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Efficacy and Environmental Effects of Peracetic Acid as a Sewage Disinfectant

Consultants in Environmental Sciences Ltd

R&D Project Record 231/6/SW

NRA
National Rivers Authority

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Statement of Use
This document reviews laboratory and field studies on the use of peracetic acid (PAA) as a disinfectant for wastewater discharges. It will be used to update NRA policy on the regulation of disinfected wastewater discharges; and to inform NRA staff of the current state of knowledge of the efficacy and environmental impact of wastewater disinfection using PAA.

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R&D Project Record 231/6/SW

CONTENTS

LIST OF TABLES

PART A

PART B

LIST OF FIGURES

PART A

PART B

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EXECUTIVE SUMMARY

i) Introduction

Consultants in Environmental Sciences Ltd was commissioned by the National Rivers Authority to conduct a comprehensive review of the programme of largely independent laboratory and field work that has been carried out on the use of peracetic acid (PAA) as a disinfectant for sewage discharges. The economic aspects of PAA treatment were not included in the terms of reference, since they are now reasonably well defined.

The objectives of this Study were to review:

- the efficacy of PAA as a sewage disinfectant; and
- the environmental effects of the use of PAA.

Evidence has been reviewed from eleven field trials and published literature.

PAA for use in sewage disinfection is supplied by Interox Chemicals Ltd as an equilibrium mixture of peracetic acid, acetic acid, hydrogen peroxide and water, marketed under the product name of *Oxymaster*. As PAA itself is consumed in reaction the equilibrium tends to shift generating more PAA and acetic acid, and depleting the peroxide.

ii) The Efficacy of PAA as a Sewage Disinfectant

The principal points to emerge concerning the efficacy of PAA as a sewage disinfectant for sewage are listed below.

Response of microorganisms

PAA can be effective against the most common indicator bacteria (thermotolerant coliforms (TTC) and total coliforms (TC)) at suitable dose levels under appropriate conditions. Table 1 presents a summary of the field data for TTC. Considerable variability has been observed, both with time and between sites. No conclusive evidence is available to explain this variability, although it is probably associated to some extent with variations in composition of the sewage flow.

Faecal Streptococci (FS) are more resistant to PAA than are thermotolerant coliforms (TTC) or total coliforms (TC).

Reductions observed for pathogenic bacteria are highly variable and have ranged from no effect to 1.8 log reduction. The best effect was observed for one run at Menagwins STW, where the contact time was 4-5 hours. For short contact times (<10 minutes) reductions have generally been <1.0 log.

Table 1 **Summary of TTC Reduction Observed in PAA Field Trials**

Location	Effluent type	PAA dose (mg l^{-1})	Contact time	Mean log reduction
Clacton	Raw	10	6 mins	2.0
		15		2.2
		20		2.7
Plymouth	Raw	15	2 mins	1.2
Trevaunance Cove	Raw	12	<2 mins	1.6
		20		2.1
Southend	Primary	12	Not known	1.9
Menagwins	Unsettled bio filter	4	4 - 5 hours	1.5
		6		3.1
Porthtowan	Settled bio filter	4*	5 mins	2.3
		6*		3.6
Unknown (Baldry & French, 1989a)	Activated sludge	2	21 hours	1.85
		8		3.28
Bexhill	Simulated S/W	10	Not known	2.2
		25		2.2

* Overdosing may have occurred

The virucidal action of PAA appears variable and inconsistent. Table 2 presents a summary of the field work carried out to date. It is clear that PAA has not been demonstrated to be effective at normal dose levels against some important enteroviruses and has been shown not to be effective against Polio virus, except at very large doses (Table 2.6).

Laboratory work has shown that bacteriophages tested respond differently from viruses to PAA. Unless a phage can be found that more closely models the response of viruses, phages do not appear to be good indicators to assess virucidal ability.

Only one study at Southend in 1989 has looked at the impact of PAA disinfection on microbial levels in marine sediments. This showed that mean TTC levels increased slightly over the disinfection period, while FS levels showed a slight decrease. No clear spatial relationship between TTC or FS levels and the location of the outfall was observed.

The effect on levels of *E. coli* and F^+ Coliphage in bivalve shellfish was investigated at Trevaunance Cove in 1990. It was found that *E. coli* counts in the water around two rafts decreased by an average of 43% and 86%. The average levels in mussels decreased by 65% and 61% respectively. In contrast, analyses for F^+ Coliphage showed a small overall reduction at raft 2 and no reduction at raft 1.

Table 2 Summary of Virus and Coliphage Reduction Observed in PAA Field Trials

Location	Effluent type	Organisms	PAA dose (mg l^{-1})	Contact time	Mean log reduction
Clacton	Raw	Enterovirus Rotavirus	10 15 20	6 mins	No consistent effect
Trevaunance Cove	Raw	Enterovirus Rotavirus F^+ Coliphage	12 20	<2 mins	No consistent effect
Southend	Primary	Enterovirus Rotavirus	12	Not known	<0.3 Little effect
Menagwins	Unsettled bio filter	F^+ Coliphage	4 6	4 - 5 hours	Little effect 1.0
Porthtowan	Settled bio filter	Enterovirus Rotavirus F^+ Coliphage	6*	5 mins	No effect No effect >0.77+

* Overdosing may have occurred
+ Mean of results obtained

Operational considerations

Most of the reduction in TC and TTC numbers appears to occur in the first ten minutes of contact. Greater than 3 log reduction of Rotavirus has been demonstrated in the laboratory after 40 minutes contact (Table 2.4). Laboratory work on Polio virus showed PAA have little effect at a dose of 20 mg l^{-1} in biological filter effluent (0.08 log reduction after 30 mins.), but showed the same dose to be more effective in activated sludge and primary settled effluent (0.36 and 0.32 log reductions, respectively).

It has been shown that PAA efficacy is related to pH and organic quality. In general PAA has been shown to be most effective in environments that do not effectively buffer pH and do not contain appreciable levels of organic matter (Tables 1.1 and 2.6). In laboratory studies PAA has been shown to be more effective for better quality sewage effluents (Tables 1.3 and 1.4).

PAA is most effective against indicator micro-organisms in secondary effluent and moderately effective in settled sewage. Stormwater and untreated sewage appear most difficult to disinfect (e.g. Table 1.8). There is some evidence that solid particles may afford protection for micro-organisms against PAA.

Reactivation of bacteria, defined as immediate resuscitation, has been observed upon dilution of PAA-treated sewage in the laboratory. Reactivation of up to 86% of the pre-disinfection TTC population in biological filter effluent has been observed. The effect was less marked for primary settled and activated sludge effluents (0.04-15.0%), but could be significant if >1.0 log reduction was sought and a similar effect occurs in the field. No field evidence on reactivation of indicators is currently available, nor are any data for pathogenic organisms.

Regrowth of disinfected organisms is unlikely to occur when reasonable dilution is available, but could possibly occur in tidal tanks or long outfalls. This is difficult to assess, since the evidence comes from laboratory studies where PAA is neutralised and samples are then stored. In the field it appears that improved disinfection has been obtained after long storage times. This may be indicative that PAA, or a substance produced by PAA that is neutralised in the laboratory studies, is long lived and therefore effectively prevents regrowth, although evidence is extremely limited.

iii) The Environmental Effects of PAA

The following conclusions have been drawn from the limited information available on the environmental effects of PAA.

Chemical and toxicological effects

PAA treatment can cause a significant increase in the organic load contained in secondary effluents. The extent of the increase would probably render PAA unacceptable for use at most secondary treatment works subject to 20 mg l^{-1} BOD consent conditions.

The effect of PAA treatment on the level of organic matter in primary settled or untreated sewage is normally insignificant.

Trace impurities in *Oxymaster* are unlikely to cause significant environmental effects.

PAA appears to reduce the pH of effluents by up to 1.0 unit at normal dose rates. No systematic effect on ammonia-N or SS has been observed, except when PAA was dosed prior to the secondary settling tank at Menagwins, where PAA was observed to inhibit the settling process.

Few by-products resulting from PAA-treatment of sewage have been identified, despite numerous investigations. However, PAA has been shown to liberate bromine from bromide, and has been observed to give rise to relatively persistent bromamine compounds. To illustrate the likely worst case, at Porthtowan STW the highest levels of bromine and bromamine observed were 2.9 mg l^{-1} and 14.2 mg l^{-1} respectively. It should be noted that overdosing may have occurred in this case. These concentrations can be biocidal and could be environmentally significant close to the outfall. There may also be a potential for bioaccumulation of persistent organic brominated compounds. However, it should be stressed that these are worst case concentrations and the levels found in other trials at other times at Porthtowan have been much lower. The potential for bioaccumulation therefore seems more significant than the potential for acute effects.

No significant increase in the toxicity of sewage has been observed after PAA treatment in the field, except at Porthtowan where overdosing may have occurred. However, there is some evidence that PAA treatment may give rise to short-lived toxic compounds, but results from laboratory studies have not been entirely consistent.

No significant increase in mutagenic effects of sewage has been observed after PAA treatment, but evidence is limited.

Oxymaster residuals have been detected in environmentally significant concentrations in two studies where sensitive analytical techniques were employed. This may have been due to overdosing in one case and short contact times in the other. In other trials testing methods have not been sufficiently sensitive with detection limits of approximately 1 mg l^{-1}. The possibility that residuals were contained in the disinfected sewage in other trials at or below the detection limits cannot be ruled out.

Total heavy metal levels have been found to be generally unaffected by PAA treatment. However, a small increase in the soluble fraction of metals has been found to occur. This suggests that the metal content may become more bio-available after disinfection with PAA. However, the increase in the dissolved fraction has been shown to be small and it is considered that while this effect has environmental significance, the impacts are unlikely to be major compared with other factors.

Ecological effects

In two separate field trials, changes in the macrofaunal community were observed at times co-incident with the summer disinfection season, and correlated, to some extent, with proximity to the discharge. In the light of later work showing significant quantities of *Oxymaster* residuals in effluents, it may be speculated that this could possibly provide a mechanism for the effects observed.

No causal relationship was established between the use of PAA and the changes in community structure in either field trial.

iv) Final Discussion

This Study is an in-depth review of the available evidence concerning PAA as a sewage disinfectant. There is evidence that disinfection with PAA can be effective in helping to achieve the coliform standards of the EC Bathing Water Directive. However, it is concluded that PAA would probably not be suitable for inland and estuarine discharges subject to secondary treatment, due to the increase in organic load due to PAA-treatment.

The work at Porthtowan gives cause for some concern as it seems that either the dosing of *Oxymaster* was poorly controlled and gave rise to biologically significant quantities of PAA, hydrogen peroxide, bromine and bromamine in the effluent, or alternatively normal doses of *Oxymaster* gave rise to these compounds. This must be balanced with most other trials where levels of by-products and residual products were generally low and dosing control appears effective.

Studies at Porthtowan have also indicated that sewage may well have a finite capacity for reaction with *Oxymaster*. If this capacity is exceeded then residual oxidants may occur in the effluent. On the other hand, if the dosage is set too low it appears that effective disinfection may not occur. The difficulty of predicting the dose of PAA required to produce an effective reduction in micro-organism numbers and not produce significant residuals should not be underestimated; this dose is likely to vary both with time (and weather in combined systems) and between sites. Given the variability observed, field testing at individual sites for residual oxidants and effectiveness of disinfection should be regarded as a minimum requirement, prior to the installation of permanent equipment. If a fixed dose was to be applied then an appropriate dose rate could be determined by careful dosing trails, taking into account variations in quality of the sewage or effluent to be disinfected. The ideal would be to have a dosing system based on residuals monitoring, but it would be unwise to rely entirely on detection of peroxide, since the correlation between peroxide and PAA concentrations in disinfected sewage is not well established. It is understood that this avenue is currently being pursued by Interox.

In terms of disinfection of stormwater, the rapid quality variations experienced during the first flush would make dose control very difficult. It is considered that a fixed dose that did not produce environmentally significant levels of residual oxidants at any time during the storm would be unlikely to be particularly effective as a disinfectant during the first flush. A secondary point is that PAA treatment of dilute stormwater could increase the BOD significantly, although no direct evidence is available.

Taking account of both the efficacy and environmental effects data, it appears that after dosing with *Oxymaster* there are a considerable number of separate and independent observations that could be explained by the presence of a reasonably persistent disinfection agent. This appears, therefore, to be a likely explanation if the information is taken as a whole, although each individual observation could also be explained by alternative hypotheses.

It is considered that an up to date comparable review of all feasible alternative disinfection systems would be useful to enable selection of the most appropriate systems for each individual set of circumstances. It appears unlikely that any one system would emerge to be clearly superior in all areas of efficacy, environmental effects, operational simplicity, reliability and economics.

1 INTRODUCTION

1.1 Background

In recent years, UK research into disinfection techniques has been somewhat fragmented. Two reviews have been conducted, a number of field trials have been carried out around the country and a programme of laboratory studies has been undertaken. The commitment by the Secretary of State for the Environment to meet the European Community (EC) Bathing Water Directive standards by 1995 and the Report of the Environment Select Committee on Pollution of Beaches in 1990 has led to greater interest in the use of disinfection techniques for sewage effluents.

As direct detection of pathogens would be extremely difficult in practical situations, the EC Bathing Water Directive sets standards for indicator bacteria and viruses, the presence of which is taken to be related to the presence of pathogenic organisms. The Directive sets a mandatory zero standard for both Salmonellae and Enteroviruses, which is now commonly accepted to be impracticable and consequently has not been widely enforced. The status of the viral standards has led to a degree of confusion about the basis on which compliance with the Directive should be judged. It is presently understood that this aspect of the Directive may be subject to review and it is expected that there will be an amendment that will set a more practicable standard for viruses. It is therefore important that disinfection techniques are effective against both pathogenic and indicator bacteria and viruses.

The scale of the bathing waters compliance programme imposes a degree of urgency on identifying acceptable disinfection processes. Strict EC timetables do not allow consecutive scheduling of field trials and laboratory work, and therefore both have been carried out concurrently. The use of chemical disinfectants as a means of satisfying EC bathing water standards has the advantage of low initial capital cost and short installation time compared with the more traditional long sea outfall approach. In some cases use of disinfection has been seen as a temporary solution while an outfall scheme is being planned and constructed, but in others it has been advocated as a permanent solution to the problem. Most experience has been gained using chlorine, but concerns have been expressed regarding the potential environmental impact of by-products of chemical reactions involving chlorine, the efficacy of chlorine against viruses and the safety of some of the systems. Consequently, interest in other disinfectants is at a high level.

The potential of peracetic acid (PAA) as a disinfectant for sewage effluents has been recognised for some time, but concerns have been raised about the possible environmental effects of PAA residuals and the efficacy of the process for sewage effluents. A number of studies have been carried out by several organisations, and field trials have been conducted at various locations since the last thorough review of this technique. Consequently, Consultants in Environmental Sciences Ltd (CES) was commissioned by the National Rivers Authority (NRA) in April 1991 to carry out a review of the available information on PAA.

1.2 Terms of Reference

This section summarises the full terms of reference. The objectives of the Study were as follows:

- to establish the efficacy of PAA as a disinfectant for sewage in respect of pathogenic and indicator organisms; and

- to establish the environmental impact of discharging PAA-dosed treated and untreated sewage into the riverine and marine environment.

These objectives were to be met by a desk study of literature published largely between 1988 and 1991 by the leading manufacturer of PAA as a disinfectant (Interox Chemicals Ltd), consultants, water companies, NRA and Water Research Centre (WRc) and by holding meetings where appropriate.

1.3 Report Structure

This Report presents basic information in the first two Chapters and is then divided into two Parts: Part A deals with the efficacy of PAA as a disinfectant for both raw and treated sewage in respect of indicator and pathogenic organisms; Part B is concerned with the environmental impact of discharging PAA-dosed effluents into the riverine and marine environment. Each Part is presented as a complete assessment containing a full literature review and conclusions, and is designed to stand in isolation from the other Part. In addition, an executive summary is presented immediately before this Chapter and gives an overall summary of the key facts and the main conclusions.

1.4 Nature of Conclusions

Biological evidence, particularly from field trials, is often equivocal due to the difficulty (or impossibility in the case of field trials) of controlling confounding variables. This is often also the case with laboratory studies when sewage effluents of a variable nature are involved. Consequently, it is difficult to draw many conclusions that could be said to be proven 'beyond reasonable doubt', given the evidence available, but it is possible to put forward more conclusions on a 'balance of probability' basis. As a corollary, a few hypotheses can now be judged to be falsified 'beyond reasonable doubt' on the latest evidence, while a greater number can now be categorised as 'unlikely'.

1.5 Definitions

A list of abbreviations used throughout the Report is presented below.

Abbreviation	Meaning
AOX	Adsorbable Organic Halogens
BOD	Biochemical Oxygen Demand
CES	Consultants in Environmental Sciences Ltd
CFU	Colony Forming Units
COD	Chemical Oxygen Demand
DWF	Dry Weather Flow
EC	European Community
E. coli	*Escherichia coli*
FDA	Federal Drug Administration
FS	Faecal Streptocci
GC/MS	Gas Chromatography/Mass Spectroscopy
NRA	National Rivers Authority
MEC	Marine Environmental Consultants
PAA	Peracetic Acid
PFU	Plaque Forming Units
SS	Suspended Solids
STW	Sewage Treatment Works
TC	Total Coliforms
TEBP	Thames Estuarine Benthic Programme
TOC	Total Organic Carbon
TTC	Thermotolerant Coliforms
WRc	Water Research Centre

2 BASIC INFORMATION

2.1 Composition and Chemistry of PAA

2.1.1 Nomenclature

Organic peroxycarboxylic acids have the general structure $R(CO_3H)_n$, where R is an alkyl, cycloalkyl or heterocyclic group and n = 1 or 2. These acids are named by prefixing the name of the parent carboxylic acid with either "peroxy" or "per". For example, CH_3CO_3H is known as either peroxyacetic acid or peracetic acid (PAA). In the widely accepted IUPAC nomenclature the systematic name ethanoic acid should be used in preference to acetic acid and hence PAA may also be known as peroxyethanoic or perethanoic acid.

2.1.2 General Properties of PAA.

PAA has a sharp, unpleasant odour and is irritating to the skin and mucous membranes. It is more water soluble than acetic acid, but is a weaker acid, due to the lack of a resonance structure in the anion, and the ease of intramolecular hydrogen bonding. PAA is miscible with water, ethyl acetate, chloroform and other halogenated solvents, acetone, acetic acid and those hydrocarbons which it does not oxidise. Peroxyacids are the most powerful oxidising agents of all organic peroxides and are used extensively in organic chemistry for specific oxidation reactions. Lower activation energies and accelerated oxidation rates occur in polar solvents (Swern, 1970). Reactions of PAA, given suitable conditions, include:

- epoxidation and hydroxylation of olefins;
- oxidation of sulphides to sulphoxides/sulphones;
- oxidation of disulphides to thiosulphinates;
- oxidation of amines to nitroso- and nitro--compounds; and
- oxidation of aldehydes to carboxylic acids.

The use of PAA in synthetic organic chemistry is generally under conditions of elevated temperature and in non-aqueous solvents to promote optimum yields. The literature details very little of the chemistry of PAA in dilute aqueous solution at ambient temperatures.

PAA is rapidly hydrolysed in sodium hydroxide solution to aqueous sodium acetate and hydrogen peroxide. It is reported that manganese, copper, iron and cobalt salts can all catalyse the decomposition of PAA (Swern, 1970).

2.1.3 Composition

PAA for sewage disinfection is supplied by Interox Chemicals Ltd in the form of a product called *Oxymaster*, that has a typical composition by weight as detailed below:

PAA	12-13%
Hydrogen peroxide	19-20%
Sulphuric acid approx.	1%
Acetic acid approx.	18%
Water approx.	49%

As PAA is an equilibrium mixture, if a constituent on one side of the equilibrium is consumed by a separate reaction, the equilibrium will re-establish to replenish that constituent. Therefore, if PAA is used up rapidly, more PAA will be generated by the reaction of hydrogen peroxide and acetic acid. Acetic acid is also an end product of reactions in which PAA is used as an oxidant. This will tend to reinforce the shift in the equilibrium. This means that a PAA dose stated in terms of the initial concentration in the mixture will tend to under-estimate the dose of PAA actually delivered. However, this is regarded as the best objective measure of PAA dose applied, since the actual dose will be dependent on the rate of direct reaction of hydrogen peroxide with the sewage.

It is clear that however the reaction proceeds, acetic acid is an end product. It is calculated that 1 mg l^{-1} of initial PAA dose will lead to a total residual acetic acid concentration of 2.3 mg l^{-1} acetic acid (Baldry *et al.*, 1990).

2.2 Field Trials

Sites at which PAA trials have been, are being or will be undertaken are summarised in Table 2.1 and discussed below. Those at which ecological aspects have been taken into account are indicated.

Table 2.1 Locations of PAA trials in UK

Date	Site	Ecological Study	Effluent Type	DWF $m^3 d^{-1}$
1977-1979	Bishopston	None	Activated Sludge	Not known
Not known	Weston-Super-Mare	None	Not known	Not known
1987	Clacton-on-Sea	None	Screened	Not known
1987-	Plymouth	None	Untreated	18,000
1988	Bexhill-on-Sea	None	Simulated Stormwater	N/A
1989-	St. Austell (Menagwins)	Freshwater	Unsettled Biological Filter	13,000
1989-	Southend-on-Sea	Estuarine /Marine	Primary	40,000
1990-	Trearddur Bay	Marine	Untreated	1,500
1990-	Mablethorpe	Marine	Secondary	Not known
1990-	Porthtowan	None	Secondary (Biological Filter + Sedimentation)	520
1990-	Trevaunance Cove	Marine	Screened	430

2.2.1 Bishopston

Trials were undertaken in 1979 to assess the viability of PAA and sodium hypochlorite treatments for effluent from an activated sludge plant discharged directly to the sea on a rocky shore on the Gower Peninsular.

Doses of approximately 4 mg l^{-1} PAA and 3 mg l^{-1} Cl_2 were compared in order to achieve a 3 log reduction in *E. coli* counts. This was designed to protect local shellfisheries. The efficacy of the PAA treatment in reducing *E. coli* counts was recorded. Both disinfectant treatments were rejected as viable options. Although effective, PAA treatment was assessed to be too expensive. It was also suggested that the potential formation of epoxylated compounds might have undesirable effects on marine communities. This was not substantiated by experimental findings.

2.2.2 Weston-Super-Mare

Trials were designed to assess the efficacy of PAA in combination with hypochlorite and as sole disinfectant to meet water quality standards during the holiday season. An environmental impact assessment was not undertaken, although studies of organochlorine residues were made using fish, gastropods (including *Patella vulgata*) and seaweeds (*Ascophyllum nodosum*) for existing chlorine treatments. The PAA treatment option was subsequently discarded on financial constraints.

2.2.3 Clacton-on-Sea

A study of PAA disinfection was undertaken in November and December 1987 by Anglian Water Authority in conjunction with Interox and WRc. Sewage is discharged into the sea from Clacton-on-Sea through a short outfall after screening and maceration. Experiments were carried out in 1987 and 1988 to determine the effect of PAA dosing on bacterial counts in the adjacent bathing areas. The results have been reported in Gould and Harrington (1988), Thomas *et al.* (1990) and Gould and Fraser (1990).

During the trials PAA was dosed at varying rates to produce concentrations of 10, 15, 20 and 25 mg l^{-1}. The periods of disinfection were preceded by an eight day period of undisinfected conditions.

2.2.4 Plymouth

Dosing of raw sewage at levels of between 15 and 20 mg l^{-1} PAA was undertaken by South West Water Authority and Interox. The Rusty Anchor Outfall discharges a Dry Weather Flow of approximately 18,000 m^3 per day of untreated sewage through a short outfall 125 m into Plymouth Sound. The discharge is intermittent, occurring for 3 hours only on the ebb tide.

Field trials were conducted during September 1988 to measure the effectiveness of PAA in reducing the bacterial concentrations in the plume from the outfall and in Plymouth Sound. Four different doses of 5, 10, 15 and 20 mg l^{-1} PAA were used. The results are reported in Atkinson *et al.* (1989). Although an extensive microbiological sampling programme was instituted, no data were collected on the region's marine communities.

2.2.5 Bexhill-on-Sea

During October 1988 a short trial was conducted by WRc and Interox in conjunction with Southern Water Authority to investigate PAA disinfection of stormwater from a Hydro-dynamic Separator (Hydro-Research and Development Ltd.), designed to remove gross solids and return them to a foul sewer. Storm conditions were simulated by blocking the normal channel causing crude sewage to enter the separator. In the second half of the trials, mains water was added to the sewage in order to dilute it. *Oxymaster* was added to the overflow after the separators in doses of 10, 15 and 25 mg l^{-1} PAA. Problems were encountered in blocking the separators and in obtaining sufficient water to dilute the sewage.

2.2.6 St. Austell (Menagwins)

A large scale trial was conducted at Menagwins STW during the summer of 1989. The works provides secondary treatment using percolating filters and has a Dry Weather Flow (DWF) of 13,000 m^3 day^{-1} during the summer. Flows up to 225 l s^{-1} (19,000 m^3 day^{-1}) receive full treatment. PAA was dosed into the final humus tanks and investigations of basic chemistry, ecology and efficacy of disinfection for various micro-organisms were undertaken.

2.2.7 Southend-on-Sea

A dry weather flow of 40,000 m^3 day^{-1} receives primary treatment before discharge. During the bathing seasons in 1989 and 1990, *Oxymaster* was dosed into the outflow from the plant to give a concentration of 12 mg l^{-1}. Studies of efficacy of disinfection for various micro-organisms and the effect of PAA dosing were carried out. The results of the trials are reported variously in Anglian Water (1989), MEC (1989), Gould (1989), Anglian Water (1990) and Joslin & Gould (1991).

2.2.8 Trearddur Bay

The outfall at Porth Gwr Mawr discharges a Dry Weather Flow of approximately 1,500 m^3 day^{-1} of untreated sewage per day into the Trearddur Bay approximately 100 m below Mean Low Water Mark. The Bay also receives septic tank effluent from a caravan site. As a result of these discharges there is an aesthetic problem and beaches in Trearddur Bay have occasionally failed to comply with the EC Bathing Directive.

Experiments were carried out in 1990-91 by Wallace Evans Ltd. to assess the impact of PAA (Wallace Evans Ltd., 1991), prior to full scale implementation.

2.2.9 Mablethorpe

Secondary effluent from Mablethorpe STW is discharged into a fresh water drain prior to discharge to the sea. During the summer months the population of the town increases and problems are experienced in the area with regard to the compliance with EC Bathing Water Directive requirements. In the summer of 1990 the works effluent was disinfected with PAA and investigations undertaken regarding efficacy of disinfection for *E. coli* and ecological effects of the discharge.

2.2.10 Porthtowan

Porthtowan STW discharges secondary effluent into a small river that then flows to the sea. The estimated resident population is 1,700 rising to 2,900 in the summer. The average DWF in winter is 302 m^3 day^{-1} and in summer is 520 m^3 day^{-1}. The site was chosen to enable more complete assessment of the results of the Menagwins trial. Extensive chemical and micro-biological work was carried out by WRc (Roddie *et al.*, 1991a).

2.2.11 Trevaunance Cove

The outfall at Trevaunance Cove discharges fine-screened sewage from the small town of St Agnes. The population in winter is 2,400 giving rise to an average DWF of 430.4 m^3 day^{-1}. In summer the population rises to 4,000 as a result of visitors. The effluent discharges directly to the sea close to a small beach through an outfall of approximately 25m in length. Extensive chemical and micro-biological work was undertaken by WRc and some work was also conducted on ecology and ecotoxicology (Roddie *et al.*, 1991b).

Part A

The Efficacy of PAA as a Sewage Disinfectant

1. BACTERIAL DISINFECTION

1.1 Introduction

Sewage effluents contain a range of pathogenic bacteria and viruses, the numbers of which are liable to vary widely, depending on the spatial and temporal patterns of diseases in the community. Micro-organisms in the digestive tract may be divided into those which are ubiquitous and cause no harm to the host and those which cause illness. The former type are often known as indicator organisms, since they may be used to indicate the presence of sewage contamination, while the latter are termed pathogenic. Pathogenic organisms are difficult to enumerate accurately because of sampling and methodological problems, hence indicator bacteria are widely used in wastewater and water analyses. The principal indicator bacteria used in the studies reviewed here are Thermotolerant coliforms (TTC), and the literature also refers to Faecal coliforms or *Escherichia coli*, Total coliforms (TC) and Faecal Streptococci (FS).

The majority of papers and reports on PAA quote data for one or more of these organisms, and the following sections attempt to summarize the most important and recent of these. It should be noted that for consistency and comparability most reductions quoted have been converted to log reductions. In cases where the mean reduction is quoted as a percentage, the log is taken of this figure to give what is termed 'arithmetic mean log reduction'. In contrast when the mean quoted is the mean of the individual log reductions, this is quoted as geometric mean log reduction or simply mean log reduction.

1.2 Thermotolerant Coliforms (TTC)

These gram-negative organisms are the most widely accepted of all the indicators. Much of the initial work on susceptibility of TTC to PAA has been undertaken by Baldry and others at Interox (Baldry & French, 1989a, 1989b, 1989c; Baldry *et al.*, 1990; Baldry *et al.*, 1991).

1.2.1 Factors Affecting Performance

A number of factors affecting the performance of PAA have been investigated as follows:

- pH;
- time of contact;
- presence of organic matter; and
- PAA dose.

a) **Effect of pH.** In a series of small scale laboratory trials (usually in 10 ml total volume) Baldry and others quantify the minimum concentrations of PAA required to give >5 log (99.999%) reduction of TTC and FS and >4 log (99.99%) reduction of phages in 5 minutes. These results are summarized in the Table 1.1, which illustrates that TTC reduction is optimum at pH 7, in contrast to FS and bacteriophages which are more susceptible to PAA at pH 5.

This table also shows that organic matter in the form of 4 g l^{-1} yeast extract exerts a protective effect on TTC, requiring approximately 5 times more PAA to achieve the same percentage kill.

Table 1.1 Concentration of PAA to pass a standardized laboratory test

	Conc. of PAA (mg l^{-1}) to achieve 5 log reduction of bacteria and 4 log reduction of bacteriophage within 5 mins.				
	DMW	pH 5	pH 7	pH 9	4 g l^{-1} Yeast Extract
E.coli	10-15	20-25	10-15	100-150	50-75
S.faecalis	10-25	10-15	75-100	500-1000	75-100
MS 2 phage	12-15	11-15	30-53	225-300	75-94
ØX174 phage	23-30	15-23	53-75	525-750	94-113

DMW = Demineralised Water Source: Baldry *et al.* (1990)

b) <u>Effect of time of contact</u>. Inactivation of TTC and other organisms in experimental systems is usually found to be most rapid in the first 10-20 minutes of contact, with little or no further inactivation after 30 minutes contact. Table 1.2 demonstrates this well for TTC in secondary sewage effluents.

Similar results which illustrate the biphasic nature of TTC inactivation are given in Thomas *et al.* (1990). These demonstrate that for 5, 10 and 15 mg l^{-1} doses of PAA to secondary effluent the majority of the reduction of TTC numbers occurs within the first 5 minutes, with very little further inactivation after 20 minutes contact (Figure 1.1). Irving (1990) and Crathorne *et al.* (1991) reiterate this finding in subsequent laboratory studies.

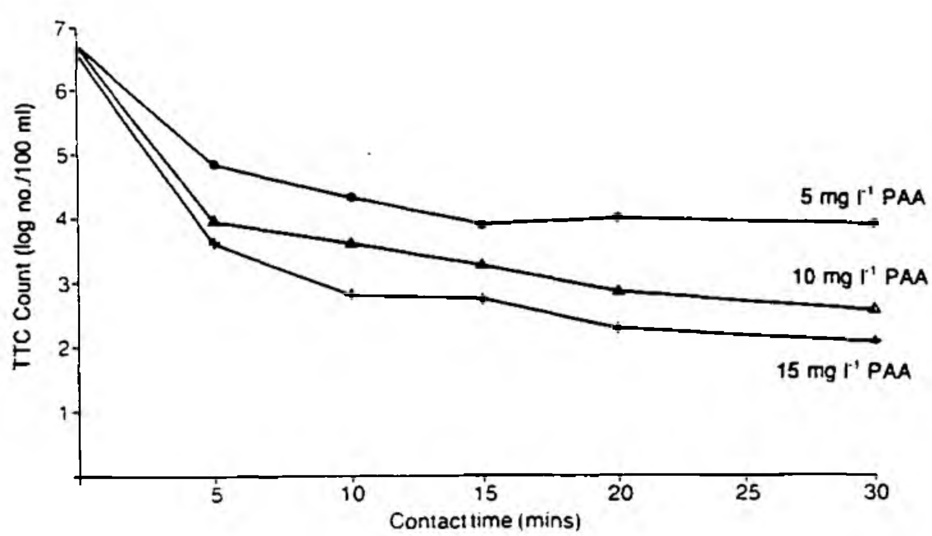


Figure 1.1 Disinfection of secondary effluent using *Oxymaster* (Thomas *et al.* (1990))

Table 1.2 Percentage survival of TTC in secondary effluent

Time (min)	PAA concentration (mg l^{-1}) 1	2	3	4	5
0	100	100	100	100	100
0.25	80	46	-	114	10
1	87	67	113	57	<0.5
2	54	-	52	4	<0.5
3	64	56	8	1	<0.5
4	67	55	6	1	<0.5
5	63	17	4	<0.5	<0.5
7	23	11	<0.05	<0.5	-
10	11	1	-	<0.5	-
15	0.3	0.4	-	<0.5	-
20	-	-	-	<0.5	-
N	$1.5x10^6$	$1.5x10^6$	$1.5x10^6$	$2.1x10^5$	$1.8x10^5$

N (cfu/100 ml) = Initial Concentration of TTC Source: Baldry and French (1989)

cfu = colony forming unit

c) <u>Effect of organic matter</u>. Despite occasional reports to the contrary (e.g. Harakeh, 1987) most of the investigations have indicated that PAA reacts with organic matter in the suspending media to some extent. Therefore higher doses of PAA and longer contact times are needed to achieve equivalent disinfection of bacteria in raw sewage than in treated effluents. This point is well illustrated in Table 1.3.

It should be noted that the bacterial levels mentioned in Table 1.3 are unusually low for raw sewage and levels of 10^7/100 ml would be more usual for TTC in UK sewage. The time/concentration combinations in Table 1.3 show a wide variation to achieve 3 log reduction in TTC count. In the case of unmacerated sewage they range from 5 minutes at 10 mg l^{-1} up to 120 minutes at 20 mg l^{-1}.

Table 1.3 Minimum conditions to achieve a 3 log reduction in bacterial numbers

Site	Type of discharge	Pre-disinfection TTC count (cfu/100 ml)	Minimum PAA conc (mg l^{-1}) to achieve a 3 log TTC reduction	Minimum contact time (minutes)
1	Raw	5.2×10^5	15	120
2	Raw	3.7×10^4	10	5
3	Raw	2.4×10^5	20	120
4	Raw	3.6×10^5	10	12
5	Macerated	9.5×10^4	10	5
6	Macerated	2.3×10^5	10	15
7	Macerated	9.8×10^4	10	15
8	Macerated	1.2×10^5	10	5
9	Macerated	9.5×10^4	10	5
10	Settled	1.0×10^5	2	50
11	Secondary (Act.S)	4.2×10^4	15	5
12	Secondary (Act.S)	3.0×10^5	1	5
13	Secondary (Act.S)	1.5×10^3	2	5
		Pre-disinfection Count of FS	Minimum conc. for 3 log FS reduction	Minimum contact time (minutes)
12	Secondary (Act.S)	1.5×10^5	10	5
13	Secondary (Act.S)	1.5×10^2	6	5

Source: Baldry and French (1989b)

Much lower concentrations of PAA and shorter times of contact are needed to produce a 3 log reduction of TTC in secondary effluent than raw sewage. Relatively few reports contain enough data on BOD, COD, TOC or SS concentrations in the effluents to be able to quantify the effect of organic matter, but Thomas *et al.* (1990) in laboratory tests dosing 10, 15 and 20 mg l^{-1} PAA for 5 minutes clearly demonstrated the poorer inactivation of TTC in stronger sewage as demonstrated in Table 1.4.

Table 1.4 Laboratory experiments showing effects of sewage strength on PAA effectiveness against bacteria

PAA dose ($mg\ l^{-1}$) 5 min contact	Arithmetic mean log reduction in TTC	Arithmetic mean log reduction in FS
Av. BOD = 196 $mg\ l^{-1}$ Av. SS = 237 $mg\ l^{-1}$	Weak Sewage	
10	0.96	0.17
15	1.42	0.91
20	2.74	1.75
Av. BOD = 301 $mg\ l^{-1}$ Av. SS = 233 $mg\ l^{-1}$	Strong Sewage	
10	0	0.14
15	0.13	0.13
20	0.14	0.10

Source: Thomas *et al.* (1990)

However, when the dose of PAA was increased to 30-40 $mg\ l^{-1}$ PAA higher levels of inactivation occurred in the case of the sewage of higher BOD, suggesting that the 'PAA demand' of sewage could be satisfied.

d) Effect of PAA dose. It would be expected that for any one sewage sample, increasing PAA dose would lead to increased TTC inactivation, as demonstrated for crude sewage in Figure 1.2.

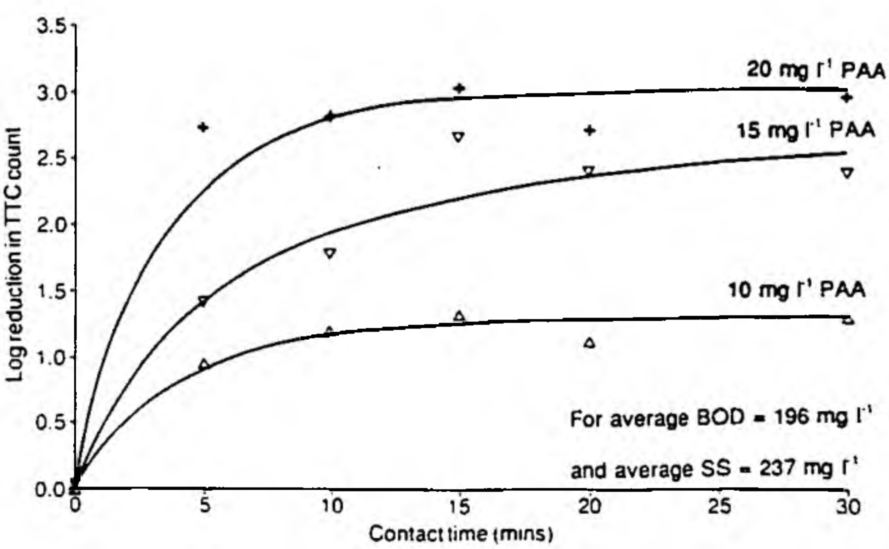


Figure 1.2 Disinfection of crude sewage (Thomas *et al.*, 1990)

1.2.2 Field Trials

Statistical analysis of field data from PAA disinfection trials at Southend sewage treatment works do indicate that reduction of TTC (and FS) in settled sewage are very dependent on PAA dose. Table 1.5 presents the significance levels of the effect on the log reduction factor of the dose, date and organism weighted mean value in the outflow. This shows that the reduction factors are dependent on dose and not other factors.

