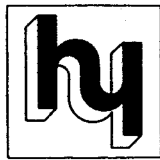
2.90



Hydraulics Research
Wallingford

OPTICAL INSTRUMENTS FOR SEDIMENT MONITORING (Algal Growth)

I E Shepherd

Contract No. PECD 7/7/194

Report No. SR 204
March 1989

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ABSTRACT

In-situ measurement of suspended solids in water is often implemented using simple light transmission measuring transducers. All such transducers are subject to fouling of the optical surfaces by various contaminant mechanisms. The evolution of sensor design has resulted in the use of solid state light sources, and the objective of this research was to try to establish any benefits accruing from the selective use of source wavelength.

After initially reviewing the mechanisms of contaminant growth, the dominant cause of light attenuation was thought to be the presence of algal blooms. It has always been recognised that bacteria, deposition of salts and detritus are present, but are considered generally less significant.

A laboratory experiment was set up with the advice of consultants to attempt controlled measurements. This consisted of generating colonised surfaces on glass slips in river waters under visible and infra-red lighting. The consultants analysed the resulting growths and presented a report on their findings.

It was concluded that ambient light, even when scattered before reaching the transducers is a major cause of surface contamination. Assuming that this effect can be minimised, the use of infra-red light instead of visible light significantly reduces algal growth. It is surprising that so few proprietary instruments use infra-red sources as they are readily available and easy to apply. Hydraulics Research Ltd manufactured their own transducers using infra-red more than 10 years ago. The results of this research suggest that further effort should be put into producing new designs of infra-red emitting transducers.

CONTENTS

		Page
Abstract		
1	INTRODUCTION	1
2	CONSULTANTS RECOMMENDATIONS	5
3	LABORATORY APPARATUS	6
4	SAMPLING REGIME	9
	4.1 Initial experiment	
	4.2 Recommended regime	
5	CONCLUSIONS AND RECOMMENDATIONS	10
6	ACKNOWLEDGEMENTS	11

FIGURES

1 Temperature control
2 Light sources
3 Light detector

Incubator photographs

APPENDICES

1 Subcontract with the Freshwater Biological Association
2 Consultants Report

1 INTRODUCTION

The objective of this project is to establish whether the use of an illumination source having a selected emission wavelength will prove beneficial in reducing fouling effects experienced with conventional filament lamp sources used in optical sediment transducers deployed in rivers and estuaries.

During the initial phase of liaison (as indicated in 4 of the schedule) it became clear that a controlled experiment in the field as suggested in the Programme of Work would be unsatisfactory due to:

a) uncontrolled flow variations;
b) effects of sunlight;
c) possible electrochemical effects at sites where experiments could be conveniently mounted;
d) cost of frequent site visits needed;
e) difficulty of eliminating source temperature effects, etc.

Our discussions at the University of Aberystwth, Freshwater Biological Association (FBA), Institution of Marine and Environmental Research and the Water Research Centre convinced us that a controlled laboratory experiment would be the appropriate method; atlhough FBA strongly advised procurement of field samples we were unable to comply.

Our Report SR 173, March 1988 described the difficulties we experienced in establishing the work on a sound scientific basis to the best advice available, including staffing problems. Our submission ref. TE/SR/39 dated 3 August 1988 requested permission to sub-contract a significant part of the project (approx. 44% of the total value) to FBA. Their proposal to analyse samples for algal, bacterial and organic deposition was presented, including clear methods for proceeding, on 24 June 1988. Subsequently permission to go ahead was granted by DOE.

Although the procedure outlined in the schedule has not been adhered to, the overall objective remained the same. We have not, however, had adequate funding to construct a prototype field transducer as originally envisaged.

The March report included a review of the environmental factors affecting algal growth, the types of algae expected in typical waters, the growth processes and the absorbtion of light.

Various designs of optical sediment monitors are used by HR Ltd to measure the in-situ concentration of mud and silt in suspension in rivers and estuaries. Developments of sensor designs have effected improvements relating to the fouling of the optical surfaces, but basic mechanisms responsible for the degradation have never been properly established. The objective of this research project is to produce an understanding of the processes involved and as a result make recommendations on the possibilities of sensor improvement.

The effort has been concentrated on the fouling caused by the growth of algae on the surfaces. It is well known that salts from the water (perhaps calicum carbonate) and fine mud particles are also deposited but it is felt that these are less dominant in many of the applications, especially in some marine environments.

Progress with the work has been frustrated for several reasons, as described below.

1.1 The staff member doing the work resigned, and it was not possible to recruit a similarly qualified person to continue the work. It was decided to transfer the project within the company to the sedimentation laboratory as the thrust of the action was oriented towards chemical/biological analyses more appropriate to that section than to the instrumentation section.

1.2 Early in 1987/88 an optical device was ordered from Oriel Scientific Ltd. This is a broad band quartz halogen source connected to an optical filter unit with a fibre optic link. The purpose of the fibre is to remove any heating effects from the culture media to eliminate any influence that the warmth of the source may have on the algal growth. The filter unit permits the transmission of selected wavelengths of light into the medium (river water) in order to investigate the effects of the different parts of the spectrum on the growth. This equipment was not received until after the departure of the staff involved, nearly five months after the order was placed.

1.3 During initial test work, the lamp source failed after about 50-60 hours. It was returned to the supplier and was sent back to HR nearly two months later.

1.4 The lamp failed again. On investigation it was discovered that the source was a projector lamp which only had a specified life of 50 hours. This was unknown to us and is not mentioned anywhere in the documentation. This was quite useless for our purposes, and it became necessary to find out if any alternative type of lamp could be fitted to the equipment. This was done but the mechanical changes needed were not implemented as later advice negated the benefits.

1.5 Subsequent to the above attempts to establish the experimental work, contact was made with the Fresh Water Biological Association (FBA) as described above. Their advice changed the direction of the work, and the method recommended is described in 2. Everything we have attempted has turned out to be far more difficult than envisaged and is explained later.

1.6 It has become quite clear that many of the problems with the type of transducers used are caused by the penetration of sunlight into the water, encouraging surface contaminant growth. The contribution to this growth produced by the transducer light source or heat generated is small in comparison with sunlight effects. However, many transducers are shielded from direct sunlight in use (the output signals are influenced by ambient lighting to a degree dependent on the type), and so the concept that reduction of growth could be achieved by selective spectral emission is still valid. However, it is clear that <u>all</u> transducers should be shielded from sunlight irrespective of whether or not they have been designed to reject ambient lighting effects from the signals they produce.

1.7 It has been noted that at least one manufacturer has claimed that the use of solid state I.R. devices instead of filament lamps reduces optical fouling. This statment has not been supported by evidence: this was the object of the research.

1.8 Other methods to reduce fouling were considered, including:

a) leaching effects from the proximity of nearby metals or materials. This was unlikely to be practical due to the constant replenishment of water near the sensor from reversing flows etc.

b) surface coatings. Any coating on the glass surfaces are bound to affect optical transmittance, and if the opacity varied with time it would have the opposite of the desired effect. Again, it is unlikely that marine inhibiters nerby would prevent the fouling of the sensor surface;

c) existing methods for automatic wiping of surfaces are available. Usually, a motor driven actuator is used. These are normally ruled out for our work because the transducers are much more expensive, and the motors consume power. In addition, moving parts underwater for long periods must give rise to concern about reliability.

2 CONSULTANTS RECOMMENDATIONS

Initial contact was made with Freshwater Biological Association at the Windermere Laboratory, and subsequently referred to the nominated expert at the Eastern Rivers Group based at the Institute of Terrestial Ecology, Monkswood Experimental Station. Discussions about our objectives resulted in the proposed sub-contract shown in Appendix I.

It was clear that our understanding of the processes involved were non-existant. Examples of the problems outlined by F.B.A. included:

a) effects of water temperature change;
b) salinity;
c) differences between population growth in daylight and in darkness;
d) seasonal variations;
e) effects of flowing or still water;
f) destruction of one type of population by another i.e. it is conceivable that a growth may appear in the short term and then be consumed by something different later.

The advice given included a strong recommendation to undertake a controlled experiment in the field so that samples obtained could be compared with those produced in the same water in a laboratory environment. Whilst the wisdom of this is acknowledged without any doubt, we were unable to implement the field test due to lack of staff, time and money. In the event, the budget was entirely consumed by the cost of the sub-contract and HR time in organisation of the laboratory experiment.

3 LABORATORY APPARATUS

The requirements were as follows:

a) The incubator should be suitable for exposing microscope slips to infra red light, and to filament lamp light having the same spectral distribution as the lamp used in a typical transducer.

b) Samples of river water taken from one of the field measurement sites should be used.

c) Facilites for loading and withdrawing slips at the required time intervals without causing undue disturbance to remaining samples.

d) The river water temperature must be controlled at or near the temperature of the water at the field site.

e) All materials used must be free from corrosion or contamination problems.

By far the most difficult requirement was (d). Generally, laboratory constant temperature water tanks use direct heating elements, thermostatic control and stirrers to minimise the temperature gradients. The large mass of water to be controlled dictates a relatively high power heater. In addition, what we needed was cooling rather than heating, to prevent warming of the water by the filament lamps or by central heating during the winter months. This would have required the purchase (or design) of a suitable tank for which no funds were available. The other problem with such a tank would be the stirring; this is usually quite vigorous and would have caused a lot of disturbance to the samples (more than would normally be experienced in river flow) and would make them very difficult to handle. The slips are very light and fragile.

The solution adopted was to use a glass fish tank suspended inside a much larger tank so that water could circulate freely around the inner tank. The arrangement is shown in the photographs, and two separate sample tanks are accommodated. Water from the mains enters the outer tank at the bottom of one end and exits at the top of the other end. The sample tanks are supported free of the base so that water circulates around the outside. Adhesive aluminium foil and a lid over the top shields the sample tanks from daylight. The tanks are divided into two sections by a p.v.c. partition with cross members to support the glass slip trays. The slips are ~~layed onto~~ placed on a perspex tray which has vertical strips used to lower them into and out of the water (not shown in the photos).

It was found that mains tap water remained at a very stable temperature throughout the experiments, and was close to the temperature of the original river samples. Thermocouples were used to monitor the relative temperatures inside the tanks and a plot is shown in Figure 1. It can be seen that the temperature gradient was low and also stable with time within about $\pm 2°C$ over long periods. The flow rate of the cooling water was adjusted empirically, and we found that the tap was an adequate pressure regulater (i.e the mains pressure did not change very much). It should be noted that the thermocouples did not have a high accuracy ($\pm 2°C$), but the intention was to demonstrate a reasonably stable temperature rather than an accurately controlled one.

