THE EFFECT OF SUNLIGHT AND OTHER FACTORS ON THE SURVIVAL OF STARVED ENTERIC BACTERIA IN NATURAL WATERS

Thesis submitted in fulfillment of
the requirements for the Degree of
Doctor of Philosophy

CHERYL MARGARET DAVIES

Environmental Engineering Section
Civil Engineering Department
December 1989

University of Newcastle upon Tyne
Newcastle upon Tyne NE1 7RU

ACKNOWLEDGEMENTS

I would like to express my appreciation of my supervisor, Mrs. L.M. Evison for her assistance and guidance throughout the course of this study.

I acknowledge the technical help provided by the laboratory staff of the Department of Civil Engineering, namely, Mr. J. Hamilton, Mrs. P. Johnston, and Mr. M. Hetherington.

I would also like to thank Mr. J. Pomfret of Northumbrian Water for his valuable assistance in the earlier stages of this study.

I am indebted to the Science and Engineering Research Council (SERC) for their financial support without which this study would not have been possible.

ABSTRACT

Results are reported from a series of experiments to determine the effects of certain environmental factors, namely, sunlight, temperature and salinity on the survival of selected enteric bacteria which are of public health importance either as indicators of faecal pollution or as pathogens.

The survival of the starved organisms in seawater and in freshwater microcosms exposed to artificial and natural sunlight, and in the dark, at a range of temperatures was investigated. An acridine orange direct viable count (AODVC) using an epifluorescent microscope was employed in addition to selective and non-selective cultural methods for estimation of decay rates of the test bacteria. The use of the AODVC allowed enumeration of those bacteria which respond to the stresses of the natural environment by entering into a viable but non-culturable form. These otherwise would not be detected, as they are, by definition, non-culturable using traditional enumeration techniques based upon the production of visible signs of growth.

Two strategies were employed by the organisms in response to the unfavourable conditions. Prior growth of those members of the Enterobacteriaceae in a nutrient-poor medium before inoculation into the microcosms allowed adaptation of the cells to low nutrient concentrations, thus extending survival and

resulting in higher resistance to other stresses such as visible light. This extension was, however, only temporary, the ultimate fate of the bacteria being death. Enterococci were particularly sensitive to low nutrient concentrations and died very rapidly in the light.

All bacteria tested were able to adopt the viable but non-culturable strategy in the dark as a temporary measure, though it was only a matter of time before viability as well as culturability was also lost. This included enterococci, for which an AODVC using the antibiotic ciprofloxacin was developed by modifying the original nalidixic acid method. Responses of bacteria in the dark were influenced by temperature.

Bright natural sunlight produced rapid death in bacteria exposed in seawater microcosms, a result of the synergistic interaction of salinity, UV light and possibly temperature too, whereas the presence of humic acids in freshwater afforded some protection to the cells by absorbing the damaging UV component of sunlight.

Low intensities of UV and visible light typical of those found below the surface of water may induce bacteria to evolve towards a viable but non-culturable form.

CONTENTS

LIST OF FIGURES AND PLATES

LIST OF TABLES

I. INTRODUCTION

Annually, billions of gallons of domestic sewage are discharged directly to sea, and into rivers which ultimately reach the coastal waters of the UK. Some is given no prior treatment though much is partially treated usually by screening, comminution, settlement, etc. As domestic sewage contains large amounts of faecal matter, an input of high numbers of enteric organisms to the environment is inevitable, including any pathogenic bacteria and also viruses which may be prevalent in the human population at that time.

The input of pathogens into waters used for bathing and other recreational purposes is potentially a health risk for those who use the waters. The risk depends on the survival, numbers, and virulence of the organism, and resistance of the bathers. Sanitary engineers have greatly reduced the number of outbreaks of waterborne diseases in the UK but outbreaks resulting from primary contact with sewage-polluted waters still occur and pathogenic agents are frequently isolated from these waters (Galbraith et al., 1987). It appears that very few water-associated outbreaks have been identified as being viral in origin but in many cases (approximately 50% of all water-associated diseases in the USA) the causal agent has remained unidentified (Jones & Watkins, 1985).

Standards exist throughout Europe (Anon., 1976) and worldwide (WHO, 1974) which are aimed at reducing the magnitude of the microbiological input to, and thereby

the health risk of, bathing waters. These are based on the procedure whereby indicator bacteria present in samples taken from bathing waters are enumerated by cultural techniques, the numbers of which are supposed to reflect the level of contamination by pathogens. However, the limitations of using traditional indicator bacteria such as total coliforms and *E.coli* to assess the health risk of natural waters have been recognised (Elliot & Colwell, 1985).

That enteric bacteria do not survive for long periods in the environment is a conclusion drawn from the steady decline in numbers with time, of bacteria which can be cultured (Grimes et al., 1986). Various causes of the decline of bacteria have been proposed: sedimentation, predation, sunlight, temperature, salinity, nutrient deficiencies, etc.

The apparent rapid die-off of coliform bacteria particularly in the marine environment is consistent with the poor correlation of their numbers with the numbers of the more persistent bacterial pathogens and enteric viruses (Cabelli, 1981). Considerable numbers of viruses have been isolated from coastal bathing waters which satisfy the criteria of World Health Organisation (WHO, 1974) standards (Lucena et al., 1982). The correlation of numbers of *E.coli* with numbers of pathogens in freshwaters is higher (Dufour, 1984a, 1984b) and their capacity for survival greater (Pike et al., 1970). This may be due to the slightly more favourable conditions in freshwater i.e., lower salinity, higher nutrient

concentrations, and the presence of sunlight-absorbing substances such as humic and fulvic acids.

To overcome the limitations of traditional indicator systems, others have been proposed (enterococci, *Clostridium perfringens*, bacteriophages) which correlate better with the numbers of pathogens present in both fresh and marine waters. Attempts have also been made to relate environmental parameters such as salinity, pH, temperature and solar radiation, to the decline of indicator bacteria in natural waters.

Examination of the survival characteristics of different species of bacteria in the laboratory has led to the discovery that some bacteria, which cannot be grown on media normally used for their enumeration/isolation, may remain viable. The evolution of bacteria towards this form, which is believed to be a response to low nutrient concentrations, enables them to survive for much longer than was previously thought. They retain the potential to reproduce under favourable conditions, and in the case of pathogenic bacteria, to cause disease, whilst remaining undetected by cultural techniques (Xu et al., 1982; Roszak et al., 1984). Recently developed microscopic techniques (Kogure et al., 1979; Roszak & Colwell, 1987b) show the substrate-responsiveness exhibited by some non-culturable bacteria, enabling their detection and confirmation of their occurrence in the environment (D.B. Roszak, Ph.D Thesis, 1986, University of Maryland, USA.; Hussong et al., 1987; Rollins & Colwell, 1986). The influence of environmental

factors on the evolution of bacteria towards this non-culturable form has been investigated to some extent (Roszak, 1986; Barcina et al., 1989) but little is known about the natural conditions required to evoke this response or the survival of bacteria in this form. Moreover, most previous studies have been carried out under conditions of darkness even though it is known that the effect of sunlight on the survival characteristics of bacteria is considerable (Gameson & Gould, 1975).

The significance of the existence of viable but non-culturable bacteria is controversial and has received widespread publicity. Whether or not this discovery constitutes an increased health risk continues to be debated (Denness, 1987; Pike, 1987).

II. LITERATURE REVIEW

CHAPTER 1

MICROBIAL IMPLICATIONS OF POLLUTION OF BATHING WATERS

1.1. Introduction.

Contamination of recreational waters by sewage discharges has been of particular concern since bathers may come into direct contact with sewage containing pathogenic organisms. The public health implication of this situation has been debated extensively (Moore, 1975; Shuval, 1975; Gameson, 1979). Contemporary public health practice for recreational waters is based on routine determinations of bacterial species, for example *Escherichia coli*, which indicate the possible presence of pathogens. This has been carried out satisfactorily for drinking water supplies and freshwater systems but it does not follow that this practice is also appropriate for saline waters. A criticism frequently directed at bacterial indicators particularly coliforms, is that their persistence in the environment is much less than the persistence of viruses and some bacterial pathogens especially in saline waters and so cannot accurately indicate their presence (Scarpino et al., 1972).

Epidemiological studies have as yet failed to clarify the situation (PHLS, 1959; Cabelli et al., 1982). Introduction of bacteriological standards for bathing waters in the UK (Anon., 1976) appears to have increased the research activity in this area and during the last decade many new ideas on the fate of pathogens in natural

waters have emerged. Generally, there are two schools of thought: the traditional concept of rapid decline of pathogens in the environment (Carlucci & Pramer, 1959; Mitchell & Chamberlin, 1978), and, the belief that pathogens may survive for significantly longer periods than previously thought (Xu et al., 1982; Roszak et al., 1984; Gauthier et al., 1987).

Accompanying the advent of new evidence on the response of bacteria to the stresses of the environment, are the realisation of the inadequacies of traditional enumeration methods, the development of new detection methods (Kogure et al., 1979; Roszak & Colwell, 1987b), and the improvement of existing methods (Rose et al., 1975; Hackney et al., 1979).

1.2. Bathing water quality.

1.2.1. The EEC Directive.

Concern about the quality of coastal waters receiving sewage discharges has led to objections to this practice on both medical and aesthetic grounds. Pike and Gameson (1970) reported that objections raised on aesthetic grounds were well founded as faeces and other objectionable matter were often left stranded on beaches. The potential health hazard of bathing in sewage polluted waters has been under investigation in Britain for many years. Following extensive outbreaks of poliomyelitis in the 1950's, a Public Health Laboratory Service (PHLS) research committee was formed to investigate the possibility of a causal relationship between the disease

and bathing. Between 1952 and 1956, this retrospective epidemiological study centred on cases of poliomyelitis and enteric fever in coastal areas. Based on what Kay and McDonald (1986) thought to be a very narrow range of evidence, the PHLS were unable to find any significant association between bathing in polluted water and risk to health (PHLS, 1959). They concluded that 'even on beaches which were aesthetically unsatisfactory, bathing in polluted sea water carries a negligible risk to health which is associated with chance contact with intact faecal matter from infected persons'. A recommendation that public health requirements would seem to be met just by improvement of grossly polluted waters was also made.

Consequently, for many years it was considered unnecessary to have microbiological standards for British bathing beaches, though concern continued to be expressed, until in 1976 the European Economic Community (EEC) Standards for Bathing Water Quality (Anon., 1976) were introduced. Member states of the Community were given 10 years to take the necessary steps to ensure that all designated bathing waters complied with the standards. The Directive provided criteria in the form of quantitative standards (see Table 1), on which remedial action could be based.

Table 1. A summary of the microbiological standards arising from the EEC Directive.

Parameters	G	I	Minimum Sampling Frequency
Total coliforms /100ml	500(80)	10000(95)	fortnightly
Faecal coliforms /100ml	100(80)	2000(95)	"
Faecal strept. /100ml	100(90)	-	Carried out if suspected
Salmonella/1000ml	-	0(95)	of being present
Enterovirus (PFU)/10L	-	0(95)	"

G - Guideline value
I - Mandatory value
(80) - percentages of samples in which the values must not be exceeded
PFU - Plaque forming unit

Britain was to have met the EEC regulations by 1985 but implementation of the standards was postponed, partly due to the great expense involved (Moore, 1977) and partly due to the evidence provided by the PHLS (1959). Many people believed that the standards were too stringent or not applicable to the British coast so there was little official support for their implementation on medical grounds, however, in recent years the attitude has changed and the standards are now strongly supported by the Department of the Environment (DOE).

Authors have based their arguments for (Shuval, 1975), and against (Moore, 1975, 1977) the use of microbial standards for bathing waters, on epidemiological evidence. Barrow (1981), in a review

of the potential microbial health hazards associated with sewage pollution of British marine and estuarine waters concluded that there is no public health evidence to suggest that bathing should be prohibited solely because of non-compliance with the Directive.

The Directive is criticised because of the ambiguous way in which it is worded, the rationale upon which it is based, and the lack of recommended methodology and monitoring procedures (Water Research Centre (WRC), 1977; Gameson, 1979).

Based on the approach of Shuval (1975), who assumed many hypothetical factors, a total coliform count of 10000/100ml could give rise to a 1 in 10000 chance of becoming sick with viral gastroenteritis from bathing, on that particular day i.e., an individual will become sick once in 10000 bathing days or one out of 10000 bathers will be sick on that particular day. Bathing in water containing 100 coliforms/100ml would accordingly result in 1 in 1000000 bathers being ill. There is no evidence to confirm that this work was the basis for the Directive, however.

In Britain, the Department of the Environment defined bathing beaches as those where 'at least 500 people should have been observed at some time during the bathing season to have been in the water, irrespective of the length of beach, with a density of at least 1500 bathers per mile'. In 1979, Britain declared that it had only 27 bathing beaches, 11 of which failed to meet the standards in 1985 (Ayrton, 1986). Pressure from the

Coastal Anti-Pollution League (CAPL) and other EEC countries persuaded the Royal Commission on Environmental Pollution to intervene and the DOE now officially recognises 379 bathing beaches all of which are coastal (Marine Conservation Society (MCS) & CAPL, 1987). In the bathing season of 1986, 234 designated beaches complied with the EEC standards, out of 379 monitored (MCS & CAPL, 1987).

The WHO standards (WHO, 1974) of 1000 *E.coli* per ml for acceptable bathing waters may be adopted by non-EEC countries which do not have their own standards.

1.2.2. Epidemiological studies.

The retrospective approach to epidemiology by the PHLS (1959) has already been discussed. Earlier in 1953, Stevenson examined a wider range of minor illnesses connected with bathing in polluted waters, in another retrospective study in the USA. A significant increase in bather morbidity rates were identified at Lake Michigan and the Ohio River. More than 50% of the symptoms derived from ear, nose and throat (ENT) infections and the relationship between water quality and disease incidence was best for freshwater sites. This study formed the basis for the US microbiological guideline value for direct contact recreational waters of a geometric mean of 200 faecal coliforms/100ml (EPA, 1976). In fact, this is a dual standard which also requires that 90% of the samples do not exceed 400 faecal coliforms/100ml.

More recently Rosenberg et al. (1976) established a relationship between an outbreak of shigellosis and bathing in a particular section of the Mississippi River. Also in the 1970's, the US Environmental Protection Agency (EPA) undertook an extensive prospective study at beaches adjacent to large urban areas in the USA and Egypt. The findings of this study are presented in a series of papers (Cabelli, 1983; Cabelli et al., 1979, 1982, 1983). It was concluded that there was a risk of contracting gastrointestinal illness by swimming in sewage polluted waters. A log-linear relationship between water quality and symptom rates existed for the study sites (Cabelli et al., 1982). An additional point to arise from this work was that enterococci gave the best correlation with the incidence of gastroenteritis symptoms. Faecal coliforms and *E.coli* correlated very poorly with the incidence of gastroenteritis. In the light of these findings, the EPA recommended a new standard for marine bathing waters of 35 enterococci/100ml and withdrew the old faecal coliform standard (EPA, 1986).

As part of the same EPA study, Dufour (1984a, 1984b) examined the relationship between bathing water quality and the incidence of gastrointestinal symptoms in freshwaters. Again, enterococci gave better correlations with the incidence of disease but *E.coli* faired better than in seawater.

Some authors have recognised the need for similar high quality epidemiological studies to be

carried out in this country (Moore, 1977; Barrow, 1981). Kay and McDonald (1986), comparing the EPA study with that carried out by the PHLS, found that although the two studies reached different conclusions about the safety of sewage-polluted bathing waters, the evidence provided is not conflicting because they examined different diseases. Some authors have commented on the misinterpretation of the conclusions of the PHLS (Agg, 1981; Kay & McDonald, 1986).

A number of small scale epidemiological studies have since been carried out in Britain and Northern Europe. Foulon et al. (1983) found a higher incidence of conjunctivitis, skin infections, colds, abdominal discomfort, puritis and nausea in swimmers than in non-swimmers off the coast of Brittany. Philipp et al. (1985) focussed attention on the health risks associated with snorkel swimming in water which complied with the EEC Directive. Brown et al. (1987) compared a number of minor ailments in swimmers and non-swimmers at two UK bathing beaches, one of which was relatively unpolluted and the other of which occasionally failed to comply with the EEC Directive. Swimmers who immersed their heads in polluted water were significantly more likely to feel ill and were more likely to show an increase in reporting stomach upset, nausea and diarrhoea after swimming.

Grimes (1986) reviewed selected cases in the USA whereby disease was derived from direct contact with either freshwater or seawater. A review of water-borne and water-associated disease in the UK between 1937 and

1986 was recently presented (Galbraith et al., 1987). The authors report more than 12 cases of gastrointestinal infections associated with direct contact with sewage-polluted river water and 2 cases associated with sea bathing, all caused by *S.typhi.* A further 12 cases associated with river water and 7 cases with sea water were caused by *S.paratyphi.* Two cases of primary tuberculosis and many cases of leptospirosis were associated with bathing or immersion in sewage-polluted waters.

1.2.3. Remedial action.

Remedial action to ensure that bathing waters comply with the EEC Directive usually takes the form of construction of a long sea outfall (DOE, 1988), though their use has a number of disadvantages. Firstly, there is a high capital cost associated with their construction. Secondly, long lead times are required for construction, and thirdly, they are not suitable for all coastal sites.

Alternatives include redirection of sewage discharges away from beaches as in the Tyneside scheme (Banks et al., 1983), provision of treatment, and disinfection of sewage effluents before they are discharged (DOE, 1988).

In 1980, a symposium on coastal discharges reviewed those schemes currently in operation and those intended for future use (ICE, 1981). The case for (Baalsrud, 1975) and against (Calvert, 1975) treatment before discharge has been debated in the Proceedings of

the International Symposium on Discharge of Sewage From Sea Outfalls (Gameson, 1975). Generally, it is agreed that some pretreatment should be given to all marine sewage discharges (Pike & Gameson, 1970).

1.3. Distribution and occurrence of pathogens.

Sewage-polluted bathing waters may contain human pathogens, the most important in terms of numbers and associated disease incidence, being viruses and bacteria. Table 2 lists pathogenic viruses and bacteria which may be present in sewage and sewage-polluted waters (Jones & Watkins, 1985).

Viruses and pathogenic bacteria have been repeatedly isolated from sewage-polluted waters in numbers constituting an infective dose (Grimes, 1986). Virulence, longevity, infective dose, and thus the ability of a particular organism to cause disease, change with changes in the environment.

The majority of infections associated with contaminated water are those which cause gastroenteritis. The infecting agent multiplies rapidly in the gut and is excreted in the faeces resulting in elevated levels of pathogens in the environment (Jones & Watkins, 1985). Routes of infection by swimming in sewage contaminated water include direct ingestion or entry via skin abrasions. Individuals may also be infected indirectly by consumption of contaminated shellfish and from aerosols.

Table 2. Pathogenic bacteria and viruses which may be found in sewage (Jones & Watkins, 1985).

Bacteria	Viruses
Salmonella typhi	Enteroviruses
S.paratyphi	Polioviruses
other spp.	Echoviruses
	Coxsackieviruses
Shigella spp.	New enteroviruses
Vibrio cholerae	Hepatitis type A
Mycobacterium tuberculosis	Norwalk virus
Leptospira icterhaemorrhagiae	Rotavirus
Campylobacter spp.	Reovirus
Listeria monocytogenes	Adenovirus
Candida albicans	Parvovirus
Yersinia enterocolitica	
Enteropath. *Escherichia coli*	
Pseudomonas aeruginosa	
Klebsiella spp.	
Staphylococcus aureus	
Aeromonas hydrophila	
Mycobacterium paratuberculosis	
Erysipellothrix rhusopathiae	
Bacillus anthracis	
Clostridium spp.	
Yersinia pestis	
Brucella spp.	

1.3.1. Salmonella.

Salmonellae are most frequently isolated from sewage-polluted waters though this may have been due to the inadequacies of detection methods available for many other pathogens. All serotypes of salmonellae are pathogenic to man to varying degrees. It has been estimated that between <1% and 3.9% of a population may be excreting salmonellae at a given time and that outwardly healthy carriers of the organism may excrete about 10^6 organisms per gram of faeces (Anon., 1987).

Yoshpe-Purer and Shuval (1972) reported isolating 229 strains (34 serotypes) of salmonellae,

including *S.paratyphi* B, from sewage and sewage-polluted waters, some of them in waters with coliforms below 2400/100ml. They also found that persistence of the bacteria in the environmnent was something which varied between strains rather than between serotypes and that those isolated most frequently were not always those which were prevalent in the population. This indicates the poor survival of some strains in the environment.

1.3.2. Shigella.

All members of this genus cause bacillary dysentery also known as shigellosis, in humans and primates. Shigellae are excreted in large numbers in the faeces of infected individuals and their presence in the population is maintained by a few symptomless carriers.

Shigellae are sensitive to temperature but are able to survive for days in cool waters. Aeration of waters however, reduces their survival significantly (Wang et al, 1966). *S.sonnei* appears to be most resistant to adverse conditions and are therefore isolated most frequently (Anon., 1987). Jamieson et al. (1976) showed survival of *S.dysenteriae* in seawater for up to 5 days. As Geldreich (1972) pointed out, until techniques for isolation of shigellae are improved or replaced, only then will the true picture of the occurrence of this organism emerge. The same can be said of many other pathogens whose detection is limited by inadequate methodology.

1.3.3. Vibrios.

This genus is composed of many species but only a handful are of medical importance, those being the ones which cause wound and enteric infections. *V.cholerae* causes cholera in man, and *V.parahaemolyticus* and nonagglutinable vibrios can cause a cholera-like disease or mild diarrhoea.

Vibrios can enter the aquatic environment in large numbers in the faeces of an infected individual. Some vibrios also occur naturally as members of the autochthonous population. The distribution of vibrios in the environment appears to be governed by salinity. *V. parahaemolyticus* has been shown to be an autochthonous member of the population of estuarine waters and shellfish (Colwell et al., 1977). *V.cholerae* and related vibrios have been isolated from brackish waters though some may be found in marine waters (Colwell et al., 1981; Singleton et al., 1982).

Vibrios survive well at low temperature but are sensitive to acidic conditions. Appropriate pH and salt concentration enable vibrios to persist in waters for weeks (Colwell & Kaper, 1977).

1.3.4. Leptospira.

Leptospira are not enteric but may be excreted in urine and usually gain access to the bloodstream by penetrating the skin. Infection may occur from swimming in sewage-polluted waters (Geldreich, 1972). It is thought that Leptospira die rapidly in seawater as they

are very sensitive to high salinities (Jamieson et al., 1976). They are also rapidly inactivated at temperatures of about 50°C and do not tolerate acidic conditions.

1.3.5. Enteropathogenic *Escherichia coli*.

The action of enteropathogenic strains of *E.coli* causes diarrhoea, especially in children, and gastroenteritis in adults (Gross & Rowe, 1985). The pathogenicity mechanism of enteropathogenic *E.coli* is not clearly understood (Koul & Panhotra, 1988).

Rosenberg et al. (1977) are amongst those who have reported water-borne outbreaks of diarrhoea due to enterotoxigenic and enteropathogenic *E.coli*. It has been reported that enteropathogenic *E.coli* does not survive long in the environment and that some serotypes are 'stronger' than others (Cooke, 1985).

1.3.6. Campylobacter.

In Britain, campylobacter enteritis is the most frequently reported form of acute bacterial diarrhoea (Skirrow, 1982). Campylobacters have been isolated from rivers (Pearson et al., 1985; Bolton et al., 1987) and from estuarine and coastal waters (Bolton et al., 1982). Bolton et al. (1987) reported worldwide water-borne outbreaks of campylobacter enteritis and Skirrow (1982) thought it too much of a coincidence that ingestion of water from lakes, streams, rivers, and the sea while engaged in recreational activities occurs often among patients suffering from campylobacter enteritis.

Campylobacters can survive in water for several weeks at low temperatures (Blaser et al., 1980) but have never been isolated in the absence of faecal coliforms (Knill et al., 1982).

1.3.7. Viruses.

Viruses are thought to be responsible for the majority of swimming-associated gastrointestinal disease (Cabelli, 1981). Over 100 enteric viruses are shed in human faeces often in large numbers (Anon., 1987). Traditionally, bacterial indicators were used to predict the presence of bacterial pathogens but it has been shown that their occurrence does not correlate well with the presence of human enteric viruses (Berg & Metcalf, 1978). Enteric viruses are more resistant to environmental stresses and may persist long after bacteria are dead (Berg, 1978; Berg & Metcalf, 1978).

Viruses have been isolated from bathing waters which were bacteriologically satisfactory according to the World Health Organisation (WHO) standards (Lucena et al., 1982). Viruses do not multiply outside a susceptible host whereas bacteria may under the appropriate conditions and therefore exaggerate the potential risk of viral contamination (Berg, 1978; Geldreich, 1978).

1.4. Indicators of recreational water quality.

The literature dealing with the historical development and rationale of indicators of water quality

is comprehensive (Bonde, 1977; Cabelli, 1978; Elliot & Colwell, 1985). Bacterial indicator species by their presence, indicate the possibility of pathogens also being present, but their absence does not guarantee that pathogens are also absent.

More specifically, Cabelli (1977) defined recreational water quality indicators as 'microorganisms (or chemicals) whose densities in water can be quantitatively related to potential health hazards resulting from recreational use, particularly those activities which expose the upper body orifices to the water'. The modern concept of an indicator of faecal pollution according to Grimes (1986) is a microorganism whose presence in a commodity suggests contamination of that commodity with animal faeces (including human faeces).

Bonde (1962) proposed the ideal requirements for indicator organisms. Cabelli (1977) went a step further and proposed criteria for the 'best indicator' of faecal pollution in recreational waters ('there is no ideal indicator'):

1) The indicator should be consistently and exclusively associated with the source of pathogens.

2) It must be present in sufficient numbers to provided an 'accurate' density estimate whenever the level of each of the pathogens is such that the risk of illness is unacceptable.

3) It should approach the resistance to disinfectants and environmental stress, including toxic

materials deposited therein, of the most resistant pathogen potentially present at significant levels in the source.

4) It should be quantifiable in recreational waters by reasonably facile and inexpensive methods and with considerable accuracy, precision, and specificity.

Obviously an indicator of faecal pollution can only be used to indicate the presence of pathogens which occur in faeces. The indicator concept breaks down when we consider the pathogens which are autochthonous to the aquatic environment such as *Vibrio* spp., *Legionella pneumophila* and *Aeromonas aeruginosa*.

Those microbial groups and taxa which have been considered as potential recreational water quality indicators are listed in Table 3. Non of those listed fulfill all of the requirements for the best indicator proposed by Cabelli (1977) though some are closer to it than others.

The advantages and disadvantages of the use of various proposed indicators have been well documented and were the subject of a symposium where each indicator was discussed separately and in detail (Hoadley & Dutka, 1977). It has recently been emphasised that those indicators originally developed for use in freshwaters (as most of them were) may not be appropriate for measuring the quality of marine and estuarine waters due to their apparent rapid decline in these waters (Elliot & Colwell, 1985).

Table 3. Potential recreational water quality indicators (Grimes, 1986)

Total coliforms	R plasmid coliforms
Faecal coliforms	Ascaris eggs
Coliphage	Entamoeba eggs
Enterococci	*Aeromonas* spp.
Faecal streptococci	*Bacteroides* spp.
Coagulase +ve staphylococci	*Candida albicans*
Bifidobacteria	*Escherichia coli*
Standard plate count	*Clostridium perfringens*
Iron and sulphur bacteria	*Klebsiella pneumoniae*
Staphylococcus aureus	Leptospira
Pseudomonas aeruginosa	*Vibrio cholerae*
Vibrio parahaemolyticus	

1.4.1. Escherichia coli and coliforms.

The rationale for the use of *E.coli* as an indicator of faecal pollution in freshwaters was established back in the 1930's, when it was discovered in high numbers in faecal discharges and that it survived longer than did the 'typhoid bacillus' (Heathman et al., 1936). Workers had already discovered the ease with which *E.coli* could be isolated and enumerated compared with pathogenic bacteria.

For many years previously total coliforms were universally employed as indicators but then it was recognised that genera such as Klebsiella, Enterobacter and Citrobacter are not always associated with faeces. The faecal coliform test enumerates bacteria from the genera Klebsiella and Escherichia, with occasional positive reactions from other genera. Only *E.coli* forms indole from tryptophan and produces gas from glucose at 44.5°C and is exclusively faecal. *E.coli* has been adopted

in preference to faecal or total coliforms in many countries.

A review of the literature on available membrane filter and most probable number (MPN) methodology has been made by Dufour (1977). He pointed out that all the methods mentioned had two common characteristics:

1) The use of elevated temperature (44.5°C) to select against non-thermotolerant coliforms.

2) A biochemical reaction to differentiate between E.coli and other thermotolerant faecal coliforms.

Standard membrane filtration and most probable number techniques recommended for routine monitoring of coliforms in drinking water supplies (DHSS, 1984) are generally used for enumerating coliforms in samples from recreational water monitoring programmes. Modifications have been made to these methods such that they involve a resuscitation step at a lower incubation temperature to enhance the recovery of sublethally stressed coliforms (DHSS, 1984; APHA, 1985).

1.4.2. Enterococci.

The term 'faecal streptococci' originally referred to Lancefield's Group D streptococci, which possess the group D antigen. These included *S.faecalis*, *S.faecium* and some species of animal origin. The term 'enterococci' has been used to describe those faecal streptococci of human origin i.e., *S.faecalis* and *S.faecium* (Schleifer & Kilpper-Balz, 1984). However, faecal streptococci of animal origin such as *S.bovis* and

S.equinis have been useful indicators of contamination of waters by animal wastes (Geldreich and Kenner, 1969).

Cabelli et al. (1982) proposed that enterococci should be employed in preference to coliforms as indicators of pollution of coastal bathing waters. As a result of this and of the study carried out by Dufour (1984a) in freshwater bathing sites, the EPA (1986) proposed marine and freshwater criteria for enterococci (Section 1.2.2.).

Enterococci appear to be more persistent than faecal coliforms and *Salmonella* spp. in the environment giving them a significant advantage over coliforms for indicating the presence of the more persistent pathogens such as viruses. High nutrient concentrations have been shown to prolong the survival of *S.faecalis* (Allen et al., 1952). The main drawbacks in using enterococci are their lower frequency in the intestine than *E.coli* and their slower growth rate so that enumeration takes longer than for *E.coli*.

1.4.3. *Clostridium perfringens*.

Clostridium perfringens appears to be one of the more promising 'alternative' indicators of faecal pollution in recreational waters and has received a great deal of attention from Cabelli and coworkers (Cabelli, 1977; Bisson & Cabelli, 1979, 1980; Emerson & Cabelli, 1981). Bonde (1962) reviewed many of the characteristics of *C.perfringens* the most important being its consistent association with human faecal wastes and the longevity of its spores.

The longevity of *C.perfringens* spores can, however, be disadvantageous in that it is impossible to tell how long ago they were released to the environment and thus there is no significant correlation with the numbers of *E.coli*. Nevertheless, Fujioka and Shizumura (1985) provided evidence of a correlation between numbers of *C.perfingens* and faecal contamination of stream water.

The DHSS (1984) provides recommended methods for enumeration of sulphite reducing clostridia and *C.perfringens* but emphasises the subsidiary role of these organisms in water examination.

1.4.4. Bacteriophages.

Bacteriophages specific for a particular bacterial species have been proposed as indicators of enteroviruses in water (Scarpino, 1975). Coliphages in particular may be useful as they persist longer than viruses in the environment (Kott et al., 1978) and their numbers correlate well with the presence of viruses (Stetler, 1984). Coliphages are also enumerated with greater ease as they occur in much higher numbers. They are, however, capable of multiplying in waters containing susceptible host cells and are therefore not always reliable indicators (Vaughn & Metcalf, 1975).

1.5. Direct detection of bacteria.

Direct detection of pathogens in the environment is not carried out routinely. Instead, monitoring of pathogen levels is done using a specific indicator system such as those discussed in the previous

section (Section 1.4.). The low concentration of pathogens, combined with their often fastidious nature has made it very difficult to culture pathogens from natural waters (Grimes, 1986).

Interest in the problems of detecting viable bacteria which may not grow on culture media, has prompted much activity in the development of direct detection methods for quantitation of pathogens. Results from trials using newly developed methods have suggested that these may be more reliable than traditional methods (Elliot & Colwell, 1985). The limitations of methods mentioned briefly in the previous section (Section 1.4) are clearly acknowledged (APHA, 1985) especially in light of the evidence of the presence of 'viable but non-culturable' bacteria (Xu et al., 1982; Roszak, 1986). Some believe that emerging direct detection methods will replace traditional cultural methods for monitoring the public health safety of recreational waters (Elliot & Colwell, 1985; Grimes, 1986).

The direct counting of bacteria using a microscope has been practised for many years (for a review see Roszak & Colwell, 1987a). Differential staining was used to show the abilities of living and dead bacteria to take up vital stains. Direct counts of bacteria collected on membrane filters and stained with a fluorescing dye have yielded higher numbers than other techniques (Daley, 1979). The principles of epifluorescence microscopy whereby cells are stained with

dyes which fluoresce on exposure to light of a particular wavelength are described by Geesey and Costerton (1981).

One of the most frequently documented methods for direct detection of bacteria is the Acridine Orange Direct Count (AODC) (Francisco et al., 1973; Daley & Hobbie, 1975). This technique allows differentiation of active (red) and inactive (green) cells. Acridine orange binds specifically with DNA and RNA under controlled conditions and, when excited with light at 436nm or 490nm, the DNA-AO complex fluoresces green and the RNA-AO complex fluoresces orange/red (Ris & Plaut, 1962; Wittekind, 1972). Both green and red cells should be counted to give a total count of bacteria. A high RNA:DNA ratio i.e., cells stained red, indicates active metabolism while a high DNA:RNA ratio i.e., cells stained green, indicates metabolic inactivity. Other factors however, may influence the ratio of nucleic acids in a cell and it is therefore inaccurate to use this method to distinguish between active and inactive cells (Hobbie et al., 1977). Several difficulties are inherent in the use of AO for direct counts. It is difficult to distinguish bacteria from non-living particles which may also take up the stain and the method requires the examination of prepared slides while still moist (Hobbie et al., 1977). Unfortunately, the AODC is not species-specific either.

The fluorescing stain 4'6-diamidino-2-phenylindole (DAPI) can be used with modifications of the standard AO technique. DAPI binds to DNA and, when excited by light at 365nm, the DNA-DAPI complex

fluoresces blue whereas DAPI bound to any non-DNA material fluoresces a weak yellow (Porter & Feig, 1980). Bacteria can be distinguished from other particulate matter more easily than with AO but does not complex specifically with RNA, thereby indicating only cells with intact, possibly functional genomes (Porter & Feig, 1980).

Zimmerman et al. (1978) first developed a method which allowed active and inactive bacteria in freshwater to be differentiated, using the reduction of 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) which acts as an electron acceptor, generating reducing power forming red INT-formazan crystals in metabolically active bacteria combined with acridine orange for the total count. These crystals can only be detected in bright-field illumination and AO stained cells can only be detected under epifluorescence. A number of modifications of this technique have since been made by Tabor and Neihof (1982) and by Dutton et al. (1986), the latter combining the INT method with malachite green in place of AO, which made it possible to determine both active and inactive freshwater bacteria under the same bright-field illumination. King and Parker (1988) combined DAPI and INT with advantages over the AO INT method of Zimmerman et al. (1978) but alternation of epifluorescence and bright-field illumination is still required.

Kogure et al. (1979) developed what has probably become the most widely used method for counting

viable bacteria in environmental samples and distinguishing them from non-viable bacteria: the direct viable count (DVC). This method involves preincubation of the samples for 6 hours at 20°C with yeast extract as a growth stimulating nutrient and nalidixic acid, a DNA gyrase inhibitor in Gram negative bacteria (Goss et al., 1964). The cells respond to the yeast extract by enlarging, but division is inhibited by the nalidixic acid. Other synthetic pathways continue to function however. Deitz et al. (1966) showed that the bactericidal effect of nalidixic acid only occurs when protein and RNA synthesis take place i.e., in the exponential phase of growth. Elongation of the cells make them easier to count under the microscope. With the use of acridine orange, this technique allows the enumeration of actively growing cells and those which are dormant but remain physiologically responsive to substrates.

Al-Hadithi and Goulder (1989) recently drew attention to the inherent problems involved in using the AODVC, i.e., how to recognise cells which have enlarged but are still smaller than many of the original cells, and, how to distinguish large non-viable cells. They proposed that an optimum critical length and a lower limit above which cells were considered to be viable, should be set.

Peele and Colwell (1981) showed that preferences of marine bacteria for a particular substrate

can affect the DVC of substrate responsive cells, the highest counts obtained using yeast extract or tryptone.

The major disadvantage of the AODVC is that nalidixic acid is only effective against some Gram negative bacteria. Modifications of this method have been made so as to increase the spectrum of bacteria which could be enumerated by the DVC (Kogure et al., 1984). In addition to nalidixic acid, two other quinolones, piromidic acid and pipemidic acid were used with an increased incubation time of 8 hours at 20°C. Although similar in structure and function to nalidixic acid, piromidic acid is effective against some Gram positive strains, for example *Staphylococcus* spp., and pipemidic acid is effective against some of the Gram negative strains which are resistant to nalidixic acid and piromidic acid, for example *Pseudomonas aeruginosa*. However, the bacteria predominantly found in the marine and estuarine environments are Gram negative (ZoBell, 1946).

Radiolabelling techniques based on the assimilation and/or respiration of radiolabelled substrates have been developed to measure the activities of heterotrophic bacteria (Wright & Burnison, 1979) often in combination with immunofluorescence. Validation that the viable cells enumerated in the DVC are metabolically active can be achieved by autoradiography (Roszak, 1986; Kogure et al., 1987; Roszak & Colwell, 1987b).

Originally developed for use in clinical microbiology, conjugation of fluorochromes to antibody

proteins provided the basis of fluorescent antibody or immunofluorescence techniques which has found application in many areas of microbial ecology (Bohlool & Schmidt, 1980). Fluorescent antibody techniques have been field tested for rapid detection of enteropathogenic *E.coli* (Pugsley & Evison, 1974a,b,c; Abshire, 1976; Roszak, 1986), *Shigella* spp. (Thomason et al., 1965), *Vibrio cholerae* (Brayton & Colwell, 1987), *Salmonella typhi* (Thomason & Wells, 1971; Cherry et al., 1972), and group D streptococci (Abshire & Guthrie, 1971; Pugsley & Evison, 1975).

Fluorescent antibodies may also be used in place of acridine orange for a species-specific direct viable count (FA DVC) of bacteria in natural waters (Xu et al., 1982, 1984). The active cells appear enlarged in the same way but the colour depends on the fluorochrome used. Rao and Melnick (1986) also described the use of fluorescent antibodies for detecting viruses. Fluorescent antibody techniques are disadvantageous in that extensive screening of bacterial strains is required to eliminate the possibility of cross-reactivity of other strains with a particular antibody, and they are also expensive to use.

Developments are also currently being made in other areas. Some of these methods have been described by Rao and Melnick (1986) and Grimes (1986) and include enzyme-linked immunosorbant assay (ELISA), radioimmunoassay (RIA), specific gene probes, cellular fatty acid analysis and bacterial restriction

endonuclease DNA analysis (BRENDA). According to Elliot and Colwell (1985) methods employing monoclonal antibodies and the advent of specific gene probes hold the brightest prospects for the future of detecting pathogens in the environment. New techniques have introduced a new precision, accuracy and reliability to detection of pathogens including viruses. The potential of the use of gene probes for screening water samples for the presence of toxin-producing genes of *V.cholerae* and enterotoxigenic *E.coli*, RNA of *Legionella* spp., and selected enteric bacteria and protozoa on a routine basis, has been demonstrated (Colwell, 1988).

1.6. Survival of bacteria in the aquatic environment.

Various mechanisms have been proposed for the 'die-off' of bacteria in natural waters and these will be discussed in Chapter 2. Previously, it was generally agreed that the ultimate fate of allochthonous pathogenic bacteria in the aquatic environment was death. This assumption was based on the the gradual decrease in the number of bacteria which could be cultured from waters using traditional cultural techniques, i.e., the production of visible growth on/in agar plates, multiple tubes and membrane filters. In the last decade evidence has been presented which suggests that those bacteria which cannot be cultured by these methods may be injured or dormant but not necessarily dead (Xu et al., 1982). The alternatives which appear to be available to bacteria when subjected to the harsh and changing conditions of

the aquatic environment are death, dormancy, or adaptation. An excellent review of literature dealing with the survival strategies employed by bacteria in the aquatic environment was recently presented by Roszak and Colwell (1987a).

1.6.1. Viable but non-culturable bacteria.

Developments in direct detection methods has led to the discovery that the number of bacterial cells enumerated in seawater by conventional methods are much lower than the actual number of viable cells present (Xu et al., 1982). Observations relating to the existence of viable but non-culturable bacteria were made previously by Jannasch (1967), Calcott and Postgate (1972), Fliersmans and Schmidt (1975), Dawes (1976), and Hoppe (1978) but the process has only recently received much attention.

Bacteria in this form have been shown to be metabolically active and capable of replication under the appropriate conditions (Roszak, 1986). 'Viable' is a term which has long been associated with those bacteria which show visible signs of growth by conventional methods, so Roszak (1986) coined the phrase 'viviform' to describe viable but non-culturable bacteria and defined them as 'that population which has the essentials for growth, i.e., metabolism and the potential for reproduction while not exhibiting cell division'.

Viable but non-culturable forms have been documented for *Vibrio cholerae* (Xu et al., 1982, 1984), *Escherichia coli* (Xu et al., 1982), enterotoxigenic

E.coli (Grimes & Colwell, 1986), *Shigella* spp. (Brayton et al., 1984), *Salmonella enteritidis* (Roszak et al., 1984), *Campylobacter jejuni* (Rollins & Colwell, 1986), and *Legionella pneumophila* (Hussong et al., 1987). It is also believed that viable but non-culturable forms of enterococci may also exist but as these organisms are resistant to nalidixic acid used in the DVC, this has yet to be confirmed (Grimes, 1986).

Although non-culturable on media which normally supports the growth of the species in question, viable but non-culturable bacteria remain physiologically responsive to substrates, as demonstrated by the DVC of Kogure et al. (1979) and autoradiography showing uptake of radiolabelled substrates (Roszak, 1986). Furthermore, epifluorescent and immunofluorescent techniques have shown that the integrity of the outer cell membrane is maintained and that cells may undergo rounding up and reduction in size (Xu et al., 1982). Recently it has been shown that β-galactosidase can be induced in non-culturable *E.coli* indicating that the transcription and translation systems also remain functional (Colwell, 1988). Palmer et al. (1984) demonstrated that the non-culturable bacteria retained plasmids coding for virulence.

Each species exhibiting the viable but non-culturable characteristics gives the same general response on exposure to natural waters. This response, as first demonstrated by Xu et al. (1982) is shown in Figure 1. The slopes of the lines can be influenced by a

number of environmental factors i.e., temperature and salinity (Roszak, 1986), and visible light (Barcina et al., 1989). The DVC decreases gradually but does not approach zero even when culturable counts do.

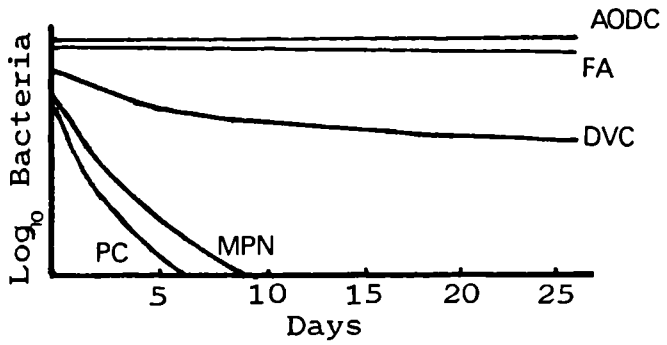


Figure 1. Characteristic response of Gram negative bacteria placed in a nutrient-poor medium (Grimes, 1986). AODC & FA - total counts, DVC - viable count, PC (plate count), MPN (most probable number) - culturable counts.

Roszak et al. (1984) found that it was possible to resuscitate viable but non-culturable *Salmonella enteritidis* 18-19 days after achieving its non-culturable state. By placing the cells in an equal volume of full strength Veal Infusion Broth with subsequent plating on to Veal Infusion Agar a few, very small atypical colonies could be grown which would normally be rejected as being *S.enteritidis*. Resuscitation was unsuccessful after the bacteria had been non-culturable for 21 days. In another case (Roszak, 1986) non-culturable *E.coli* cells could be cultured after incubation for several hours in 1/10 strength nutrient broth with subsequent plating on nutrient agar of corresponding strength. This was however, only effective for a limited time after losing

culturability, after which time the cells could only be recovered by animal passage.

Viable but non-culturable *E.coli* and *V.cholerae* have been recovered by passing them through ligated rabbit ileal loops with simultaneous detection of enterotoxigenicity (Colwell et al., 1985) using the method of Spira et al. (1981). *Shigella flexneri* was recovered by passage through a mouse gut. Colonies were formed when the contents of the mouse caecum were plated out (Brayton et al., 1984). Trials with human volunteers showed that human passage could also be effective in inducing culturability in previously non-culturable forms (Colwell et al., 1987).

Table 4 provides a summary of the characteristics of viable but non-culturable bacteria.

The viable but non-culturable phenomenon has been described as a form of dormancy, adopted by some of the allochthonous bacteria which are unable to form spores or cysts in response to unfavourable environmental conditions, especially low nutrient concentrations (Grimes, 1986). Barcina et al. (1989) showed that low intensity visible light was able to induce the evolution of *E.coli* towards their non-culturable form in river water. Xu et al. (1982) emphasised the need for reevaluation of the concept of bacterial decay in the aquatic environment and suggested that dormancy would be more appropriately considered.

Table 4. Selected characteristics of viable but non-culturable bacteria (Grimes et al., 1986).

Culture
1) No growth on or in standard culture media.
2) Resumption of growth under appropriate conditions.
Cytology
1) Cells appear intact when stained by AODC and/or FAC methods.
2) Cells are substrate-responsive, demonstrated by DVC and autoradiography.
3) Cells may undergo changes in size and/or shape.
Virulence
1) Viable but non-culturable cells remain virulent.

The implications of this phenomenon are that human pathogenic bacteria may be present in the aquatic environment yet remain undetected. However, the relevance of the results obtained by Colwell and coworkers to real conditions in the marine environment has been questioned. The main criticisms were voiced by Pike (1987):

1) Pure cultures were used in the experiments, and these do not decay in the same way as fresh faecal bacteria.