Table 1.5 Effects of variables on log reduction factors in Southend field trials

	Significance level for variables specified		
Variable	TC	TTC	FS
Dose		0.0001 (very dependent)	0.0001 (very dependent)
Date (includes effect of dosing)	0.12 (no significance)	0.001 (very dependent)	0.035 (little dependence)
Organism weighted mean value in outflow	0.07 (no real dependence)	0.024 (no real dependence)	0.044 (no real dependence)

Source: Anglian Water (1989)

In these trials with 12 mg l^{-1} PAA the overall reduction of TTC was usually of the order of 1.92 log, compared with 1.75 log for TC and 0.41 log for FS. These reductions were poorer than expected, possibly due to an increase in sewage strength because of dry weather over the trial period. Further trials in the 1990 bathing season at Southend gave 2 log TTC reductions for two short periods and very varied performance at other times (Anglian Water, 1991). Appendix A1.1 presents further details of this field trial.

Disinfection trials at Clacton (Appendix A1.2) also examined the effectiveness of different PAA doses in inactivating TTC and FS in raw sewage, although statistical analysis of the results was not attempted. As Table 1.6 shows, the 'worst' percentage reductions showed correlations with the PAA dose, but the 'best' reductions were virtually the same for each dose.

Table 1.6 **Reduction of TTC and FS in raw sewage by PAA in Clacton field trials (contact time ≈ 6 mins.)**

PAA dose (mg l^{-1})	Log reduction in TTC			Log reduction in FS		
	Best	Worst	Arithmetic Mean	Best	Worst	Arithmetic Mean
20	5.0	1.5	2.7	4.5	0.3	1.4
15	5.0	1.3	2.2	4.4	0.1*	0.8*
10	4.0	1.0	2.0	4.3	0.1+	0.6*

* One value (zero reduction) excluded
+ Three values (zero reduction) excluded

Source: Gould and Harrington (1988)

The Trevaunance Cove field trial on screened sewage showed log reductions in TTC from 1.6 to 2.1 with a wide standard deviation for dose rates from 12-20 mg l^{-1} PAA (Roddie *et al.*, 1991b). However, it was noted that the contact time was below 2 minutes for much of the sewage. This may have contributed to the relatively poor performance observed.

Other field studies investigated the effectiveness of TTC removal from secondary effluents. Baldry and French (1989a) reported that for a small percolating filter plant, a 2 mg l^{-1} dose of PAA into the humus tank effluent, discharged to a lagoon with 14.5 hours retention, led to enhanced TTC removal (1.3 log compared with 0.46 log in the absence of PAA). In a similar system for an activated sludge plant discharging to a lagoon with 21 hours retention, 2 mg l^{-1} PAA reduced TTC levels by 1.85 log. Increasing the dose to 8 mg l^{-1} reduced TTC levels by 3.28 log.

Similar reductions in TTC were found using 6 mg l^{-1} PAA for unsettled final effluent treatment at Menagwins sewage treatment plant (Realey & Brogden, 1990). They reported 2.2-3.7 log reduction of TTC after 4-5 hours contact, with the target 3 log reduction generally being achieved. However, on some occasions no reduction was recorded. At Porthtowan settled final effluent was disinfected using dose rates ranging from 4-6 mg l^{-1} PAA. Log reductions observed ranged from 2.5->4 with a contact time of around 5 minutes (Roddie *et al*, 1991a). The reductions achieved were reported to be variable, but were always greater than 2.5 log for the higher dose and greater than 1.5 log for the lower dose.

Finally field trials of simulated stormwater at Bexhill investigated the addition of 10, 15 and 25 mg l^{-1} PAA and reported 2-3 log reductions of TTC (Realey, 1989).

1.2.3 Laboratory Studies

Recent reports by WRc have summarized laboratory data on the efficiency of PAA for disinfection of crude sewage, primary settled sewage, activated sludge effluent, biological filter effluent and storm water. The experiments with crude sewage (Irving, 1990) indicated that 10 mg l^{-1} PAA gave 2-2.5 log reductions for TTC and 20 mg l^{-1} PAA gave 3-4 log reductions, usually within the first 15 minutes of contact. Details were not supplied on the chemical characteristics of the sewage. In parallel experiments on the same crude sewage chlorine doses of 10 mg l^{-1} gave 5-6 log reduction of both TTC and TC.

Samples of 3 different sewages in the Trearddur Bay area have also been treated with 10, 12 and 15 mg l^{-1} PAA and bacterial reduction monitored for up to 40 minutes in the laboratory. It was found that 2 log reductions of TTC occurred after 5 minutes, with slightly longer times required for similar reductions of TC (Wallace Evans Ltd., 1990).

PAA doses were also applied to primary settled sewage and secondary effluents, as summarized in Table 1.7 below (Crathorne *et al.*, 1991). The table indicates 2-3 log reductions for TTC at 10 mg l^{-1} PAA, and 3-4 log reductions at 20 mg l^{-1} PAA. The data indicate similar performance for the settled sewage and secondary effluents, which is unexpected, and possibly attributable to the apparent poor quality of secondary effluents (BOD 195 and 253 mg l^{-1} and SS 67 and 101 mg l^{-1})

Table 1.7 Inactivation of TTC and FS by PAA
(Figures are log reductions after 30 mins. contact)

	PAA dose applied mg l^{-1}	Log Reduction	
		Geometric Mean	Range
a) Activated Sludge eff.			
TTC	10	2.6	1.7-3.4
	20	4.1	4.0-4.3
FS	10	0.51	0.2-0.9
	20	1.8	-
b) Biological Filter eff.			
TTC	10	3.2	2.4-3.6
	20	3.7	3.2-4.2
FS	10	1.6	1.0-3.2
	20	3.8	3.1-4.3
c) Primary Settled Sewage			
TTC	10	3.3	2.4-4.0
	20	4.0	3.4-4.7
FS	10	1.6	1.0-2.0
	20	4.4	4.2-4.5

Source: Crathorne *et al.* (1991)

In a laboratory evaluation of stormwater disinfection (Brogden and Realey, 1991), reductions of TTC and FS were measured in Menagwins and Stevenage sewage at 5, 10 and 15 mg l^{-1} PAA doses. Crude sewage was diluted to give a range of simulated stormwater conditions, and bacterial counts determined after 5 minutes contact. Results are summarized in Table 1.8 for TTC and show that neither 5 nor 10 mg l^{-1} PAA could achieve a 3 log reduction of TTC under these conditions, but at 15 mg l^{-1} PAA a 3 log reduction of TTC was achieved for Menagwins sewage at 3 DWF and above. The same dose was rather less effective for Stevenage sewage, 3 log reductions being achieved only at 6 DWF and above. This may be as a result of the higher BOD (300-400 mg l^{-1}).

Table 1.8 Log reductions in TTC in simulated stormwater (5 mins. contact time)

Site	PAA dose mg l^{-1}	Log Reduction 1DWF	3DWF	6DWF	12DWF	18DWF
Menagwins	5	0.32	1.08	1.61	0.94	1.52
	10	1.86	2.62	2.70	2.57	2.53
	15	1.63	3.04	3.93	3.14	3.34
Stevenage (June)	5	0.07	-0.02	0.11	0.59	1.43
(Runs 1 + 2	10	0.18	0.03	1.14	2.67	3.45
Averaged)	15	0.29	-0.04	2.97	4.24	4.70
Stevenage (Nov.)	5	0.69	2.24	1.95	3.14	2.28
(Run 3)	10	1.89	2.03	1.67	1.88	1.72

Source: Brogden and Realey (1991)

1.2.4 TTC in marine sediments

All the studies referred to above have been concerned with reduction of TTC by PAA in sewage effluents of various types. Relevant bathing water TTC counts are reviewed in Appendix A1. Only one investigation has been carried out to determine whether PAA treatment of effluents has any effect on the marine sediments near to an outfall. Joslin and Gould (1991) reported data from a survey carried out in 1989 in which TTC and FS counts were made in sediments before and after PAA dosing at Southend on Sea. The results showed no clear cut spatial relationship with the outfall source. Mean TTC results showed a slight increase over the period of PAA dosing, while FS results showed a slight decrease. No clear trend was observed, and these investigations were not continued in 1990.

1.3 Total Coliforms (TC)

The majority of reports reviewed do not have any data on the response of TC to PAA, most concentrating on TTC and FS. However, the EC Bathing Water Directive specifies maxima for TC, and these counts are made weekly throughout the bathing season.

Such evidence as is available indicates that TC are slightly more resistant to PAA than TTC. The 1989 field trials at Southend (Anglian Water, 1989) demonstrated a 1.75 log reduction for TC, while under the same conditions the TTC reduction was 1.92 log at the plant.

At various sites in Plymouth, Atkinson *et al.* (1990) found that a 20 mg l^{-1} dose of PAA into raw sewage produced reductions of between 0.62 to 1.30 log for TC and between 0.39 to 2.00 log for FC. However, reductions in TTC in bathing waters was measured to be between 0.28 and 0.59, possibly due to other discharges or some degree of reactivation or regrowth.

Laboratory studies of dosing PAA to crude sewages in the Trearddur Bay area (Wallace Evans Ltd 1990) also showed that the TC were very slightly more resistant to inactivation. Generally TC counts are about 1 log higher than TTC counts in raw sewage samples and this differential tends to be maintained in PAA-treated samples.

A series of experiments by Irving (1990) followed the inactivation of TC, TTC and FS in the presence of PAA and the relative resistance of the three types of organisms is illustrated in Figure 1.3. TC are usually found to be intermediate in resistance to PAA, with TTC more sensitive to PAA and FS more resistant.

The Irving study (1990) also demonstrated clearly the tendency for coliform organisms to regrow following disinfection with PAA. Figure 1.4 illustrates that the coliform count increased to more than the initial count 24 hours after disinfection with 10 mg l^{-1} PAA, and for 20 mg l^{-1} PAA the 24-hour count was even higher. However, this tendency to regrow was greatly reduced if the treated sewage was diluted tenfold in sea water.

1.4 Faecal Streptococci (FS)

1.4.1 Introduction

Faecal Streptococci are gram-positive organisms which are known to be generally more resistant than TTC to PAA. Indeed, results indicate that FS disinfection is variable, poor or even absent when effluents are treated with PAA (Tables 1.6 and 1.7). Although the EC Bathing Water Directive only requires that FS are enumerated when the water quality is suspected of deterioration, FS are often enumerated in bathing water monitoring programmes. FS are much more tolerant of high salinity than TTC or TC.

1.4.2 Laboratory Studies

From the data of Baldry *et al.* (1990) (Table 1.1) it would appear that pH 5 is optimal for FS inactivation, in common with bacteriophage and virus inactivation. FS inactivation also appears to be affected by the organic matter present in the effluent as shown in Table 1.4 (Thomas *et al.*, 1990). A typical time course of FS inactivation is shown in Figure 1.3 (Irving, 1990), with reduction occurring in the first 20 minutes of contact, but any survivors persisting for several hours. In crude sewage dosed with 10 mg l^{-1} PAA 40 percent of FS survived (0.4 log reduction) after 2 hours contact, and even with 20 mg l^{-1} PAA 2 percent survived (1.7 log reduction). Little evidence of regrowth of FS was found, in contrast to TC.

FS reductions in laboratory experiments on crude sewage from Penybont, Trearddur Bay and Wick treated with 10, 12 and 15 mg l^{-1} PAA were variable, generally ranging from 0 to 0.4 but occasionally reaching 2 log reduction. These were generally much poorer than for TTC or TC (Wallace Evans Ltd., 1990).

In the primary settled sewage and secondary effluents of poor organic quality used in the WRc (Crathorne *et al.*, 1991) study (10 and 20 mg l^{-1} PAA, 30 mins. contact) 3-4 log reductions of FS were usually obtained with the 20 mg l^{-1} dose, which compared well with TTC reductions at that dose (Table 1.7).

Lower doses of PAA tend not to be very effective against FS even in final effluents. Realey and Brogden (1990) reported 1.3-2.0 log reductions for FS in Menagwins sewage with 4-5 hours contact for dose of 6 mg l^{-1}.

In the simulated stormwater tests (Brogden & Realey, 1991) (Table 1.9) 2-3 log reduction of FS were observed with 15 mg l^{-1} PAA in all simulated Menagwins stormwaters. In the case of stronger Stevenage sewage 2 log reductions were only obtained at 12 or 18 DWF (12 to 18 fold dilution).

1.4.3 Field Studies

As might be expected, the field trials also show much greater variability for FS than for TTC inactivation. The Clacton field trials reported by Gould and Harrington (1988) demonstrated that 10-25 mg l^{-1} PAA achieved reductions of FS ranging from 0.08-4.00 log compared with 2-3 log reduction for TTC (Table 1.6). In the Southend field trials (Anglian Water, 1989) FS reductions of only 0.41 log were achieved. In the Trevaunance Cove field trial FS reductions were around 1-1.5, with wide variations, for doses of 12-20 mg l^{-1} PAA. However, in the Porthtowan field trial on settled secondary effluent, FS reductions were variable but averaged >3 log reductions for a 6 mg l^{-1} dose rate and the difference between TTC and FS reductions were less marked than in other cases. This could be explained by overdosing of PAA, which was suggested by some of the chemical results, but was not definitely shown to be the case.

Table 1.9 presents results for reductions in FS observed for simulated stormwater by Brogden & Realey (1991). The results are highly variable, and reductions are less than comparable results for TTC. From the studies reviewed for this Report it appears that, although FS levels are normally reduced by PAA treatment, the percentage reduction is often less than for TTC, and usually significantly less. The factors contributing to the variability are not elucidated.

Only one field trial, the Bexhill stormwater trial (Realey, 1989), has demonstrated greater reductions of FS than TTC. In that study doses of 10, 15 and 25 mg l^{-1} PAA gave 2-3 log reduction of TTC but 4 log reduction of FS. However it was recognized that these results were not consistent with previous studies, and the unexpected results were attributed to possible discharges from a hospital affecting the resistance of FS, although this seems unlikely.

Table 1.9 **Log reductions in faecal streptococci in simulated stormwater (5 mins. contact)**

Site	PAA Dose mg l^{-1}	Log Reduction 1DWF	3DWF	6DWF	12DWF	18DWF
Menagwins	5	0.98	0.79	0.89	0.60	0.64
	10	2.10	3.48	3.48	3.13	3.39
	15	1.94	3.49	3.85	3.82	3.54
Stevenage (June)	5	-0.03	-0.07	0.21	0.12	0.07
(Runs 1 + 2	10	0.01	0.02	0.14	0.40	1.22
Averaged)	15	0.03	-0.06	0.35	2.52	2.39
Stevenage (Nov.)	5	0.09	0.18	1.08	0.91	0.87
(Run 3)	10	-0.64	1.56	1.02	2.03	1.58

Source: Brogden and Realey (1991)

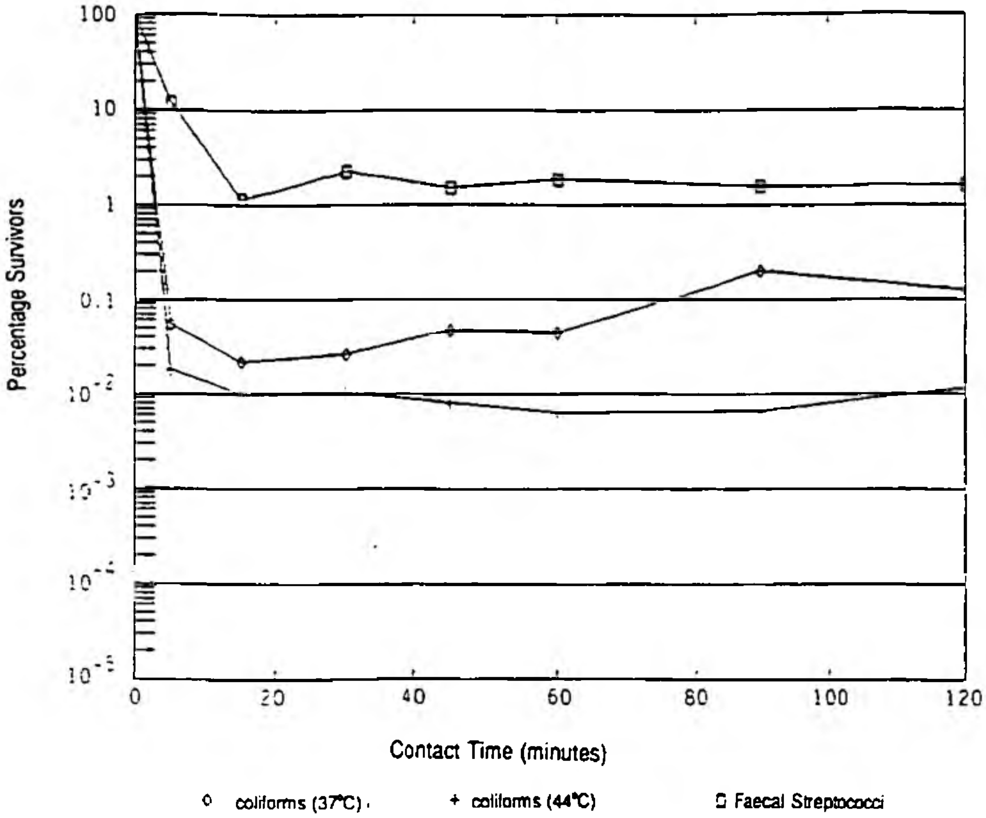


Figure 1.3 **Inactivation of total (37°C) and thermotolerant (44°C) coliform bacteria and faecal streptococci in sewage by Oxymaster. Initial dose 20 mg l^{-1} peracetic acid (Irving, 1990)**

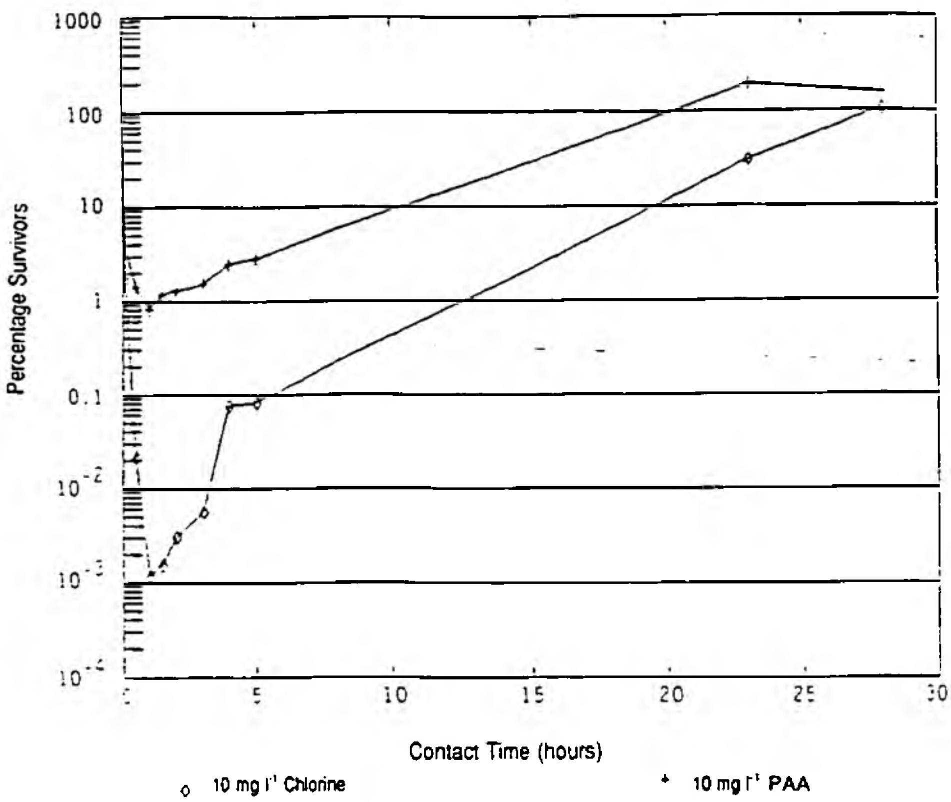


Figure 1.4 **Regrowth of total coliform bacteria in sewage disinfected with 10 mg l^{-1} chlorine or peracetic acid (Irving, 1990)**

1.5 Pathogenic Bacteria

Despite the importance of pathogenic bacteria in assessing the suitability of discharges to bathing waters, there appear to be even fewer data than for viruses. Four reports provide quantitative data on Salmonella. The relative lack of data is due to the practical difficulties in terms of laboratory procedures for the enumeration of pathogenic bacteria and the low, variable concentration of Salmonella in sewage.

The study at Trearddur Bay reported very limited historical data for Salmonella in beach samples and results from a single die-off experiment. In this study *Salmonella enteritidis* was added to screened raw sewage which was then incubated for 4 hours at room temperature, before dosing with 12 mg l^{-1} PAA.

The results of this brief experimental test programme, presented in Table 1.10, showed no conclusive evidence of disinfection.

Table 1.10 Effect of PAA on Salmonella

Time after dosing (min.)	Salmonella MPN/10 ml	% Reduction
0	16	0
2	18	-12.5
5	9	43.75
10	9	43.75
10	16	0

Source: Wallace Evans Ltd (1990)

In the field trials at Menagwins almost all the samples contained less Salmonella than the detection limit (2 per 100 ml). Thus no conclusions could be drawn. In the Porthtowan study a main and a duplicating laboratory were employed. It was shown by Roddie *et al.* (1991a) that these laboratories used differing methods of enumeration and that the uncertainty was high. However, the observed reductions for Salmonella were low. In the Trevaunance Cove study no clear effects were observed for any 'non-routine' microorganisms (Roddie *et al.*, 1991b).

The only other evidence is from the Anglian Water Authority report on disinfection trials at Southend (1990). The Report does not analyze the data but merely comments that Salmonella reductions followed a similar pattern to those for coliforms (which were very variable but gave an overall arithmetic mean reduction of 2 log). Calculation of the geometric mean reduction gives 0.52 mean log reduction (70%).

Gould and Fraser (1990) quote an unnamed site where a dose of 10-12 mg l^{-1} PAA eradicated Salmonella which were present at levels of 10-60/100 ml. At present this report is the only evidence to support the view that PAA at doses around 10 mg l^{-1} is an effective disinfectant for Salmonella.

1.6 Other Bacteria

There have been a very limited number of trials involving other types of bacteria, notably:

a) *Campylobacter*;

b) *Staphylococcus aureus*; and

c) *Pseudomonas aeruginosa*.

Campylobacter is a principal cause of bacterial diarrhoea and *Staph. aureus* is a skin pathogen. *Pseudomonas aeruginosa* can cause infections of the respiratory and urinary tracts. The limited evidence available from Realey & Brogden (1990) and Gould & Fraser (1990) suggests that *Campylobacter* is similar to FS in its response to PAA and that the other two organisms are significantly more resistant. Roddie *et al* (1991a) report low reductions for *Pseudomonas aeruginosa*, but were unable to obtain any clear data for *Staphylococcus aureus* or *Campylobacter*, and highlight the difficulties of enumeration. In their report on disinfection of raw sewage the same authors (Roddie *et al.*, 1991b) were unable to demonstrate any clear effects, but concluded that pathogenic microorganisms are generally less affected by PAA than are TTC.

1.7 Regrowth and Reactivation

The phenomenon of 'regrowth' after disinfection has been observed on many occasions e.g. Shuval *et al.* (1973), but has in recent years become intertwined with 'reactivation' (Colwell, 1987 and Davies, 1990). The former process refers to the regrowth of the survivors, while the latter refers to cells damaged by disinfection recovering their ability to grow through some resuscitation procedure. In general, it is only possible to distinguish experimentally between the two processes in terms of time for recovery of bacterial numbers to take place. It is assumed that regrowth is relatively slow and reactivation relatively fast.

Both phenomena may be of importance in the context of disinfection of sewage by PAA, but there is a dearth of information on this topic. The literature reviewed contained only two papers that described experiments (Irving, 1990 and Crathorne *et al.*, 1991) and one paper which referred to the possibility of regrowth affecting results (Realey & Brogden, 1991). Additionally, comments from Plymouth Marine Laboratory (1988) indicated their concern about the possible effects of regrowth and reactivation. Interox has not published any data on regrowth but commented simply that it does not occur at operational levels.

The regrowth experiments carried out by Irving (1990) on crude sewage showed an increase in numbers of both TC and TTC in less than 5 hours (see Figure 1.4) with significant increases occurring over longer periods. FS did not show any regrowth. These tests were extended to examine the effect of dilution on the response of bacteria that had been subjected to disinfection with PAA. The results are summarised in Figure 1.5 which shows that at ten-fold dilution there is still a slight tendency for regrowth of TC, less so for TTC and no regrowth of FS. In the case of a hundred-fold dilution there was no regrowth.

This work has been subsequently extended to examine the potential for regrowth of bacteria from secondary effluents (Crathorne *et al.*, 1991). The results shown in Table 1.11.

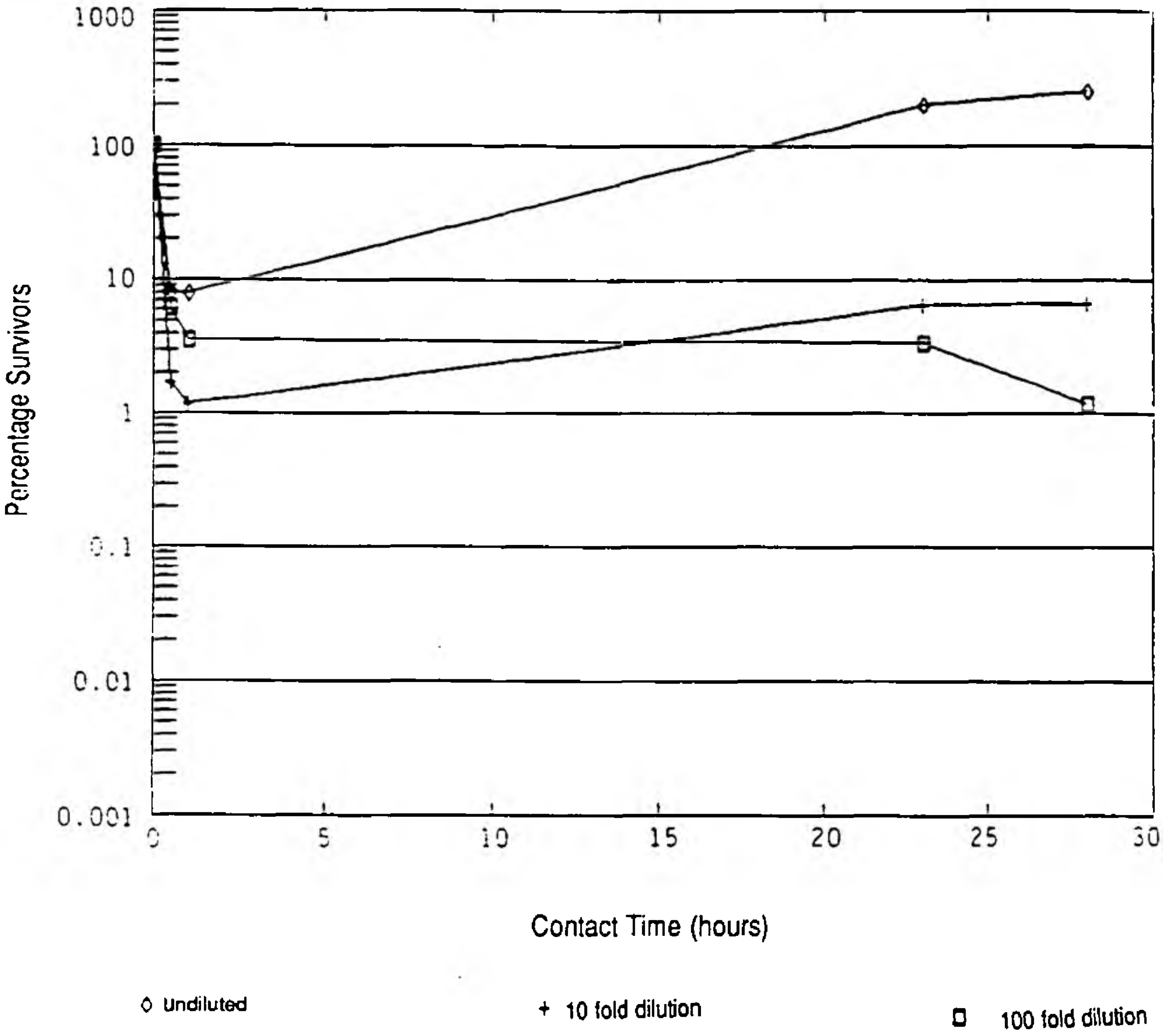


Figure 1.5 **Effect of dilution in sea water on regrowth of total coliforms in sewage disinfected with 10 mg l^{-1} PAA (Irving, 1990)**

Table 1.11 Apparent regrowth of indicator organisms following disinfection with 10 mg l^{-1} PAA

Negative values indicate an increase in count over pre-disinfection levels

			Log reduction after 2 or 24 hours storage			
			TTC		FS	
Effluent type	Water type	Dilution	2 h	24 h	2 h	24 h
AS	River	Nil	1.3	-0.60	0.08	0.05
		10x	1.3	-0.74	0.10	0.08
		100x	1.1	-0.65	0.13	0.05
AS	River	Nil	2.4	-0.22		
		10x	2.0	0.34		
		100x	1.4	1.1		
AS	Sea	Nil	1.8	0.55	0.09	0.27
		10x	1.6	1.9	0.20	0.35
		100x	1.3	1.8	0.10	0.44
BF	River	Nil	3.5	1.9	1.9	3.0
		10x	2.7	2.4	0.74	0.89
		100x	0.18	0.64	0.27	0.06
BF	Sea	Nil	3.0	2.3	0.96	1.2
		10x	2.4	2.5	0.24	0.47
		100x	0.57	1.6	-0.08	0.21
PS	Estuarine	Nil	3.0	0.80	0.64	0.38
		10x	2.9	1.3	0.95	0.47
		100x	2.0	1.1	0.85	0.68
PS	Sea	Nil	3.5	1.1	1.2	0.89
		10x	3.0	2.4	0.66	0.66
		100x	1.5	1.9	0.04	0.21

Key to effluent types:
AS Activated sludge
BF Biological filter
PS Primary settled

Source: Crathorne *et al.* (1991)

It was observed in the course of these experiments that much of the increase in bacterial numbers occurred immediately upon dilution and must be due to reactivation rather than regrowth. This phenomenon was further examined by taking samples after 30 minutes of contact with PAA and then diluting these and sampling immediately. The results of these tests are presented in Table 1.12 in terms of the percentage of the pre-disinfection population which reappeared after dilution.

Table 1.12 Reactivation of indicator bacteria after dilution of disinfected sewage effluents

Effluent type	Water type	Dilution	Increase in count observable immediately after dilution (% of pre-disinfection pop.that recovered immediatly) TTC	FS
AS	River	10x	1.9	39
		10x	1.1	
		100x	6.2	53
		100x	4.7	
AS	Sea	10x	2.5	0
		100x	8.1	12
BF	River	10x	63	63
		100x	68	67
BF	Sea	10x	86	78
		100x	55	96
PS	Estuarine	10x	0.04	-11
		100x	1.0	11
PS	Sea	10x	0.9	70
		100x	15	84

For key to effluent types, see Table 1.11. Source: Crathorne *et al.* (1991)

The results imply that PAA is acting, at least partly, as a bacteriostatic agent. However, attempts to remove the bacteriostatic effect by adding neutraliser failed to cause a corresponding increase in numbers. There is a possibility that PAA produces a bacteriostatic compound which is not removed by sodium thiosulphate and catalase (the neutraliser employed), but is also seems that this effect is partly due to the nature of the secondary effluent (Table 1.12).

2. PAA AS A DISINFECTANT FOR VIRUSES AND ANIMAL PARASITES

It is extremely difficult to assess the health risk from viruses in sewage, but there is no doubt that some viruses, such as enteroviruses, human Rotavirus, hepatitis A virus and Norwalk agent, are pathogenic and are well adapted to transmission via water. The possibility that contact with sewage may result in viral infection must be taken seriously, especially where it enters recreational waters or commercial shellfishery areas and receives little dilution.

The isolation or enumeration of human viruses from sewage, effluents and natural waters is still a complex and expensive procedure. For this reason bacteriophages have occasionally been used as indicators of the behaviour of human viruses. However, it has been established that there are wide variations in their resistance to disinfection, so that the data on bacteriophages need to be interpreted with caution.

2.1 Bacteriophages

Some of the early data concerning the effects of PAA on bacteriophages are summarised in Tables 2.1 and 2.2.

Table 2.1 Effectiveness of PAA for bacteriophages

	Conc. of PAA required to give 4 log reductions within 5 mins.				
Phage	DMW	pH 5	pH 7	pH 9	4 g l^{-1} Yeast Extract
MS2 Ø	12-15	11-15	30-53	225-300	75-94
ØX174	23-30	15-23	53-75	525-750	94-113

(DMW = Demineralised Water) Source: Baldry *et al.* (1990)

Table 2.2 presents results for bacteriophage disinfection tests at Menagwins STW (Realey & Brogden, 1990). The sampling points referred to are situated as follows:

- 1 before disinfection;
- 2a in humus tanks; and
- 2b at outfall.

From Table 2.2 it can be seen that the average reduction of F^+ coliphage at 4 mg l^{-1} PAA was zero but at 6 mg l^{-1} PAA there was a reduction of around 1 log.

From Table 2.2 it can be concluded that PAA will reduce the level of F^+ coliphage at concentrations of 6 mg l^{-1}, but has little effect below 4 mg l^{-1}. The extent of the reduction appears to be significantly affected by the type of bacteriophage. For example ØX174 requires twice the concentration of PAA to obtain the same level of disinfection as MS2 Ø (Table 2.1).

Table 2.2 Results of bacteriophage disinfection tests in the humus tanks at Menagwins STW

Sampling Run	Time from Start (hours)	Conc. of F^+ Coliphage (PFU/100 ml) Sampling point 1	2a	2b
5	0	15315	2523	-
	4	-	-	1261
6 mg l^{-1} PAA	8	11712	3063	-
	12	17117	1982	450
	16	-	-	1441
	20	12612	1455	-
	24	-	-	545
	Average	14189	2256	924
6	4	1261	200	-
	7	-	-	300
4 mg l^{-1} PAA	12	1414	100	-
	15	-	-	3909
	16	1300	4775	-
	19	-	-	1622
	23	1900	901	-
	26	-	-	1364
	Average	1469	1494	2049

Source: Realey & Brodgen (1990)

The level of disinfection also appears to be influenced by chemical factors, notably pH and organic mattter concentration. Table 2.1 shows that PAA is much more effective at a pH below 7. Unfortunately no details of pH are given for DMW or Yeast extract experiments, but it seems reasonable to assume that the latter is well buffered and that DMW would have a pH around 5 due to PAA addition (Interox, pers. comm.).

The effect of organic matter in the form of yeast extract seems to exert a protective action, so that approximately two to three times as much PAA is required to obtain similar reductions (Baldry *et al.*, 1991). In the absence of any further data it is impossible to translate this result numerically into the context of disinfection of bacteriophage in sewage. It is clear, however, that higher dose levels will be required than those which are effective in demineralised water.

Measurements of reductions in F⁺ coliphage were carried out by Roddie *et al.* (1991a & 1991b). For settled secondary effluent doses of 4-6 mg l^{-1} PAA produced measured reductions of between >0.2 to >1.3 logs, while for raw sewage no clear reduction was observed.

With bacteriophages there is also the problem inherent for all indicators, namely how closely they model the response of their pathogenic counterparts, in this case human viruses. The evidence of tests such as those by Realey & Brogden (1990), and Roddie *et al.* (1991a & 1991b), in which both phages and human viruses have been tested simultaneously, suggests that their model behaviour has not yet been sufficiently established for them to be useful criteria by which to assess the efficacy of PAA as a viral disinfectant.

2.2 Human Viruses

Techniques for quantitative recovery of viruses from sewage are laborious and expensive. For this reason relatively few data are available to characterise the response of human viruses to disinfectants including PAA. There have, however, been a small number of laboratory and field trials using PAA in the past 10 years, and these are reviewed below.

2.2.1 Laboratory Studies

The pioneer work in this field was by Butler & Harakeh (1982) and Harakeh (1984) who reported on the virucidal action of PAA compared with chlorine and other disinfectants. The results showed that concentrations of >140 mg l^{-1} PAA were required to give 3-4 log reduction in 30 minutes. This work has been criticised (Baldry *et al.*, 1990) for the use of animal sera in the challenge tests. More recent work has suspended the virus particles in sewage or effluent prior to dosing with PAA. There have been recent reports of laboratory-based studies by Baldry & French (1989a,b), Baldry *et al.* (1991), Gould & Fraser (1990), Marine Environmental Consultants (1989), Crathorne *et al.* (1991) and Harakeh (1987).

Work at the Interox laboratory is summarised in Table 2.3 taken from Baldry *et al.*, 1991. Interox have also collaborated with MEC and the virus laboratory of Welsh Water (now Wallace Evans Ltd.) to examine the effect of PAA on human Rotavirus. The results of this work are given in Table 2.4 taken from MEC (1989).

The results presented in Table 2.4 show that human Rotavirus is much more susceptible to PAA than the other viruses tested. The greater resistance of Polio virus to PAA has been confirmed by recent work by Crathorne *et al.* (1991). Experiments showed poor disinfecting action at doses of 10 and 20 mg l^{-1} as shown in Table 2.5.

Table 2.3 Effectiveness of PAA for viruses

		Concentration of PAA ($mg\ l^{-1}$) to give 4 log reduction	
Virus	Contact Time (minutes)	Demineralised Water	Yeast Extract
Poliovirus	10	1500-2250	>2250*
	15	750-1500	1500-2250
	30	>750*	750-1500
	60	150-375	375-750
Echovirus	60	100-375	100-750
Coxsackievirus	15	250-500	500-1000
	60	100-375	100-375

* Just failed, giving 3.89 log reduction

Source: Baldry *et al.* (1991)

Harakeh (1987) has more recently published further details of experiments on virus disinfection carried out at the University of Surrey. The technique used seems to have avoided the criticism of the earlier work in that viruses were suspended in activated sludge effluent prior to reaction with PAA. However, there is still an anomaly, as the PAA concentration did not fall during the 30 minutes disinfection, contrary to other experience. The results are also in direct contradiction to those of Gould & Fraser (1990) since they show human Rotavirus to be highly resistant to PAA, requiring a dose of 140 $mg\ l^{-1}$ to give 4 log reduction.