One half of each tank was illuminated by an infra red (I.R.) solid state lamp (Figure 2) and the other half by a group of 6 miniature filament lamps of the type used in typical sensors. The filament lamp voltage was set at 11V (for 12V lamps) as this method is used to extend lamp life in the typical sensors. The spectral distribution of the emitted light should then be similar to that generated by the field transducers. Since the I.R. source is invisible, a method for checking that the emission was present was needed. The illumination test circuit of Figure 3 was used for this. The detector is very sensitive so a neutral density filter was used to restrict the intensity to within the dynamic range. The emitter has a narrow band output of 950 nm with half power points at $\pm$50nm, but the detector has a relative response at $\pm$50% from about 600nm to 1000nm, with a peak at about 920nm.

An initial trial experiment had to be abandoned as aluminium trays were used to support the slips, and gross contamination was found due to some form of corrosion occurring. This happened even though no other metals were present in the water, and no electrical connections were made to them. The other materials were only p.v.c. and glass. Perspex trays were used later to avoid the problem.

Handling of the microscope slips was difficult due to our lack of experience in the use of them. We were advised to mark the tops of the slips with a diamond tipped tool so that the surface to be examined could be easily identified later. The slips used were:

a) cover glass, Chance Propper No.1 16mm diameter, thickness 0.12 to 0.17mm.

b) As above, 22mm diameter.

They are very fragile and we found that the marking on the surface set up stresses which, after immersion in water for several weeks, caused fracturing. As with the aluminium contamination problem, it was only discovered after some time but resulted in the re-start of the tests yet again. After several false starts, the definitive tests were begun, regrettably rather late in the season; it was not until the second week of November that the river water samples were collected for the tests (we were advised not to use any of the previously retained samples, even though they had been kept in the dark, due to warming in the laboratory and the possible multiplication of organisms not found in fresh samples). We understand that the presence of various micro organisms is seasonal, and it would have been much better to use samples collected in the summer. However, due to the various problems encountered this was not possible, and we were constrained to the tests finally in the winter months.

Two samples of river water were used, one obtained from a tidal reach of the Thames at Tilbury Power Station, and the other from the Severn at Bristol Docks. The Thames sample was collected at low water and had a high concentration of sediment which settled out in the test tank and covered the surface of the slips. The Severn water, although again from a tidal location and near to low water had a very low concentration of suspended silt.

A simple test of leaving some water in a beaker in sunlight demonstrated that the growth of organisms was greatly accelerated compared with the artificial lighting in the test tanks.

In order to correlate the microscopic analysis to be done by F.B.A. with the occlusion of transmitted light, it was intended to measure the relative attenuation of a light beam by each of the sample slips before they were sent for analysis. The detector shown in Figure 3 was used. The simple method evolved was as follows:

a) place clean slips between filament lamp source and detector;
b) adjust lamp voltage to provide a reference detector output of 10.0 volts;
c) remove the clean slip and interpose the sample slip;
d) record the reduced detector output voltage.

The method worked well, but the results were not worth considering as the surface growth was not sufficiently homogeneous and was affected by the deposited silt such that repeatable readings were almost impossible to achieve.

4 SAMPLING REGIME

4.1 Initial experiment

The samples were to be analysed for:

a) algal growth
b) organic carbon deposits
c) bacteria

12 slips were immersed, and 4 collected after 3, 7 and 13 days. Slips a) and c) were stored in small vials containing 2% formalin, and slips b) were air dried and stored. After 14 days they were hand delivered to F.B.A. for analysis.

The results of this lead to the conclusion that the growth was slower than first expected, and that the samples would need to be immersed for longer periods. This is perhaps not surprising, because the slips were illuminated by lamps above the surface of the water tanks, so it is likely that the light intensity AT THE LENS of a submerged transducer would be higher than in this experiment. Some light is bound to be lost by reflection and refraction at the air/water interface in the test tank.

The other problem was that insufficient carbon had been collected to permit a sensible measurement, so the sampling was modified in the later experiment to provide a greater surface area.

4.2 Recommended regime.

The following was repeated for visible light and I.R. light for both the Thames samples and the Severn samples where possible, but subject to a limitation of larger diameter slips and the space needed to accommodate them on the sample trays.

Microbiological samples			Algal samples		Carbon samples	
Immersion after	16mm slips		16mm slips		22mm slips	
3 weeks	2 slips,	2 pots	2 slips,	2 pots	6 slips,	3 pots
6 weeks	2	2	2	2	6	3
9 weeks	2	2	2	2	6	3
TOTALS	6 LARGE pots each with 1 slip in 2% formalin. Storage possible		6 SMALL pots each with 1 slip in freshwater. Must deliver immediately		9 LARGE pots each with 2 air dryed slips. Storage possible.	

As the fresh water stored samples had to be delivered as soon as the slips were removed from the tanks, all the slips were delivered together by hand at the required time intervals.

5 CONCLUSIONS AND RECOMMENDATIONS

The conclusions of the analysis are provided in the Consultants Report (Appendix II). These, together with our comments about the experimental work, are summarised below.

5.1 The experimental work we were able to do was less thorough than was planned. The main reasons for this are described in the introduction. In the event, we were only able to do the laboratory investigation.

5.2 There were many problems involved with setting up the controlled experiment (see section 3). The Consultants final recommendations include a suggestion that the sample slips be suspended vertically in the incubation tanks. We accept this point but the slips were mounted horizontally initially to catch the floating detritus as it was deposited. The advice originally suggested that this would help to encourage the algal growth. In the event, there was far too much detritus and this, in fact, inhibited the growth by preventing light penetration onto the slips.

5.3 It was not possible to sensibly correlate the measured attentuation of light by the contamination due to the very patchy nature of the deposits. Uneven fouling was found to be very significant. Transducers that offer compensation for uneven fouling should be very advantageous.

5.4 Bacterial, chemical and detrital effects are present irrespective of any illumination sources.

5.5 Natural light penetration to the sensor (even by scattering rather than direct illumination) is very significant in causing algal growth. All ambient light should be excluded, even where transducers incorporate electronic methods for eliminating the effects of ambient light on the direct measurement.

5.6 Infra red sources reduce algal growth.

5.7 Future designs of transducers should:

a) offer compensation for uneven fouling;
b) use I.R. light sources;
c) exclude direct and scattered ambient light AS A DIRECT CONSEQUENCE of their design. Bolt-on light shields are not considered adequate.

6 ACKNOWLEDGEMENTS

Dr A F H Marker of the Freshwater Biological Association has been a much appreciated advisor and mentor throughout. We would not have achieved sensible results without his valuable assistance. I am also grateful to Ed Walker who organised the experimental work and retrieved the field samples. He also made several trips to Huntingdon to transport the samples.

I acknowledge the cooperation of Mr C E Wright of D.O.E. who organised the project in such a way that we could pursue the evolving objectives during the course of the work.

FIGURES

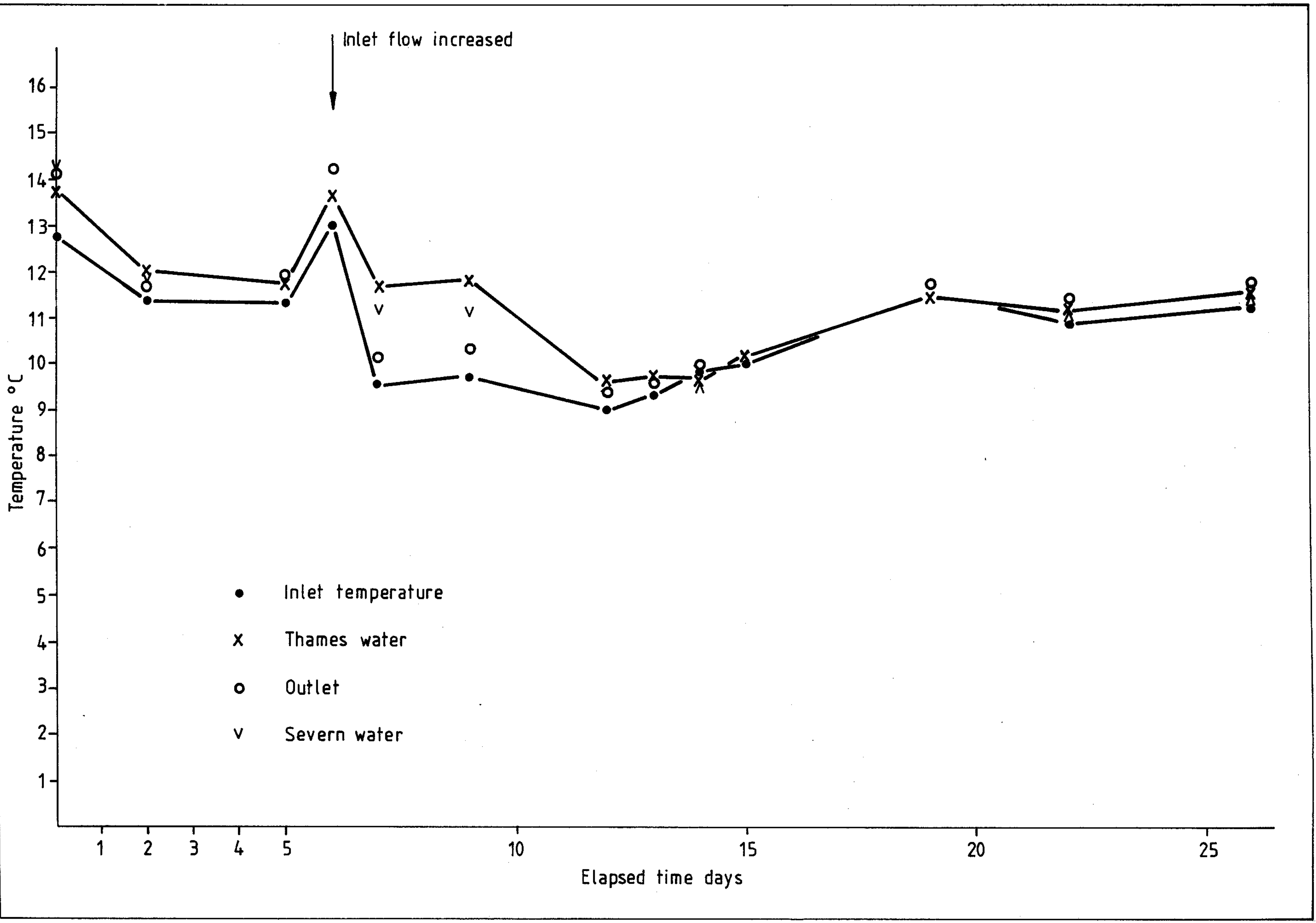
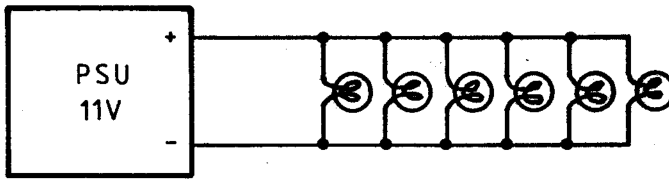
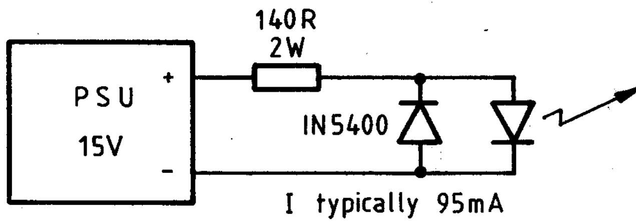