2) The waters used were not fully saline.

3) The influence of sunlight on bacterial decay was ignored.

4) No resuscitation steps were included in enumeration methods.

5) Selective media which are inhibitory to stressed bacteria were used.

The serious public health implications of the existence of viable but non-culturable pathogens in bathing waters has been dismissed on the grounds that cultural methods have been used satisfactorily for monitoring the bacteriological quality of bathing waters for many years and the discovery of this not entirely new phenomenon does not increase the health risk of bathing in such waters (Pike, 1987). However, in other situations the implications of the occurrence of non-culturable pathogens may be more serious. *Vibrio cholerae* 01 were detected by the FA DVC in all 16 samples taken from rivers and ponds in Bangladesh where cholera is endemic. Only 1 of these samples gave culturable counts of *V.cholerae* 01 (Brayton et al., 1987). Similar observations were also made by Colwell et al. (1985).

In the UK, failure to culture *Legionella pneumophila*, the causative agent of Legionnaire's disease

from public water supplies when its presence can be detected by FA techniques may be cause for concern (Colbourne et al., 1988). The discovery of the viable but non-culturable form of *Legionella pneumophila* by Hussong et al. (1987) has shed some light on some of the peculiar features of this organism and the nature of legionnaires disease. The causative agent of an outbreak of campylobacter enteritis, also in the UK, remained undetected by cultural methods but on passage through chickens gave rise to colonies of *Campylobacter jejuni* on a solid medium (Colwell, 1988).

The examples quoted above suggest that the viable but non-culturable phenomenon may hold the key to many questions relating to diseases which are not very well understood (Colwell, 1988).

1.6.2. Injury.

Coliforms which are exposed to the stresses of the environment may become injured or debilitated (Bissonette et al., 1975; Dawe & Penrose, 1978; McFeters et al., 1982) and may require special steps in addition to the usual procedures employed for their enumeration to enable their recovery. These cells temporarily lose their ability to multiply on selective media but remain part of the viable population and able to multiply on non-selective media (Hoadley & Cheng, 1974; Bissonette et al., 1975). Injury can be thus defined as the differential between the number of colony forming units on non-selective media and the number of colony forming units on selective media (Domek et al., 1984). Ray

(1979) estimated that between 56% and 95% of a surviving population could be injured.

Injured cells also lose their normal resistance and become sensitive to chemicals including those present in selective media (Anderson et al., 1979). Impaired resistance to high incubation temperatures has also been reported (Ray, 1979). Increased sensitivity to selective agents has been found to be due to structural injury (Ray & Speck, 1973). Various sites have been implicated in association with structural damage, the favourites being the permeability barriers in the surface structures i.e., teichoic acids in Gram positive bacteria and lipopolysaccharides in Gram negative bacteria (see Ray, 1979).

Because of their inadequacies, developments have been made in improving existing methods to recover injured cells allowing their detection and enumeration. Some of these improved methods were reviewed by Ray (1979) and the revival of injured bacteria was also the subject of a symposium (Andrew & Russell, 1984). Methods designed to allow recovery of injured bacteria by membrane filtration were reviewed by Hoadley (1981). Generally recovery methods involve an enrichment step in dilute, non-selective media and preincubation at a lower temperature (Hurst, 1977; Ray, 1979).

The implication of the injury of bacteria in the aquatic environment, because they do not grow on selective media, is that they may escape detection and are potentially hazardous as they are capable of repair

and toxin production (Singh & McFeters, 1986). Dawe and Penrose (1978) proposed that injury was a survival mechanism employed under adverse conditions, because a high injury rate corresponds to a high survival rate.

1.6.3. Dormancy.

Dormancy was first described for autochthonous species of the aquatic environment (Novitsky & Morita, 1978) but is now evident in many Gram negative bacteria including allochthonous species, on entering nutrient-poor waters (Xu et al., 1982). However, unlike allochthonous species, dormant autochthonous bacteria can be cultured (Novitsky & Morita, 1978; MacDonell & Hood, 1982).

Novitsky and Morita (1978) demonstrated the existence of bacterial populations which under nutrient-poor conditions, undergo morphological changes resulting in ultramicrobacteria. These are very small, rounded forms some of which are less than 0.3µm in diameter (Torrella & Morita, 1981; Tabor et al., 1981). These small forms survive as vegetative cells by using their internal energy reserves at a lower metabolic rate (Novitsky & Morita, 1978; Morita, 1985), a strategy also believed to be employed by allochthonous bacteria under adverse conditions (Roszak et al., 1984).

1.6.4. Adaptation.

Gauthier et al. (1987) showed that Enterobacteriaceae have a higher survival rate in seawater when they have been previously grown on a salted

medium and that this can avoid the evolution of the cells towards the viable but non-culturable form. The detrimental effects of high salt concentrations on bacterial mortality will be discussed in Chapter 2. It seems that only Dawe and Penrose (1978) have previously reported evidence for the role of salts in enhancing survival by the repair of injured coliforms.

Highest recoveries of salt-adapted *E.coli* have been observed using non-selective media supplemented with sodium chloride. Gauthier et al. (1987) found that media prepared with seawater appeared to be less effective than unsalted media. However, Bahkrouf et al. (1988) reported that the addition of sodium chloride to media is less effective in culturing salt-adapted *E.coli* as the addition of seawater.

It has been suggested that adaptation of *E.coli* to the marine environment could be possible in the presence of salts and natural organic matter such as those found in raw sewage (Munro et al., 1987b). This may also explain the increased survival of coliforms in marine sediments wherein both salts and organic matter are encountered (Gerba & McLeod, 1976; LaLiberte & Grimes, 1982).

Munro et al. (1987b, 1989) suggested that adaptation may result from the development of an osmoregulatory mechanism induced by salts due to the intracellular accumulation of organic compounds. According to Chai (1983) adaptation may be due to structural modifications in the outer membrane of cells

grown on salted media. This idea is supported by evidence of modifications in sensitivity to antibiotics, colicins, heavy metals and bacteriophages of cells grown in seawater or sewage presented by Munro et al. (1987a). The disappearance of β-galactosidase activity which is normally responsible for acidification and fermentation of lactose in standard enumeration techniques, and the increased activity of other enzymes, in non-salt adapted *E.coli* cells starved in seawater was also noted by Munro et al. (1987a). This supports the idea of reversible adaptation to starvation through enhancement of certain metabolic pathways.

The survival of *E.coli* in nutrient-free seawater is dependent on the age of the cells at the time of harvesting and exposure (Gauthier et al., 1989). Chai (1983) showed that even natural seawater could support the weak growth of *E.coli* under laboratory conditions and that the sensitivity of these cells to certain chemicals was different to that of cells grown in a rich medium.

II. LITERATURE REVIEW

CHAPTER 2

FACTORS AFFECTING DIE-OFF OF BACTERIA IN THE AQUATIC ENVIRONMENT

2.1. Introduction.

The death of a microbe will occur only as a result of an environmental stress, otherwise it is able to live and divide indefinitely (Postgate, 1977). As previously discussed (Section 1.6.), recent work shows that bacteria do not necessarily die, even as a result of environmental stresses but may remain dormant for long periods or adapt to the stressful conditions. Nevertheless, considerable attention has been given over the last century, to assessing the rates of die-off of bacteria, usually indicators, in the aquatic environment and as a consequence an enormous literature exists on the subject. For public health reasons, most investigations have dealt with the survival of bacterial indicators.

As a result of such a large number of investigations being carried out, much of the literature available is contradictory, mainly because of the differences in experimental protocols used. Crane and Moore (1985) suggested that the greatest need for future research was for the unification of the data relating environmental parameters to bacterial survival, and they proposed that the model of Chick (1908) should be used to unify the existing data from past investigations. The reader is directed to their calculations of die-off rate coefficients using values in the literature, assuming

first order decay kinetics which appeared to accurately describe bacterial mortality under all the conditions specified. The most striking feature of these calculations is the high variability of die-off rate coefficients for a given species due to the interaction of different environmental factors. The lack of correlation between environmental parameters and die-off rate coefficients is thought to be due to i) the non-linear effects of some parameters e.g., pH, and temperature, and ii) the failure of many investigators to specify experimental conditions for some of the important variables (Crane & Moore, 1985).

The influence of environmental factors on the survival of bacteria in natural waters is still not well understood despite the large number of studies which have previously been carried out. Nevertheless, several factors have been proposed as making a significant contribution to the decline of bacteria in the aquatic environment, though their relative importance is not always agreed upon by the various authors.

At the risk of duplicating the efforts of many previous investigators, the most frequently documented factors are discussed in this chapter. Existing reviews on this subject are numerous (Waksman & Hotchkiss, 1937; Greenberg, 1957; Carlucci & Pramer, 1959; Jones, 1971; Mitchell & Chamberlin, 1975, 1978; Elliot & Colwell, 1985). Carlucci and Pramer (1959) summarised the major factors which influence the decline of enteric bacteria

in seawater. These are:

i) Adsorption and sedimentation.

ii) Sunlight.

iii) Nutrient deficiencies.

iv) Toxic substances.

v) Salinity.

vi) pH.

vii) Bacteriophages.

viii) Predation.

ix) Competition and antagonism.

Temperature was later added to this list (Pike et al., 1970; Mancini, 1978). A number of other factors have also been proposed by various authors as making contributions to bacterial mortality in the sea and include dissolved oxygen (Hanes et al., 1964) and turbidity (Gameson et al., 1973; Fujioka & Narikawa, 1982).

Light is considered by many to be the single most important contributor to bacterial die-off in the sea (Fujioka et al., 1981; Chamberlin & Mitchell, 1978; Gameson & Gould, 1975; Kapuscinski & Mitchell, 1983) and has been implemented as an agent of sublethal injury (Kapuscinski & Mitchell, 1981).

2.2. Factors affecting bacterial mortality.

2.2.1. Adsorption and sedimentation.

Reduction in counts of bacteria in seawater has been attributed, in part, to adsorption of bacteria to organic and inorganic particles with subsequent settlement and deposition on the ocean floor. Consequently bacteria may accumulate in sediments. Russell (1892), Weiss (1951), and Rittenberg et al. (1958) recorded greater numbers of bacteria in marine sediments than in the overlying waters. Mitchell and Chamberlin (1978) pointed out the significance of sedimentation and adsorption in removing coliforms from both fresh and marine surface waters under conditions of low vertical mixing. A significant reduction in numbers may also occur in moving waters with 50-100mg/l suspended solids through flocculation (Evison & Morgan, 1982).

Although adsorption of bacteria to sand, silt and clay particles, and flocculation contribute to their removal from the water column, it is generally believed that this also increases their persistence and protects them from environmental stresses in sediments. Extended survival of bacteria has been reported in marine (Chan et al., 1979; Gerba & McLeod, 1976), estuarine (Hood & Ness, 1982), and freshwater (LaLiberte & Grimes, 1982; Burton et al., 1987) sediments and has been attributed to the higher concentration of nutrients in sediments (Gerba & McLeod, 1976) and the protective effect of sorption (Roper & Marshall, 1974, 1979).

In view of these observations, it has recently been suggested that it would be more appropriate to monitor numbers of faecal bacteria in sediments rather than in the overlying waters in primary-contact and shellfishing waters (Elliot & Colwell, 1985). However, resuspension of sediment organisms back into the water column may result from wave and tide action, storms, run-off, dredging, and some recreational activities (Grimes, 1975, 1980, 1982).

2.2.2. Nutrient deficiencies.

Alexander (1986) suggested that in addition to susceptibility to antagonistic and predative agents, nutrient deficiencies may be responsible for the decline of those bacteria which are relatively resistant to the effects of abiotic factors.

Increased survival of bacteria has been attributed to the nutrient content of the water (Slanetz & Bartley, 1965; Hendricks, 1972; Yanagita & Takagi, 1980). When seawater was supplemented with inorganic salts and organic matter, the decline of *Escherichia coli* was reduced (Carlucci & Pramer, 1960). This effect was found to be even more pronounced in freshwater (Hendricks, 1972). The addition of amino acids to freshwater increases survival of *E.coli* up to 3 fold. The addition of ammonium or nitrate has a limited effect but no carbon or phosphorus source is effective to the same degree (Lim & Flint, 1989).

The chemical composition of water greatly influences the survival of enteric bacteria. The

composition of seawater varies much less than that of freshwater (Mitchell & Chamberlin, 1978). The type and concentration of nutrients available have different effects on different species, for example, the addition of peptone to *E.coli* in seawater increases its survival but the addition of glucose reduces its survival (Carlucci & Pramer, 1960). *E.coli* and other coliforms require only low concentrations of organic matter to grow, whereas faecal streptococci require much higher concentrations (Allen et al., 1952).

The difference in survival rates of different species of bacteria may be due to their varying abilities to find and compete for growth limiting nutrients in a particular environment (Alexander, 1986). The presence of autochthonous bacteria competing for growth limiting nutrients reduces the availability of nutrients for allochthonous bacteria, and reduces the response of the latter to any nutrients which may then be added (Scheuerman et al., 1988). The most successfully competitive organism is likely to be the one with the fastest growth rate under the prevailing conditions (Alexander, 1986). For this reason, enteric bacteria are unable to compete adequately with autochthonous bacteria for low concentrations of nutrients. However if bacterial predators preferentially consume nutrients, then the effect of predation on bacterial mortality will be reduced (Sinclair & Alexander, 1984).

Evidence exists which shows that some allochthonous bacteria are capable of growth after being

discharged into rivers and marine waters (Hendricks & Morrison, 1967) depending on the presence of appropriate nutrients. Under conditions of high nutrient concentrations, the adverse effects of salinity and temperature are less pronounced for coliform bacteria (Anderson et al., 1979). This is also true for *Vibrio cholerae* (Singleton et al., 1982).

Fresh waters often contain humic materials derived from the decomposition of plants and animals. Humic substances have been accredited with the stimulation of various processes of cell metabolism. At low concentrations they can stimulate the growth of phytoplankton but at high concentrations may inhibit growth altogether (Prakash & MacGregor, 1983). The utilisation of humic materials by some microorganisms as an energy source has been reported (Shapiro, 1957) and the increased growth of *Pseudomonas* spp. in some freshwater systems has been attributed to the metabolism of fulvic acids (De Haan, 1974). Phosphorus and nitrogen occur as functional groups and structural components of humic acids which may be released as soluble forms (Malcolm, 1985). Seawater also contains humic acids but in very low concentrations (Harvey & Boran, 1985).

It is believed that the main reason for the death of allochthonous bacteria in the aquatic environment is their inability to lower their metabolic requirements in a situation of low nutrient availability (Klein & Casida, 1967).

2.2.3. pH.

Generally, a neutral pH environment (5.5-7.5) extends the survival of bacteria (McFeters & Stuart, 1972).

Carlucci and Pramer (1960) found that the death of *E.coli* at acidic pHs was less rapid than at alkaline pHs and that the pH of natural seawater contributed to the rapid death of *E.coli* cells which enter the oceans. It was later suggested that although pH contributed to the death of *E.coli* in seawater, it did not appear to be of primary significance (Mitchell & Chamberlin, 1978). Since the normal pH of seawater lies between the range pH 7.9 and pH 8.2, any variation within these values would have a negligible effect on bacterial survival (Morgan, 1984). Freshwater environments are more susceptible to changes in pH than marine waters.

2.2.4. Salinity.

The influence of salinity appears to be due to either an osmotic effect, or to toxicity of specific ions (Carlucci & Pramer, 1960). The salinity of surface sea water is approximately 3.5% (ZoBell, 1946), suggesting a high concentration of potentially toxic inorganic salts. The physiological effects of salinity are very diverse, as discussed by Rheinheimer (1985).

It has been shown repeatedly that most bacteria investigated are able to survive longer in waters of low salinity than in waters of high salinity, both in the presence (Fujioka et al., 1981), and in the

absence of sunlight (Faust et al., 1975; Evison & Morgan, 1982; Gameson, 1984). Pike et al. (1970) found that the T90 values for coliforms in a river were approximately double those for seawater.

Early laboratory studies reported by Gameson (1984) also showed that T90 values for total coliforms in the dark had a direct correlation with salinity. An inverse, linear relationship was also found when faecal coliforms were exposed in water of various salinities, to daylight and to an artificial light source (Gameson & Gould, 1985). Evison and Morgan (1982), however, provided evidence which suggested that the relationship between die-off rate and salinity was not linear for most bacteria. Maximum survival was recorded for those bacteria tested at 0.05% salinity. At 0%, survival time was shorter.

Mancini (1978) expressed the influence of light on coliform mortality as a function of salinity. Chojnowski and Mancini (1979) and Sieracki (1980) also noted the synergistic effect of sunlight and salinity. It was also found that sublethal stress could develop in *E.coli* exposed to waters of high salinity (Kapuscinski & Mitchell, 1981).

2.2.5. Temperature.

Extremes of temperature seem to be most disruptive to bacterial survival (McFeters et al., 1974; Flint, 1987). Several investigators have noted that at lower temperatures bacteria survive longer than at higher temperatures, in fresh waters (McFeters & Stuart, 1972;

Auban et al., 1983; Barcina et al., 1986a), in estuarine waters (Faust et al., 1975; Vasconcelos & Swartz, 1976) and in marine waters (Pike et al., 1970; Evison & Tosti, 1980).

Pike et al. (1970) and Mancini (1978) commented on the direct relationship which appeared to exist between temperature and die-off rates of coliforms in fresh and sea water laboratory experiments under conditions of darkness. Mortality rate increases linearly with increasing temperature between 4°C and 24°C for faecal coliforms in seawater in the dark (Gameson, 1984). Evison and Morgan (1982) confirmed this and found that a similar relationship existed for faecal streptococci and salmonellae. Rhodes and Kator (1988) however found no significant correlation between mortality of salmonellae and temperature in estuarine waters.

That survival is inversely proportional to temperature has been reported over a number of temperature ranges (McFeters & Stuart, 1972; Faust et al., 1975; Evison & Tosti, 1980). However, conflicting evidence demonstrating prolonged survival of *E.coli* and salmonellae in sterile and non-sterile estuarine waters at warm temperatures (>18°C) has been presented (Rhodes & Kator, 1988) suggesting that survival is directly proportional to temperature, an observation which supports those previously made by Anderson et al. (1983).

The influence of temperature on the survival of enteric bacteria in marine and fresh waters is thought

to be more significant in the dark (Gould & Munro, 1980), or where light penetration is poor, for example in turbid waters (Evison & Morgan, 1982), but its influence is negligible in sunlight. However, Gameson and Gould (1985) suggested that it was likely that the mortality rate of coliform bacteria in sunlight increases with temperature, but with a significantly smaller temperature coefficient than in the dark. Earlier, Hollaender (1943) had found that the lower the temperature the longer the T_{90} of *E.coli* exposed to radiation between 350 and 400nm.

It is believed that temperature exerts its effect on survival through its influence on other factors (EPA, 1985) and also through its control of the metabolic rate of cells (Jones, 1971). The influence of temperature on the survival of *E.coli* only exists when the natural aquatic community are present and it is thought, therefore, to be exerted through the predatory activity of protozoa. In sterile waters, the inverse relationship between survival of *E.coli* and temperature is not apparent (Barcina et al., 1986a,b). Anderson et al. (1983) found that survival of *E.coli* is directly related to temperature in the absence of eukaryotes. These workers concluded that the interactions between experimental bacteria and autochthonous organisms and the occurrence of sublethal stress may account for the discrepancies in temperature-related survival data. Evidence in support of this was obtained by Flint (1987) who concluded that though temperature was a major factor affecting the survival of bacteria introduced into the

natural environment, its importance is secondary to that of biotic influences.

Undoubtedly, temperature is still considered by many to contribute significantly to bacterial die-off in the environment. The influence of this factor should be considered in connection with the time of year and location of release of bacteria into the environment. Water temperature tends to vary according to season and latitude and is a function of sunlight intensity (ZoBell, 1946).

2.2.6. Biological factors.

The observation that heat sterilisation destroys much of the bactericidal effect of sea water has been made repeatedly (ZoBell, 1936; Orlob, 1956; Carlucci & Pramer, 1959; Mitchell & Morris, 1969) indicating the influence of a factor which shows thermal instability. A biological influence on bacterial die-off rates has also been demonstrated in fresh waters (Purdy & Butterfield, 1918).

2.2.6.1. Bacteriophages.

Bacteriophages have been suggested as a factor contributing to the death of enteric bacteria entering the environment (Mitchell, 1968). However, it is believed that their effect is negligible compared with the effect of many of the other factors (Mitchell & Chamberlin, 1978; Scheuerman et al., 1988).

2.2.6.2. Antagonism and competition.

Autochthonous marine bacteria (Rosenfeld & ZoBell (1947) and planktonic algae (Sieburth, 1959, 1960) produce chemicals which are antagonistic to enteric bacteria. Many antagonists have been identified, as noted by Mitchell and Chamberlin (1978), but there is little evidence to suggest that these are effective in killing allochthonous bacteria in the sea and their significance remains unclear.

It has also been suggested that some algal toxins may act as photosensitisers in photodynamic action thus rendering the cell more susceptible to the lethal effect of sunlight (Jørgensen, 1962).

2.2.6.3. Predation.

The importance of predation as a factor affecting bacterial survival in the sea was emphasised early on by a number of authors (Enzinger & Cooper, 1976; Roper & Marshall, 1977; Mitchell & Chamberlin, 1978) and more recently by Evison and Morgan (1982) and Barcina et al. (1986). Mitchell and Chamberlin (1978) mentioned 3 groups of organisms which may be important in reducing the numbers of *E.coli* in sea water: i) cell wall-lytic marine bacteria, ii) certain marine amoebae, and iii) marine bacterial parasites, e.g., *Bdellovibrio bacteriovorus*.

A number of workers observed that the indigenous protozoa were more effective than predaceous bacteria in removing coliforms from marine (Mitchell &

Morris, 1969; McCambridge & McMeekin, 1979) and estuarine waters (Enzinger & Cooper, 1976), and that parasites such as *Bdellovibrio* and other lytic bacteria made no significant contribution to the death of *E.coli* in fresh water compared with the predatory activity of protozoa.

Mitchell and Chamberlin (1978) and McCambridge and McMeekin (1981) recognised the synergistic interaction of the influences of predation and sunlight whereby the solar radiation may injure the bacteria making them more susceptible to predation, thus more significantly reducing the numbers of bacteria than each factor acting independently.

2.2.7. Specific ion toxicity.

Nabbut and Kurayiyyah (1972) found that certain inorganic salts were more bactericidal to *S.typhi* than others and concluded that the variation in bactericidal action of sea water is partly due to fluctuations in toxic ion concentrations. Iodate has been proposed as the main toxic agent in sea water (Johannesson, 1957). Heavy metal toxicity has also been implicated for *E.coli* in sea water (Jones,1964).

Although the presence of toxic ions do not appear to be of primary significance, their presence is not favourable for the survival of *E.coli* in sea water and will clearly make some contribution to their death.

2.2.8. Dissolved oxygen.

Dissolved oxygen (DO) levels of water vary with the season of the year, lower DO levels occurring in summer and higher DO levels during winter. The survival of *E.coli* is directly proportional to the DO concentration of the water, the decline in cell numbers being lowest at high DO levels (Faust et al., 1975). However, the shock of leaving an oxygen-poor medium such as sewage and entering an oxygen-rich medium like sea water promotes rapid decay of coliform bacteria (Kott, unpublished work).

2.2.9. Turbidity.

The effect of turbidity is exercised mainly by way of its influence on light penetration and availability of nutrients. Turbidity is usually associated with the suspension of inorganic and organic matter in water. Suspended particles adsorb nutrients to their surfaces, presenting bacteria with a more favourable nutritional environment. Toxic substances may also be rendered harmless by adsorption to particles (Rheinheimer, 1985).

Penetration of light is less in turbid waters, lethal radiation being absorbed by particles (Gameson et al., 1973). High turbidity may therefore, be one of the major factors responsible for increased survival of bacteria in estuarine environments and other turbid waters (Evison & Morgan, 1982; Fujioka et al, 1981).

2.3. The effect of light on bacterial mortality.

2.3.1. Mechanisms of inactivation.

Discussion of all the papers which describe mechanisms for the lethal effects of light is beyond the scope of this review for indeed there are many. Previously Harrison (1967), Eisenstark (1971), Krinsky (1977), Chamberlin and Mitchell (1978), and more recently Gameson and Gould (1985) have reviewed the literature describing the mechanisms of light inactivation of bacteria by U-V and visible light.

The lethal effects of sunlight on bacteria, in particular the ultra-violet component of sunlight were established very early on (Ward, 1893a,b; Gates, 1930; Gaarder & Sparck, 1931; Kelner, 1949). Many types of cell damage have been induced by the photodynamic action of UV light, including loss of colony-forming ability, damage to DNA, damage to the cell membrane, and inactivation of enzymes (Smith & Hanawalt, 1969). Photodynamic action requires the presence of a photosensitising pigment and oxygen.

The effect of light in the near visible and visible part of the spectrum (370-500nm) on bacteria in sea water received little attention for many years. Greenberg (1956), and Carlucci and Pramer (1960) believed that the effect of sunlight on bacterial mortality was negligible. This was due in part, to the high attenuation coefficients of the U-V and visible components in sea water, and partly to the misconceptions that only far-

ultraviolet light (wavelength <280nm) was harmful to bacteria and that nucleic acids were the only sites of damage.

It was later confirmed that visible light was lethal to many bacteria and that the addition of photosensitisers such as fluorescent dyes, and the presence of oxygen enhanced mortality (Eisenstark, 1971). Only U-V light can react directly with DNA. The effects of visible light are due to the presence of natural endogenous photosensitisers which behave analogously to exogenous photosensitisers involved in photodynamic action. In an extensive review of the literature Eisenstark (1971) compared the effects of photodynamic action and the effects of visible light. His observations were:

i) The oxygen demand for killing by U-V light was low compared to that for visible light.

ii) Photoreactivation occurred after exposure to U-V light but not to visible light.

iii) The action spectrum for visible light was 300-380nm for division delay and 338nm for growth inhibition (c.w. 260nm).

iv) DNA does not undergo rapid degradation when exposed to visible light.

2.3.1.1. Inactivation by ultraviolet light.

Inactivation of bacterial cells by U-V light is principally due to localised lesions in DNA caused by the formation of pyrimidine dimers particularly thymine, by direct absorption of U-V light, resulting in mutation or

loss of the ability to replicate (Smith & Hanawalt, 1969). Ultimately, damage to the DNA results in death of the cell. Setlow and Pollard (1962) provided indirect evidence for the involvement of DNA in the lethal action of U-V light by comparing the U-V adsorption spectrum of DNA and the action spectrum for U-V inactivation. Both spectra peaked at 260nm.

2.3.1.2. Repair of U-V-inactivated cells.

2.3.1.2.1. Photoreactivation.

Damage caused by U-V light is often reparable. Kelner (1949) demonstrated that the survival of bacteria exposed to U-V light was increased by subsequent exposure to visible light (<510nm). This is a phenomenon known as photoreactivation. This repair mechanism is thought to involve an enzyme which utilises thymine dimers as substrate, the absorption range for this enzyme being 300-500nm (Setlow & Setlow, 1963).

Jagger et al. (1969) demonstrated another type of photoreactivation which was thought to operate by delaying growth and promoting processes which eliminate dimers from DNA, thus allowing repair. A detailed review of these and other mechanisms for recovery from photochemical damage can be found in Smith and Hanawalt (1969).

2.3.1.2.2. Dark recovery.

The studies of Setlow (1967, 1968) provided experimental evidence for repair of U-V-damaged cells in the dark. Defective regions in the DNA strand are excised and replaced with normal nucleotides, using the

information on the complementary, intact strand for base pairing. This process is known as excision-repair and occurs without the aid of light. The steps involved in excision-repair are discussed in detail by Setlow (1968) and Smith and Hanawalt (1969).

Dark repair is not usually as effective as photoreactivation (Setlow, 1968).

2.3.1.3. Inactivation by visible light.

The photodynamic effect is initiated by a photon of visible light (hv) exciting a suitable sensitiser (S) forming an electronically excited species (S'), which may be converted to a metastable excited species (^1S) which has a sufficiently long lifetime to interact with other chemicals to initiate photochemical reactions (Krinsky, 1977).

Several mechanisms have been proposed by which the excited sensitiser may cause the oxidation of the substrate molecules and these are listed by Smith and Hanawalt (1969). The role of singlet oxygen ($^1O_2$) as a major intermediate in photosensitised oxidations has been confirmed on numerous occasions, as reported by Krinsky (1977). However, this author also noted there is evidence suggesting that there is no single, universal intermediate in photosensitised reactions.

Eisenstark (1971) suggested that target cells or chromophores exist in bacteria, which can be photosensitised by visible light and may be capable of mediating damage to the DNA. A number of workers have demonstrated that mutation may occur in bacteria exposed

to light of wavelengths greater than 300nm (Webb & Tai, 1969; Webb & Malina, 1967). This would seem to implicate DNA as a chromophore but there appears to be little experimental evidence to support this.

Thymine dimers were found in far smaller numbers in cells exposed to visible light than in those exposed to U-V light. The lower limit of the daylight spectrum is 293nm (Henderson, 1970) and the absorption spectrum of DNA is almost zero at 320nm so it is most unlikely that DNA is the direct target of visible light (Eisenstark, 1971). Other chromophores which have been suggested are ubiquinones and naphthoquinones, cytochrome a_1, riboflavin, NADH, porphyrins, and tryptophan. Eisenstark (1971) observed that many suspected chromophores were an integral part of the bacterial membrane.

2.3.1.4. Photosensitisers.

Certain substances exist which enhance the cellular damage caused by light. These are known as photosensitisers and may be exogenous compounds such as the fluorescent dyes, methylene blue and acridine orange (Spikes & Straight, 1967). Sistrom et al. (1956) showed that inactivation of bacteria in the presence of light also occurred in the absence of exogenous sensitisers and they proposed that natural components of the cell may be sensitisers in some bacteria.

Almost every single biological molecule which absorbs light between 320nm and 900nm has been proposed as a potential photosensitising compound (Krinsky, 1977).

Eisenstark (1971) proposed carotenoids, porphyrins, cytochromes, cytochrome oxidase, NAD, NADH, flavins, haemproteins and other pigmented components of cells as endogenous photosensitisers, though as Chamberlin and Mitchell (1978) pointed out, the presence of these photosensitising compounds does not necessarily indicate light sensitivity. Gameson and Gould (1985) observed that differences in pigment densities and enzyme systems may explain the variations in light sensitivity of different species of bacteria.

2.3.1.5. Sites of lethal action.

Death or failure to produce colonies on culture media has been used to assess the effects of photosensitisation though the exact mechanism by which death occurs is not known (Krinsky, 1977). However, the failure to produce colonies does not necessarily indicate the death of a cell.

Many cellular chromophores are components of the cell membrane, therefore the effect of photodynamic action may result in membrane damage. Mathews and Sistrom (1960) and Sprott et al. (1976) have noted the destruction of membrane-associated enzymes by visible light. Increased sensitivity of *E.coli* to inorganic salts present in laboratory media and detergents, after exposure to near U-V light are general characteristics of membrane damage (Moss & Smith, 1981).

Krinsky (1977) reported that specific bases and amino acids were also sites for the destructive action of light. Studies using methylene blue as a sensitiser have

shown that tyrosine, tryptophan, histidine, methionine and cystine are susceptible to photodynamic action (Smith & Hanawalt, 1969).

Sunlight is also an agent of sublethal injury but repair can be brought about by supplementation of the culture media with pyruvate. It is believed that cells exposed to sunlight become sensitive to peroxide and that pyruvate acts as a peroxide scavenger. This implements the catalase system as a site of sunlight-induced injury (Kapuscinski & Mitchell, 1981). It is likely that the initial stress of sunlight is sublethal but that continuous exposure causes death (Fujioka et al., 1982).

2.3.1.6. Repair.

Gameson and Gould (1985) pointed out that there is a lack of demonstrable evidence for the repair of cells exposed to visible light, under natural conditions.

Webb and Lorenz (1970) reported that repair of cell damage due to visible light occurs to a much lower extent than that caused by U-V light.

2.3.1.7. Protection against photodynamic effects.

It is also believed that damaging forms of oxygen in cells may be transformed into harmless species by various enzymatic protection systems which scavenge excited oxygen forms (Morris, 1975). In addition, Bitton et al. (1972) proposed that colloidal suspensions of clays and the presence of humic acids which absorb some of the lethal radiation, protect bacteria from the harmful effects of U-V light.

2.4. Previous light studies.

With the exception of Reynolds (1964), who quite by chance observed differences in coliform mortality rates between day and night, most workers dismissed the effect of sunlight on bacterial mortality as being insignificant for reasons previously mentioned (Section 2.3.1.). It was not until 1967 that higher mortality rates of coliforms were found when exposed to daylight than when kept in the dark (Gameson & Saxon, 1967). Consequently, after numerous shore-based, submerged bottle, and *in situ* experiments had been performed, the conclusion was that sunlight had an important effect on bacterial mortality in sea water.

About half the lethal effect of sunlight can be attributed to wavelengths below 370nm, a quarter to the near-visible ultraviolet (370-400nm) region and a quarter to the blue-green region of the visible spectrum (400-500nm). However, this observation may be invalid at lower depths of the sea because of the greater attenuation of U-V light by sea water (Gameson and Gould, 1985). Hollaender (1943) demonstrated that as wavelength increases, the probability of inactivation decreases, and concluded that 265nm is perhaps the most lethal wavelength for *E.coli*. However, light of wavelength 293nm is the lowest found at sea level (Henderson, 1970) and 300nm is probably the lowest wavelength of light occuring at British latitudes (Gould & Munro, 1980).

Between 1965 and 1975, more than 300 laboratory beaker experiments on the mortality of coliform bacteria exposed to sunlight and a further 400 on the mortality of coliforms in the dark were carried out at the WRC, the results of which were published in a series of technical reports by WRC (Gameson, 1984; Gameson & Gould, 1985). The conclusions formed as a result of these experiments being carried out were discussed by Gameson and Gould (1975) and are as follows:

1) The mortality curves for coliform bacteria in natural waters were approximately log-linear, following first-order kinetics.

2) In laboratory experiments, it was frequently found that *E.coli* NCTC 9002 exhibited a lag phase or steadily increasing mortality rate.

3) It was found that the T90 value of bacteria in the dark decreased with increasing temperature.

4) T90s as short as 20 minutes were recorded in bright summer sunshine.

5) It was found that the rate of decay (K) is approximately proportional to light intensity but decay rates were not found to be significantly different for bright sunshine and over cast sky.

6) The temperature does not exert a significant effect on the value K between 2 and 29°C in sunlight.

Mitchell and Chamberlin (1975, 1978) used the following mathematical model to summarise the observations made by Gameson and Gould (1975). Data from the WRC field experiments and from Foxworthy and Kneeling (1969) were used to test the model.

$$\frac{dC}{dt} = -Kl_0 e^{-\alpha z} C$$

where C = concentration of coliform bacteria at time t and depth z,

K = proportionality coefficient,

l_0 = light intensity just below water surface,

α = effective attenuation coefficient.

The influence of sunlight on mortality rate of *E.coli* is related to depth with an effective attenuation coefficient of approximately $0.22m^{-1}$, directly proportional to light intensity, and a first order reaction with respect to coliform concentration according to Mitchell and Chamberlin (1978). They assumed that light intensity decreased exponentially with depth. In conclusion, the variability of coliform decay rates in sea water can be attributed to the variability of factors influencing the depth profile of light intensity, and bacterial concentration.

Mancini (1978) developed a decay model for coliforms which also took the contribution of the dark mortality rate into consideration.

Mitchell and Chamberlin (1978) believed that light was the single most influential factor acting upon bacterial decay rate but not to the exclusion of the role of all other factors. These workers also pointed out that only actively metabolising coliforms are sensitive to light so that the effect of light would be substantially reduced in nutrient-poor waters and it is conceivable that light only injures coliforms rendering them more susceptible to other factors.

It has been suggested that light intensity becomes an increasingly important influence on bacterial die-off above a certain threshold intensity, and that this value may be quite low (Anson & Ware, 1975; Sieracki, 1980).

2.4.1. Methods of measuring bacterial mortality.

There are two types of methods of evaluating the survival of bacteria in water, namely *in situ* studies and *in vitro* studies. True *in situ* studies involve monitoring the survival of bacteria actually in the body of water itself. Indirect *in situ* methods are carried out with the bacteria in a permeable or an impermeable container, in or on the body of water. Under *in vitro* conditions, the bacteria and water are stored in a container and exposed to a specified set of conditions. Zanoni and Fleissner (1982) reported that small

variations in test conditions can significantly effect the survival pattern of bacteria in *in vitro* studies.

2.4.1.1. *In situ* methods.

Conditions in the sea cannot be accurately reproduced in laboratory experiments indicating the preference of studies carried out in the sea itself. The WRC carried out a number of *in situ* experiments , the results of which are reported by Gameson and Gould (1975). Gameson (1985) reviewed the various techniques which have been used to measure bacterial mortality in the sea. He concluded that *in situ* studies are difficult and costly to carry out and a lot of replication is needed because values vary so much. Also, *in situ* studies have failed to provide evidence of a quantifiable relationship between incident solar radiation and mortality rate (Foxworthy & Kneeling, 1969; Gameson & Gould, 1975). Mancini (1978) observed a wide scatter in reported coliform mortality rates measured *in situ* under supposedly similar conditions.

Reported *in situ* mortality rates for coliforms have been summarised for sea water (Gunnerson, 1975; Chamberlin & Mitchell, 1978) and fresh water (Chamberlin & Mitchell, 1978). They ranged from T90 values of 0.2 to 5.47 hours in seawater and 2.1 to 279 hours for freshwater. Harremoes (1975) concluded that the variability of T90 values from different investigations were due to errors and inaccuracies rather than actual differences.

2.4.1.2. Indirect *in situ* methods.

2.4.1.2.1. Submersed containers.

Significant developments in equipment, and techniques to overcome some of the problems involved in studying the behaviour of bacteria in natural bodies of water have recently been made (McFeters & Stuart, 1981). After examining previous attempts to hold captive a population whilst exposing it to the aquatic environment McFeters and Stuart (1972, 1981) developed the membrane diffusion chamber which has since been used by many other workers (Vasconcelos & Swartz, 1976; Grimes & Colwell, 1986; Rhodes & Kator, 1988)).

Bottles (Gameson & Saxon, 1967), polyethylene bags (Stewart et al., 1971; Bellair et al., 1977; Sieracki, 1980), and dialysis bags (Head & James, 1970; Bianchi & Bensoussan, 1977) have previously been used as submersed containers with varying degrees of success.

The main disadvantage of submersed bottles is that they do not allow the full range of wavelengths to pass through. The correlation between mortality rates observed *in situ* and those observed in dialysis bags is poor (Occhipinti, 1975). This has been attributed to the fact that dialysis tubing provides an ideal substrate for growth of marine heterotrophs and so their use is limited to 3 days in summer and 9 or more days in winter (Vargo et al., 1975). It is believed that the use of polyethylene bags has overcome the problem of lost radiation through bottles and bacterial growth on dialysis tubing (Stewart et al., 1971)

2.4.1.2.2. Floating containers.

Confinement of water in a floating container eliminates the dilution which occurs in the sea, but otherwise is probably the closest approach to simulating natural conditions. Rigid containers are easier to use than flexible containers but radiation is lost through the walls. Flexible containers require calm conditions which may be unrepresentative (Gameson, 1985).

2.4.1.3. *In vitro* studies.

Previously beakers have been used to expose bacteria to sunlight in laboratory-based experiments. A matt black background can minimise reflected and scattered radiation from other sources (Gameson, 1985). A controlled artificial light source provides a good alternative to natural sunlight and overcomes the problems which the weather often creates. Henderson (1970) reported that artificial light sources are 'adequate but, on the whole, not very impressive'. Gameson and Gould (1985) described several types of solar simulator which have been designed for this kind of experiment.

In carefully controlled beaker experiments simulating the discharge of sewage into the marine environment, Fujioka et al. (1981) found that even on a cloudy day, exposure to daylight resulted in 99% inactivation of faecal coliforms, but that faecal streptococci remained stable after 4 hours. The bacteria were inactivated to nearly the same extent whether they

were exposed to sea water or, for that matter, to polyphosphate buffer indicating that the high salt content was not necessary for the bactericidal action of sunlight. However, a fresh water environment was found to be much more favourable to the survival of bacteria in which the populations of faecal streptococci and faecal coliforms remained stable for up to 3 days. They concluded that sunlight can penetrate clear glass, translucent plastic and at least 3.3m of clear water, confirming the role of visible light as the bactericidal component of sunlight in seawater

In the period of April 1978 to March 1981, an investigation was carried out in the Department of Civil Engineering, University of Newcastle upon Tyne under contract to WRC, part of which was to compare the survival of pure cultures of selected enteric bacteria and natural sewage bacteria in waters exposed to an artificial light source and to natural sunlight. The results of this investigation are reported in various sources (Morgan, 1984; Evison & Morgan, 1982; Evison, 1988; Gameson & Gould, 1985). A surprisingly small difference was found between the mortality rates of faecal streptococci and faecal coliforms considering that previous workers suggested that the former survives significantly longer than the latter. The salmonellae survived longer than *E.coli* or faecal streptococci, but *Yersinia enterocoliticus*, *Shigella sonnei*, and *Shigella flexneri* were found to be more sensitive to light than

E.coli. The overall conclusions of this extensive study were as follows:

1) Survival of bacteria with varying light intensity is best assessed using light intensity readings at 400nm. The relationship between this and decay is generally linear.

2) Bacterial survival decreases with increasing light intensity and survival is greater in fresh water than in sea water.

3) Faecal streptococci generally survive longer in fresh water and sea water than *E.coli*, though their survival is poorer than some of the salmonellae in sea water.

4) Instead of sewage and faecal pathogens, pure cultures would appear to be a reasonable substitute.

In conclusion, *in vitro* studies may be of some use in certain situations but should not be used to establish coefficients to be used in calculations involving natural water bodies. However, laboratory experiments are relatively easily carried out and allow the relevance of individual factors to be examined, while avoiding the high costs associated with appropriate field work.

2.4.2. Light studies on bacteria other than coliforms.

As a result of selecting this group as an indicator of faecal pollution, the majority of work discussed so far have been concerned with coliform bacteria and to a lesser extent faecal streptococci. As

McCambridge and McMeekin (1981) pointed out, susceptibility of bacteria to sunlight-induced mortality varies from one organism to another. Grigsby and Calkins (1980) also reported that different strains of *E.coli* showed substantial differences in radiation sensitivity.

S.flexneri, *S.sonnei*, *S.dysenteriae* (McFeters et al., 1974), *S.typhi* (Wait & Sobsey, 1980), enterococci (McFeters et al., 1974; Fujioka et al., 1981), and *Legionella pneumophila* (Dutka, 1984) have all been shown to survive longer than *E.coli* and coliforms in studies involving exposure to natural and artificial sunlight.

2.4.3. Determination of mortality in the dark.

Bacterial mortality in the dark is very slow compared with that in daylight (Gameson & Saxon, 1967). T90 values obtained in the dark have usually been in the order of days or weeks rather than minutes or hours (Gameson, 1984; El-Sharkawi et al., 1989).

It is very difficult to carry out *in situ* experiments in darkness and these are unlikely to produce very reliable results (Gameson, 1984). However, numerous laboratory experiments have been carried out which may provide relevant information as to mortality of bacteria in the sea during the night (Pike et al., 1970, Slanetz & Bartley, 1965). Most experiments carried out in the dark appear to have been done so with the aim of investigating the effects of one or more of the other factors on bacterial mortality though some have been carried out purposely to show the difference in decay rates in the dark compared with in the light.

Comparative studies at the University of Newcastle upon Tyne were discussed by Gameson (1984) along with other dark experiments carried out at the WRC. The main conclusions were:

1) Faecal streptococci survived approximately twice as long in the dark as faecal coliforms and salmonellae.

2) Microbial mortality in the dark is usually a log-linear relationship expressed by first order kinetics but occasionally in the Newcastle study a departure from first order kinetics was observed. This was attributed to the effect of predation on the bacteria.

3) Departure from log-linear relationships introduced difficulties in interpreting the data and that repetition and replication of experiments was necessary.

4) Variability in results made it impossible to accurately establish the effects of temperature and salinity, though it is accepted that total coliforms survive better in fresh water than in sea water and that T90(dark) is dependant on temperature.

5) Only mortality at the early stages of an experiment is representative of conditions in the natural environment. Prolonged confinement of bacteria within a vessel is not representative of true conditions.

III. SUMMARY OF LITERATURE AND RESEARCH OBJECTIVES

A survey of the literature has indicated that the primary focus of research has been on the assessment of survival of indicator bacteria in the aquatic environment using cultural techniques. The following observations have been based on this concept: in seawater, a combination of sunlight and salinity are mainly responsible for the rapid decline of *E.coli* (Gameson & Saxon, 1967; Fujioka & Narikawa, 1982) though temperature and sedimentation also contribute (Mitchell, 1968; Mitchell & Chamberlin, 1975). Survival is greater in estuarine waters (Fujioka et al., 1981), probably because of reduced salinity and the protective effect of turbidity against sunlight. Temperature and predation are also considered to be important (Faust et al., 1975; McCambridge & McMeekin, 1979). Survival in freshwater is less clearly understood but temperature (Mancini, 1978) and predation (Flint, 1987) may be important. Sedimentation and predation have clearly less influence on the physiological responses of bacteria in the environment which may result in non-culturability.

That not all viable bacteria in natural waters can be detected using traditional cultural techniques is evident (Xu et al., 1982). Therefore many previous studies have not taken into account the presence and effects of environmental factors on those non-culturable bacteria. Only a few such studies have been carried out. However, it is already apparent that the rate at which bacteria evolve towards the viable but non-culturable

form is influenced by temperature and salinity (Roszak, 1986) and by low intensity visible light (Barcina et al., 1989).

The present study was undertaken with the broad objective of investigating the influence of some of the more important environmental factors, i.e., temperature, salinity and sunlight, on the survival of enteric bacteria under conditions of low nutrient concentrations. In addition to cultural techniques, a direct microscopic technique allowed the study to be extended to those bacteria which are viable but non-culturable but restricted the study to the use of pure cultures as it is not selective.

The specific objectives of the study were:

1) To investigate the combined effects of sunlight, temperature and salinity on the survival of selected enteric bacteria using direct detection and cultural techniques.