Table 2.4 **Summary of results of disinfection experiment carried out with Gowerton sewage and added human Rotavirus**

		Log reduction observed			
		Contact time (minutes)			
* Product code	PAA concentration (mg l^{-1})	0	10	20	40
A	15	0	>2.5	>3.5	>3.5
B	15	0	2.1	3.7	>3.7
C	15	0	1.1	1.4	>3.3
Mean			>1.6	>1.7	>3.5
A	20	0	1.9	2.2	2.5
B	20	0	1.2	2.4	>3.7
C	20	0	>4.0	>4.0	>4.0
Mean			>1.6	>2.4	>3.7
A	25	0	1.9	1.2	>3.3
B	25	0	1.2	>3.4	>3.4
C	25	0	1.8	1.8	>3.5
Mean			1.5	>1.6	>3.4
Overall Mean			>1.6	>1.8	>3.2

* Product Code A Oxymaster (5% PAA), B Oxymaster (12% PAA) and C Proxitane 4002 (38-40% PAA)

Source: MEC (1989)

Table 2.5 **Action of PAA on Polio virus**

Source	PAA Applied Dose (mg l^{-1})	Log Geometric Mean Reduction of Poliovirus after 30 mins.
Activated Sludge Effluent	10 20	0.40 0.36
Biological Filter Effluent	20	0.08
Primary Settled Sewage	20	0.33

Source: Crathorne *et al.* (1991)

2.2.2 Field Data for Human Viruses

The results of the field trials are reviewed in Appendix A1.1-A1.6 but the conclusions may be briefly summarised as follows:

a)	Clacton	-	no consistent virucidal effect
b)	Southend	-	results too variable for firm conclusion, but showed a reduction of around 50% in enterovirus
c)	Menagwins	-	results too variable to draw any conclusions
d)	Trearddur	-	results variable but showed 0.55-1.10 log reduction
e)	Unnamed (Gould & Fraser)	-	0.17-0.52 log reduction of Polio 1, 2 and 3, Coxsackie B and Echovirus
f)	Porthtowan	-	results too variable to draw any conclusions
g)	Trevaunance Cove	-	results too variable to draw any conclusions

The difficulties in obtaining useful data in the field trials stems mainly from the low and variable viral content of wastewater. However, in the Trearddur Bay study it was also concluded that some variability was due to the slow release of virus particles from sediments by wave action.

2.4 Reactivation

No experiments appear to have been carried out to test the possibility that viruses may recover from disinfection by PAA, although the possibility is mentioned in comments by Plymouth Marine Laboratory (1988). Experience of disinfection by chlorine suggests that the observed reduction in numbers may be reversible to some extent.

2.5 Effects of Organic Matter

The results quoted in Table 2.6 and Table 2.7 for Baldry *et al.* (1991) show that for both bacteriophage and human viruses, the presence of organic matter can exert a protective action such that greater concentrations of PAA are required to obtain the same reduction. Echovirus, however, appears to be an exception. This further reinforces the information which indicates that PAA is unlikely to be particularly effective as a virucidal agent in the disinfection of sewage in the normal range of dosage.

2.6 Protozoa and Metazoa

Reduction in numbers of animal parasites by PAA disinfection is not relevant to this review, except for the presence of organisms in sewage or effluents which may transmit disease. Two species of amoeba, namely *Naegleria gruberi* and *N. fowlen*, are implicated in waterborne infections. Tests on these organisms gave a minimum inhibitory concentration of 1-10 mg l^{-1}

PAA and an amoebicidal concentration of around 10 mg l^{-1} (Kilvington, 1989).

The only trials on metazoans appear to be tests on the ova of *Taenia saginata* in sludges. Trials reported by Fraser *et al.* (1984) showed that PAA concentrations of 250-1000 mg l^{-1} caused up to 99% inhibition of hatching and up to 100% loss of viability in embryos suspended in raw and digested sludges.

No information was found regarding *Giardia* or *Cryptosporidium*.

Table 2.6 Comparison of PAA effectiveness for clean and organically enriched media

		PAA conc. (mg l^{-1}) to achieve 4 log reduction	
	contact time (min)	demineralised water	yeast extract
E. coli	5	10-15	50-75
S. faecalis	5	10-25	75-100
phage MS2	5	12-15	75-94
phage Øx174	5	25-30	94-113
Poliovirus	10	1500-2250	>2250*
Poliovirus	15	750-1500	1500-2250
Poliovirus	30	>750*	750-1500
Poliovirus	60	150-375	375-750
Echovirus	60	100-375	100-375
Coxsackievirus	15	250-500	500-1000
Coxsackievirus	60	100-375	100-375

* These concentrations just failed to meet the test requirements, giving a 3.89 log reduction in numbers.

Source: Baldry *et al.* (1991)

Table 2.7 Effectiveness of PAA for other media

	PAA conc. (mg l^{-1}) to achieve 4 log reduction			
	pH5 buffer	pH7 buffer	pH9 buffer	bovine serum albumin
E. coli	20-25	10-15	100-150	ND
S. faecalis	10-15	75-100	500-1000	ND
phage MS2	11-15	30-53	225-300	35-45
phage Øx174	15-23	53-75	525-750	39-65

ND - not determined

Source: Baldry *et al.* (1991)

3. REVIEW OF INTERNATIONAL EXPERIENCE OF USING PAA AS A DISINFECTANT

This review is based upon a computerised literature search using Aqualine and Chemical Abstracts databases. It includes all references to PAA (under all identified pseudonyms) published in English in the past 5 years, but excluding the British literature, which has been used in compiling the remainder of this Report. Work by Baldry and others from Interox, although published in foreign journals, has not been included, as it is similar in content to that already reviewed in the British publications. With the above restrictions, and limiting the area to PAA disinfection a total of 8 references were identified and are briefly summarised below.

Mathieu *et al.* (1990) carried out laboratory tests which gave approximately 2 log TTC reduction at a dose of 2.5 mg l^{-1} PAA after 60 minutes at 30°C. Microtox assay showed that there was low toxicity in the disinfected culture.

Giodani *et al.* (1989) also confirmed the toxicity of PAA for coliforms in sewage effluents at low levels of suspended solids (5-8 mg l^{-1}). They found that PAA remained in the disinfected wastewater for periods of up to 60 minutes, contrary to most other studies. The idea of continuing protection has also been mentioned in work by Hajenian (1981) on the disinfection of F2 bacteriophage.

Comparative studies on a range of disinfectants have been carried out by a number of workers, notably Hugues (1987) and Harakeh (1984). The results have generally tended to show PAA to have a similar efficacy to that reported in British laboratory studies. Poffe (1978) found that PAA in concentrations of 5-10 mg l^{-1} gave between 1.4 log reduction and complete removal of FS with 15 minutes contact. This suggests a higher effectiveness than usually reported and he also suggested that a concentration of 1 mg l^{-1} PAA could give similar results with 60 minutes contact. Depre (1982) reported results much closer to British experience, suggesting 5 mg l^{-1} at 15 minutes to give 2 log coliform reduction.

The only American reference (Maltais and Stern, 1990) deals with the use of PAA for the cleaning of reverse osmosis membranes. It is surprising that PAA does not appear to have been researched or applied in North America for sewage disinfection. However, one American supplier (Mintech Corporation, Minneapolis) sells a formulation of PAA and hydrogen peroxide for disinfection of membranes in the food industry. The lack of work in the US was attributed by Interox to lack of marketing effort. It may also be that other disinfection systems are well established and that there is no perceived need for a product like PAA, since secondary treatment is normal for sewage discharges.

4. CONCLUSIONS

4.1 Reduction of micro-organisms

Table 4.1 summarises the results obtained in field trials for TTC reduction. It can be seen that mean reductions vary considerably and it should be noted that the variation of individual results from which the means are calculated is even greater. Indeed, at times during some field trials little or no reduction was observed. FS have generally proven rather more resistant to PAA than TTC.

Table 4.1 Summary of TTC Reduction Observed in PAA Field Trials

Location	Effluent type	PAA dose (mg l^{-1})	Contact time	Mean log reduction
Clacton	Raw	10 15 20	6 mins	2.0 2.2 2.7
Plymouth	Raw	15	2 mins	1.2
Trevaunance Cove	Raw	12 20	<2 mins	1.6 2.1
Southend	Primary	12	Not known	1.9
Menagwins	Unsettled bio filter	4 6	4 - 5 hours	1.5 3.1
Porthtowan	Settled bio filter	4* 6*	5 mins	2.3 3.6
Unknown (Baldry & French, 1989a)	Activated sludge	2 8	21 hours	1.85 3.28
Bexhill	Simulated S/W	10 25	Not known	2.2 2.2

* Overdosing may have occurred

The data on pathogenic bacteria are rather sparse due to the difficulty of obtaining meaningful results. However, the results observed for: Salmonella, *Campylobacter*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*; range from no effect to 1.8 log reduction, and have been observed to be highly variable. The best effect was observed on one run at Menagwins, where the contact time was a matter of hours. For short contact times (<10 minutes) reductions have generally been <1.0 log.

Table 4.2 summarises the results obtained in the field for reduction of viruses by PAA. It is clear that PAA has not been demonstrated to be effective against some important enteroviruses. Furthermore, it appears from the Trearddur Bay Study that viruses associated with solids may not be affected and may be subsequently released to the environment after discharge. Laboratory work indicates that PAA is not effective against Polio virus.

Table 4.2 Summary of Virus and Coliphage Reduction Observed in PAA Field Trials

Location	Effluent type	Organisms	PAA dose (mg l^{-1})	Contact time	Mean log reduction
Clacton	Raw	Enterovirus Rotavirus	10 15 20	6 mins	No consistent effect
Trevaunance Cove	Raw	Enterovirus Rotavirus F^+ Coliphage	12 20	<2 mins	No consistent effect
Southend	Primary	Enterovirus Rotavirus	12	Not known	<0.3 Little effect
Menagwins	Unsettled bio filter	F^+ Coliphage	4 6	4 - 5 hours	Little effect 1.0
Porthtowan	Settled bio filter	Enterovirus Rotavirus F^+ Coliphage	6*	5 mins	No effect No effect >0.77+

* Overdosing may have occurred
\+ Mean of results obtained

It is apparent from the laboratory work on bacteriophages that their response to PAA disinfection is significantly different from that of human viruses. Unless some bacteriophage can be found that more closely models the response of enteroviruses to PAA, phage does not appear to be a very useful indicator.

In summary, PAA appears to be most effective against indicator organisms in secondary sewage effluents, but has not been shown to be consistently effective against some important pathogenic bacteria and viruses.

4.2 Regrowth and reactivation

The overall conclusion regarding regrowth is that it seems unlikely to occur under practical circumstances. Coliforms generally require a combination of temperatures above 15°C and a BOD concentration above 20 mg l^{-1} to show significant growth rates. This may occur where raw or partly treated sewage is stored in tidal tanks and/or discharged through long outfalls. Any such scheme must take this possibility into account.

It appears that reactivation is a phenomenon which is more likely to produce significant increases in bacterial indicators after treatment with PAA. It is perhaps surprising that this has only been observed in the laboratory, although an examination of the sampling programme at Southend and other sites shows that they are unlikely to detect reactivation, because of the siting of sampling stations. A second complicating factor is that other sources of contamination are often cited as reasons for higher counts of indicator bacteria; in field trials this observation could also be explained by reactivation.

In the laboratory, up to 86% of the pre-disinfection TTC population in percolating filter effluents showed immediate recovery after dilution. The effect was less marked for primary and activated sludge effluents (0.04-15.0%), but could be significant if >1.0 log reduction was sought and a similar effect occurs in the field. No field data or data on reactivation of pathogenic organisms are available.

4.3 Practical Considerations

In practice, the disinfection capability of PAA appears to be variable from site to site and over time at each site, possibly due to variable organic content of wastewater and different ability of wastewaters to buffer pH. It would therefore appear that a careful preliminary investigation would be advisable at any site where PAA disinfection is considered. In general it appears that PAA-treatment is more effective for secondary effluent than for primary effluent, stormwater or raw sewage.

An area of particular concern for stormwater disinfection would be the highly variable nature of stormwater. In particular, successful disinfection of the first flush would require very careful dose control.

Appendix A1

The Results of Field Trials

APPENDIX A1 - THE RESULTS OF FIELD TRIALS

A number of full-scale trials on disinfection of discharges using PAA have been carried out over the past 2-3 years. The results of these are briefly reviewed below. However, in reviewing this work it must be appreciated that most trials are incomplete and therefore cannot yet serve as a definitive guide.

A1.1 Field Trials at Southend

A1.1.1 Introduction

A dry weather flow of 40,000 m^3 per day undergoes grit removal, screening and primary sedimentation. During the bathing seasons in 1989 and 1990, *Oxymaster* was dosed into the outflow from the plant to give a PAA concentration of 12 mg l^{-1}. The results of the trials are reported variously in Anglian Water (1989), MEC (1989), Gould (1989), Anglian Water (1990) and Joslin & Gould (1991).

A1.1.2 Results

During 1989 samples were collected before and after PAA dosing and analyzed for *E. coli*, TC and FS, with occasional samples analysed for Enterovirus and Rotavirus. The results showed 1.92 log reduction for *E. coli*, 1.75 log reduction for TC (see Figure A1.1) and 0.41 log for FS. Virological data were more ambiguous with reductions in Enterovirus varying from 0 to 50% and those for Rotavirus from negative to 70%.

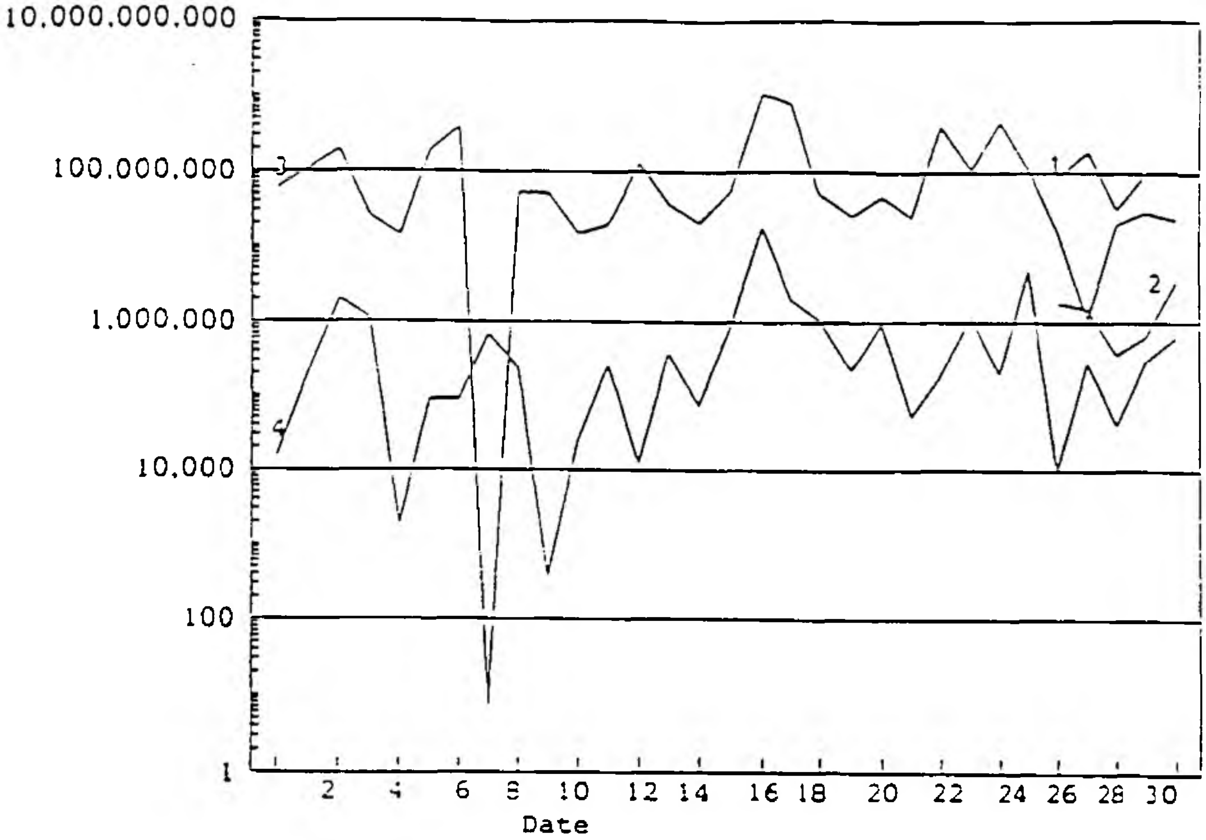
Disinfection during 1990 produced considerably lower reductions than in the previous year. During May and part of August, Anglian Water demonstrated 2 log reduction in *E. coli*, but during the remainder of the bathing season the reductions were lower (Figure A1.2).

Field samples taken in 1989 showed reduction in the concentration of TTC and FS in the central channel of the Thames as given in Table A1.1.

Table A1.1 Reduction in concentration of TTC and FS in the Thames central channel

	Concentration of TTC/FS	
	Without Disinfection (cfu/100 ml)	With Disinfection (cfu/100 ml)
TTC	131-290	5-121
FS	37-72	3-37

Source: Gould (1989a)



Key: 1 Total coli Pre-dose 2 Total coli Post-dose
3 E. coli. Pre-dose 4 E. coli Post-dose

Figure A1.1 1989 Results for disinfection using PAA at Southend

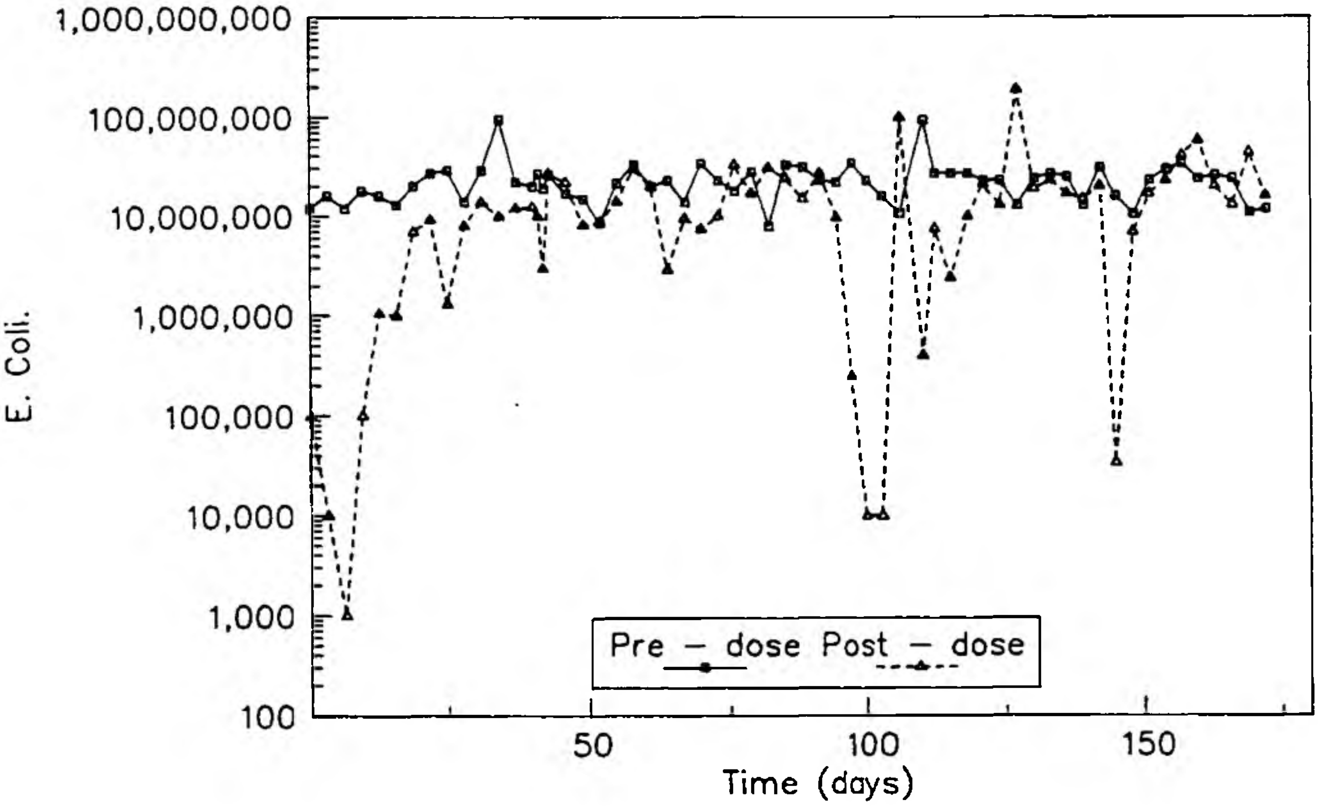


Figure A1.2 1990 Results for disinfection using PAA at Southend

Bacterial examination was extended to give weekly concentrations for Salmonella. Anglian Water comment that these show similar reductions to those for TTC but calculation of the geometric mean gives only 0.52 log reduction (70%). Results for 1990 of the viral sampling programme were inconclusive due to low virus levels in the sewage and the toxicity of the sewage.

A1.2 Disinfection Experiments at Clacton-on-Sea

A1.2.1 Introduction

At Clacton sewage is discharged into the sea through a short outfall after screening and maceration. Experiments were carried out in 1987 and 1988 to determine the effect of PAA dosing on bacterial counts in the adjacent bathing areas. The results have been reported in Gould and Harrington (1988), Thomas *et al.* (1990) and Gould and Fraser (1990) and are summarised below.

During the trials PAA was dosed at concentrations of 10, 15, 20 and 25 mg l^{-1}. The periods of disinfection were preceded by an eight-day period of with no disinfection.

A1.2.2 Results

The results are presented in detail in Gould and Harrington (1988). The data for indicator bacteria are summarised in Figs. A1.3 and A1.4 and show an overall reduction of 2-3 log for TTC. In the case of FS the reductions are somewhat less and show considerable variation.

The results of the tests for Enterovirus and Rotavirus are presented in Tables A1.2 and A1.3. It was concluded that no consistent virucidal effect was observed. In reviewing the results Gould and Fraser (1990) commented that certain operational factors at Clacton precluded sampling a combined undisinfected effluent stream. The absence of flow measurement made assessment of initial virus numbers uncertain, and comminution may have caused an increase in the initial concentration.

A1.3 Disinfection Experiments at Plymouth

A1.3.1 Introduction

The Rusty Anchor Outfall discharges a DWF of approximately 18,000 m^3 d^{-1} of untreated sewage through a short outfall 125 m into Plymouth Sound. The discharge is intermittent, occurring for 3 hours only on the ebb tide.

Field trials were conducted during September 1988 to measure the effectiveness of PAA in reducing the bacterial concentrations in the plume from the outfall and in Plymouth Sound, Four different doses, 5, 10, 15 and 20 mg l^{-1} of PAA were used. The results are reported in Atkinson *et al.* (1989) and are summarised in Tables A1.5 and A1.6

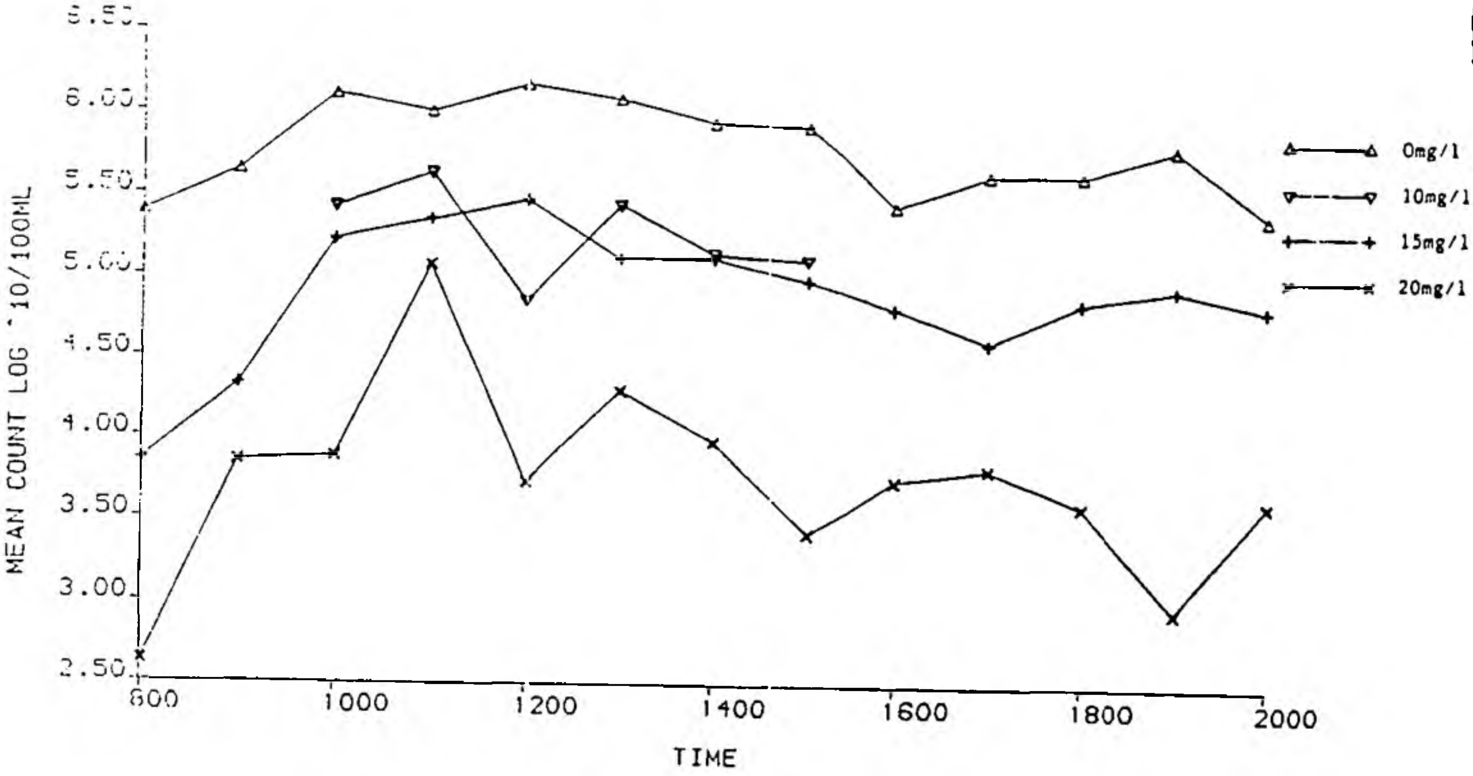


Figure A1.3 Results for faecal streptococci from the Clacton disinfection trial

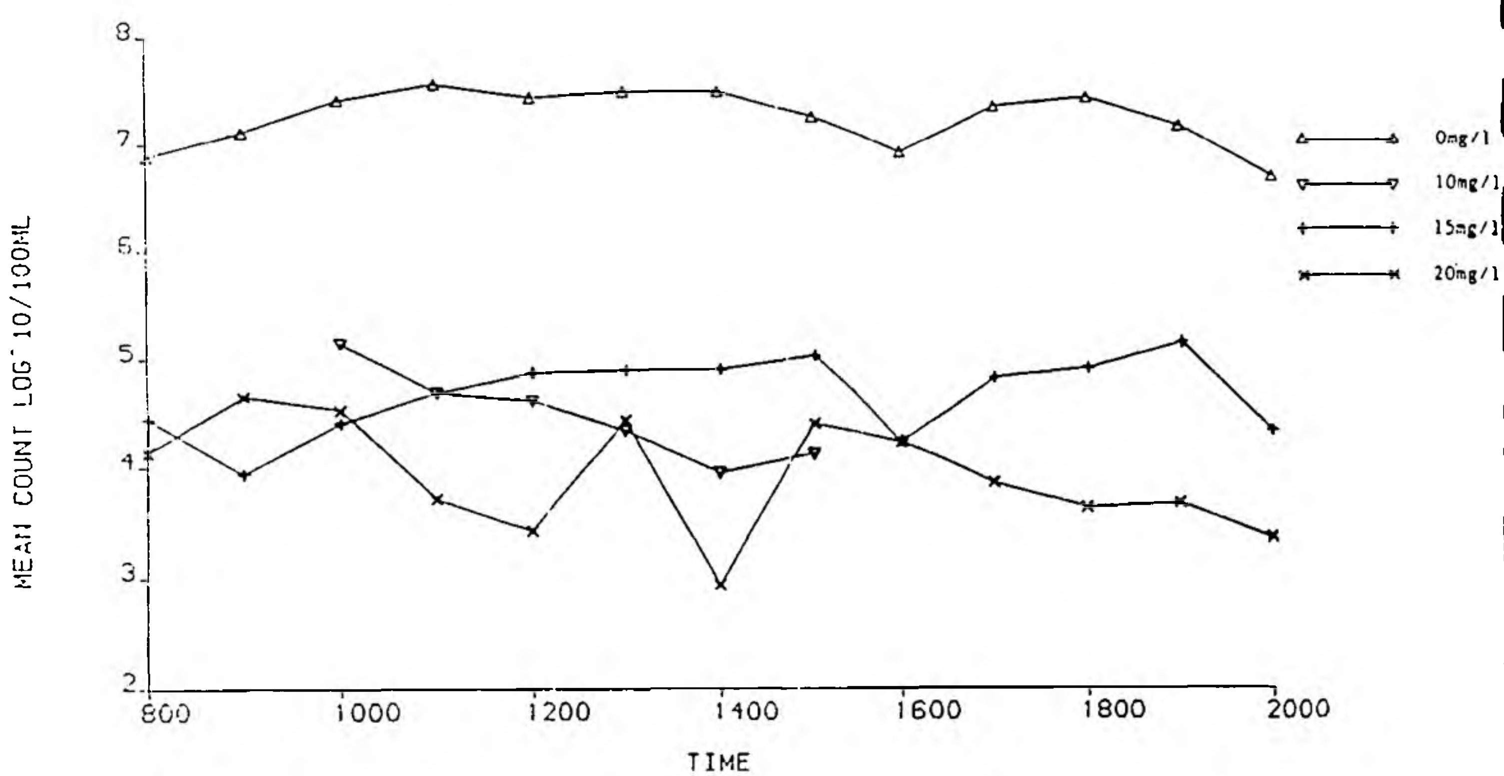


Figure A1.4 Results for thermotolerant coliforms from the Clacton disinfection trial

Table A1.2 Clacton disinfection trial results: Enteroviruses PFU/100 ml

Dose	Date	Clacton*	Thorpe*	Post+
20 mg l^{-1}	3/12	3.0	1.0	7.5
	4/12	0.0	3.0	5.0
15 mg l^{-1}	8/12	3.5	5.0	4.0
	9/12	3.0	12.0	19.0
	10/12	0.0	2.0	1.0
	11/12	1.0	0.0	0.5
10 mg l^{-1}	15/12	0.0	0.0	1.0
	16/12	3.0	0.0	4.0
	17/12	10.0	5.0	6.0

* Untreated
\+ Treated combined flow

Source: Gould & Harrington (1988)

PFU = Plaque Forming Unit

Table A1.3 Clacton disinfection trial results: Rotavirus FC/100 ml

Dose	Date	Clacton*	Thorpe*	Post+
20 mg l^{-1}	3/12	4.0	5.0	20.0
	4/12	8.0	7.0	6.0
15 mg l^{-1}	8/12	6.0	14.0	20.0
	9/12	10.0	CD	10.0
	10/12	4.0	CD	CD
	11/12	CD	CD	6.0
20 mg l^{-1}	15/12	CD	6.0	6.0
	16/12	6.0	6.0	6.0
	17/12	8.0	8.0	4.0

* Untreated
\+ Treated combined flow

Source: Gould & Harrington (1988)

FC = Fluorescing cells
CD = Cells dead (sample toxic to monolayer)

A1.3.2 Results

The survey attempted to measure the impact of PAA in 3 ways as follows:

a) In reducing the coliform concentration in the raw sewage prior to discharge. The results of this are summarized in Table A1.4.

b) In reducing coliform levels in the plume from the sewage discharge. The original data for these results are not given in Atkinson *et al.* (1989) but Table A1.5 summarises their findings.

c) In reducing coliform levels in bathing waters - shown in Table A1.6.

Table A1.4 Average reduction in coliforms with a dose of 20 mg l^{-1} PAA in raw sewage

Site	Arithmetic Mean Log Reduction	
	Total Coliform	Faecal Coliform
Low Level Tanks	0.62	0.57
Dead Lake	1.30	2.00
Rusty Anchor	0.89	0.39

Source: Atkinson *et al.* (1989)

The data show that at doses of 15 and 20 mg l^{-1} it was possible to obtain substantial reductions in numbers of both TC and faecal coliforms prior to discharge and that this reduction substantially improved water quality in the immediate vicinity of the discharge. Coliform concentrations in bathing waters were less affected, possibly due to other discharges, regrowth or reactivation.

Table A1.5 Effect of PAA treatment on bacterial levels near Rusty Anchor Outfall

Site	Percentage Samples Failing EC Directive	
	No Treatment	PAA Treatment
Plume - All samples	70	46
- surface	84	83
- middle	84	29
- bottom	42	25

Source: Atkinson *et al.* (1989)

Table A1.6 Effect of PAA treatment on TTC levels in bathing waters

Beach	Log Reduction of TTC
Devils Point West	0.28
Devils Point	0.51
Rusty Anchor	0.28
Pebbleside	0.40
Tinside	0.31
Lions Den	0.38
East Hoe	0.59

Source: Atkinson *et al.* (1989)

A1.4 Disinfection Experiments at Trearddur Bay

A1.4.1 Introduction

The outfall at Porth Gwr Mawr discharges a dry weather flow of 1500 m^3 day^{-1} of untreated sewage into the Trearddur Bay approximately 100 m beyond Mean Low Water Mark. The Bay also receives septic tank effluent from a caravan site. As a result of these discharges there is an aesthetic problem, and beaches in Trearddur Bay have occasionally failed to comply with the EC Bathing Water Directive.

Experiments were carried out in 1990-91 to assess the impact of PAA (Wallace Evans Ltd., 1991) prior to full scale implementation. The results are summarized below.

A1.4.2 Results

The laboratory trials involved collecting 2 litre samples, which were inoculated with *Salmonella enteritidis* and poliovirus 2 (10 ml, tissue culture derived suspension). These were stirred for 4 hours at room temperature and then dosed with PAA at doses of 10, 12 and 15 mg l^{-1} and sampled after 0, 2, 5, 10 and 15 minutes.

Results showed >2 log reduction in *E.coli* and TC after 10 minutes or more contact with 10, 12 or 15 mg l^{-1}. FS results were variable with up to 2 log reductions achieved. Initial results for Salmonella were inconclusive, but an amended methodology suugests that 2 log reduction was consistently achieved at 12 mg/l (Welsh Water, pers. comm.).

A1.5 Field Trials at Menagwins STW

A1.5.1 Introduction

The treatment plant at Menagwins serves a population of about 20,000 and treats a dry weather flow of 8,600 m^3 day^{-1}, with 50% higher flows in the summer. Flows up to 225 l s^{-1} receive secondary treatment by percolating filters. The effluent is discharged to the St Austell river which reaches the sea at Pentewan Beach, 4 km downstream.

PAA dosing into the flow from the biological filters upstream of the humus tanks was carried out during the period 10 August - 4 December 1989 at a concentration which varied from 4-6 mg l^{-1}. Samples were collected at points in the effluent, the river and the waters close to the beach to monitor the impact on the concentrations of TTC, FS, viruses and Salmonella.

A1.5.2 Results

The results for TTC are summarised in Table A1.7.

Table A1.7 Results for TTC at Menagwins STW

Sample Run	PAA Dose (mg l^{-1})	Arithmetic Mean Log Reduction
2	6	2.2
3	6	3.7
5	6	3.5
6	4	1.5

Source: Realey & Brogden (1990)

It was concluded that the relatively low reduction in Sample Run 2 was due to a peak coliform load passing through the system and that PAA at 6 mg l^{-1} could be expected to reduce TTC numbers by 3 orders of magnitude. It was noted, however, reductions were variable and on some occasions no reduction was recorded.

The results for FS are summarised in Table A1.8.

Table A1.8 Results for FS at Menagwins STW

Sample Run	PAA Dose (mg l^{-1})	Arithmetic Mean Log Reduction
3	6	2.0
5	6	1.3
6	4	0.4

Source: Realey & Brodgen (1990)

These results indicate that FS appear more resistant than TTC to PAA. Levels of TTC and FS close to the beach did not appear to be greatly affected by the PAA disinfection, which suggests that Menagwins STW may not be the main source of the bacterial pollution. However, reactivation could provide an alternative explanation. Due to the low numbers of viruses and Salmonella in the sewage, it was not possible to draw any conclusions about reductions due to PAA.

Occasional tests on other bacteria showed that *Pseudomonas sp.* responded in a similar way to FS and *Staph. aureus* and *Campylobacter* were more resistant. Results for F^+ Coliphage indicated that a dose of 4 mg l^{-1} PAA had little effect, while a dose of 6 mg l^{-1} PAA achieved a 1 log arithmetic mean reduction.

A1.6 Field Trials at Bexhill-on-Sea

A1.6.1 Introduction

Experiments were carried out in a sewer in London Road, Bexhill-on-Sea where suspended solids removal is carried out by Hydro-dynamic separators. Storm conditions were simulated by blocking the normal channel, causing crude sewage to enter the separator. In the second half of the trials, mains water was added to dilute the sewage. *Oxymaster* was added to the overflow after the separators in doses of 10, 15 and 25 mg l^{-1} PAA. Problems were encountered in blocking the separators, and in obtaining sufficient water to dilute the sewage.

A1.6.2 Results

The results of the trials are summarised in Table A1.9.

Table A1.9 Effects of PAA at Bexhill-on-Sea

Experiment Number	PAA dose mg l^{-1}	Arithmetic Mean Log Reduction for TTC	Arithmetic Mean Log Reduction for FS
1	25	1.9	>4.0
2	15	1.6	>2.7
3	10	2.2	>4.0
4	25	2.4	>4.0
5	15	2.0	>4.0
6	10	2.1	>4.0

Source: Realey (1989)

The results for TTC are in agreement with other observations that a 2-3 log reduction can be obtained in crude sewage using around 15-20 mg l^{-1} PAA. It is surprising and interesting to note that a dose of 10 mg l^{-1} achieved a similar result.

