gals/min) will effectively remove debris from up to 100mm mains, the effect on larger mains is unreliable. Therefore, in order to obtain comparable results samples are taken using the following standard procedure.

Procedure

- (a) Select sampling points with care, preferably with the assistance of a waterman with local knowledge. Animals are most likely to be found in real or hydraulic dead ends of the system. The governing principle is to select sampling points supplied by discrete, identifiable lengths of 75 to 100mm diameter main. A suitable length would be 150–200m and water should preferably approach the hydrant from one direction only. "Valve operations" may be necessary to achieve this.
- (b) Flush briefly (by opening and closing as quickly as is safely possible) to clear any debris from the hydrant.
- (c) Fit a Vernon Morris flow-gauge (Fig 1) and attach the WRC net over the neck of the gauge.
- (d) Flush at 7.5 l/s (100 gals/min) for 5 minutes. Any deviation from this standard must be recorded on the sample label. When reading the Vernon Morris gauge, the sampling nets should be held out horizontally to minimize the effects of back pressure. An alternative is to flush at full bore for a set period of 5 minutes with the net attached and subsequently measure the actual flow with the gauge (but see 'Precautions' below). The important point is to arrange that conditions are standardized in order to achieve comparable results.

¹ These nets are available from Henry Simon Limited, Cheadle Heath, Stockport, Cheshire, under the description 'WRC Modified Plankton Nets'.

- (e) Shut off, remove net to polythene bag, attach a suitable label to draw-string of net and record location, data, rate of flow, and period flushed, diameter of main, material of main and sampler's name.
- (f) It may be necessary to carry out further flushing to maintain a clean supply. A further sample may be taken to confirm that flushing has been satisfactory.
- (g) If it is necessary to store samples, retain the samples in their nets and store at 1–4°C.
- (h) Before re-use, nets should be washed in weak detergent shortly after use, shaken out and hung up to dry. (See also Section III for precautions to be taken against further spread of infestation).

Precautions

To prevent nets becoming clogged or split, the duration of flushing and the flow rates have to be adjusted to suit the particular conditions. When clogging or splitting seems likely to occur it is recommended that a flow rate of 7.5 l/s (100 galls/min) is not exceeded. As it may present hazards in freezing conditions or waste of water in drought conditions, flushing should be avoided in these circumstances. The effects of flushing on the rest of the main should always be considered, particularly with reference to the risk of disturbing material which might give rise to further complaints.

Experience has shown that it is possible to obtain samples when air-scour cleaning methods are employed but the risk of damage to the sample collection bag is increased, due to greater quantities of material being dislodged and greater velocities of ejected material.

The above details refer primarily to survey sampling. When monitoring the efficiency of infestation control measures, samples should be taken from an already flushed length of main prior to, and immediately following a treatment.

Flushing may be carried out with or without simultaneous chemical treatment of the main under investigation.

i) *Flushing without chemical treatment*

a) **Simple flushing**

The material removed from the main by the flushing will be influenced by the type and amount of deposit and the organisms present. For example, animals such as *Asellus* and snails clinging to the inside wall may be more difficult to remove than chironomid larvae. In order to achieve comparable results it is important that the standard conditions for taking a sample should be applied.

b) **Flushing with swabbing**

Where flows are inadequate for simple flushing or the main is likely to be corroded or contain large quantities of solids, more efficient removal of animals may often be achieved by the use of a sterile foam swab. It is necessary, of course, to have a suitable access point.

ii) *Flushing with the assistance of chemicals*

Removal of animals which cling tenaciously to pipe walls or solid debris may be facilitated by the use of chemicals to kill or stun, followed by flushing. This method is aimed at investigating the animal infestation and is not to be used as a method of mains treatment. The use of chemicals to remove infestation is covered elsewhere (eg Sands, 1969), although such treatment should be monitored (Objective 4) by obtaining net flushing samples before, during and after chemical injection takes place.

Chemicals may be injected using a small metering pump or hand pump into short lengths of main isolated from consumers, to sample the population of infestation organisms. Doses of up to 0.1 mg/l pyrethrins (for crustaceans and chironomid larvae) or 10 mg/l chlorine (for snails and flat worms) can be used with a contact period of 30–60 min.