Fig 1 Temperature control



Filament lamps
L.E.S. type
R.S. 587-939
12V 1W



I.R. Emitter Type
TSUS 5402 λ = 950nm
θ/2 = 50°

Fig 2 Light sources

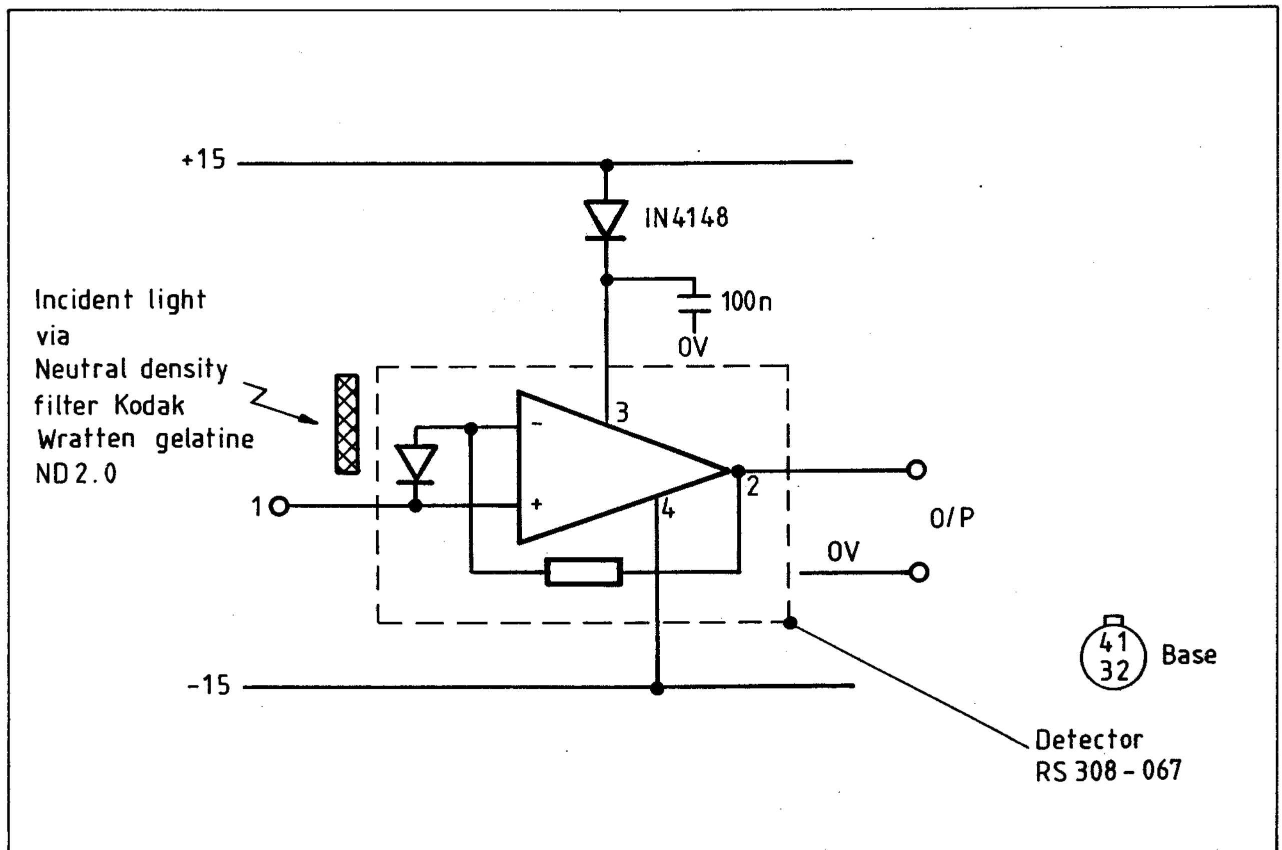
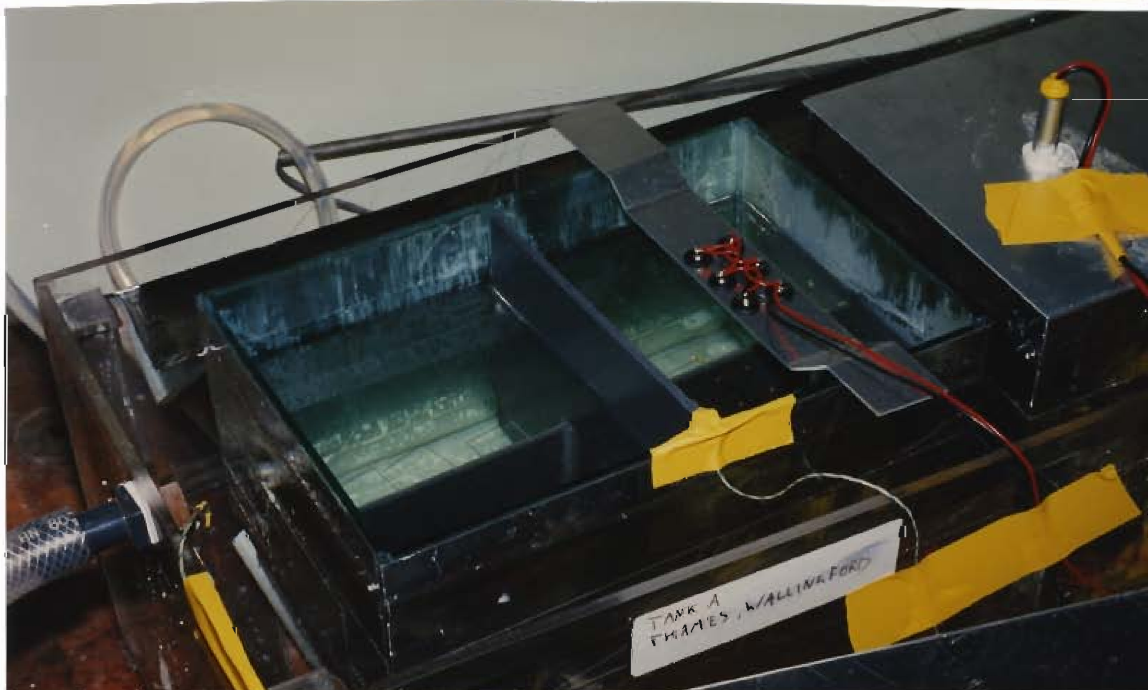


Fig 3 Light detector

PHOTOGRAPHS

INCUBATOR CONSTRUCTION


TANK A
THAMES, WALLINGFORD

APPENDIX 1

Subcontract from Hydraulics Research Ltd to the Freshwater Biological Association. The examination of biological organisms in deposits that contaminate the optical surfaces of turbidity sensors.

Background

The optical surfaces of turbidity sensors are contaminated by microorganisms rapidly in natural waters and consequently give progressively faulty results. Traditionally the light source emits in the visible part of the spectrum and thus provide a light source for surface growing algae. Although IR sensors have been developed recently to avoid this problem, heterotrophic organisms do not require light and will still contaminate the surfaces. Moreover inorganic deposition takes place under a wide variety of conditions.

Objectives

1. To identify the major algae present on sensor surfaces.
2. To estimate numbers of microorganisms growing on these surfaces.
3. To estimate the organic content of the deposits growing on the optical surfaces.

Methodology

1. (i) The main contractor will construct an incubator fitted with irradiances sources with wavelength emissions comparable to those in the turbidity sensors. Irradiance levels should also be comparable. The incubator temperature should be at maintained at the current river temperature.

   (ii) The simulated optical surfaces may be either microscope slides or microscope cover slips.

   (iii) The main contractor will develope a method of measuring the transmission of light through the simulated optical surfaces before and after immersion in river water, and provide this data for the subcontractor.

   (iv) The main contractor will be responsible for transporting the samples to the subcontractor by a mutually agreed method.

2. (i) The subcontractor will examine the slides or

coverslips and report on contamination by algae and other microorganisms.

(ii) The subcontractor will estimate the organic biomass present. If sufficient material is present chlorophyll a will be estimated, otherwise oxidizable organic carbon will be estimated by standard wet combustion.

(iii) Results will be compared with transmission data provided by the contractor.

Reporting

At the end of the subcontract a report will be provided to the main contactor.

Time Scale

Costs

Samples will be analysed at the rate of 1 sample per day. This is based on the assumption that samples will be provided as a time series and that it will not be possible to store biological degradable material. The samples will be analysed by Scientists of the Freshwater Biological Association at Monks Wood and at Windermere.

15 days work: £5300

Nominated Officers

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APPENDIX II

CONSULTANTS REPORT

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Report date: March, 1989.
Report to: Hydraulics Research Ltd.
Contract No: Order No. 28198
FBA Report Ref: NEX ERG/T040398a1/5
TFS Project No: T04039a1

Sub-contract from Hydraulics Research Ltd
in relation to:

"Optical instruments for sediment monitors
(algal growth)."

This is an unpublished report and should not be cited without permission, which should be sought through the Director of the Freshwater Biological Association in the first instance.

The Freshwater Biological Association is part of the Terrestrial and Freshwater Sciences Directorate of the Natural Environment Research Council.

Contents

including 34 pages, 11 tables and 8 plates

1. Summary

2. Background and Introduction

3. Methods

4. Results

5. Discussion, conclusions and recommendations

Acknowledgements

References

Tables 1 - 11

Plates 1 - 8

1. Summary

1.1 Incubators were constructed by Hydraulics Research Ltd to simulate the light quality and intensity provided by Partech suspended solids sensors. Microscope coverslips were used to simulate the glass optical surfaces of the sensors. Experiments were carried out at Hydraulics Research Ltd and transported to the Freshwater Biological Association's Eastern Rivers Group at Monkswood by staff of Hydraulics Research Ltd.