The results for FS are also surprising and are not consistent with any other published data set. In comparison laboratory tests with simulated stormwater by Realy & Brogden (1991) gave FS reductions of 2-3.5 log reduction for diluted Menagwins sewage and 0-2.4 log for diluted Stevenage sewage.

A1.7 Field Trials at Porthtowan

A1.7.1 Introduction

Porthowan STW discharges a secondary treated effluent to a small stream that flows into the sea approximately 2 km downstream. The works consists of primary sedimentation tanks followed by biological filters and serves 1,700 people in the winter. The winter average DWF is quoted as 302 m^3 day^{-1} (Roddie *et al.*, 1991a), and the sewage is described as mainly domestic with a moderate level of infiltration.

The trial was designed to answer some of the questions raised by the Menagwins trial. A contact tank was therefore installed just prior to the works discharge. The tank was designed to achieve a residence time in excess of five minutes. Lithium tracer studies confirmed that the tank performance was satisfactory at flow rates below 2.9 times winter DWF.

Dosing was carried out in early November and late December 1990 at doses of 4 and 6 mg l^{-1} PAA. Samples were collected at various points and analyses carried out routinely for TTC and FS. In addition non-routine analyses were carried out for enterovirus, rotavirus, F^+ coliphage, *Pseudomonas aeuruginosa*, *Staphylococcus aureua*, *Campylobacter* and Salmonella. In some cases samples were duplicated and sent to separate laboratories to compare enumeration methodologies.

A1.7.2 Results

Caution should be exercised in interpretation of the results since Roddie *et al.* (1991a) report that the nominal PAA doses may have been exceeded in some cases.

The results summarised in Table A1.10 indicate that the efficacy of the disinfection system was somewhat related sewage flow, since, comparing runs 2a and 3 to run 4, higher flows appear to be associated with lower reductions for the same PAA dose. In addition, the median flow when TTC is less than the limit of detection is lower than when TTC is detected in runs 2a and 3. Table A1.11 summarises information on the efficacy of PAA against TTC and FS. As numbers were below detection limit for a large number of samples taken in runs 2a and 3, mean reduction figures could not be produced in these cases.

Table A1.10 Results for TTC at Porthtowan STW

Run	Nominal dose (mg l^{-1} PAA)	Median flow when TTC<LOD ($l\ s^{-1}$)	Median flow when TTC>LOD ($l\ s^{-1}$)	Median flow over run ($l\ s^{-1}$)	% of samples where TTC>LOD
1	0	-	-	3.00	-
2a	6	2.5	7.0	3.75	25
2b	4	-	4.0	4.00	100
3	6	2.5	4.0	3.25	42
4	6	-	9.0	9.0	100

LOD = Limit of Detection

Source: Roddie *et al.* (1991a)

The results indicate that PAA is more effective against TTC than against FS, but that the reductions achieved are variable. The authors report that this could not be related to sewage variables. It is possible that it is related to variations in retention time, which the authors warn may not be closely related to flow, presumably because of short-circuiting.

The results of the analyses for non-routine organisms showed considerable variation between laboratories, indicating that further work on standardising methods of enumeration is required and that the level of uncertainty in the results is high. The duplicating laboratory detected consistently higher numbers for F^+ Coliphage, *Pseudomonas* and Salmonella than did the main laboratory, but detected enterovirus and rotavirus less often. Only F^+ Coliphage and *Pseudomonas* were detected with high frequency in the samples. A summary of results of all the runs are presented in Table A1.12.

Table A1.11 Efficacy of PAA against TTC and FS at Porthtowan STW

Run	TTC		FS	
	Log reduction	% in range	Log reduction	% in range
2a (6 mg l^{-1} PAA dose)	2.5-3	25	2.5-3	25
	3-3.5	31.25	3-3.5	18.75
	3.5-4	12.5	3.5-4	0
	>4	31.25	>4	56.25
2b (4 mg l^{-1} PAA dose)	2.30 mean log reduction	-	1.54 mean log reduction	-
3 (6 mg l^{-1} PAA dose)	2.5-3	4.75	1.5-2	4.75
	3-3.5	14.25	2-2.5	4.75
	3.5-4	0	2.5-3	0
	>4	81	3-3.5	0
			>3.5	90.5
4 (6 mg l^{-1} PAA dose)	3.60 mean log reduction	-	3.35 mean log reduction	-

Extracted from Roddie *et al.* (1991a)

Table A1.12 Results for pathogenic bacteria and viruses (log reduction)

	Trial							
	2a		2b		3		4	
Determinand	M	D	M	D	M	D	M	D
Rotavirus	N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R
Enterovirus	=	N/R	=	N/R	=	N/R	=	N/R
F+ Coliphage	N/R	>0.7	N/R	>0.3	N/R	>0.2	=	>1.3
Pseudomonas	N/R	>>>	N/R	>0.2	>>>	0.15	>0.3	>1.2
Staphylococcus	N/R	N/R	N/R	N/R	>1.0	N/R	>>>	N/R
Campylobacter	N/R	>>>	N/R	N/R	N/R	N/R	N/R	N/R
Salmonella	N/R	N/R	N/R	=	>1.0	>>>	N/R	>1.0

Source: Roddie *et al.* (1991a)

>>> numbers reduced to below limit of detection
> log reduction greater than indicated value
= no clear effect on numbers
N/R no results
M main laboratory
D duplicating laboratory

It can be seen that the log reductions observed are frequently low in comparison with those for TTC and FS, but that the uncertainties involved are too great to allow definitive conclusions to be drawn.

A1.8 Field Trials at Trevaunance Cove

A1.8.1 Introduction

Sewage from St Agnes is discharged into the sea via a short sea outfall approximately 25 m in length at Trevaunance Cove. The sewage flow passes through a 1.5 mm fine screen prior to discharge, and is intermittent because all the sewage is pumped to the outfall. After screening the flow passes to a holding tank. The winter DWF is given as 430.4 m^3 day^{-1} in the report by Roddie *et al.* (1991b). This is from an estimated winter population of 2,400, rising to approximately 4,000 in summer.

PAA dosing was carried out in July and August 1991. PAA was added downstream of the screening plant prior to the holding tank. A lithium tracer study indicated that the contact time was around 2 minutes for much of the influent sewage. However, it was found that most of the disinfectant was eliminated even in this short period.

A1.8.2 Results

The results observed are summarised in Table A1.13.

Table A1.13 Efficacy of PAA against TC and FS at Trevaunance Cove

Sampling run	Dose (mg l^{-1} PAA)	TTC Log reduction		FS Log reduction	
		Mean	Standard deviation	Mean	Standard deviation
2a	12	1.6	0.8	1.0	0.7
2b	14	2.1	0.6	1.1	0.4
2c	16	2.1	0.5	1.1	0.4
3	20	2.1	0.9	1.9	0.9
4	20	2.0	0.5	1.5	0.5
5	20	2.1	0.9	1.5	0.7

Compiled from Roddie *et al.* (1991b)

Once again FS was found to be more resistant than TTC to PAA and considerable variation in performance was found. It should be noted that the relatively poor performance in this trial may be at least partially due to the short retention time obtained in the contact tank.

Data on 'non-routine' microbiology were gathered in a similar way to those for Porthtowan. However, it was concluded from the duplicate analyses conducted that the data acquired could only be used to indicate presence or absence. It was stated by Roddie *et al.* (1991b) that, on the basis of the limited data from this trial, PAA did not appear to be effective in reducing the numbers of pathogenic bacteria and viruses. Evidence for this statement was observed for enterovirus, *Pseudomonas aureginosa*, *Staphylococcus aureus*, Salmonella and F^+ Coliphage, but the results appear variable and inconsistent.

Microbiological analyses were conducted for mussels placed in two rafts approximately equidistant from the outfall and for water samples at these sites. *E. Coli* counts in the water decreased by an average of 43% post-disinfection at raft 1 and by 86% at raft 2. For the mussels the average reductions observed were 65% and 61% respectively. Analyses for F^+ Coliphage showed a small reduction at raft 2, but no reduction at raft 1.

Relationships between sewage chemistry and disinfection efficiency were examined. It was found that low levels of BOD, COD, SS and ammonia were associated with more effective disinfection. It was also found that increasing peroxide concentrations were related to better kill rates, possibly indicating that the presence of peroxide in the effluent can be used as a dose rate controller.

Part B

The Environmental Effects of PAA

1 CHEMISTRY OF PAA

1.1 *Oxymaster* Formulation and Trace Constituents

PAA is supplied by Interox as *Oxymaster*, which has a typical composition by weight as detailed below:

PAA		12-13%
Hydrogen peroxide		19-20%
Stabiliser	approx.	1%
Acetic acid	approx.	18%
Water	approx.	49%

The stabiliser used in *Oxymaster* is sulphuric acid (Interox, pers. comm.). Other materials may be used as stabilisers in the hydrogen peroxide in small quantities, but commercial considerations preclude detailed specification (Interox, pers. comm.). Raw materials specifications made available by Interox indicate that the impurities detailed in Table 1.1 may be present in the hydrogen peroxide.

Table 1.1 Impurities in *Oxymaster*

Chemical	Typical concentration (mg l^{-1})
Cl^-	<1
PO_4^{3-}	150 - 250
NO_3^-	70 - 150
$Fe^{2+ \text{ and } 3+}$	0.1 - 0.25
Ni^{2+}	0.02 - 0.05
Non-volatile matter (described as "salts")	300 - 500

Source: Interox pers. comm.

Impurities present in the acetic acid are listed in Table 1.2 from data supplied by Interox. It is likely that the acetaldehyde and formic acid will be at least partially oxidised to PAA and performic acid respectively on addition of hydrogen peroxide. Without a knowledge of the exact formulation for the preparation of *Oxymaster* it is not possible to quantify the concentrations of these impurities being added to the effluent stream. However, at a dose rate of 20 mg l^{-1} PAA a dilution of 6 x 10^3 will be achieved in the effluent stream.

Table 1.2 **Impurities Present in Acetic Acid**

Chemical	Concentration
acetaldehyde	<0.05%
formic acid	<0.15%
heavy metals	<1 mg l^{-1}

Source: Interox pers. comm.

1.2 Disinfection Residuals

1.2.1 *Oxymaster* Residuals

This Section is intended to discuss the residuals arising from the chemicals in the *Oxymaster* formulation rather than the potential for by-product generation, which is discussed in Section 1.6.

As *Oxymaster* is an equilibrium mixture, if a constituent on one side of the equilibrium is consumed by a separate reaction, the equilibrium will move to replenish that constituent. Therefore, if PAA is used up rapidly, more PAA will be generated by the reaction between hydrogen peroxide and acetic acid. Interestingly, acetic acid is also produced when oxidation is effected by PAA. This will therefore tend to reinforce the shift in the equilibrium. This means that a PAA dose stated in terms of the initial concentration in the mixture will tend to under-estimate the dose of PAA actually delivered. However, the initial PAA concentration is regarded as the best objective measure of PAA dose applied, since the actual dose will be dependent on the rate of direct reaction of hydrogen peroxide with the sewage, relative to that for PAA.

It is clear that, however the reaction proceeds, acetic acid is an end product. It is calculated that 1 mg l^{-1} of initial PAA dose will lead to a total acetic acid residual concentration of 2.3 mg l^{-1} (Baldry *et al.*, 1990). It is therefore expected that the main residual would be acetic acid with possible traces of PAA and hydrogen peroxide, and possibly very small quantities of sulphuric acid.

1.2.2 Testing Methods

To date no tests have been carried out specifically for acetic acid residuals, because, although it is clear that this is the primary residual product (Baldry *et al.*, 1990), the presence of acetic acid can be inferred from some of the reported BOD and COD results, as discussed in Section 1.3.1. The main points of discussion in the literature concern testing for residual PAA, and, to a lesser extent, for hydrogen peroxide.

In many of the field trials conducted on PAA the residual levels of peroxygen have been measured using a PAA test kit patented by Interox. This is a colorimetric method based on the production of free chlorine by the oxidation by PAA of added chloride and then the use of N,N-diethyl-phenylenediamine (DPD) to produce a coloured complex (Fraser, 1987). The claimed limit of detection is approximately 1 mg l^{-1} total peroxygen (excluding hydrogen peroxide). This lack of sensitivity is attributed by Interox to the fact that PAA does not produce a stoichiometric release of chlorine (i.e. there is no energy gradient driving the reaction). A method based on the stoichiometric release of iodine from iodide is currently under development by Interox, and has shown a limit of detection for PAA of approximately 0.1 mg l^{-1} in laboratory trials with fresh water. However, spurious results have been found when the method is used in sewage effluents (Interox pers. comm.). Crathorne *et al.* (1991) have questioned the use of the chloride-based technique because of the slow rate of liberation of free chlorine by PAA and have used an iodide-based technique similar to that under development by Interox.

Interox claim that the competitive removal by hydrogen peroxide of the free chlorine generated is negligible, as the rate of reaction between chlorine and DPD is much faster. This can only be confirmed or otherwise by comparison of the relevant rate constants and consideration of the relative concentrations. However, it is claimed that the rate of removal of chlorine by hydrogen peroxide is sufficiently fast to prevent significant levels of free chlorine being produced under operational conditions (Baldry *et al.*, 1990). Section 1.4.3 presents further discussion on the production of free chlorine by the reaction of PAA with chloride ions.

During field trials at Plymouth (Atkinson *et al.*, 1989) levels of residual peroxygen were measured using a Merckoquant 100 11 test kit manufactured by Merck BDH . This method is understood to be based on the liberation of oxygen from both hydrogen peroxide and PAA by the action of peroxidase and then the formation of a coloured oxygen complex. The manufacturers claim a limit of detection of approximately 0.25 mg l^{-1} total peroxygen.

At both Trevaunance Cove and Porthtowan (Roddie *et al.*, 1991a & 1991b) an iodine-based technique similar to the described by Crathorne *et al.* (1991) was used to detect residual PAA. The authors point out that a weakness of this technique is that monochloramine (MCA) cannot be distinguished from PAA. Therefore the inherent assumption in the results quoted for PAA residuals is that MCA is not formed because PAA cannot rapidly oxidise chloride. The presence of free halogens including bromamines is also detected by this test, but at a separate stage, so that due allowance can be made. Another problem is that, since the technique is photometric, a relatively clear sample is required. This means that the samples must be centrifuged prior to analysis. The consequent delay could have led to the decay of some PAA. The detection limit is quoted as 0.01 mg l^{-1}.

Hydrogen peroxide may be detected by a variety of methods of varying sensitivity. During field trials at Menagwins (Realey & Brogden, 1990), indicator strips were used to give a qualitative assessment of the level of hydrogen peroxide present in the effluent. A method based on potassium titanium oxalate, with a detection limit of 1 mg l^{-1} has been used at some (unspecified) trials (Interox, pers. comm.). In the field trials at Porthtowan and Trevaunance Cove hydrogen peroxide was measured using a standard technique involving the peroxidase catalysed reaction of DPD. The detection limit of this method was quoted (Roddie *et al.*, 1991b) as 0.01 mg l^{-1}. It is stated that an experiment was conducted to establish that PAA did not interfere with this method.

1.2.3 Results for PAA and Hydrogen Peroxide

Field trials of PAA disinfection at several coastal sites (Thomas and Dillon, 1989) showed no detectable residual peroxygen in the sewage plumes at PAA doses of 10, 15 and 20 mg l^{-1}. However, the minimum level of detection for the residual peroxygen is variously reported as 0.5 mg l^{-1} (Gould & Fraser, 1991) and 1.0 mg l^{-1} (Interox, pers. comm.).

During field trials at the Menagwins sewage treatment works (Realey and Brogden, 1990) the levels of hydrogen peroxide were measured on several occasions during a single day's sampling. It is reported that low levels of hydrogen peroxide persisted in the effluent discharge some 4-5 hours after PAA dosing. However, the discussion is qualitative only and the method of detection used (indicator strips) is not particularly precise. In addition, no control experiments were undertaken to check for the absence of hydrogen peroxide in the untreated effluent stream, although the presence of peroxide would be unusual.

It has been generally assumed that PAA is rapidly consumed when it is added to the sewage, but Harakeh (1987) found that PAA did not react rapidly with secondary effluent. After 30 minutes contact time no measurable change was found in PAA levels compared with the initial concentration. This is surprising considering that PAA was observed to have disinfectant action within this time period. No details of the testing procedure for PAA were given.

In field trials at Porthtowan STW, Roddie *et al.* (1991a) found significant quantities of PAA and peroxide in the effluent as shown in Table 1.3.

Table 1.3 Residual Oxidants in sewage at Porthtowan - PAA dose = 6 mg l^{-1}

Date - Time	Site	Conc. of PAA (mg l^{-1})	Conc. of Peroxide (mg l^{-1})
4/12/90 - 1800	Pre-disinfection	0	0
	Post-disinfection	9.1	39
5/12/90 - 0600	Pre-disinfection	0	0
	Post-disinfection	2.2	79
11/12/91 - 1600	Post-disinfection	10	76
11/12/91 - 1800	Post-disinfection	16	
11/12/91 - 2000	Post-disinfection	11	41
12/12/91 - 0600	Post-disinfection	12	51
12/12/91 - 0900	Post-disinfection	9.3	39

Source: Roddie *et al.* (1991a)

These results show that high concentrations of PAA and peroxide were present in the effluent. Taking into consideration the nominal dose rate of 6 mg l^{-1} PAA and the 5-minute contact time these results are surprising. However, they are confirmed to a large extent by measurements in the river that show PAA concentrations ranging from 0.17 to 0.69 mg l^{-1} and measurements of significant bromine and organic bromamine concentrations in the effluent.

It is clear that these results cast doubt on the performance of the dosing system and mean that the results for reductions achieved in microbial numbers for this trial may not correspond to the nominal dose rate. Indeed, Roddie *et al.* (1991a) show a strong correlation between TTC kill and peroxide in the effluent. It is therefore possible that some of the variation in reductions achieved may be related to variations in the actual dose rate and that residual peroxide could be used as a marker for dose rate. However, there was a poorer relation between concentration of peroxide and concentration of PAA, so that it would probably not be wise to rely entirely on peroxide concentration as an indicator of residual oxidants.

The results reported by Roddie *et al.* (1991b) for residual PAA and hydrogen peroxide at Trevaunance Cove are shown in Table 1.4.

Table 1.4 Residual disinfectants at Trevaunance Cove - PAA Dose 20 mg l^{-1}

Date - time	Site	Conc. of PAA (mg l^{-1})	Conc. of hydrogen peroxide (mg l^{-1})
15/8/90 - 0600	Pre-disinfection	<0.01	<0.01**
	Post-disinfection	2.0	12.3
15/8/90 - 0900	Pre-disinfection	<0.01	<0.01**
	Post-disinfection	0.02	3.1
11/9/90 - 1600	Post-disinfection	<0.01	9.9
11/9/90 - 1900	Post-disinfection	<0.01	2.8
12/9/90 - 0645	Pre-disinfection	<0.01	<0.1
	Post-disinfection	0.45	16.3
12/9/90 - 0915	Pre-disinfection	<0.01	<0.1
	Post-disinfection	<0.01	<0.1

** measured in undiluted sewage

Source: Roddie *et al.* (1991b)

These results show much lower concentrations of disinfectant residuals than the results at Porthtowan, despite the short retention time of the contact tank (less than 2 minutes for much of the sewage). This may be indicative of better performance by the dosing system, and/or the greater capacity of raw sewage to react with PAA and hydrogen peroxide.

1.3 The Effect of PAA Addition on Principal Components of Final Effluent Chemical Quality

The treatment of an effluent stream with PAA may affect the major indicators of chemical quality of the discharge in terms of the following:

- organic quality (BOD and COD);
- pH;
- suspended solids; and
- ammonia content.

1.3.1 Organic Quality

Observed effluent organic quality in terms of BOD could be affected by PAA influencing the real BOD (and COD) of the effluent and/or treatment with PAA affecting the test employed. It is postulated that PAA treatment could affect the BOD test by producing a residual reduction in microbial activity leading to an apparent reduction in the BOD of the sample, unless the procedure for BOD testing involved reseeding of the sample. In addition both the BOD and the COD of samples could be affected by the following factors:

- residual acetic acid contributing to the organic load;
- oxidation of organic matter by PAA; and
- oxidation of refractory compounds to more bio-degradable compounds.

Acetic acid is reported in standard texts to exert a BOD_5 of approximately 62% w/w. Clearly for dose rates ranging from 4-20 mg l^{-1} PAA there is potential for elevation of BOD levels in final effluent and also for the lowering of final pH. Given that for 1 mg PAA added 2.3 mg acetic acid arise in the final effluent (Section 1.2), this effect in isolation would give an increase in BOD_5 of approximately 1.4 mg BOD per mg PAA added. Experimental work by Interox has confirmed that *Oxymaster* does increase the BOD_5 of secondary effluent (Corner and Fraser, 1991). However, Interox has also conducted experiments indicating that *Oxymaster* can reduce the BOD of raw and settled sewage (Interox, pers. comm.).

In controlled laboratory experiments undertaken by Interox it has been found that the addition of *Oxymaster* increased the BOD_5 of a final effluent with an initial BOD_5 of 35 mg l^{-1} as detailed below in Table 1.5. Thomas *et al.* (1989) reported that in laboratory trials for the evaluation of *Oxymaster* for secondary effluent disinfection increased levels of COD and TOC were observed and BOD levels varied erratically. The COD and TOC are reported as increasing by 2.5 and 1.2 mg l^{-1} respectively, per mg l^{-1} of added PAA.

Realey and Brogden (1990) also noted that dosing of PAA prior to the humus tanks caused an increase in filtered BOD in the Menagwins STW (secondary) effluent. Typically the BOD increased by 6-7 mg l^{-1} for a 6 mg l^{-1} PAA dose.

Roddie *et al.* (1991a) in their report on Porthtowan measured the changes in routine chemical determinands in the effluent between point 1 (pre-disinfection) and point 2 (post-disinfection) are shown in Table 1.6.

Table 1.5 **Effect of PAA on the BOD of Secondary Effluent**

Dose of PAA ($mg\ l^{-1}$)	Increase in BOD_5 ($mg\ l^{-1}$)
4	4.1
6	9.4
8	16
10	28

Source: Interox pers. comm.

Table 1.6 Changes in routine chemical determinands after disinfection at Porthtowan

Run	Dose ($mg\ l^{-1}$)	BOD ($mg\ l^{-1}$)	COD ($mg\ l^{-1}$)	PO_4 ($mg\ l^{-1}$)	pH	SS ($mg\ l^{-1}$)
1	0	21.00	102.00	7.60	7.00	30.00
		% change	% change	% change	% change	% change
2a	6	284	88	7.7	-14.9	6.7
2b	4	100	28	3.7	-4.5	-23.0
3	6	291	101	1.1	-17.8	-20.0
4	6	233	102	-14.5	8.9	-23.0

Source: Roddie *et al.* (1991a)

It can be seen that the main effect was a very large increase in BOD and COD that appeared to be related to nominal dose. This effect was more dramatic than previously reported, despite the use of similar methods for BOD analysis and could possibly be explained by overdosing.

At Trevaunace Cove Roddie *et al.* (1991b) carried out the most detailed study to date on the effect of PAA disinfection on raw sewage. On average the results for BOD and COD showed a repeated but non-significant increase in BOD and COD values. However, it was observed that the BOD and COD tended to increase after treatment with PAA during periods of low flow and decrease during periods of high flow. It is difficult to associate quality differences with flow, but in this case it appears that low flow is normally associated with better quality.

The Interox measurements indicate a non-linear response of BOD to added PAA. Simple addition of acetic acid to the effluent alone cannot explain this. A plausible, but untested hypothesis could be that compounds that would otherwise not be oxidised in the BOD test may be initially oxidised by *Oxymaster* to more easily degraded substances which then exert a BOD. The mechanism of changes in BOD in primary settled and raw sewage is difficult to explore, since the background BOD for raw and settled sewage is much greater than for secondary effluent.

1.3.2 Other Indicators of Chemical Quality

Realey and Brogden (1990) report that PAA treatment prior to humus tanks increased the suspended solids and ammonia levels in the final effluent, and lowered the final pH value. Interox dispute some of these findings and highlight the lack of control sampling during the trial (Interox Environmental Services Team, 1990). It is difficult to make meaningful comparisons of the effluent quality before and after PAA dosing, since the trial was inadequately controlled for normal variations in the effluent quality at the works.

Realey and Brogden (1990) suggested that the SS increase may have been due to the disinfectant altering the particle size distribution within the effluent, either physically or by reducing microbial activity, and thence reducing the efficiency of the humus tanks. Interox suggested that the increase in suspended solids may be due to the flotation of the sediment by oxygen released from PAA and hydrogen peroxide within the humus tank (Interox Environmental Services Team, 1990).

Realey and Brogden (1990) report a drop in final effluent pH in the order of 0.5-1 pH units at a dose rate of 6 mg l^{-1} PAA. A controlled laboratory experiment indicated a pH reduction of 0.5 units. However, examination of the field data indicate that the pH of the effluent was unusually low even without PAA dosing and, on the three occasions when the pH was measured both prior to the dosing point and after the point of disinfection, considerable variation in pH was observed. In one case a lowering by 0.1 unit was reported and in another a 0.8 unit reduction. In the third case an increase in pH of 0.5 units was reported, casting some doubts on the accuracy of the pH results from the trial. Thomas *et al.* (1989) report that in laboratory tests on secondary effluent pH levels were also reduced by approximately 0.5 units over the range examined. Interox believes that a reduction in pH of approximately 0.1-0.2 units would be expected for secondary effluent dosed at 6 mg l^{-1} PAA (pers. comm.). Clearly, there is potential for the pH of sewage effluent to be lowered by *Oxymaster* addition, with the actual reduction depending upon the initial pH and buffering capacity of the sewage used (e.g. water hardness).

A rise in the ammonia-N present after dosing is also reported for the Menagwins trial. No explanation for this phenomenon is offered. It is possible that this is associated with the apparent impairment of settling in the humus tank, but this depends on the extent to which the ammonia in the effluent is in solid form.

Table 1.6 shows the effect of disinfection on phosphate, pH and SS at Porthtowan. It can be seen that phosphate concentrations increased slightly on three of four trials and pH and SS concentrations decreased in 3 of 4 trials. The authors suggest that the increase in pH observed was probably anomalous and should be treated with caution. The maximum reduction in pH observed was 1.2 units, while SS decreased by up to 6.9 mg l^{-1}. Ammonia concentrations decreased by 3.8% in run 2a, showed no change in run 2b, increased by 1.3%

in run 3 and showed no change in run 4. It therefore appears unlikely that ammonia levels exhibit any systematic change.

Roddie *et al.* (1991b) state that at Trevaunance Cove the values of pH in the treated effluent were generally lower by 0.2-0.4 units, although considerable variation outside that range was observed from time to time. On average ammonia decreased in all six runs, SS decreased in five of six runs and orthophosphate decreased in three out of five runs. It should be noted that some of these quality changes are probably due to the screening plant, and so it is difficult to draw any firm conclusions about the effect of PAA on these variables except that it is not very significant in this context.

1.4 By-Product Formation

1.4.1 Theoretical Considerations

Oxymaster is an aqueous equilibrium mixture containing two strong oxidising agents, PAA and hydrogen peroxide. CES (1988) reported that concern had been expressed regarding harmful by-products that may be formed in reactions with the constituents of sewage during disinfection. Research into the formation of by-products has been fragmented and has tended to concentrate on the possible reactions of PAA and hydrogen peroxide with organic chemicals present in sewage, and also the oxidation of halides to halogens and thence the formation of halogenated organic species. There has also been limited consideration of the effect upon heavy metal concentration and bio-availability in final effluents.

It has been suggested (Crathorne *et al.*, 1991) that by-products could arise from reactions of PAA with organic compounds in sewage, including the possibility, in principle at least, that amines could be oxidised to N-oxides and hydroxylamines and that epoxides may be formed from the unsaturated compounds present. In addition it is suggested that further peracids and other peroxides could theoretically be generated.

Interox refutes these suggestions (Baldry *et al.*, 1990), suggesting that PAA is unlikely to react with organic compounds in waste water at the typical dose rates and conditions. It is emphasised that, although PAA is used for epoxidation and oxidation in synthetic organic chemistry, specific conditions of temperature, solvent, pH and/or catalysts are required which are not encountered in sewage. Whilst this is true when high yields are desired, there is very little information in the literature on the dilute aqueous phase chemistry of PAA, and it is not possible to discount entirely the formation of by-products from reaction with organic chemicals. However, GC/MS analysis of field samples has provided some evidence on this aspect, which is discussed in Section 1.4.2. It is clear from theoretical considerations that there will be competing reactions for both PAA and hydrogen peroxide occurring in sewage media, including: disinfection by PAA; molecular decomposition of hydrogen peroxide by enzymes; and the oxidation of highly oxidisable species such as hydrogen sulphide and mercaptans.

Interox literature states that the formation of toxic organic derivatives through the action of *Oxymaster* on biological organic substrates is extremely unlikely to occur at the application concentrations used and under typical operational conditions (Fraser, 1987). It is suggested that at low concentrations *Oxymaster* will be incapable of the formation of epoxides, peroxyaldehydes, peroxy-acids or keto-peroxides and that the only reaction products likely to be detected are acetic acid and oxygen (Atkinson *et al.*, 1989). However, no controlled laboratory experiments appear to have been undertaken by Interox.

It is clear that PAA in aqueous solution will oxidise halide ions to halogens which then exist in solution as hypohalogous acids or anions, commonly known as free available halogen. Any free available chlorine produced will react quickly with bromide or iodide ions in solution to give chloride and free available bromine or iodine. Hence, in any solution of mixed halides it is most likely that free available bromine and iodine will be found. Hypobromous and hypoiodous acids can in turn react with organic compounds to form organohalogen derivatives (Crathorne *et al.*, 1991).

Baldry *et al.*, (1990) state that the potential for formation of chlorinated organic compounds from the reactions of free chlorine generated by PAA use is small. The rapid rate of decline in PAA concentration in sewage, combined with the presence of hydrogen peroxide, and the reaction of the latter with available chlorine is cited as meaning that there are unlikely to be any significant levels of free chlorine existing under operating conditions.

Whilst it is accepted by Interox (Baldry *et al.*, 1990) that reactions between amines and peroxygen are known to occur, the normal reaction conditions involve high concentrations and elevated temperatures. Again, little information is available in the literature on the chemistry of such reactions in dilute aqueous conditions.

1.4.2 Organic By-products

Very few studies of the reaction products of PAA with organic materials typically found in sewage have been undertaken. The reactions of both humic and fulvic acid with PAA were determined by Schnitzer and Skinner (1974). Reaction products were predominantly phenolic acids, benzenecarboxylic acids and aliphatic acids. However, the oxidation was undertaken in glacial acetic acid at 40°C over 8 days.

Crathorne *et al.* (1991) studied the reaction products of PAA in raw sewage in controlled laboratory tests using GC/MS analysis. An apparent increase in the adsorbable organic halogen (AOX) concentration of approximately 29 $\mu g\ l^{-1}$ in the liquid fraction of PAA-treated sewage was reported. It was also stated that a large number of organic compounds were detected in the solvent extracts of sewage prepared for analysis, but there was little difference between the extracts before and after disinfection. The compounds responsible for the increased AOX were not identified by GC/MS, but were considered likely to be organobromine compounds.

It was suggested by the same authors that a wider range of disinfection by-products was not detected for reasons including:

i) the presence of a large number of compounds at high concentrations which were unaffected by the disinfectant and may have masked the presence of by-products at low concentrations;

ii) failure to extract products with the technique employed; and

iii) non-detection of by-products by GC/MS.

Analysis by GC/MS was also undertaken at Porthtowan (Crathorne *et al.*, 1991), and Menagwins (Realey and Brogden, 1990), Trevaunance Cove (Roddie *et al.*, 1991b) and further analysis was carried out at Porthtowan (Roddie *et al.*, 1991a). No major by-products were detected in any of these trials.

1.4.3 Studies of Halogen Liberation and the Formation of Halogenated Organics

Crathorne *et al.* (1991) have undertaken laboratory studies in which PAA (20 mg l^{-1}) was reacted with humic acid solutions (20 mg l^{-1}) in solutions containing potassium bromide or potassium chloride at concentrations ranging from 0.1 to 10 mg l^{-1}, buffered to pH 7. No significant concentrations of AOX or trihalomethanes were determined in the chloride solutions, but bromoform in concentrations up to 10.5 μg l^{-1} was detected in the bromide solutions and other organobromine compounds were indicated by AOX concentrations of up to 139 μg l^{-1}, expressed as chlorine, or up to 313 μg l^{-1} if entirely expressed as bromine. The authors interpret the lack of organochlorine compounds as evidence that PAA does not oxidise chloride to hypochlorous acid. In addition a similar unquantified effect was reported by the same authors for raw sewage treated with PAA.

In laboratory experiments reported by Baldry *et al.* (1990), the addition of 4 mg l^{-1} PAA to a solution of 20 g l^{-1} of chloride produced 0.3 mg l^{-1} of free chlorine in 20 minutes in the presence of the fast chlorine scavenger DPD. Investigations using raw sewage and doses of up to 15 mg l^{-1} PAA yielded <0.2 mg l^{-1} free chlorine, whilst the treatment of secondary effluent with 6 mg l^{-1} PAA yielded a maximum concentration of 0.6 mg l^{-1} of free chlorine. The initial chloride concentrations in the effluent are not given.

Testing for chlorine during trials at the Menagwins sewage treatment works (Realey and Brogden 1990) indicated that traces of chlorine were found just prior to dosing on two occasions and in a single river sample, possibly indicating the presence of free chlorine in the effluent or difficulties with the detection test. However, the discussion is not quantitative although a detection limit of 10 μg l^{-1} is quoted.

Analysis was undertaken for halogenated by-products during the Menagwins trial (Realey and Brogden 1990) with dosing of PAA at 6 mg l^{-1} and 4 mg l^{-1}. No evidence was reported of increases in the levels of five trihalomethanes (THMs), polychlorinated biphenyls (PCBs), polychlorinated phenols (PCPs) or hexachlorocyclohexane (γ-HCH) after disinfection. However, the only limit of detection quoted is 0.1 ng l^{-1} for PCBs.

Roddie *et al.* (1991a) carried out analyses for phenol and chlorinated phenols, and for bromine and organic bromamines. Significant quantities of bromine and bromamines were found in the effluent as shown in Table 1.7. The highest levels measured are known to affect bacteria and could be environmentally significant, depending on dilution, chemical conditions in the river and organisms present. Local ecological studies would have been required to attempt to quanify the potential effect. Analyses were also carried out for phenols, chlorinated phenols, adsorbable organohalides (AOX) and volatile organohalide compounds (VOCl's). It was found that, where phenol and chlorinated phenols were detected prior to disinfection, their concentrations were reduced by the disinfection process. The concentrations of VOCl's were consistently below the detection limits, which varied from 0.1 to 0.4 $\mu g\ l^{-1}$. The AOX measurements provided no evidence that AOX compounds were formed in the disinfection process.

At Trevaunance Cove bromine and bromamines concentrations prior to disinfection were less than detection limits, while after disinfection they ranged from 0.06-0.35 $mg\ l^{-1}$ and <0.02 to 0.45 $mg\ l^{-1}$ respectively, expressed in $mg\ l^{-1}$ as Br_2. The levels of phenolic compounds did not display a consistent pattern between pre and post-disinfected sewage and VOCl compounds (mainly chloroform) were present in similar concentrations both pre and post-disinfection.

Table 1.7 Residual bromine and organic bromamine in sewage at Porthtowan - PAA dose = 6 $mg\ l^{-1}$

Date - Time	Site	Conc. of Bromine ($mg\ l^{-1}$)	Conc. of Organic Bromamines ($mg\ l^{-1}$)
4/12/90 - 1800	Pre-disinfection	0	0
	Post-disinfection	0.61	2.5
5/12/90 - 0600	Pre-disinfection	0	0
	Post-disinfection	2.9	14.2
11/12/91 - 1600	Post-disinfection	0.45	6.2
11/12/91 - 1800	Post-disinfection	861.8**	1.3
11/12/91 - 2000	Post-disinfection	1	1.3
12/12/91 - 0600	Post-disinfection	0.89	6.2
12/12/91 - 0900	Post-disinfection	0.8	1.3

Source: Roddie *et al.* (1991a)

** The concentration of PAA was sufficiently high that a reaction with DPD occurred before the addition of KI. After two minutes, an appreciable pink colour had evolved without KI addition. Consequently the bromine concentration may be overestimated due to a contribution of the PAA colorisation of the DPD.

1.4.4 The Effect of PAA Addition on the Heavy Metal Content of Final Effluents

It has been suggested that dosing with PAA may alter the concentration, speciation and bio-availability of metals in sewage. Possible mechanisms by which this could arise include:

- change in oxidation state of metal ions;
- oxidation of organic complexes;
- reaction with insoluble metal sulphides in sewage;

- change in pH; and
- change in bio-availability of metals through reduced microbial activity.

The oxidation state of certain metals may be altered and metals bound in organic matrices could be released through PAA oxidation and destruction of the organic matrix (Wallace Evans, 1990). The results of analysis for metals at Menagwins sewage treatment works (Realey and Brogden, 1990) were inconclusive. Although they report no indication that PAA affected partitioning of metals, it is unclear whether the methods used were sufficiently sensitive to detect the level of metals found in typical domestic sewage.

In the Porthtowan field trial samples were analyzed for cadmium, chromium, copper, nickel, lead and zinc. PAA treatment was found consistently to increase the soluble fractions of cadmium, copper, lead and zinc by a small amount, although the total concentrations were generally not significantly affected. This is consistent with lowering of the pH of the effluent and possible break up of organo-metallic complexes.

Similar analyses were carried out on a limited number of samples at Trevaunace Cove. Roddie *et al.* (1991b) state that little difference was seen in the total concentrations with the exception of those of lead, which were consistently higher post-disinfection. In the baseline trial with no disinfection the proportion of metals in the soluble phase tended to decrease. In contrast the results from the disinfection trials indicate a greater portion of metals in the soluble phase, with certain exceptions. This is consistent with the results from Porthtowan.

Overall the increase in soluble metals observed was small and the change in the effect on the environment is likely to be commensurate with this change. This means that the discharge would have a marginally more detrimental effect on the environment during PAA-treatment.

1.4.5 Ecological Effects of Potential By-products

From an ecological standpoint halogenated organic compounds are of particular interest in view of their persistence in the environment. They are not readily degraded by chemical oxidation or bacterial action and are viewed by certain ecologists as "permanent" additions to the environment (Clark, 1989). Insolubility and high lipophilicity enables partition gradients to be established across biological membranes and creates the potential for bioaccumulation and biomagnification in aquatic food webs. This is aggravated by the capacity of organohalogens to adsorb on to suspended solids that might subsequently be ingested by suspension-feeding organisms (Section 3.4). The effects of bio-accumulation are difficult to determine or predict.