Adequate flushing of treated water must follow to ensure the complete removal of the chemical from the main, ie at least four times estimated mains capacity. The appropriate health authority must be informed before introducing pyrethrins. Pyrethrins are toxic to fish; aquarists must be warned if there is any likelihood that treated water could be supplied to premises where fish are kept. It is necessary to inform consumers of the shut-

off. Consideration must be given to analytical chemical confirmation that the chemical has been removed after treatment and also to the destination of the waste treated water from the operation, since if discharged to a watercourse, pollution may be caused.

1.2 Inserting traps into the mains (in-line trapping)

Deacon meter traps (Fig 2) (Holland, 1956) are cones of stainless steel mesh inserted into the Deacon meter access points in the distribution system when they are not being used for metering. The cones have a mesh size of 0.5mm and are 5 inches (127mm) at the open end, tapering to 2 inches (51mm). A brass plate retains the trap in line with all water passing through the metering point. Used for qualitative sampling they may be left in place for periods of one to seven days after which they are removed and the contents washed into a sampling jar. These traps indicate qualitatively the range of large organisms present and also the movement of organisms along these mains.

1.3 Direct observation

Points of possible access to the distribution system include water towers, service reservoirs and tanks and large trunk mains. Every opportunity should be taken to examine these sites internally when they are drained. This should be carried out, in the case of reservoirs and tanks, with a small amount of water remaining and before any other personnel have entered. Samples of deposit are obtained with a fine mesh (265 μ m) hand net for qualitative analysis and the number of organisms is estimated and recorded.

Sampling for ingress includes the investigation of the species and the number of infestation organisms in the treatment works, before, during and after treatment and in the final water. It should help identify the source of organisms and whether or not they are reproducing in the treatment works.

Principal means of sampling are:—

1. Traps and filters in line
2. Filters in conjunction with pump sampling
3. Core samples for sand filters
4. Insect attractors
5. Direct observation

11.1 Traps and filters in line

Suitable sampling points include the entrance to inspection boxes after filtration and along continuously flowing sampling pipework. Traps will have to be designed to fit the particular situation. A suitable filter with a 50 μ m sintered bronze filter element for insertion along the final water pipework is available commercially² (Fig 3).

If the filters are fitted together with a flow meter, when the filter is removed and the washings examined microscopically, the number of the organisms may be related to the volume of water filtered.

11.2 Filters in conjunction with pump sampling

The Water Research Centre has developed a plankton pump for sampling large volumes of water from various points in treatment works or from service reservoirs and tanks (Evins & Greaves, 1979). It incorporates an expansion chamber in order that flows and head-losses across the filtering mesh are reduced.

11.3 Core samples for sand filters

Evins & Greaves (1979) used 1m long PVC hemi-cylinders of 37.5mm radius with removable aluminium sides fitted across the diameter to investigate organisms present in

² A suitable filter is marketed by Norgren Pneumatic Products, Shipston on Stour, Warwickshire.

11 Sampling for Ingress to the Distribution System

sand filters. A similar method could be used to investigate the colonization of both rapid gravity and slow sand filters.

II.4 Insect attractors

In microstrainer houses, water towers, and similar structures, qualitative sampling of flying insects may be facilitated by the use of an attracting/destroying system. Specific pieces of equipment are widely available³, and are basically similar in overall design, incorporating electrified grids surrounding an ultra-violet light source. Flying insects are attracted by the emissions, which are 310nm and therefore harmless to humans. The grids carry high voltages (up to 4500V) but low current (less than 10mA).

Contact or near contact with the grid electrocutes the insects which then fall into collecting trays suspended underneath the equipment. Insects may, therefore, be sampled and identified.

This method acts as a secondary line of defence against ingress to the system and against the build-up of flying-insect instars in the distribution network, since both adults hatching from the water surface and those entering the building may be attracted and destroyed before they can reproduce. It should be stressed that all such buildings should be effectively sealed to prevent the ingress of flying adults.

II.5 Direct observation

Potential points for direct observation include raw water inlets to the works and, when drained, the clean water tanks. Examination of clean water tanks is similar to that for service reservoirs. Every opportunity should be taken to examine by direct observation.