1.2 Preliminary experiments were carried out in October 1988.

1.3 Water was taken from the Rivers Thames and Severn in December 1988 and the main experiments were carried out between December 1988 and the end of February 1989. The microscope coverslip samples from these experiments were taken from Thames water after 3, 6 and 9 weeks and from Severn water after 6 and 9 weeks.

1.4 Although bacterial numbers were very variable, there did not appear to be consistent differences between visible and infra-red treatments.

1.5 Water from the River Thames was taken during a period of high turbidity in the estuary and a thick layer of detritus was deposited on the coverslip which largely inhibited algal growth, due to the blocking of the light path. The dominant alga was a marine centric diatom, probably a spore of *Thalassiosira*, but was present in very small numbers compared with the detritus.

1.6 Samples from the clear water of the River Severn showed considerable algal growth in visible but very little in infra-red radiation. There was significantly greater quantities of organic matter under visible radiation. Under infra-red radiation organic matter was still 40-60% of that found under visible radiation.

1.7 Conclusions

1.7.1 Detrital deposition will occur on glass surfaces irrespective of the quality of the light source. Bacterial numbers are likewise unaffected.

1.7.2 Patchy distribution of these deposits may also affect the long term stability of the sensors.

1.7.3 Direct daylight falling on *in-situ* sensors will iteself generate algal growth. Light shields should always be kept in place and the sensor and shield kept in as low light as is reasonably practical.

2. Background and Introduction

Towards the end of May 1988 the Freshwater Biological Association (FBA) was formally approached by Hydraulics Research Ltd for advice on the interference of the light path of optical transducers by algae and other forms of contamination. This problem is particularly severe when instruments are immersed in water bodies to monitor turbidity and suspended solids loads over prolonged periods. Specifically the FBA was asked to analyze samples, which would be provided by Hydraulics Research Ltd, for:

A) type of growth,

B) relative weight / unit area or thickness,

C) possible information on growth rate.

After discussions during June and July 1988 the FBA agreed to become biological consultants to Hydraulics Research Ltd, experts in instrument design. In outline:

A. (i) Hydraulics Research would construct an incubator fitted with irradiance sources with wavelength emissions and irradiance levels comparable to those in the turbidity sensors. The incubator would be maintained at current river temperatures when the samples were taken.

(ii) The simulated optical surfaces would be either microcsope slides or coverslips.

(iii) Hydraulics Research Ltd would develop a method of measuring the transmission of light through the simulated optical surfaces before and after immersion in river water, and provide this data for the FBA.

(iv) Hydraulics Research would transport the samples to the FBA.

B. (i) The FBA would examine the slides or coverslips, report on contamination by algae and other micro-organisms and estimate the organic biomass present.

(ii) Results would be compared with transmission data provided by Hydraulics Research.

After a period during which the apparatus was constructed by Hydraulics Research preliminary experiments were carried out in late September 1988 and extended for two to three weeks into October. Based on very limited algal growth over this period, and the need for modifications to the apparatus the main experiments were started in December and then extended for 9 weeks.

3. Methods

3.1 Samples

Since the contaminating surfaces on the optical transducers were clear, plane-flat glass, excellent substitute surfaces were provided by clean coverslips. Coverslips were carefully transported from Hydraulics Research at Wallingford to the FBA Eastern Rivers Group at Monkswood near Huntingdon.

3.2 Algae

Coverslips were placed on glass slides and a second coverslip placed on top to prevent evaporation. Numbers of live algae were estimated by direct microscopic counts. Macroparticulate organic and inorganic detritus and algal abundance were estimated as percentage cover by the method described by Greig-Smith (1983). Clean, uncolonized areas were also estimated in the same way and included the thin covering of bacteria since they were not visible using this simple microscopic technique In three sets of samples (Thames water: 3, 6 and 9 weeks) so much detritus was present that samples could not be estimated directly. In these cases loose superficial material was washed off and the remaining material estimated directly as above. The washings, however, were made up to a known volume, and sedimented in Lugol's iodine in sedimentation chambers and counted under an inverted microscope (Lund, Kipling and LeCren, 1958).

3.3 Bacteria

The bacterial biofilm was allowed to develop on glass coverslips at Hydraulics Research Ltd. The coverslips

were fixed immediately in the 0.2µm membrane filtered 2% formaldehyde solution provided by the FBA. The surface to be analysed was marked prior to colonization by scratching the upper surface.

The bacteria colonizing the surface were enumerated using a direct epifluorescence microscpoe technique (Porter and Feig, 1980). The coverslips were immersed in 4'6-diamidino-2-phenylindole (DAPI) for at least 5 minutes to stain the bacteria with fluorochrome. The individual bacteria were counted using a Leitz Orthoplan microscope fitted with a ploempack illuminator and selecting ultra-violet light for the incident excitation wavelength. The fluorescing bacteria were counted at a total magnification of 1200x.

Each coverslip was counted along three transects of 20 fields (60 fields in total) and the field area was selected according to the concentration of bacteria in the biofilm. The number of bacteria was calculated per cm^2 of surface.

3.4 Organic matter was estimated by measuring its reducing capacity. Pairs of dried coverslips were placed in a solution of $K_2Cr_2O_7$ in concentrated H_2SO_4 at 100°C for one hour. The decrease in oxidant was measured by titration

against ferrous ammonium sulphate. The method was essentially that described by Mackereth, Heron and Talling (1978, p102) except that a solution of glucose was used as a standard and the titration end point was detected using 1,10-Phenanthroline-ferrous sulphate-complex solution as the indicator.

4. Results

4.1. Bacteria

4.1.1. Problems encountered. a) Container for sample No 20 was empty; b) The samples numbered 28, 42, 43, 56 and 57 were not marked to indicate the surface to be counted; c) Samples 21, 22, 28, 29, 35, 36, 42 & 43 all contained a large amount of particulate detritus and this caused some interference with the counting procedure. Only bacteria attached to particles on the glass surface and those directly attached were enumerated.

4.1.2. Results are given in Table 1. Widely variable numbers of bacteria were encountered, making statistical interpretation difficult. However, it can be seen that there is no evidence for either increasing numbers of bacteria during the period of immersion or for a difference between visible and IR irradiance. The highest count was 5.92×10^{6} cm^{-2}. If it is assumed that one bacterium occupies 1 $micron^{2}$ (probably an overestimate) then the surface cover by bacteria would be less than 6%.

4.2. Algae

4.2.1. River Thames

There was little algal material in these samples at any time during the 9 weeks of immersion. Clearly there was much suspended material in the water when it was taken, probably because the river was in spate. Consequently the coverslips became covered with a thick layer of detritus (Plates 1 and 2) which must have largely obscured the light path and prevented algal growth. To make adequate counts samples were separated into material which closely adhered to the coverslip (Tables 2 - 4) and the overlying

suspension (Tables 5 - 7). Initially the dominant diatom was marine, a resting spore, probably a Thalassiosira sp or a close relative (Plate 5). At the end of 9 weeks there were fewer individuals with cellular contents but plenty of empty frustules, suggesting that they had germinated and either died or left the immediate vicinity of the coverslip. Phormidium spp. (Plates 7 and 8) began to occur towards the end of the period of immersion in visible but not IR radiation. Throughout the period of immersion detritus occupied between 10 -20% of the coverslip while algae were less than 1% (Tables 2-4).

4.2.2. River Severn

The suspended solids load was much lower in the water from the River Severn and hence the light path was not obstructed. Although 3 week immersion samples were not provided, coverslips exposed to visible radiation showed a large increase in algal growth between 6 and 9 weeks (Tables 8 and 9). At the end of 9 weeks algae were the most important component interfering with the light path, although detritus remained a significant component throughout (Table 9). In contrast coverslips exposed to IR radiation showed very sparse algal growth although the detrital component remained. In the samples exposed to visible radiation diatoms (Bacillariophyceae) and Phormidium spp (Cyanophyceae) predominated. The community which developed was clearly associated with the glass either as strongly motile forms (Nitzschia spp., Plates 6 and 8; Cymatopleura sp., Plate 7) or weakly motile (Phormidium spp., Plates 7 and 8) or attached (Pinnularia sp., Plate 7).

4.2.3. Table 10 shows the distribution of organic matter between the material closely adhering to the coverslip and the loosely associated suspended material (River Thames). Most of the material did not closely adhere to the coverslip. Bearing in mind that between 10-20% of the coverslip was covered by detritus <u>after</u> the overlying material had been removed, the light path to the original coverslip must have been largely obscured. Curiously the amount of detritus in the samples exposed to visible radiation progressively dropped until, after 9 weeks, the difference was statistically significant. Why this should be so is unclear and no explanations are offered here since they would be pure conjecture.

The large increase in the algal component of samples exposed to visible radiation in Severn water was reflected in the organic mass analyses (Table 11). Both at 6 and 9 weeks there was significantly more organic matter in samples exposed to visible radiation. However, in spite of low algal numbers under IR radiation, organic matter levels were still 60% and 50% lower than those exposed to visible radiation levels after 6 and 9 weeks respectively.

5. Discussion, conclusions and recommendations

Fouling of submerged artifacts in natural waters is widespread and well documented. Indeed glass surfaces, generally microscope slides, have been widely used to assess microalgal growth in natural waters for decades (see Butcher, 1932; Tippett, 1970; Munilanu and Maly, 1981, for example). Since the purpose of these investigations was to examine microbial growth on optical glass surfaces, it was natural to use an artificial glass surface for these experiments. We chose to use microscope coverslips for ease of later analyses, and their use in estimating periphyton is also documented (Dilks and Meir, 1981).

Since the primary purpose of these investigations was to identify the significance of biological contamination on optical glass surfaces, no attempt was made to produce a complete species list. The most important criterion was whether contamination was due to growing organisms, passive deposition of detritus or chemical corrosion and precipitation.

Three main types of micro-organisms were found growing on the glass surfaces. Firstly, very small numbers of protozoa and even smaller numbers of rotifers, secondly a thin covering of bacteria directly on the glass and thirdly a range of algae (in which we include the Cyanophyceae, otherwise known as Cyanobacteria).

Three main types of "attachment" were observed:

(1) Organisms loosely associated with detritus (Plates 1 and 2) but not directly attached in any way:

(i) Centric diatoms (Plate 5)

(2) Motile organism growing directly on the glass surface:

(i) Nitzschia spp. (diatoms). Plate 6 and 8,

(ii)Cymatopleura sp. (diatom). Plate 7,

(3) Organisms attached directly to the glass surface or only weakly motile:

(i) Vorticella (Protozoa), Plates 3,

(ii)Phormidium spp. Plates 7 and 8.

(iii)Tube-dwelling diatoms, eg Pinnularia sp, Plate 7

(iv) Rotifers (Plate 4).