2) To determine experimentally the effects of sunlight on viable but non-culturable forms of these bacteria.

3) To determine on the basis of experimental evidence the mechanism(s) responsible for the longer survival of bacteria in freshwater.

4) To develop a method by which viable but non-culturable enterococci can be enumerated using epifluorescent microscopic techniques.

5) To evaluate the importance of sunlight as a bactericidal factor in British bathing waters.

IV. MATERIALS AND METHODS

4.1. Bacterial cultures.

All dehydrated, commercial media and reagents for maintenance and isolation of bacteria were prepared according to the manufacturers' instructions. The procedures for preparation of other media appear in Appendix 1.

4.1.1. Maintenance media.

All salmonellae, *Escherichia coli* NCTC 9001, *Streptococcus faecalis* NCTC 775, and environmental strains of *E.coli* were maintained on Nutrient Agar (made with distilled water, see Appendix 1) slopes at 4°C. Environmental strains of *Streptococcus faecalis* and *Streptococcus faecium* were maintained in Cooked Meat Medium, pH 7.2 (CM82, Oxoid Ltd., Basingstoke, Hants.) at 4°C (Cowen & Steel, 1974). Subcultures to fresh media were made monthly.

4.1.2. Isolation and identification of test organisms.

All salmonellae, except for strains of *S.anatum*, were isolated in this department from sewage-contaminated *Mytilus edulis* (M.M.F. Mesquita, 1988, Ph.D Thesis, University of Newcastle upon Tyne.) and identified tentatively by the Public Health Laboratory, Newcastle General Hospital, Newcastle upon Tyne, before being sent to the PHLS, Colindale, London, for phage typing.

All environmental strains of *E.coli* were isolated from settled sewage (Howdon Sewage Treatment Works) on membrane filters using the selective medium Membrane Lauryl Sulphate Broth (MM615, Oxoid Ltd.) (details of this method are given in Section 4.4.). Some of the isolated yellow colonies appearing on the filter after incubation at 44.5°C for 18 hours were picked off using a nichrome loop and were identified as *E.coli* by the production of characteristic colonies on MacConkey Agar (CM7, Oxoid Ltd.), growth and production of gas at 44.5°C in Brilliant Green Bile Broth (CM31, Oxoid Ltd.), and the IMViC tests of indole production (Tryptone water, see Appendix 1), citrate utilisation (Koser Citrate Medium, CM65, Oxoid Ltd.) and Methyl Red-Voges Proskauer reactions (MR-VP Medium, CM63, Oxoid Ltd.). According to the IMViC scheme, a ++-- response to these tests is characteristic of *E.coli*.

All environmental strains of enterococci were isolated from Howdon settled sewage on membrane filters using Slanetz and Bartley Medium (CM377, Oxoid Ltd.) (details of this method are given in Section 4.4.). Some of the isolated red/maroon colonies were picked off the membrane after incubation at 44.5°C for 48 hours using a nichrome loop and were streaked onto Bile Aesculin-Azide Agar (0525-01-5, Difco Laboratories, East Molesey, Surrey.) plates. Blackening of the medium indicated the hydrolysis of aesculin in the presence of bile (DHSS, 1984) which is characteristic of group D streptococci. Further differentiation of the organisims was carried out

using the following tests: i) reduction of tetrazolium and ii) fermentation of sorbitol (Tyrosine Sorbitol Thallous Acetate Agar, see Appendix 1), iii) fermentation of arabinose (Peptone Water Sugar, see Appendix 1), and iv) growth at pH 9.6 (Glucose Phenolphthalein Broth, see Appendix 1). The relative responses to these tests were ++-+ for *S.faecalis*, --++ for *S.faecium*, and --+- for other Group D faecal streptococci.

In addition, *Escherichia coli* NCTC 9001, *S. faecalis* NCTC 775 and, *S.anatum* NCTC 3072 and NCTC 5779 were obtained from the National Collection of Type Cultures, Central Public Health Laboratory, London, and one strain of *S.anatum* was kindly provided by Dr. Lightfoot of the PHL, Newcastle General Hospital.

4.2. Preparation of microcosms.

4.2.1. Collection and sterilisation of waters.

Seawater was collected from open sea about 7-8 miles off the North East coast at Blyth, a site well away from sludge dumping grounds. The salinity of the water was measured using a salinometer (Model M.C.5, Electrical Instruments Ltd., Kent.). The pH of the seawater was measured using a pH meter (Model 7, Corning Ltd., Stone, Staffs.).

Freshwater was collected from Derwent Reservoir, used for water supply and also for recreational activities. The salinity and pH were measured as above and 1M NaOH solution was added to

adjust the pH to that of the seawater used in the same set of experiments.

Treated, unchlorinated water was collected from Mosswood Water Treatment Works. Similarly, the salinity and pH were measured and 1M NaOH solution was used to adjust the pH.

Synthetic sewage was prepared according to the formula given by Lim and Flint (1989), listed in Appendix 1.

All waters were sterilised by passing them through a 0.2µm, 47mm diameter membrane filter (Sartorius, V.A. Howe & Co. Ltd., London) and autoclaving.

Settled sewage was collected from Howdon Sewage Treatment Works and was sterilised by passing it through a 0.2µm, 47mm filter and distributed into 250ml conical flasks in 100ml aliquots. The flasks were stoppered with cotton wool and covered with aluminium foil before autoclaving.

Humic substances extracted from peat were obtained from Mr W. Hunter, Mosswood Laboratory, Sunderland and South Shields Water Company. The extract had been prepared by soaking peat in reservoir water, allowing the humic acids to leach out and passing through a 0.45µm filter. The humic acid extract was sterilised by filtering and autoclaving as above.

4.2.2. Preparation of microcosm vessels.

Microcosms for dark experiments were prepared using 250ml Erlenmeyer flasks or 100ml medical flat bottles with screw caps. These were washed with dichromate solution and rinsed very thoroughly (at least 20 times) with sterile deionised water. The flasks were stoppered with cotton wool and covered with aluminium foil and the medical flat bottles were sealed with their caps, and autoclaved. 100mls of sterile filtered microcosm water was added to the Erlenmeyer flasks prior to inoculation.

Microcosms for light experiments were prepared using 1000ml and 500ml beakers washed with dichromate solution, rinsed in sterile deionised water and autoclaved together with a magnetic follower, each beaker covered with aluminium foil. 1000mls or 500mls of sterile microcosm water were added to the vessels prior to inoculation.

4.2.3. Growth and harvesting of bacteria.

The test organisms were grown overnight in 100ml of sterile filtered settled sewage or Tryptone Soya Broth (CM129, Oxoid Ltd.) at 37°C. The cells were then harvested by centrifugation (MSE Scientific Instruments, Crawley, West Sussex.) at 3000 rpm for 20 minutes and resuspended in sterile water similar in composition to that intended for the microcosm. This procedure was

repeated 3 times, finally resuspending the cells in the appropriate water.

4.2.4. Inoculation of microcosms.

For light experiments, an appropriate volume of the cell suspension was added to a sterile beaker containing filtered sterilised microcosm water to give the desired concentration of cells. After thorough stirring on a magnetic stirrer, about 60mls were removed from the vessel and transferred to a sterile medical flat bottle as a dark control. For experiments which were only carried out in the dark, the cell suspension was added directly to a sterile Erlenmeyer flask containing sterile filtered microcosm water.

4.2.5. Incubation and removal of samples.

For dark experiments, the microcosms were placed in the dark at 5°C, 15°C, or 25°C. The incubators used were either temperature-regulated water baths in the case of 25°C, or dry air fan-assisted incubators (Gallenkamp, Loughborough, Leics.) in the case of 5°C and 15°C.

For light experiments, the beakers were exposed (aluminium cover removed) with a cellophane film cover, to light from various sources under controlled conditions.

Periodically, 10ml or 1ml aliquots were removed aseptically from the microcosms with a sterile pipette,

after stirring for 5 minutes on a magnetic stirrer (if beaker) or shaking thoroughly (if flask or bottle).

4.2.6. Addition of humic acids to microcosm water.

In certain experiments, the sterile humic acid extract was added to sterile microcosm water such that the absorbance of the water at 315nm (SP6-300 Spectrophotometer, Pye Unicam, Cambridge.) reached the desired level i.e., in most cases the same absorbance at 315nm as freshwater from Derwent Reservoir. After adjusting the pH to that of the seawater with 1M NaOH solution, the water was refiltered and autoclaved.

4.3. Light sources.

4.3.1. Growth cabinet.

A cabinet (Model 600G3/TL, Fisons Scientific Instruments, Loughborough, Leics.) designed for growing plants and algae was used to attain lower light intensities for some microcosm experiments (see Plate 1). This consisted of 13 warm white fluorescent lamps (1.2m x 40 watts). Light intensity was varied using different combinations of light settings which altered the number of fluorescent lamps lit. Separate controls allowed the lighting period and temperature to be controlled (see Figure 2).

All experiments in the growth cabinet were carried out with the lighting period set at 8 hours dark,

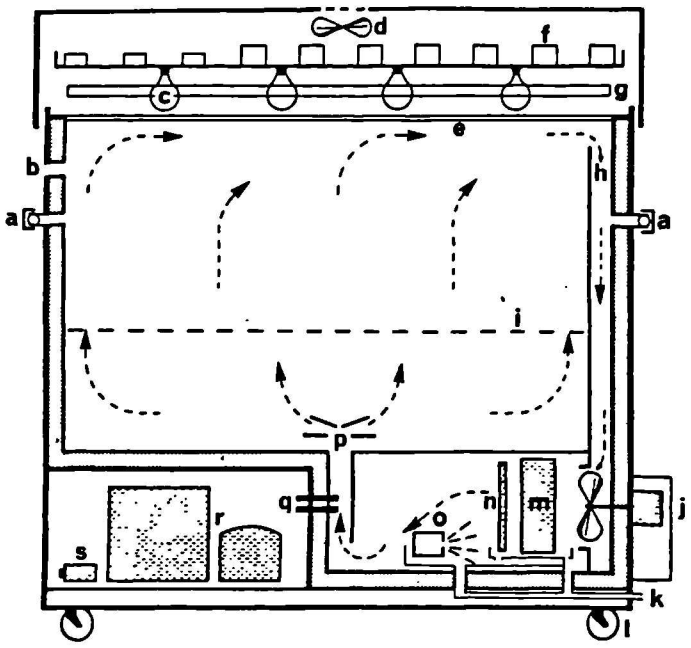


FIGURE 2. Schematic diagram of the growth cabinet

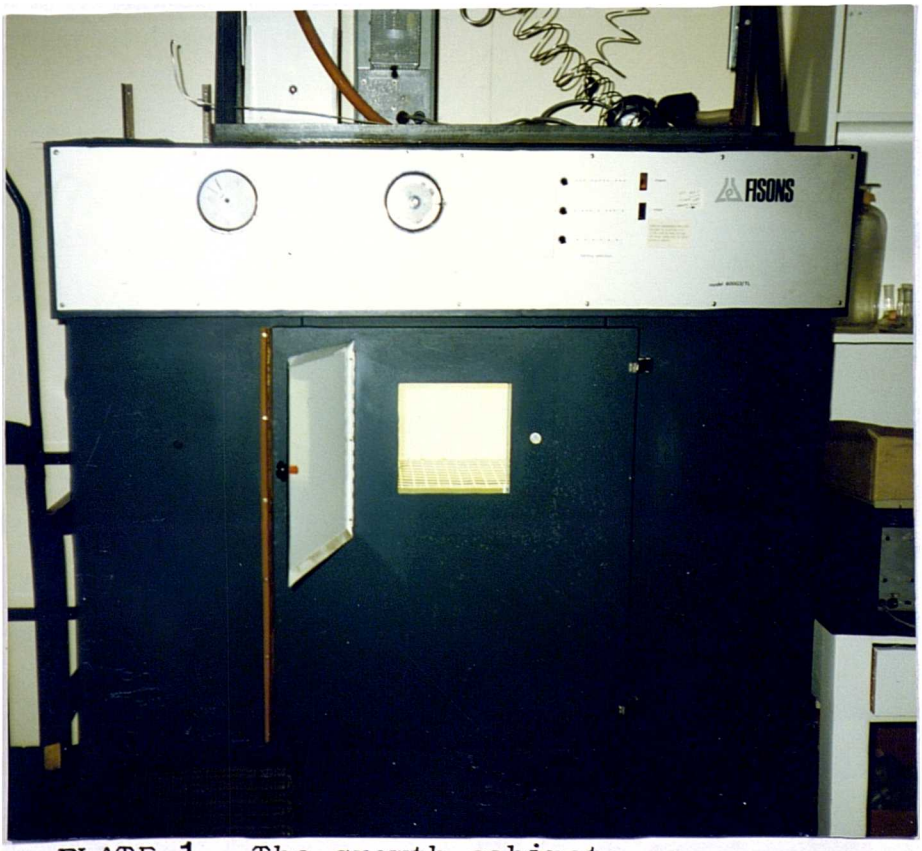


PLATE 1. The growth cabinet

16 hours light with 12 or 6 lamps lit, and the temperature at 15°C. The microcosms were placed on a mesh shelf within the cabinet a distance of 40cm below the lamps. Light intensity readings were taken before and after the experiments using a Kipp and Zonen solarimeter (Type CM 5, F.T. Scientific Instruments Ltd., Gloucester.) and three Fleming sensors which measure intensities of light at 315nm, 350nm, and 400nm (Fleming Instruments Ltd., Stevenage, Herts.).

4.3.2. 1600W solar simulator.

An artificial light source, designed and built by the Water Research Centre, Stevenage and identical to the one described by Gameson (1985), was used to expose test bacteria to higher light intensities (see Plate 2). The use of this light source has been described in a number of previous reports (Morgan, 1981; C. Okuofu, 1985, Ph.D Thesis, University of Newcastle upon Tyne.).

Basically, the light source consists of 3 plates, each attached to a rigid aluminium frame by means of clamps (see Figure 3). A 5L experimental vessel, from hereon referred to as the vessel, surrounded by a perspex water jacket sits on a magnetic stirrer on the base plate. A chiller-thermocirculator (Churchill Instrument Co. Ltd., Perivale, Greenford, Middlesex.) circulates water at a constant temperature through the water jacket. An experimental beaker was placed inside the vessel surrounded by tap water to transfer the temperature of the water in the water jacket through the walls of the vessel to the experimental beaker. In some

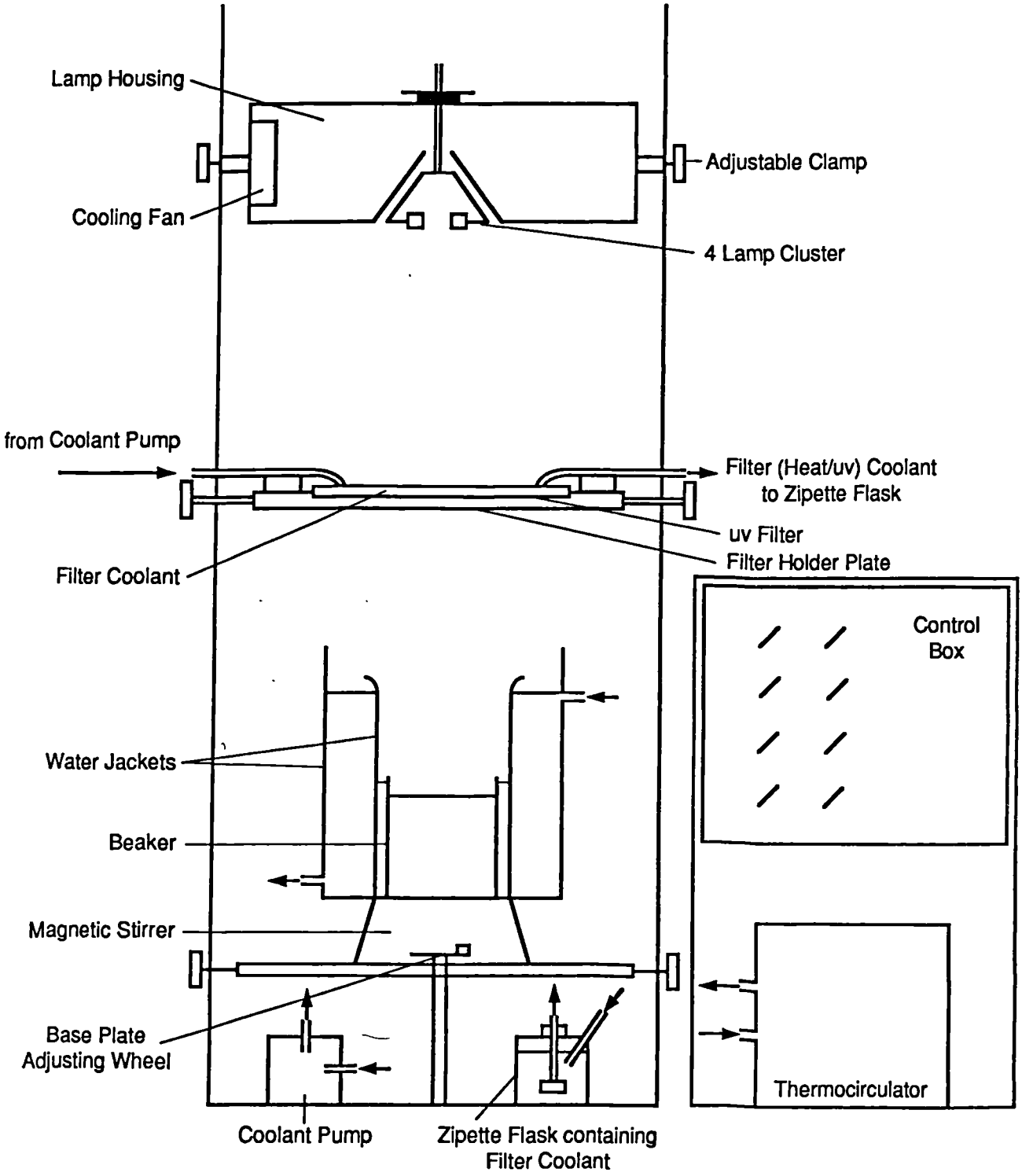


FIGURE 3. Schematic diagram of the 1600W solar simulator

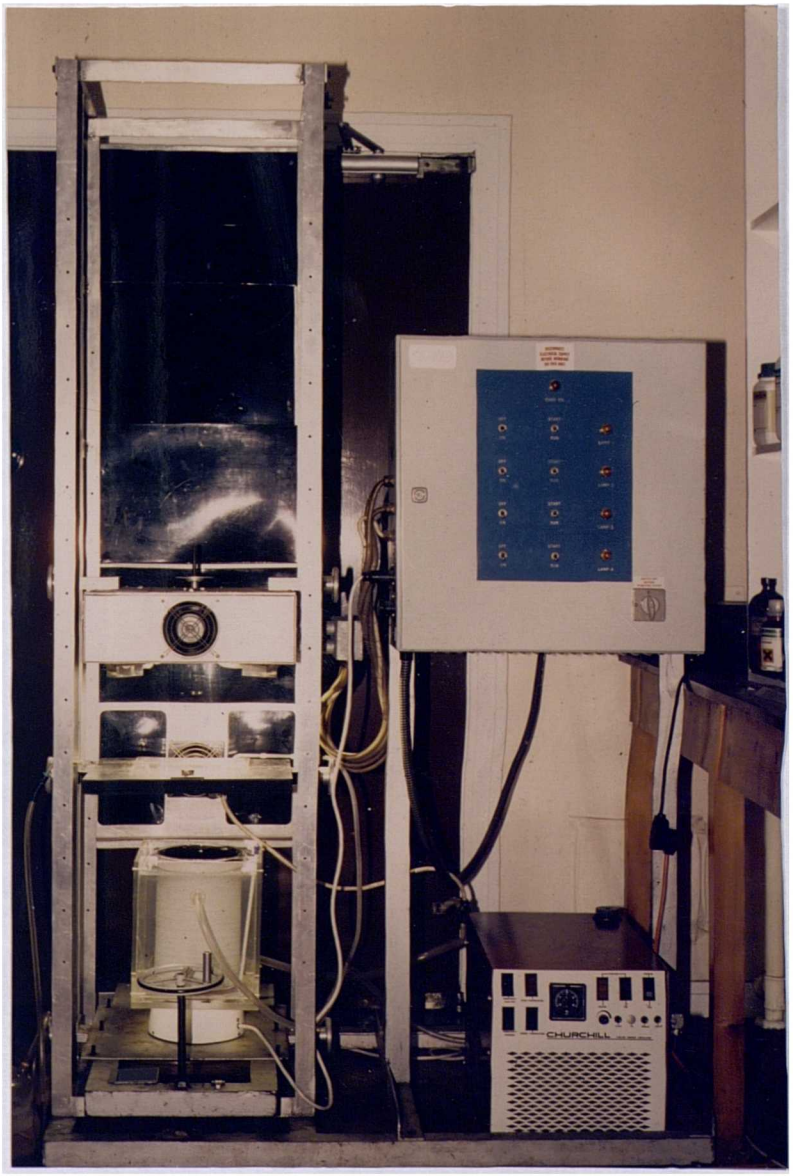


PLATE 2. The 1600W solar simulator

experiments where high light intensities were required, it was necessary to raise the level of the experimental beaker nearer to the lamps by placing it on top of an inverted small wire basket within the vessel. Under these conditions, the magnetic stirrer on the base plate could not be used.

Above the experimental vessel is the filter holder plate. This holds a bio-assay dish containing a coolant solution of 0.05% Teepol (BDH Chemicals Ltd., Poole, Dorset.) to a depth of 1cm. The coolant solution is circulated between the bio-assay dish and a sealed zipette flask by a medium rate peristaltic pump. Cooling of the coolant, which filters out excess heat and U-V light from the lamps, may be necessary. This can be achieved by placing the zipette flask in a water jacket.

Above the filter-holder plate is the bulb housing, equipped with a cooling fan. A cluster of 4 x 400W mercury iodide projector lamps (Thorn Lighting Ltd., Wortley, Leeds) are fitted into the lamp housing, arranged in a cruciform below a floodlight reflector. The spectral distribution of light emitted from the lamps is similar to that of sunlight. Focussing of the lamps may be adjusted by means of a focussing wheel above the lamp housing. In all experiments the lamps were focussed according to the instructions of S.D. Willson, WRC (see Appendix 2).

The light source is connected, via a switch-gear control box (designed and built at the Water Research Centre, Stevenage) to the mains electricity

supply. The light source was used in accordance with the instructions provided by S.D. Willson (Appendix 2), except that a sterile experimental beaker was used within to contain the test bacteria, as there was no satisfactory way to sterilise the 5L vessel with a water jacket attached to it. Consequently, lamp calibration was carried out with the Kipp and Zonen solarimeter in one position only i.e., in the position of the experimental beaker.

Intensity was varied by altering the distance between the lamps and the surface of the water in the experimental beaker and by altering the distance between the lamps and the filter. The intensity of the light emitted from the lamps was measured before and after each experiment using the Kipp and Zonen solarimeter. The lamps were cleaned with methylated spirits before each experiment and the test bacteria were exposed to the light for 5-8 hours.

Experiments were carried out at two light intensity levels both within the range of solar radiation intensities as measured by the Meteorological Office for the East of Britain (0-3MJm^{-1} integrated over a hourly period) and are, therefore, likely to be representative of intensities of sunlight received on the NE coast.

4.3.3. Natural sunlight.

Some microcosms were exposed to natural sunlight on the rooftop of the Department of Civil Engineering, University of Newcastle upon Tyne. In experiments where the temperature was kept constant, the

microcosms were placed in a temperature-regulated water bath on the rooftop at 15°C.

In experiments where the temperature was not controlled (Summer 1988), the microcosms were placed on a matt black board to minimise reflected light. Temperature measurements were taken at intervals and the mean temperature calculated. Light intensity was also measured at intervals using the Kipp and Zonen solarimeter and three Fleming sensors which measure the intensities of light at 400nm, 350nm and 315nm. Microcosms were usually exposed for 4-8 hours.

In the summer of 1989, when some experiments were temperature regulated, the cumulative intensity of the sunlight throughout the duration of the experiment was measured using the Kipp and Zonen solarimeter. Temperature measurements were taken at intervals.

4.3.4. Expression of light intensity readings.

Intensity has the dimensions of energy/area/time. Convenient units of light intensity are ergs/cm^{-2}/sec, watts/cm^{-2}/sec, and photons/cm^{-2}/sec (Setlow and Pollard, 1962). The Meteorological Office recommend the use of the SI unit for energy, the joule(J) (a power of one watt(W) lasting for one second). Solar radiation data is thus expressed in joules per square metre (Jm^{-2}), but in practice it is more convenient to use the megajoule per square metre (MJm^{-2}).

Integration of the signal from the Kipp and Zonen solarimeter by a digital volt-time integrator (Model Mk IV, Lintronic Ltd., London.) produces a

measurement of light intensity in counts per mV-hr, each count corresponding to one deflection of the integrator needle. 100 counts are equal to 1mV-hr. From the Kipp and Zonen calibration certificate, 120mV-hr are equal to 1W-hr cm^{-2}. 1W-hr m^{-2} is equal to 3600Jm^{-2}, therefore 1mV-hr is equal to 0.3MJm^{-2}.

4.4. Enumeration methods.

4.4.1. Cultural methods.

All dehydrated, commercial enumeration media and reagents were prepared according to the manufacturers' instructions. The procedures for preparation of other media appear in Appendix 1.

10-fold serial dilutions of collected samples were made using sterile quarter strength Ringer solution (pH 7.0) (BR 52, Oxoid Ltd.).

4.4.1.1. The standard plate count.

Standard plate counts from all organisms were made in duplicate by the pour plate method on Nutrient Agar (see Appendix 1) made with water of the same composition as the water in the microcosm from which the samples were taken (D.B. Roszak, 1986, Ph.D Thesis, University of Maryland, College Park, USA.) i.e., either seawater, freshwater or treated, unchlorinated water. Preliminary experiments showed that higher counts were obtained on this medium than on nutrient agar made with tap or distilled water. Inoculated plates were incubated inverted in plastic bags at 20°C for 7 days after which

time all colonies were counted. The geometric means of the duplicate counts within the recommended limits i.e., between 30 and 300 colonies (DHSS, 1984), were calculated and the final counts expressed per millilitre.

4.4.1.2. Enumeration of *E.coli*.

E.coli 9001 and environmental strains of *E.coli* in microcosms were enumerated in duplicate using Membrane Lauryl Sulphate Broth (MLSB) (MM615, Oxoid Ltd.) and the membrane filter technique. The counts using this method were found comparable to counts using MPN multiple tube techniques but the membrane filtration method was used in preference because of the number of samples involved. 20ml aliquots of the serially diluted samples were passed through cellulose nitrate membrane filters (Whatman Labsales Ltd., Maidstone, Kent.) of pore size 0.45µm and diameter 47mm, using a sterile filtration unit and with the aid of a vacuum pump. The filters were each transferred aseptically from the filtration apparatus onto sterile pads (Whatman Labsales Ltd.) presoaked in MLSB, in sterile tins. These were incubated inverted, in plastic bags at 30°C ±0.5°C for 4 hours followed by 44.5°C ±0.5°C for 14 hours after which time all yellow colonies on the surface of the membrane were counted. No confirmation tests were carried out. The geometric means of duplicate counts within the recommended limits i.e., between 10 and 100 colonies (DHSS, 1984), were calculated and the final counts expressed per millilitre.

4.4.1.3. Enumeration of enterococci.

S.faecalis 775 and environmental strains of enterococci were enumerated using Slanetz and Bartley's Medium (CM377, Oxoid Ltd.) by the membrane filtration technique, again in preference to the MPN multiple tube technique. Similarly, 20ml aliquots of the diluted samples were filtered were passed through cellulose nitrate filters (Whatman Labsales Ltd.) of pore size 0.45µm and diameter 47mm and transferred aseptically on to a 15ml layer of Slanetz and Bartley's Medium. They were then incubated inverted in plastic bags at 30°C ±0.5°C for 4 hours followed by 44.5°C ±0.5°C for 44 hours after which time all red/maroon colonies were counted. No confirmation test were carried out. The geometric means of duplicate counts within the recommended limits i.e., between 10 and 100 colonies (DHSS, 1984), were counted and the final counts expressed per millilitre.

4.4.1.4. Enumeration of salmonellae.

All salmonellae were enumerated in duplicate by the pour plate method using Xylose Lysine Decarboxylase Agar (XLD) (CM469, Oxoid Ltd.) containing 7µg/l novobiocin (N-1628, Sigma Chemical Company, Poole, Dorset.) (Restaino et al., 1982) which had been found preferable in preliminary experiments to Bismuth Sulphite Agar (CM201, Oxoid Ltd.), Desoxycholate Citrate Agar (Hynes modification) (CM227, Oxoid Ltd.), or Brilliant Green Agar (Modified) (CM329, Oxoid Ltd.). The plates were incubated inverted in plastic bags at 37°C ±0.5°C for 24 hours after which time all red colonies with black

centres were counted. All counts within the range of 30 and 300 were used to find the geometric mean of the duplicates and the final counts were expressed per millilitre.

4.4.2. Direct microscopic methods.

4.4.2.1. Acridine orange direct viable count.

In addition to selective and non-selective culturable counts on each organism, a direct viable count (DVC) was carried out using a standard epifluorescent microscope (Karl Zeiss, Oberkochen, West Germany.). All reagents used in the DVC were sterilised by passing them through a cellulose nitrate filter of pore size 0.2µm and diameter 47mm (Sartorius) followed by autoclaving.

For experiments where *E.coli* and salmonellae were used, 10mls of the serially diluted sample were incubated with 0.002% (w/v) nalidixic acid (N-4382, Sigma Chemical Company.) and 0.025% (w/v) yeast extract (L21, Oxoid Ltd.) for 6 hours at 20°C (Kogure et al., 1978). Between 1ml and 10mls were then passed through a polycarbonate membrane filter (Nuclepore, Sterilin Ltd., Teddington, Middlesex.) of pore size 0.2µm and diameter 25mm, shiny side facing upwards. The membrane filter had been soaked in Irgalan black dye for about 2 hours to give a better contrast to the stained cells. About 2mls of 0.01% (w/v) acridine orange (BDH Chemicals Ltd.) were placed on the surface of the membrane on which the cells had been collected and allowed to stain the cells for 2-3 minutes. The acridine orange was then washed through the

membrane filter with sterile filtered distilled water. The stained membranes were mounted on a glass microscope slide with a drop of water. A cover slip was placed on top of the membrane filter with a small drop of immersion oil at each corner to ensure adhesion of the cover slip to the membrane. The slides were then examined by epifluorescence microscopy using a water immersion objective at 630x magnification.

4.4.2.2. Modified AO DVC for enterococci.

Except where indicated, a DVC of enterococci was carried out by modifying the original method of Kogure et al. (1979) and using 1.0mg/l ciprofloxacin (Ciproxin, Bayer UK Ltd., Newbury, Berks.), a quinolone like nalidixic acid with a similar structure and mode of action (Smith, 1985), but effective against a wide range of Gram positive bacteria (King & Phillips, 1986).

Preliminary experiments were carried out to establish the optimum concentrations of ciprofloxacin and yeast extract solution and the length of the optimum incubation period. Actively growing cells were incubated with 0, 0.1, 1.0, 10.0, and 25.0mg/l ciprofloxacin and 0.038% yeast extract solution. 1.0mg/l ciprofloxacin was found to inhibit division of *S.faecium* and *S.faecalis* cells such that the number of active bacteria did not increase or decrease. 1.5 times the concentration of yeast extract solution (i.e., 0.038% (w/v)) used in the AO DVC for Gram negative bacteria, was required for enlargement of cells. 6-10 hours incubation time was

found sufficient for differentiation of enlarged cells from non-enlarged cells. These conditions were used in all experiments for a AODVC of enterococci, unless indicated otherwise.

4.4.2.3. Conversion of counts.

The number of red, elongated cells or red, enlarged cells (in the case of enterococci) per field of view were counted in 20 fields of view for each sample. The final count was expressed per millilitre as follows:

$$n = \frac{Y.A.d}{a.v}$$ (Jones, 1979)

where n = number of organisms per ml of sample

Y = mean count per field

A = effective filtration area of membrane filter

a = area of field

v = volume of sample filtered

d = dilution factor.

4.5. Statistical analysis.

4.5.1. Calculation of decay rate and T90 values.

The linear least squares regression method using the Minitab statistical computing system (Minitab Inc., State College, Pennsylvania, USA.) was employed for computing the rate of decay of bacteria under each set of conditions and by each method of enumeration.

Bacterial decay can be modelled by a first order reaction of the type $\frac{Nt}{No} = 10^{-kt}$ (Chick's Law)

where Nt = number of bacteria after time t

No = number of bacteria at time 0

t = time

k = decay rate constant

This can also be written as $\log \frac{Nt}{No} = -kt$

and allows k to be readily calculated by linear regression. By regressing log Nt/No against time, k was computed as the slope of the line of best fit for the data.

T90 is the time taken for the bacterial population to decay by 90%, in which case, Nt will equal 10 and No will equal 100.

Therefore $\log \frac{Nt}{No} = \log 0.1$

and $\log 0.1 = -kt_{90}$

T90 was calculated from $\frac{\log 0.1}{-k}$ and was expressed in hours.

4.5.2. Other statistical analyses.

Mean bacterial counts for all of the experiments carried out can be found in Appendices 3-12. All statistical analyses were carried out on the data using the the statistical computing package Minitab. Analyses were carried out on the decay rate constants (k) or on the T90 values, whichever was found most appropriate and include the computation of analysis of variance, of

correlation coefficients, and of lines of best fit through linear regression. Significance testing was carried out at the 95% confidence level except where indicated (then at the 90% confidence level) using tabulated values of the appropriate statistical distribution as given by White et al. (1985), to compare the computed values with. Logarithms used are log base 10.

Minitab was also used to compute the 95% confidence intervals for replicate counts as the confidence intervals for the line of best fit when all the counts obtained were used. These can be found along with the raw data in Appendices 3-12. Because such a large number of data were obtained it was inconvenient to compute confidence intervals for each individual count.

4.6. Northumbrian Water survey.

Initially the present study took the form of a survey of the removal of faecal indicator bacteria from sewage discharges by various preliminary treatment units at existing points along the North East coast of England. This was carried out for a period of about 6 months in collaboration with Northumbrian Water Authority. This survey was not directly related to subsequent studies on bacterial survival and thus the findings, in the form of a report submitted to Northumbrian Water, are placed in Appendix 14. However, this study also provided an opportunity to select the most appropriate media and enumeration methods, in terms of reliability and

feasibility, for subsequent laboratory-based survival studies.

V. EXPERIMENTS AND RESULTS

5.1. Dark experiments

5.1.1. Survival of different species of bacteria

5.1.1.1. Aims

The aim of this experiment was, first of all, to verify the existence of viable but non-culturable forms of bacteria by exposing them to unfavourable conditions, i.e., in this case, high salinity and low nutrient concentrations, and secondly, to compare the survival of different species, both culture collection and environmental isolates in the dark, using the three different enumeration methods detailed in Section 4.4. The test bacteria were selected due to their universal use as an indicator or their pathogenicity.

5.1.1.2. Experimental procedure

Selected indicator and pathogenic bacteria were grown in Tryptone Soya (TS) Broth and inoculated into seawater flask microcosms following the washing and harvesting procedures outlined in Section 4.2.3. and also used in all subsequent experiments. The microcosms were incubated at 15°C in the dark.

5.1.1.3. Results

The decay rates of these bacteria in seawater expressed as the T90 values are summarised in Table 5. Individual counts can be found in Appendix 3.

The R-sq values for the linear least squares regression analysis to compute the decay rate constants (k) (see Section 4.6.1.) using culturable counts were between 90 and 100% indicating that the data points fitted the regression line well and that log-linear decay following first order kinetics accurately described the data. For AODVCs, the R-sq values were generally between 50 and 90% indicating that the fit of the line was not so good but that most of the data is described by log-linear decay.

Table 5. Comparison of decay of selected bacteria in seawater in the dark at 15°C.

	T90 (hours)		
	AODVC	SL	NL
E.coli (NCTC 9001)	358	22	-
E.coli (sewage)	448	45	89
**S.faecalis* (NCTC 775)^	276	161	-
**S.faecium* (sewage)^	658	156	-
S.oranienburg	230	125	150
S.virchow	301	89	117
S.typhimurium	318	47	347
S.newport	289	31	108

* not non-culturable within the first 19 days of the experiment
AODVC Acridine orange direct viable count
SL Selective count
NL Non-selective count
- no counts
^ red cells counted for DVC

A rapid decline in numbers of detectable bacteria was observed when selective and non-selective methods were employed. Generally, one way analysis of variance shows that the decline is significantly less rapid at the 95% confidence level when the AODVC is

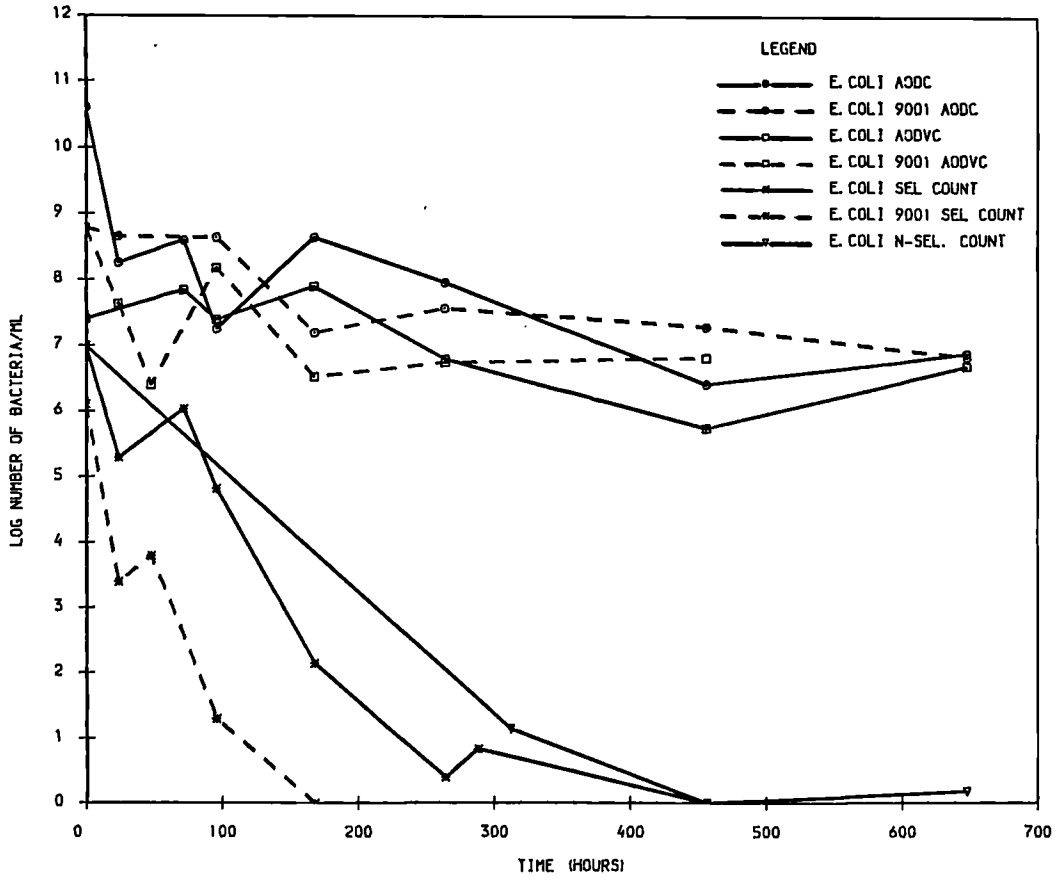


FIGURE 4. SURVIVAL OF TWO ISOLATES OF E.COLI IN THE DARK IN SEAWATER AT 15C

employed than when either selective ($F=27.70$ (1,14)) or non-selective ($F=10.94$ (1,11)) methods are used.

T_{90} values estimated using the two cultural methods are not significantly different from each other.

Figure 4 shows the results represented graphically for the two *E.coli* isolates. At first sight, the sewage isolate of *E.coli* appears to remain culturable longer than its culture collection counterpart though more data would be required to verify this statistically.

5.1.1.4. Discussion

The difference between decay (as T_{90} value) estimated using the AODVC and those using cultural methods indicates that when culturable counts fall to zero, a high number (approx. 10^6-10^7 per ml) of viable bacteria can still be enumerated by the AODVC. Only in the case of the two enterococci, did the selective culturable counts fail to fall to zero within the first 19 days of the experiment. The findings of this experiment are in accordance with the observations made by Colwell and coworkers, that exposure of some Gram negative bacteria grown in rich culture media, to a low nutrient milieu, promotes the rapid evolution of these bacteria towards a viable but non-culturable form. The general characteristics of viable but non-culturable bacteria, as shown in Figure 1 (Section 1.5.1.) were exhibited by the Gram negative bacteria in this experiment. That is not to say that the enterococci would not also have become viable but non-culturable with

time, but without a true AODVC this is limited to general supposition.

Confidence intervals given in graphical form for the sewage isolate of *E.coli* (see Appendix 3) show that the variation in replicate counts made by the AODVC do not differ all that much from the variation in replicate counts made using cultural methods. The non-selective cultural method appears to be subject to least variation.

5.1.2. Survival of *E.coli* at different temperatures and salinities

5.1.2.1. Aims

The possibility of growing the test bacteria in a less rich medium than TS Broth prior to inoculation into microcosms, and the influence of this on their survival characteristics was considered. Filtered, sterile settled sewage was chosen as the culture medium, it being nutritionally rich enough to support the growth of *E.coli* but not as rich as specially formulated media in which nutrients are plentiful and unrepresentative of the levels actually found in the environment. The influence of different temperature and salinity regimes on the survival of *E.coli* was also investigated.

5.1.2.2. Experimental procedure

A sewage isolate of *E.coli* which had been grown in filtered, sterile settled sewage was incubated in 10 flask microcosms at 10 different salinity and temperature regimes, in the dark. Different salinities were achieved by mixing freshwater and seawater in the appropriate proportions. This experiment was carried out in duplicate.

5.1.2.3. Results

Data obtained from two replicate experiments are summarised in Table 6 in the form of the T90 values of *E.coli* under the specified conditions of temperature and salinity. The T90 values from the two replicate experiments were not significantly different from each other. Individual counts can be found in Appendix 4.

Table 6. Decay of *E.coli* under different temperature and salinity regimes in the dark.

		T90(hours)					
		EXPT. A			EXPT. B		
T	S	AODVC	SL	NL	AODVC	SL	NL
4°C	0.05%	185	207	208	280	500	356
20°C	0.05%	180	213	195	204	353	356
37°C	0.05%	93	163	146	72	176	-
4°C	1.75%	167	216	226	198	568	170
20°C	1.75%	171	230	287	104	394	-
20°C	1.75%	162	247	265	-	-	-
37°C	1.75%	67	67	68	46	90	139
4°C	3.50%	155	199	251	176	196	247
20°C	3.50%	160	210	264	210	231	247
37°C	3.50%	37	56	69	23	102	252

A 1st Experiment
B 2nd Experiment
T Temperature
S Salinity

Contrary to the observations of the previous experiment, the T90 values of bacteria as enumerated by the AODVC were not significantly higher than those estimated using culturable counts, suggesting that previous growth in a dilute medium influences the survival characteristics of *E.coli* in such a way that

they do not evolve towards a viable but non-culturable form. *E.coli* was detectable by cultural methods after around 40 days of incubation, in all cases except at 37°C. At a temperature of 37°C *E.coli* were not detectable by cultural methods after 16-20 days. Detection of *E.coli* using the AODVC method was impossible shortly afterwards. Only the decay rates estimated from selective counts were found to be significantly different between this and the previous experiment (A $F=14.27$ $(1,15)$, B $F=10.61(1,16)$) though few data are available for non-selective counts in the previous experiments.

On examination of the T90 values obtained under different regimes, it is obvious that at 37°C the decrease in numbers of *E.coli* is more rapid than at 20°C or 4°C. This is evident using all three methods of enumeration. However, the influences of these two lower temperatures and of salinity on the decay rates are less clear without further analysis.

In an attempt to show more clearly the combined effects of temperature and salinity on the survival characteristics of *E.coli* in the dark, a 3D response surface was fitted to data for each enumeration method in experiments A and B, using the decay rate constant (k) as the response. Fitting a response surface involves the prediction of the response to intermediate temperature and salinity regimes not tested experimentally. A similar response was obtained for each set of data, i.e., the shape of the response net being similar but the decay

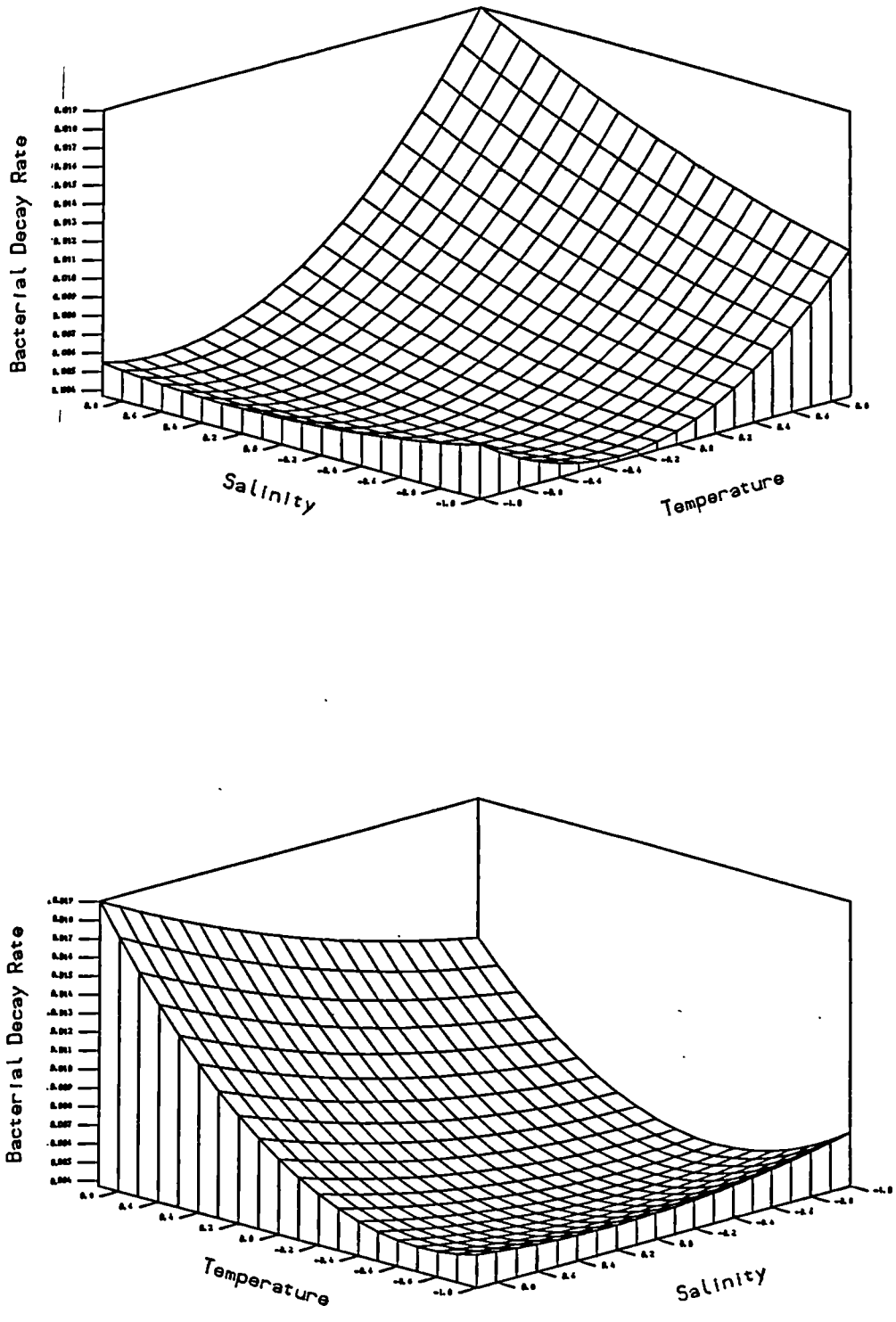


FIGURE 5 - 3D RESPONSE SURFACE FOR BACTERIAL DECAY RATE

rates on the z axis differing slightly. Figure 5 shows the general response, from two different views, using the decay rate constants obtained by the AODVC method in Experiment A which is typical of all the sets of data. Table 7 allows conversion of the axis values to actual readings of temperature and salinity. The use of -1, 0, and +1 to represent the low, medium and high temperatures and salinities respectively, are for simplification in the process of fitting the response surface.