III Laboratory Examination

Wash the sample into a white tray and allow to settle. A sieve may be used to remove coarse debris. All large organisms are counted but sub-samples of small organisms from a known area of the tray may be removed and examined in a small petri dish to estimate approximate (relative) abundance. The approximate total number of small organisms in the whole sample may then be estimated by counting distinct parts, for example, the head capsules of chironomid larvae. A simple scale of abundance may be employed rather than absolute numbers.

Diagrams for the identification of most infestation organisms are given by Sands (1969).

Care should be taken when dealing with chironomid larvae and pupae. A form of *Paratanytarsus inquilinus* (Kruger) produces eggs in the pupal and larval stages, without the necessity of hatching and emergence. Its presence has been confirmed in many distribution systems (Langton, 1974). Whenever its presence is suspected or known, all bags, nets, swabs and samples must be sterilized with boiling water or bleach to reduce the possibility of infecting laboratories and works. To aid identification a diagram of the pupal integument is included. (Fig 4) Possession of characters in the combination shown is diagnostic for this species.

The relevant composition of the deposit with particular reference to frass (invertebrate faecal pellets), rust particles, floc and other material should be noted on a simple scale of abundance. The total volume of deposit is poured into a suitable measuring cylinder, allowed to settle for one hour and the volume measured. This may be related to the volume flushed to allow comparison between samples. If required, the percentage chemical composition of the deposit may be measured. This could include percentage organic matter, iron, aluminium, and manganese.

The results which are obtained are, of course, not strictly quantitative and serve only as a guide to the intensity of infestation or as a relative measure of the degree of control which has been secured.

As the eggs and immature forms of some organisms can be mistaken for flakes of rust, scale or sand, careful examination of such material is advisable.

³ For example, the 'Insect-o-cutor' range from Henry Simon Limited, Stockport, Cheshire.

References

- Durand, R & Cordolias, E. (1952). Proceedings of a colloquium on the hydraulic transport of coal. (London, National Coal Board, Scientific Dept.)
- Evins, C & Greaves, G. F. (1979). Penetration of water treatment works by animals. WRC TR 115. Medmenham.
- Holland, G.J. (1956). The eradication of *Asellus aquaticus* from water supply mains. J.Inst.Wat.Engrs. 10: 221-241.
- Langton, P.M. (1974) On the biology of the parthenogenetic *Paratanytarsus* breeding in the Essex Water Company distribution system. Appendix in Williams, D.N. An infestation by a parthenogenetic chironomid. Water Treat. Exam 23: 215-231.
- Sands, J.R. (1969). The control of animals in water mains. Water Research Association, Medmenham TP 63.

Additional Useful Reference

- Smalls, I.C. & Greaves, G.F. (1968). A survey of animals in distribution systems. Proc.Soc.Wat.Treat.Exam. 17: 150-186.