Associated with the organisms were varying amounts of detritus. It is unfortunate that the sample of Thames water, used for the main experiment, was taken during spate conditions when the suspended solids load was exceptionally high. This lead to the deposition of a thick layer of detritus on the coverslip, obscured the light path and effectively prevented the algal growth which would otherwise would have occurred. Coverslips immersed in Severn water, which carried a more normal suspended solids load, showed a much more vigorous growth of algae. Infra-red radiation does indeed inhibit the growth of algae but makes no difference to the deposition of detritus or the growth of bacteria, both of which must affect the correct functioning of the in situ probes. Thus switching away from the visible spectrum will only partially alleviate the problem. Furthermore variability between replicates suggest that uneven fouling on the sensor optics may be profoundly important, perhaps as important as the level of fouling itself.

Antifouling by prevention of adhesion due to the texture of the surface is worth investigating but the new surface must be transparent to the wavelengths of radiation utilized by the sensor (visible or IR) and at least as smooth or smoother than glass. Most antifouling

coatings are toxic (eg TBT- or Cu-based paints) and are generally opaque as well (French and Evans, 1986).

Our own experience with Partech sensors in the FBA suggests that direct daylight may have an important role. We have found that direct light affects the output by several points, even with the light-shield protector in place. Without the shield the effect is very large indeed. We suggest, therefore, that algae will grow over the optical surfaces of IR sensors stimulated by the penetration of natural daylight.

At the time of writing this report Hydraulics Research Ltd had not provided light transmission data for the samples analyzed. Hence we provide no comparison. Hydraulics Research Ltd did not find it possible to immerse sensors in the river concurrently with the experiments reported on in this report. Fouling materials from *in situ* sensors would have formed a valuable control in these studies and would be worth considering in future studies. Due to the late start of the studies the main experiment was carried out in mid-winter. Clearly mid-summer is more suitable for biological studies. Should these studies be repeated in the future, it would be worth considering modifications to the laboratory apparatus, firstly to provide lateral light so that the coverslips could be suspended vertically and secondly to provide some form of recirculation to simulate the flow of a river.

Acknowledgments

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References

Butcher, R.W. (1931) An apparatus for studying the growth of epiphytic algae with special reference to the River Tees. Trans. North. Naturalist's Union, 1, 1-15.

Dilks, D.W. and Meir, P.G. (1982) The use of coverslips in estimating periphyton acrual. J. Freshwat. Ecol., 1, 321-326.

French, M.S. and Evans, L.V. (1986) Fouling on paints containing copper and zinc. In: Algal Biofouling. Studies in Environmental Science 28, pp 79-100. Eds L.V. Evans and K.D. Hoagland. Publs Elsevier.

Greig-Smith, P. (1983) Studies in ecology, Vol 9. Quantitative plant ecology. Blackwell Sci. Publs, 3rd edn.

Lund, J.W.G., Kipling, C. and Le Cren, E.D. (1958) The inverted microscope method of estimating algal numbers and the statistical basis of estimating by counting. Hydrobiologia, 11, 143-170.

Mackereth, F.J.H., Heron, J. & Talling, J.F. (1978) Water analysis: some revised methods for limnologists. Freshwater Biological Association, Scientific Publication No.36.

Munilanu, N. and Maly, E.J. (1981) The effect of current on the distribution of diatoms settling on submerged glass slides. Hydrobiologia, 78, 273-282.

Porter, K.G. and Feig, Y.S. (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr., 25, 943-948.

Tippett, R. (1970) Artificial surfaces as a method of studying populations of benthic micro-algae in freshwater. Br. phycol. J., 5, 187-199.

Table 1

Bacterial numbers expressed as numbers cm^{-2} and their standard deviation (SD).

Preliminary experiment			Sample number	numbers cm^{-2} $x10^{-6}$	SD
Thames	vis	3 days	3	2.00	6.83
			4	1.23	6.97
	vis	7 days	7	0.77	1.02
			8	1.81	3.00
	vis	14 days	11	1.49	3.42
			12	1.49	1.36
Severn	vis	14 days	15	0.23	0.38
			16	0.22	0.54
Thames	IR	14 days	19	0.81	0.85
Main experiment					
Thames	3 weeks	vis	21	0.29	0.21
			22	0.46	0.36
		IR	28*	0.49	0.44
			29	0.32	0.25
	6 weeks	vis	35	1.11	0.78
			36	1.06	0.96
		IR	42*	1.21	0.94
			43*	0.51	0.44
Severn	6 weeks	vis	49	4.87	2.54
			50	4.01	2.46
		IR	56*	1.45	1.56
			57*	5.92	3.28

* Samples were not marked to indicate the surface to be counted.

Table 2

Numbers of live algae ($cm^2 \times 10^{-2}$) found on coverslips (excluding the loose covering of detritus) in River Thames water.
3 weeks immersion.

	visible		infra red	
	23	24	30	31
Bacillariophyceae (diatoms)				
Thalassiosiraceae spores	4.78	0.54	1.13	0.66
Other centric diatoms	0.85	1.18	0	0
Pennate diatoms	0.09	0.42	0.24	0.05
Chlorophyceae				
Encrusted colonial forms	0	0.80	0	0.23
Cyanophyceae	0	0.12	0	0
Flagellates	0.33	0.09	0	0

Percentage cover on the coverslip between detritus, algae and no cover (clear area)

	visible		infra red	
	23	24	30	31
no cover	85.6	83.8	82.8	83.9
detritus	14.0	16.2	17.1	16.0
algae	0.4	0.4	<0.1	<0.1

Table 3

Numbers of live algae ($cm^2 \times 10^{-2}$) found on coverslips (excluding the loose covering of detritus) in River Thames water.
6 weeks immersion.

	visible		infra red	
	37	38	44	45
Bacillariophyceae (diatoms)				
Thalassiosiraceae spores	4.73	3.41	2.13	1.42
Other centric diatoms	3.60	1.89	0	0
Pennate diatoms	0	0	0.52	0.09
Chlorophyceae				
Encrusted colonial forms	0.76	0	0	0.09
Cyanophyceae				
Phormidium spp.	37.9	22.73	0	0
Other Cyanophyceae	5.3	2.08	0	0

Percentage cover on the coverslip between detritus, algae and no cover (clear area)

	visible		infra red	
	37	38	44	45
no cover	86.1	93.0	86.4	83.7
detritus	13.2	6.8	13.5	16.0
algae	0.7	0.2	0.1	0.3

Table 4

Numbers of live algae ($cm^2 \times 10^{-2}$) found on coverslips (excluding the loose covering of detritus) in River Thames water.
9 weeks immersion.

	visible		infra red	
	65	66	72	73
Bacillariophyceae (diatoms)				
Thalassiosiraceae spores	0.14	0.50	1.33	0.76
Other centric diatoms	0.19	0.05	0	0
Pennate diatoms	0	0	0.09	0
Chlorophyceae				
Encrusted colonial forms	1.56	1.14	0.05	0
Cyanophyceae				
Phormidium spp.	11.08	73.20	0	0
Other Cyanophyceae	0.33	4.59	0	1.65

Percentage cover on the coverslip between detritus, algae and no cover (clear area)

	visible		infra red	
	65	66	72	73
no cover	71.1	77.6	81.3	71.9
detritus	28.8	22.3	18.6	28.0
algae	<0.1	0.1	<0.1	<0.1

Table 5

Numbers of live algae ($cm^2 \times 10^{-2}$) found within the suspension of detritus covering coverslips in River Thames water.
3 weeks immersion.

	visible		infra-red	
	23	24	30	31
Bacillariophyceae (diatoms)				
Thalassiosiraceae spores	67.59	176.32	11.75	20.57
Other centric diatoms	8.82	26.45	0	0
Pennate diatoms	2.94	41.14	0	0
Chlorophyceae				
Encrusted colonial forms	8.82	2.94	0	0

Table 6

Numbers of live algae ($cm^2 \times 10^{-2}$) found within the suspension of detritus covering coverslips in River Thames water.
6 weeks immersion.

	visible		infra red	
	37	38	44	45
Bacillariophyceae (diatoms)				
Thalassiosiraceae spores	29.39	18.81	19.98	22.92
Other centric diatoms	2.35	2.35	0	0.59
Pennate diatoms	7.05	4.69	0	1.18
Chlorophyceae				
Encrusted colonial forms	1.17	0	0	0

Table 7

Numbers of live algae ($cm^2 \times 10^{-2}$) found within the suspension of detritus covering coverslips in River Thames water.
9 weeks immersion.

	visible		infra red	
	65	66	72	73
Bacillariophyceae (diatoms)				
Thalassiosiraceae spores	2.35	2.93	5.29	8.23
Other centric diatoms	1.76	3.53	0	0
Pennate diatoms	6.47	5.87	1.77	2.35
Cyanophyceae	0	0	1.00	0

Table 8

Numbers of live algae ($cm^2 \times 10^{-2}$) found on coverslips in River Severn water.

6 weeks immersion.

	visible		infra red	
	51	52	58	59
Bacillariophyceae (diatoms)				
Nitzschia sp. (<20u)	30.30	31.81	1.99	0
Nitzschia sp. (20-50u)	5.87	10.23	1.04	0.57
Cymtopleura sp.	0	2.84	0	0
Pinnularia sp.	0.19	31.43	0	0
Melosira sp.	2.08	1.14	1.23	0.09
Other diatoms	2.47	7.96	0	0.09
Cyanophyceae				
Phormidium spp.	247.92	494.13	0	0
Other Cyanophycese	23.30	0	0	0.19

Percentage cover on the coverslip between detritus, algae and no cover (clear area)

	visible		infra red	
	51	52	58	59
no cover	84.0	80.3	94.6	92.3
detritus	12.4	9.9	5.3	7.6
Bacillariophyceae	2.3	4.1	0.1	0.1
Cyanophyceae	1.3	5.7	0	0

Table 9

Numbers of live algae ($cm^2 \times 10^{-2}$) found on coverslips in River Severn water.