The potential of PAA to form hydroxylamines, amine oxides and epoxides has also been suggested on the basis of the standard non-aqueous chemistry of PAA, but evidence for their formation under normal field conditions is non-existent (Horth *et al.*, 1990). However, based on known toxicological data (for example, American Conference of Governmental Industrial Hygienists threshold limit values), should such compounds be produced, it would appear highly undesirable to introduce them into aquatic ecosystems.

In the Porthtowan trial (Roddie *et al.*, 1991a) bromine was not detected in the receiving water, although it was found in the effluent. This is consistent with the rapid decay expected.

However, bromamine concentrations in the receiving water did not show any evidence of decay, but were consistent with dilution only. This may be indicative of a potential for bioacumulation of such compunds.

1.5 Safety Considerations in the Dosing of Oxymaster

1.5.1 Operational Considerations

It is the stated objective of Interox that Oxymaster shall at no time be dosed into an effluent stream at a rate above the safe operational levels by a failure or malfunction of dosing equipment.

To meet this objective various features are incorporated into any system design, including:

- non-return valves and negative suction head to prevent syphoning from tanks;
- flowmeters between pumps and the point of application;
- effluent level monitors to assess the volume requiring treatment;
- single loop control systems to achieve proportional dosing;
- float switches to activate level monitors in storm systems.

A "closed loop" monitoring function can be operated between the level monitor and Oxymaster flowmeter with a feedback signal being supplied to the dosing pump. In addition, an on-line titrimetric analysis system is being developed to monitor and respond to the presence of residuals at the headworks prior to discharge.

Equipment is monitored by computer or programmable logic controller and will generate a suitable alarm signal in the event of a failure. Failure of level monitors, flowmeters or pump modulating equipment results in a shutdown of the system. In the event of a pump failure standby pumps can be brought on-line, or the system be shut down.

The sensitivity of the control system to variations in signals can be adjusted to the requirement of the operator. Interox state that in the interests of safety and efficiency they should be present during commissioning and calibration at each individual site.

In the light of the precautions taken, it seems surprising that at Porthtowan consistent overdosing appears to have occurred. Roddie *et al.* (1991a) do not comment on calibration procedures or describe efforts to identify the problem. It is therefore difficult to form any definitive conclusion from this trial about the performance of dosing systems.

1.5.2 The Release of Oxymaster Due to Major Leakage

Interox has examined the potential of leakage from an 18 tonne ISO tank, which would contain approximately 2160 kg of PAA, 3420 kg of hydrogen peroxide and 3240 kg of acetic acid. In this exercise Interox assumed that the tanker would be voided in one hour and all of the Oxymaster would find its way into an effluent stream flowing at approximately 50,000 m^3 per day. The resulting concentration of PAA and hydrogen peroxide would be 1,037 mg l^{-1} and 1,556 mg l^{-1}, respectively, if all the *Oxymaster* spilled went into the effluent stream. Clearly these concentrations would be greater if the effluent stream were of a lower volume. However, these concentrations of active ingredients would apply only at the end of any outfall and would decrease with dilution beyond the end of the outfall. This dilution would be dependent on water depth, jet velocities at any diffusers and current velocities.

The containers in which the solution is stored on-site are normally those in which it is transported. It appears more likely that an accident involving breach of a tank would occur in transit rather than when the tank is stationary at the site.

2 ECOLOGICAL STUDIES

2.1 Introduction

Despite concern expressed about the possible effects of PAA-dosed effluents on aquatic ecosystems, little direct scientific evidence exists to support or refute the cases made. In the few instances that ecological investigations have been undertaken in association with PAA trials in the UK, the data are either limited or have not been available for examination. This Chapter reviews the ecological information gained from studies in the UK.

2.2 St. Austell (Menagwins Sewage Treatment Works)

Trials were instigated to assess PAA disinfection of secondary effluent from the Menagwins STW as a means of achieving compliance with EC Bathing Water and Shellfisheries Directives. Commencing in July 1989, samples were collected for chemical and microbiological analyses from sites at the works, in St. Austell River ("White River") and on the beach at Pentewan, approximately 4 kilometres downstream. This study included a biological monitoring programme designed to identify effects that might relate to the discharge of PAA-treated effluents into riverine receiving waters. The location of the sampling stations relative to the sewage outfall is illustrated in Figure 2.1. Examination of aquatic organisms did not extend into the marine environment.

Two macroinvertebrate surveys were carried out, the first in June 1989, prior to PAA dosing, and the second towards the end of November 1989 after 3 months of the trials (Realey and Brogden, 1990). Species lists and faunal data sets for the sites sampled were not published. This is unfortunate, as a knowledge of the biology of the individual species and the structure of the communities that they form can greatly assist in the interpretation of prevailing environmental conditions (Hynes, 1960; Trett, 1989; Newell *et al.*, 1990a; 1990b). Instead, macrofaunal data for each site in both surveys were presented as BMWP (Biological Monitoring Working Party) scores. These are given in Tables 2.4 and 2.5. In essence low BMWP scores are held to reflect assemblages of tolerant species, similar to those found in organically enriched conditions, and high BMWP scores to indicate more sensitive communities susceptible to the effects of increased environmental stress (such as increased organic loadings) (HMSO, 1985).

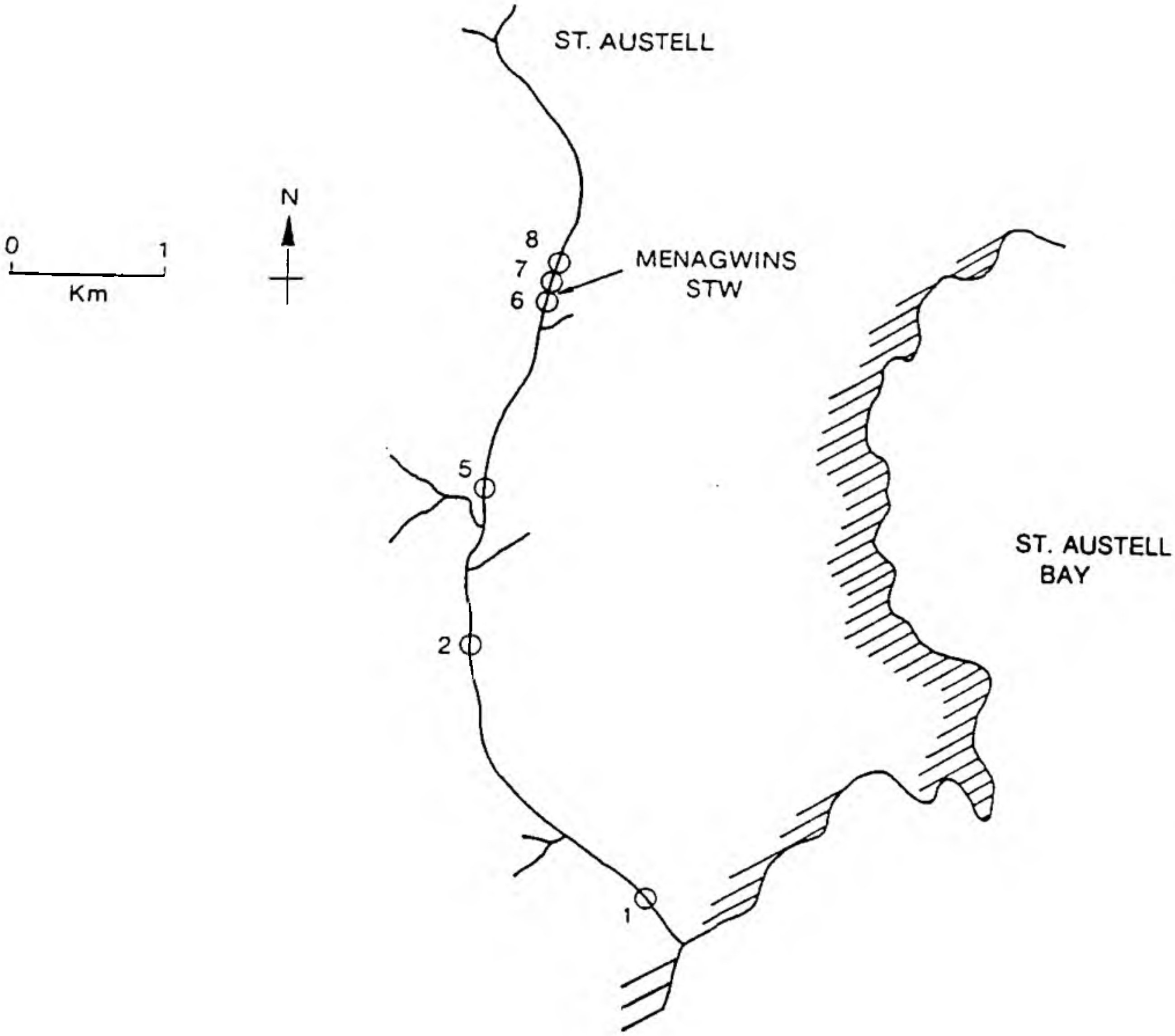


Figure 2.1 Sampling points for biological surveys during the Menagwins trial

Table 2.1 Macroinvertebrate study on St. Austell River, 19th. June 1989

Site No.	Site Name	Taxa	BMWP Score	ASPT*	SF**
1	Pentewan Bridge	9	23	3.29	-
5	Molingey Gauging Stn.	6	12	2.4	slight
8	Iron Bridge	7	13	2.6	-

* - ASPT: average score per taxon;
** - SF: "sewage fungus". See text for details.

Source: Realey and Brogden (1990)

Table 2.2 Macroinvertebrate study on St. Austell River, 30th. November 1989

Site No.	Site Name	Taxa	BMWP Score	ASPT	SF
1	Pentewan Bridge	9	38	4.2	rare
2	Peckhill Woods	10	43	4.3	common
5	Molingey Gauging Stn.	8	34	4.25	abundant
6	-	3	7	2.3	v. abundant
7	Upstream of STW	5	23	4.6	-
8	Iron Bridge	4	16	4.0	-

Source: Realey and Brogden (1990)

See Table 2.1 for key to abbreviations used.

In their report, Realey and Brogden (1990) noted the presence of the crustacean family Asellidae (the only British species being *Asellus aquaticus*, the hog louse) upstream of the outfall. The presence of this species, which is tolerant of low oxygen concentrations, was equated with organic enrichment of the river upstream of the sewage treatment works. "Sewage fungus" was also recorded and caused some concern as it was observed to extend up to 2.5 km downstream of the works once disinfection trials commenced. The "sewage fungus" was subsequently noted to disappear when the trials ceased.

The data presented in this study are insufficient to enable the effects, if any, of the PAA treatment on the river's macrofaunal assemblages to be assessed fully. The principal reasons can be summarised as follows:

i.) In general, biotic indices lead to a loss of information (e.g. Mason, 1981). Markedly different environmental conditions can produce similar scores and natural, non-anthropogenic changes in habitats, such as seasonal changes in waterplant communities or sediment scouring following heavy precipitation, can alter the observed score values. Species lists and raw data for each station should be provided along with the biotic scores;

ii.) In procedural recommendations for the use of the BMWP score it is stated that "scores are intended to be representative of the site for the *season* sampled" (NWC, 1981; HMSO, 1985). However, sampling was carried out first in early summer and again in late autumn/early winter. Due to the preponderance of larval insects with free-flying adults in the spring and summer months, the composition of freshwater macrobenthic communities can vary significantly between seasons. The appearance of newly hatched nymphs and larvae in the late summer/early autumn from eggs oviposited during the summer months can give the false impression of improving environmental conditions. This effect may be intensified by the phenomenon of *invertebrate drift* whereby organisms from other, possibly cleaner habitats upstream in the catchment are transported into the survey area during the first heavy rains of autumn (Hynes, 1970; Bayly and Williams, 1973; Moss, 1988). Resources permitting, sampling could have been undertaken at one or two-weekly intervals to determine whether changes in the composition of the faunal community were *gradual and unidirectional*;

iii.) Because of the sampling regime adopted, the only sites that can be compared directly are sites 1 (Pentewan Beach), 5 (Molingey Gauging Station) and 8 (Iron Bridge). Sites 2, 6 and 7 were not sampled in the earlier survey;

iv.) It is implied that the occurrence of the "sewage fungus" in some way resulted from the PAA disinfection trials. No indication is given as to whether the "sewage fungus" extended upstream of the sewage treatment works. The presence of *Asellus aquaticus* at the upstream stations suggests that organically enriched and/or low dissolved oxygen conditions were present at these stations. These are frequently associated with true sewage fungus communities and would indicate extrinsic factors that might invalidate the observations made;

v.) The report notes that silt/clay deposition had occurred in the survey area as a result of unspecified activities in the catchment. This was suggested to be responsible for low numbers of fauna (Realey and Brogden, 1990). Unfortunately it is not clear whether the authors are referring to species richness, species diversity or abundance of individuals. This factor alone would invalidate comparisons between surveys;

vi.) Because of the need to achieve the required exposure and residence times in this trial, *Oxymaster* dosing was carried out upstream of the humus tanks. This may have produced two environmental stresses not usually associated with PAA disinfection processes or the normal operation of the sewage treatment works:

a) elevated concentrations of ammonia were recorded in the effluent. Many aquatic organisms are sensitive to ammonia and ammonium ions and would suffer accordingly;

b) settlement processes in the humus tank may also have been affected. This would account for the increase in solids noted in the effluent. The effects of suspended solids on aquatic plants and animals are many and varied and are mostly detrimental (Section 3.4). For example, decreased light penetration affects primary production processes, respiratory and feeding structures become clogged and toxic elements and compounds adsorbed to sediment particles can enter food webs at sites removed from the normal, primary impact zone.

These factors make generalisation of the studies of the effects of PAA-treated effluent on macroinvertebrate communities in St. Austell River extremely difficult. Further studies were proposed for 1990, but the results of these have not been obtained.

Interox were invited by South West Water to examine and comment on the WRc report (Realey and Brogden, 1990). Their comments (Fraser, 1990), which address many aspects of the trial, raise the question of the identification of the "sewage fungus". It would be of considerable interest to know the composition of this community and, if it did conform to that of sewage fungus, the factors responsible for its proliferation.

2.3 Southend Outfall

To date the Southend PAA disinfection programme includes the most comprehensive ecological study made of PAA-treated effluents discharged to any receiving water. As such it deserves close attention.

2.3.1 The Surveys

In May 1989 Anglian Water (now Anglian Water Services Ltd (AWSL)) implemented seasonal PAA disinfection to meet the microbial standards of the EC Bathing Water Directive for beaches at the mouth of the Thames Estuary. Disinfection was designed to cope with primary sewage effluents and storm discharges. The modified discharge consent permitted the use of PAA concentrations of up to 12 mg l^{-1} (AWSL, 1991). Immediately prior to treatment (April 1989) a subtidal sampling programme was commissioned by Interox. This was undertaken by Marine Environmental Consultants (MEC), to assess the macrobenthic assemblages at 20 fixed sampling stations (Gould, 1989a). The survey was repeated in October 1989 towards the end of the treatment season (Gould, 1990). In the following year, the April and October surveys were replicated by MEC (subsequently Acer Environmental) for Anglian Water Services Ltd and the survey grid expanded to 22 stations (Joslin and Gould, 1991). The sampling grid and the distribution of the sampling stations with respect to the outfall are illustrated in Figure 2.2.

The original survey included consideration of *Oxymaster* disinfection on bivalve molluscs in terms of growth, fecundity and sanitary quality (Gould, 1989a). However, it is understood that this aspect of the study was not undertaken because of the possibility of other sources of pollution in the region.

2.3.2 Results

The macrofaunal species observed in each survey are summarised in Table 2.3. Full species lists for each of the four surveys undertaken to date are presented in Joslin and Gould (1991).

Table 2.3 Summary of numbers of benthic macrofaunal taxa observed in ecological surveys of Southend outfall

Survey	Annelida	Mollusca	Crustacea	Others	Total
April 1989	20	9	11	2	42
October 1989	32	14	15	8	69
April 1990	23	4	4	5	36
October 1990	17	9	10	5	41

Source: Joslin and Gould (1991)

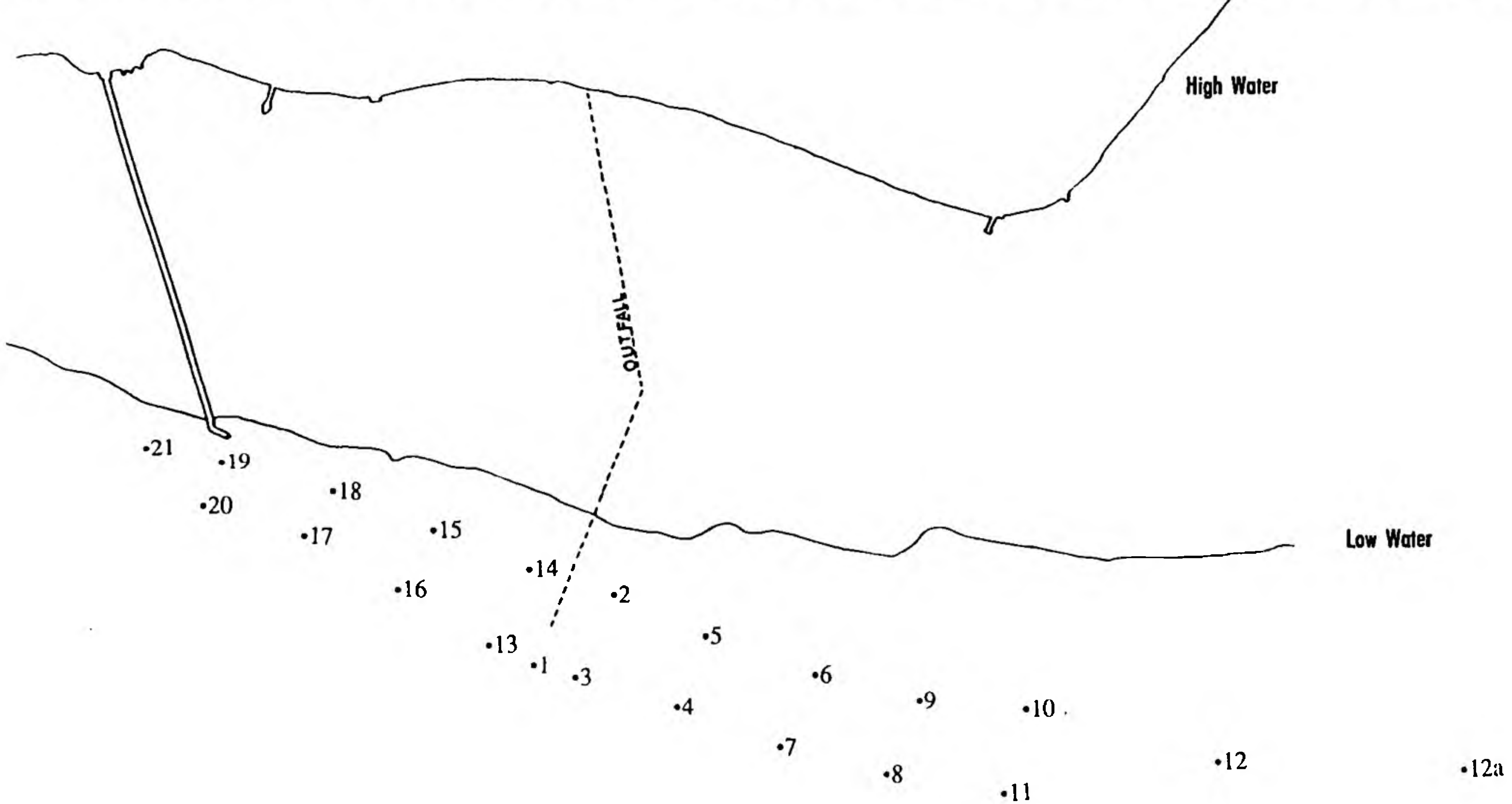


Figure 2.2 Sampling Points for the Benthic Survey at Southend

Examination of Table 2.3 reveals that annelids (principally polychaete species) were the dominant macrobenthic group, followed by the Crustacea. With the exception of "others", which comprised several miscellaneous marine groups, and the annelids in 1990, *total* species richness (number of species observed within the survey area) for each group increased between the April and October surveys of a given year. This is a typical pattern of recruitment of marine species into outer estuarine sites during the summer months (Green, 1968; Newell, 1979; Barnes and Hughes, 1988). In the case of the Thames during the 2 years studied, this process was enhanced by reduced freshwater flow (Trett *et al.*, 1990; Attrill, 1990a; 1990b). The conclusion of Joslin and Gould (1991) that the increase in species observed was indicative of "no effect on benthic communities" is not supported by the data.

Although there is some discrepancy between the species identified in these surveys and those recorded at the outer Thames Estuary subtidal sites in the quarterly surveys of the NRA Thames Estuarine Benthic Programme (TEBP; cf. Joslin and Gould, 1991 and Attrill, 1990), these differences appear to be consistent between surveys. Further, the structure of the species assemblages reported broadly agrees with that observed in association with other organically enriched sediments (see Pearson, 1975; Pearson and Rosenberg, 1978; Newell *et al.*, 1985; CES, 1987, 1990; Rainbow *et al.*, 1988). Non-selective detritivores and deposit feeders predominate in the muddy sediments in the immediate vicinity of the outfall and are progressively replaced by less tolerant, more mobile macrofaunal species at increasing distances from the point of discharge.

2.3.3 Critique

In their report, Joslin and Gould (1991) conclude that:

> "The results indicate that no major changes have taken place. The animal community has not been adversely affected during this period and the number of species has remained largely unchanged apart from seasonal variations".

In this study the communities were compared using diversity (Shannon-Weiner) and evenness indices. Changes in community structure at the same site, in the same month, in consecutive years, were assessed using Spearman's rank correlation test and Sorensen's similarity index. These techniques are all valuable when applied with care. The Shannon-Weiner diversity index, for example, is a "3-dimensional" statistic that depends on species richness (number of species present), dominance and abundance. Consequently, widely differing communities can give rise to similar diversity values.

Examination of the total numbers of species in the four surveys reported to date (see Table 2.3) shows that in each case there has been a *decline* in the numbers observed in the April and October surveys since the treatment commenced. This is despite the inclusion of two extra sampling stations at the extremities of the survey area equivalent to four additional samples per survey (2 replicates per station). This is also evident when site by site comparisons are made for the same months in consecutive years (Table 2.4).

If the data for the April surveys are considered (Table 2.4), after the first year of treatment *fewer* species were observed at 19 of the stations sampled. The total densities of animals at the start of the new season were also lower at 13 stations. Similarly, the number of species observed towards the end of the treatment periods in the October surveys declined between 1989 and 1990 at 10 stations. However, for the same surveys the densities of animals observed *increased* at 15 stations. Depending on evenness (a measure of the distribution of individuals amongst the species), this might imply increased dominance in the macrofaunal communities surrounding the Southend outfall. A *change* in dominance is commonly associated with new environmental stress factors (see Newell *et al.*, 1991). Stress factors can include natural physical disturbance, changes in chemistry and/or granulometry of sediments as well as the entire range of anthropogenic perturbation that might be classed as pollution. Numerically, the polychaete annelid, *Aricidea minuta*, was the dominant species at many of the sites in the survey area, especially at sites around the mouth of the outfall (Table 2.5). *Aricidea minuta* is a small sedentary polychaete with a high biotic (reproductive) potential which appears to enable it to rapidly exploit suitable, possible transient, conditions. The gut contents indicate that it is a deposit feeder which may benefit from organically enriched sediments and/or their high microbial populations. With the onset of PAA disinfection, the abundance of this species was affected, as noted by Joslin and Gould (1991), and other, opportunistic groups gained in importance. This in itself indicates a change in the dominance structure of the macrofaunal communities.

Table 2.4 Numbers of stations at which changes in species richness (numbers of species) and total densities of individuals have occurred in the April and October surveys of Southend macrofauna for 1989 and 1990.

	Species			Densities		
Survey	Rose	Same	Fell	Rose	Same	Fell
April 1989/1990	1	0	19	7	0	13
October 1989/1990	8	2	10	15	0	5

Source: Joslin and Gould (1991)

To assess the extent to which such changes had occurred in the dominance-diversity characteristics of the assemblages surrounding the outfall, data sets from Joslin and Gould (1991) were re-examined for this Study using k-dominance analyses. The % cumulative abundance was calculated for the communities at stations 1, 2, 3, 13, and 14 around the outfall and station 17 at the western end of the survey area (see Figure 2.2) for all 4 surveys reported. The resulting plots are shown in Figures 2.3 to 2.7 and the dominant species for each station are shown in Table 2.5. To determine the effect on the macrofaunal community, it is important to examine the changes in dominance along with the biology of the species present, rather than simply the dominant species shown in Table 2.5.

Table 2.5 **Dominant species in macrofaunal communities at Southend sampling stations 1, 2, 3, 13, 14 and 17 in 1989 and 1990 Acer Environmental surveys**

	1989		1990	
Station	April	October	April	October
1	*Aricidea minuta*	*Retusa obtusa*	*Aricidea minuta*	*Aricidea minuta*
2	*Aricidea minuta*	*Mytilus edulis*	*Aricidea minuta*	*Aricidea minuta*
3	*Aricidea minuta*	*Pseudocuma longicornis** *Telina fabula**	*Aricidea minuta*	*Aricidea minuta*
13	*Aricidea minuta*	*Telina fabula*	*Aricidea minuta*	*Bathyporeia elegans*
14	*Barnea candida*	*Anthozoan sp.*	*Pseudocuma longicornis*	*Spiophanes bombyx*
17	*Aricidea minuta*	*Retusa obtusa*	*Aricidea minuta*	*Aricidea minuta*

* - co-dominant species (18.2%). Source: Joslin and Gould (1991)

Figures 2.3 and 2.4 illustrate the k-dominance curves for the April surveys in 1989 and 1990 respectively. These relate to the macrofaunal communities present prior to the treatment season, following winter periods. In April 1989, before PAA treatment was adopted at Southend, the maximum number of species observed at the sampling stations was 17 at Station 14 (Figure 2.3). In April 1990 the maximum number of species observed declined to 12 at Station 14. The dominance-diversity characteristics of certain of the macrofaunal communities had changed considerably over the year. Compare, for example, the communities at Station 13 (Figures 2.3 and 2.4). Dominance increased from 19.6% in April 1990 (Figure 2.3) to 84.7% in April 1990 (Figure 2.4) with a concomitant fall in the number of species from 14 in 1989 to 4 in 1990. Dominance also increased less dramatically at Stations 3 and 14. In contrast at Stations 1 and 2 dominance declined. At Station 1 this change was accompanied by a decline in species richness from 13 species in April 1989 to 8 in April 1990 (Figures 2.3 and 2.4). At Station 2 species richness remained unaltered (11 species). Examination of the species complements showed that the dominant species remained unaltered, although the sub-dominant species changed.

Comparison of the k-dominance curves for macrofaunal communities in October 1989 and 1990 indicates fundamental changes in the community structure (dominance - species richness characteristics; Figure 2.5 and 2.6). With the exception of Station 14 dominance increased at each of the outfall stations. The community at Station 14, in common with most of the other outfall stations, exhibited a decline in the number of species from 24, in October 1989, to 16 in October 1990 (Figures 2.5 and 2.6). Comparison with stations beyond the immediate vicinity of the discharge (e.g. Station 17) indicates that the change in community structure in October are atypical of the survey area (Figure 2.7). This appears to override complications in interpretation that might arise from seasonal variation in invertebrate recruitment or the effects of changes in sediment granulometry. Apart from April 1990, when *Aricidea minuta* became unusually abundant at many stations throughout the survey area, dominance and species richness at Station 17 remained remarkably consistent (Figure 2.7).

Comparisons of co-dominance values (the cumulative % abundance of the 2 most common species at a given sampling station) emphasise the degree of change in community structure. In the October surveys, following PAA treatment, variation in the co-dominance is greater at the stations close to the outfall than for example at Station 17, for example (Figure 2.8). The same is also true for co-dominance measured in April 1989 and 1990. However, the trend is not so marked, as a result of the overall increased abundance of *Aricidea minuta*, see below.

Instability of community structure indicated by the variation in co-dominance is commonly associated with variable environmental stress. This appears to be seasonal and centred around the Southend outfall. The mechanism is uncertain, but there is evidence to suggest that the stress results in a change in the trophic structure of the communities. At Station 13 *Bathyporeia elegans*, a mobile amphipod crustacean that does not form permanent burrows, became dominant, and at Station 14 *Spiophanes bombyx*, a sedentary polychaete annelid commonly associated with organically enriched sediments, was the most abundant species (Table 2.5).

The causes of changes in the macrofaunal communities described in the Acer surveys are not entirely clear. Their correlation with the occurrence of PAA treatment could be coincidental. It has been postulated that the presence or absence of one species may have directly influenced the abundance of several other species producing misleading changes in densities and species richness. In this case, it was suggested that the unique occurrence of the sedentary polychaete, *Sabellaria spinulosa*, at four stations in the October 1990 survey only might have produced an artificially high species richness and be responsible for a disproportionately large decline in species numbers when it disappeared from the survey area during the following year. Whilst this might be true for the closely related species *Sabellaria alveolata* (a reef-building polychaete not recorded in the outer Thames Estuary, it is unlikely that the loose, sandy tubes of *Sabellaria spinulosa* would account for the presence of the 35 species that were found exclusively at these stations in the October 1989 survey.

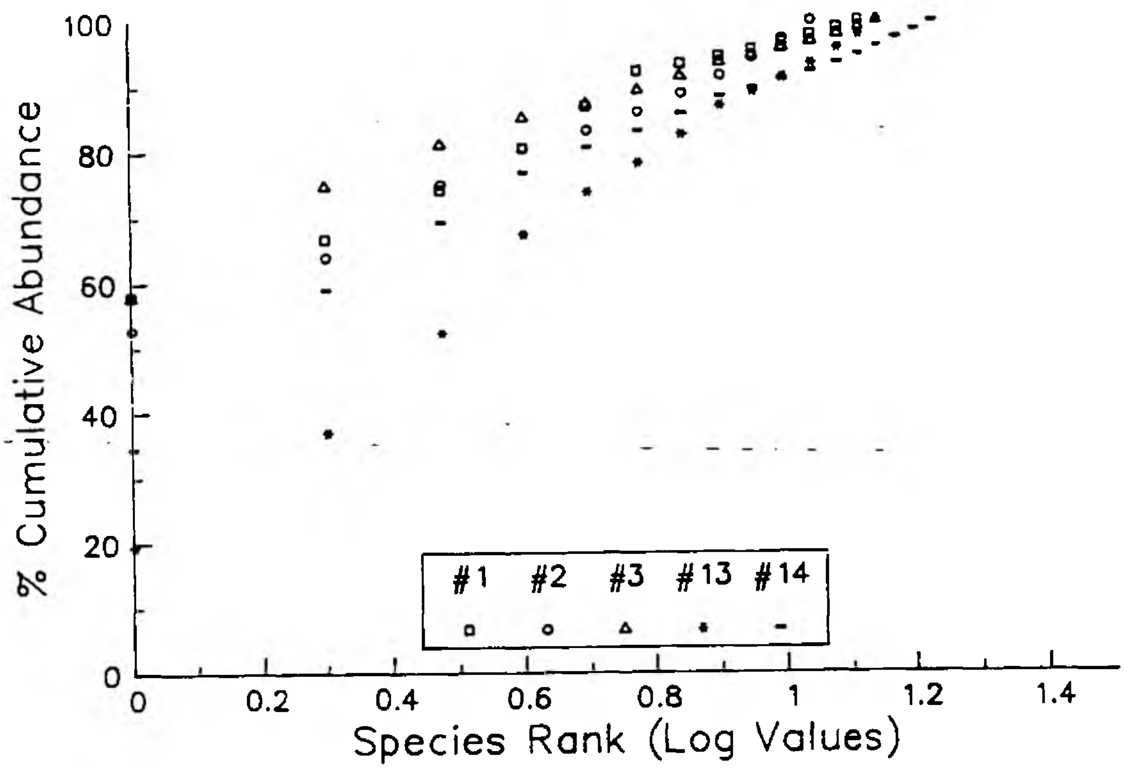


Figure 2.3 April 1989 k-dominance curves for macrofaunal assemblages at Southend

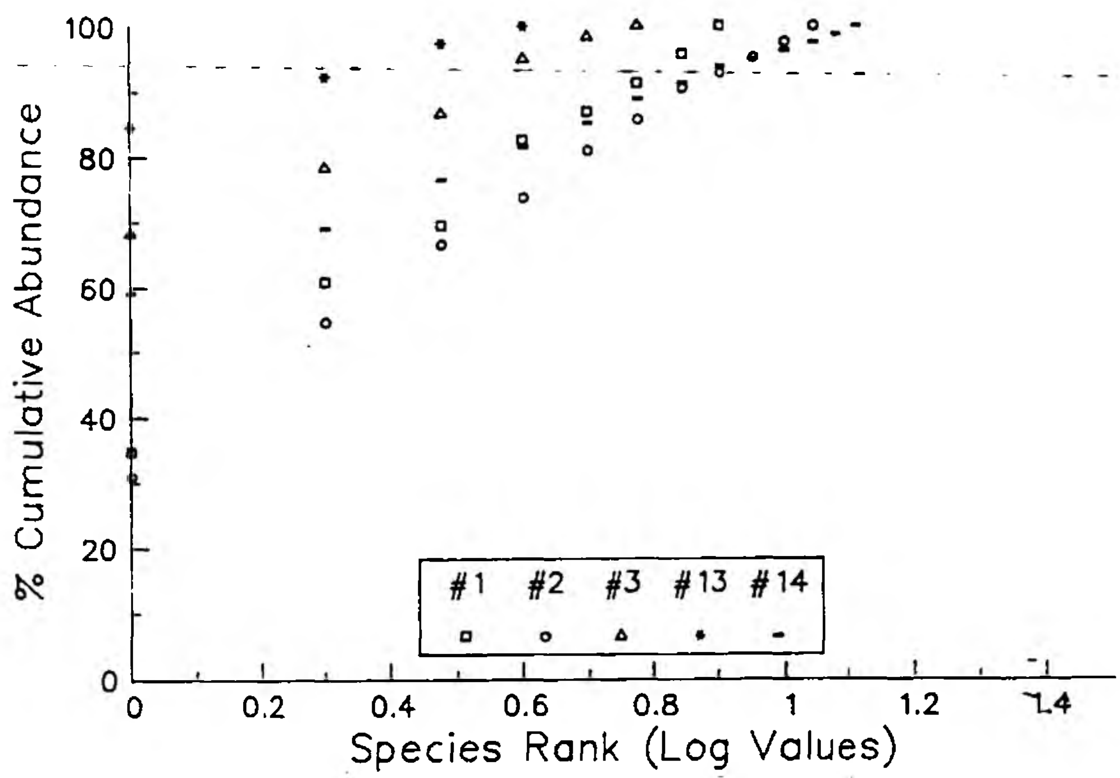


Figure 2.4 April 1990 k-dominance curves for macrofaunal assemblages at Southend

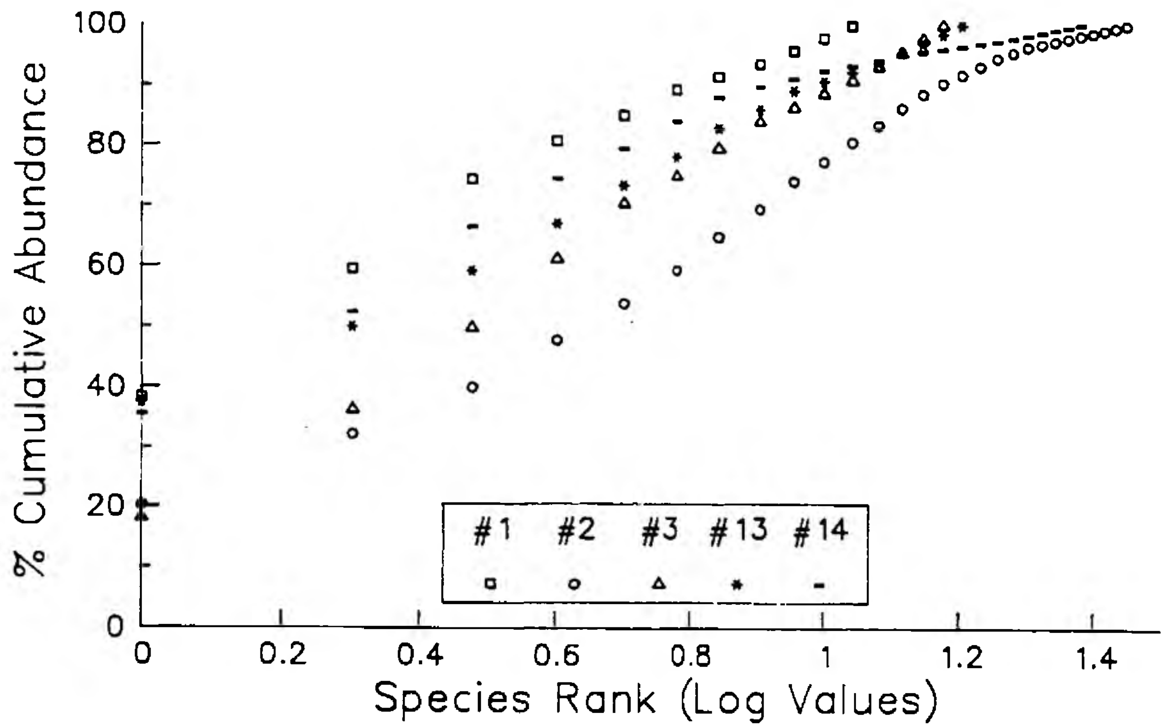


Figure 2.5 October 1989 k-dominance curves for macrofaunal assemblages at Southend