Table 7. Conversion of x and y axis values in Figure 5 to actual values of salinity and temperature.

X-axis	Temperature	Y-axis	Salinity
-1.0	4.0°C	-1.0	0.05%
-0.8	7.2°C	-0.8	0.39%
-0.6	10.4°C	-0.6	0.73%
-0.4	13.6°C	-0.4	1.07%
-0.2	16.8°C	-0.2	1.41%
0.0	20.0°C	0.0	1.75%
0.2	23.4°C	0.2	2.10%
0.4	26.8°C	0.4	2.45%
0.6	30.2°C	0.6	2.80%
0.8	33.6°C	0.8	3.15%
1.0	37.0°C	1.0	3.50%

The main observations, on examination of the response surfaces were:

1) At lower temperatures, the effect of salinity on the decay rate is negligible but is much more pronounced at higher temperatures.

2) The effect of temperature is non-linear, the lowest decay rates being exhibited around 15°C at low salinity, and 10-13°C at high salinity.

3) At the highest salinity, the effect of temperature is linear above 10°C.

4) The combined effect of high salinity and high temperature results in more rapid decay than either factor acting independently though temperature appears to be exerting the more significant influence.

5.1.2.4. Discussion.

At 37°C, the fate of *E.coli* is quite clearly death. A heavy inoculum was introduced into the microcosms which probably caused the initial rapid decay observed with each of the three methods which made the T90 values appear to be not significantly different. This was probably due to competition for the existing nutrients during which many of the weaker cells die leaving the stronger cells to adopt a survival strategy following depletion of the nutrient, in this case the viable but non-culturable strategy. This is only evident from examination of the counts or graphically which are not shown here. Therefore, statistical analysis especially of T90 values can be misleading.

5.2. Artificial light source experiments

5.2.1. Aims

The use of an artificial light source (ALS) as a means of carrying out individual light experiments under similar conditions was investigated. The ability of the light source to accurately simulate the bactericidal action of natural sunlight was also

evaluated. Five species of bacteria were selected for exposure to the ALS. Enterococci have been studied very little in connection with survival under low nutrient concentrations. Two sewage isolates, one of *S.faecalis* and one of *S.faecium*, were used in this and subsequent experiments. Two species of salmonellae were chosen for their prevalence in the human population and their frequency of isolation from sewage polluted waters, *S.montevideo* being one of the more prevalent and more frequently isolated species and *S.oranienburg* being less so. A sewage isolate of *E.coli* was also employed for these experiments.

5.2.2. Experimental procedure

The selected bacteria, grown in filtered, sterile settled sewage, were exposed to the ALS in seawater and freshwater beaker microcosms at two light intensity levels as described in Section 4.3.2. and at three different temperatures. Dark controls allowed exposure of the test bacteria in medical flat bottles to similar conditions in the dark.

5.2.3. Results

Figures 6-10 show the results from some of the individual light experiments represented graphically. It appears that there is very little decay of bacteria exposed to the ALS but the time scale is very short and positive T_{90} values were obtained (see Table 8) and do, in fact, indicate decay. No effect of temperature or

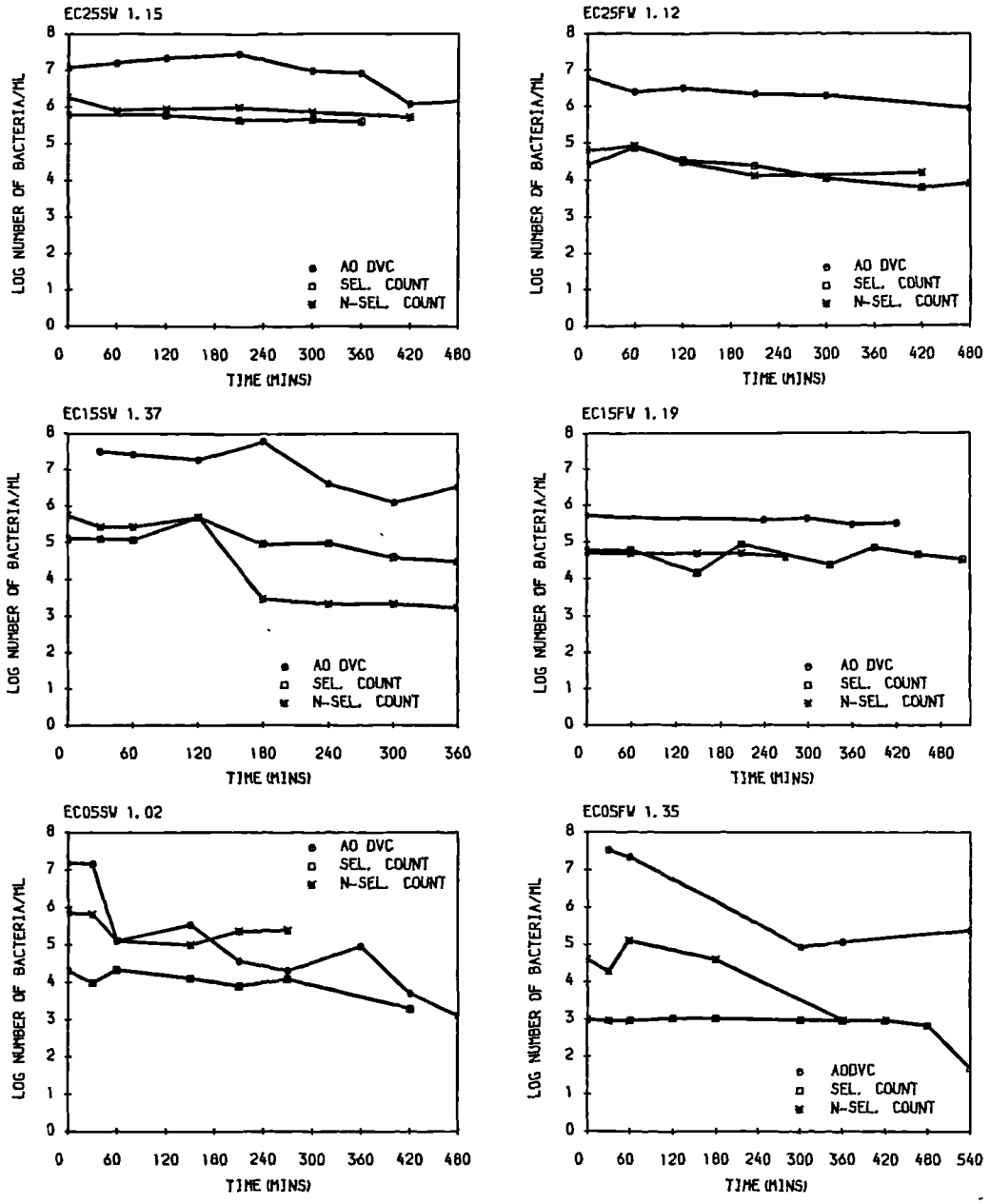


FIGURE 6. ARTIFICIAL LIGHT SOURCE

EXPERIMENTS - E.COLI 1.2MJm-2

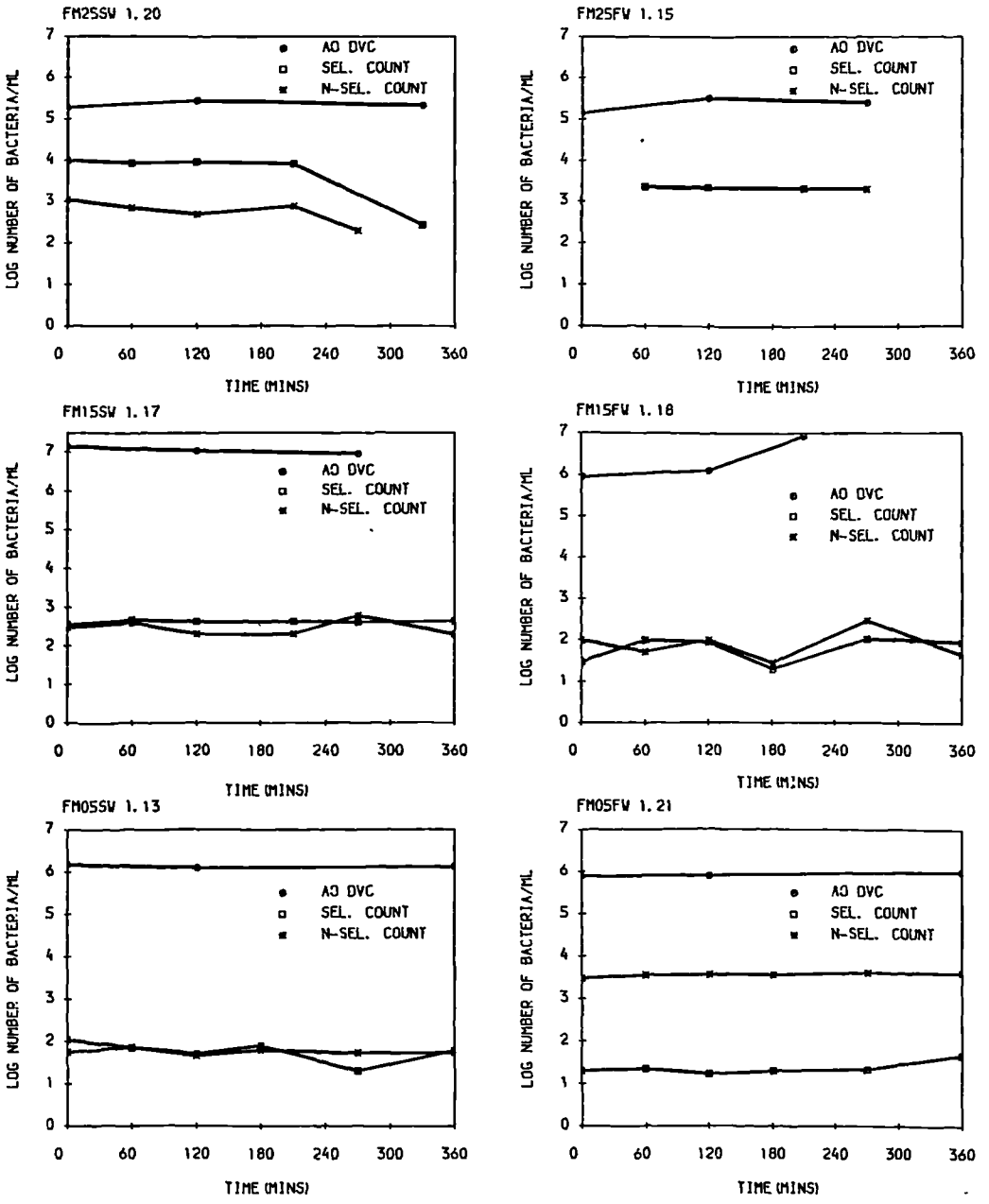


FIGURE 7. ARTIFICIAL LIGHT SOURCE EXPERIMENTS - S. FAECIUM 1.2MJm-2

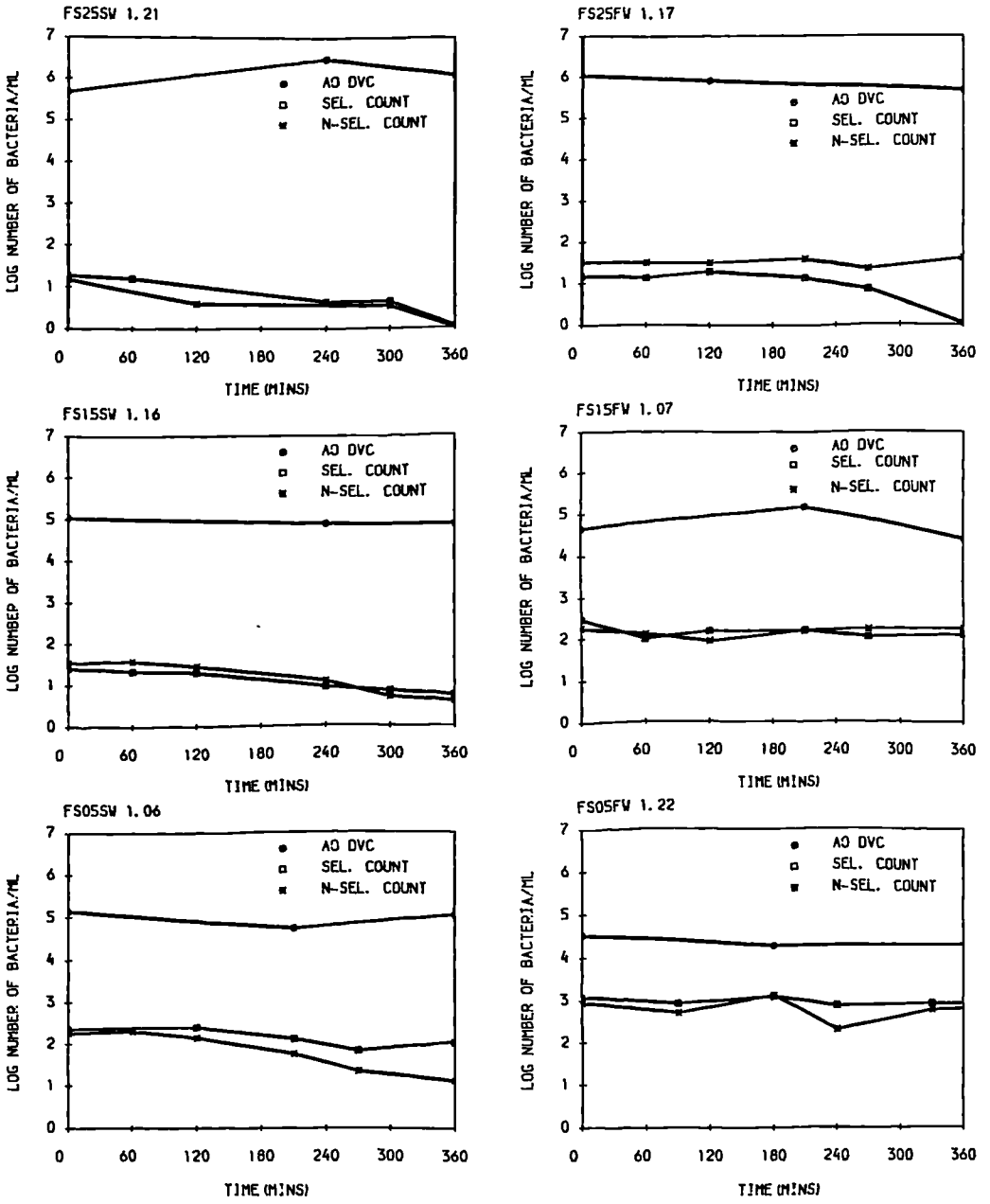


FIGURE 8. ARTIFICIAL LIGHT SOURCE EXPERIMENTS - S. FAECALIS 1.2MJm-2

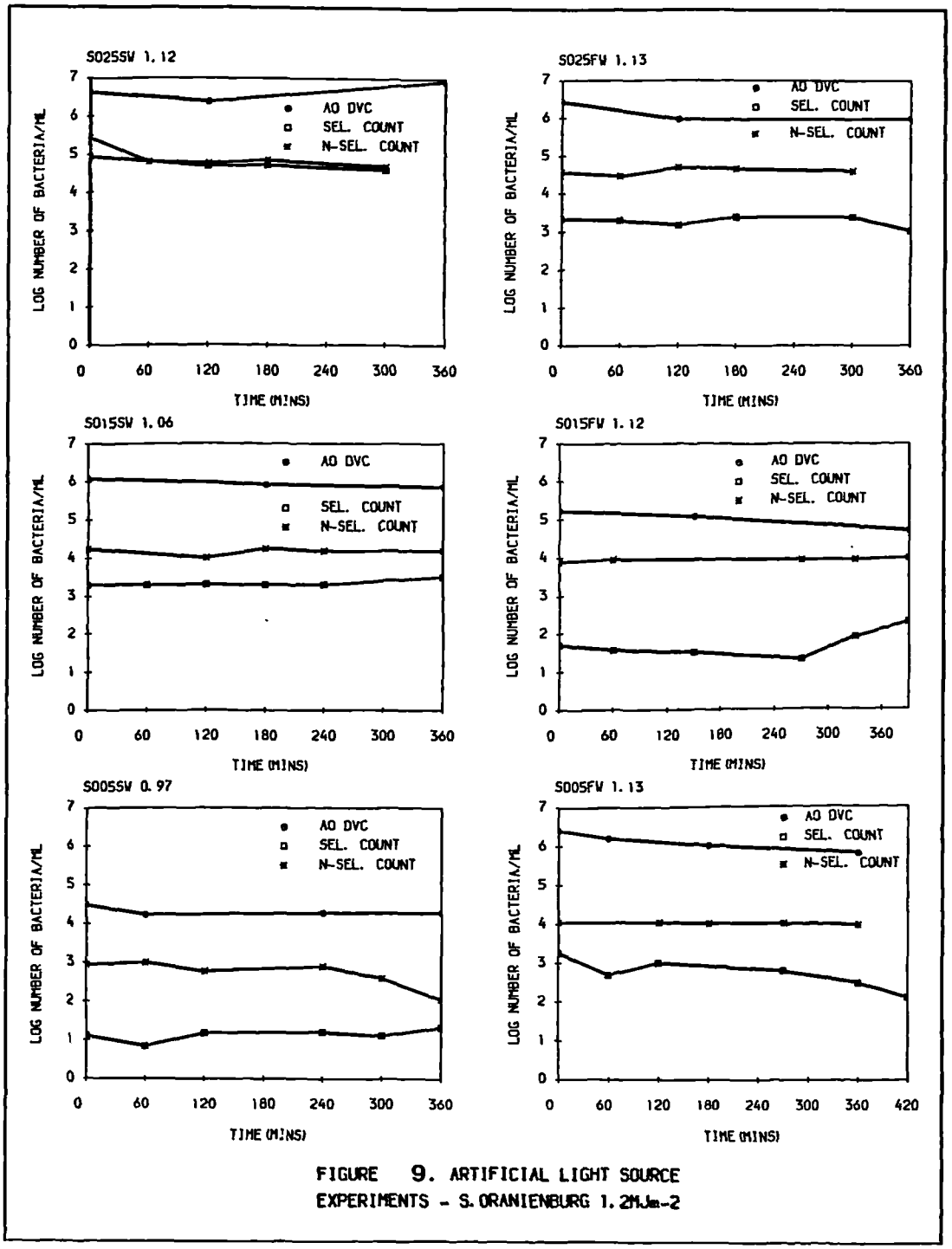


FIGURE 9. ARTIFICIAL LIGHT SOURCE EXPERIMENTS - S. ORANIENBURG 1.2MJm-2

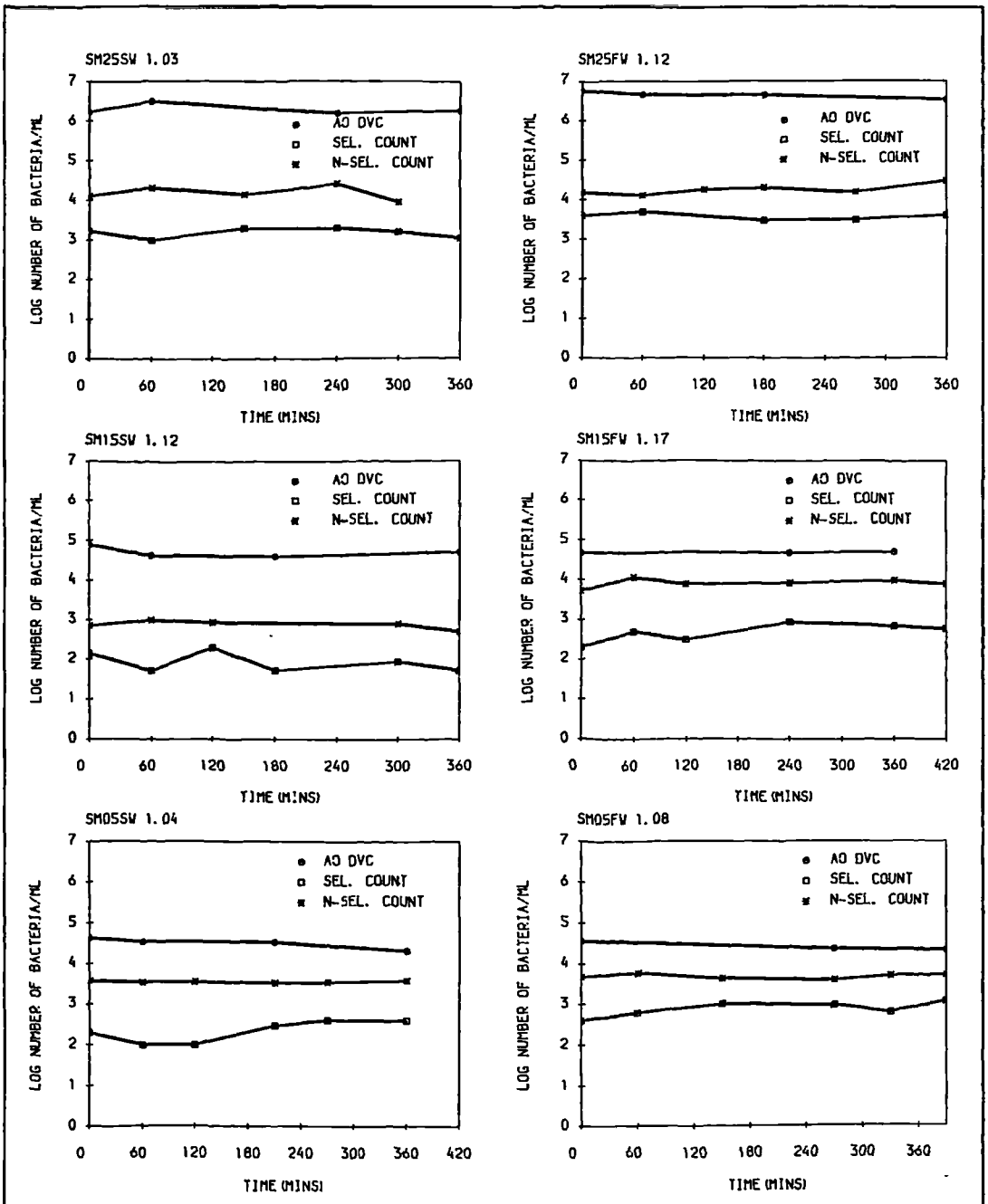


FIGURE 10. ARTIFICIAL LIGHT SOURCE EXPERIMENTS - S. MONTEVIDEO 1.2MJm-2

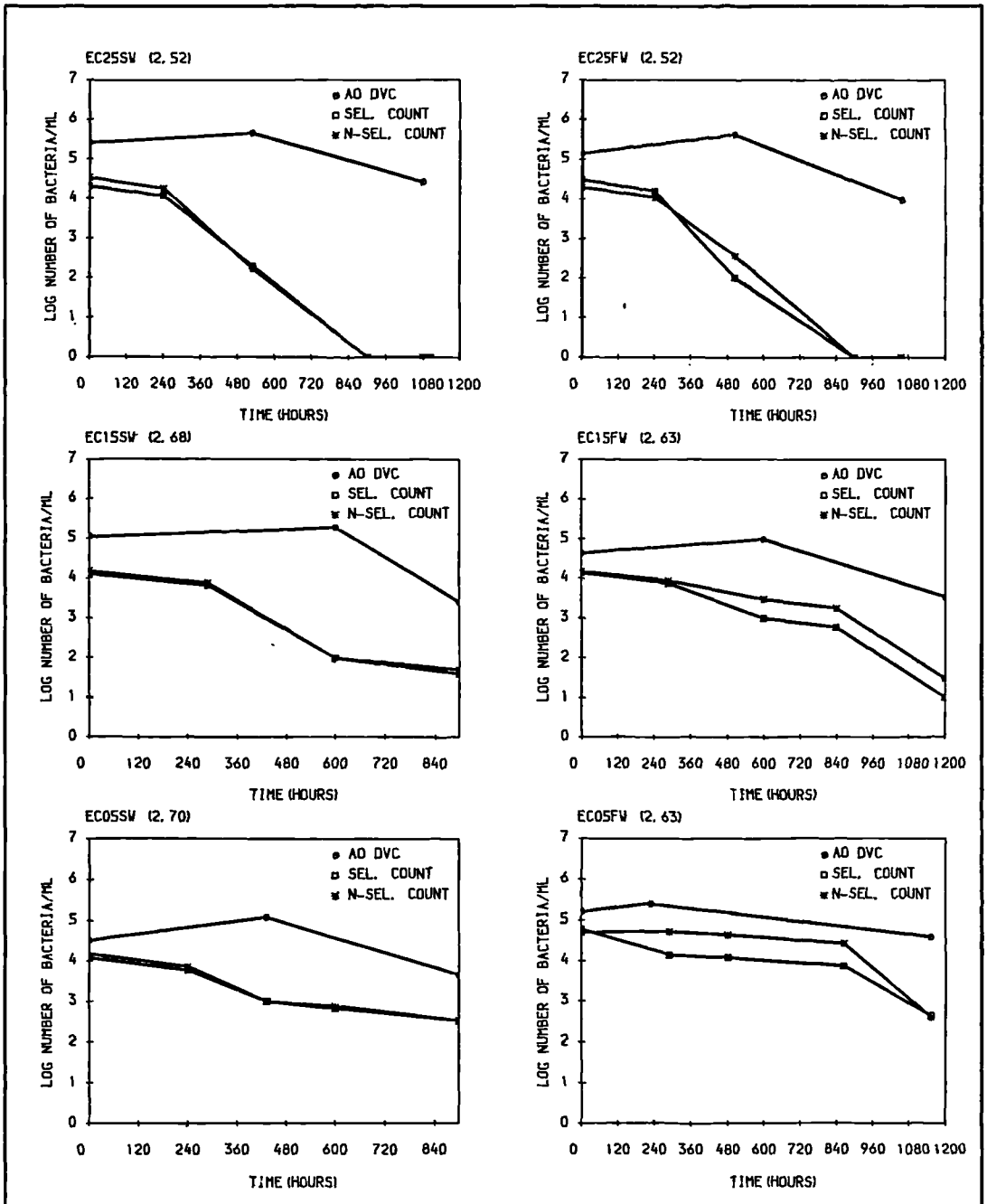


FIGURE 11. ARTIFICIAL LIGHT SOURCE EXPERIMENTS - E.COLI DARK (2.6MJm-2)

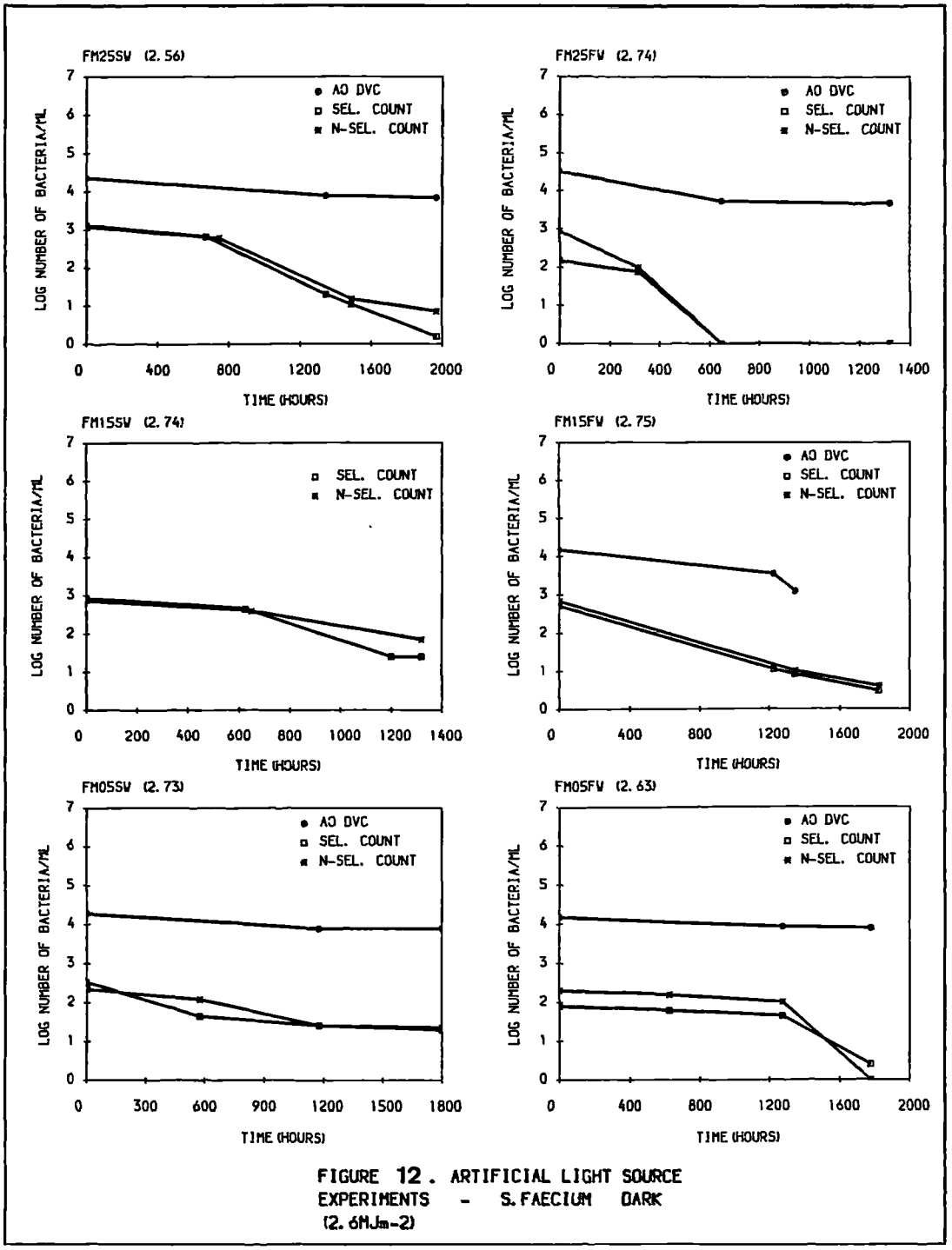


FIGURE 12. ARTIFICIAL LIGHT SOURCE EXPERIMENTS - S. FAECIUM DARK (2.6MJm-2)

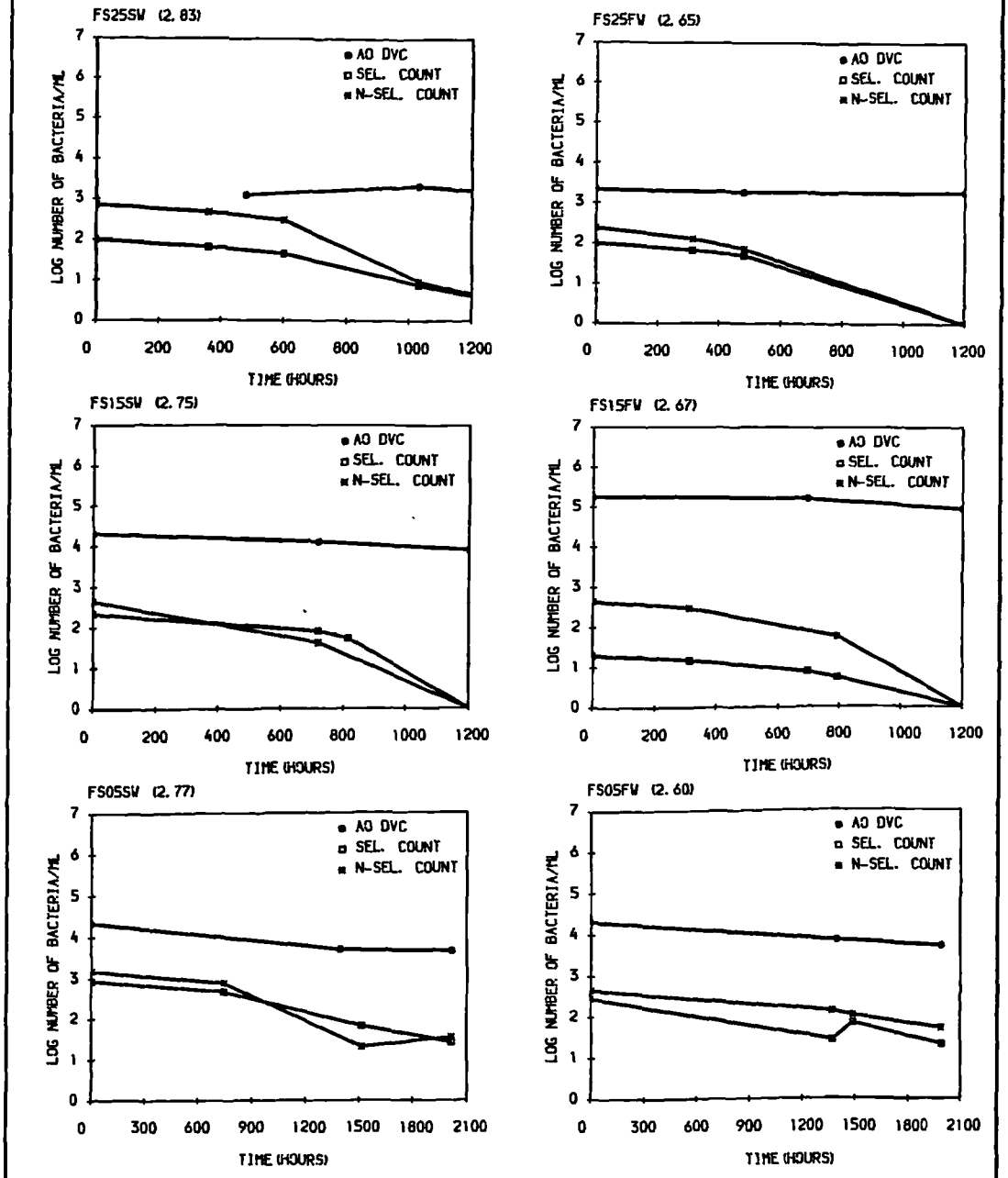


FIGURE 13. ARTIFICIAL LIGHT SOURCE EXPERIMENTS - S. FAECALIS DARK (2.6MJm-2)

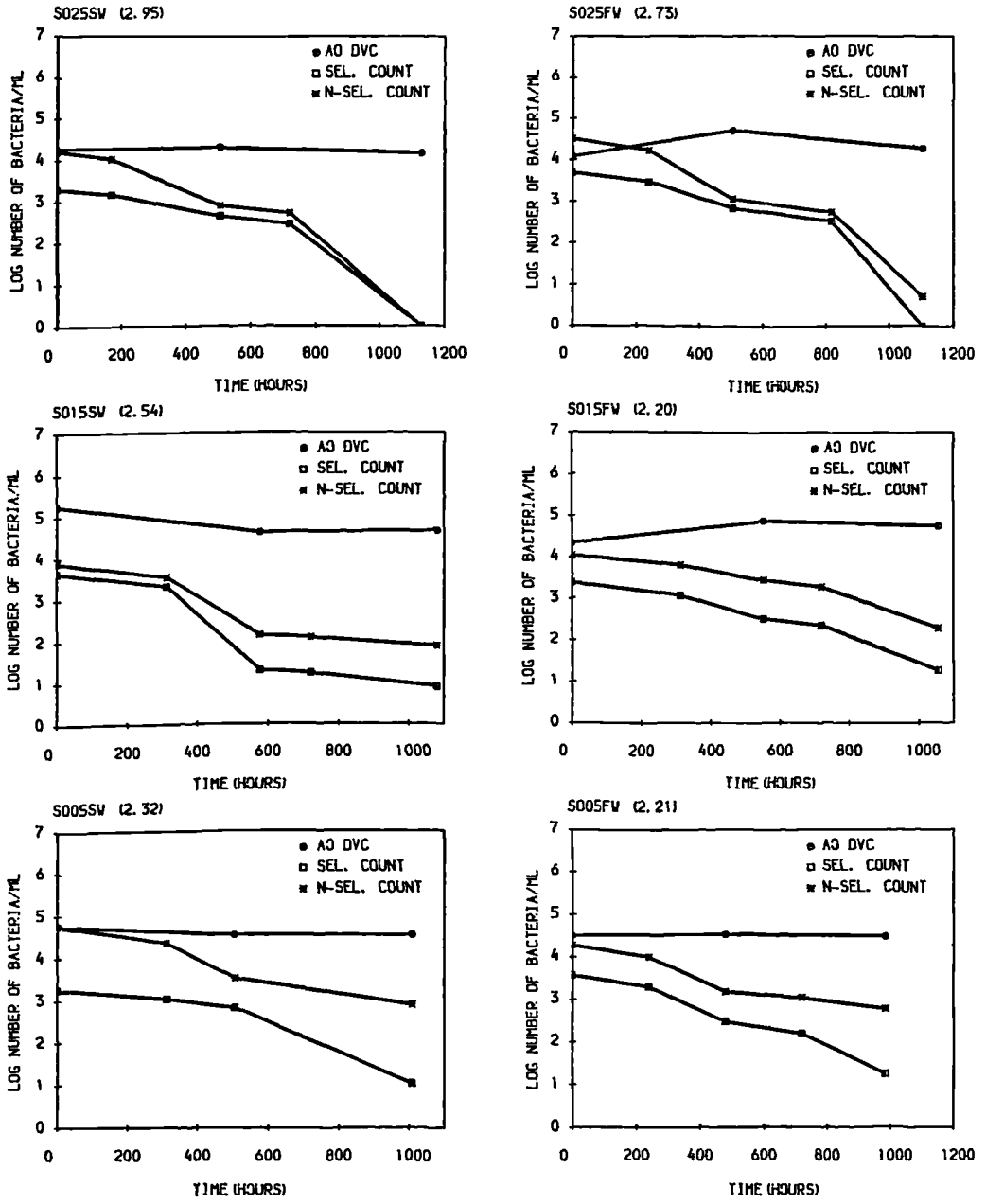


FIGURE 14. ARTIFICIAL LIGHT SOURCE EXPERIMENTS - S. ORANIENBURG DARK (2.6MJm-2)

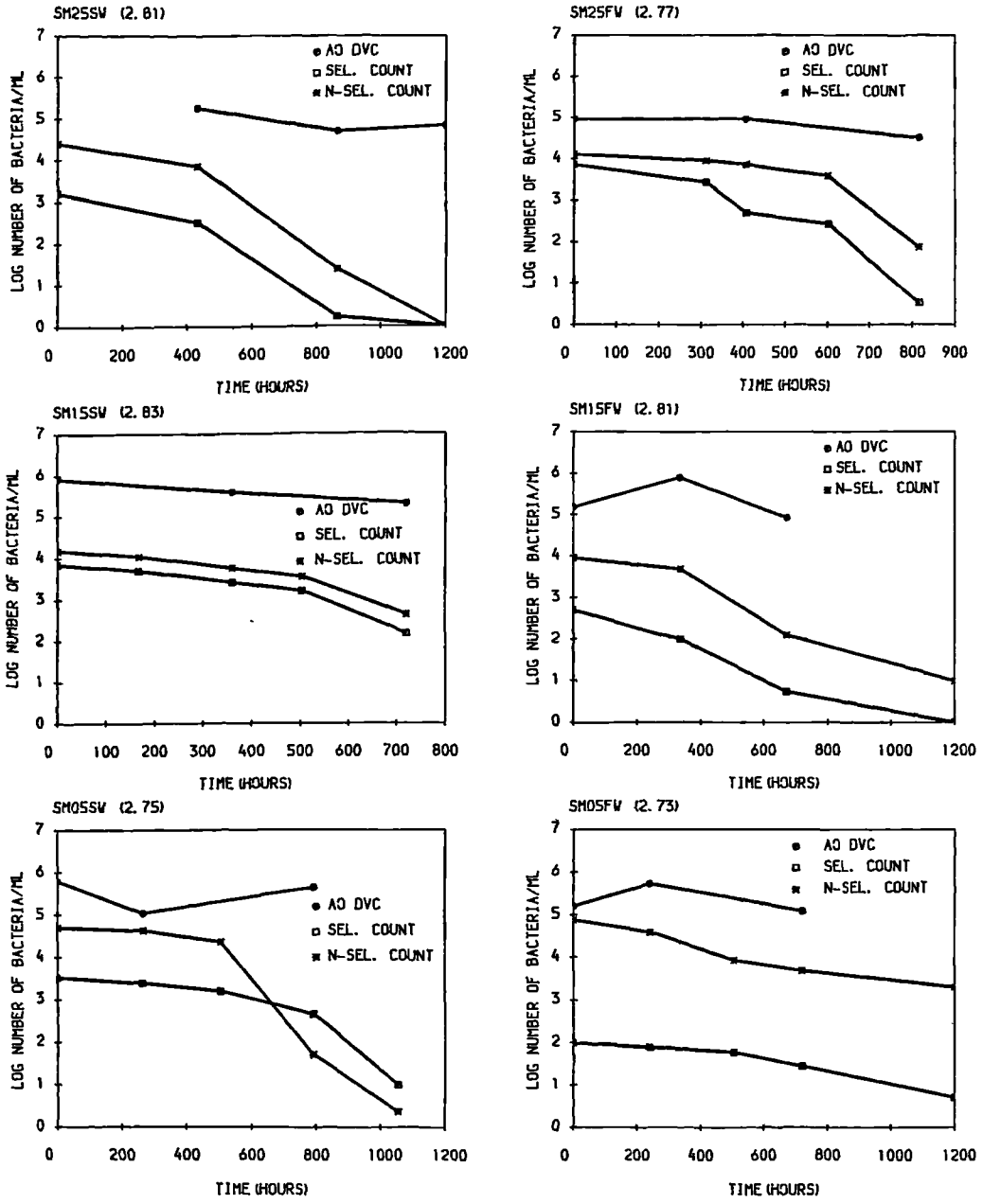


FIGURE 15. ARTIFICIAL LIGHT SOURCE EXPERIMENTS - S. MONTEVIDEO DARK (2.6MJm-2)

salinity is evident from the plots. Selective and non-selective counts for enterococci are very close together as they are for *E.coli* at higher temperatures indicating that the cells are not injured by exposure to the light source. A much greater difference exists between selective and non-selective counts for the salmonellae indicating injury of cells probably brought about by the incorporation of novobiocin as a selective agent into the medium.

Similarly, Figures 11-15 show results from some of the individual experiments in the dark (dark controls of 2.6MJm^{-1}) over a much greater time scale. Two distinct phases of decay can be seen from the culturable counts consisting of an initial slow decay followed by a rapid decline in counts. The AODVCs decline much more slowly indicating the evolution of cells towards a viable but non-culturable stage. The rate of the second decay phase appears to be influenced by temperature, the slope of the line being greatest at 25°C.

Individual light intensities (integrated over a 1 hour period) for each experiment are given in Table 8 and Appendix 5 but for ease of discussion the two levels used are described from hereon by the mean value all the light intensities measured at each level, i.e., 1.2MJm^{-1} and 2.6MJm^{-1}. Individual counts can also be found in Appendix 5.