9 weeks immersion.

	visible		infra red	
	79	80	86	87
Bacillariophyceae (diatoms)				
Nitzschia sp. (<20u)	528.5	271.0	0.66	2.60
Nitzschia sp. (20-50u)	45.6	481.0	0.33	0.39
Melosira sp.	23.4	81.0	0.09	0.05
Other diatoms	0	0	0.09	0.05
Chlorophyceae (filamentous)	112.2	0	0	0
Cyanophyceae				
Phormidium spp.	*****	*****	6.16	0
Other Cyanophyceae	243.2	145.0	0	0

***** VERY large masses ----- too difficult to estimate by counting. See table below.

Percentage cover on the coverslip between detritus, algae and no cover (clear area)

	visible		infra red	
	79	80	86	87
no cover	70.0	59.7	93.0	89.1
detritus	3.7	7.7	6.9	10.8
Bacillariophyceae	10.1	16.1	<0.1	0.1
Cyanophyceae	16.2	16.5	<0.1	0

Table 10

Organic mass ($\mu g\ cm^{-2}$) found on coverslips immersed in Thames water for three weeks. Samples were separated into adhering material and loose debris.

		coverslip		suspension		total	
		data	mean	data	mean	data	mean
Vis	25	54.7		125.4		180.1	
	26	66.3	49.9	125.4	125.4	191.7	175.3
	27	28.8		125.4		154.2	
IR	32	15.9		177.3		193.2	
	33	17.4	14.0	174.4	178.3	191.8	192.3
	34	8.7		183.1		191.8	

Table 11

Organic mass ($\mu g\ cm^{-2}$) analysis of material found on coverslips, together with statistical comparison between visible and IR illumination.

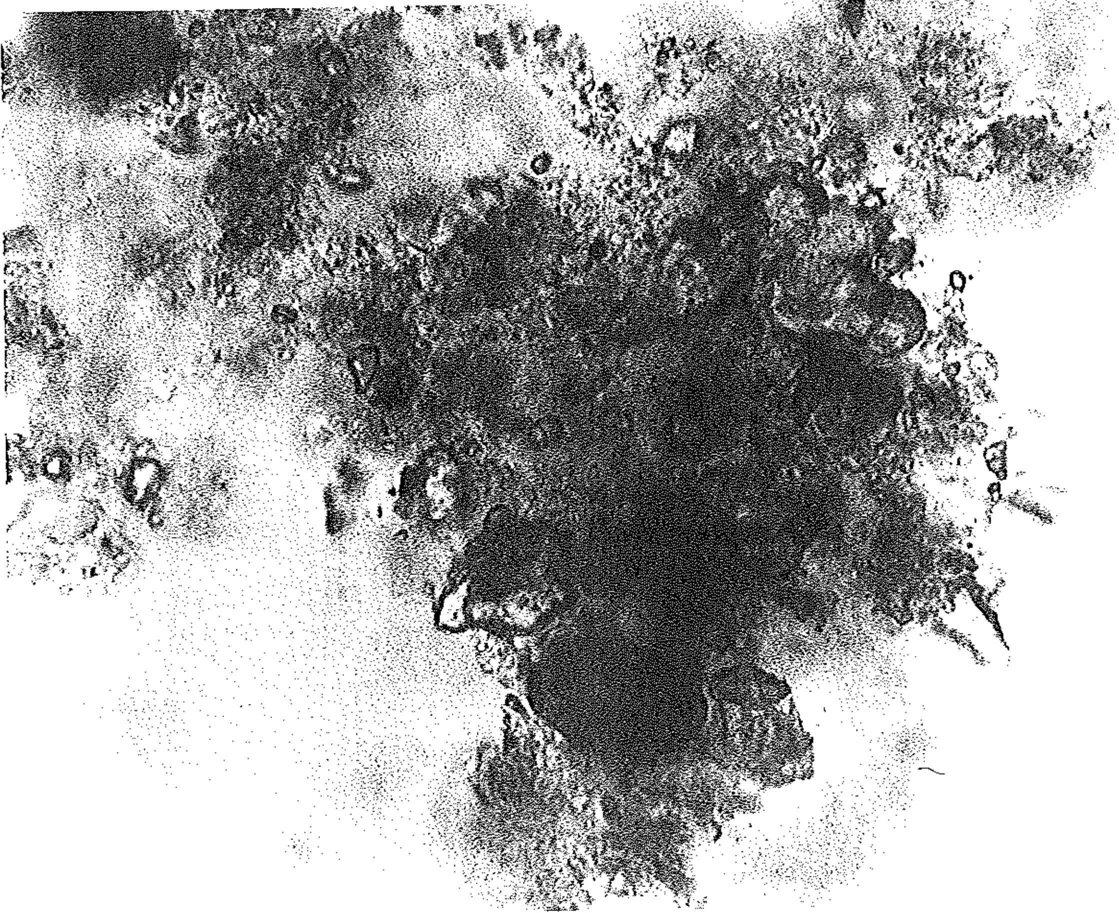
		sample	data	mean	SD	"t"	significance
River Thames							
3 weeks	vis	25	180.1				
		26	191.6	175.3	19.16		
		27	154.2			2.17	ns
	IR	32	193.2				
		33	191.8	192.3	0.81		
		34	191.8				
6 weeks	vis	39	121.7				
		40	169.3	156.0	29.92		
		41	176.9			2.72	ns
	IR	46	184.5				
		47	244.2	205.7	33.42		
		48	188.3				
9 weeks	vis	67	134.9				
		68	114.7	115.9	18.34		
		69	98.3			11.34	>99%
	IR	74	221.6				
		75	236.0	222.2	13.50		
		76	209.1				
River Severn							
6 weeks	vis	53	61.8				
		54	60.9	63.7	4.14		
		55	68.5				
	IR	60	36.1			12.01	>99%
		61	41.8	39.0	2.85		
		62	39.0				
9 weeks	vis	81	114.7				
		82	88.6	98.0	14.50		
		83	90.6				
	IR	88	48.2			8.08	>95%
		89	42.4	47.5	4.85		
		90	52.0				

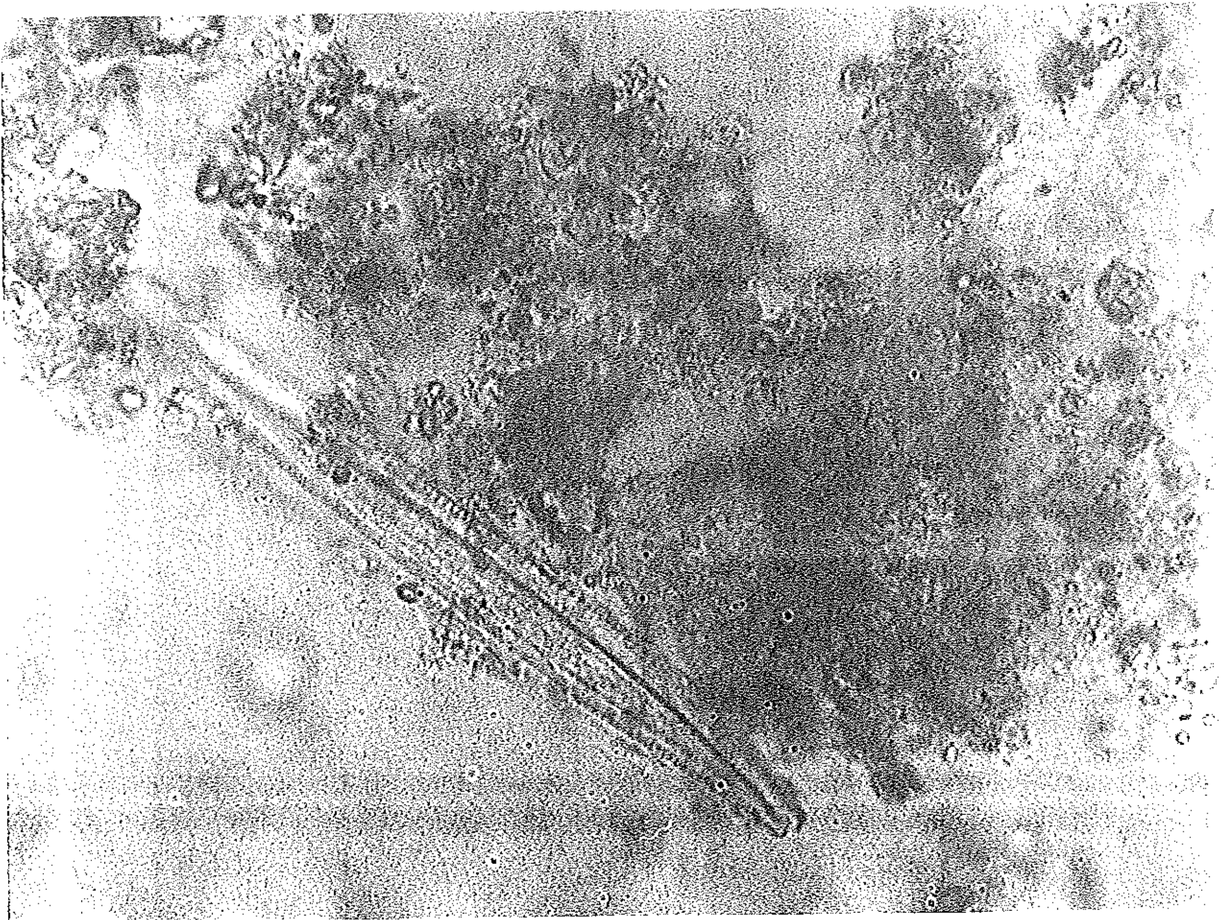
Plate 1

Particulate and largely amorphous detritus, but showing clearing the empty remains of a diatom frustule (*Nitzschia* sp.)

Plate 2

Particulate detritus, showing quartz particles (small sand grains).