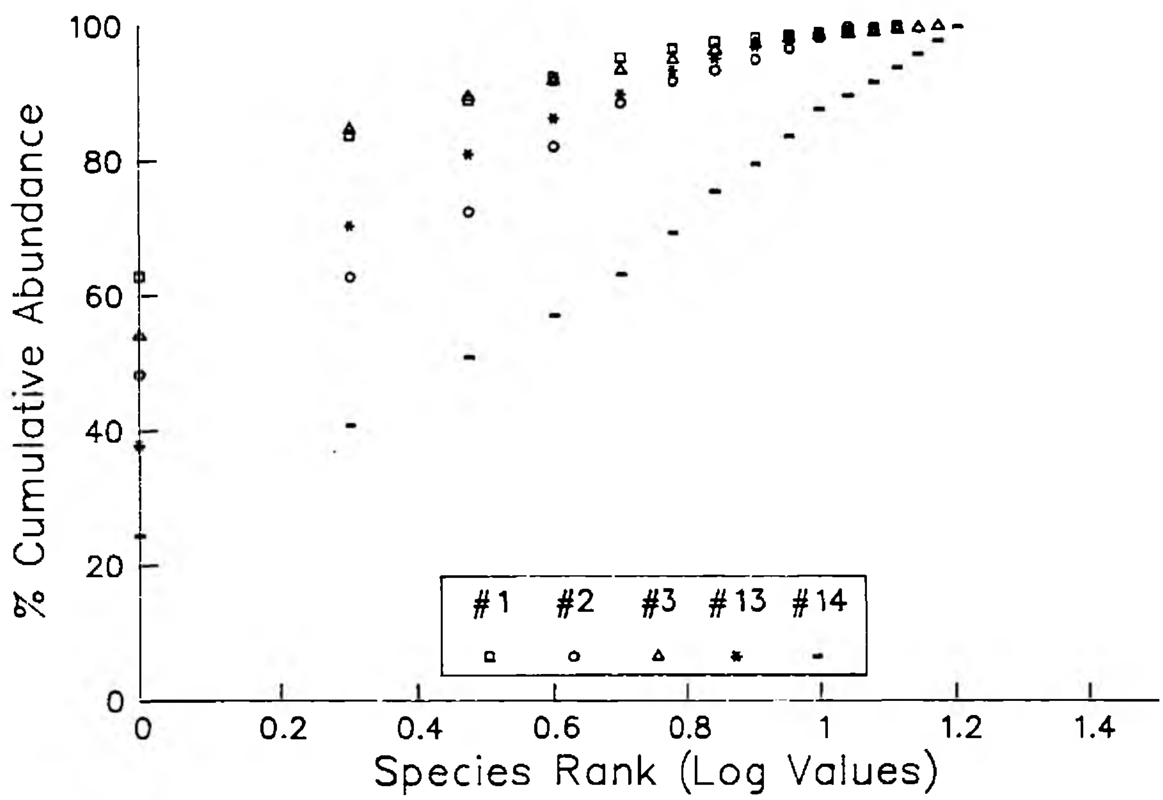


Figure 2.6 October 1990 k-dominance curves for macrofaunal assemblages at Southend

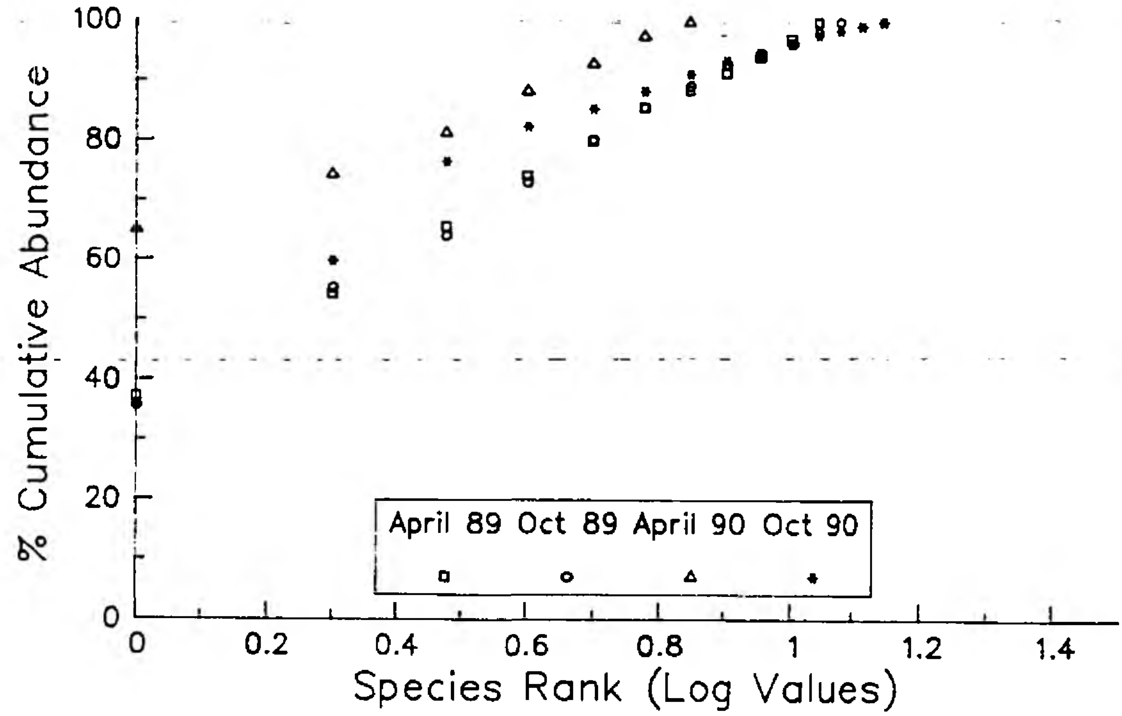


Figure 2.7 k-dominance curves for macrofaunal assemblages at station 17 at Southend 1989-90

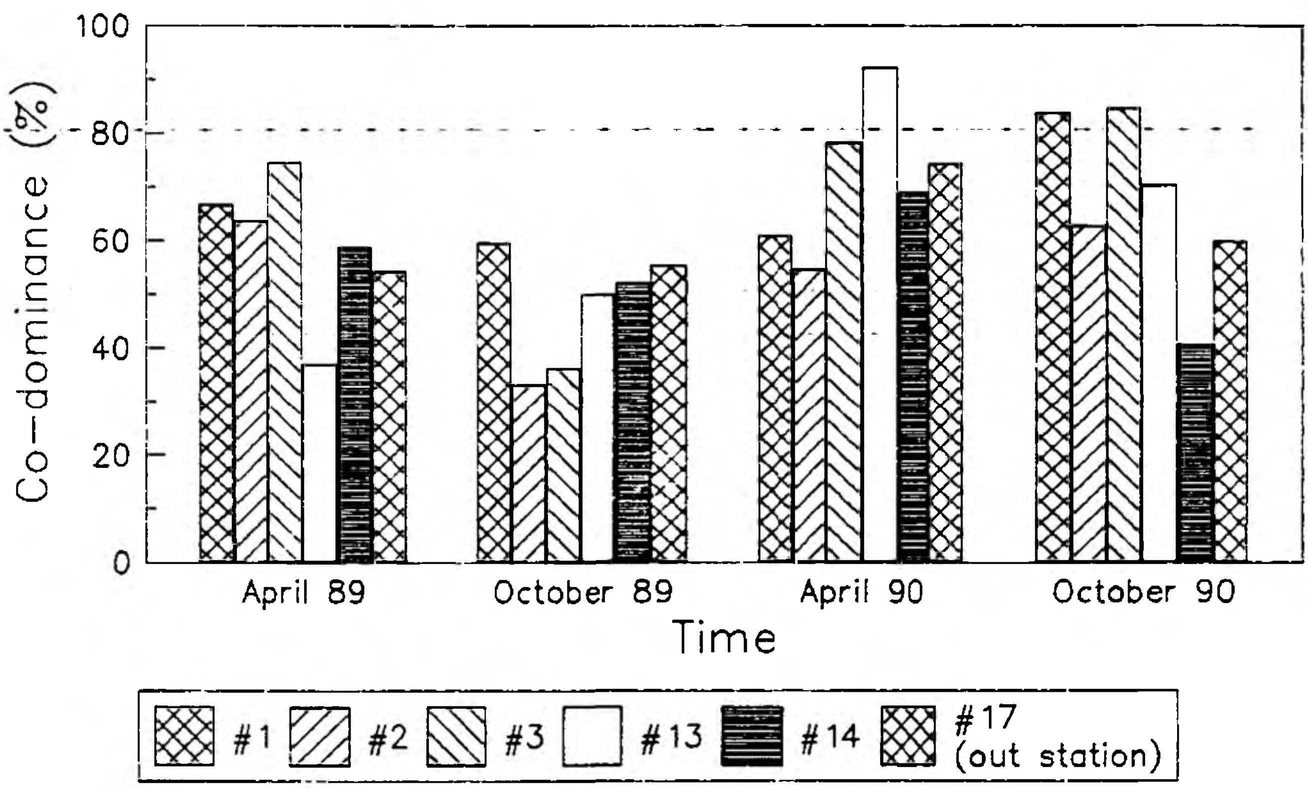


Figure 2.8 Abundance of co-dominant species at Southend

2.3.4 Complementary Macro- and Meiofaunal Studies

The results of the analyses shown above appear to indicate that environmental stresses during the period following the initiation of PAA disinfection have led to the establishment of further modified macroinvertebrate communities. However, as is commonly observed in surveys of macrofaunal invertebrates, the degree of variation between replicate samples was high and might invalidate any conclusions drawn. Confirmation that at least some of the effects observed are not entirely attributable to sample variation may come from examination of certain of the macrofaunal data from the Thames Estuary Benthic Programme (TEBP) carried out by the NRA, Thames Region. Not all the sampling sites are relevant to the present study. However, the quarterly data sets for the Southend subtidal sampling station by the outfall (Attrill, 1990a; 1991) are of central interest and are given in full in Appendix B1. The dominance-diversity characteristics of the communities sampled are illustrated in Figure 2.9. The macrofauna in the vicinity of the Southend outfall exhibit the highest dominance values during the second and third quarters of the year, coincident with the periods of PAA disinfection (Figure 2.9). The lowest dominance value was recorded in the first quarter of 1990, a period outside the bathing season. Examination of TEBP data for other subtidal outer estuarine sampling stations does not reveal similar patterns of change in dominance. Indeed, dominance values for macrofaunal assemblages were predominantly higher during the winter months.

A further point of note from the NRA studies are the biomass statistics (Attrill, pers. comm.). Those for the Southend outfall macrofauna over four quarters are compared with the data for the subtidal sites at Grain Flats and Canvey Beach in Figure 2.10. Data are also given for the intertidal sampling site at Southend for completeness.

In contrast to the situation at other sampling stations, the macrofaunal biomass for the Southend outfall (Southend subtidal) declined gradually from the second quarter of 1989 onwards, but rose again partially in the first quarter of 1990 and declined again thereafter. In most cases, biomass in aquatic ecosystems would be expected to increase over the summer months as a result of increased primary production (Barnes and Hughes, 1988). The outfall station was the only site at which biomass was lower at the end of 1990 than at the start of the sampling period.

In general macrofauna are held to be moderate to poor indicators of environmental conditions in aquatic habitats (Platt and Warwick, 1980; Barnes and Hughes, 1988; Clark, 1989; see also Gay *et al.*, 1991). The comparative insensitivity of macrofauna to changes in prevailing conditions, the relatively low natural densities and the lack of similar changes at other estuarine sampling stations away from the outfall make the changes described above all the more notable.

In contrast, meiofaunal indices are particularly sensitive to any perturbation that affects conditions within aquatic sediments (Lambshead, 1986; Trett *et al.*, 1990b; Newell *et al.*, 1991; Appendix B2). Annual surveys of subtidal benthos carried out over the past ten years in relation to outfalls discharging to a variety of receiving waters have demonstrated their value in detecting and delineating impact zones (Newell *et al.*, 1990b). The NRA survey programme included a study of meiofaunal assemblages throughout the Thames Estuary.

Quarterly data for the Southend outfall sampling station for the period April 1989 to March 1990 are given in Appendix B1. Data for intertidal sampling sites are also included for comparison. These comprise data for the principal meiofaunal groups (Nematoda, Harpacticoid Copepoda and Acari). As with the macrofauna, dominance amongst the nematode assemblages towards the mouth of the outfall was greatest in the second and third quarters of 1989, when a non-selective detritivore species (*Richtersia inaequalis*) was exceptionally abundant (Figure 2.11). This was not observed at surrounding sampling stations at Grain Flats, Canvey Beach, Chapman Buoy, Shoeburyness East or, indeed, at the intertidal sampling station towards the landward end of the Southend outfall (Trett *et al.*, 1990c; Figure 2.12 and Appendix B1). Data from subsequent meiofaunal surveys have not been released as yet, but are known to support the observations above (Forster, pers. comm.; Forster *et al.*, in preparation).

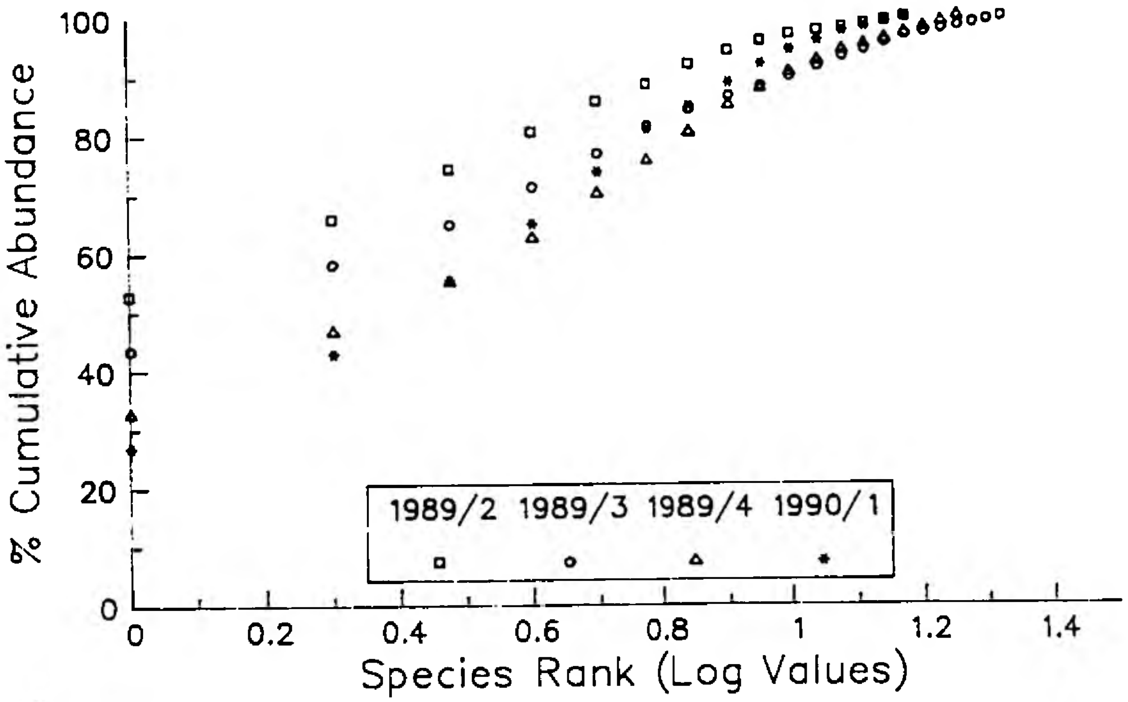


Figure 2.9 TEBP k-dominance curves for macrofaunal assemblages

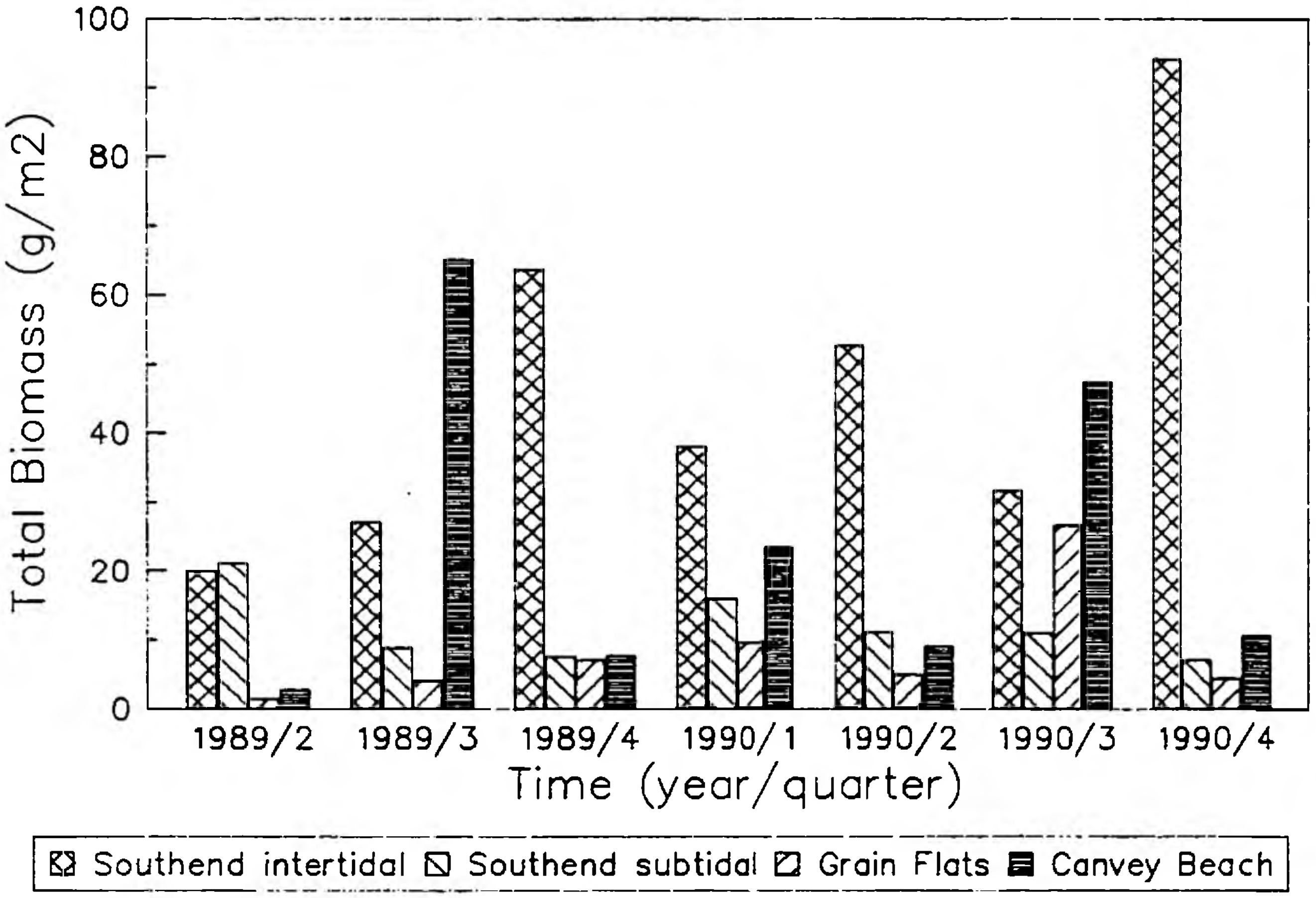


Figure 2.10 TEBP total biomass observations

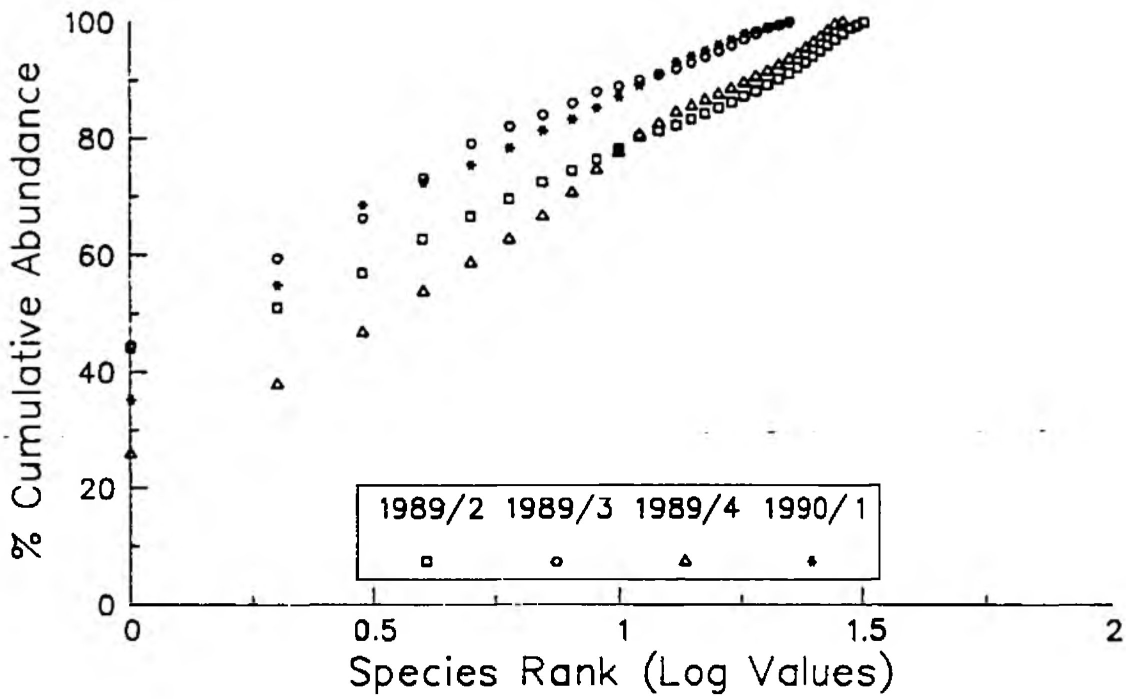


Figure 2.11 k-dominance curves for nematode assemblages at Southend (subtidal)

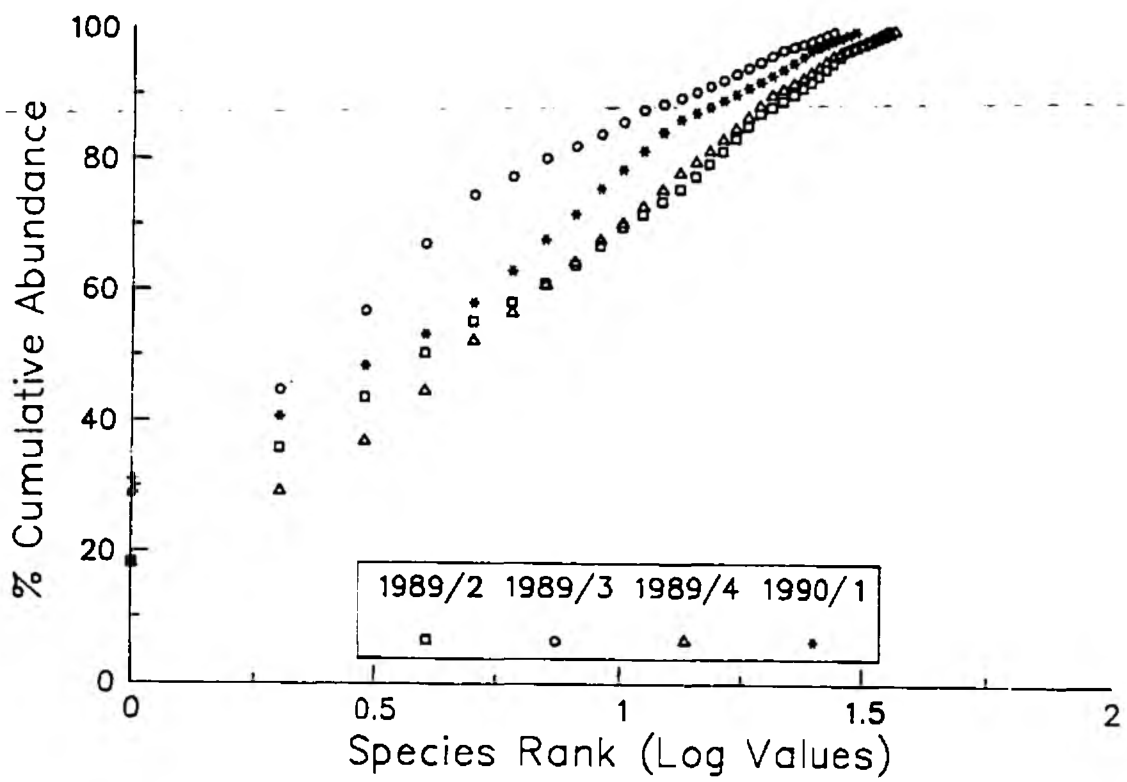


Figure 2.12 k-dominance curves for nematode assemblages at Southend (intertidal)

2.4 Trearddur Bay, Wales

Following studies undertaken by Welsh Water of the outfall at Porth Gwr Mawr that currently discharges raw sewage to Trearddur Bay south of Holyhead, fine screening and PAA disinfection were proposed as a means of improving aesthetics and maintaining compliance with the EC Bathing Water Directive Standards. In 1990 a protocol was agreed upon by the NRA, Dwr Cymru Cyf. and Wallace Evans Ltd to detect and monitor any changes that might occur following the introduction of PAA disinfection. In addition to standard bacteriological and virological studies, three permanent rocky shore transects, between 15 and 30 m long, have been established to enable any changes in community structure to be determined (Wallace Evans, 1990). The transects were examined in August 1990 and re-surveyed in April 1991 prior to commissioning of the scheme in May. The results of the most recent survey have not been published, and until the summer survey it will not be possible to comment on ecological changes.

2.5 Mablethorpe

Trials of PAA disinfection for secondary effluent commenced in May 1990 at Mablethorpe Sewage Treatment Works on the Lincolnshire coast under the auspices of Anglian Water Services Ltd in collaboration with Interox (NRA, 1991). The discharge is to freshwater (Wold Grift Drain) that ultimately flows across a beach in an open culvert to the lower shore. At low tide the effluent pools on the beach and drains to the sea via a narrow channel. Ecological studies were undertaken by Anglian Water Services Ltd and Acer Environmental of the freshwater and marine benthos respectively (NRA, 1991; Acer, 1991).

2.5.1 Freshwater Surveys

As in the Study at Menagwins sewage treatment works (section 2.2), details of the invertebrate taxa and observed abundances have not been published for the freshwater surveys. Instead, BMWP scores are presented for surveys dating from June 1988 to April 1991 at three sampling stations along the drain; one upstream of the STW and two downstream. Immediately prior to the PAA disinfection programme, a specific survey was conducted to determine the ecological effects of PAA treatment. This was undertaken at two sites in May 1990 and was repeated in November 1991, following the treatment period. Relevant BMWP scores have been extracted from these surveys and are presented in Table 2.6.

Table 2.6 BMWP scores for invertebrate assemblages in the Wold Grift Drain upstream and downstream of Mablethorpe STW prior to and following PAA disinfection of secondary effluent

Survey	BMWP	ASPT*
December 1988:		
Upstream Mablethorpe STW	58	3.63
Bambers Bridge	38	3.45
December 1989:		
Upstream Mablethorpe STW	27	3.86
Bambers Bridge	18	3.60
April 1990:		
Upstream Mablethorpe STW	57	3.73
Bambers Bridge	33	3.70
- PAA Treatment - - - - - - - - - -		
November 1990:		
Upstream Mablethorpe STW	30	4.24
Bambers Bridge	27	3.86

* - ASPT: average score per taxon

Source: NRA (1991)

Further sampling sites downstream of the treatment works were included in the surveys, (NRA, 1991) but the appearance of crabs and ragworms (probably *Nereis diversicolor* and *Carcinus maenas*) in the samples indicated the influence of salt water. This gave rise to artificially low BMWP scores as the index is designed to assess freshwater communities. The downstream stations were subsequently omitted from the surveys. This is a disadvantage of the BMWP score system not shared by detailed surveys of macro- and meiofaunal assemblages.

For the reasons discussed in Section 2.2, comparisons cannot be drawn from scores that relate to different seasons. The fall in scores between April and November 1990 (Table 2.6) most probably reflected seasonal changes in the invertebrate communities. In the autumn, many species are present as eggs or newly hatched nymphs that are either missed or are too small to be identified, which leads to lower BMWP scores. Comparison of the November 1990 data with those of December 1989 suggests that BMWP scores have been little affected by the PAA-treated effluent. However, examination of the 1988 data indicates that the 1989 results may themselves be depressed, possibly as a result of the exceptionally dry conditions in 1989. BMWP scores alone are insufficient to resolve these questions, and detailed consideration would have to be given to the biologies of the individual species present and their relative abundances before conclusions could be drawn about the causes of changes that may have occurred in the invertebrate assemblages.

2.5.2 Marine Surveys

The marine ecological surveys, carried out in April and November 1990 (Acer, 1991), included subtidal and intertidal (littoral) sampling sites. The distribution of these sites, with respect to the Wold Grift outfall, is shown in Figures 2.13 and 2.14. In each case, macrobenthos was collected for analysis. Consideration was not given to meiofaunal assemblages. In both the April and November surveys the subtidal sites were sampled using a 0.1 m^2 Day grab. In contrast the intertidal survey was undertaken using core samplers. The low densities of macrofauna present in the coarse-grained, littoral sediments caused problems in the April survey, as the paired samples taken at each site, using 7 cm diameter corers, yielded low numbers of macrofauna. Consequently in the November survey five replicates were taken at each site using 13 cm diameter corers.

The increased sampling effort (862% by volume using cores to a depth of 15 cm replicated five times) in the November survey of the intertidal sites undermines any comparison between the April and November data sets (McIntyre *et al.*, 1984). Despite this, histograms are presented in the Acer report using raw data to compare total numbers of species and individuals found at the intertidal sites in the April and November surveys. These, not surprisingly, appear to indicate strong recruitment (Section 2.2.3), and the growth of existing populations are used to support the conclusions that no deleterious effects could be observed in the intertidal macrobenthic communities (Acer, 1991; NRA, 1991). It is not possible, using simple techniques, to determine comparable numbers of *species* that would have been observed if similar sampling efforts had been used. This would require consideration of species discovery curves for which there are insufficient data. However, numbers of individuals (abundance data) can be compared using standardised data. For the purposes of this review, the raw intertidal data have been transformed to numbers of individuals per unit area (number m^{-2}). These are compared in Figure 2.15.

From Figure 2.15, it can be seen that corrected densities of macrofauna declined at seven of the intertidal sampling stations and that the magnitude of this reduction was greatest in the immediate vicinity of the outfall (Stations 17, 18 and 19). This is all the more notable as it followed a summer growing period, when densities would be expected to rise as a result of increased primary production and elevated ambient temperatures. In this connection, densities did rise at the north end of the survey area away from the outfall.

Table 2.7 summarises the species that were dominant in the macrofaunal assemblages at the intertidal and subtidal stations before and after PAA disinfection. The polychaete *Nephthys cirrosa*, not noted in the April survey, became the dominant species at subtidal stations 6 to 12 in November. It is not known whether this is a natural seasonal change in macrofaunal community structure. The results of further surveys may elucidate this.

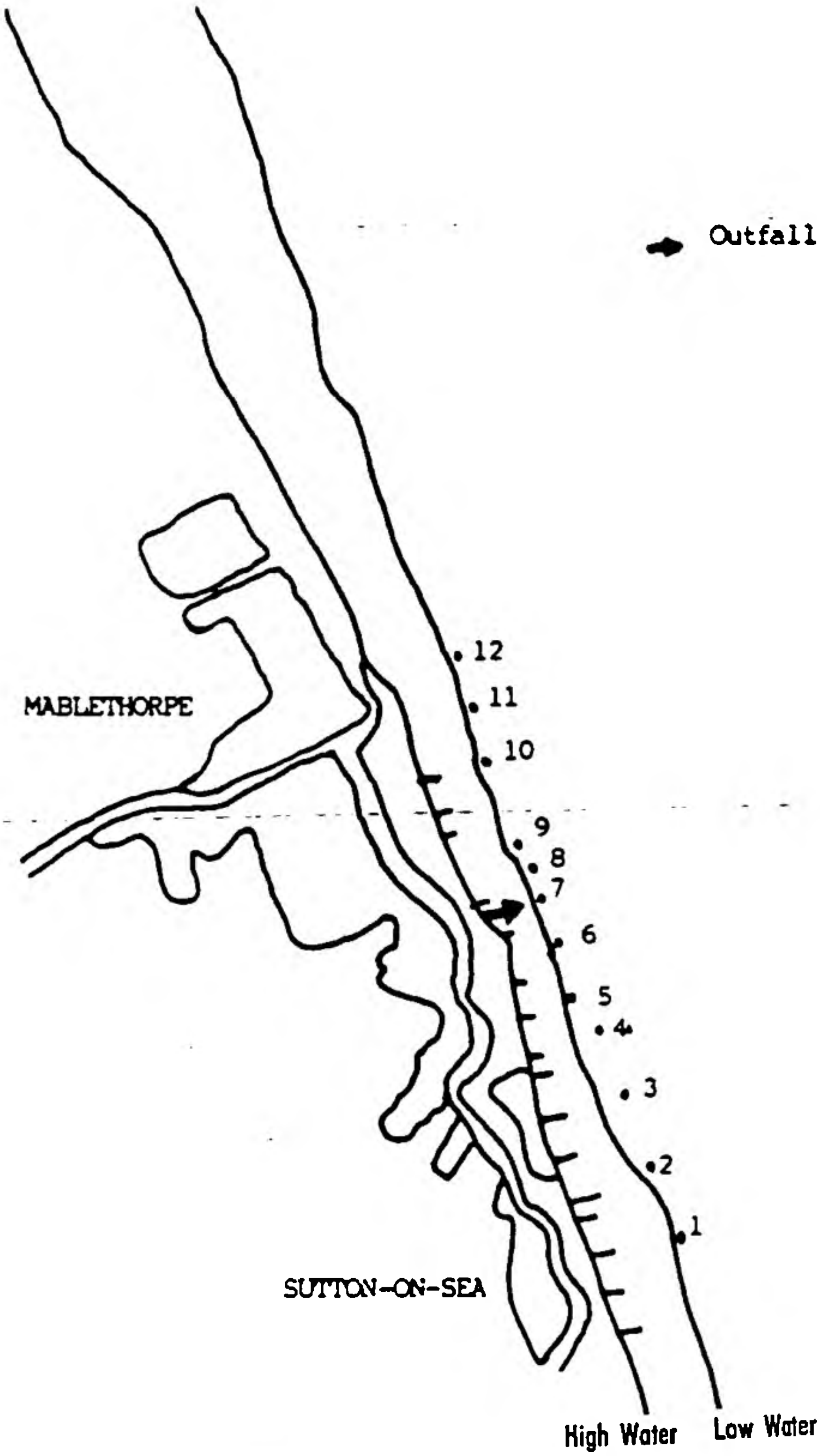


Figure 2.13 Mablethorpe benthic survey - position of subtidal sites

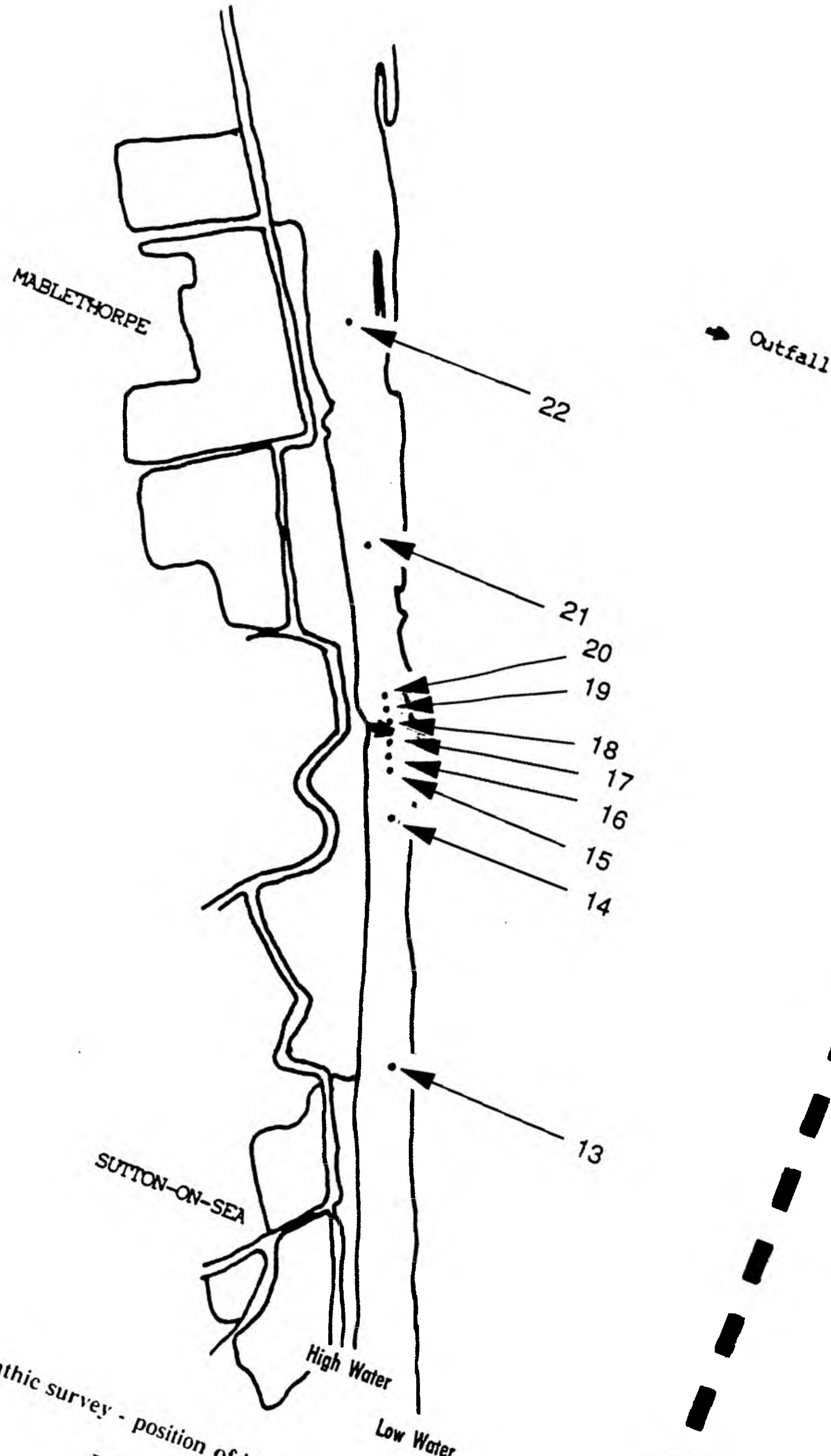


Figure 2.14 Mablethorpe benthic survey - position of intertidal sites

As with the Southend data (Section 2.2), the Mablethorpe macrofaunal assemblages can be examined in terms of their dominance-diversity characteristics. However, k-dominance analyses can only be undertaken for the subtidal communities due to the non-standardised sampling methods employed in the intertidal surveys. As might be expected, dominance values at stations 1 and 12, outside the zone of influence of the outfall, declined during the summer months as species richness increased (Figure 2.16). This results from recruitment as other marine communities release eggs and larvae into the plankton. At Stations 6, 7 and 8, immediately around the outfall, dominance remains high although a few extra species were observed in November at Station 7 (Figures 2.17 and 2.18).