Decay rates in the form of the T90 values of the test bacteria in seawater and in freshwater, and at

Table 8. Summary of ALS experiments (Light)

EXPT	LI	T90 (hours) AODVC	SL	NL
EC25SW	1.15	7.5	31.0	17.8
EC25SW	1.31	10.8	39.6	43.3
EC25FW	1.37	5.2	7.3	17.3
EC25FW	1.12	11.5	8.8	9.2
EC15SW	1.37	5.3	3.9	2.7
EC15SW	1.16	5.1	8.9	2.1
EC15FW	1.17	2.3	29.4	-
EC15FW	1.19	n	62.4	9.0
EC05SW	1.25	34.4	6.3	4.2
EC05SW	1.02	4.2	5.8	10.2
EC05FW	1.19	5.5	-	14.0
EC05FW	1.35	3.4	14.2	3.8
EC25SW	2.97	34.1	9.0	7.1
EC25SW	2.52	18.6	14.1	39.1
EC25FW	2.70	28.2	22.6	-
EC25FW	2.52	10.1	50.0	14.5
EC15SW	2.52	11.4	45.0	25.0
EC15SW	2.68	21.2	3.6	14.0
EC15FW	2.47	9.9	20.1	-
EC15FW	2.63	13.8	15.4	15.2
EC05SW	2.61	63.4	35.7	-
EC05SW	2.70	7.7	8.1	9.5
EC05FW	2.63	-	43.5	18.4
EC05FW	2.54	10.8	42.1	9.1
FM25SW	1.20	n	3.9	8.4
FM25FW	1.15	n	62.9	104
FM15SW	1.17	16.7	69.4	25.0
FM15FW	1.18	n	16.2	64.1
FM05SW	1.13	131	15.7	127
FM05FW	1.21	n	28.3	46.6

EXPT	LI	T90 (hours) AODVC	SL	NL
FM25SW	2.56	66.7	10.0	9.8
FM25SW	2.77	9.4	11.9	5.8
FM25FW	2.74	14.6	47.3	12.0
FM25FW	2.48	16.2	108	10.7
FM15SW	2.74	-	3.6	31.4
FM15SW	2.66	n	6.3	6.2
FM15FW	2.75	n	7.3	16.0
FM15FW	2.77	11.6	32.2	76.1
FM05SW	2.73	53.6	7.9	13.9
FM05SW	2.48	88.7	12.2	6.0
FM05FW	2.63	7.6	11.7	13.4
FM05FW	2.63	n	4.0	4.0
FS25SW	1.21	n	5.4	8.5
FS25FW	1.17	16.7	6.0	26.8
FS15SW	1.16	29.0	8.4	5.6
FS15FW	1.07	34.0	27.1	6.3
FS05SW	1.06	41.7	12.5	4.6
FS05FW	1.22	42.8	49.5	52.4
FS25SW	2.83	-	2.4	2.1
FS25SW	2.70	23.1	4.0	1.1
FS25FW	2.65	n	14.1	6.6
FS25FW	2.66	-	7.1	9.6
FS15SW	2.75	n	1.8	2.4
FS15SW	2.81	9.6	4.5	9.4
FS15FW	2.67	n	11.0	7.5
FS15FW	2.68	n	12.3	12.0
FS05SW	2.77	n	4.1	4.9
FS05SW	2.70	11.8	2.5	2.4
FS05FW	2.60	26.0	5.3	19.4
FS05FW	2.72	19.2	4.7	17.0

Table 8. continued.

SO25SW	1.12	8.6	14.9	8.7
SO25FW	1.13	16.5	83.8	n
SO15SW	1.06	27.1	8.8	n
SO15FW	1.12	12.3	n	n
SO05SW	0.97	n	n	8.2
SO05FW	1.13	10.4	8.2	72.5
SO25SW	2.52	14.9	53.8	44.7
SO25SW	2.95	10.0	23.9	34.5
SO25FW	2.30	63.1	91.6	68.0
SO25FW	2.73	20.3	14.5	20.1
SO15SW	2.88	14.7	24.3	3.2
SO15SW	2.54	31.6	6.8	-
SO15FW	2.48	15.6	32.1	58.5
SO15FW	2.20	5.3	11.9	15.8
SO05SW	2.61	7.6	29.7	50.3
SO05SW	2.32	20.8	12.8	33.5
SO05FW	2.84	-	-	25.9
SO05FW	2.21	13.3	14.7	41.8
SM25SW	1.03	51.3	268	65.4
SM25FW	1.12	28.1	61.1	n
SM15SW	1.12	52.7	26.5	38.1
SM15FW	1.17	n	n	n
SM05SW	1.04	22.3	n	n
SM05FW	1.08	24.6	n	125
SM25SW	2.81	-	15.6	65.6
SM25SW	2.52	26.2	35.4	38.6
SM25FW	2.77	6.1	26.8	58.9
SM25FW	2.81	-	26.5	23.2
SM15SW	2.83	12.6	62.4	31.1
SM15SW	3.02	17.3	45.5	28.1
SM15FW	2.81	37.9	20.6	18.7
SM15FW	2.82	10.9	45.3	45.9
SM05SW	2.75	8.7	18.0	34.9
SM05SW	2.86	19.4	25.7	67.2
SM05FW	2.73	19.6	n	120
SM05FW	2.58	8.9	44.6	15.4

LEGEND:

EC E.coli
FM S.faecium
FS S.faecalis
SO S.oranienburg
SM S.montevideo

SW Seawater
FW Freshwater

05
15 Temperature (°C)
25
LI Light Intensity (MJm^{-2})

AODVC Acridine Orange Direct Viable Count
SL Culturable count on selective media
NL Culturable count on non selective media

EXPT Experimental conditions

- Results lost in processing or not determined

n No decay

Table 9. Summary of ALS experiments (Dark Controls)

	T90 (hours)		
EXPT.	AODVC	SL	NL
EC25SW	4505	62	68
EC25SW	2237	101	99
EC25SW	1143	243	247
EC25SW	1086	173	166
EC25FW	2045	188	126
EC25FW	1595	280	361
EC25FW	885	165	173
EC15SW	6098	368	234
EC15SW	3195	224	327
EC15SW	617	345	347
EC15FW	935	228	465
EC15FW	1192	503	397
EC15FW	877	382	444
EC05SW	n	470	625
EC05SW	3021	360	376
EC05SW	1053	433	546
EC05FW	n	676	847
EC05FW	1590	633	641
EC05FW	752	699	769
FM25SW	3571	450	415
FM25SW	3571	689	862
FM25SW	7519	249	461
FM25FW	2232	352	366
FM25FW	1603	202	163
FM25FW	6667	131	144
FM15SW	17857	752	575
FM15SW	-	820	-
FM15SW	4464	769	725
FM15FW	4405	568	336
FM15FW	1441	787	794
FM15FW	4132	781	769
FM05SW	4739	1198	869
FM05SW	4525	1468	1669
FM05SW	4202	1351	2268
FM05FW	-	-	-
FM05FW	5952	1387	649
FM05FW	12500	1314	2494

	T90 (hours)		
EXPT.	AODVC	SL	NL
FS25SW	n	426	-
FS25SW	-	552	418
FS25SW	1119	338	355
FS25FW	2632	89	77
FS25FW	20408	385	344
FS25FW	-	372	355
FS15SW	n	561	730
FS15SW	3012	420	362
FS15SW	1577	474	840
FS15FW	n	257	521
FS15FW	4149	575	360
FS15FW	-	346	599
FS05SW	n	1764	1538
FS05SW	2688	1316	1088
FS05SW	-	592	2500
FS05FW	n	478	621
FS05FW	3086	1739	2092
FS05FW	1012	787	1727
SO25SW	4464	182	188
SO25SW	1866	239	235
SO25SW	17543	299	266
SO25FW	2841	230	365
SO25FW	-	287	262
SO25FW	158	272	291
SO15SW	n	265	214
SO15SW	n	322	571
SO15SW	1751	386	526
SO15FW	1718	352	330
SO15FW	2985	448	426
SO15FW	n	505	610
SO05SW	n	588	521
SO05SW	990	532	543
SO05SW	4587	455	353
SO05FW	11236	459	389
SO05FW	34483	613	585
SO05FW	-	426	667

Table 9. continued.

SM25SW	n	300	256
SM25SW	1855	202	189
SM25SW	1706	272	219
SM25FW	1757	613	575
SM25FW	4505	297	221
SM25FW	1838	245	308
SM15SW	730	556	649
SM15SW	1166	429	379
SM15SW	-	-	-
SM15FW	5263	503	633
SM15FW	5025	488	398
SM15FW	2703	316	379
SM05SW	n	617	667
SM05SW	4065	450	495
SM05SW	n	459	595
SM05FW	840	529	485
SM05FW	4184	980	917
SM05FW	3436	719	752

see legend for Table 8.

different temperatures, for 96 individual experiments, are summarised in Table 8. Similarly, Table 9 shows the decay rates of 91 dark controls of light experiments. The decay rate constants for both light and dark data were found to be log-normally distributed.

R-sq values for the linear least squares regression lines for computating k values were between 50 and 100% for light and dark experiments.

Table 10. Analysis of variance in decay rates obtained using different enumeration methods (ALS).

		AODVC vs SL			AODVC vs NL			SL vs NL		
	LI	F	DF		F	DF		F	DF	
EC	1.2	3.03	(1,21)	N	0.15	(1,21)	N	1.76	(1,21)	N
EC	2.6	2.80	(1,22)	N	0.04	(1,21)	N	2.50	(1,21)	N
EC	Dark	21.08	(1,36)	S	14.39	(1,36)	S	2.07	(1,36)	N
FM*	1.2	6.04	(1,10)	S	2.69	(1,10)	N	0.96	(1,10)	N
FM	2.6	9.66	(1,21)	S	8.68	(1,21)	S	0.34	(1,21)	N
FM^	Dark	13.91	(1,31)	S	16.64	(1,30)	S	0.61	(1,31)	N
FS*	1.2	6.45	(1,10)	S	7.81	(1,10)	S	0.16	(1,10)	N
FS	2.6	17.85	(1,20)	S	11.89	(1,20)	S	0.43	(1,20)	N
FS^	Dark	13.79	(1,32)	S	7.02	(1,31)	S	0.12	(1,32)	N
SO	1.2	0.04	(1,10)	N	0.07	(1,10)	N	0.04	(1,10)	N
SO	2.6	1.14	(1,21)	N	0.09	(1,21)	N	0.00	(1,21)	N
SO	Dark	30.20	(1,33)	S	26.04	(1,33)	S	0.08	(1,33)	N
SM	1.2	5.14	(1,10)	S	4.60	(1,10)	N	0.53	(1,10)	N
SM	2.6	4.08	(1,18)	N	3.31	(1,17)	N	0.80	(1,18)	N
SM	Dark	18.06	(1,32)	S	38.02	(1,32)	S	0.60	(1,32)	N

Legend:
S significant difference at 95% confidence level
N no significant difference at 95% confidence level
LI mean light intensity for set of experiments (MJm^{-2})
DF degrees of freedom
F computed F-ratio
* red cells counted
^ red cells count for $1.2MJm^{-2}$ dark control

One way analysis of variance was performed on the decay rate constants of light and dark data to investigate the differences between decay rates estimated using the AODVC and decay rates estimated using cultural methods. The decay rate constant was used in preference to the T90 value in this analysis because positive k values could then be used to represent those instances where growth rather than decay had occurred. The computed F-ratios for the analysis are shown in Table 10. *These were tested for* significance by comparing them with tabulated F-ratios at the 95% confidence level as given by White et al. (1985).

Analysis of variance indicated that there are no significant differences between decay rate constants estimated using selective counts and those using non-selective counts under any of the test conditions (see Table 10). Decay rates (k) estimated using AODVC were, however, *significantly higher than those using* culturable counts, but only in the dark for the Gram negative species, i.e., *E.coli*, *S.oranienburg*, and *S.montevideo*. For *S.montevideo* at the lower intensity ($1.2MJm^{-2}$) a significant difference was observed between decay rates (k) estimated by AODVC and by selective culturable counts, in the light, which is not in accordance with the observations for the other Gram negative bacteria. This observed difference was probably due to the small number of data available for this particular analysis. The

computed F-ratio at 1 and 10 degrees of freedom was only slightly higher than the tabulated F-ratio (4.96 (1,10)).

For enterococci, decay rates estimated using AODVC are significantly higher than those using culturable counts in both light and dark experiments.

Analysis of variance was also carried out on the decay rate constants for the two light intensity levels. A significant difference between decay rates at the 1.2MJm^{-1} level and at the 2.6MJm^{-1} level was found for *E.coli*, *S.faecalis* and *S.montevideo* (see Table 11).

Table 11. Analysis of variance in decay rates at two intensity levels.

1.2MJm^{-1} vs 2.6MJm^{-1}			
	F	DF	
EC	*12.97*	*(1,67)*	*S*
FM	0.09	(1,46)	N
FS	4.52	(1,50)	S
SO	0.58	(1,51)	N
SM	9.25	(1,45)	S

Legend: see Table 10.

E.coli and *S.faecalis* appear to decay more rapidly at 1.2MJm^{-1} whereas *S.montevideo* appears to decay more rapidly at 2.6MJm^{-1}. However, there is no clear relationship between decay rate and the two intensity levels used in these experiments, and correlation analysis indicates that there are no significant associations between decay rate and light intensity except, perhaps, for *S.montevideo*.

TABLE 12. CORRELATION BETWEEN DECAY AND ENVIRONMENTAL PARAMETERS FOR ARTIFICIAL LIGHT SOURCE EXPERIMENTS.

	DECAY RATE CONSTANT (K)														
	E.COLI			S.FAECIUM			S.FAECALIS			S.ORANIENBURG			S.MONTEVIDEO		
	AODVC	SEL.	N.SEL.	AODVC	SEL.	N.SEL.	AODVC	SEL.	N.SEL.	AODVC	SEL.	N.SEL.	AODVC	SEL.	N.SEL.
TEMPERATURE (LIGHT)	0.239	0.001	0.225	-0.443	0.048	-0.012	0.328	0.100	-0.122	0.211	0.179	-0.105	-0.026	-0.414	0.217
	(24)	(23)	(22)	(17)	(18)	(18)	(16)	(18)	(18)	(17)	(18)	(18)	(15)	(17)	(16)
TEMPERATURE (DARK)	-0.253	-0.644*	-0.541*	-0.320	-0.708*	-0.625*	0.017	-0.472*	-0.556*	-0.326	-0.829*	-0.668*	-0.002	-0.020	-0.778*
	(19)	(19)	(19)	(16)	(17)	(16)	(16)	(18)	(17)	(17)	(18)	(18)	(17)	(17)	(17)
SALINITY (LIGHT)	0.020	-0.408	-0.356	0.062	-0.104	-0.218	0.001	-0.364	-0.284	0.283	-0.025	-0.335	-0.097	-0.272	-0.001
	(24)	(23)	(22)	(17)	(18)	(18)	(16)	(18)	(18)	(17)	(18)	(18)	(15)	(17)	(16)
SALINITY (DARK)	-0.050	-0.314	-0.193	0.359	0.293	0.438	-0.155	0.392	0.321	0.252	-0.194	-0.271	0.126	0.130	-0.236
	(19)	(19)	(19)	(16)	(17)	(16)	(16)	(18)	(17)	(17)	(18)	(18)	(17)	(17)	(17)
LIGHT INTENSITY (1)	-0.220	-0.562*	-0.274	-0.477*	-0.639*	-0.721*	-0.055	-0.762*	-0.662*	-0.648*	-0.575*	-0.496*	-0.680*	-0.402*	-0.680*
	(43)	(42)	(41)	(33)	(35)	(34)	(32)	(36)	(35)	(32)	(36)	(35)	(32)	(34)	(33)
LIGHT INTENSITY (2)	0.597*	-0.000	-0.397	-0.124	-0.079	-0.453	0.068	-0.472*	-0.367	-0.067	0.199	-0.220	-0.409	-0.539*	-0.651*
	(24)	(23)	(22)	(17)	(18)	(18)	(16)	(18)	(18)	(16)	(18)	(18)	(15)	(17)	(16)

(1) includes dark readings (as zero intensity)

(2) does not include dark readings

* Significant association at 95% confidence level

numbers in parentheses below coefficients - numbers of pairs

The ALS data was analysed for the simple product moment correlation coefficient using the decay rate constants, to test for associations between the decay rates and 1) temperature, 2) salinity, 3) light intensity. The correlation coefficients (r) were tested for significance at the 95% confidence level by comparing the computed r values with the tabulated critical r values at n-2 degrees of freedom as given by White et al. (1985), and are summarised in Table 12.

The correlation analysis of the data shows that there is:

1) no evidence of a significant association between decay rate and either temperature or salinity in ALS light experiments,

2) no evidence of a significant association between decay rate and salinity in the dark,

3) no evidence of a significant association between decay and light intensity at the two different intensity levels,

4) a significant negative correlation between decay rate estimated using culturable counts and temperature in the dark,

5) in general, a significant negative correlation between decay rate and light intensity when data at zero intensity (dark controls) are used in the analysis.

It appears from the results of the correlation analysis that the effect of sunlight on the decay rate

overrides the effects of both temperature and salinity in light experiments as these factors are poorly correlated with decay, and accordingly, temperature appears to be the dominant factor in the dark. Based on these assumptions, the relative order of importance of these factors from most important to least important, as bactericidal agents in natural waters may be:

light - temperature - salinity

However, a significant correlation may result from the combined influence of several factors.

A multiple regression analysis was carried out on the decay rate constants from light and dark experiments to evaluate the combined effect of temperature, salinity and light intensity on decay rates of bacteria exposed to the ALS (not shown). The R-sq values (mostly below 50%) for the regression lines indicated a poor fit of the data to the line, supporting the idea of the influence of a dominant factor rather than a concerted effect from all of the factors, both in the light and in the dark. There is no evidence of a significant difference between R-sq values for light experiments and R-sq values for dark experiments.

That some species survive better than others in the light was shown by analysis of variance of decay rates for different species (AODVC $F=6.27(4,84)$, SL $F=9.39(4,84)$, NL $F=7.05(4,87)$) whereas survival in the dark appeared not to vary significantly between species.

5.2.4. Discussion

The results of analysis of variance on decay rates measured by different enumeration methods indicate that the survival characteristics of enterococci under low nutrient conditions and in the light may be different from those exhibited by Gram negative bacteria. However, one must also consider that a true AODVC for enterococci has not yet been developed and that * and ^ (Table 10) indicate an estimation of viable cells which was made merely by counting the number of red cells stained by acridine orange. The use of ciprofloxacin to give an AODVC of enterococci (not indicated by * or ^) is detailed in Section 5.6. Interestingly, the growth of enterococci in filtered settled sewage was weak compared to the growth of the other test organisms.

In dark experiments, the significantly lower decay rates (k) obtained using the AODVC compared with decay rates (k) obtained using culturable methods suggest the evolution of Gram negative bacteria towards a viable but non-culturable form. As culturability and viability decrease at a similar rate in the light for Gram negative bacteria it is supposed that the ultimated fate of these bacteria is death though prolonged survival is observed compared to previous studies indicating initial adaptation to low nutrient conditions. The rate of decay in artificial light does not appear to be influenced by temperature or salinity. That the picture concerning the

effect of different light intensities on decay rates is unclear is probably a result of the two chosen intensities being not sufficiently different to produce a different effect.

5.3. Natural sunlight experiments

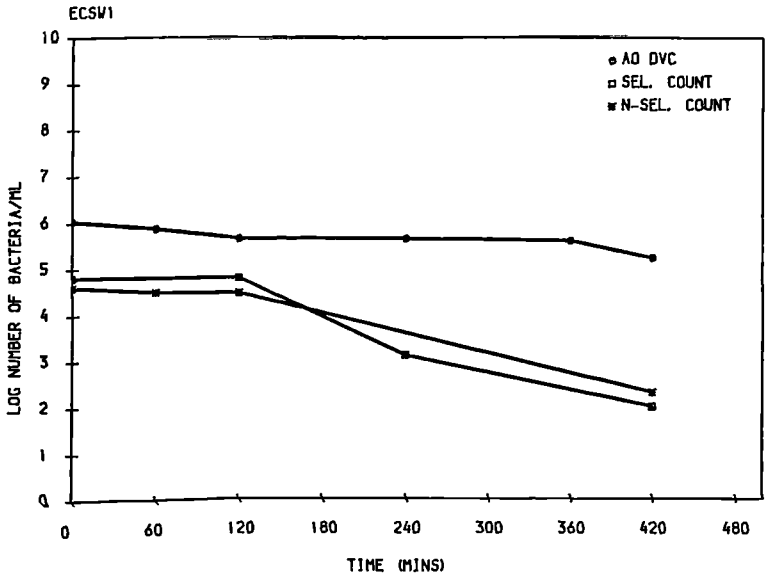
5.3.1. Effect of temperature and salinty on survival of bacteria in natural sunlight

5.3.1.1. Aims

To compare the bactericidal effect of light from the ALS and natural sunlight the five species used in the ALS experiments were employed for two series of experiments. In addition, *S.newport* and *S.typhimurium* were exposed to natural sunlight. A comparison of decay of selected bacteria exposed to natural sunlight in water of two different salinities was carried out in the first series. In the second series, the effect of temperature on the decay of the selected bacteria when exposed to natural sunlight was evaluated.

5.3.1.2. Experimental procedure

The first series was carried out during the summer months of 1988 with no temperature regulation. *E.coli*, *S.faecalis*, *S.faecium*, *S.typhimurium*, and *S.newport* were grown in filtered, sterile settled sewage and each species was exposed concurrently in seawater and freshwater beaker microcosms, to natural sunlight, on a matt black board.



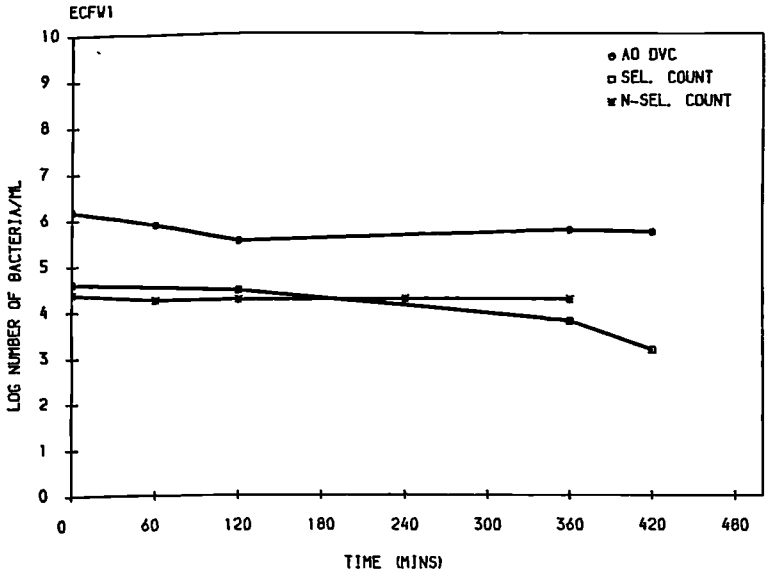
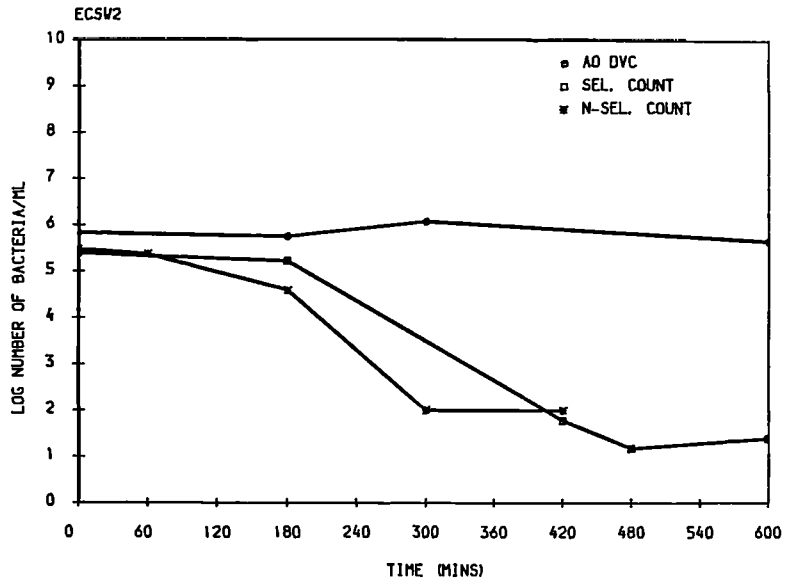


FIGURE 16. SURVIVAL OF E.COLI EXPOSED TO NATURAL SUNLIGHT IN SEAWATER AND FRESHWATER A



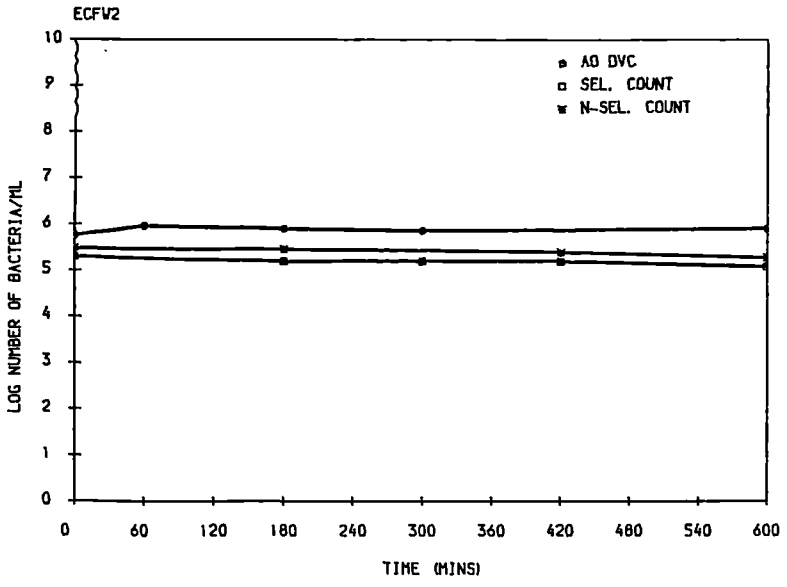
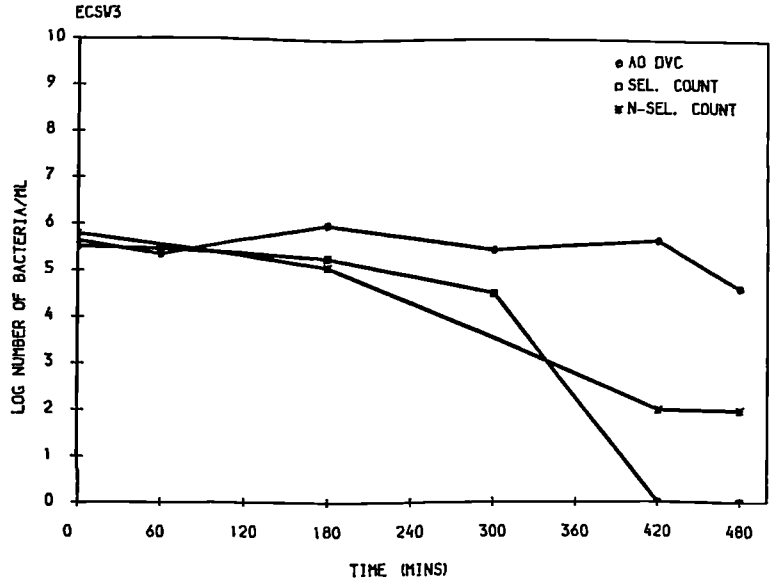


FIGURE 17. SURVIVAL OF E.COLI EXPOSED TO NATURAL SUNLIGHT IN SEAWATER AND FRESHWATER B



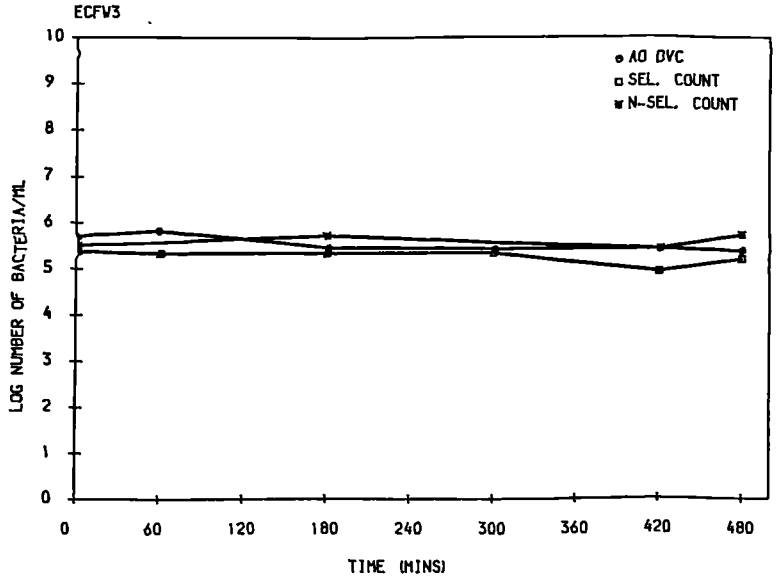
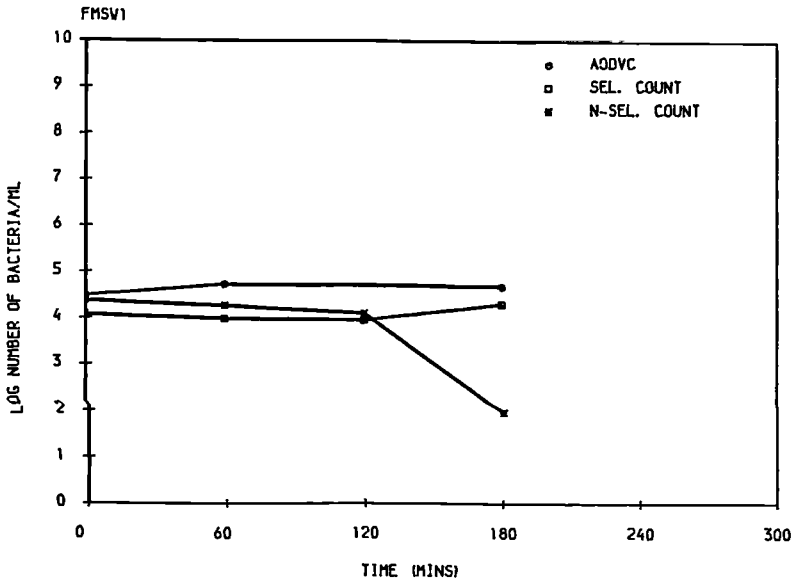


FIGURE 18. SURVIVAL OF E.COLI EXPOSED TO NATURAL SUNLIGHT IN SEAWATER AND FRESHWATER C



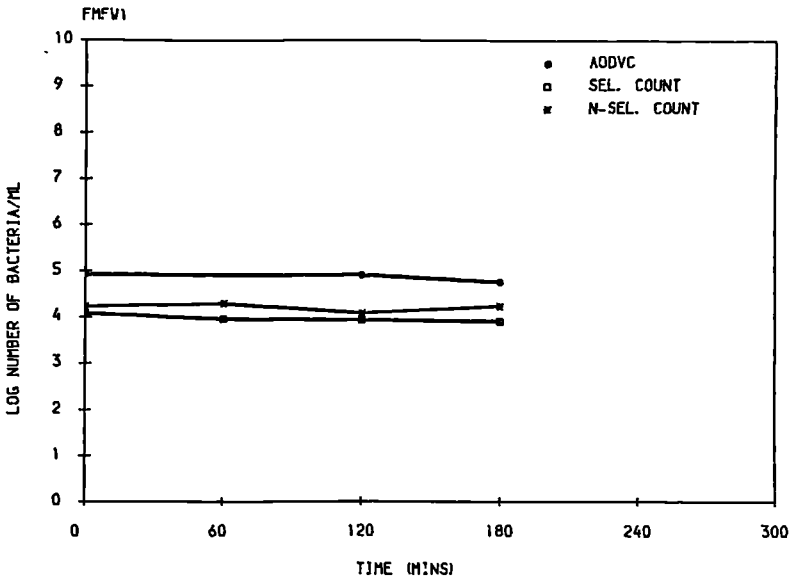
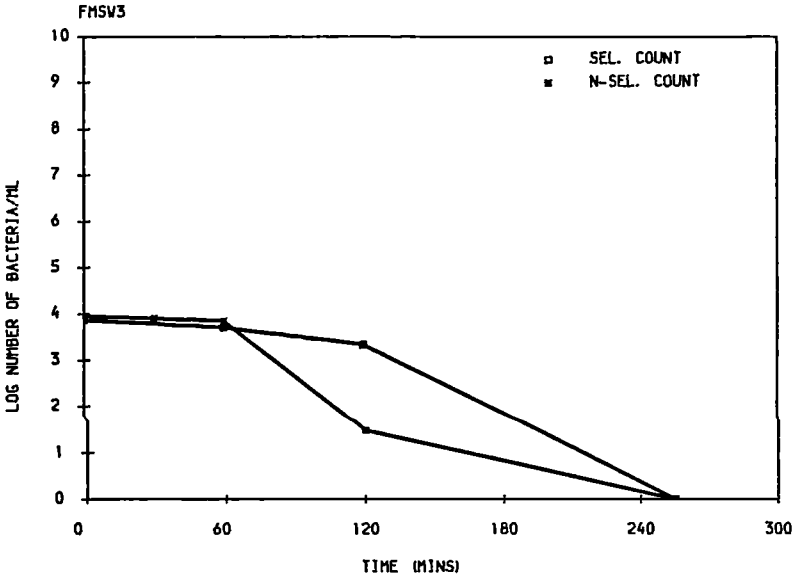


FIGURE 19. SURVIVAL OF S. FAECIUM EXPOSED TO NATURAL SUNLIGHT IN SEAWATER AND FRESHWATER A



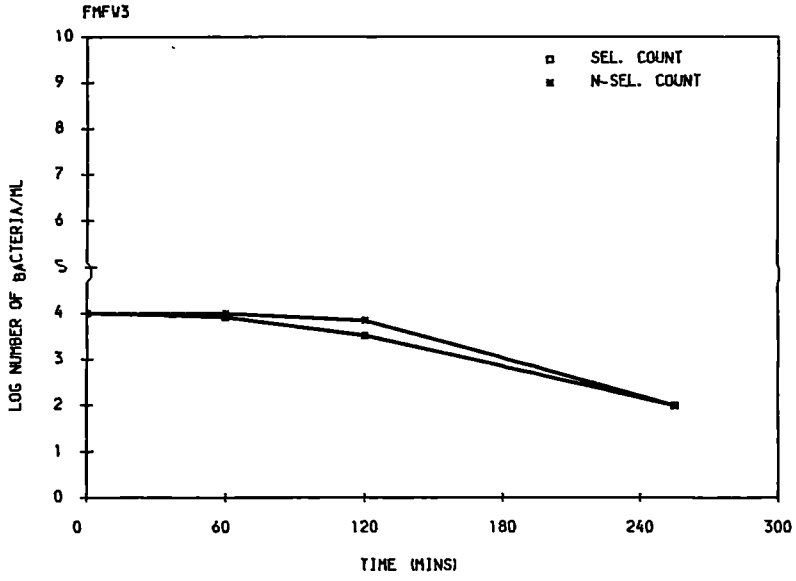
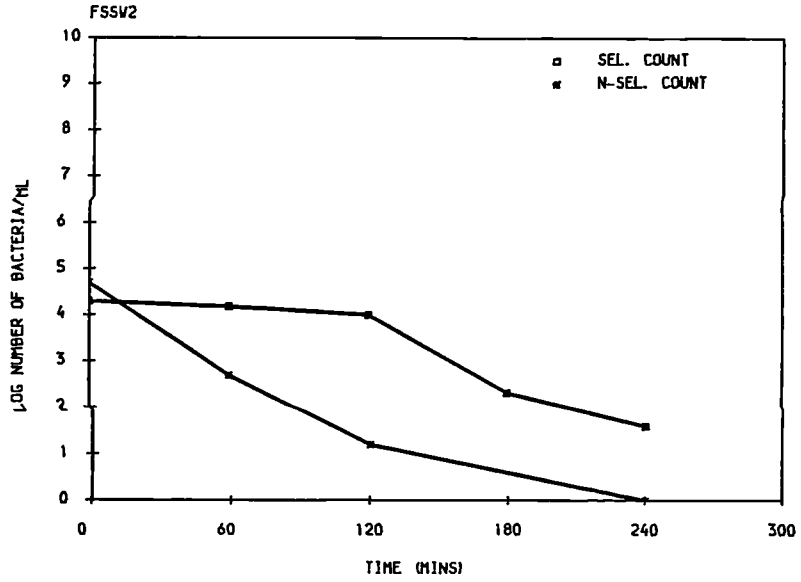


FIGURE 20. SURVIVAL OF S. FAECIUM EXPOSED TO NATURAL SUNLIGHT IN SEAWATER AND FRESHWATER B



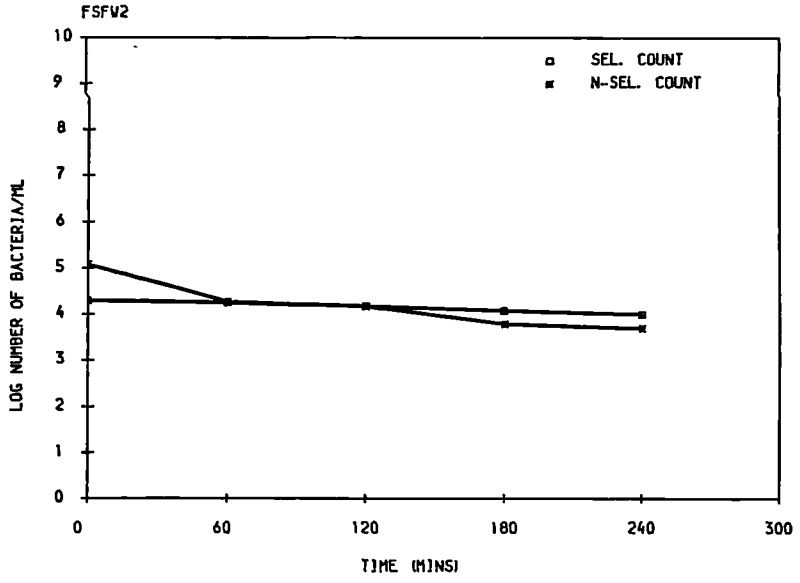
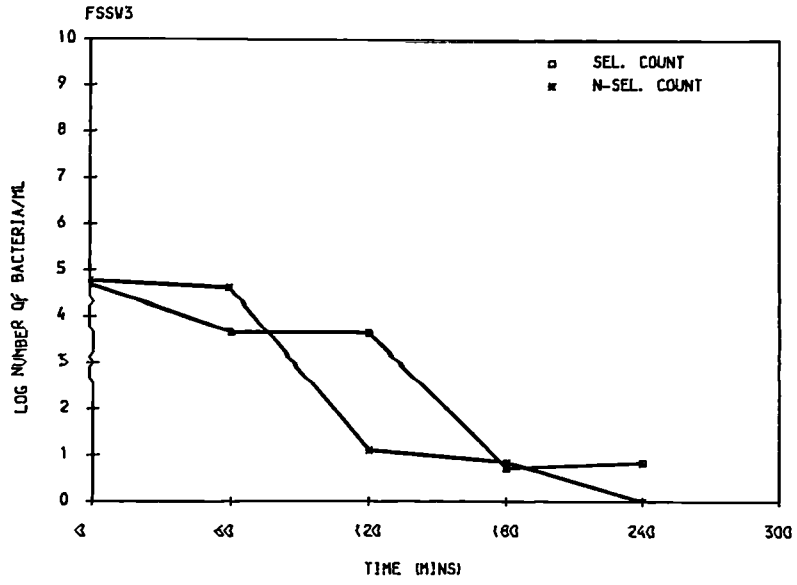


FIGURE 21. SURVIVAL OF S. FAECALIS EXPOSED TO NATURAL SUNLIGHT IN SEAWATER AND FRESHWATER A



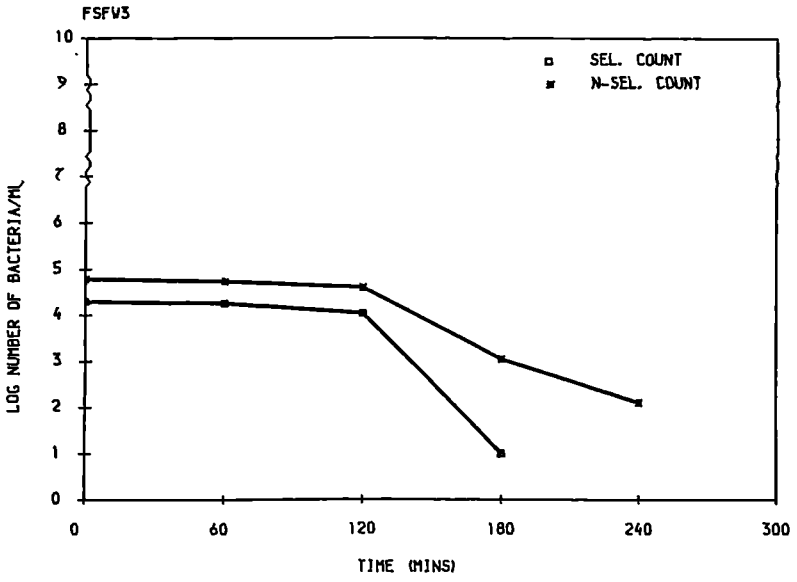
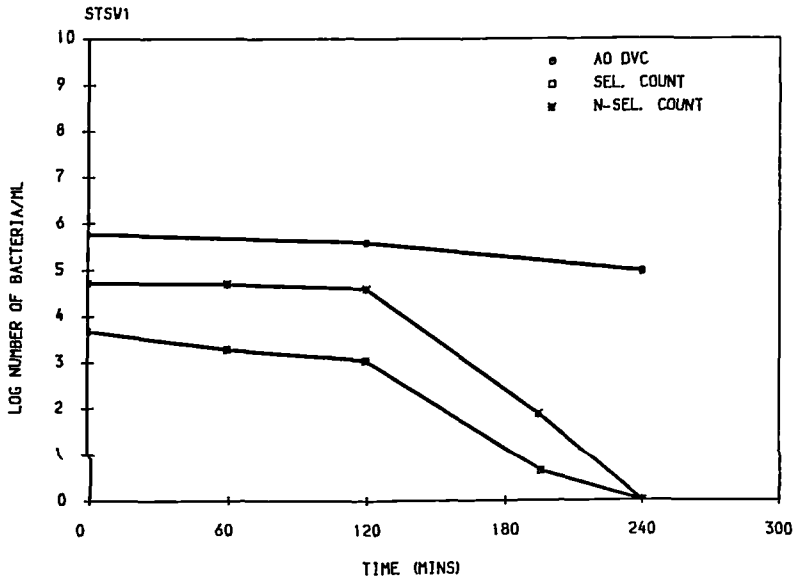


FIGURE 22. SURVIVAL OF S. FAECALIS EXPOSED TO NATURAL SUNLIGHT IN SEAWATER AND FRESHWATER B



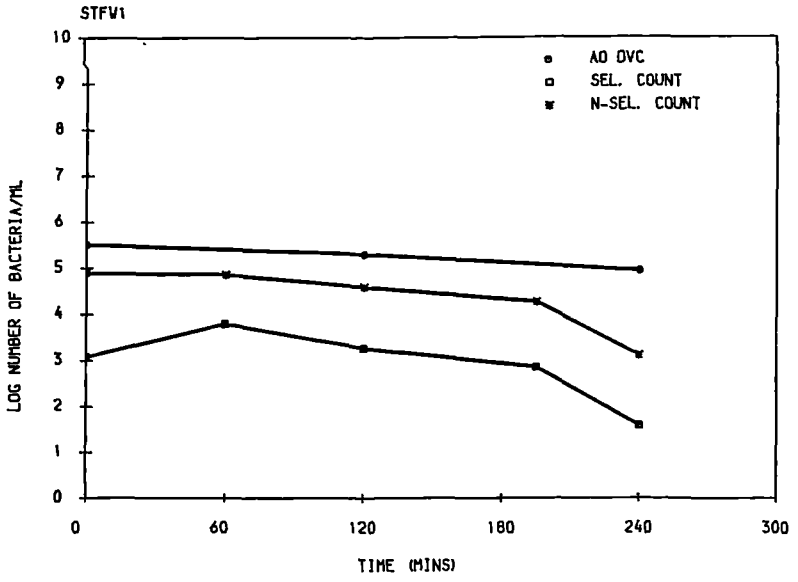
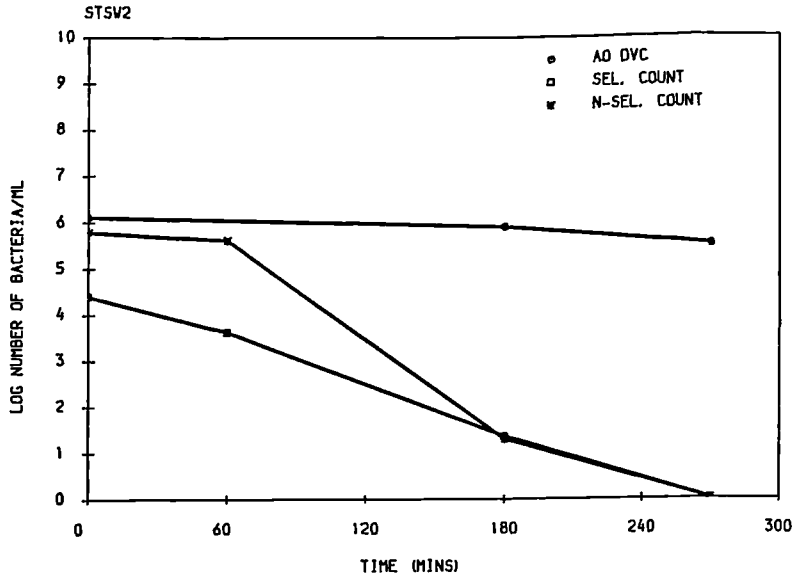


FIGURE 23. SURVIVAL OF S. TYPHIMURIUM EXPOSED TO NATURAL SUNLIGHT IN SEAWATER AND FRESHWATER A



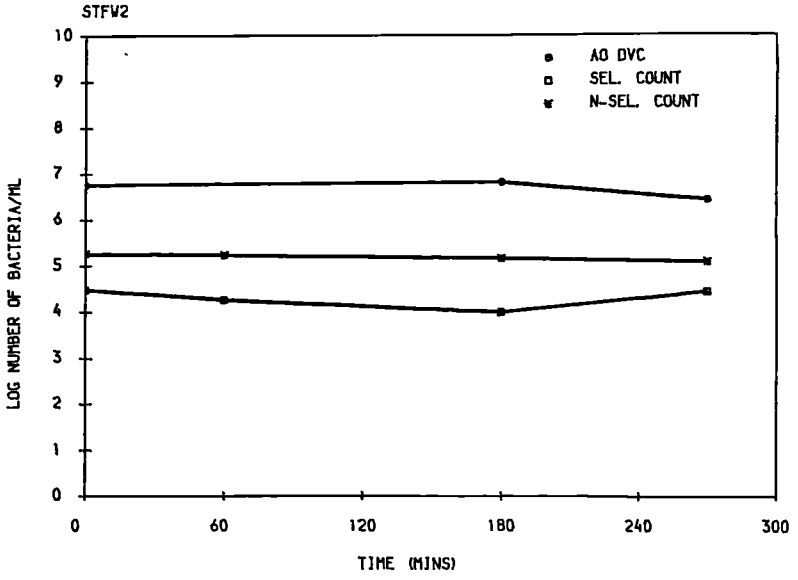


FIGURE 24. SURVIVAL OF S. TYPHIMURIUM EXPOSED TO NATURAL SUNLIGHT IN SEAWATER AND FRESHWATER B

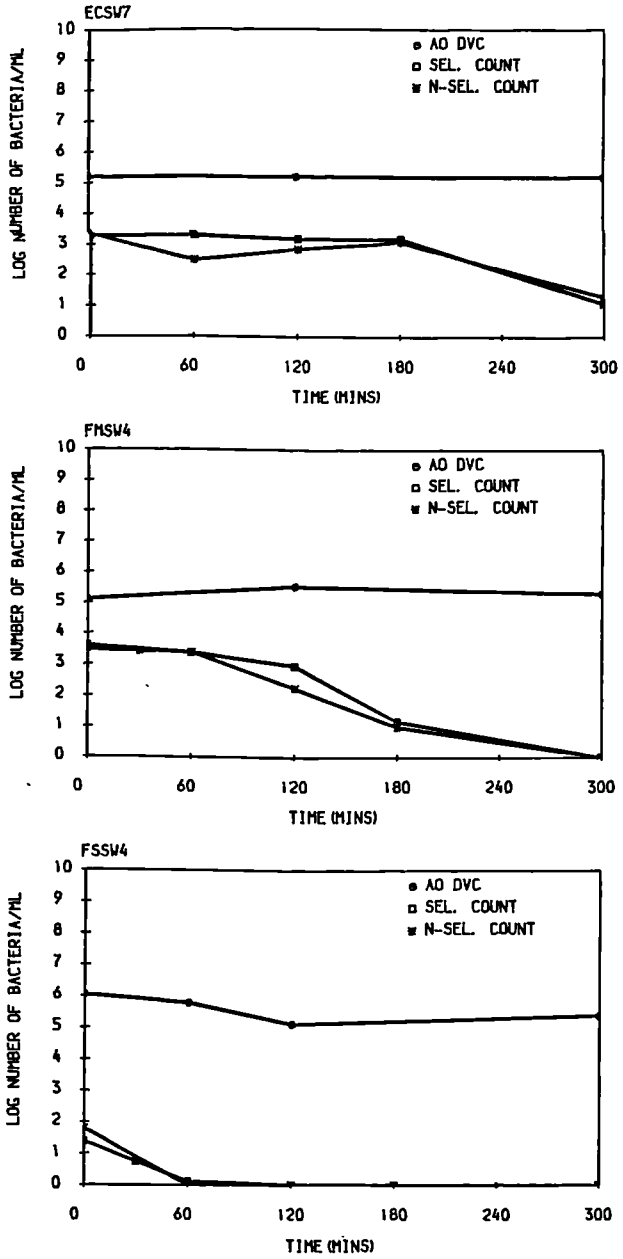


FIGURE 25. COMPARATIVE SURVIVAL OF BACTERIA EXPOSED TO NATURAL SUNLIGHT IN SEAWATER

The second series of experiments were carried out during the summer of 1989, in which the effect of regulating the temperature of the microcosms at 15°C compared with no temperature regulation, was examined. *E.coli*, *S.faecium*, *S.faecalis*, *S.montevideo*, and *S.oranienburg* were grown in sterile, filtered settled sewage and exposed concurrently, either in seawater or freshwater beaker microcosms in a temperature-regulated water bath or as a control, with no temperature regulation, to natural sunlight.