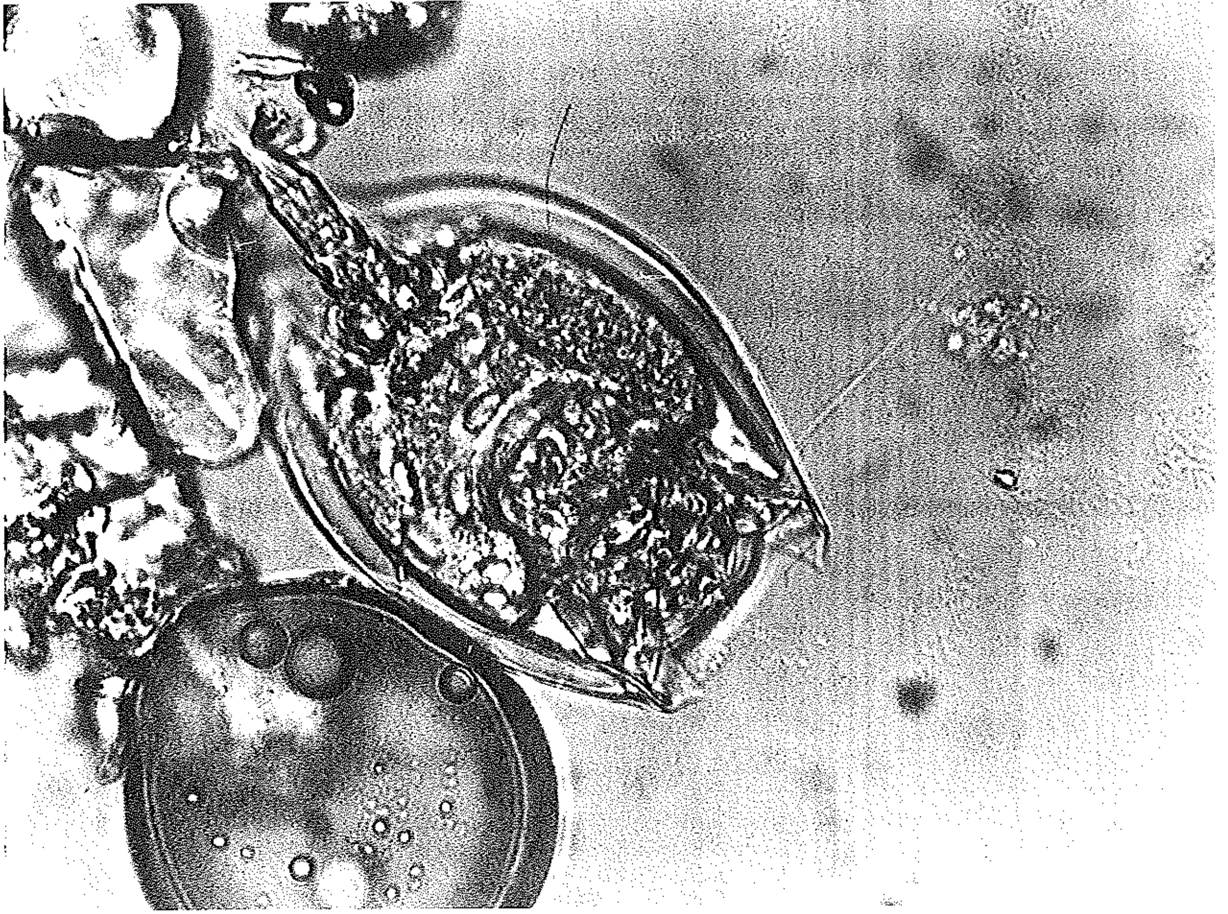


Plates 3

Vorticella (a protozoan) attached to coverslip.

Plate 4

A rotifer attached to a coverslip



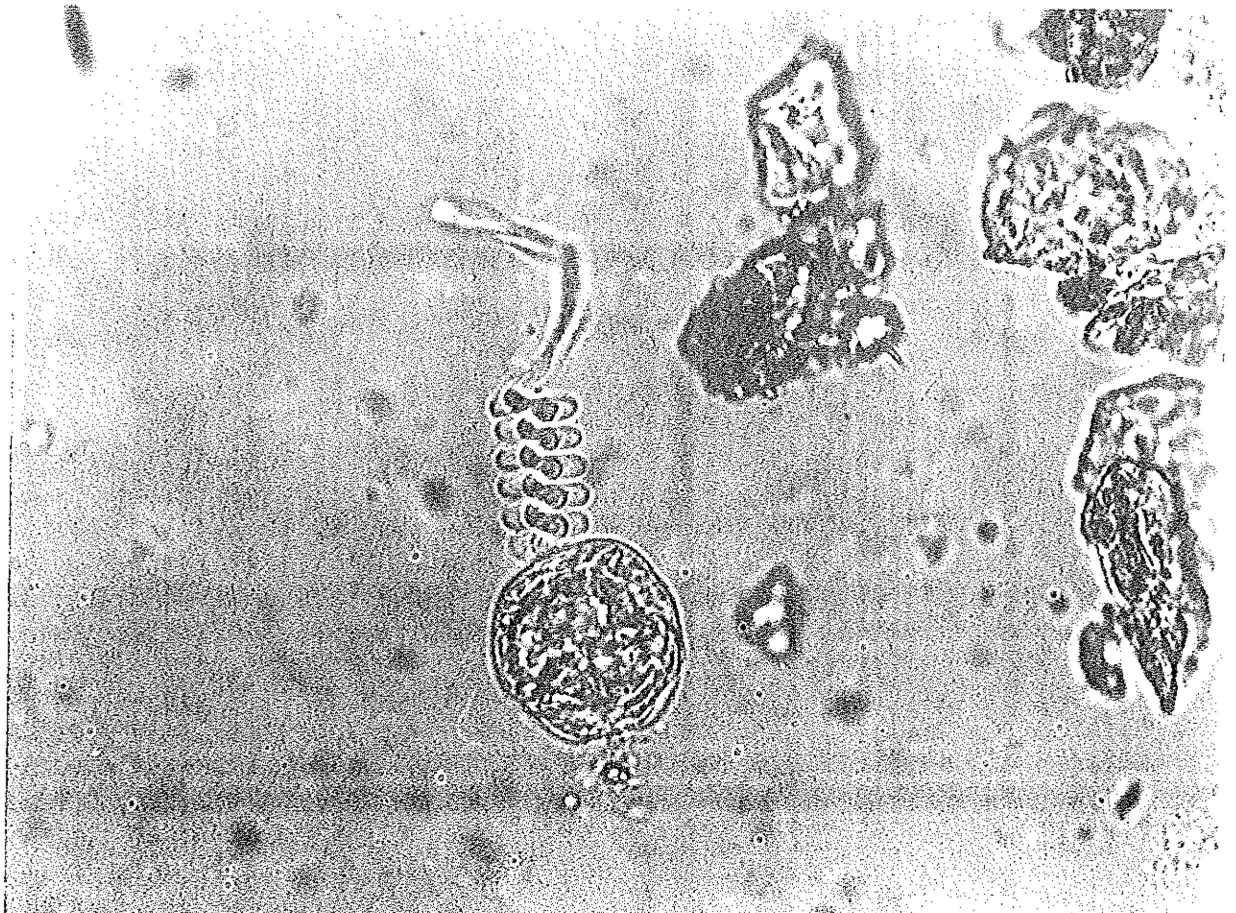
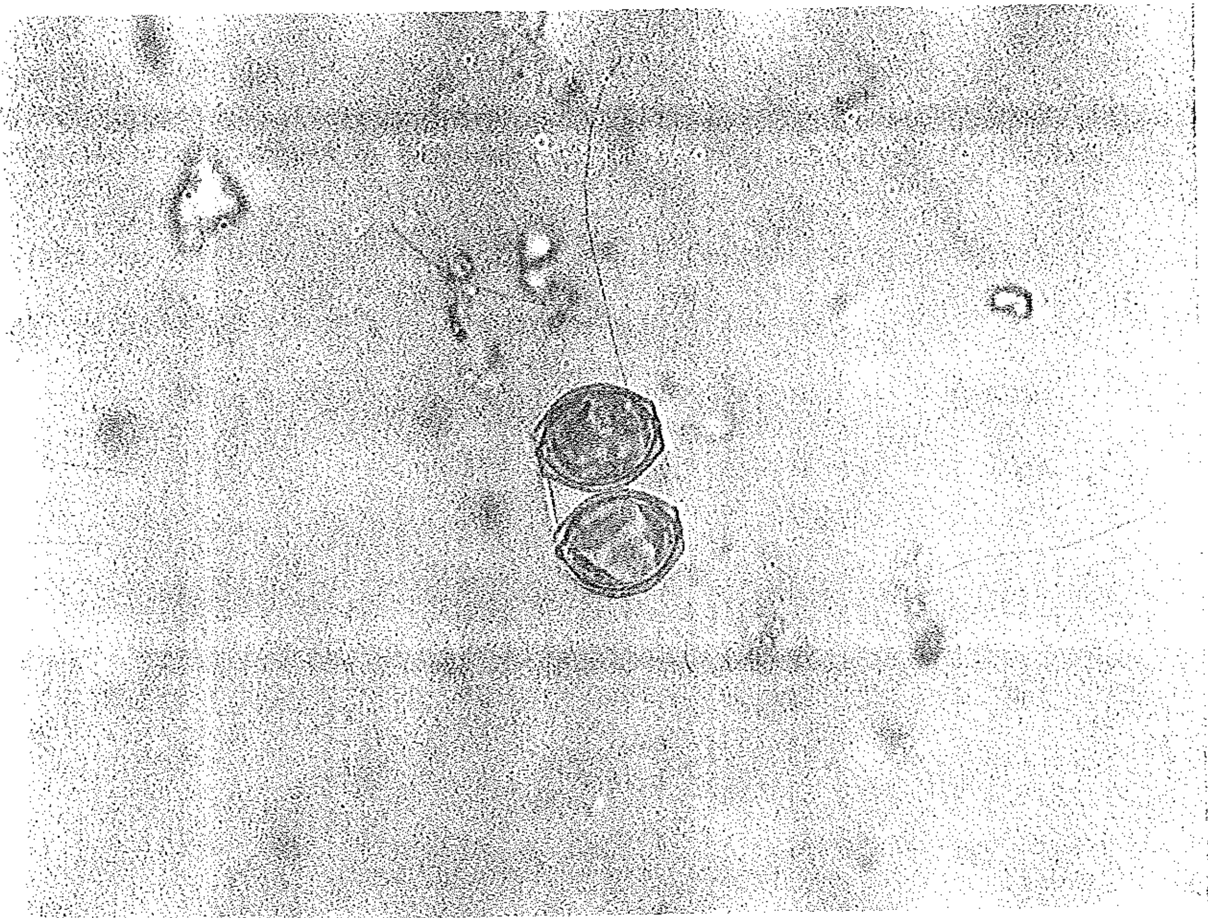


Plate 5

Centric diatom spores found in flocculent detrius. Thames water.

Plate 6

A small species of Nitzschia (Bacillariophyceae), adhering to but mobile on the surface of the coverslip.