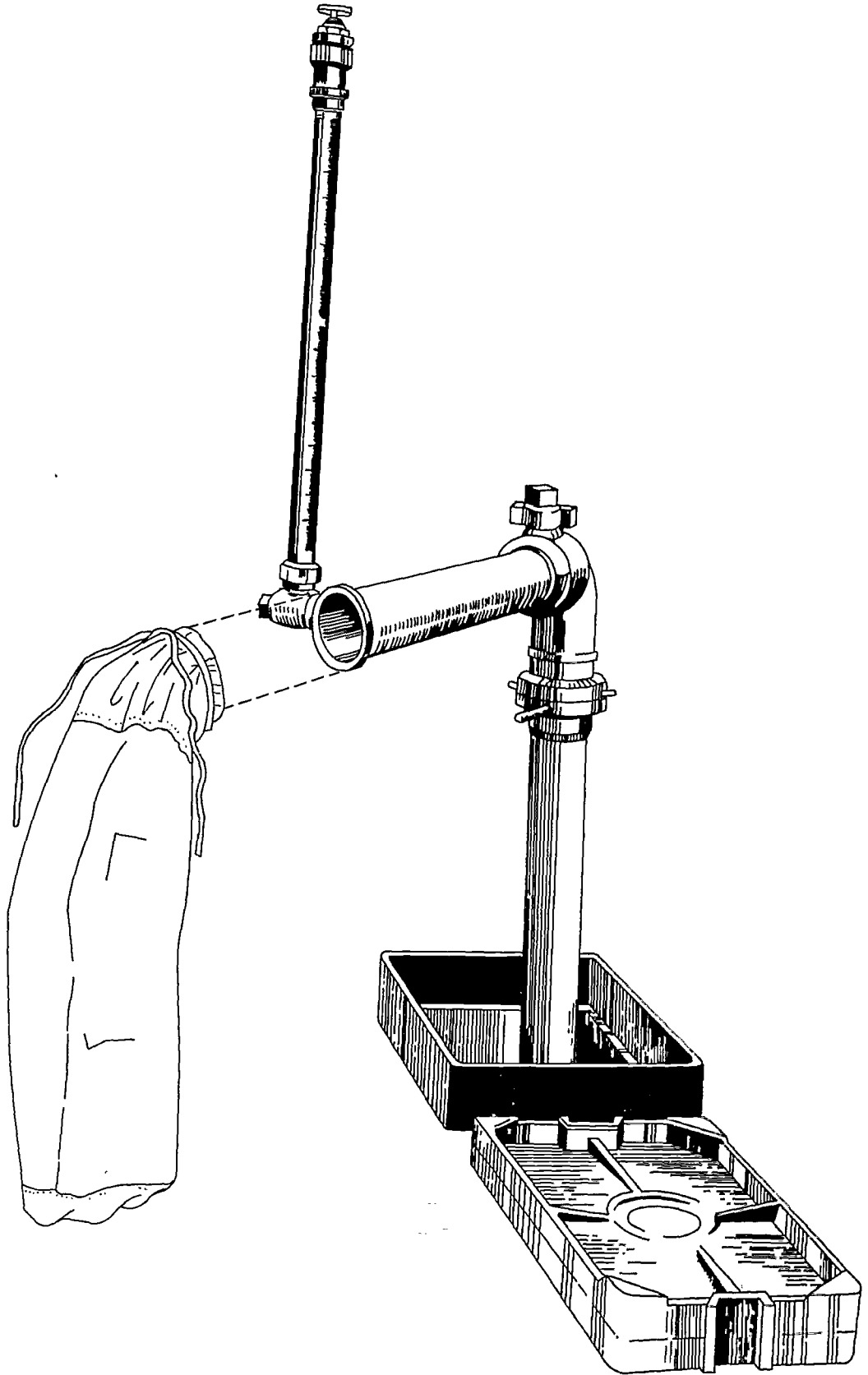


Fig.1 VERNON - MORRIS FLOW GAUGE

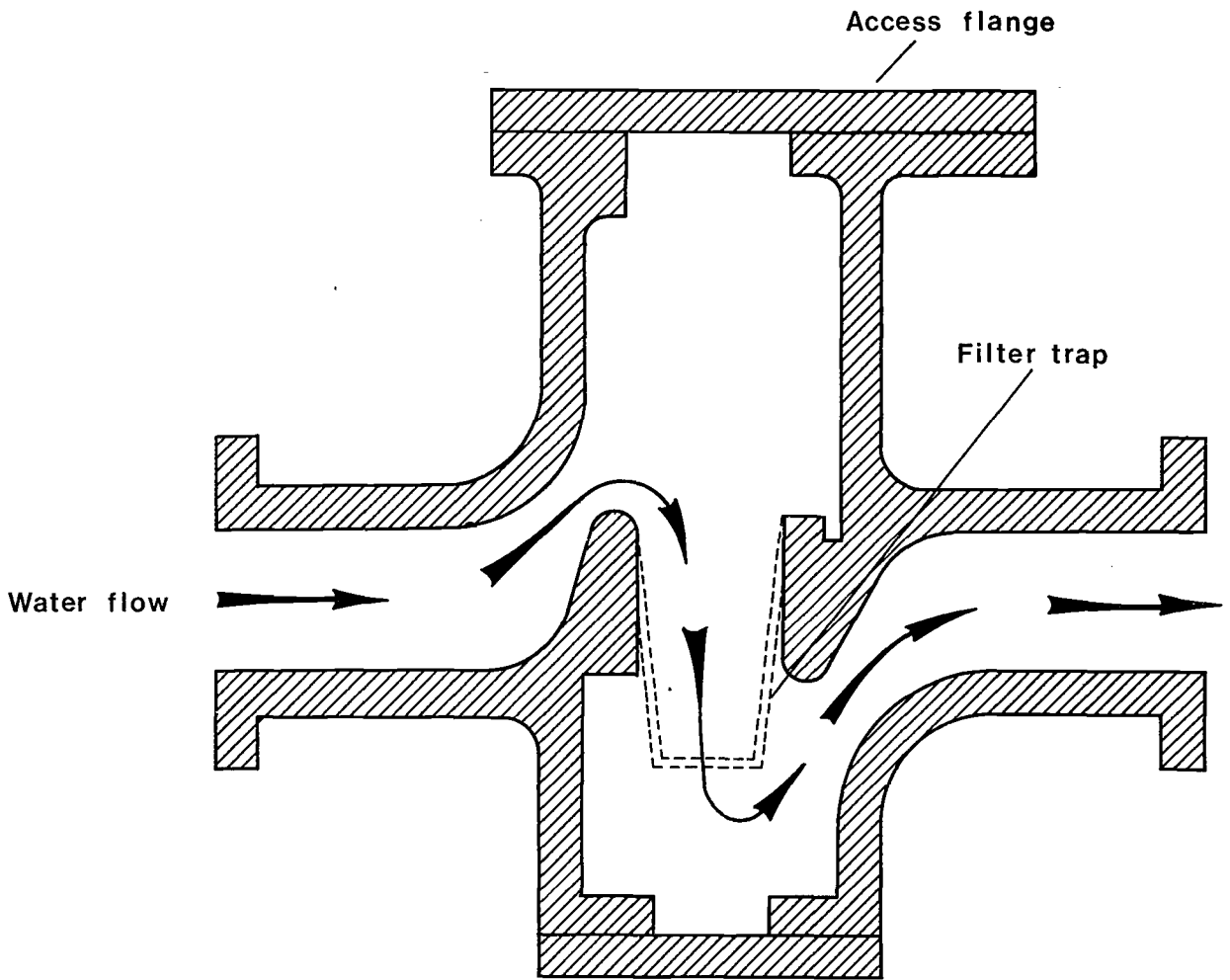


Fig. 2 'DEACON' METER TRAP IN POSITION - CROSS SECTION
(after Holland 1956)