5.3.1.3. Results

Figures 16-25 show results of some of the individual 1988 experiments represented graphically. These clearly show that decay in seawater is more rapid than in freshwater for all species tested. Non-selective counts for *E.coli* and *S.typhimurium* are close to selective counts in seawater indicating the lack of injury. AODVCs and culturable counts are close together in freshwater indicating very little stress.

Table 13 is a summary of the T_{90} values obtained when the test bacteria were exposed to natural sunlight in the summer of 1988. Each pair or threesome of experiments indicate that these were results from microcosms exposed simultaneously, and therefore, under the same conditions. Light intensity (integrated over a 30 minute period and expressed as the mean of all the readings taken) and temperature measurements for each experiment can be found along with the individual counts in Appendix 6. A

significant difference was found in light intensities measured on different days (F=10.63 (8,29)) in 1988. Intensities measured in 1988 were significantly lower (F=9.64 (1,128)) than intensities measured in 1989.

Figures 26-29 show the results of some of the individual 1989 experiments represented graphically.

Table 13. T90 values from natural sunlight experiments - temperature not regulated (Summer 1988).

	T90 (hours)				T90 (hours)		
EXPT.	AODVC	SL	NL	EXPT.	AODVC	SL	NL
ECSW1	12.6	2.3	2.9	STSW1	5.8	1.2	0.9
ECFW1	19.2	5.2	71.5	STFW1	8.8	3.2	3.1
ECSW2	4.1	2.0	1.7	STSW2	7.9	0.8	0.6
ECFW2	n	52.2	52.0	STFW2	17.0	41.6	25.8
ECSW3	14.5	5.1	1.9	ECSW6	13.8	4.0	15.7
ECFW3	20.0	26.0	n	SNSW1	7.4	23.5	28.3
ECSW4	39.5	9.6	2.6	ECSW7	n	2.4	3.1
ECFW4	n	29.0	22.0	FMSW4*	n	3.2	12.6
				FSSW4*	8.7	0.8	0.7
ECSW5	8.8	1.7	2.3				
ECFW5	13.1	38.6	n	ECSW8	37.7	11.3	13.9
				FSSW5	-	1.9	0.9
FMSW1*	n	n	1.4	FSSW6^	-	0.7	0.7
FMFW1*	20.4	16.7	45.0				
FMSW2	-	1.6	1.9				
FMFW2	-	4.7	5.9				
FMSW3	-	3.8	0.8				
FMFW3	-	2.0	2.0				
FSSW1	-	0.9	2.7				
FSFW1	-	12.9	n				
FSSW2	-	8.0	1.3				
FSFW2	-	16.0	2.2				
FSSW3	-	0.9	0.8				
FSFW3	-	0.8	1.2				

Legend:
n no decay
FS^ S.faecalis NCTC 775
EC E.coli
FM S.faecium
FS S.faecalis
ST S.typhimurium
SN S.newport
SW seawater
FW freshwater
* red cells counted

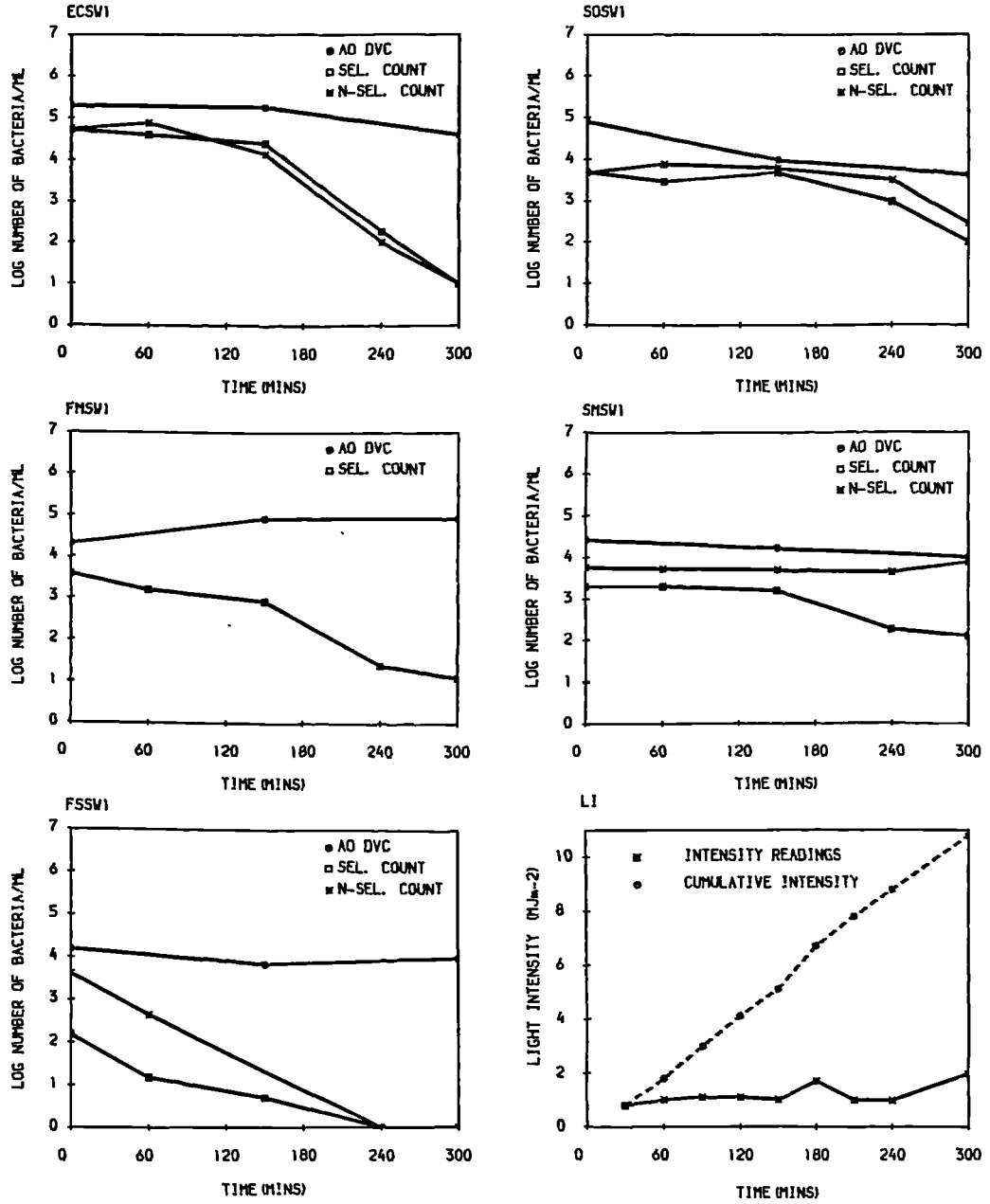


FIGURE 26. SURVIVAL OF BACTERIA EXPOSED TO NATURAL SUNLIGHT IN SEAWATER - TEMPERATURE UNREGULATED

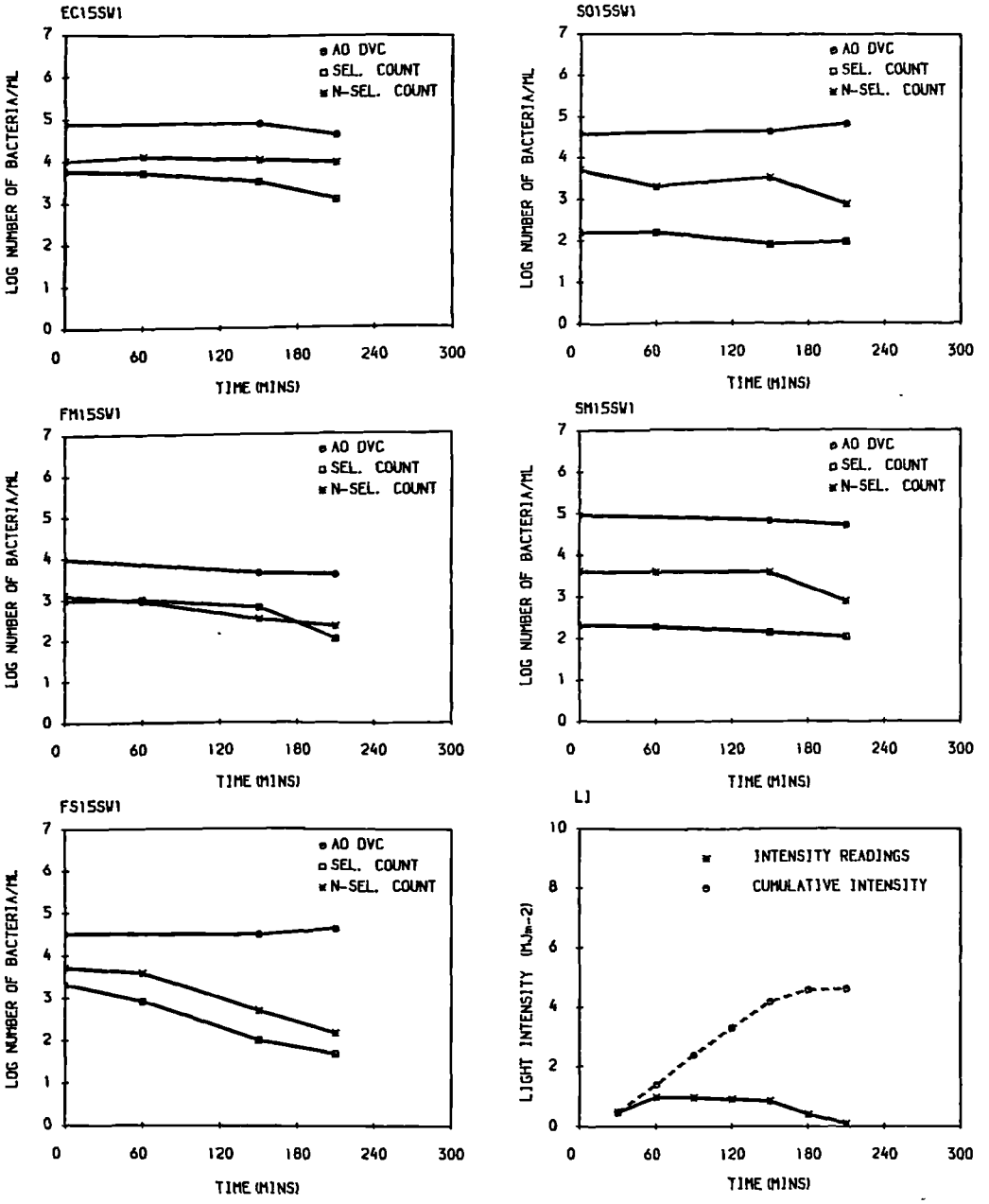


FIGURE 27. SURVIVAL OF BACTERIA EXPOSED TO NATURAL SUNLIGHT IN SEAWATER - TEMPERATURE REGULATED AT 15 C

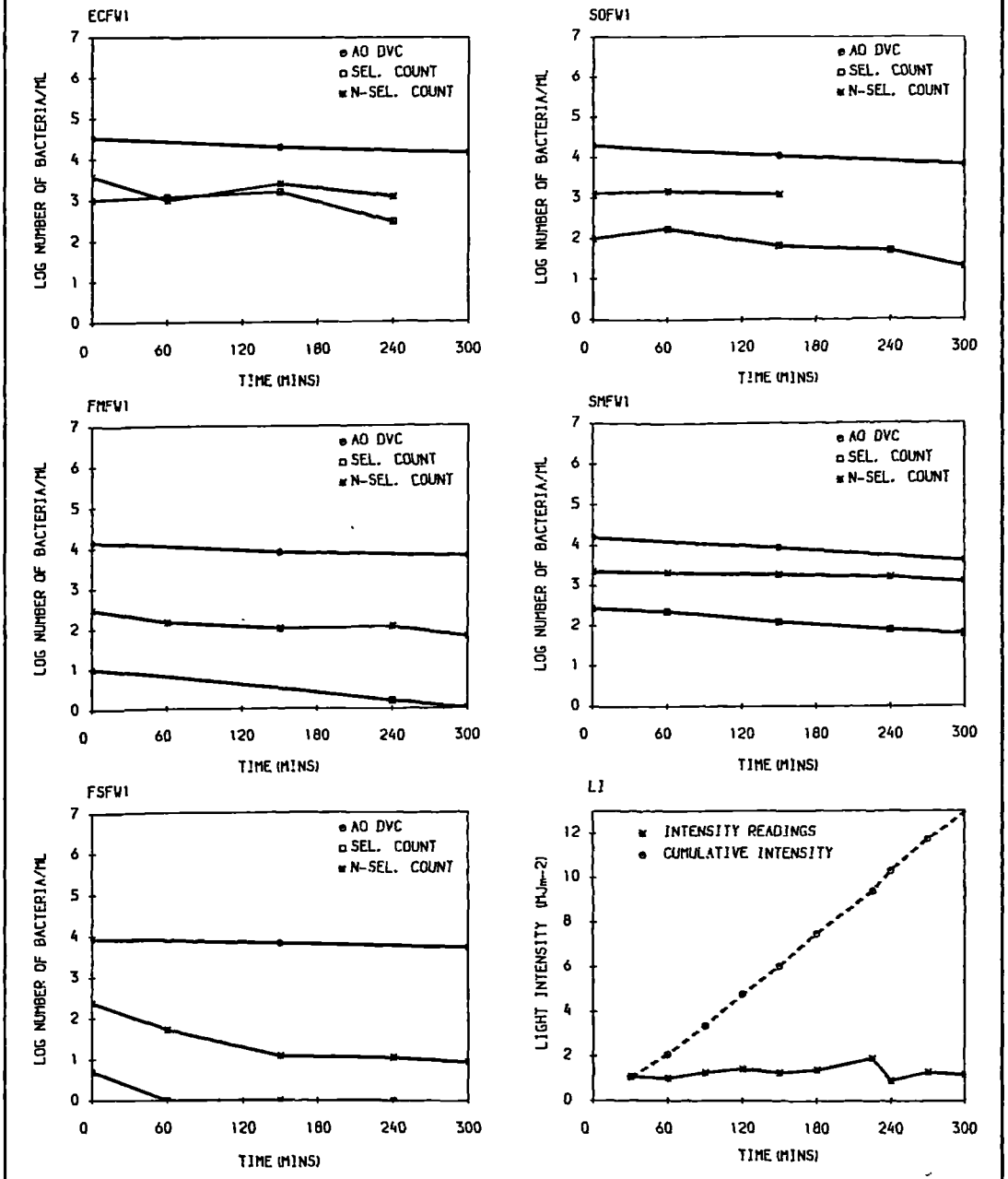


FIGURE 28. SURVIVAL OF BACTERIA EXPOSED TO NATURAL SUNLIGHT IN FRESHWATER - TEMPERATURE UNREGULATED

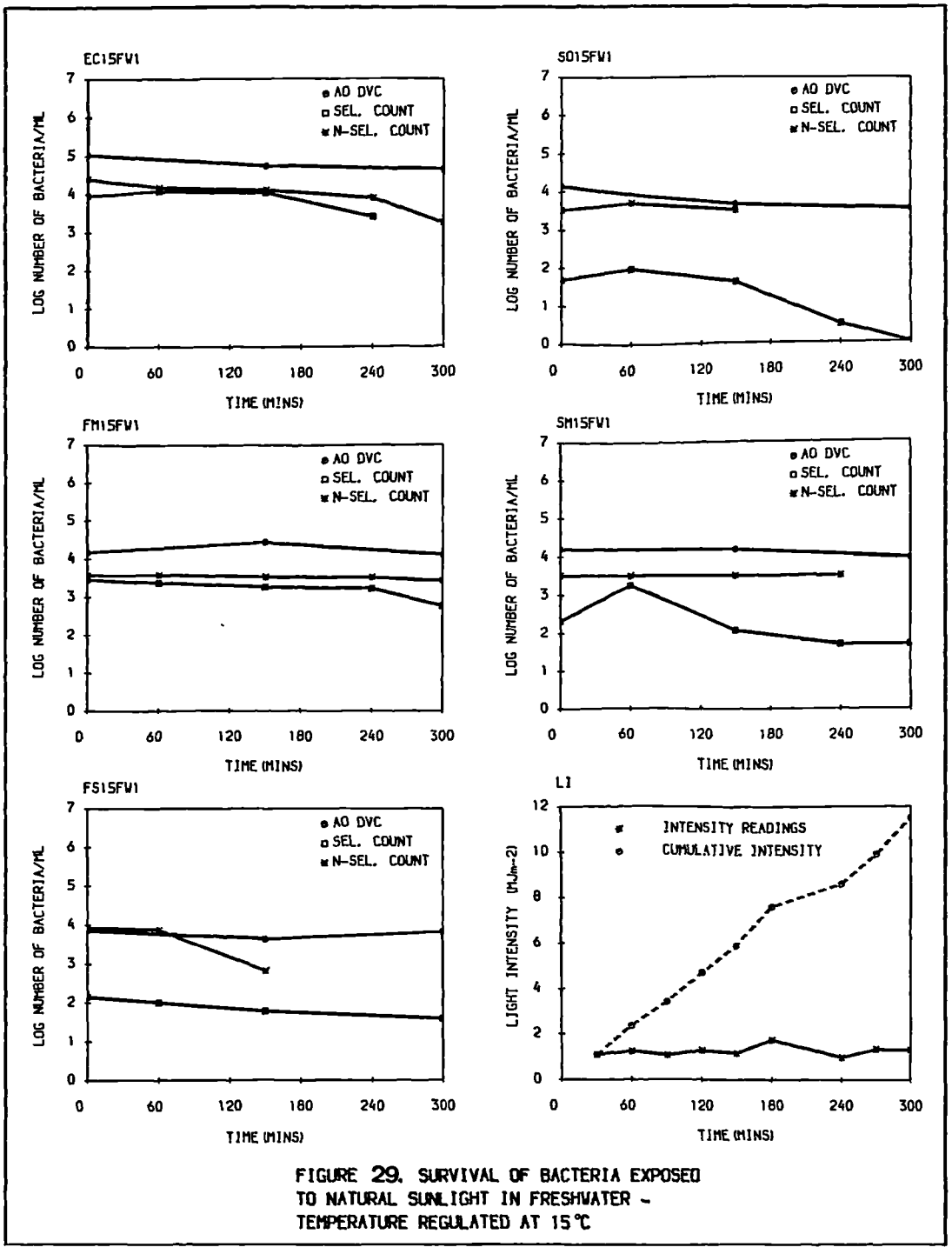


FIGURE 29. SURVIVAL OF BACTERIA EXPOSED TO NATURAL SUNLIGHT IN FRESHWATER - TEMPERATURE REGULATED AT 15°C

TABLE 14. SUMMARY OF NATURAL SUNLIGHT EXPERIMENTS - 1989

	T90 (HOURS)														
	E.COLI			S.FAECIUM			S.FAECALIS			S.ORANIENBURG			S.MONTEVIDEO		
	AODVC	SEL.	N.SEL.	AODVC	SEL.	N.SEL.	AODVC	SEL.	N.SEL.	AODVC	SEL.	N.SEL.	AODVC	SEL.	N.SEL.
SW 1	7.0	1.3	1.2	3.6	1.9	-	23.9	1.5	1.0	4.0	3.4	4.6	11.6	3.7	7.3
SW 2	3.7	1.7	3.0	16.7	2.2	1.1	11.7	1.8	1.8	7.3	4.7	3.2	18.8	6.1	14.0
SW 15 1	17.9	5.3	54.0	12.5	4.0	4.3	no decay	2.1	2.2	no decay	11.4	5.7	14.9	12.3	5.7
SW 15 2	no decay	3.9	2.4	22.0	2.1	1.7	20.4	1.7	2.2	24.9	1.6	4.3	66.1	6.1	5.2
FW 1	13.4	8.5	14.1	14.1	3.3	7.8	24.4	2.7	3.7	11.0	6.6	56.0	8.3	7.4	19.2
FW 2	7.3	12.7	9.6	6.9	4.1	4.2	8.8	1.7	3.9	9.2	4.1	10.8	9.9	4.3	4.9
FW 15 1	12.5	7.6	5.1	79.4	8.7	37.7	132.3	9.1	2.2	7.8	2.3	35.5	19.3	7.0	242.0
FW 15 2	no decay	7.1	8.1	7.5	4.4	7.8	no decay	1.0	1.0	7.9	5.1	43.9	29.6	4.7	5.6

Selective counts for *E.coli* were close to the non-selective counts, whereas for other species, the counts were further apart.

Table 14 is a summary of the T90 values obtained when the test bacteria were exposed to natural sunlight in the summer of 1989. Individual counts can be found in Appendix 7. The five species were simultaneously exposed to sunlight. The light intensity (integrated over a 30 minute period) was measured continuously throughout the duration of the experiments and the temperature, in unregulated experiments was measured every 30 minutes. These can also be found in Appendix 7.

R-sq values for the linear least squares regression lines for computing k values were 70-100% for 1988, with a few AODVC decay rates outside this range, and 50-100% for 1989 with many below 50% for the enterococci.

There was no significant difference in light intensities measured on different days in 1989. The decay rate constants for both series of experiments were found to be log-normally distributed.

One way analysis of variance was performed on the decay rate constants from both series of experiments to examine the differences between decay rates estimated using AODVC and using culturable counts, the results of which are shown in Table 15. For the 1988 experiments there was no evidence of a significant difference between

decay rates estimated using counts from each of the three enumeration methods. This indicates that the loss of culturability and the loss of viability occur at the same rate, in other words the fate of the bacteria exposed to natural sunlight was death.

Table 15. Analysis of variance in decay rates obtained using different enumeration methods (Natural Sunlight).

1988	AODVC vs SL			AODVC vs NL			SL vs NL		
	F	DF		F	DF		F	DF	
EC	4.21	(1,18)	N	4.18	(1,18)	N	0.02	(1,18)	N
FM	3.14	(1,6)	N	3.25	(1,6)	N	0.19	(1,6)	N
ST	4.16	(1,6)	N	3.25	(1,6)	N	0.12	(1,6)	N
1989									
EC	4.07	(1,14)	N	2.98	(1,14)	N	0.03	(1,14)	N
FM	18.02	(1,12)	S	4.02	(1,12)	N	0.03	(1,12)	N
FS	20.14	(1,14)	S	22.35	(1,14)	S	0.01	(1,14)	N
SO	6.76	(1,14)	S	0.44	(1,14)	N	3.90	(1,14)	N
SM	19.82	(1,14)	S	4.36	(1,14)	N	2.00	(1,14)	N

Legend:
S significant difference at 95% confidence level
N no significant difference at 95% confidence level
DF degrees of freedom
F computed F-ratio

For experiments carried out in 1989, the results are less easily interpreted. There is no evidence of a significant difference between decay rates estimated by different enumeration methods for *E.coli*, except in two cases where AODVCs indicated that no decay had occurred, indicating the death of cells exposed to natural sunlight. For enterococci, the only significant difference is observed for *S.faecium* between the decay

estimated using AODVC and using a non-selective culturable method. Examination of the T90 values in Table 14 indicates the presence of a value (37.7) which is higher than the other T90 values for this organism. Had this value not been used in the analysis, there would have been no significant difference between decay rates estimated using the AODVC and using non-selective counts for *S.faecium* and even with the inclusion of this value, the computed F-ratio for this particular analysis is only slightly higher than the tabulated F-ratio (F=4.75 (1,12)). This pattern, however, was also evident for *S.faecium* in the ALS experiments and perhaps should not be dismissed.

For the salmonellae, a significant difference between decay rates estimated using AODVC and using selective culturable counts is evident. There is no evidence, however, of a difference in decay rates estimated using AODVC and using non-selective culturable counts indicating more rapid loss of culturability on selective media than on non-selective media suggesting that the cells may be injured by exposing them to natural sunlight. This observation was only evident for *S.montevideo* at the 1.2MJm^{-1} level.

Further analysis of variance on decay rates estimated using AODVCs and using culturable counts was carried out on each organism for seawater and for freshwater. In most cases where the previous analysis

had shown a significant difference (see Table 15) it had been due to freshwater decay rates which masked the difference in seawater decay rates as estimated using the AODVC and cultural methods.

Analysis of variance in the decay rate constants revealed that decay in seawater was significantly faster than decay in freshwater (see Table 16) for *E.coli* and *S.typhimurium* exposed to natural sunlight in 1988. This was only evident for *E.coli* in 1989. The influence of light, though apparently not dependent on intensity, is observed merely by comparing decay rates in natural sunlight with those from previous dark experiments.

Table 16. Analysis of variance in decay rates in seawater and in freshwater for natural sunlight experiments.

		SW vs FW		
		F	DF	
1988	EC	25.80	(1,28)	S
	FM	1.75	(1,12)	N
	FS	1.74	(1,10)	N
	ST	7.76	(1,16)	S
1989	EC	7.01	(1,22)	S
	FM	7.88	(1,21)	S
	FS	0.03	(1,22)	N
	SO	1.50	(1,22)	N
	SM	0.41	(1,22)	N

Legend: see Table 14.

No evidence of significantly different decay rates for different species was obtained by analysis of variance though examination of plots revealed that salmonellae survive better than *E.coli* which in turn

TABLE 17. CORRELATION BETWEEN DECAY AND ENVIRONMENTAL PARAMETERS FOR NATURAL SUNLIGHT EXPERIMENTS

DECAY RATE CONSTANT (K)

1989	E.COLI			S.FAECIUM			S.FAECALIS			S.ORANIENBURG			S.MONTEVIDEO		
	AODVC	SEL.	N.SEL.	AODVC	SEL.	N.SEL.	AODVC	SEL.	N.SEL.	AODVC	SEL.	N.SEL.	AODVC	SEL.	N.SEL.
SALINITY	-0.231 (8)	-0.706* (8)	-0.562^ (8)	-0.163 (8)	0.245 (8)	-0.761 (7)	-0.120 (8)	0.044 (8)	-0.151 (8)	0.122 (8)	-0.148 (8)	-0.922* (8)	0.327 (8)	0.106 (8)	-0.242 (8)
TEMPERATURE	-0.750* (8)	-0.501 (8)	-0.356 (8)	-0.516 (8)	-0.468 (8)	-0.327 (7)	-0.724* (8)	0.110 (8)	0.117 (8)	-0.577^ (8)	0.316 (8)	-0.250 (8)	-0.709* (8)	-0.492 (8)	0.128 (8)
TOTAL INTENSITY	-0.105 (8)	0.210 (8)	0.061 (8)	-0.175 (8)	-0.270 (8)	0.459 (7)	-0.128 (8)	-0.089 (8)	-0.096 (8)	-0.632^ (8)	0.038 (8)	0.670^ (8)	-0.366 (8)	-0.514 (8)	0.356 (8)

1988	E.COLI			S.FAECIUM			S.FAECALIS			S.TYPHIMURIUM		
	AODVC	SEL.	N.SEL.	AODVC	SEL.	N.SEL.	AODVC	SEL.	N.SEL.	AODVC	SEL.	N.SEL.
SALINITY	-0.554 (10)	-0.735* (10)	-0.961* (10)	-	-0.049 (6)	-0.766^ (6)	-	-0.296 (6)	-0.489 (6)	-0.792^ (4)	-0.921^ (4)	-0.947^ (4)
TEMPERATURE	-0.631* (10)	-0.376 (10)	0.011 (10)	-	0.468 (6)	-0.332 (6)	-	-0.794^ (6)	-0.681^ (6)	-0.607 (4)	0.069 (4)	0.089 (4)
MEAN INTENSITY												
OVERALL	-0.271 (10)	0.037 (10)	-0.118 (10)	-	0.197 (6)	-0.493 (6)	-	-0.833* (6)	-0.393 (6)	0.607 (4)	-0.069 (4)	-0.089 (4)
400nm	-0.346 (10)	-0.175 (10)	-0.039 (10)	-	0.213 (6)	-0.486 (6)	-	-	-	-0.607 (4)	0.069 (4)	0.089 (4)
350nm	0.515^ (10)	0.196 (10)	-0.062 (10)	-	0.427 (6)	-0.362 (6)	-	-	-	0.607 (4)	-0.069 (4)	-0.089 (4)
315nm	-0.045 (10)	-0.239 (10)	0.097 (10)	-	0.654^ (6)	-0.149 (6)	-	-	-	0.607 (4)	-0.069 (4)	-0.089 (4)

* Significant association at 95% confidence level

^ Significant association at 90% confidence level

numbers in parentheses below coefficients - number of pairs

survive better than enterococci. Decay in natural sunlight was significantly higher than in light from the ALS for each the following five test species (EC F=6.17 (1,118); FM F=21.24 (1,88); FS F=21.63 (1,86); SO F=20.36 (1,67); SM F=49.59 (1,65)).

The correlation coefficients for the natural sunlight data are shown in Table 17. The decay rate constants for natural sunlight experiments were correlated with salinity, temperature and light intensity. For 1988 experiments, correlation of decay rate with light intensity measured at different wavelengths was also carried out. Correlation analysis of the data indicates that:

1) there is a significant association between decay rate and salinity for *E.coli* at the 95% confidence level and for *S.typhimurium* at the 90% confidence level, in the 1988 experiments,

2) there is a significant association between decay rates and salinity for *E.coli* at the 95% confidence level for selective counts and at the 90% confidence level for non-selective counts, in the 1989 experiments,

3) there is a significant association between temperature and decay rates estimated using the AODVC for 4 out of 5 test bacteria, in the 1989 experiments.

No evidence was provided by the correlation analysis for any other associations between decay rate and the factors in question suggesting the possibility of

a combined effect of sunlight and salinity for Gram negative bacteria.

A multiple regression analysis was performed on the decay rate constants to evaluate the combined effect of temperature, salinity and light intensity on the decay rates in natural sunlight (not shown). The R-sq values for the fitted lines were in the range 60-100% for the 1988 experiments, and 50-100% for the 1989 experiments, indicating that the regression equation adequately described most of the data analysed and that decay was probably a result of a combined effect of all or some of the factors considered. No significant difference was found between R-sq values for 1988 experiments and R-sq values for 1989 experiments though both were significantly higher than R-sq values for ALS experiments (1988 $F=36.3$ (1,23); 1989 $F=10.89$ (1,28).

5.3.1.4. Discussion

In freshwater, decay occurs at a similar rate irrespective of the enumeration method used. In seawater initial decay rates are similar but after a period of time the culturable counts decrease rapidly. Several possible reasons for this will be discussed in the next Chapter though the obvious one is that the bacteria evolve towards a viable but non-culturable stage. This observation was only made in the first series of experiments which were carried out in 1988 during which most days were quite dull. For 1989 experiments, the most rapid decline was observed in seawater where

temperature was unregulated indicating the synergistic interaction of salinity, temperature and sunlight.

The absorbance of the UV component of sunlight by humic acids in freshwater may account, in part, for increased survival of bacteria in freshwater. The absorption spectrum for freshwater is given in Table 23, Section 5.5.4 and is discussed further therein.

5.3.2. Exposure of enterococci to natural sunlight with added nutrients

5.3.2.1. Aims

The numbers of enterococci, especially of *S.faecalis*, had decreased much more rapidly in the light throughout this study than had been expected considering that most previous workers have reported that their survival was usually better than that of *E.coli*. This was thought to be due to the nutrient concentrations in the microcosms being too low to enable enterococci, which usually require quite high nutrient concentrations, to withstand the pressure of exposure to light. The addition of 0.25% (typical concentration of settled sewage in the vicinity of an outfall (Morgan, 1984)) filtered, sterile synthetic sewage on the decay of enterococci exposed to natural sunlight was examined.

5.3.2.2. Experimental procedure

Filtered, sterile synthetic sewage was added to the microcosms to give a final concentration of 0.25% (v/v) prior to their inoculation with *S.faecalis* and *S.faecium* which had been grown in filtered, sterile settled sewage.

TABLE 18. EFFECTS OF ADDITION OF SYNTHETIC SEWAGE ON DECAY OF ENTEROCOCCI EXPOSED TO NATURAL SUNLIGHT.

T90 (HOURS)

	S.FAECIUM			S.FAECALIS		
	AODVC	SEL.	N.SEL.	AODVC	SEL.	N.SEL.
SW + 0.25% SS1	12.2	4.1	2.2	30.2	1.7	1.6
SW + 0.25% SS2	17.4	3.7	3.2	9.6	21.9	27.3
SW1	no decay	3.0	3.9	no decay	1.0	1.0
SW2	64.1	2.8	2.6	18.9	5.3	4.2
FW + 0.25% SS1	75.0	8.8	7.1	121.0	22.6	39.1
FW + 0.25% SS2	no decay	5.3	8.0	30.4	3.6	9.2
FW1	29.0	5.4	5.0	no decay	4.8	6.2
FW2	no decay	4.0	5.6	no decay	1.7	6.6

5.3.2.3. Results

Decay rates for bacteria in this experiment can be seen in Table 18. Individual counts and light intensity readings can be seen in Appendix 7.

One way analysis of variance in the decay rate constants showed that the addition of nutrients in the form of synthetic sewage had no significant effect on the decay rates of enterococci exposed to natural sunlight (not shown). Light intensities measured were significantly different on different days ($F=3.98$ $(3,33)$).

5.3.2.4. Discussion

The use of microscopic counts in estimating the decay rates demanded the use of synthetic sewage because it contains no organisms or particles which are likely to interfere with the enumeration of bacteria introduced into the microcosm. It is probably not as nutritionally beneficial to the bacteria as unfiltered settled sewage would have been, however. The poor growth of enterococci in filtered settled sewage has already been noted (Section 4.2.).

5.4. Growth cabinet experiment

5.4.1. Aims

The rapid decay of bacteria exposed to natural and artificial sunlight has already been shown (Sections 5.2. and 5.3.). However, the light intensities

encountered in these experiments are unlikely to be encountered below the surface of a body of water such as the sea or a lake as many processes are involved in reducing the transmission of light through water. The growth cabinet provides a light source more representative, therefore, of subsurface sunlight intensities and was used in this experiment to examine the survival characteristics of bacteria exposed to low intensities of light. It also allows exposure of bacteria to a period of darkness simulating night time conditions.

5.4.2. Experimental procedure

Environmental isolates of *E.coli*, *S.typhimurium* and *S.faecalis* grown in TS Broth were exposed to light from the growth cabinet (6 lamps lit) in flask microcosms for 16 hours of light plus 8 hours of darkness per day (experiment carried out prior to the use of settled sewage as a culture medium). Dark controls were placed in the dark for 24 hours per day.

5.4.3. Results

The overall light intensity as measured by the Kipp and Zonen solarimeter was approximately 1/10-1/20 of the intensity of light emitted by the ALS. However, the proportion of the light emitted at 315nm measured using a Fleming sensor, as a percentage of the overall intensity, was greater than that emitted by the ALS and approximately the same proportion of the overall light

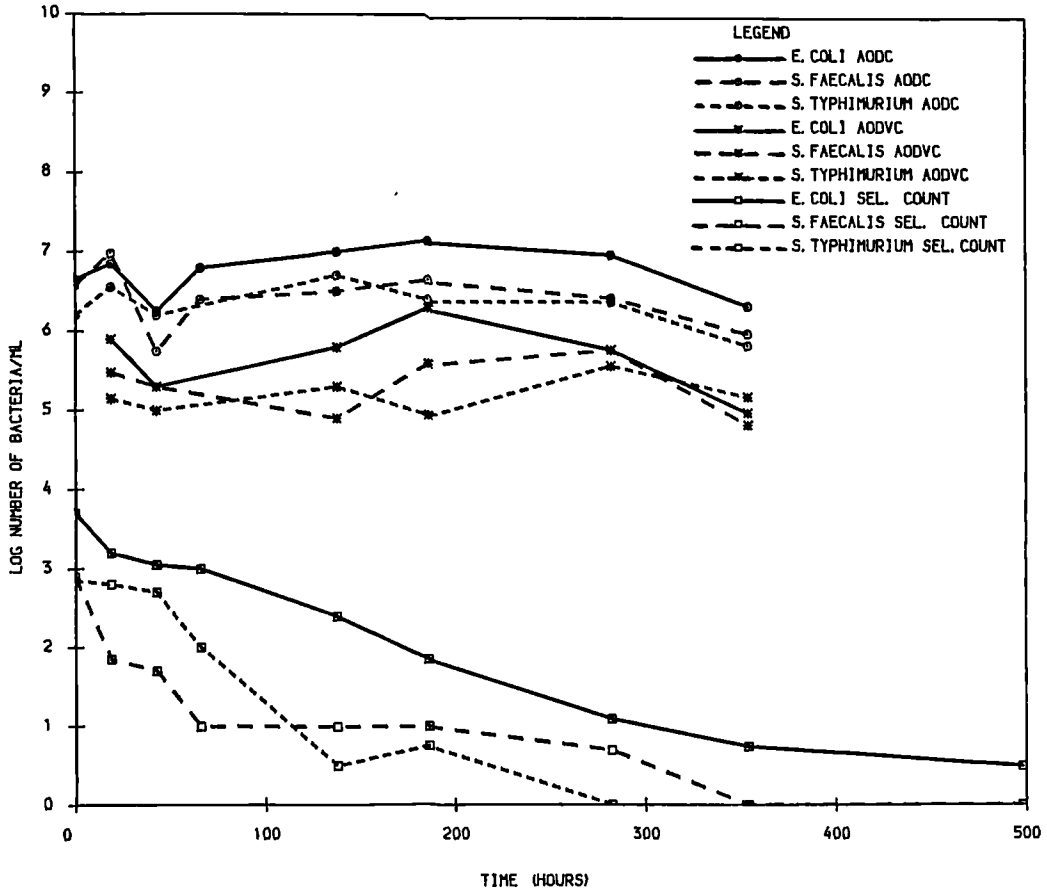


FIGURE 30. EFFECT OF LOW INTENSITY LIGHT ON THE SURVIVAL OF BACTERIA IN SEAWATER

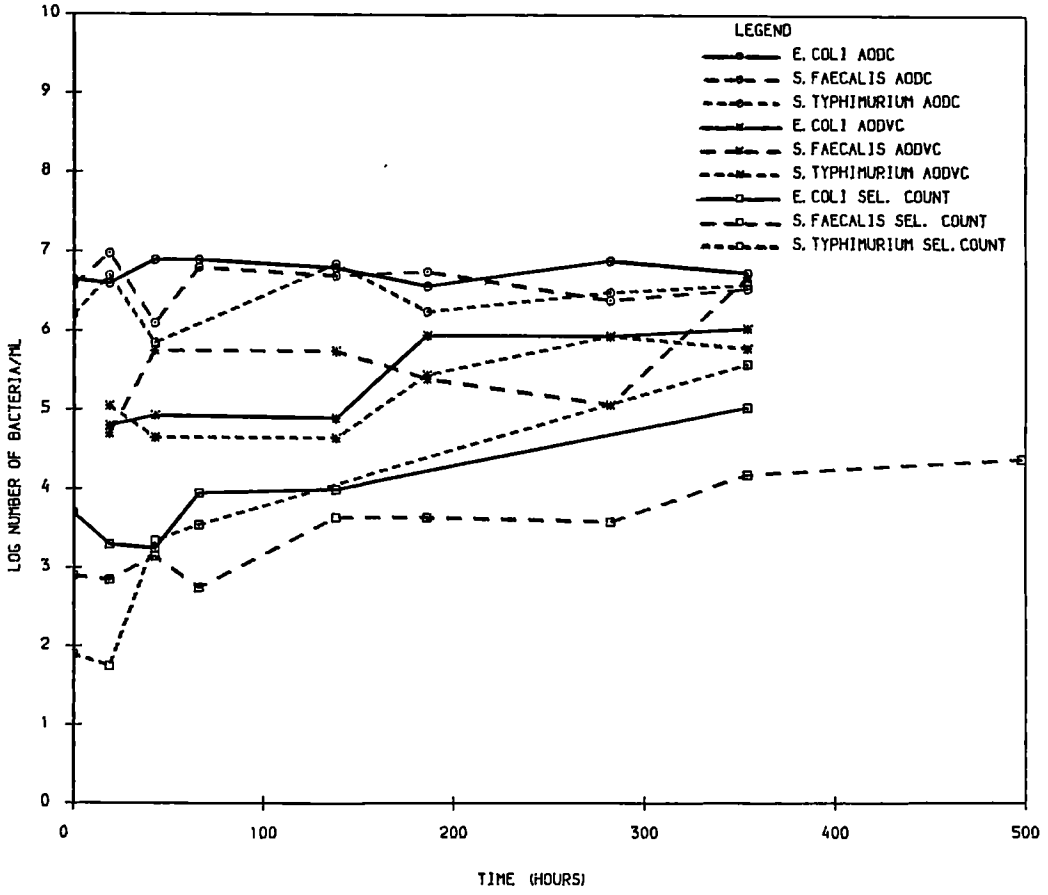


FIGURE 31. SURVIVAL OF BACTERIA IN THE DARK IN SEAWATER

intensity as is emitted in natural sunlight at 315nm (approximately 14% of the overall intensity compared to 1% by the ALS). The overall intensity measured was 0.1MJm^{-2} (integrated over a 1 hour period) and at 315nm, 0.014MJm^{-2}, which did not change over the duration of the experiment.

Figures 30 and 31 show the results represented graphically, of the three selected bacteria exposed to light from the growth cabinet, and in the dark respectively, where AODC, AODVC and selective culturable counts were made. Individual counts can be found in Appendix 8. Decay is less rapid than when exposed to natural sunlight or the ALS but more rapid than in continuous darkness.

5.4.4. Discussion

A decline in the numbers of culturable bacteria is observed for all three species exposed to the light. The AODVCs, on the other hand, remained constant for about 12 days with a slight decrease in numbers towards the end of experiment. The AODCs remained constant for the duration of the experiment. Figure 30 shows the general characteristics exhibited by viable but non-culturable forms of bacteria indicating the inducement of these forms by low intensity light such as that able to penetrate below the surface of a body of water. These results are in accordance with those obtained by Barcina et al. (1989) who exposed *E.coli*, also grown on a rich

culture medium, to low intensity visible light in river water.

In the dark, the direct enumeration methods indicate that the numbers of bacteria remain constant with time, whereas the cultural method indicates an increase in numbers, i.e., return to culturability. An increase in number of culturable bacteria would not be shown immediately by the AODVCs because the number of viable bacteria remains the same. One explanation for this is the possibility of carryover of nutrients from TS Broth in spite of the intensive washing procedure followed. Barcina et al. (1989) also found growth of *E.coli* in the dark but in freshwater, as opposed to seawater, which has higher nutrient levels. All subsequent cultures for inoculation into microcosms for exposure to light in the growth cabinet were grown in filtered sterile settled sewage.

5.5. Humic acid experiments

5.5.1. Aims

It is believed that the presence of humic acids in freshwater may contribute to the increased survival of most bacteria in freshwater, either as a metabolisable substrate or as a light-absorbing substance (see Section 2.2.2.) and may, therefore, account for differences in freshwater and seawater mortality rates. This experiment was carried out to determine which theory could be supported by experimental evidence.