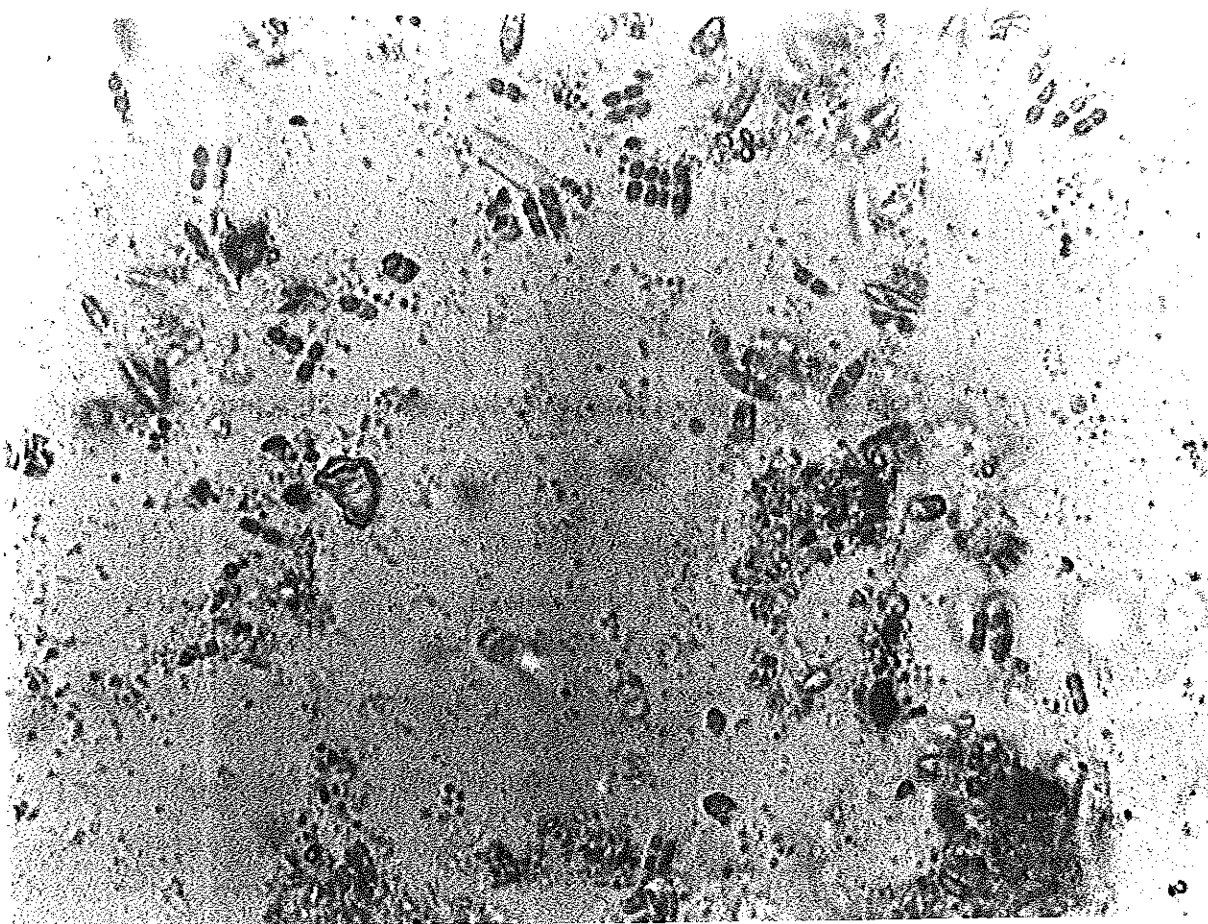


Plate 7

Photograph showing three different forms of algal growth.

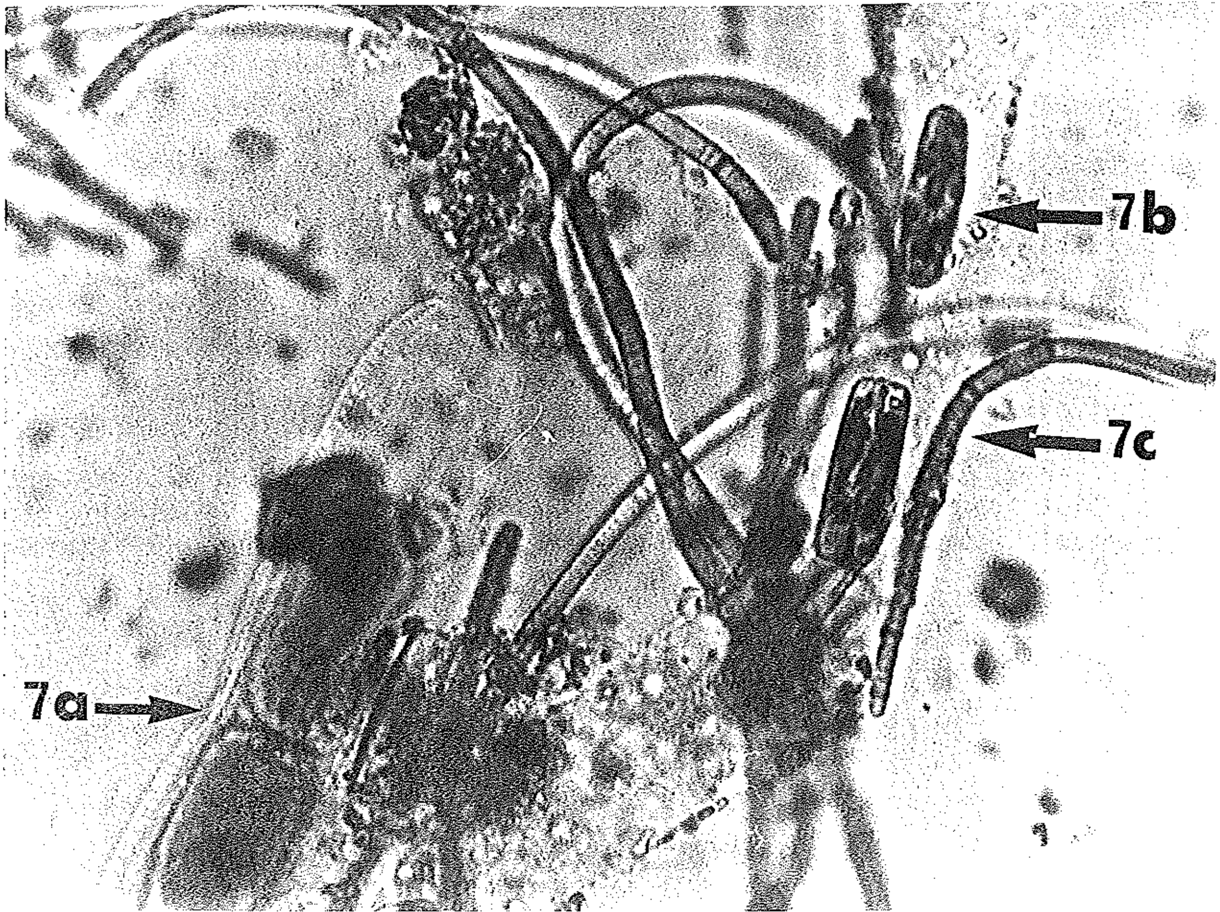
7a Motile diatom, Cymatopleura.

7b Diatom (Pinnularia sp.) motile only within a gelatinous tube which, itself in firmly attached to the substratum.

7c Filaments of Phormidium sp. ---- very slightly motile.

Plate 8

The same species of Phormidium (8d) as in Plate 7 but with a another motile diatom (Nitzschia sp. --- 8e).


7b
7c
7a

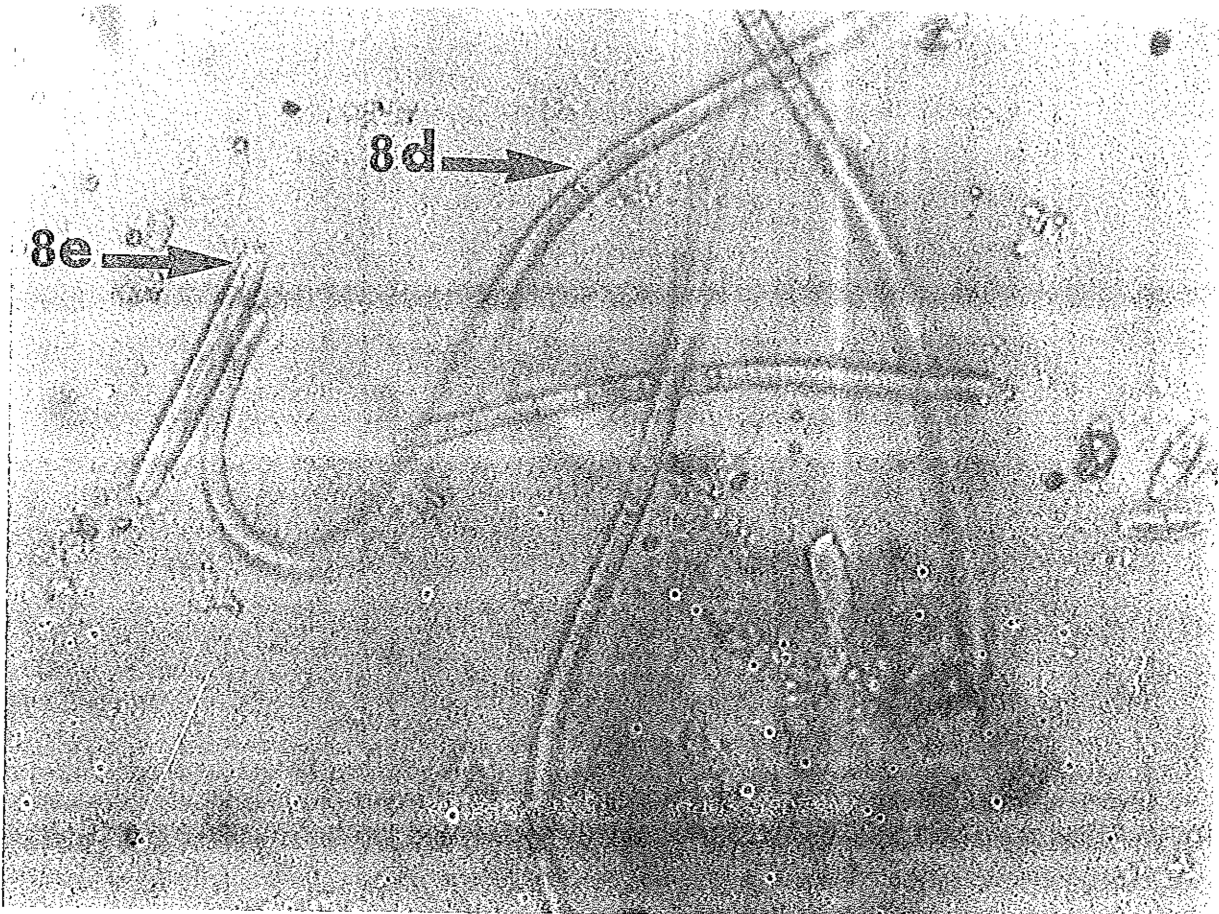

8d
8e

Plate 5

Centric diatom spores found in flocculent detrius. Thames water.

Plate 6

A small species of <u>Nitzschia</u> (Bacillariophyceae), adhering to but mobile on the surface of the coverslip.

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7a Motile diatom, *Cymatopleura*.

7b Diatom (*Pinnularia* sp.) motile only within a gelatinous tube which, itself in firmly attached to the substratum.

7c Filaments of *Phormidium* sp. ---- very slightly motile.

Plate 8

The same species of *Phormidium* (8d) as in Plate 7 but with a another motile diatom (*Nitzschia* sp. --- 8e).

7b
7c
7a
8d
8e