Variability in dominance values of the Mablethorpe data appears to result from the small size and numbers of samples collected, a feature of macrofaunal as opposed to meiofaunal surveys (Appendix 2). To minimise this effect co-dominance values have also been calculated from the Mablethorpe data. These are the summation of the dominance values of the two most abundant species at each station. If co-dominance is related to the distance of the sampling stations from the outfall, a trend of increasing values of co-dominance is apparent with closer proximity to the discharge, following the treatment period (Figure 2.19). Straight line regression analysis reveals a weak correlation ($R^2 = 0.138$) after the treatment season, compared with no correlation ($R^2 = 0.034$) in April, prior to the start of the trial. Despite the usual stresses associated with the outfall (including organic enrichment, elevated BOD and suspended solids; CES, 1990), this trend was not obvious in the April survey (Figure 2.20). Macrofaunal data for the present season are necessary before conclusions can be drawn about the nature of these changes observed.

Table 2.7 Dominant species present at subtidal (1 - 12) and intertidal (13 - 22) stations at Mablethorpe 1990

	Dominant Species	
Stn.	April 1990	November 1990
1	*Bathyporeia elegans*	*Polydora ligni*
2	Anthozoan species	*Sabella pavonina*
3	*Bodotria arenosa*	*Phyllodoce mucosa*
4	Anthozoan species	*Lanice conchilega*
5	Anthozoan species	*Phyllodoce mucosa*
6	*Nephthys caeca*	*Nephthys cirrosa*
7	*Bathyporeia elegans*	*Nephthys cirrosa*
8	*Bathyporeia elegans*	*Nephthys cirrosa*
9	*Bathyporeia elegans*	*Nephthys cirrosa*
10	*Nephthys caeca*	*Nephthys cirrosa*
11	*Bathyporeia elegans*	*Nephthys cirrosa*
12	*Nephthys caeca*	*Nephthys cirrosa*
13	*Hydrobia ulvae*	*Scololepis squamata*
14	*Spiophanes bombyx*	*Spio martinensis*
15	*Magelona mirabilis*	*
16	**	*Harpinia antennaria*
17	*Scololepis squamata*	*
18	*Bathyporeia elegans*	*Hydrobia ulvae*
19	*Bathyporeia elegans*	*Nephthys cirrosa*
20	*Hydrobia ulvae*	*Scololepis squamata*
21	**	*Harpinia antennaria*
22	*Bathyporeia elegans*	*Tubificoides benedeni*

Source: Acer (1991)

* - single individuals of several species

** - no species observed

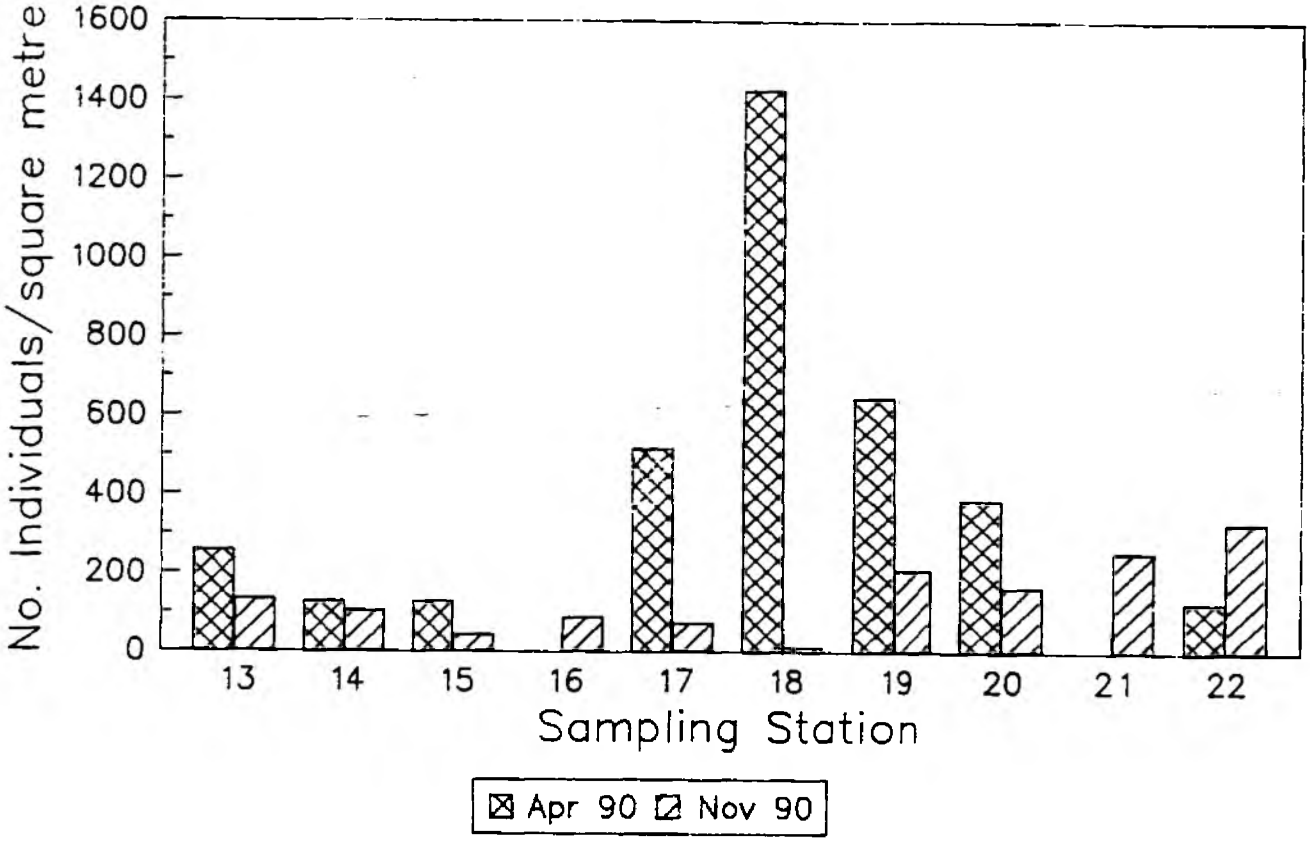


Figure 2.15 Densities of macrofauna at intertidal sites, Mablethorpe 1990

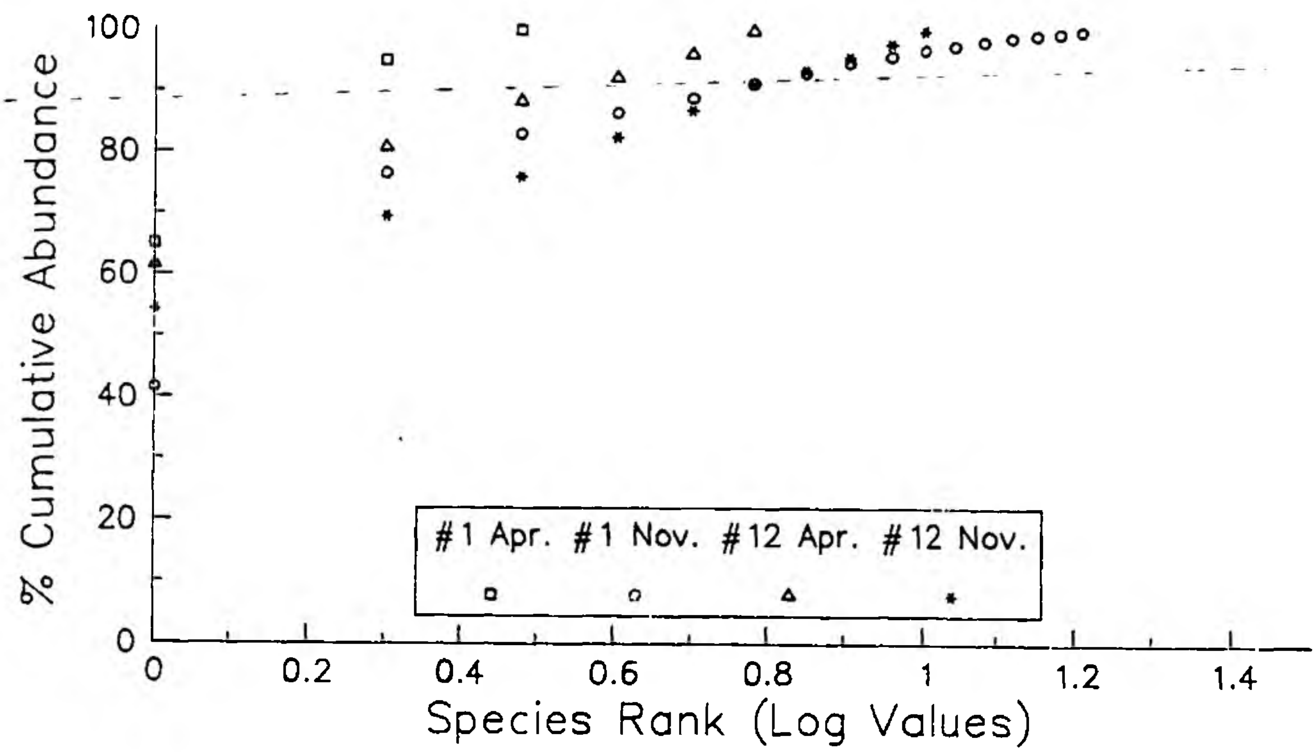


Figure 2.16 k-dominance curves for macrofaunal assemblages at Mablethorpe (Stations 1 & 12)

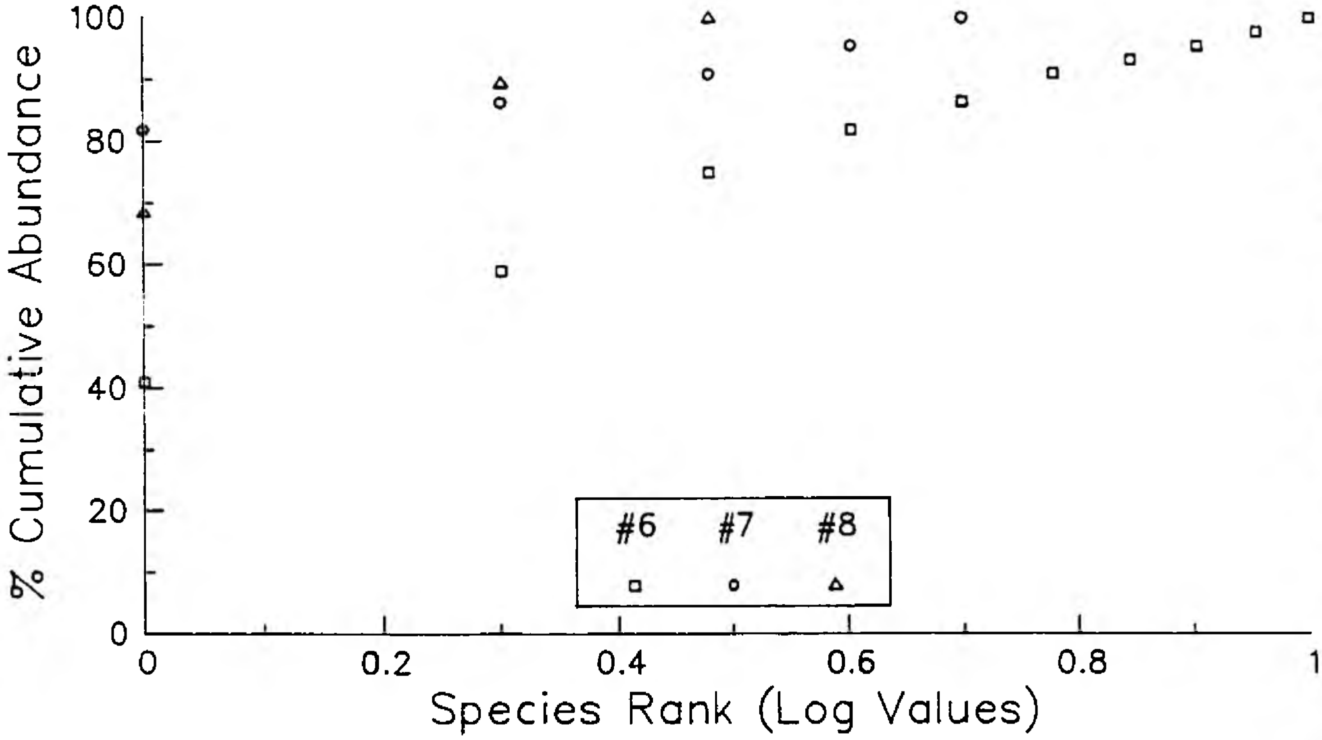


Figure 2.17 **April 1990 k-dominance curves for macrofaunal assemblages at Mablethorpe**

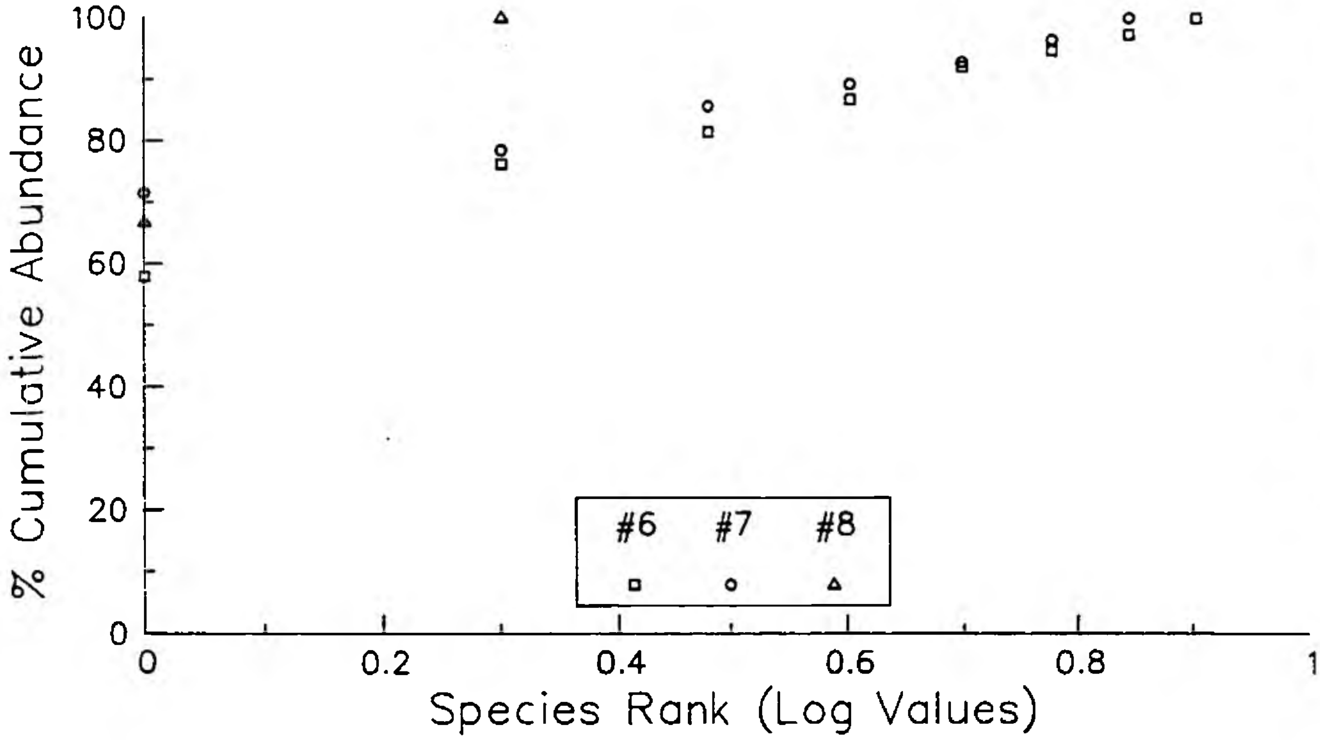


Figure 2.18 **November 1990 k-dominance curves for macrofaunal assemblages at Mablethorpe**

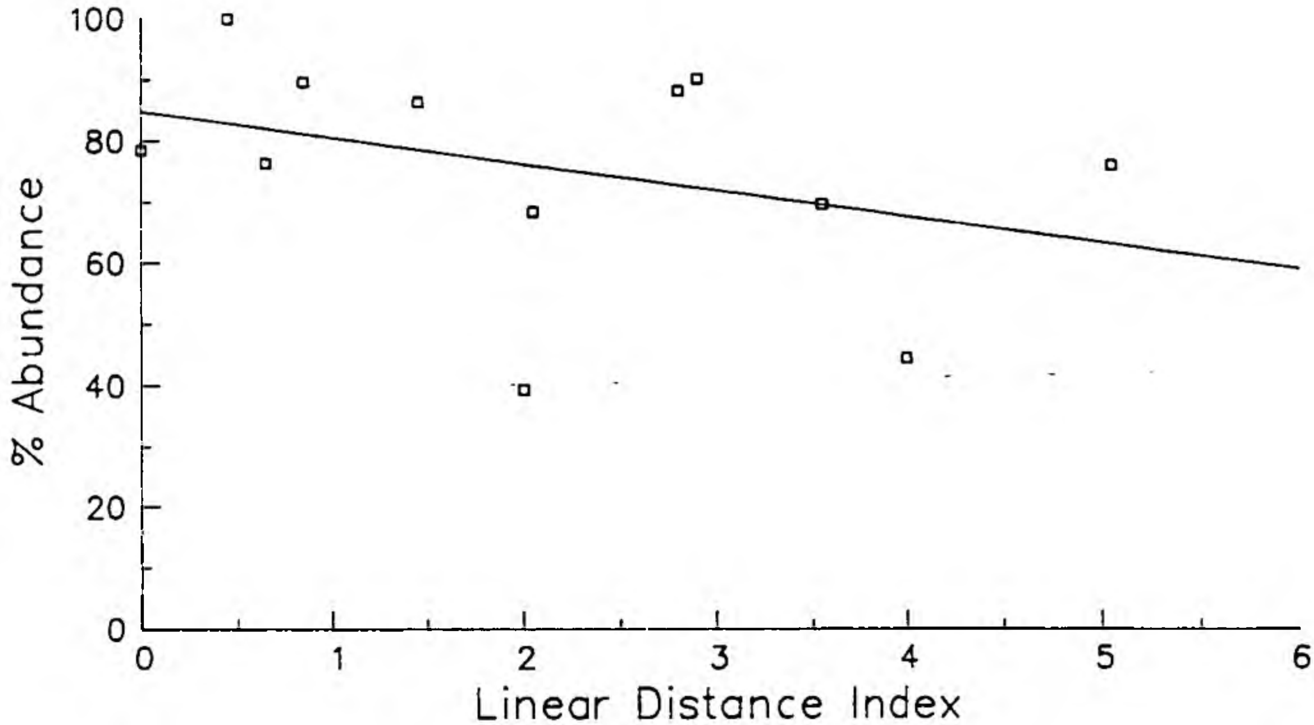


Figure 2.19 **Macrofaunal co-dominance values vs. distance from outfall at Mablethorpe (November 1990)**

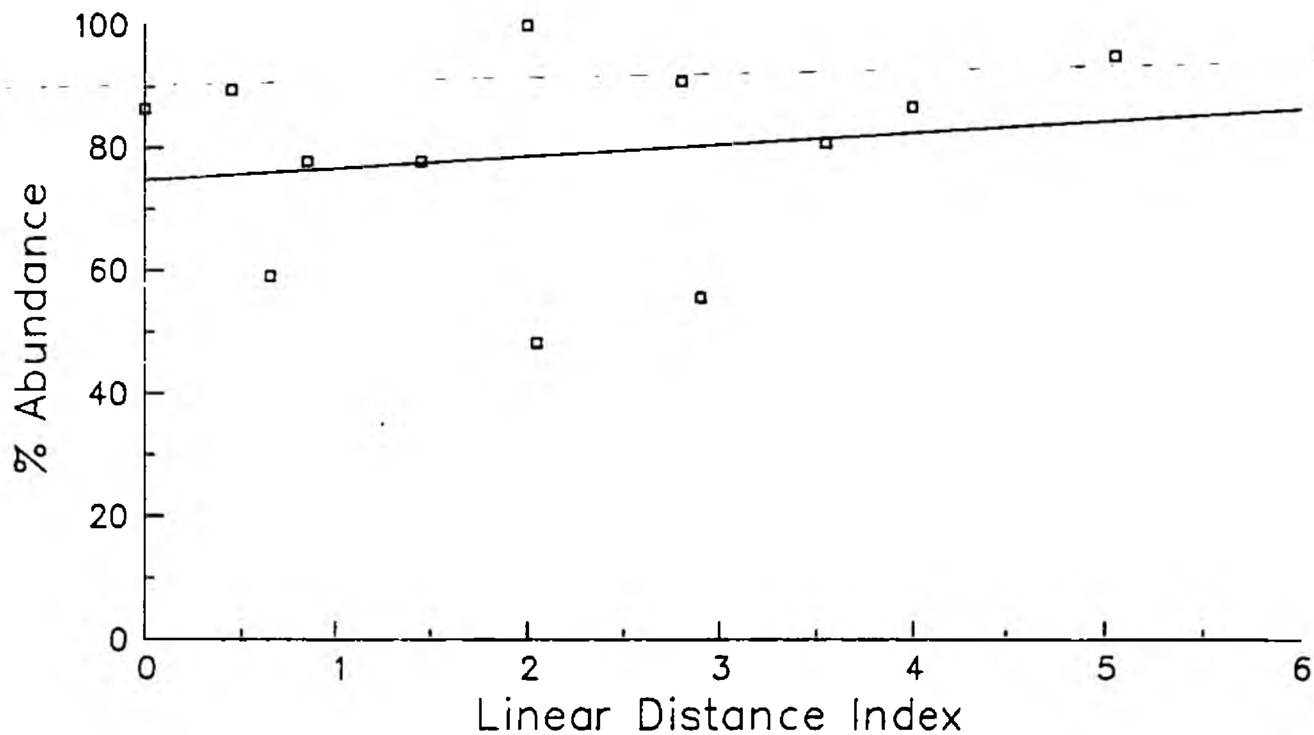


Figure 2.20 **Macrofaunal co-dominance values vs. distance from outfall at Mablethorpe (April 1990)**

2.6 Trevaunance Cove

The biological surveys compared the macrofauna and flora present in 5, randomly positioned, rocky shore quadrats at the mouth of the outfall before and after the trials, with a further 5 quadrats at a control site (Trevellas Porth). It was concluded that no effects of the disinfection trials could be detected. There are several points that undermine this conclusion.

a) The exact location of the quadrats with respect to the outfall was not given. From the data presented it was apparent that 2 of the 5 quadrats at the mouth of the outfall were directly influenced by the low salinity effluent (quadrats 1 and 2). In June, these supported a luxuriant growth (90% cover) of a macroalga *Enteromorpha* species which are capable of growing well in saltwater. However, its distribution is restricted to the immediate vicinity of the outfall by gastropod molluscs (principally Littorinidae) that graze the alga off the surface of rocks. The molluscs are unable to tolerate the low salinity and cannot consume the alga from the intertidal zone that is directly influenced by the effluent. This is emphasised by the very low densities of littorinids in quadrats 1 and 2. This shows that the quadrats at the mouth of the outfall are subject to differential environmental stresses that may invalidate comparisons.

b) Many of the organisms whose abundance appeared to be unaffected by the trials would not necessarily have been directly exposed to the transient stress of the disinfection. The bivalve mollusc, *Mytilus edulis*, the gastropod molluscs (littorinids, *Nucella lapillus* and *Patella* species) and the barnacles (Cirripedia; *Chthamalus* species and *Semibalanus balanoides*) are all capable of sealing themselves off from adverse environmental conditions for prolonged periods. The collembolan (springtail), *Lipura* (now *Anurida*) maritma is covered in a waxy cuticle and is found supported on the surface of water. It does not come into direct (physiological) contact with the seawater. The numbers of the few species that are unable to isolate themselves from contact with the seawater appear to reduce. These include the amphipod crustacean, *Hyale nilssoni*, which has exposed gill surfaces, the mesogastropod, *Lamellaria perspicua*, which completely covers its shell with soft body tissue and the unidentified, soft-bodied, nemertean worm. Each of these species was observed in the vicinity of the outfall prior to the treatment but was either not observed after the treatment (*H. nilssoni*, *L. perspicua* and nemertean) or, in the case of one quadrat, was present in markedly reduced numbers (*Hyale nilssoni*).

c) Differing degrees of exposure and differential recruitment rates will affect comparisons between the observed communities of flora and macrofauna at the 2 sites. These may have invalidated control observations.

d) Seasonal variation prevents accurate comparison between intertidal communities in June and October except in the case of long lived species.

In the absence of suitable particulate substrates enabling meiofaunal studies to be undertaken, sensitive epiphytic species such as hydroids and Bryozoans, present on the fronds of macroalgae, could have been used as indicator species.

In conclusion, it would appear that, despite the short periods of the trials, the more sensitive macrofaunal species that have not evolved to withstand exposure to conditions on rock surfaces at low tides may have been adversely affected by the disinfection treatments. However, further or more detailed studies would have been necessary to have confirmed this.

3 ECOTOXICOLOGY

3.1 Introduction

Toxicological assessments of *Oxymaster* and PAA-treated effluents have been undertaken for few marine and freshwater invertebrate and vertebrate organisms. These studies are summarised in Table 3.1.

Table 3.1 Summary of species used in toxicity testing studies of PAA formulations

Species	Reference	Habitat
Daphnia magna (Water flea)	HRC (1985a)	Freshwater
Crangon crangon (Brown shrimp)	Tinsley and Sims (1987a)	Marine
Mytilus edulis (Edible mussel)	Fairhurst (1987) Flower *et al.* (1991)	Marine/Estuarine
Crassostrea gigas (Pacific oyster)	Butler (1987) Flower *et al.* (1991)	Marine
Salmo gairdneri (Rainbow trout)	HRC (1985b)	Freshwater
Pleuronectes platessa (Plaice)	Tinsley and Sims (1987b)	Marine

Before examining the findings of these studies it is necessary to consider certain aspects of the experimental techniques.

i.) In certain cases (for example, Tinsley and Sims, 1987a, 1987b), LC_{50}^{*} and EC_{50}^{**} values are quoted as mg l^{-1} of *Oxymaster*. In other reports (CES, 1988; Fawell *et al.*, 1989; Horth, 1990) the LC_{50} and EC_{50} values are cited as mg l^{-1} PAA. This can lead to confusion. In the latter cases PAA concentrations have been derived by calculating from the known concentration of *Oxymaster* based on the equilibrium mixture composition:

12-13% PAA, 19-20% hydrogen peroxide, 18% acetic acid, 49% water.

(* - concentration required to kill 50% of test population; ** - concentration required to induce a given effect in 50% of test population (e.g. arrested development in bivalve larvae).

ii.) The existence of an equilibrium mixture means that the effective concentrations of PAA used in the tests are notional. PAA concentrations will be buffered by the equilibrium kinetics replacing PAA molecules that may be consumed by other reactions. This would invalidate comparisons with toxicity studies of other disinfectant systems where the concentration of the active molecule is depleted by reaction.

iii.) The toxic effects of the other components of the equilibrium mixture are inevitably being examined, since they are necessarily present when PAA is tested. This can lead to synergistic toxicity where the observed effects are greater than the sum of the toxicities of the individual components.

iv.) The validity of laboratory-based toxicity tests only holds for comparative toxicity studies. Their relevance to field conditions is minimal. Stressed animals under artificial conditions are generally more susceptible to the effects of toxic materials than unstressed animals.

3.2 Laboratory Studies of *Oxymaster* Toxicity

The lethal concentration values for *Pleuronectes platessa*, *Crangon crangon*, *Daphnia magna* and *Salmo gairdneri* are summarised in Table 3.2.

As would be expected, the freshwater organisms are more sensitive to PAA than their marine counterparts (Mason; 1981, Clark, 1989; Green and Trett, 1989). The underlying mechanism is uncertain but it is suggested to relate to water solutes inhibiting the action of toxic materials at exposed membrane surfaces (Hutchinson, pers. comm.). Although the LC_{50} values for *P. platessa* of between 10 and 12 mg l^{-1} PAA are close to the field dose rates applied for disinfection of sewage, field conditions will differ markedly from those in the laboratory and it is highly unlikely that plaice will ever encounter these concentrations given the rapid degradation of the active components and mixing in the environment.

Experiments have also examined the median effective concentrations (EC_{50} values) for PAA on the development of bivalve larvae to the D-stage (the stage at which protovalves first appear). This has enabled comparisons to be drawn with toxicities of several other disinfectant agents. Butler (1987) examined the effects of PAA on development of oyster larvae and Fairhurst (1987) the effects on mussel larvae. Both species were exposed to 0.006, 0.012, 0.12, 0.6 and 1.8 mg l^{-1} PAA. The results assessed after 48 hours exposure were remarkably similar (Table 3.3).

Table 3.2 **LC_{50} values (mg l^{-1} PAA calculated from known concentrations of *Oxymaster*) for the brown shrimp, *Crangon crangon* (after Tinsley and Sims, 1987a), plaice, *Pleuronectes platessa* (after Tinsley and Sims, 1987b), the water flea, *Daphnia magna* (after HRC, 1985a) and rainbow trout, *Salmo gairdneri* (after HRC, 1985b).**

	C. crangon		*P. platessa*	
Time (hours)	LC_{50}	95%ile limits	LC_{50}	95%ile limits
24	35.3	39.8-54.7	11.8	10.7-13.5
48	20.7	17.9-24.0	10.7	9.6-11.9
72	16.6	14.1-19.4	10.7	9.6-11.9
96	15.2	12.9-17.9	10.7	9.6-11.9

	D. magna		*S. gairdneri*	
Time (hours)	LC_{50}	95%ile limits	LC_{50}	95%ile limits
3	-	-	10.2	7.4-14.4
6	-	-	4.7	4.2- 5.3
24	0.79	0.71-0.89	2.6	2.4- 3.0
48	0.40	0.32-0.47	2.2	1.7- 2.6
72	-	-	1.7	1.4- 2.0
96	-	-	1.6	1.2- 2.2

Sources: HRC (1985a 1885b), Tinsley and Sims (1987a & 1987b)

Fawell *et al.* (1989), Horth *et al.* (1990) and Crathorne *et al.* (1991) used larvae from oysters (species unspecified) from Whitstable, Kent, to assess the relative toxicity of PAA-treated and untreated effluents of varying organic quality as opposed to the *Oxymaster* formulation. They were unable to demonstrate increased toxicity of treated effluents, although untreated "strong sewage" (high BOD) was shown to be up to 20 times as toxic as "weak" (low BOD) sewage. However, it was discovered that the inactivator used to neutralise unreacted *Oxymaster* reduced the toxicity of the untreated sewage. The inactivator comprised a solution of 5% sodium thiosulphate and 0.025% catalase. It was believed that this may have masked certain effects of PAA-treated effluents (Table 3.5 and 3.6). However, this was not found in a study by Horth et al. (1990), which found that sewage and neutraliser was significantly more toxic than unameneded sewage (Table 3.4). This was attibuted to variations in the sewage chemistry.

Table 3.3 Effects of PAA concentrations on larval survival and development for the edible mussel, *Mytilus edulis* (after Fairhurst, 1987) and the Pacific oyster, *Crassostrea gigas* (after Butler, 1987).

Concentrations PAA expressed as mg l^{-1} (determined from known concentrations of *Oxymaster*).

	M. edulis	*C. gigas*
Highest no effect concentration	0.12	0.12
Lowest concentration giving complete failure	1.8	1.8
EC_{50}	0.26 ± 0.05	0.27 ± 0.02

Sources: Fairhurst (1987) and Butler (1987)

Table 3.4 Concentrations of sewage solutions with inactivator, disinfected and untreated required to produce highest no observed effect (HNOEC) and lowest observed effect (LOEC) on the development of oyster larvae (Horth, 1990).

	Sewage + Inactivator	PAA Treated Sewage	Untreated Sewage
LOEC	3.2%	10.0%	20.0%
HNOEC	1.0%	3.2%	10.0%

Source: Horth *et al.* (1990)

Gould (1989b) cites data from several sources comparing the toxicity of different disinfectants to oysters belonging to the genus *Crassostrea* (*C. gigas* and *C. virginica*). However, the effects quoted differ, and the PAA EC_{50} value for *C. gigas* larvae (see above) is compared (favourably), for example, to the bromine chloride LC_{50} values for *C. virginica*. It is not valid to compare different threshold effects on different species for different compounds.

The Microtox test involving effects on bio-luminescent bacteria has been used by Fawell *et al.* (1989) to assess the potential toxicity of sewage disinfected with PAA. The results are summarised in Table 3.5. It should be noted that the samples treated with PAA were samples of domestic sewage, treated with the appropriate dose of PAA, which had then been neutralised.

Table 3.5 Microtox results expressed as mean percentage reduction in bacterial bioluminescence for high and low-BOD sewage samples.

	Percentage reduction in bacterial bioluminescence	
Sample	Low BOD	High BOD
Untreated Sewage	31	85
Sewage + Neutraliser	24	64
10 mg/l PAA at 5 minutes	18	55
10 mg/l PAA at 30 minutes	24	60
15 mg/l PAA at 30 minutes	23	70
20 mg/l PAA at 30 minutes	26	48
30 mg/l PAA at 30 minutes	19	74
40 mg/l PAA at 30 minutes	24	69

Source: Fawell et al. (1989)

The results show that untreated sewage caused a greater reduction in bacterial bioluminescence than disinfected neutralised sewage. The addition of the neutraliser appeared to reduce the toxicity in the disinfected samples, a similar result to that obtained from the oyster larvae experiments.

Under operational conditions neutraliser would not be added to disinfected sewage but the sewage would be diluted upon discharge to the environment. It is therefore difficult to use the results presented in Table 3.5 to define the toxic effect of a discharge of PAA-disinfected sewage. Fawell *et al.* (1989) have also carried out experiments to relate the Microtox results to dilution as shown in Table 3.6. The dilutions are expressed as percentages i.e. 1% dilution is equivalent to 100 fold dilution. It should be noted that the value for 20 mg l^{-1} PAA at 30 minutes is a calculated value.

Table 3.6 **Microtox 15 minute EC_{50} as percentage dilution of sewage in seawater with 95% confidence limits calculated using the moving average method, for the High BOD samples*.**

Sample	Dilution to reach EC_{50} (15 minute)	95% Confidence limits on dilutions
Untreated sewage	25x	11 - 330
Sewage + neutraliser	8x	Cannot be calculated
10 mg/l PAA at 5 minutes	1.4x	0.92 - 1.9
10 mg/l PAA at 30 minutes	2.2x	1.6 - 3.0
15 mg/l PAA at 30 minutes	3.8x	2.9 - 5.6
20 mg/l PAA at 30 minutes	0.96x	Cannot be calculated
30 mg/l PAA at 30 minutes	8.3x	5 - 33
40 mg/l PAA at 30 minutes	4x	2.9 - 6.3

Source: Fawell *et al.* (1989)

* EC_{50} cannot be calculated for low BOD samples as none gave more than 50% reduction in the Microtox test.

These results do not show a clear link between the addition of PAA in increasing amounts and toxicity. In all cases PAA-treated sewage with added neutraliser was observed to have lower toxicity than untreated sewage. However, in two of the three cases the addition of neutraliser alone was also found to reduce the apparent toxicity of the sewage. Crathorne *et al.* (1991) found some evidence from Microtox testing that PAA-treatment may produce short-lived inhibitory or toxic effects, but found that studies on oyster larvae did not reflect this.

Flower *et al.* (1991) carried out a comprehensive series of field and laboratory studies of the effects of PAA-treated effluents on bivalve molluscs *Mytilus edulis* and *Crassostrea gigas*. These studies were undertaken for Anglian Water Services Ltd in 1990 and centred on the Southend *Oxymaster* treatment programme. They can be divided into 3 areas:

- laboratory investigations of the toxicity of PAA-treated wastewater;
- mutagenicity studies of PAA-treated wastewater; and
- field and laboratory investigations of the effects of a PAA-treatment programme on the quality of the receiving water.

The field studies are considered in Section 3.3 and the studies of mutagenicity of PAA-treated effluents are considered in Section 3.4.

In the laboratory studies no evidence was found of depressed feeding rates in mussels exposed to disinfected and non-disinfected sewage at comparable concentrations (0.1% and 10%). Results of EC_{50} studies (concentrations of disinfected or undisinfected sewage required to produce 50% failure of *C. gigas* embryos reaching the D-stage) were less clear. There was some indication of increased toxicity of PAA-treated sewage effluents. However, on the basis of temporal variability of quality of sewage effluent samples, Flower *et al.* were unable to correlate this solely with PAA treatment.

3.3 Field Studies

3.3.1 Southend Receiving Water Quality Studies

In the water quality survey, caged mussels were deployed at five sublittoral sites in the immediate vicinity of the Southend discharge and at two further sites, 2 km seaward (Figure 3.1). A set of mussels was held in the receiving waters for four weeks prior to the end of the PAA treatment period. A further set was exposed for four weeks following treatment. Feeding rates were assessed and body condition indices (BCI) calculated for mussels before and after their respective exposure periods.

The observed feeding rates for mussels in each deployment are shown in Figures 3.2 and 3.3. Work undertaken at the Plymouth Marine Laboratories has shown that feeding rates in mussels from pollution-stressed environments are lower than those of specimens from pollution-free conditions. In the first deployment study, during PAA treatment (Figure 3.2), it can be seen that mean feeding rates in mussels from the Sites 3 to 7 around the mouth of the outfall were lower than the pre-exposure values and lower than those at Sites 1 and 2[1]. However, feeding rate differences between sites and before and after exposure were not statistically significant at the unspecified level tested. In contrast, feeding rates in the second deployment, after PAA treatment, rose significantly ($p<0.01$; Figure 3.3). However, differences between sites were not significant.

These studies do not provide information to suggest that PAA treatment of sewage increases pollution stress in the receiving waters, as indicated by *Mytilus edulis* feeding rates. However, by the same virtue, the data do not indicate that the non-disinfected effluent from the Southend outfall is exerting pollution stress on the bivalves (Figure 3.1). A further observation is that differences between pre- and post-deployment feeding rates will reflect seasonal changes in the physiology of mussel populations. Cycles of reproductive activity, for example, will have marked effects on body reserves and condition which, in turn, will

[1] It should be noted that Tables 3 and 4 in Flower *et al.* (1991) are incorrectly labelled; Site 1 in each table should be labelled 1 and 2 and remaining columns numbered 3 to 7 in sequence.

affect feeding rates. This might invalidate comparisons made between feeding rates of mussel stocks at different times.