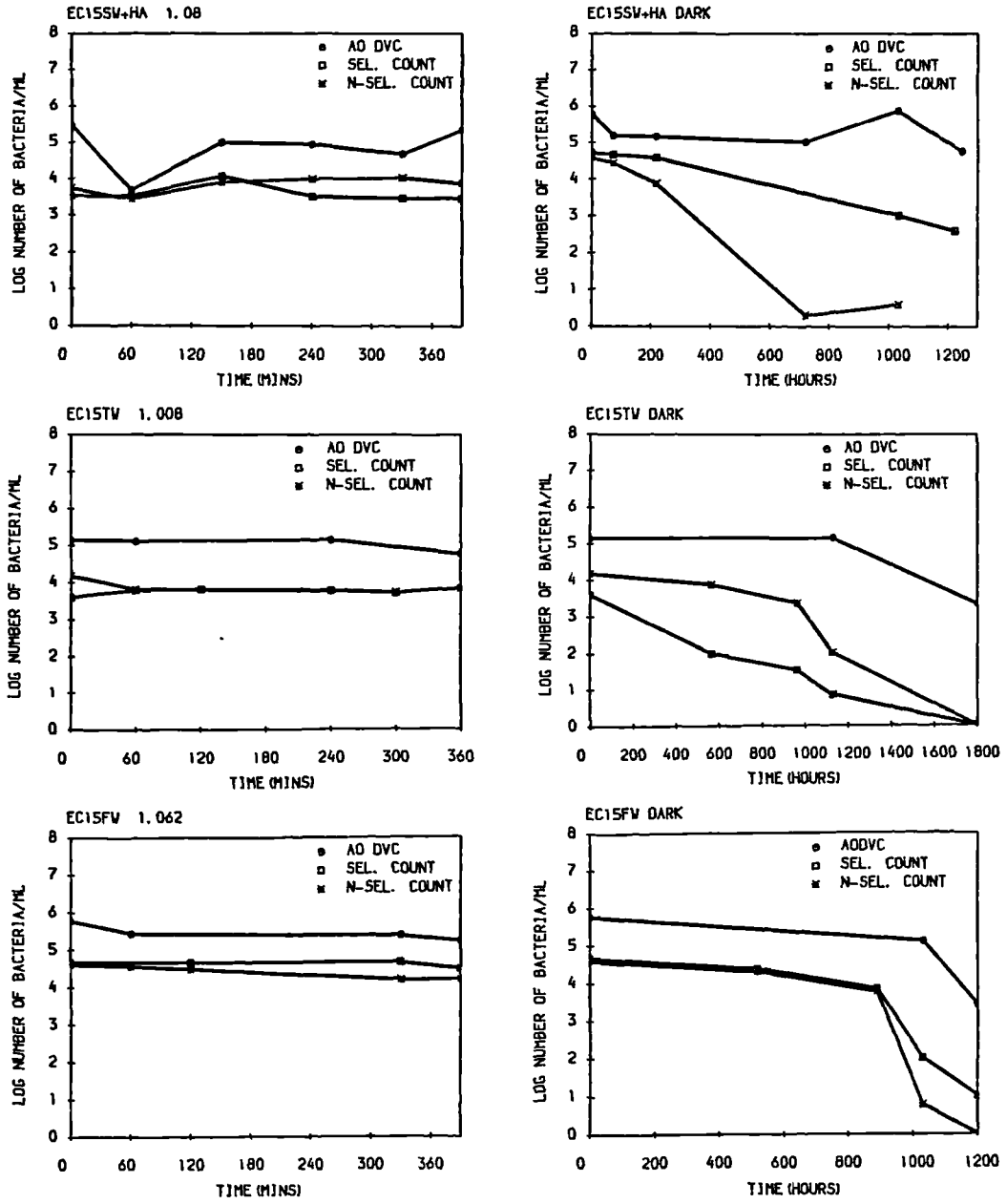


FIGURE 32. SURVIVAL OF E.COLI EXPOSED TO THE ALS AND IN THE DARK IN DIFFERENT WATERS AT 15 C

5.5.2. Experimental procedure

Humic acid extract was added to the microcosm water prior to inoculation (see Section 3.2.6.). *E.coli* was exposed in beaker microcosms, to light from the growth cabinet (12 lamps lit) for 16 hours per day with the remainder of the time being in darkness, and to the ALS, in a variety of natural waters with and without humic acid extract added. Dark controls were also carried out. Waters used include seawater, freshwater and treated, unchlorinated water with a very low concentration (<5 Hazen) of coloured compounds.

5.5.3. Results

5.5.3.1. ALS experiments

Figure 32 shows typical results from ALS experiments and dark controls represented graphically. The plots represent those experiments in which *E.coli* was exposed to the ALS at 15°C in different waters and corresponding dark controls.

Table 19 shows the decay of *E.coli* in various natural waters when exposed to the ALS and in the dark. Light intensities are given with the data and in Appendix 9. Decay is in the form of the T90 values.

For ALS experiments, R-sq values were mostly below 50% in the light, and 70-100% in the dark.

One way analysis of variance was performed on the decay rate constants from the ALS experiments to evaluate:

1) the difference in decay rate constants estimated using different enumeration methods.

2) the difference in the decay rates of *E.coli* in the different waters.

3) the difference in decay rates for light and dark experiments.

Tables 21 and 22 show the results of the analysis on ALS data.

There is no evidence of a significant difference in decay rates estimated by different enumeration methods for *E.coli* exposed to the ALS. In the dark, however, there is a significant difference between decay rates estimated using the AODVC and using culturable counts indicating evolution of *E.coli* towards a viable but non-culturable form. There is also a significant difference between decay rates in the light and in the dark ($F=4.68$ (1,46)).

Analysis of variance also shows that there is no evidence of a difference in decay rates for *E.coli* in different waters exposed to the ALS. Similarly, there is no evidence of a difference in decay rates for *E.coli* in different waters in the dark.

5.5.3.2. Growth cabinet experiments

Figure 33 shows typical results from the growth cabinet experiments represented graphically. The plots represent one of the sets of experiments in which *E.coli* was exposed concurrently in different waters to light from the growth cabinet.

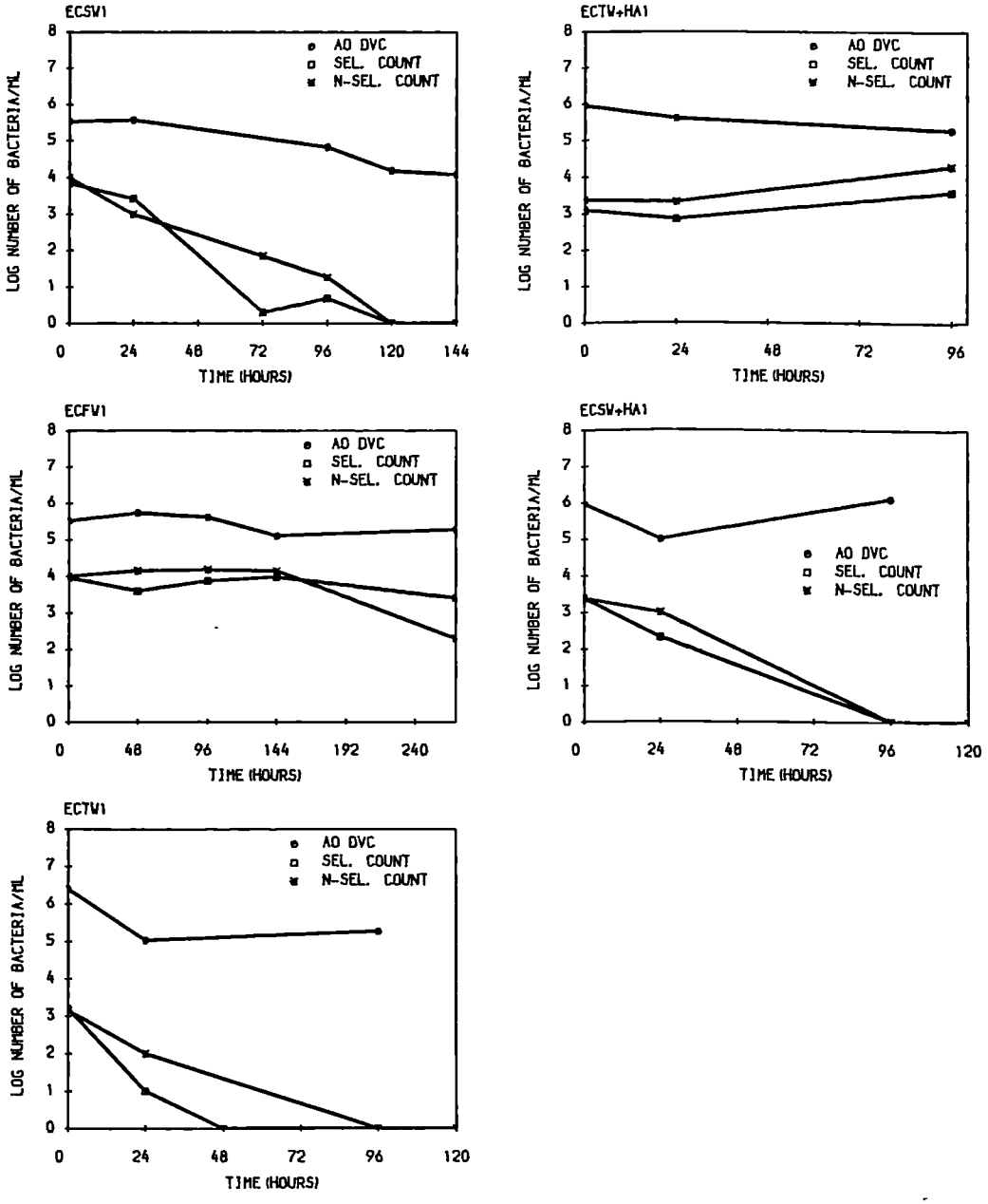


FIGURE 33. SURVIVAL OF E.COLI EXPOSED IN DIFFERENT WATERS TO LIGHT FROM THE GROWTH CABINET AT 15 C

In the dark, however, there is no significant difference between decay rates estimated using AODVC and using non-selective culturable counts. The non-selective counts increase in waters with no humic acids added, whereas, in the same waters the selective counts decrease (see Table 20). This probably indicates that these particular microcosms may have been contaminated with an organism which was not *E.coli*.

Table 19. Effects of addition of humic acids on the survival of *E.coli* exposed to the artificial light source and in the dark.

		T90(hours)		
EXPT.	LI	AODVC	SL	NL
ALS				
EC25SW+HA	-	-	-	-
EC25TW	1.032	17.9	19.7	5.2
EC25FW	1.044	27.3	43.4	34.3
EC15SW+HA	1.080	n	72.4	n
EC15TW	1.008	18.8	n	14.9
EC15FW	1.062	15.6	41.7	13.8
EC05SW+HA	1.021	n	48.4	6.2
EC05TW	1.053	21.9	27.6	29.5
EC05FW	0.981	61.5	83.3	89.6
DARK CONTROLS				
EC25SW+HA		-	-	-
EC25TW		588	288	340
EC25FW		826	592	450
EC15SW+HA		1808	571	220
EC15TW		610	410	339
EC15FW		435	321	243
EC05SW+HA		n	526	231
EC05TW		775	461	331
EC05FW		562	352	249

Legend:
SW seawater
TW treated, unchlorinated water
FW freshwater
EC E.coli
HA humic acid extract
n no decay
25, 15, 05 temperatures (°C)
LI light intensity (MJm^{-1})

Table 20. Effects of addition of humic acids on the survival of *E.coli* exposed to light in the growth cabinet at 15°C and in the dark.

	T90 (hours)		
EXPT.	AODVC	SL	NL
GC			
ECFW1	n	125	95
ECFW2	92	57	n
ECFW3	578	758	167
ECFW4	1282	704	581
ECSW1	104	33	33
ECSW2	120	33	31
ECTW1	122	36	74
ECTW2	118	30	28
ECTW3	180	11	30
ECSW+HA1	n	32	28
ECSW+HA2	266	26	24
ECSW+HA3	259	17	31
ECSW+HA4	102	26	32
ECTW+HA1	153	n	n
ECTW+HA2	n	n	n
ECTW+HA3	163	46	109
ECTW+HA4	538	46	128
DARK CONTROLS			
ECFW	n	714	n
ECTW	2653	471	n
ECTW+HA	n	272	273
ECSW+HA	24390	182	175

see legend for Table 19.
1,2,3,4 similar numbers indicate concurrent exposure

Table 20 shows the decay of *E.coli* in various waters in similar experiments carried out in the growth cabinet and in the dark. The overall light intensity measured was 0.126MJm^{-1} and at 315nm, 0.018MJm^{-1} (integrated over a 1 hour period), which did not change over the duration of the experiment. Individual counts can be found in Appendix 10.

R-sq values for the linear least squares regression lines for computing k values were 80-100% for culturable counts and 0-50% for AODVCs in the dark. In the light, R-sq values were 0-70%.

One way analysis of variance was performed on the decay rate constants from the growth cabinet experiments to evaluate:

1) the difference in decay rate constants estimated using different enumeration methods.

2) the difference in the decay rates of *E.coli* in the different waters.

3) the difference in decay rates for light and dark experiments.

Tables 21 and 22 show the results of the analysis on growth cabinet data.

Decay rates estimated using AODVC are significantly slower than the decay rates estimated using selective culturable counts for the growth cabinet experiments, indicating the evolution of *E.coli* towards a viable but non-culturable form when exposed to low intensities of light.

Table 21. Analysis of variance in decay rates obtained using different enumeration methods for Growth Cabinet and Artificial Light Source experiments.

	AODVC vs SL			AODVC vs NL		
	F	DF		F	DF	
ALS	0.06	(1,32)	N	0.56	(1,14)	N
DARK CONTROL	4.60	(1,14)	S	4.60	(1,14)	S
GC	11.15	(1,32)	S	8.75	(1,32)	S
DARK CONTROL	5.99	(1,6)	S	0.75	(1,6)	N

Legend:
S significant difference at 95% confidence level
N no significant difference at 95% confidence level
F computed F-ratio
DF degrees of freedom
GC growth cabinet

Table 22. Analysis of variance in decay rates of *E.coli* in different waters.

		F	DF	
ALS	FWvsTWvsSW+HA	1.76	(2,21)	N
DARK	CONTROL	0.26	(2,21)	N
GC	FWvsTWvsSWvsTW+HAvsSW+HA	5.56	(4,46)	S
DARK	CONTROL	1.79	(3,8)	N
GC	TWvsTW+HA	5.99	(1,19)	S
GC	SWvsSW+HA	0.10	(1,15)	N

see legend for Tables 19 and 21.

Analysis of variance shows a significant difference in decay rates in different waters (F=5.56 (4,46)) and in saline and in non-saline waters in this experiment (F=9.41 (1,49)) for decay of *E.coli* in the growth cabinet. There is also a significant difference in decay in the light and decay in the dark (F=5.01 (1,55)).

5.5.4. Discussion

The addition of humic acids had no effect on the decay of bacteria in microcosms exposed to light from the ALS.

In the dark, the decay rates for waters without humic acids would have been expected to be higher than the decay rates for waters with humic acids if the protective role of humic acids is as a substrate. They would have been expected to be the same if the protective role of humic acids is light absorbance.

In the presence of light from the growth cabinet, the addition of humic acid to unchlorinated, treated water resulted in an increase in T90 values of *E.coli* such that survival in this water was similar to their survival in freshwater. The addition of humic acids to seawater does not have a similar effect. This may be due to the synergistic effect of salinity and UV light (also seen in the natural sunlight experiments) overriding any beneficial effects which the addition of humic acids may afford.

The general lack of influence of humic acids in the dark and in ALS experiments indicate that the role of humic acids in prolonging survival of bacteria is only evident in the presence of light of shorter wavelengths. Absorbance of light by humic acids does not occur to the same extent with light in the visible part of the spectrum. Evidence for this is shown in Table 23, which shows the absorbance of light at different wavelengths by the three waters used in this study. The absorbance of light by seawater and treated water, at each of the wavelengths measured, is much lower than by freshwater, particularly at longer wavelengths. The greater absorbance of light by freshwater, at all wavelengths measured, but particularly at shorter wavelengths is thought to be due to the presence of humic acids.

Table 23. Absorbance of light in the UV-visible part of the spectrum by three types of water.

	Absorbance		
Wavelength (nm)	Freshwater	Seawater	Treated
400	0.041	0.004	0.004
380	0.071	0.008	0.008
360	0.079	0.009	0.007
340	0.105	0.011	0.011
320	0.154	0 014	0.016
312	0.205	0.014	0.014

Further evidence for the absorbance of UV light by humic acids is provided by examining the emission spectra of the various light sources used. The emission spectra for the ALS and for natural sunlight can be found

in Gameson and Gould (1985) but may not be reproduced. The emission spectrum for the fluorescent lamps in the growth cabinet, provided by Thorn Lighting Ltd., can be found in Appendix 13. The intensity of UV light emitted by the artificial light source as a proportion of the overall intensity is very low compared with the proportion emitted from the fluorescent tubes in the growth cabinet and natural sunlight. Only in ALS experiments is there no significant difference between survival in seawater and survival in freshwater, except for *S.faecalis* (F=7.32 (1,50)) which would seem to connect the action of UV light with the influence of salinity.

Incidently, correlation analysis was performed on freshwater decay rates from all freshwater experiments to determine whether or not the varying absorbance and colour of different freshwaters used in the natural sunlight and ALS experiments could account for differences in observed freshwater decay rates. Colour and absorbance data were provided by Mr Thompson, Sunderland and South Shields Water Company for water in Derwent Reservoir at the time of collection of waters for the experiments. The waters used in this study had colours in the range of 22-51 Hazen and absorbances at 254nm in the range of 0.218-0.353. Correlation of freshwater decay rates from natural sunlight and ALS experiments with the corresponding colour and absorbance of the water used yielded no significant associations

(not shown) except of course between colour and absorbance. This suggests that the absorbances and colours of different freshwaters used cannot account for observed differences in decay rates in freshwater experiments carried out under supposedly similar conditions.

5.6. *Salmonella anatum* experiments

5.6.1. Aims

An experiment was carried out to investigate the effect of light from the ALS on survival of three different strains of the same species of bacteria. The ALS was chosen for this experiment because it allows different species or strains of bacteria to be exposed to light under similar conditions. *S.anatum* was chosen be cause it is frequently isolated from waters receiving sewage discharges and has been shown to be particularly resistant to light in the past (Morgan, 1984).

5.6.2. Experimental procedure

S.anatum NCTC 3072, NCTC 5779, and a strain from Newcastle General Hospital (from hereon referred to as GH) were exposed in freshwater and seawater beaker microcosms to the ALS at an average light intensity of 2.68MJm^{-2} and in the dark, at 15°C.

5.6.3. Results

Decay rates of *S.anatum* for light and dark experiments in the form of the T90 values are shown in

Table 24. Individual counts can be found in Appendix 11.

R-sq values for the linear least squares regression lines for computing k values were 80-100% in the light and 90-100% in the dark.

One way analysis of variance was performed on the decay rate constants for *S.anatum* under the conditions specified, the results of which are summarised in Table 25. A significant difference in decay rates estimated by the AODVC and by cultural methods is evident both in the light and in the dark for all three strains, indicating the evolution of *S.anatum* towards a viable but non-culturable form, both in the light and in the dark. Although the T90 values for *S.anatum* are lower than the T90 values obtained for other species of salmonellae in earlier experiments at a similar light intensity level, it appears that *S.anatum* is able to survive exposure to light from the ALS by becoming viable but non-culturable, whereas the other species were killed.

Table 24. Decay of three strains of *S.anatum* exposed to the artificial light source at 15°C.

EXPT.		LI	T90 (hours) AODVC	SL	NL
ALS					
SA (GH)	SW	2.63	11.0	1.2	1.4
	SW	2.63	3.9	1.2	2.2
	FW	2.66	4.3	2.9	2.4
	FW	2.59	9.4	7.4	3.6
SA NCTC 5779	SW	2.66	11.1	4.2	2.8
	SW	2.81	5.3	2.7	3.5
	FW	2.70	45.7	8.9	8.4
	FW	2.70	8.4	9.1	5.4
SA NCTC 3072	SW	2.66	29.0	7.8	4.1
	SW	2.81	758	5.0	4.4
	FW	2.59	n	5.2	8.8
	FW	2.70	33.0	6.4	4.7
DARK CONTROLS					
SA (GH)	SW		515	31	27
	SW		150	30	28
	FW		328	28	40
	FW		359	30	44
SA NCTC 5779	SW		549	147	45
	SW		4098	81	42
	FW		606	81	76
	FW		917	53	35

Legend:
SA S.anatum
SW seawater
FW freshwater

Table 25. Analysis of variance in decay rates of three different strains of *S.anatum* and by three enumeration methods.

	AODVC vs SL			AODVC vs NL		
	F	DF		F	DF	
ALS	7.13	(1,22)	S	14.42	(1,22)	S
DARK CONTROL	24.15	(1,14)	S	70.49	(1,14)	S

		F	DF	
ALS	GH vs NCTC 5779 vs NCTC 3072	7.58	(2,33)	S
DARK CONTROL	GH vs NCTC 5779	4.32	(1,22)	S

Legend:
S significant difference at 95% confidence level
N no significant difference at 95% confidence level
DF degrees of freedom
F computed F-ratio

5.6.4. Discussion

A significant difference between decay rates for NCTC strains (NCTC 5779 and NCTC 3072) and decay rates for the GH strain is evident in the light, and between NCTC 5779 and GH in the dark (there was no dark control for NCTC 3072). This suggests that different strains as well as different species may have different survival capabilities. No difference was found between NCTC 5779 and NCTC 3072 in the dark or in the light. The implications of this is that survival may be determined by the conditions under which the culture has been kept previously, as both of these are culture collection strains and previously kept under similar conditions. Also these two culture collection strains were isolated more than 50 years ago, whereas the GH strain was more recently isolated.

5.7. AODVC for enterococci

5.7.1. Aims

A search of the literature was made for information on the action of various quinolones on enterococci and their potential as a DNA gyrase inhibitor in place of nalidixic acid in an AODVC for enterococci. Ciprofloxacin was chosen for its activity against a wide spectrum of Gram positive and Gram negative aerobes, enterococci included, as well as some anaerobes. Ciprofloxacin has a Minimum Inhibitory Concentration for 50% of the population (MIC_{50}) of 1mg/l for enterococci compared with an MIC_{50} of >512mg/l for nalidixic acid against enterococci (King & Phillips, 1986). Experiments were carried out to determine the optimum concentration of ciprofloxacin and the optimum incubation period.

5.7.2. Optimum concentration of ciprofloxacin

5.7.2.1. Experimental procedure

Actively growing *S.faecium* were incubated at 20°C with ciprofloxacin at various concentrations and 0.038% (w/v) yeast extract solution. Total direct counts (TDC) were made every 2 hours for 6 hours and are shown in Figure 34.

5.7.2.2. Results

Individual counts can be found in Appendix 12. Most of the cells enumerated throughout the experiment were red. 1.0mg/l ciprofloxacin inhibited division such that the

number of cells did not increase or decrease. 0.1mg/l allowed a slight increase in the number of cells, 10mg/l also inhibited division but is clearly well above the MIC_{II}, and 25mg/l caused a sharp decline in the number of cells. 1.0mg/l, therefore, after checking that *S.faecalis* also reacted suitably to this concentration, was used in the AODVCs of enterococci in experiments unless otherwise indicated. The use of 0.038% yeast extract solution produced suitably enlarged cells after 6-10 hours incubation.

5.7.3. Optimum incubation period

5.7.3.1. Experimental procedure

S.faecium which had been grown in filtered, sterile settled sewage then exposed to seawater for 1 week was incubated with 1mg/l ciprofloxacin (final concentration) and 0.038% yeast extract solution. TDCs and DVCs were carried out every 2 hours. TDCs and DVCs were also carried out on a control incubated with 0.038% yeast extract solution but without ciprofloxacin.

5.7.3.2. Results

Individual counts can be found in Appendix 12. The ratio of DVC to TDC(%) for *S.faecium* with and without ciprofloxacin were plotted against time and can be seen in Figure 35. After an initial increase in the DVC/TDC ratio due to an increase in enlarged cells, a point is reached where the ratio remains constant i.e., no more cells are enlarging. It is reasonable to assume

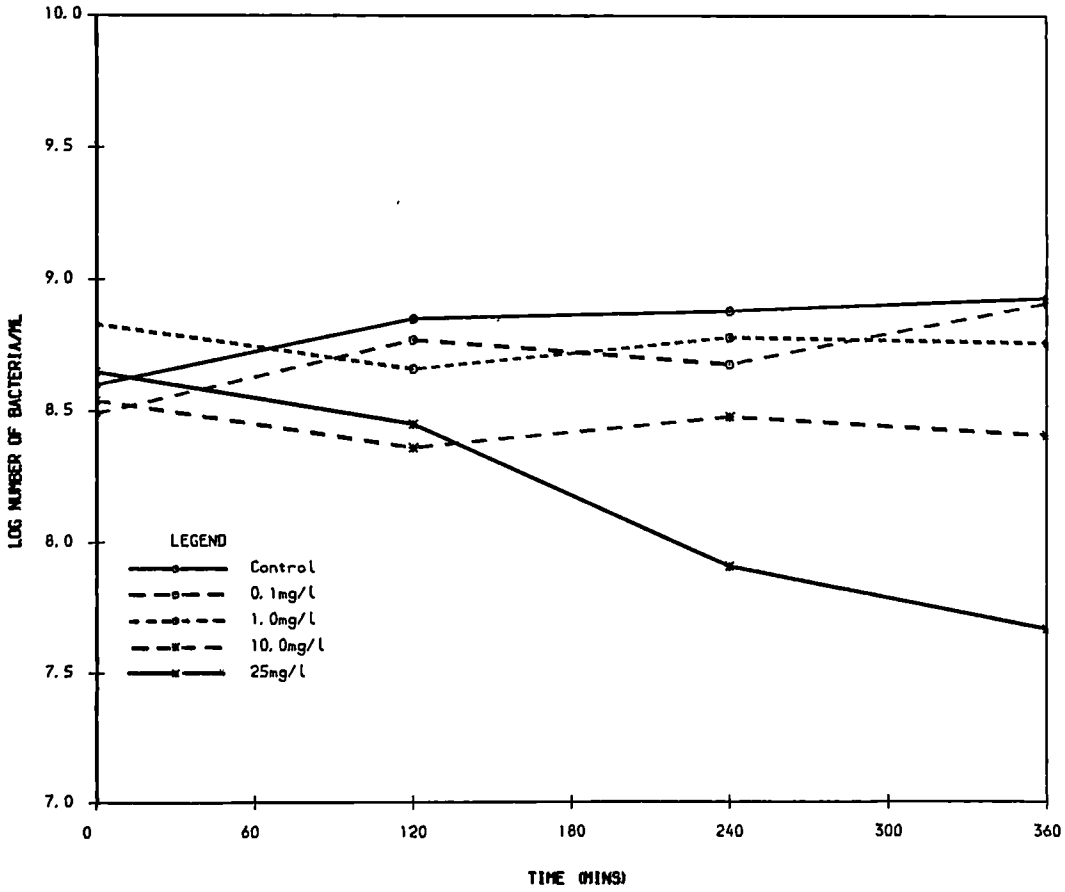


FIGURE 34. DETERMINATION OF OPTIMUM CONCENTRATION OF CIPROFLOXACIN FOR AODVC OF ENTEROCOCCI

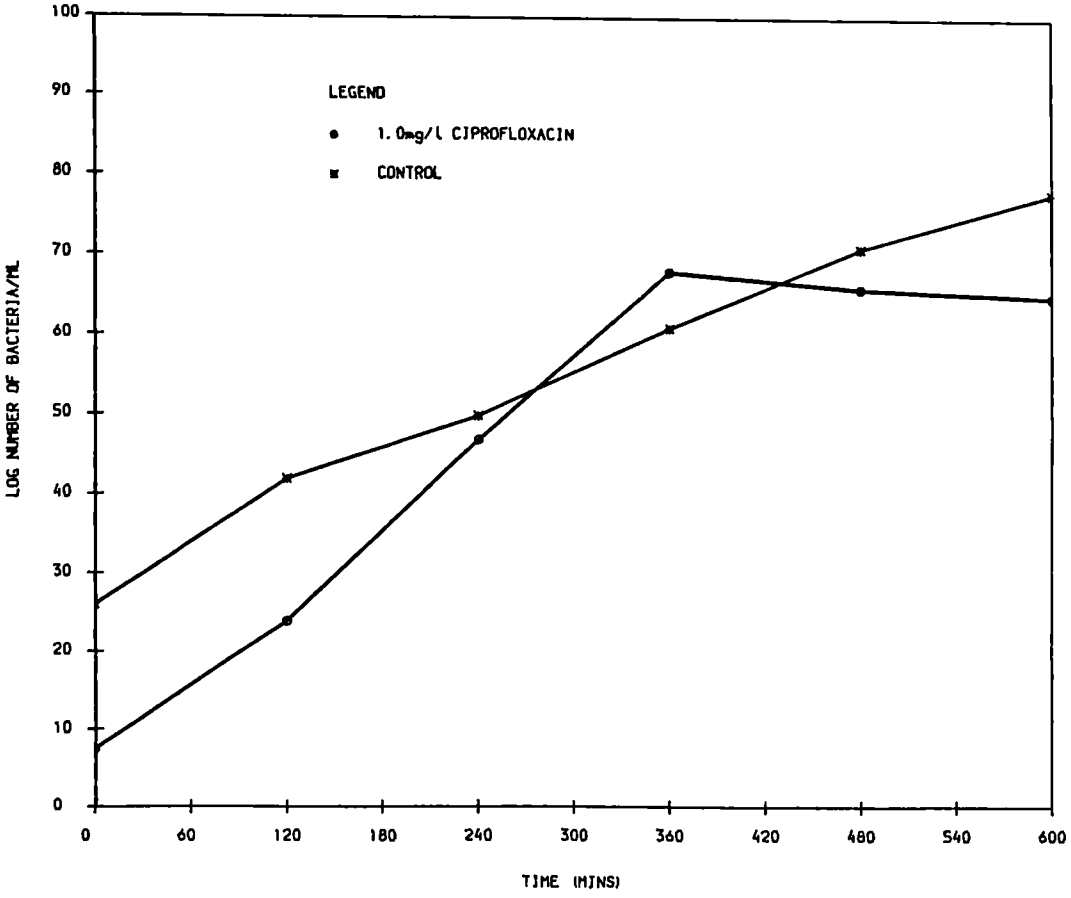


FIGURE 35. DETERMINATION OF OPTIMUM INCUBATION PERIOD FOR AODVC OF ENTEROCOCCI

therefore, that 6 hours is a long enough incubation period for all viable cells to respond to the action of ciprofloxacin by enlarging. The control shows that cells free of the influence of ciprofloxacin continue to divide and multiply after 6 hours.

Plates 3-5 show *S.faecium* under the microscope at an actively growing stage, a starvation stage, and enlarged following incubation with 1mg/l ciprofloxacin and 0.038% yeast extract solution for 6 hours.

5.7.4. Discussion

The use of ciprofloxacin as a DNA gyrase inhibitor was successful in inducing enlargement of enterococci cells. The size of enlarged cells varied greatly, however. Enlargement of cells up to twice their original size was often observed. Attempts to take photos of enlarged cells were, on the whole, unsuccessful. This was mainly due to lack of time towards the end of the present study. Improvements must be made to this method before it can be claimed that it is a AODVC for enterococci. The future use of this method, however, is quite promising in that the number of enlarged cells is consistently higher than counts made by cultural methods. This indicates that under certain conditions a proportion of the viable enterococci population cannot be cultured.

The limits of detection for both the ciprofloxacin and nalidixic acid AODVC methods were as follows: based on counts from 20 fields of view, the lowest number of bacteria which can be detected when 1ml is filtered is approximately 10^1 per ml, and when 10mls are filtered, approximately 10^1 per ml. The AODVC technique is, therefore, less sensitive than culturable techniques which can detect 1 organism per ml.

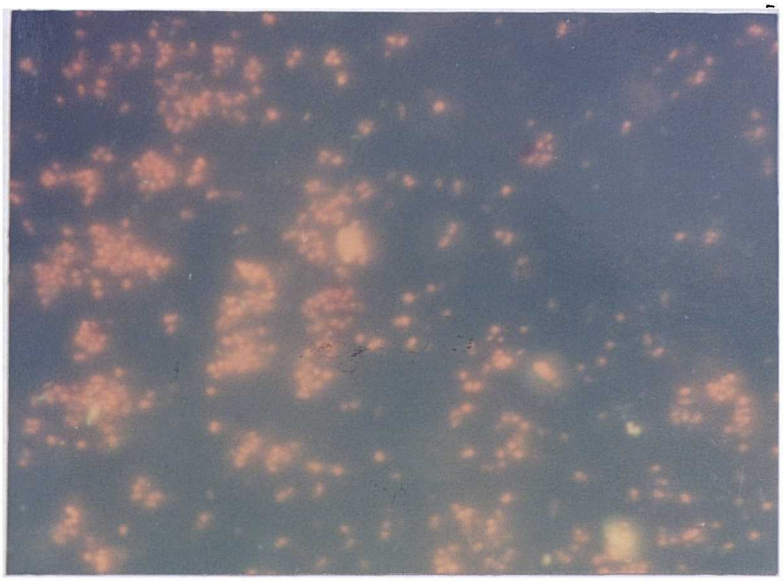


PLATE 3. Actively growing S.faecium

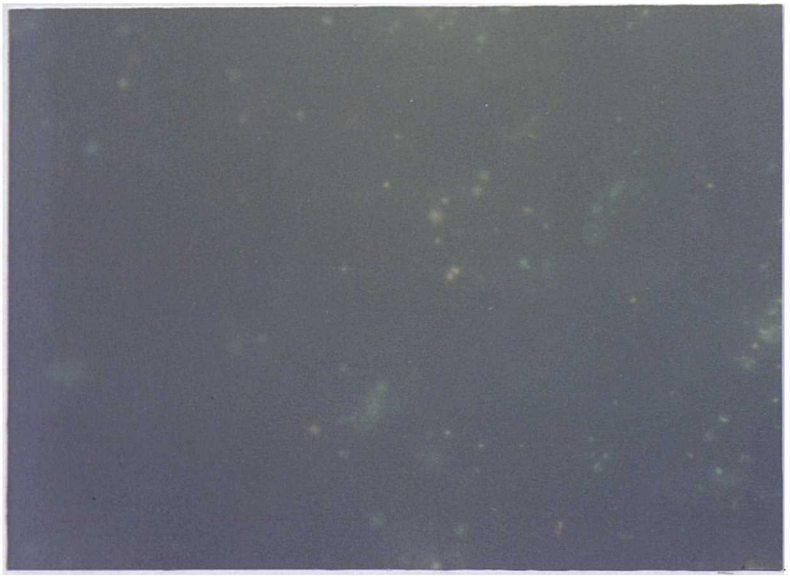


PLATE 4. Starved S.faecium

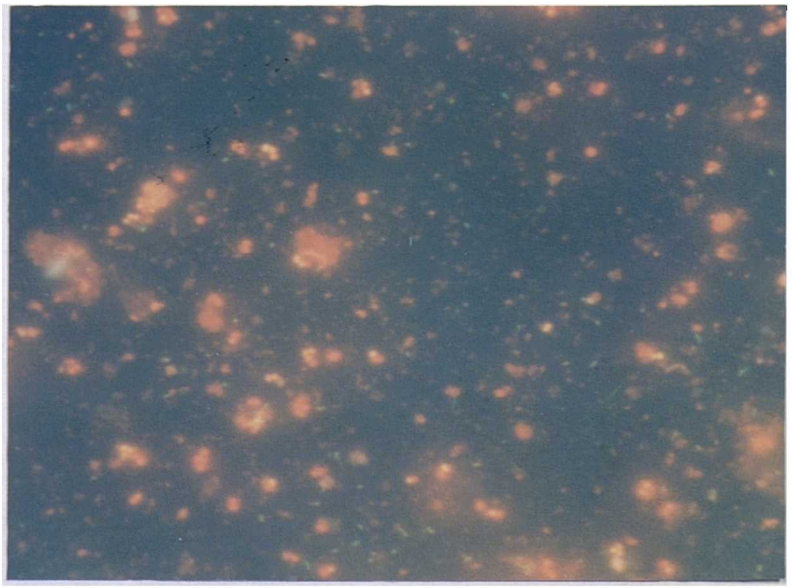


PLATE 5. Enlarged S.faecium in AODVC

VI. DISCUSSION

6.1. Comparison of present study with previous studies

Convincing evidence has been provided by previous studies on the survival characteristics of enteric bacteria which has led to the belief that enteric bacteria are delicate organisms outside their natural habitat and die very quickly on exposure to the stresses of natural waters (Carlucci & Pramer, 1959; Chamberlin & Mitchell, 1978). Evidence which suggests that *E.coli* dies much more rapidly than enterococci and pathogenic bacteria on exposure to the environment has also been presented (Gameson, 1984; Evison & Morgan, 1982). This has led to a gradual decrease in confidence in the usefulness of *E.coli* as an indicator of public health risks involved in bathing in natural waters to the extent that the USEPA now uses solely enterococci for indicating faecal pollution of coastal bathing waters (Cabelli, 1983).

Relatively few workers have indicated the contrary, i.e., that *E.coli* may persist for long periods of time (Gauthier et al., 1987) and in certain situations, outlive supposedly more robust species such as enterococci (Okuofu, 1985).

There is a striking difference between rates of decay of *E.coli* and enterococci in the present study and those reported in other studies also using traditional cultural methods for enumeration. Morgan (1984), in a study employing the same ALS as used in the present study reported much shorter T90 values for *E.coli* both

exposed to the ALS and in dark controls under similar conditions of temperature and salinity.

In the present study, filtration of the settled sewage used for growing the test bacteria in, prior to inoculation into microcosms, was carried out to remove indigenous bacteria and particulate matter which may have inhibited the microscopic enumeration of the test bacteria introduced into the medium. Effectively, by filtration, a fairly low nutrient environment was created which the test bacteria are exposed to, prior to being exposed to the test conditions. This appears to have afforded *E.coli*, at least, with an opportunity to adapt to low nutrient conditions thus extending their survival in filtered seawater and freshwater microcosms under the stresses to which they were then exposed.

Adaptation to low nutrient conditions has been reported (Chai, 1983; Gauthier et al., 1987) which results in physiological changes within adapted cells which apparently increases resistance to environmental stresses. Furthermore, Munro et al. (1989) have presented experimental evidence for the long term protection provided for cells exposed to seawater by increasing the osmotic pressure of the cells by previous growth on a medium containing NaCl. Extended survival is due, in part, to the possession of genes which regulate osmotic pressure and the ability of these bacteria to express these genes in the environment. Adaptation of cells to high salinities is not specific to *E.coli* and

has been shown in other Enterobacteriaceae, e.g., *Salmonella typhimurium* (Munro et al., 1987).

Sewage contains quite high concentrations of NaCl which are present in urine and may, therefore, afford protection to cells grown in it, as do culture media containing NaCl. However, *E.coli* cells grown anaerobically in urine (to simulate the conditions found *in vivo*) result in cells which are more sensitive to seawater than those grown on any bacteriological medium (Munro et al., 1989). This would, in part, explain why decay rates measured *in situ* have often been reported to be higher than those occurring *in vitro*, and suggests that perhaps we should start to reconsider the way in which we culture microorganisms in the laboratory before we try to simulate die-off in the environment.

Unlike *E.coli*, enterococci appear to be unable to adapt to low nutrient concentrations. In fact, even in settled sewage their growth is very weak compared to the growth of *E.coli* and salmonellae. Sinclair and Alexander (1984) provided evidence which suggested that *S.faecalis* declined very rapidly in sterile lake water and that this was probably a result of an inability to obtain nutrients. In a parallel experiment their numbers in settled sewage, which contains higher concentrations of nutrients, remained constant for some time before declining. Bacteria which are sensitive to low nutrient concentrations may also be less able to withstand other environmental stresses than those species which are more resistant to starvation. This would, perhaps, lead to

the supposition that starvation may result in increased sensitivity to light by enterococci, and would explain why the numbers of enterococci in the present study declined so rapidly in the light compared with other studies, and also why they did not survive better in seawater than in freshwater as was previously reported. Their survival in the dark compared quite favourably with the results obtained by Morgan (1984) in similar ALS experiments, whereas survival of *E.coli* in the present study was much greater than their survival as reported by Morgan (1984).

One possible explanation for the differences between decay rates for *E.coli* observed in the present study and those observed in other studies may be that different strains of *E.coli* were used. Many hundreds of strains of *E.coli* have previously been isolated which have shown substantial differences in sensitivity. Indeed, the present study provides evidence of differences in decay rates of two *E.coli* isolates (see Section 5.1.1.3.). Previously reported T90 values for *E.coli* have covered a wide range.

To suit the purposes of this study, i.e., to study the effects of sunlight, temperature and salinity on the survival of starved enteric bacteria in natural waters, it was necessary to remove the influence of a number of bactericidal agents which would otherwise be present and exerting an effect on the survival characteristics of the bacteria under natural conditions. Filtration of microcosm waters removes the indigenous

flora of the water which may be competitors, antagonists or predators of the test bacteria, thereby removing several bactericidal agents which would otherwise be present, and also particulate organic matter which may have a protective effect on the bacteria. Adjustment of pH to a level which was considered to have a negligible effect on bacterial survival (pH 8.0) was also necessary. These modifications may also account for some of the discrepancies between decay rates observed in this study and those observed in other studies.

6.2. Variation in decay rates

Decay rates observed in the present study for replicate ALS experiments are very variable (see Tables 8 and 9, Section 5.2.3.), a surprising observation considering that experimental conditions under which the tests were conducted are supposed to be similar. Gameson (1984) reported that marked changes in decay rates can occur at different times in different experiments, and he suggested that comparisons of different species of bacteria should be made with all the test bacteria being exposed at the same time, together in the same vessel. However, when considering bacterial decay using the AODVC techniques, experiments using more than one species cannot be carried out due to the fact that the method is not selective.

Variations in the nutrient and toxin concentrations of the sewage, used as a culture medium for the test bacteria, may be the main reason for such variable decay rates. Although in the present study,

sewage was always collected from the same location, the composition may vary greatly at different times. Even when the same sewage is used for a number of experiments, only one experiment can be carried out at any one time with the ALS, and so the age of the sewage used in each test will vary, and probably the chemical composition will also change. In natural sunlight and growth cabinet experiments in which many tests can be carried out concurrently, this variation in decay rates is less apparent. Different nutrient concentrations, as shown in this study, greatly affect the responses of bacteria to environmental stresses. Variations in initial proportions of culturable bacteria i.e., at time zero of the exposure period, probably also reflect differences in nutrient concentrations of sewage used for their growth prior to inoculation into microcosms.

Other factors which may affect the response of bacteria to environmental stresses are: salinity, temperature, light intensity, light wavelength, initial number of cells, age of cells, predisposition of cells, nutrient concentration and age of culture medium, and nutrient concentration and age of microcosm waters. Anson and Ware (1975) found that aging of seawater reduced its bactericidal effect.

Zanoni and Fleissner (1982) reported that slight variations in *in vitro* conditions could lead to wide variations in decay rates of test bacteria. This and observations reported herein demonstrate the difficulties involved in controlling all influential

factors to present reproducible conditions to test bacteria and serves to illustrate the magnitude of problems which may be encountered in *in situ* experiments which are subject to much more variation than *in vitro* tests. It is suggested, bearing in mind the observations made in the present study, that a defined dilute medium be used for growth of bacteria prior to their inoculation into microcosms which will not vary in composition between experiments. In conclusion, reproducible decay rates are difficult to obtain but survival trends generally do recur.

6.3. Limitations of enumeration methods

One of the greatest limitations of the present study was that the use of the AODVC restricted the experiments to investigations on pure cultures of bacteria as the AODVC, unlike cultural methods, cannot be made selective. Attempts were made in the present study to develop a selective AODVC for *E.coli* using the selective agents present in bacteriological media, i.e., lauryl sulphate, Teepol 610, and bile salts, but with little success. The results, therefore, are not reported herein. In an attempt to determine whether or not the above agents would select against other organisms such that only *E.coli* cells would enlarge, a mixed culture of *E.coli* and *Vibrio parahaemolyticus* were exposed to them. *V.parahaemolyticus* was chosen as an organism which could be easily distinguished from *E.coli* under the microscope by its morphology.

Fluorescent antibody techniques which are selective, have been developed for detection of *E.coli*, enterococci, salmonellae and other pathogens and which can be modified to produce a DVC of bacteria, but there are several problems inherent in their use. Because so many different serotypes of a particular species, for example *E.coli*, exist, a pooled antiserum would be required to detect as many different serotypes as possible. One cannot be sure even then that all serotypes present are detected. This has, in the past, only proved successful with enteropathogenic strains of *E.coli*. Furthermore, there is a danger of cross-reactivity of the serum with other species, the elimination of which requires extensive screening. As yet fluorescent antibody techniques for enumeration of bacteria remain rather expensive and are still used mainly for diagnostic purposes.

Gene probes for DNA are being developed for a wide range of microorganisms though they do not provide information as to the viability of the cells detected. Moreover, the procedure requires that cells must be grown before DNA can be detected (Grimes, 1986) and, therefore, will not be detected if non-culturable. Unless further developments are made to improve direct detection methods, then studies of viable but non-culturable bacteria will be limited to the use of pure cultures.

An alternative is to attempt to improve existing cultural methods for recovery of all or most of the viable bacteria. Roszak (1986) recovered 'non-

culturable' *E.coli* cells in media diluted 1/10 of its normal laboratory strength. Apart from the use of a resuscitation step at a lower temperature (30°C) for selective counts of *E.coli* and enterococci, and the use of a non-selective medium made up with microcosm water, little attempt was made in the present study to modify existing bacteriological techniques for recovery of stressed bacteria, but this is suggested as an area for further research.

Periodically, non-selective culturable counts fall below the selective culturable counts, usually towards the end of the duration of the experiment, e.g., for *E.coli* in seawater microcosms exposed to natural sunlight and enterococci exposed to natural sunlight. This is probably due to the use of the pour plate method for non-selective counts whereby the bacteria is dispersed in fairly warm agar. Stressed cells are more sensitive to high temperatures than non-stressed cells and may be killed by the warm agar. Enterococci, are particularly sensitive to temperature (Morgan, 1984). An alternative method to use for a non-selective count is the spread plate method, involving spreading the bacteria over the surface of cooled, solidified agar using a bent glass rod. Nevertheless, experience shows that this also results in lowered counts of bacteria because some adhere to the glass rod.