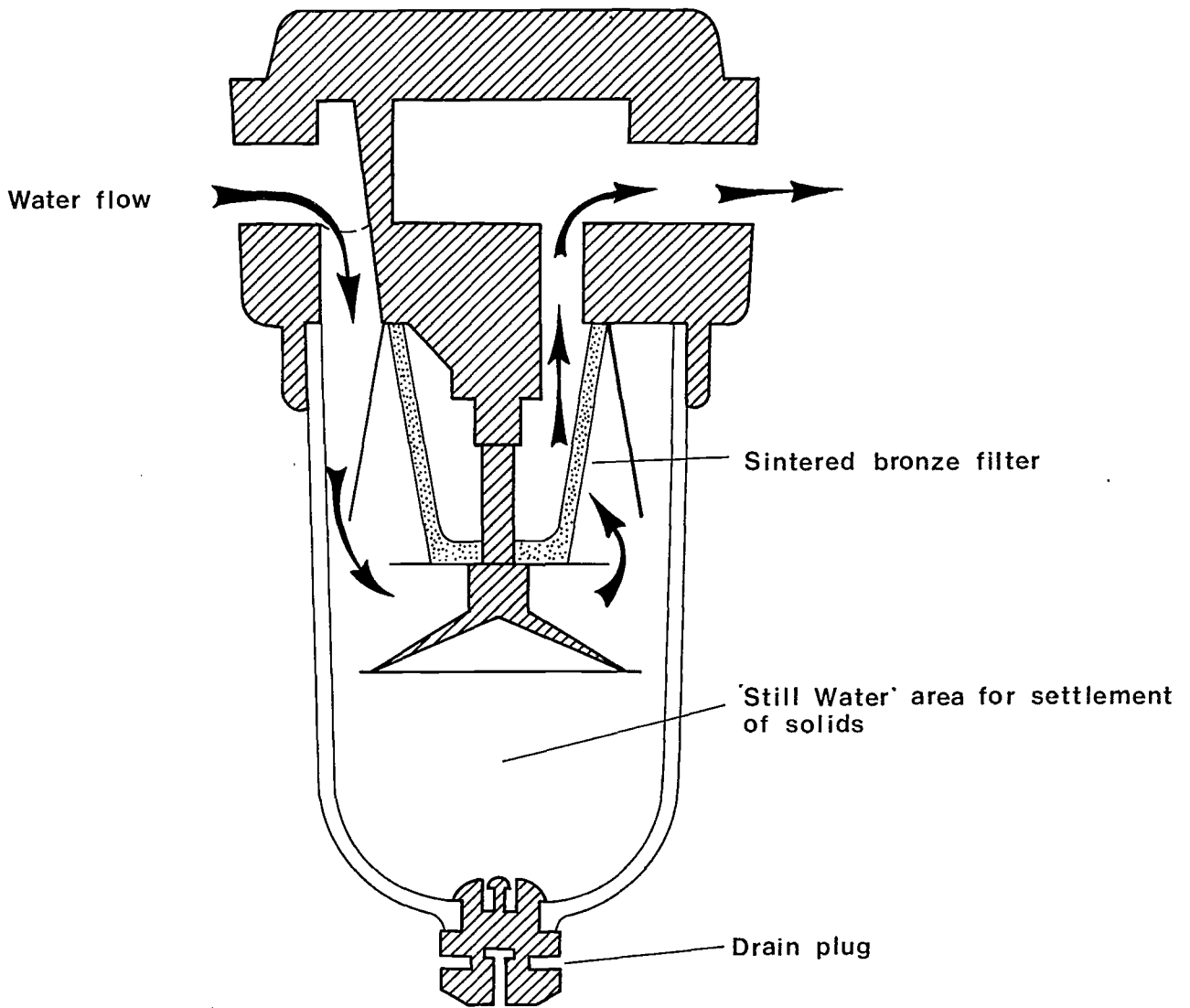


Fig.3 NORGREN TYPE WATER FILTER - CROSS SECTION

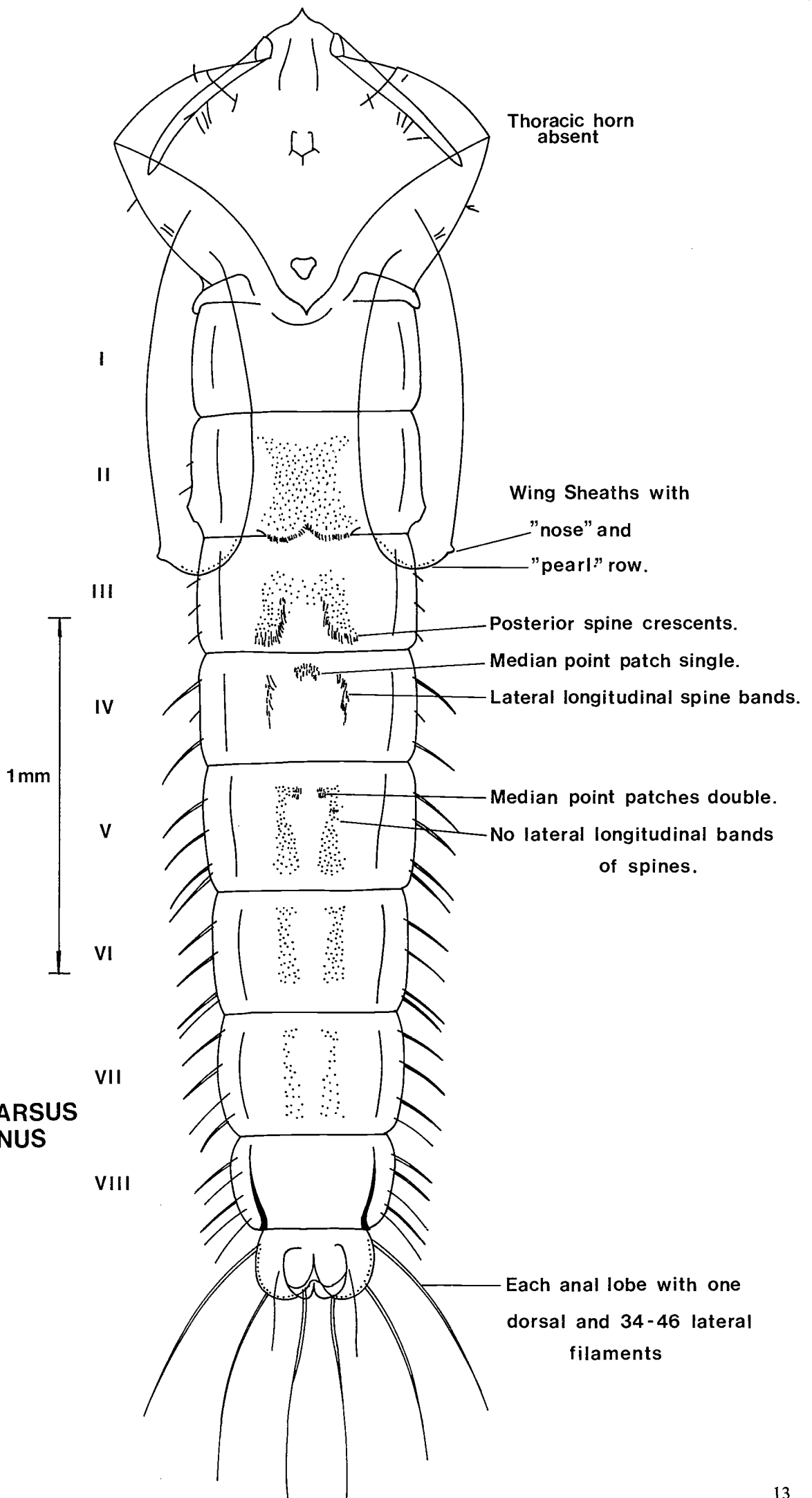


Fig.4
PARATANYTARSUS
INQUILINUS

**Address for
Correspondence**

No matter how thoroughly a procedure is tested there is always the possibility of a user discovering a new problem. Users with queries or information about these methods should contact:

The Secretary
The Standing Committee of Analysts
Department of the Environment
Romney House
43 Marsham Street
LONDON SW1P 3PY
England

Department of the Environment

Standing Committee of Analysts

Members responsible for the production of this booklet are:

Mr J S Alabaster	2	Mr R Lloyd	2
Dr G I Barrow	3	Mr P J Long	3
Dr G A Best	3	Mr A F Lott	2
Mr P M Bolas	2	Mr J C McCullins	3
Mr A Bufton	2	Mr A H Nield	3
Mr P A Chave	3	Dr H A Painter	2
Mr B E P Clement	3	Mr L R Pittwell	2,3
Mr T Clough	1	Dr J E Portmann	3
Dr B T Croll	1	Mr L D Purdie	3