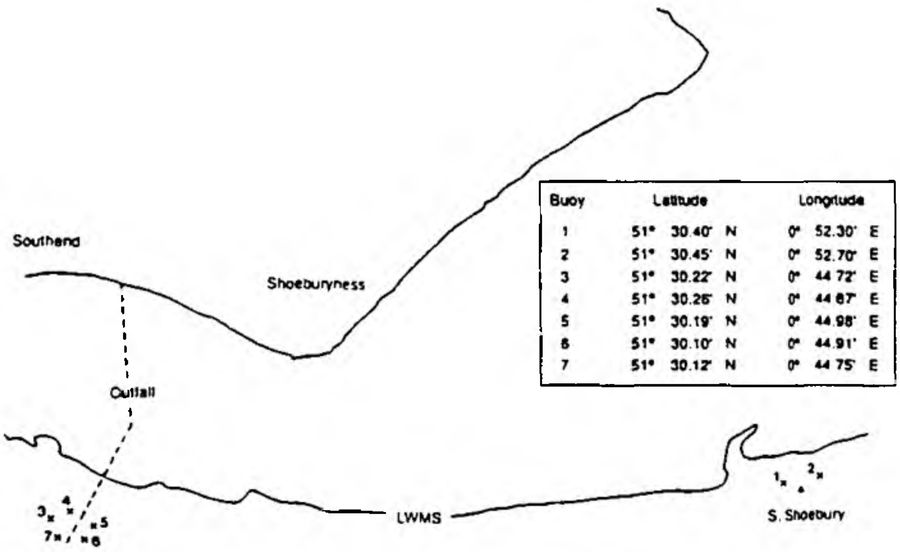


Figure 3.1 Location of Mussel Deployment Buoys

BCI values relate body mass (dry weight) to shell cavity volume and can be used to assess net assimilation and growth of bivalves, such as *M. edulis*, over a given period. These studies also failed to reveal deleterious effects of the PAA-treated effluents. In the first deployment, pre- and post-exposure BCI values cannot be compared, as the data provided by Flower *et al.* (1991) relate to different size classes. Significant increases, however, were observed in the mean shell lengths of mussels at all sites over the exposure period during PAA treatment, and this was taken to indicate normal growth. There was no evidence of reduced growth in the vicinity of the outfall. During the second exposure period shell length did not increase significantly at any of the sites and BCI values fell. This was attributed to possible changes in the quality of food material (Flower *et al.*, 1991). A seasonal decline in ambient water temperatures would produce similar effects.

Water samples, collected concurrently to the *Mytilus* deployment studies at Southend, were used in oyster (*Crassostrea gigas*) embryo-larval toxicity tests for laboratory assessment of water quality. The results were highly variable. On two occasions, samples collected from Sites 1 and 2, 2 km away from the outfall, proved more toxic than samples from sites around the outfall. The reasons for this are uncertain. However, such findings would seem to call into question the validity of this aspect of the study and might cast doubt on the factors affecting mussels in the cage studies described above.

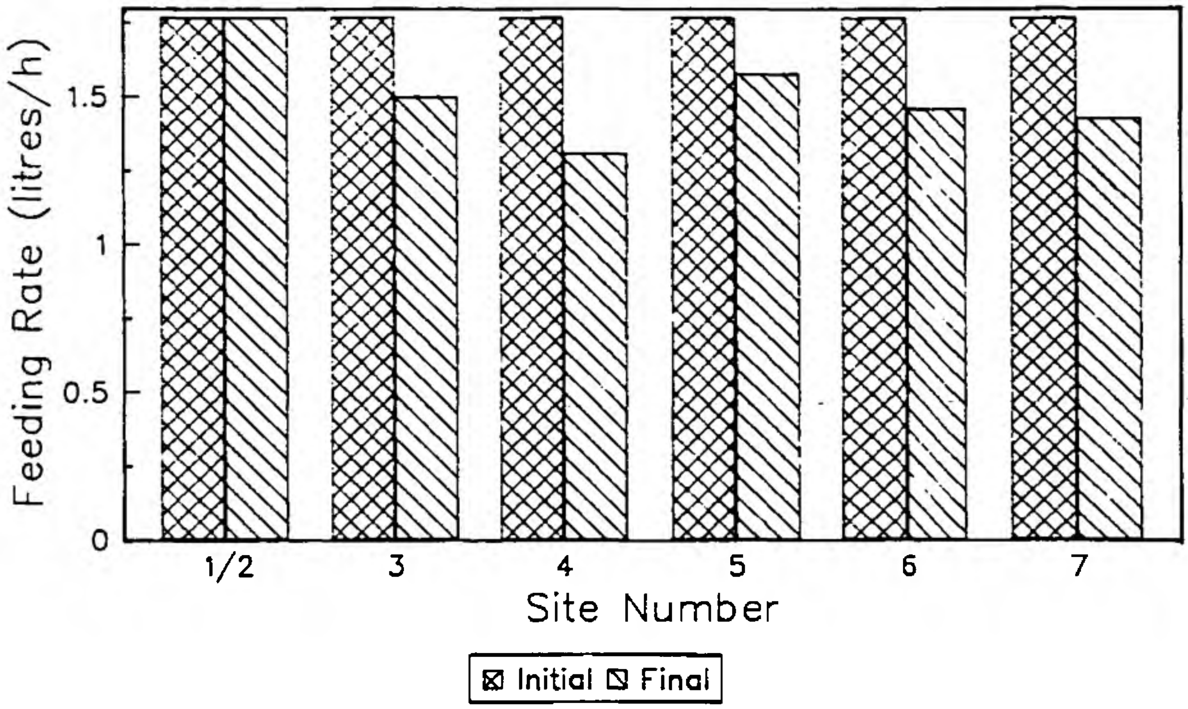


Figure 3.2 Deployment 1 at Southend 1990, Mussel Feeding Rates

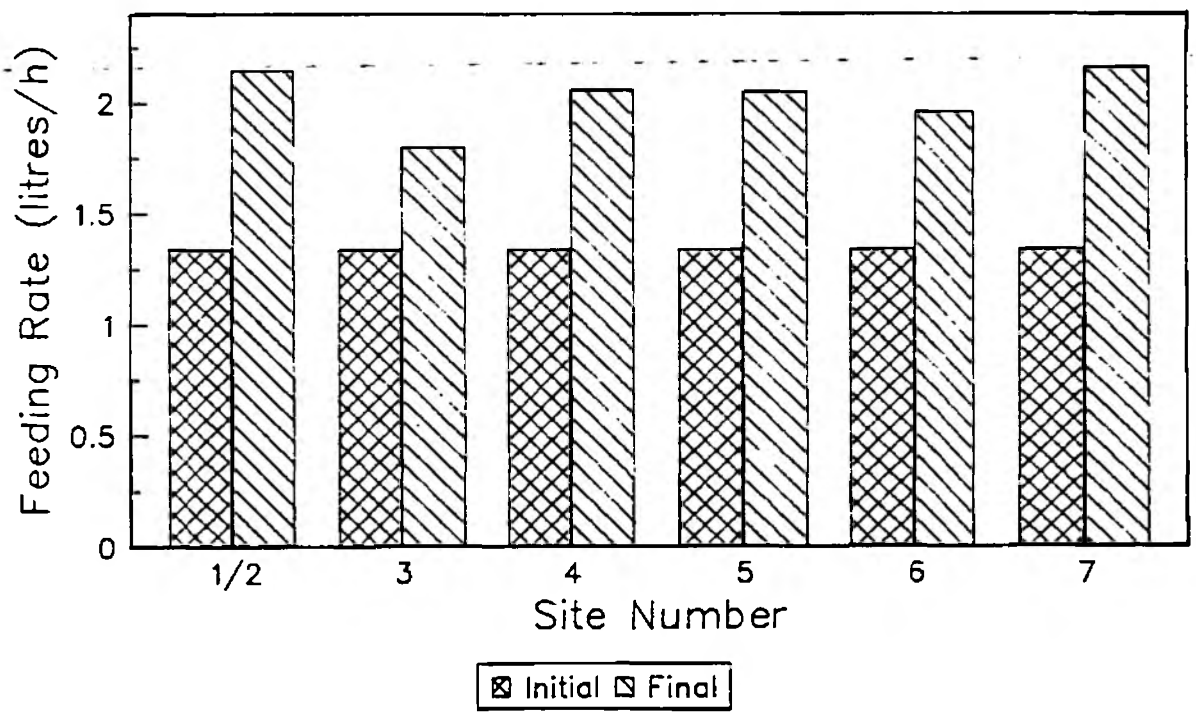


Figure 3.3 Deployment 2 at Southend 1990, Mussel Feeding Rates

3.3.2 Trevaunance Cove Field Trials

The trials at Trevaunance Cove included toxicity testing of treated and untreated wastewater effluents as well as a biological survey programme (Roddie *et al.*, 1991b). The results of the toxicity studies (oyster embryo development and Microtox studies) were inconclusive.

i) Oyster Embryo Studies

Whilst differences could not be detected using this method between the toxicities of treated and untreated sewage samples collected within the treatment works, significant differences were shown between samples collected at periods of low flow and high flow. Highest toxicity correlated with high flow sewage samples. Effluent samples in seawater collected at 2 sites in the dispersion plume proved to be highly toxic to oyster embryos and, with one exception, completely arrested larval development. The exception was a sample taken at a low flow period in the absence of PAA treatment at the site furthest downstream of the outfall. This reduced development of oyster larvae to 45% of the control value.

ii) Microtox Studies

Samples collected within the treatment works failed to show significant differences between treated and untreated sewage but, in general, supported the findings that samples collected at low flow periods were less toxic than those at times of high flow. However, the results were not always self-consistent. The results for effluent plume samples contrasted with those of the oyster embryo studies and indicated similar or lower toxicity (EC_{50}) values than the sewage samples collected within the works.

3.3.3 Porthtowan Field Trials

The PAA disinfection trials at Porthtowan (Roddie *et al.*, 1991a) did not include a floral survey or examination of the macro- and meiofaunal assemblages in the receiving waters. Toxicity studies examined effects in the laboratory, using oyster larval development and Microtox studies, and *in situ*, using feeding rates of caged freshwater shrimps, *Gammarus pulex* (Amphipoda). For reasons beyond the control of the investigators, the results of both the field and laboratory studies were inconclusive. However, the oyster embryo and Microtox studies showed that the treated sewage was more toxic than the corresponding untreated samples. Examination of toxicity of effluents in river water was confounded by variable water quality. Samples of water from upstream of the outfall had differing, inhibitory effects on bioluminescence (Microtox) and larval development. This appeared to relate to background metal concentrations in the stream water. Careful assessment was made of the relative concentrations of metals and organic compounds in the river water and in the treated and untreated sewage effluents. This leads to the suggestion that the observed increase in toxicity arose from the disinfectant mixture itself (residual PAA and/or peroxide).

The caged *Gammarus* feeding studies may have also been affected by the background toxicity of the river water arising from natural metal contaminants. High mortality was observed in cages deployed upstream of the outfall. In all cases low feeding rates were recorded. Again, this was suggested to reflect poor water quality which would appear to be the most likely explanation. Following prolonged exposure to toxic heavy metal ions, crustaceans, including Amphipoda, are known to produce characteristic detoxifying proteins (metallothionines; Rainbow and White, 1989). The *Gammarus* used in the study were collected from the hard chalk waters of the River Darenth in North Kent. These would not have been formerly exposed to the heavy metals present in the soft waters of the Cornish stream. It was of interest to note that feeding rates increased in the presence of the treated sewage effluents. However, this was not statistically significant given the large variability between replicates.

In summary, little can be inferred about the toxicology of the treated, as opposed to untreated, effluents in the Porthtowan trials due to the prevailing environmental conditions. An ecological study of organisms already adapted and selected to live in the stream in the presence of the heavy metals such as Nematoda (Howell, 1984) would have revealed more about the impacts of PAA disinfection than the methods adopted.

3.4 Identification of Bioaccumulated PAA By-products

Samples of *Cerastoderma* (= *Cardium*) *edule* (common cockles) were collected from the area in the vicinity of the Southend outfall for GCMS examination at the MAFF Burnham-on-Crouch Laboratories. The aim of the investigation was to determine whether potential PAA by-products had accumulated in the bivalves. However, it was not possible to identify metabolites of potential by-products (Waldock, pers. comm.). With new information from the WRc studies concerning the possible formation of brominated compounds in the presence of PAA (Crathorne *et al.*, 1991) it is hoped that this line of research can be resumed.

3.5 Mutagenicity

Flower *et al.* (1991) attempted to assess the mutagenicity of PAA-treated sewage using gill tissue from the mussel, *Mytilus edulis*. The tests, based on a technique to evaluate the incidence of micronuclei formation (Majone *et al.*, 1987; Brunetti *et al.*, 1988 and Scarpato *et al.*, 1990), involved the exposure of mussels to serial dilutions of PAA-treated sewage in the laboratory and to the receiving waters at Southend during and following *Oxymaster* treatment. For technical reasons the method could not be employed using gill tissue, and the attempt was abandoned. Alternative mussel tissues, including haemolymph, were suggested as more suitable alternatives, but it is not known whether these are to be adopted in any future studies.

There have been a limited number of reports relating to the mutagenic properties of PAA or sewage disinfected with PAA. Agnet *et al.* (1976) gave some data on mutagenicity for PAA, but this work has been criticised by Baldry *et al.* (1990) as being contrary to more recent findings. They and Corner & Fraser (1990) point to two recent studies which show no significant mutagenic activity. These studies were:

a) Ames tests carried out on PAA-disinfected sewage at Southend. The tests were carried out for Interox by Severn Trent Water.

The results showed no significant mutagenic activity either in the sewage before disinfection or after disinfection with PAA. They also showed no increase in toxicity to *Salmonella typhimurium* after disinfection with PAA.

b) Ames tests carried out on PAA by Microbiological Associates Inc. Corner & Fraser (1990) do not give details of the test technique but merely indicate that PAA did not show mutagenic activity either with or without exogenous metabolic activation.

There is only one reference in the literature to attempts to measure the carcinogenic activity of PAA. This work (Bock *et al.*, 1975) has been criticised by Baldry *et al.* (1990), for the methodology employed. In the absence of any other work on mammalian cells, it is not possible to draw any definitive conclusion about the carcinogenic potential of PAA.

4 REVIEW OF INTERNATIONAL EXPERIENCE

In an interview with Interox it was stated that the UK is the centre of their strategic development because of the problems associated with compliance with EC bathing water quality standards and the high UK population densities. The UK is also the centre for their research and development activities. While they expect countries outside the UK to adopt oxyacid disinfection treatments in time on the grounds of its "environmental acceptability", the current widespread use of cheaper alternatives, such as chlorine and chlorine donors, has prevented this.

Extensive searches of the international literature failed to reveal reports of ecological studies relating to the use of PAA formulations or other higher oxyacids (e.g. perpropionic acid). One American supplier (Minntech Corporation, Minneapolis) was found to use a reaction mixture of PAA and hydrogen peroxide, under the trade name of Minncare, as a disinfectant for reverse osmosis membranes in place of formaldehyde (Maltais and Stern, 1990). The FDA status of PAA is not known, but hydrogen peroxide is on the GRAS (generally recognised as safe) list of compounds for use in cleaning tanks and surfaces in the food industry.

5 CONCLUSIONS

5.1 Observed Impact on Benthic Flora and Fauna

To date, insufficient field data have been gathered concerning the effects of PAA-treated effluents on marine, estuarine and freshwater ecosystems to enable definitive conclusions to be drawn. Ecological data available from the Southend trials (Joslin and Gould, 1991) strongly suggest that changes occurred resulting in the development of modified assemblages of macroinvertebrates (Section 2.2.3). The cause of these effects is unknown. Although meiofaunal samples have been examined from the sediments close to the Southend outfall, it would be wrong to draw inferences from the increased dominance observed during the periods of treatment from a single sampling station examined once every quarter (Trett *et al.*, 1990a, b, c). This aspect needs closer attention, as these organisms are nearer to the base of food webs than macrofauna and respond more rapidly to changes in the nature and quality of their food supply. As this can range from diatoms to interstitial and episammic bacteria, such studies provide a sensitive means of integrating fundamental changes in sediment microbiology.

Close inspection of the data for Southend would indicate that the statement that "no major changes" had occurred at Southend, and that those changes observed in the macrobenthic communities were purely seasonal (Joslin and Gould, 1991), cannot be substantiated. The downward trends in macrofaunal species richness and biomass alone would seem not to support this and should provide grounds for closer investigation in future. Multivariate analyses, such as cluster analyses, might prove valuable in identifying homologous species assemblages both within and between surveys and aid in the identification and delineation of impact zones. This in turn would enable key species groups to be recognised and could focus attention on sites for chemical monitoring studies. It is understood that multivariate analysis may be used at a later date when more data is available. In this connection, meiofauna might also prove to be valuable in increasing the resolution of the surveys.

At Mablethorpe (Section 2.5.2) there appears to be some indication that co-dominance increases with proximity to the outfall more significantly following the period in which PAA disinfection took place than prior to PAA treatment. This may point towards additional stress on the organisms close to the outfall during the bathing season. However, the change in inter-tidal sampling methods means that it is not possible to infer a great deal about variation in species richness. The density of individuals at most intertidal sites appears to decline between April and November, an effect that could be indicative of some additional stress on the organisms in that period but there is insufficient data to substantiate this conjecture. It is expected that the possible changes occurring in the macrofauna in the vicinity of the outfall will become more apparent when sampling is carried out in later years.

It should be emphasised that the demonstration of a correlation between the timing of PAA disinfection as well as the location of the point source discharge with the changes observed in the benthic communities is not proof of a causal relationship. In the present case a plausible mechanism has yet to be established and demonstrated. Other factors, such as seasonal changes in the nature of trade effluents, might be implicated. Delaying the start of PAA disinfection in one season might allow this point to be resolved by using surveys to determine whether a "recovery" or "improvement" in benthic communities resulted. Further benthic studies are currently in progress, and it is to be hoped that these will enable longer term trends to be described.

It is possible to refute the suggestions put forward at both Southend and Mablethorpe that no changes appear to be taking place. The current indications from the surveys at Southend and Mablethorpe are that changes in relatively insensitive macrofaunal communities are observed at times co-incident with the use of PAA disinfection. These changes also appear to be correlated to some extent with proximity to the outfalls in question. It therefore appears possible that there is a direct or indirect effect, which exerts some influence on the macrofaunal communities in the vicinity of outfalls discharging PAA-treated sewage. However, in the absence of a mechanism for this action it is not possible to refute the hypothesis that the apparent changes could be co-incidental.

The relatively limited data available for Trevaunance Cove may also indicate that an effect of PAA may be present, but more data would be required to confirm this.

5.2 Effect on Effluent Chemical Quality

5.2.1 Organic Quality

It is clear that addition of PAA can increase both the BOD and the COD of secondary effluents. Acetic acid is the main residual and this would theoretically increase the BOD of the effluent by 1.4 mg per mg PAA. At the indicative dose rate of 6 mg l^{-1} given by Interox this would lead to an elevation of effluent of 8.4 mg l^{-1} BOD. In experiments by Interox an increase of 9.4 mg l^{-1} in BOD was observed.

In the context of usual consent standards for BOD in secondary effluents, the increase in BOD caused by the addition of PAA at the suggested dose rates would appear to be unacceptable in the case of secondary treatment.

The effect on untreated and primary settled sewage is not so clear. Interox has observed a fall in BOD for both untreated and settled sewage, but trials at Trevaunance Cove generally showed an insignificant increase. In the context of a BOD of approximately 300 mg l^{-1} for raw sewage and 200 mg l^{-1} for primary effluent the effect is unlikely to be of major significance.

5.2.2 Other Indicators of Chemical Quality

The following conclusions can be drawn regarding pH, ammonia-N and suspended solids:

- it is considered unlikely that PAA treatment would consistently lower the pH of sewage by more than about 1.0 unit, if the dosing system is effective;

- no systematic effect on ammonia-N was observed; and

- no systematic effect on SS was observed.

5.3 Trace Constituents of *Oxymaster*

From the information examined it would appear that any potential environmental impacts arising from the use of PAA as a disinfectant are likely to be associated with residuals of the major constituents of *Oxymaster* degradation products and by-products of its reaction with sewage, rather than with trace impurities reported as present in *Oxymaster*.

5.4 Disinfection Residuals and Ecotoxicity

The major residual after the use of PAA is acetic acid, the effect of which is discussed in Section 1.3.1. The other major residuals that may be present are unreacted PAA and hydrogen peroxide. From the experimental data reviewed, residual PAA was observed in the most recent field trials, where sensitive detection methods were employed, but not otherwise. In one case very high concentrations of up to 16 mg l^{-1} were observed in the final effluent. However, since the nominal dose rate was 6 mg l^{-1} this appears to indicate that the dosing system was ineffective. These results serve to demonstrate that sewage may have a finite capacity for PAA reaction.

Hydrogen peroxide has been observed in three cases, at levels of up to 76 mg l^{-1}. Peroxide levels tend to be high when PAA levels are high and it is possible that the presence of peroxide in the effluent could be used to highlight the point at which the sewage is unable to react with further *Oxymaster*. However, the correlation between these concentrations is not such that high confidence could be placed on the use of peroxide alone as an indicator for dose control. The variable nature of sewage effluent may well mean that the reactive capacity of effluent at a given site is variable, empasising the potential usefulness of a control system based on residual detection.

The 24 hour LC_{50} for *Oxymaster* for the water flea are 0.79 mg l^{-1} PAA and the EC_{50} for development of edible mussel to the D-stage is 0.26 mg l^{-1} PAA. However, it is not possible to translate laboratory values of LC_{50} and EC_{50} direct to PAA residual concentrations because:

- organisms are often already under stress and so may be more susceptible to the effects of toxic materials;

- dilution is available, although disinfection systems are often required at old short sea outfalls where initial dilutions at certain parts of the tidal cycle may be very poor; and

- many other compounds are present in sewage that may have inhibitory or synergistic effects on PAA toxicity.

5.5 By-product Formation and Effect on Heavy Metal Content

The identification of by-products in field conditions has proved extremely difficult. The only substances positively identified as arising from PAA treatment of sewage are bromine, bromamines and small amounts of adsorbable organic halogens (probably organobromides). The first two products were detected at Porthtowan, where significant overdosing may have occurred. It is impossible to exclude the possibility that other compounds arose as by-products, but the concentrations arising must have been below analytical detection limits. The possibility of increasing the bio-availability of heavy metals in effluents has been suggested and was confirmed to some extent by work at Porthtowan and Trevaunance Cove. These studies showed that the dissolved fraction of metals tended to increase post-disinfection, but that the total amount of metals did not vary significantly. At Trevaunance Cove the same effect was not so clearly demonstrated due to screening taking place concurrently with disinfection. However, in both cases the magnitude of this effect was small.

Environmental effects of the evolution of brominated compounds are difficult to assess, since they depend on many factors. The highest levels found at Porthtowan could cause an acute effect close to the outfall, but would tend to dilute and disperse relatively quickly and were only present transiently in the effluent. At other trials levels of by-products have been generally lower and it is therefore concluded that levels of disinfection residuals are more likely to cause significant environmental effects. The small increase in soluble metals may also have a small detrimental effect on the environment. However, once again it is concluded that this effect is likely to be overshadowed by other factors.

Comparative toxicity studies of untreated sewage and PAA-treated sewage which have not shown any significant increase in toxicity, except at Porthtowan, although the inactivator used to remove residual PAA has been shown to suppress apparent toxicity in some cases.

5.6 Mutagenicity

It may be concluded that there is little evidence of any mutagenic risk arising from the disinfection of sewage with PAA, especially in view of the dilution and dispersion after the sewage is discharged.

Regarding carcinogenicity little evidence is available, but evidence from the Ames test, while by no means definitive, suggests that it is not likely to be a serious risk.

5.7 Bioaccumulation

It is clear that bio-accumulation of by-products is a potentially serious problem where bio-accumulable compounds are discharged, and consequently this should be kept under review so that firm conclusions can be drawn in the future. Even very small concentrations can be magnified by this effect, but no field studies are available. Given that brominated compounds have been found to occur in the Porthtowan effluent, it is suggested that bioaccumulation studies could now provide useful data on this question.

6 SUGGESTIONS FOR FUTURE WORK

This review has included all the research carried out into the environmental effects of PAA in the available literature. It is apparent that there are still some uncertainties about these effects, and some suggestions for further research to reduce these uncertainties are provided in the list below.

i) Much has been written about the effects of suspended solids on aquatic ecosystems (Moore, 1976; CES, 1990). However, little work has been carried out regarding possible effects of PAA on the particle size distribution, surface charges and the flocculation and sedimentation characteristics of suspended organic/inorganic matter in sewage effluents. This is important because these factors will have an effect on the already modified communities of benthic organisms present in the vicinity of a sewage outfall. Changes in the distribution pattern of materials in sewage effluents can lead to the concentration of adsorbed pollutants, already present in the effluents, at new sites in the receiving waters, with consequent effects on communities of burrowing and filter-and-deposit feeding species.

ii) Comparative study of the ecological and chemical effects of various disinfection processes could provide some insight into the relative environmental acceptability of various processes and may help clarify the possible mechanisms for the observed effects in ecology at Southend and Mablethorpe.

iii) Little appears to be known about the dilute aqueous phase chemistry of PAA. It has proved extremely difficult to investigate the chemistry in real situations where *Oxymaster* is reacting within the complex organic matrix contained in sewage. It is suggested that some more controlled experiments in the aqueous phase would be useful to establish whether any of the suggested by-products can be formed and give a firmer foundation for the theory of PAA reaction kinetics.

iv) The effect of pH and the influence of buffering of the sewage components and the carriage water in hard water areas has not been fully investigated. In principle it seems that PAA should be most effective in soft water areas, but there are no reports confirming this in practice.

v) Accumulation studies of organobromides, such as orgainc bromamines, for a number of aquatic species could be very useful. It is important to consider species with different feeding strategies (e.g. browsers, filter-feeders, deposit feeders, scavengers and predators) and species with different lipid contents.

vi) The variation in sewage quality during the daily cycle means that differing doses of PAA appear to be required at diffent times, rather than the constant dose currently applied. The development of a dosing control system based on the detection of *Oxymaster* residuals, such as hydrogen peroxide and PAA, would be a major improvement in dose control.

7 SUGGESTIONS FOR IMPROVEMENTS IN METHODOLOGY OR INTERPRETATION

i) BMWP scores can provide a useful index of the ecological quality of communities, but can be misleading where ambient conditions change significantly. It is recommended that where such scores are used, the species lists from which BMWP scores are derived are also included.

ii) If, as is usual, time does not permit long term gathering of base-line ecological data prior to the start of field trials, monitoring the benthic community after the end of the trials or reducing the length of time for which PAA-treatment takes place could provide useful data to determine if any changes are caused by the use of PAA at an individual outfall. To date, surveys have been rather short term, although it is understood that more work is being carried out at Southend.

iii) Laboratory toxicity studies of particular compounds are difficult to extend to field conditions where by-products may be formed, synergistic effects may occur, and many species are present under varying degrees of stress. Such studies are useful to determine comparative toxicities of individual compounds under similar conditions, but cannot be simply extrapolated to field conditions. The definitive determination of environmental effects is only possible by field ecological surveys.

iv) It is recommended that consideration be given to the use of meiofauna to determine environmental effects, since they form the food for larger organisms and are therfore closer to the base of food webs. They therefore respond more rapidly to changes in the environment than do macrofauna.

v) Where meiofauna are unavailable due to the physical nature of the habitat, epifaunal species could be used as sensitive indicators of changes in conditions.

vi) Much effort has been devoted to the study of the effects of PAA on indicator organisms. It is suggested that the primary focus of future research should be on the effects on pathogenic organisms and, in particular, viruses. It would be useful, therfore, to commence future investigations on disinfection products with laboratory studies on sewage samples with artificially enhanced virus levels and to agree a protocol for the assay of viruses in sewage.

Appendix B1

Meiofaunal and Macrofaunal Data Sets from the Thames Estuarine Benthic Programme, Southend April 1989 - March 1990

Southend Outfall Subtidal Meiofaunal Assemblages

Species	1989/2	1989/3	1989/4	1990/1
Nematoda				
Metachromadora suecica	81		81	+
Metachromadora sp.	81			
Chaetonema riemanni	27			
Richtersia inaequalis	1221	220	700	3591
Halalaimus longicaudatus	27			
Leptolaimus sp. 2	54			
Sabatieria punctata	27	49	108	
Dichromadora sp.	163			
Desmodorid sp. 1	163			
Aegialoalaimid sp. 1	54	343		199
Linhomoeus sp. 2	27			
Theristus sp. 3	54			
Desmodora sp. 1	190			
Calamicrolaimus honestus	27	98	54	
Prochromadorella ditlevseni	27	49		
Diplopeltid sp. 1	27	49		
Ptycholaimellus ponticus	27			
Halalaimus isaitshikovi	27	49	27	299
Cyatholaimus gracilis	27			
Spilophorella candida	27			299
Daptonema normandica	27			
Onyx perfectus	54	147	135	199
Hypodontolaimus balticus	27			
Paracanthonchus sp.	27			
Camacolaimus tardus	109			
Leptolaimus papilliger	27	49		
Spirinia parasitifera	27		27	+
Pseudonchus sp.	27			
Oncholaimus brachycercus	27			
Ascolaimus elongatus	+	49		
Mesacanthion diplechma	+	98		199
Spilophorella paradoxa		294	27	100
Cyatholaimid sp. 1		736		
Odontophora setosa		343	27	
Oncholaimellus calvadosus		49	+	
Xyalid sp. 2		98		
Trefusia longicaudata		49		
Ceramonematid sp. 1		49		

continued ...

Species	1989/2	1989/3	1989/4	1990/1
Camacolaimus barbatus	27		27	119
Microlaimus marinus		49	27	
Cricolaimus sp.		+		
Daptonema setosa		+		
Dichromadora cephalata			242	1396
Sigmophoranema rufum			81	
Xyalid sp. 1			323	
Viscosia viscosa			108	
Daptonema sp. 2			27	
Rhabditid sp. 2			27	
Paralongicyatholaimus sp.			189	
Cyatholaimid sp. 2			108	1995
Microlaimus conothelis			27	
Desmoscolex falcatus			27	+
Theristus sp. 3			27	
Enoplolaimus vulgaris			54	
Chromadorita tentabunda			27	
Odontophora villoti			27	299
Sabatieria longiseta			27	
Viscosia glabra				299
Oncholaimus skawensis				100
Paracanthonchus caecus				199
Eleutherolaimus sp.				100
Neochromadora trichophora				100
Leptolaimus sp. 3				100
Halichoanolaimus robustus				+
Indet.	326	343	215	499
1B:2A	1.49	1.36	1.44	0.69
N	3063	5247	2911	10471
S	32	22	29	22
Copepoda				
Ectinosoma melaniceps	80			
Halectinosoma sp.	16		245	
Copepodites	16		9	
Stenhelia giesbrechti		8		
Canuella perplexa		25	9	18
Cletodes longicaudatus			18	
N	112	33	281	18
S	3	2	3	1

continued ...

Species	1989/2	1989/3	1989/4	1990/1
Acari				
Copidognathus dentatus				9
N	0	0	0	9
S	0	0	0	1

Southend Outfall Intertidal Meiofaunal Assemblages

Species	1989/2	1989/3	1989/4	1990/1
Nematoda				
Odontophora villoti	1587			
Chromadora macrolaima	5555			15039
Ptycholaimellus ponticus	15078	10468	7821	7520
Tripyloides sp.	1587	13689	869	1504
Metachromadora riemani	794		1738	48125
Ascolaimus elongatus	2381	805		7520
Desmolaimus zeelandicus	6349			
Theristus sp. 3	794		869	
Microlaimus marinus	3968	24962		
Metachromadora sp.	14284	1610		
Monoposthia costata	2381	2416	2607	1504
Linhomoeid sp. 1	2381		2607	
Anoplostoma viviparum	1587	1610	7821	7520
Paracanthonchus heterodontus	2381	805		
Terschellingia longicaudatus	1587	805	869	1504
Calomicrolaimus honestus	794	805	4345	
Richtersia inaequalis	794	+	+	+
Axonolaimus paraspinosus	794	805		
Praeacanthonchus sp.	1587	1610	869	
Calyptronema maxweberi	794			+
Linhomoeus sp. 2	794			1504
Eleutherolaimus sp.	794	+	869	
Daptonema normandica	1587	6442	7821	
Daptonema tenuispiculum	1587	805	1738	
Sabatieria punctata	1587	8858	4345	4512
Oncholaimellus calvadosicus	1587			
Viscosia sp. 2	2381			
Spirinia parasitifera	794	1610	1738	6016
Daptonema sp. 2	794			+
Hypodontolaimus balticus	+		+	3008
Sphaerolaimus balticus	+		+	1504
Sabatieria longicaudatus	+			
Nemanema cylindraticaudatum	+		+	
Quadricoma sp.	+			
Desmoscolex falcatus	+			
Chromadorita tentabunda		2416	1738	1504
Viscosia glabra		805		
Diplopeltid sp. 1		805		

continued ...

Species	1989/2	1989/3	1989/4	1990/1
Nematoda continued				
Oncholaimus skawensis		805	1738	1504
Daptonema setosa		805	1738	
Comesomatid sp. 1		+		
Euchromadora vulgaris		+		
Parasphaerolaimus paradoxa		+		
Dichromadora cephalata			11296	
Leptolaimus sp. 3			2607	
Aegialoalaimid sp. 1			3476	1504
Theristus acer			869	
Xyalid sp. 1			1738	
Microlaimus conothelis			18247	
Monoposthia mirabilis			869	
Anticoma acuminata			+	
Mesacanthion diplechma			+	
Halalaimus isaitshikovi			+	
Viscosia elegans			+	
Setosabatieria hilarula			+	
Sigmphoranema rufum				6016
Paracanthonchus caecus				7520
Odontophora setosa				4512
Microlaimus robustidens				12031
Oxystomina asetosa				1504
Viscosia cobbi				1504
Tripyloides marinus				4512
Praeacanthonchus opheliae				1502
Oxystomina elongata				+
Thalassoalaimus tardus				+
Indet.	6349	4026	4345	3008
1B:2A	0.40	0.34	0.54	0.26
N	85711	87767	101670	153403
S	35	27	36	30
Copepoda				
Bryocamptus sp.	709			
Pseudomesochra latifurea	205			
Schizopera clandestina	103			
Laophonte sp.	1829			
Copepodites	308	10	53	
Ectinosoma sp.		80		

continued ...

Species	1989/2	1989/3	1989/4	1990/1
Copepoda continued				
Stenhelia geisbrechti		760		
Paramphiascella sp.		10		
Longipedia sp.		70		
Longipedia coronata			441	
Cletodid sp.				83
Stenhelia palustris				100
Amphiascus sp. 1				75
N	3154	930	494	258
S	5	5	2	3
Acari				
Oribatid sp.	17		18	8
N	17	0	18	8
S	1	0	1	1

Macrofaunal Species Recorded at Southend Outfall, 1989-1990; Thames Estuary Benthic Programme (Attrill, 1990).

Species	1989/2	1989/3	1989/4	1990/1
HYDROZOA				
Sertularia colonies	10	10		
ANTHOZOA				
Sagartia troglodytes			20	
NEMERTINA				
?*Cephalotrix* sp.		3		
OLIGOCHAETA				
Monopylephorus rubroniveus	2.5	3		12.5
Tubificoides benedeni				2.5
POLYCHAETA				
Nephthys caeca	57.5	87	37.5	50
Glycera convoluta	2.5	10	20	7.5
Caulleriella sp.	7.5		12.5	10
Eteone longa		10		
Aricidea ?minuta	232.5	37	87.5	85
Scoloplos armiger	37.5		5	30
Capitellides giardi		33		
Unidentified poly SEBBA			2.5	
Unidentified poly SR2C			2.5	
Neoamphitrite figulus	2.5		2.5	
Magelona papillicornis			5	
Pygospio elegans		3	22.5	40
CRUSTACEA				
Diastylis bradyi		13	12.5	
Melita sp.			2.5	
Periculodes longimanus	15	27		
Stenothoe marina	5	40		
Microprotopus maculatus	2.5			
Bathyporeia elegans	27.5	7	15	22.5
Bathyporeia guilliamsoniana				2.5
?*Dexamine setosa*		17		
Caprella linearis	22.5	257		
Crangon crangon		7	2.5	
Eupagurus bernhardus			2.5	
Carcinus maenus		3		
PYCNOGONIDA				
Nymphon rubrum		3		

Macrofauna Species Recorded at Southend Outfall, 1989-1990 continued

Species	1989/2	1989/3	1989/4	1990/1
MOLLUSCA				
Retusa obtusa				5
Macoma balthica	2.5	10	7.5	12.5
Tellina fabula	12.5	7	7.5	27.5
Abra sp. (?*alba*)				2.5
Cerastoderma edule		3		
TOTALS	440	590	267.5	315

Community Data

Total No. Spp.	15	21	18	15
Mean No. Spp.	8.25	11	9.75	9.50
Diversity (H'e)	1.67	2.13	2.30	2.20
Evenness (J)	0.62	0.69	0.78	0.81
Temperature (°C)	12	20	15	8

Appendix B2

The Value of Meiofaunal Assemblages in the Detection of Change in Aquatic Habitats

Meiofauna are sediment-dwelling animal species that measure less than 1 mm. They are of fundamental importance in marine, estuarine and freshwater ecosystems, forming food for larger, macrofaunal invertebrates as well as fish and possibly certain bird species such as waders (Feil, 1989; Gee, 1989). Meiofaunal studies have become accepted as the most sensitive indicators of disturbance or stress in aquatic habitats. They offer many distinct advantages over macrofaunal studies in the assessment of prevailing environmental conditions (Newell *et al.,* 1991). Essentially, being nearer to the base of food webs in aquatic ecosystems they respond rapidly to any changes in environmental conditions that affect the quality and nature of their food supply. Unlike other larger animals, the less mobile meiofauna are subjected continuously to the effects and constraints of any "foreign" materials that enter their environment and this is reflected in the composition of their communities.

The advantages of using meiofauna as biological indicator organisms in monitoring environmental conditions can be summarised as follows:

i. the existence of inherently stable populations enables changes in the structure of meiofaunal assemblages to be related more easily to changes in environmental conditions;

ii. short generation times and high species diversity, especially in the Nematoda, enable communities to respond more rapidly than macrofauna to changing conditions;

iii. certain meiofaunal species are amongst the last to survive in grossly polluted conditions; consequently meiofaunal indices can be used to assess the entire range of polluted conditions;

v. the high densities of certain meiofaunal species in a given environment make statistically valid sampling simpler than for other groups; sampling macrofauna on a comparable scale would itself have a marked impact on benthic communities;

vi. given the equivalent effort required to gain the same resolution using macrofaunal indices, the costs of meiofaunal surveys and analyses in terms of time, effort and expenditure are relatively low. This has proved to be an important consideration where industries and authorities have had to strike a balance between competing demands on limited resources.

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