Dr H Egglshaw	1,2	Mr B D Ravenscroft	3
Dr J M Elliott	1	Mr L F Reynolds	2
Mr H Fennell	2	Mr L A Richards	3
Mr M C Finniear	3	Prof J P Riley	3
Dr R Gardiner	3	Mr M S Rolfe	2
Mr G I Goodfellow	3	Dr D Taylor	3
Mr T R Graham	1,2,3	Dr K C Thompson	3
Dr J Hargreaves	2	Dr A M Ure	3
Dr S M Haslam	2	Dr D A Williams	3
Mr H A Hawkes	1,2	Dr R Wood	3
Dr J M Hellowell	1,2		
Mr E Hodges	3		
Dr R J Huggins	1	¹ Panel Member	
Dr D T E Hunt	3	² Working Group Member	
Dr H Knopp	2	³ Main Committee Member	
Mr J S Leahy	3		

HER MAJESTY'S STATIONERY OFFICE

Government Bookshops

49 High Holborn, London WC1V 6HB

13a Castle Street, Edinburgh EH2 3AR

41 The Hayes, Cardiff CF1 1JW

Brazenose Street, Manchester M60 8AS

Southey House, Wine Street, Bristol BS1 2BQ

258 Broad Street, Birmingham B1 2HE

80 Chichester Street, Belfast BT1 4JY

*Government publications are also available
through booksellers*

ISBN 0 11 751789 5