6.4. Viable but non-culturable bacteria

The results of the present study confirm the limitations of cultural methods used for detecting viable

bacteria of public health significance but no convincing evidence for a stable viable but non-culturable state has been provided, except where bacteria were grown previously on a nutrient-rich medium and then exposed to a nutrient-poor medium. That is not to say that this may not occur under natural conditions where sewage is discharged into the environment, but generally progressive dilution of nutrients would occur through gradual dispersion and, therefore, dilution of the sewage into the environment. The survival characteristics of bacteria present in sewage discharges may be dependant on the quality of the receiving waters and how frequently sewage is discharged to them. Exposure of bacteria to progressively lower concentrations of nutrients which may occur during dispersion of sewage discharges into the environment, however, may result in increased survival of the bacteria through adaptation.

The present study has confirmed the existence of those bacteria which under certain conditions cannot be detected by cultural techniques (see Figures 4 and 30). That most of the cells enumerated by the AODVC are metabolically active and potentially able to replicate has been proved in previous studies (Roszak, 1986; Roszak & Colwell, 1987b). In the present study, numbers of bacteria enumerated by the AODVC were consistently higher than numbers enumerated using cultural techniques, an observation also made by numerous other workers (Kogure et al., 1979; Xu et al., 1982).

After sampling a range of waters from pristine to polluted harbour and sewage outfall waters, Roszak (1986) found that those which provided optimum conditions for growth, in terms of nutrients, temperature and salinity, were also those waters having the highest proportion of culturable bacteria.

6.5. Survival and environmental parameters

6.5.1. Nutrient concentrations

It has been shown in this and in previous studies (Xu *et al.*, 1982; Roszak *et al.*, 1984) that bacteria grown in nutrient-rich media and exposed to an environment in which nutrient concentrations are very low, may evolve towards a dormant stage, generally known as the viable but non-culturable form which, by definition, implies that the cells are viable but undetected by cultural methods. Previous growth in a less rich medium, however, appears to induce adaptation of the cells to low nutrient concentrations which allow them to remain culturable and survive longer.

The present study has shown that a heavy inoculum of bacteria into the microcosms inevitably results in an initial rapid rate of decay (see Section 5.1.2.3.). This may be due to competition by a large number of bacteria for very few nutrients. This results in the weaker cells dying very rapidly and the stronger cells surviving and being able to adopt the viable but non-culturable strategy, following depletion of available nutrients. This latter stage is only apparent when

environmental conditions are not too stressful, i.e., in the absence of light and at low temperatures. Novitsky and Morita (1978) maintain that the study of bacteria at low population densities provide a more realistic approach to the study of starvation.

In dark experiments with a lower inoculum, two distinct phases of decay as measured using cultural methods can be frequently observed (see Figures 11-15, Section 5.2.3.). Initial slow decay is followed by rapid decay to zero in both freshwater and seawater microcosms. The rapid decay phase may be due either to accumulation of inhibitory metabolic products or to the developement of bacteria towards a viable but non-culturable form. A much less rapid decline in AODVCs demonstrates that the bacteria are becoming viable but non-culturable. Further evidence is provided by the present study to support this assumption in that the slope of the line for the second phase of decay is influenced by temperature (see Figures 11-15, Section 5.2.3.). This two phase decay pattern was also observed by Evison and Morgan (1982) but was attributed by them to the effect of protozoal predation. The departure from log-linear decay means that the computation of the decay rate constant (k) by linear regression in the way described in Section 4.5.1. may not then be valid.

Statistical analyses of decay rates where departures from log-linear decay occurs, often yield misleading results on survival characteristics. It is

preferable, therefore, to examine plots of the data before any conclusions are made.

6.5.2. Sunlight, temperature and salinity

Generally, bacteria exposed to light from the ALS do not show viable but non-culturable characteristics (see Figures 6-10, Section 5.2.3.), but show increased resistance to the effect of the light predominantly from the visible part of the spectrum, probably through the physiological changes accompanying adaptation to low nutrient concentrations. The resistance is, however, not permanent, and both viability and culturability appear to decrease at the same rates resulting ultimately in the death of the population. Different temperatures and salinities do not appear to influence the rate of decay in this case. The ALS emits light of almost entirely longer wavelengths, the readings taken at 315nm being very low compared with similar readings for the other light sources, i.e., natural sunlight and the growth cabinet. This is evident from the higher decay rates obtained in natural sunlight compared with those obtained at similar light intensities for the ALS and supports the observations made by Gameson and Gould (1985) that about half the lethal effect of sunlight could be attributed to wavelengths lower than 370nm. Considerable bacterial mortality is, however, still caused by light of wavelengths above 370nm.

The present study shows that natural sunlight appears to act synergistically with salinity and possibly with temperature to cause rapid bacterial mortality in

seawater (see Figures 26-29, Section 5.3.1.3.), an observation not made with light emitted from the ALS (death indicated by similar rates of decline of AODVCs and culturable counts). A few exceptions to these observations were made, in fact, whereby the culturable counts of some Gram negative species of bacteria declined more rapidly than the AODVCs did on exposure to natural sunlight in seawater (see Figures 16-25, Section 5.3.1.3.).

One possible explanation for the above observation is that the DNA of the cells may have been damaged by the UV component of sunlight in a way such that they cannot replicate, whilst retaining the ability to take up yeast extract and acridine orange in the AODVC allowing their enlargement and subsequent detection under the microscope. It is thought that the uptake of yeast extract in the AODVC is brought about by changes in the permeability of the membrane induced by nalidixic acid (Roszak, 1986) and so it may still be possible for DNA-damaged cells to enlarge in the AODVC. Moreover, exposure of bacteria to low doses of UV light may result in filamentous growth of the bacteria which then lose their viability (Goss et al., 1964) implying that the enlarged cells counted in the AODVC may have been produced as a result of exposure to UV component of sunlight which may have a similar inhibitory effect as nalidixic acid. However, this is unlikely as this pattern of survival (more rapid decline of culturable

counts than of AODVCs) is also observed in the dark (see Figures 11-15, Section 5.2.3.).

A more likely explanation is that instead of causing death, the light intensities were low enough to allow the bacteria to survive in viable but non-culturable forms. This observation was only made in 1988 experiments when light intensity measurements taken were significantly lower than those taken in 1989 (Section 5.3.1.3.). Most experiments carried out in 1989 were done so in uninterrupted bright sunshine, and rapid death of the test bacteria was observed as opposed to loss of culturability.

No statistically significant relationship was found in the present study between decay rates and light intensity measurements, either expressed as total light intensity received or as mean intensity received, for natural sunlight. It is obvious from the present study, however, that bacteria decay more rapidly at higher intensities (see natural sunlight experiments, Section 5.3.1.3.) than at lower intensities (see growth cabinet experiments, Section 5.5.3.2.). Other workers have reported in laboratory based experiments that the decay rates of *E.coli* in seawater, as estimated using culturable counts, is positively related to the cumulative radiant exposure received (Sieracki, 1980; Gameson & Gould, 1975). Freshwater decay rates for *E.coli* were also found to be related to cumulative radiant exposure received (Sieracki, 1980). No other such relationships have been recorded for salmonellae or

enterococci. This discrepancy demonstrates the difficulties in measuring intensities of natural sunlight. Many different measurements of the emission spectrum of sunlight are given by Henderson (1970), the composition of which are influenced by latitude, atmospheric conditions, time of day, etc. Also, measured radiation is not necessarily radiation contributing to mortality.

It is likely that it is not only the intensity of light which is important but also the wavelengths of light which the bacteria are exposed to. Experimental evidence is provided in the present study to support this. Decay rates of bacteria in natural sunlight were significantly faster than decay rates measured in artificial light source experiments carried out at similar overall light intensities (see Section 5.3.1.3.).

Interestingly, Sieracki (1980) also reported that below a threshold value of light intensity of 12-14 Langleys (or approximately 0.5MJm^{-2}), the decay rate of *E.coli* was not influenced by solar radiation. This is based on decay rates as estimated using cultural techniques. Anson and Ware (1975) also reported that this threshold value may exist but gave no indication of its magnitude except in stating that it would probably be quite low. In the present study light at 0.126MJm^{-2} exerted a significant effect on the mortality of *E.coli* compared with decay in the dark. This indicates that the threshold value must be below 0.126MJm^{-2} (see Section 5.5.3.2.).

The present study employed the use of an equation for a first order reaction (see Section 4.5.1.) in the computation of decay rate constants for both light and dark experiments. This equation has been used in many studies to estimate bacterial mortality rates (Mitchell & Chamberlin, 1978; Morgan, 1984). However, Gameson and Gould (1985), assuming that bacterial decay was a function of both time and light intensity acting independently, incorporated a factor representing cumulative radiation during the period of exposure, into the equation. The use of this equation for the computation of decay rate constants was considered inappropriate in the present study for the following reasons:

1) it assumes that all light causes decay which is not always the case, especially if a threshold intensity exists, as some workers have indicated,

2) it assumes that all mortality is due solely to the effect of light unless decay rates in the dark are measured separately and subtracted from the decay rates estimated using this equation. This means that dark controls must be carried out in every study, which is not always possible, particularly *in situ*,

3) it assumes that the overall intensity measured is that responsible for mortality.

Relationships between environmental parameters, i.e., light, temperature and salinity and the decline in numbers of culturable bacteria in the present study, on

the whole, conform with the observations made in other investigations. These are:

1) that bacterial decay is much faster in the light than in the dark (Gameson & Saxon, 1967; Kapuscinski & Mitchell, 1983),

2) that bacterial decay occurs more rapidly at higher temperatures than at lower temperatures in the dark (McFeters & Stuart, 1972; Faust et al., 1975; Pike et al., 1970),

3) that temperature does not exert a significant effect on bacterial decay in the presence of light (Gameson & Gould, 1975),

4) that the effect of salinity is reduced in low nutrient concentrations (Mitchell & Chamberlin, 1978; Morgan, 1984),

5) that salinity and natural sunlight may act synergistically in reducing the numbers of bacteria (Chojnowski & Mancini, 1979; Sieracki, 1980),

6) that bacterial decay is more rapid at higher light intensities than at lower light intensities (Gameson & Gould, 1975),

7) that enterococci survive longer than E.coli in the dark (Gameson, 1984),

8) that pathogens such as salmonellae survive longer than *E.coli* both in the light and in the dark (Evison & Morgan, 1982; Evison, 1988).

Relationships between environmental parameters and the decline in numbers of bacteria as estimated using the AODVC in the present study are as follows:

1) numbers of bacteria decline more rapidly in the light than in the dark,

2) there appears to be no significant relationship between decline in numbers of bacteria and temperature,

3) there appears to be no significant relationship between decline in numbers of bacteria and salinity,

The latter two observations are consistent with the findings of Roszak (1986). No previous observations on the use of the AODVC in light studies were available to compare the findings of this study with. Roszak (1986) hypothesised that those relationships observed between environmental parameters and decline in numbers of culturable bacteria do hold between environmental parameters and decline in the numbers of viable bacteria (as estimated by the AODVC) because there are many stages to the viable but non-culturable form depending on prevailing conditions. Cells in the earlier stages of this form may regain culturability and the ability to replicate and, therefore, the AODVC does not enumerate a constant proportion of the viable population.

Furthermore, Roszak (1986) noted that up to 28% of the total cell populations consisted of small cells which did not enlarge but which showed metabolic activity and are, therefore, considered as viable. This and other problems in distinguishing viable cells from non-viable cells when using the AODVC are discussed by Al-Hadithi & Goulder (1989) who proposed that a critical length should be decided upon above which cells could be considered viable.

Previously it has been reported that different species of bacteria have different survival capabilities in the dark and in the light (Evison & Morgan, 1982). Although no statistically significant difference was found in the present study between decay rates for different species, examination of the plots for dark experiments (Figures 11-15, Section 5.2.3.) implies that there is a difference in survival of different species and that enterococci and salmonellae survive better than *E.coli* in the dark. On the other hand, in the light (see Section 5.2.3.), a difference in decay rates for different species is statistically evident with salmonellae surviving longer than *E.coli* which, in turn, survive longer than enterococci. Results from the present study also suggest that different strains of a particular species, in this case *S.anatum*, may have similar decay rates if they have a similar life history (see Section 5.6.3.).

6.5.3. Humic acids

The extended survival of bacteria in freshwater compared with in seawater has, in the past, been attributed to low salinity (Carlucci & Pramer, 1960). In low nutrient environments, however, as seen in the present study, the effect of salinity is not observed unless in the presence of UV light with which it may act synergistically (see natural sunlight experiments, Section 5.3.1.3.). Extended survival of bacteria in freshwater compared with their survival in seawater was observed in the present study in natural sunlight and in

the growth cabinet. Morgan (1984) noted that comparatively little difference between seawater and freshwater decay rates was observed at low nutrient concentrations (0.025% settled sewage) for both enterococci and *E.coli*. According to observations made in this study, this extended survival may be due to the presence of UV-absorbing substances including humic acids, which protect cells from possible damage to the DNA by UV light. Absorbance of visible light by humic acids also occurs but to a much lower extent and clearly the effect is not sufficiently significant as to result in lower decay rates of bacteria in freshwater microcosms exposed to the ALS (see Figures 6-10, Section 5.2.3.).

6.5.4. Low light intensities

Survival of selected bacteria at low light intensities in the present study are in accordance with observations made by Barcina et al. (1989) in freshwater microcosms exposed to low intensity visible light (see Section 5.4.). Culturable counts of bacteria decline more rapidly than viable counts in the light, which remain more or less constant, implying loss of culturability whilst retaining viability (see Figure 30). In the dark, however, an increase in culturable counts was observed in the present study (see Figure 31), whereas, Barcina et al. (1989) observed that counts from AODC, AODVC, and cultural methods, remained constant. In the present study, this discrepancy is attributed to the carryover of nutrients from the use of a rich culture medium (in this case TS Broth), and probably a result of

not washing the cells thoroughly. This problem was not encountered at all with the use of filtered settled sewage as a culture medium as it contains much lower concentrations of nutrients than specially formulated culture media such as TS Broth, which if carried over would probably have a negligible effect on survival of bacteria in the microcosms.

Table 26. Penetration of sunlight through seawater and freshwater.

SEAWATER (ZoBell, 1946)

Depth (m)	Transmission (%) Orange 600nm	Green 530nm	Blue 480nm
0	100	100	100
5	18	35	26
10	1.8	16	7.8
15	0.53	7.6	3.9
20	0.27	5.6	2.3
30	0.012	0.12	0.082

FRESHWATER (Westlake, 1965)

Depth (m)	Transmission (%) Red		Green		Blue		Violet	
	A	B	A	B	A	B	A	B
0	100	100	100	100	100	100	100	100
0.1	94	90	97	90	92	85	72	-
0.2	90	82	93	82	90	70	62	34
0.3	88	76	89	76	82	55	55	-
0.4	81	70	84	70	71	45	40	-
0.5	75	65	-	65	-	37	-	13
0.7	-	54	-	55	-	25	-	6-7

Legend: A data for River Frome
B data for River Test

Many processes are involved in reducing the intensity of the light as it penetrates a body of water. Table 26 shows the transmission of light of different

wavelengths through natural waters (values taken from the literature).

Based on percentage transmission values from the literature, the overall intensity measurements taken during the present study in the natural sunlight experiments would be reduced to the overall intensity of the light emitted from the fluorescent tubes in the growth cabinet, in between 5 and 15m in seawater, and probably in less than 1m in freshwater. Although no values in the literature were found, it can be estimated that light at 315nm in natural sunlight would be reduced to the intensity of the light at 315nm emitted in the growth cabinet in the first 5m of seawater. In freshwater, values in the literature indicate that within 0.5-0.7m, the intensity of natural sunlight light at 315nm would be reduced to the intensity of light at 315nm in the growth cabinet.

The implications of experimental evidence given in the present study are that bacteria in freshwater may survive longer than those in seawater because of the protective effect of humic acids present therein. Bactericidal intensities are reduced in freshwater, in less than one metre depth, to intensities which allow the survival of bacteria by inducing dormancy, i.e., viable but non-culturability. In the sea this will occur only over a greater depth. This emphasises the advantages of discharging sewage so that it will rise rapidly to the surface of the water where the bacteria will encounter bactericidal intensities and wavelengths of sunlight.

Discharges made below the thermocline, where this exists, will not be brought to the surface and, therefore, will not be exposed to sunlight.

Nevertheless, one must consider that all experiments carried out in this study were made in filtered waters, whereas under natural conditions both freshwaters and seawaters will most probably contain large amounts of particulate organic matter, especially close to the outfall, which will reduce transmission of light even further. Moreover, the figures given are an overestimation of the penetrative ability of bactericidal UV and visible light. Visible light penetrates much deeper than UV light though the results of this study suggest that it is not as effective a bactericidal agent as UV light. In reality though, it is probably visible light which is responsible for the majority of bacterial mortality in natural waters. The findings of the present study serve to emphasise the importance of sunlight as a bactericidal factor which cannot be repudiated, even for British coastal waters which are often quite turbid.

6.6. The use of ciprofloxacin for AODVCs

One of the limitations of the AODVC is that it can only be used for bacteria which are relatively susceptible to the antibiotic nalidixic acid. This excludes a range of Gram positive bacteria, enterococci among them.

The use of ciprofloxacin as an antibiotic in an AODVC for enterococci brings the viable counts lower, i.e., nearer to the culturable counts, compared with

counts obtained by counting the number of cells stained red by acridine orange. This implies that counting the red cells (carried out in some of the earlier experiments) is an overestimation of the true number of viable enterococci. The modified AODVC using ciprofloxacin, always gives counts which are higher than the culturable counts, indicating that a proportion of the population remains non-culturable.

The size of the 'enlarged' enterococci varies greatly under the microscope indicating that further work may be needed, as with the nalidixic acid AODVC, to determine the critical size above which cells can truely be considered viable. Whether or not a cell has enlarged is not as clear cut a decision as previous reports have implied (Kogure et al., 1979). At times Gram negative cells observed after incubation with nalidixic acid are quite clearly filamentous, whereas at other times cells would only enlarge slightly. A wide variation in sizes of enlarged cells may also be seen in one field of view. Observations made in the present study and by previous workers suggest that improvements must be made in the AODVC technique if it is to provide a reliable indication of numbers of viable cells present.

6.7. Implications of work presented herein

It is impossible to reproduce natural conditions accurately in the laboratory and, indeed, the present study did not set out to do so. The intention was to allow assessment of specific responses of bacteria to

different environmental conditions which may then, with due care, be extrapolated to natural conditions.

The implications of the results of work presented herein are many. The observations made in this study are important with respect to the concern which continues to be expressed about reported outbreaks of disease associated with swimming in waters supposed to be safe according to the criteria of microbiological standards (Cabelli et al., 1982). It is believed that most of these are minor ailments and that many others may go unreported.

Experimental evidence presented herein implies that only those bacteria exposed to sunlight in the surface layers of water will be killed by sunlight. Those which are allowed to remain in the lower layers may remain dormant until favourable conditions for growth prevail, providing they are not killed or removed by the influence of other factors which were eliminated in the present study, i.e., predation, antagonism, competition, etc. These bacteria may be resuspended into the water column by various recreational activities and ingested by bathers whereupon the ability to grow may return, and produce disease if pathogenic.

Gameson (1986) recommended a T_{90} value for culturable total coliforms of 10 hours as a basis for design of outfalls discharging sewage to British coastal waters. This value is based on mortality in daylight in water which is not visibly turbid. Generally, T_{90} values of less than 10 hours (using culturable counts and

AODVCs) are reported in the present study for *E.coli* in seawater exposed to natural sunlight. However, one must also consider the following:

1) The T90 values obtained in the present study were measured in the absence of some of the bactericidal factors which would be present under natural conditions. This indicates that under natural conditions the T90 values may be even lower than the present study suggests.

2) The experiments were carried out in low nutrient concentrations which appears to result in extended survival of some bacteria. This indicates that under natural conditions the T90 values may be lower than the present study suggests.

3) Light intensities encountered in a shallow vessel such as the beakers used in some of the experiments in the present study, are higher than those which would be encountered under natural conditions. This indicates that under natural conditions the T90 values may be higher than the present study suggests.

4) Particulate matter which, under natural conditions has a protective effect on bacteria exposed to sunlight was removed in the present study. This indicates that under natural conditions the T90 values may be higher than the present study suggests.

5) *E.coli* has been found to survive appreciably longer than total coliforms (Gameson & Gould, 1975).

The observations made in the present study do not disagree with the recommendation that a T90 value of 10 hours provides a suitable basis for outfall design.

The recognition of viable but non-culturable bacteria has not increased the health risk of bathing in waters which receive sewage discharges, since these organisms have previously existed (Jannasch, 1967; Hoppe, 1978). On the contrary, this discovery may have decreased the risk by increasing the awareness of the shortcomings of the bacteriological techniques traditionally used in monitoring bathing water quality. But one must also consider that the numbers of culturable bacteria, as a proportion of those bacteria which are potentially able to cause disease, may vary with varying environmental conditions, in particular, nutrient concentrations. This should be given due consideration when making inferences from results of monitoring programmes.

Although it is widely believed that the setting of the EEC standards for bathing waters was not based on scientific or epidemiological evidence, a margin of safety between culturable counts (on which the EEC standards are based) and the true viable counts of bacteria may have been afforded unintentionally. In view of the findings of the present study, indicating that unless sewage bacteria are exposed to bright sunlight they may not be killed but may remain viable and non-culturable, it is believed that lowering the existing standards may be a better safeguard of the health of bathers if the present risk is considered unacceptable.

To suggest the incorporation of standards based on direct detection methods into the existing legislation

is, as yet, out of the question as methods are inadequately selective for individual species, not entirely reliable, and too expensive to use on a routine basis. But to remain aware that those bacteria measured by plate counts, membrane filtration and multiple tubes may be a small proportion of those which are viable, and the ways in which changing environmental conditions can alter the relative proportions of culturable bacteria, is advisable. The importance of the use of resuscitation steps in existing cultural enumeration techniques i.e, employing non-selective media and low temperatures to improve recovery of culturable bacteria, must be emphasised.

The occurrence of viable but non-culturable forms of bacteria is widely reported (Xu et al., 1982; Rollins & Colwell, 1986; Roszak et al., 1984) and bacteria in this form have been likened to the dormant forms of autochthonous marine bacteria and also to the spore-like structures which are produced by sporing bacteria (Roszak & Colwell, 1987a). However, in each of these two cases, the ability to grow on or in culture media returns with favourable conditions irrespective of the time period involved. Viable but nonculturability may be reversible in the early stages but after a certain period the cells can only be cultured after animal passage. As yet no convincing explanation for this has been proposed.

A number of authors have recently noted the formation of new membrane proteins in starved, non-

culturable cells which are not present in actively growing cells (Smigielski et al., 1989; Colbourne et al., 1988). These have been found to be similar to heat-shock proteins and it has been suggested that the formation of heat-shock proteins may indicate the existence of a temperature-linked survival mechanism which may be a feature of all Gram negative bacteria found in the environment (Colbourne et al., 1988). This warrants further investigation.

Investigations into the mechanisms involved in development of cells towards a viable but non-culturable form on a molecular level are clearly beyond the scope of the present study but are suggested for future research.

VII. SUMMARY AND CONCLUSIONS

The present study has served to bring together and review much of the published literature on the survival of bacterial species which are of public health significance, and has pointed out the limitations of some of the previous investigations in this field of study.

Results are reported of experimental work carried out in an attempt to contribute to present knowledge as regards the decay of bacterial indicators and pathogenic bacteria in recreational waters. These experiments represent decay over a wide range of environmental conditions and many of the results obtained conform to the observations made by others. Other observations and the conclusions drawn from them form part of an original contribution to the field.

The following conclusions were made from the work presented herein with consideration of the specific objectives put forward in Chapter III:

1) The UV component of sunlight acts synergistically with salinity and possibly with temperature to cause rapid death of enteric bacteria in seawater. The visible proportion of sunlight alone also causes death but less rapidly, and salinity and temperature do not appear to be involved in this process. In the dark, cells may adapt initially to low nutrient concentrations and resist some of the other stresses. However, after a time when the few existing nutrients have been depleted, they may lose

their culturability at a rate which is influenced by temperature, though they remain viable for some time.

2) Below the surface of a body of water where many processes are involved in reducing the intensity of transmission of sunlight, the intensity may only be a proportion of that of full sunlight and insufficient to be bactericidal. In deeper waters where light intensities are low, bacteria are able to survive by employing the viable but non-culturable strategy.

3) Humic acids in freshwater protect cells from the bactericidal effect of sunlight by absorbing the damaging UV component. Death of bacteria is observed, therefore, at a lower rate than in seawater. Extended survival in freshwater is also due to the absence of high salinity which aggravates the influence of sunlight producing a synergistic effect. Freshwater in the dark may afford bacteria with the opportunity to become viable but non-culturable at low nutrient concentrations.

4) The use of the antibiotic, ciprofloxacin, when incubated for several hours with the enterococcal bacteria and yeast extract, induces some cells to enlarge, the number of which are consistently higher than the number of bacteria enumerated by cultural techniques, thereby indicating the presence of viable but non-culturable enterococci cells.

5) Sunlight has been shown to be the single most important factor affecting the survival of enteric bacteria in natural waters. Although its bactericidal effect is reduced in turbid waters, and its intensity is not particularly high in the UK, on a sunny day it considerably reduces the numbers of enteric bacteria at the surface of marine and fresh waters. However, low intensities of light such as on a dull winters' day will be reduced even further on passage through the water and will, therefore, have reduced effectiveness.

6) A stable viable but non-culturable state only exists in bacteria which are are transferred straight from a nutrient-rich environment to a nutrient-poor environment.

Development of a selective AODVC method is required before this method can be used as routinely as traditional cultural methods are. Also, there is much room for improvement in the AODVC so that one can be sure that enlarged cells represent all, and only, viable cells.

Meanwhile, there is also an urgent need for improvement of existing cultural methods so that they can be used to recover much higher proportions of the viable bacteria present.

Investigations into the possible mechanisms involved in viable but non-culturability, for example the formation of heat-shock proteins, have already been suggested for further research in Chapter VI.

Developments in this area will be much welcomed by public health scientists, and may end the controversy surrounding it. A greater understanding of the problem is needed before more effective bacteriological standards for recreational waters can be set in the future. A problem understood may be a problem avoided.

VIII. REFERENCES

ABSHIRE,R.L. (1976) Detection of enteropathogenic Escherichia coli strains in wastewater by fluorescent antibody. Can. J. Microbiol. 22, 363-378.

ABSHIRE,R.L.& GUTHRIE,R.K. (1971) The use of fluorescent antibody techniques for the detection of Streptococcus faecalis as an indicator of faecal pollution of water. Water Res. 5, 1089-1097.

AGG,A.R. (1981) Cleaning up the coastline. Water 39, 26-28.

AL-HADITHI,S.A., & GOULDER,R. (1989) An alternative approach to the yeast extract-nalidixic acid method for determining the proportion of metabolically active aquatic bacteria. Lett. Appl. Microbiol. 8, 87-90.

ALEXANDER,M. (1986) Survival and growth of bacteria. Environ. Manage. 10, 464-469.

ALLEN, L.A., PASLEY, S.M., & PIERCE, M.S.F. (1952) Conditions affecting the growth of Bacterium coli on bile salts media. Enumeration of this organism in polluted waters. J. Gen. Microbiol. 7, 257-267.

ANDERSON,I.C., RHODES, M., & KATOR,H. (1979) Sublethal stress in Escherichia coli: A function of salinity. Appl. Environ. Microbiol. 38(6), 1147-1152.

ANDERSON,I.C., RHODES,M.W., & KATOR,H.I. (1983) Seasonal variation in survival of Escherichia coli exposed in situ in membrane diffusion chambers containing filtered and nonfiltered estuarine water. Appl. Environ. Microbiol. 45(6), 1877-1883.

ANDREW,M.H.F. & RUSSELL,A.D. (ed.) (1984) The Revival of Injured Microbes. Academic Press: London.

ANON. (1976) Council Directive of 8 December 1975 Concerning the quality of bathing water (76/160/EEC). Off. J. Eur. Communities, L31, 1-7.

ANON. (1987) Drinking Water Microbiology (ed. Cliver,D.O., & Newman, R.A.) Chem-Orbital: Illinois.

ANSON,A.E., & WARE,G.C. (1975) Laboratory studies on the effect of the container on the mortality of E.coli in seawater. Water Res. 9, 895-899.

APHA (1985) Standard Methods for the Examination of Water and Waste Water, 16th Edition. APHA, AWWA, WPCF.

AUBAN, E.G., RIPOLLES, A.A., & DOMARCO, M.J.P. (1983) Relative frequencies and significance of faecal coliforms as indicators related to water temperature. Zb. Mikrobiol. 138, 329-336.

AYRTON,R. (1987) Environmental health and the coastal discharge of sewage. Environ. Hlth. 95(7), 17-28.

BAHKROUF,A., GAUTHIER,M.J., JEDDI,M., & BOUDABBOUS,A. (1988) Starvation survival of Pseudomonas aeruginosa in sea water before and after adaptation to salinity. Lett. Appl. Microbiol. 7(3), 59-62.

BAALSRUD,K. (1975) The case for treatment. In Discharge of Sewage From Sea Outfalls (ed. Gameson,A.L.H.) pp 165 -172. Pergamon Press: Oxford.

BANKS,W.,McPATE,G.R., & DAVISON,I.A. (1983) Howden sewage treatment works. J. Inst. Water Eng. Sci. 37 (6), 485-505.

BARCINA,I., ARANA,I., IRIBERRI,J., & EGEA,L. (1986a) Influence of light and natural microbiota of the Butron River on E.coli survival. Antonie van Leeuwenhoek 52, 555-566.

BARCINA,I., ARANA,I., IRIBERRI,J., & EGEA,L. (1986b) Factors affecting the survival of E.coli in a river. Hydrobiologia 141, 249-253.

BARCINA,I., GONZALEZ,J.M., IRIBERRI,I.J., & EGEA,L. (1989) Effect of visible light on progressive dormancy of Escherichia coli cells during the survival process in natural fresh water. Appl. Environ. Microbiol. 55(1), 246-251.

BARROW,G.I. (1981) Microbial pollution of coasts and estuaries: The Public Health Implications. Water Pollut. Control 80(2), 221-230.

BELLAIR,J.T., PARR-SMITH,G.A., & WALLIS,I.G. (1977) Significance of diurnal variations in fecal coliform die-off rates in the design of ocean outfalls. J. Water Pollut. Control Fed. 49, 2022-2030.

BERG,G. (1978) The indicator system. In Indicators of Viruses in Water and Food (ed. Berg,G.) pp 1-14. Ann Arbor Science: Michigan.

BERG,G. & METCALF,T.M. (1978) Indicators of viruses in water. In Indicators of Viruses in Water and Food (ed. Berg,G.) pp 267-296. Ann Arbor Science: Michigan.

BIANCHI,A.J.M., & BENSOUSSAN,M.G. (1977) Non-marine bacteria in dialysis bags, in seawater. Mar. Pollut. Bull. 8(12), 282-283.

BISSON,J.W. & CABELLI,V.J. (1979) Membrane filter enumeration method for Clostridium perfringen. Appl. Environ. Microbiol. 37, 55-66.

BISSON,J.W. & CABELLI,V.J. (1980) Clostridium as a water pollution indicator. Appl. Environ. Microbiol. 52, 241-248.

BISSONETTE,G.K., JEZESKI,J.J., McFETERS,G.A. & STUART,D.G. (1975) Influence of environmental stress on enumeration of indicator bacteria from natural waters. Appl. Environ. Microbiol. 29, 186-194.

BITTON,G., HENIS,Y., & LAHAV,N. (1972) Effect of several clay minerals and humic acid on the survival of Klebsiella aerogenes exposed to ultraviolet irradiation. Appl. Microbiol. 23, 870-874.

BLASER,M.J., HARDESTY,H.L., POWERS,B., & WANG,W.L.L. (1980) Survival of Campylobacter fetus subsp. jejuni in biological milieus. J. Clin. Microbiol. 11, 309-313.

BOHLOOL, B.B., & SCHMIDT, E.L. (1980) The immuno-fluorescent approach in microbial ecology. Adv. Microb. Ecol. 4, 203-241.

BOLTON,F.J., COATES,D., HUTCHINSON,D.N., & GODFREE,A.F. (1987) A study of thermophilic campylobacters in a river system. J. Appl. Bacteriol. 62, 167-176.

BOLTON,F.J., HINCHCLIFFE,P.M., COATES,D., & ROBERTSON,L. (1982) A most probable number method for estimating small numbers of campylobacters in water. J. Hyg., Camb. 89, 185-190.

BONDE,G.J. (1962) Bacterial Indicators of Water Pollution pp 13-22. Teknisk Forlag: Copenhagen.

BONDE,G.J. (1977) Bacterial indication of water pollution. In Advances In Aquatic Microbiology (ed. Droop,M.R. & Jannasch,H.W.) pp 273-364. Academic Press: London.

BRAYTON,P.R., & COLWELL,R.R. (1987) Fluorescent antibody staining method for enumeration of viable environmental Vibrio cholerae 01. J. Microbiol. Methods 6, 309-314.

BRAYTON,P.R., ROSZAK,D.B., HUQ,S.A., PALMER,L.M., GRIMES,D.J., & COLWELL,R.R. (1984) Public health significance of human pathogens in the ocean. Oceans '84 Marine Pollution Sessions: Washington D.C.

BRAYTON,P.R., TAMPLIN,M.L., HUQ,A., & COLWELL,R.R. (1987) Enumeration of Vibrio cholerae 01 in Bangladesh waters by fluorescent-antibody direct viable count. Appl. Environ. Microbiol. 53(12), 2862-2865.

BROWN,J.M., CAMPBELL,E.A., RICHARDS,A.D., & WHEELER,D. (1987) Sewage pollution of bathing water. Lancet 2(8569), 1208-1209.

BURTON,G.A. Jr., GUNNISON,D., & LANZA,G.R. (1987) Survival of pathogenic bacteria in various freshwater sediments. Appl. Environ. Microbiol. 53(4), 633-638.

CABELLI,V.J. (1977) Indicators of recreational water quality. In Bacterial Indicators/Health Hazards Associated With Water (ed.Hoadley,A.W. & Dutka,B.J.) pp 222-238. ASTM STP 635. American Society for Testing and Materials: Philadelphia.

CABELLI,V.J. (1978) New standards for enteric bacteria. In Water Pollution Microbiology vol.2, (ed. Mitchell, R.) pp 233-271. Wiley: New York.

CABELLI,V.J. (1981) Epidemiology of enteric viral infections. In Viruses and Wastewater Treatment (ed. Goddard,D.M., & Butler,M.) pp 291-304. Pergamon Press: Oxford.

CABELLI,V.J. (1983) Health effects criteria for marine recreational waters. US Environmental Protection Agency. EPA-600/1-80-031. Cincinnati: Ohio.

CABELLI,V.J., DUFOUR,A.P., LEVIN,M.A., McCABE,L.J., & HABERMAN, P.W. (1979) Relationship of microbial indicators to health effects at marine bathing beaches. Am. J. Publ. Hlth. 69(7), 690-696.

CABELLI,V.J., DUFOUR,A.P., McCABE,L.J., & LEVIN,M.A. (1982) Swimming-associated gastroenteritis and water quality. Am. J. Epidemiol. 115(4), 606-616.

CABELLI,V.J., DUFOUR,A.P., McCABE,L.J., & LEVIN,M.A. (1983) A marine recreational water quality criterion consistent with indicator concepts and risk analysis. J. Water Pollut. Control Fed. 55(10), 1306-1313.

CALCOTT,P.H., & POSTGATE,J.R. (1972) Substrate-accelerated death in Klebsiella aerogenes. J. Gen. Microbiol. 70, 115-122.

CALVERT,J.T. (1975) The case against treatment. In Discharge of Sewage From Sea Outfalls (ed. Gameson,A.L.H.) pp 173-177. Pergamon Press: Oxford.

CARLUCCI,A.F., & PRAMER,D. (1959) Factors affecting the survival of bacteria in sea water. Appl. Microbiol. 7, 388-391.

CARLUCCI,A.F., & PRAMER,D. (1960) An evaluation of factors affecting the survival of Escherichia coli in sea water. II. Salinity, pH, and Nutrients. Appl. Microbiol. 8, 247-250.

CHAI,T-J. (1983) Characteristics of Escherichia coli grown in bay water as compared with rich medium. Appl. Environ. Microbiol. 45(4), 1316-1323.

CHAMBERLIN,C.E., & MITCHELL,R. (1978) A decay model for enteric bacteria in natural waters. In Water Pollution Microbiology vol.2, (ed. Mitchell,R.) pp 325-348. Wiley: New York.

CHAN,K-Y., WONG,S.H., & MAK,C.Y. (1979) Effects of bottom sediments on the survival of Enterobacter aerogenes in seawater. Mar. Pollut. Bull. 10, 205-210.

CHERRY,W.B., HANKS,J.B., THOMASON,B.M., MURLIN,A.M., BIDDLE,J.W., & CROOM,J.M. (1972) Salmonellae as an index of pollution of surface waters. Appl. Microbiol. 24(3), 334-340.

CHICK,H. (1908) Investigation of the law of disinfection. J. Hyg. 8, 92-158.

CHOJNOWSKI,K.J., MANCINI,J.L., & JERIS,J.S. (1979) Influence of light on coliform mortality rates in fresh water and seawater. J. Water Pollut. Control Fed. 51 (10), 2538-2539.

COLBOURNE,J.S., PRATT,D.J., SMITH,M.G., FISHER-HOCH,S.P., & HARPER,D. (1984) Water fittings as sources of Legionella pneumophila in a hospital plumbing system. Lancet 1, 210-213.

COLBOURNE,J.S., DENNIS,P.J., TREW,R.M., BERRY,C., & VESEY,G. (1988) Legionella and public water supplies. Water Sci. Technol. 20(11/12), 5-10.

COLWELL,R.R. (1988) The need for interaction between microbiologists and engineers. Water Sci. Technol. 2(11/12), 1-4.

COLWELL,R.R., BRAYTON,P.R., GRIMES,D.J., ROSZAK,D.B., HUQ,S.A., & PALMER,L.M. (1985) Viable but non-culturable Vibrio cholerae and related pathogens in the environment: implications for release of genetically engineered microorganisms. Bio/Technology 3, 817-820.

COLWELL,R.R. & KAPER,J. (1977) Vibrio species as bacterial indicators of potential health hazards associated with water. In Bacterial Indicators/Health Hazards Associated With Water (ed. Hoadley,A.W. & Dutka,B.J.) pp 115-125. ASTM STP 635. ASTM: Philadelphia.

COLWELL,R.R., KAPER,J., & JOSEPH,S.W. (1977) Vibrio cholerae, Vibrio parahaemolyticus, and other vibrios. occurrence and distribution in Chesapeake Bay. Science 198, 394-396.

COLWELL,R.R., TAMPLIN,M.L., BRAYTON,P.R., GAUZENS,A.L., TALL,B.D., HERRINGTON,D., LEVINE,M.M., HALL,S., HUQ,A., & SACK,D.A. (1987) Environmental aspects of Vibrio cholerae in transmission of cholera. Paper presented at the US-Japan Cholera Conference, Williamsburg, Virginia.

COOKE,E.M. (1985) Escherichia coli - an overview. J. Hyg., Camb. 95, 523-530.

CRANE, S.R., & MOORE J.A. (1986) Modeling enteric bacterial die-off: A Review. Water Air Soil Pollut. 27, 411-439.

DALEY,R.J. (1979) Direct epifluorescent enumeration of native aquatic bacteria: uses, limitations, and comparative accuracy. In Native Aquatic Bacteria: Enumeration, Activity, and Ecology (ed. Costerton,J.W., & Colwell,R.R.) pp 14-23. ASTM STP 695. American Society for Testing and Materials.

DALEY,R.J., & HOBBIE,J.E. (1975) Direct counts of aquatic bacteria by a modified epifluorescence technique. Limnol. Oceanogr. 20, 875-882.

DAWE,L.L. & PENROSE,W.R. (1978) "Bactericidal" property of seawater: death or debilitation? Appl. Environ. Microbiol. 35(5), 829-833.

DAWES,E.A. (1977) Endogenous metabolism and the survival of starved prokaryotes. Symp. Soc. Gen. Microbiol. 26, 19-53.

DE HAAN,H. (1974) Effect of a fulvic acid fraction on the growth of a Pseudomonas from Tjeukemeer (The Netherlands). Freshwater Biol. 4, 301-310.

DEITZ,W.H., COOK,T.M., & GOSS,W.A. (1966) Mechanism of action of nalidixic acid on Escherichia coli. III. Conditions required for lethality. J. Bacteriol. 91, 768-773.

DENNESS,B. (1987) Sewage Disposal to Sea. Sons of Neptune Ltd. : Scarborough.

DHSS/HMSO (1984) Bacteriological Examination of Drinking Water Supplies 1982. Methods for the Examination of Waters and Associated Materials. Report 71. HMSO.

DOE (1988) Review of operational and experimental techniques for the removal of bacteria, viruses and pathogens from sewage effluents. PECD 7/7/260. C.E.S. Ltd.

DOMEK,M.J., LECHEVALLIER,M.W., CAMERON,S.C., & McFETERS,G.A. (1984) Evidence for the role of copper in the injury process of coliform bacteria in drinking water. Appl. Environ. Microbiol. 48(2), 289-293.

DUFOUR,A.P. (1977) Escherichia coli: the fecal coliform. In Bacteria Indicators/Health Hazards Associated With Water (ed. Hoadley,A.W. & Dutka,B.J.) pp 48-58. ASTM STP 635. ASTM: Philadelphia.

DUFOUR,A.P. (1984a) Health effects criteria for fresh recreational waters. EPA-600/1-84-004. U.S. Environmental Protection Agency: Cincinnati,Ohio.

DUFOUR,A.P. (1984b) Bacterial indicators of recreational water quality. Can. J. Publ. Hlth. 75,49-56.

DUTKA,B.J. (1984) Sensitivity of Legionella pneumophila to sunlight in fresh and marine waters. Appl. Environ. Microbiol. 48(5), 970-974.

DUTTON, R.J., BITTON, G., & KOOPMAN, B. (1986) Application of a direct microscopic method of the determination of active bacteria in lakes. Water Res. 20(11), 1461-1464.

EISENSTARK,A. (1971) Mutagenic and lethal effects of visible and near-ultraviolet light on bacterial cells. Adv. Genet. 16, 167-198.

EL-SHARKAWI,F., EL-ATTAR,L., GAWAD,A.A., & MOLAZEM,S. (1989) Some environmental factors affecting survival of fecal pathogens and indicator organisms in seawater. Water Sci. Technol. 21(1), 115-120.

ELLIOT,E.L., & COLWELL,R.R. (1985) Indicator organisms for estuarine and marine waters. FEMS Microbiol. Rev. 32, 61-79.

EMERSON, D.J., & CABELLI,V.J. (1982) Extraction of Clostridium perfringens spores from bottom sediment samples. Appl. Environ. Microbiol. 44, 1144-1149.

ENZINGER,R.M., & COOPER,R.C. (1976) Role of bacteria and protozoa in the removal of E.coli from estuarine waters. Appl. Environ. Microbiol. 31, 758-763.

EPA (1976) Quality criteria for water. EPA/440/9-76/023. U.S. Environmental Protection Agency: Washington.

EPA (1985) Coliform bacteria. In Rates, Constants, and Kinetics Formulations in Surface Water Quality Modelling, 2nd Edition. pp 424-455. EPA/600/3-85/040. U.S. Environmental Protection Agency: Washington.

EPA (1986) Ambient water quality criteria for bacteria. EPA/440/5-84/002. Environmental Protection Agency: Washington.

EVISON,L.M. (1985) Bacterial pollution of coastal waters in the UK and Mediterranean. J. Appl. Bacteriol. Symp. Supp. 81S-93S.

EVISON,L.M. (1988) Comparative studies on the survival of indicator organisms and pathogens in fresh and sea water. Water Sci. Technol. 20(11/12), 309-315.

EVISON,L.M., & MORGAN,S. (1982) Survival of pathogens and indicators in sea water. Paper presented at a meeting of IWPC and IPHE, 3-11-82.

EVISON, L.M., & TOSTI, E. (1980) An appraisal of bacterial indicators of pollution in sea water. Prog. Water Technol. 12, 591-599.

FAUST,M.A., AOTAKY,A.E., & HAGADON,M.T. (1975) Effect of physical parameters on the in situ survival of Escherichia coli MC-6 in an estuarine environment. Appl. Microbiol. 30, 800-806.

FLIERMANS,C.B., & SCHMIDT,E.L. (1975) Autoradiography and immunofluorescence combined for autecological study of single cell activity with Nitrobacter as a model. Appl. Microbiol. 30(4), 676-684.

FLINT,K.P. (1987) The long-term survival of Escherichia coli in river water. J. Appl. Bacteriol. 63(3), 261-270.

FOXWORTHY,J.E., & KNEELING,H.R. (1969) Eddy diffusion and bacterial reduction in waste fields in the ocean. University of Southern California, Los Angeles, Report 69-1.

FOULON, G., MAURIN, J., NGUYEN NGOC QUOI, & MARTIN-BOUYER,G. (1983) Relationship between the microbiological quality of bathing water and health effects. A preliminary survey. Revue Francaise des Sciences de L'Eau 2, 127-143.

FRANCISCO,D.E., MAH,R.A., & RABIN,A.C. (1973) Acridine orange-epifluorescence technique for counting bacteria in natural waters. Trans. Am. Microsc. Soc. 92(3), 416-421.

FUJIOKA,R.S., HASHOMOTO,H.H., SIWAK,E.B., & YOUNG, R.H.P. (1981) Effect of sunlight on survival of indicator bacteria in seawater. Appl. Environ. Microbiol. 41(3), 690-696.

FUJIOKA,R.S., & NARIKAWA, O.T. (1982) Effect of enumeration of indicator bacteria under field conditions. Appl. Environ. Microbiol. 44(2), 395-401.

FUJIOKA, R.S., & SHIZUMURA,L.K. (1985) Clostridium perfringens, a reliable indicator of stream water quality. J. Water Pollut. Control Fed. 57(10), 986-992.

GAARDER,T. & SPARCK,R. (1931) III. Biochemical and biological investigations of variation in the productivity of the West Norwegian oyster pools. Conseil. Perm. Internat. p. L'Explor. de la Mer. 75, 47-58.

GALBRAITH,N.S., BARRETT, N.J., & STANWELL-SMITH,R. (1987) Water and disease after Croyden: A review of water-borne and water-associated disease in the UK 1937-86. J. Inst. Water Environ. Manag. 1(1), 7-21.

GAMESON,A.L.H. (1975) Discharge of Sewage From Sea Outfalls. Pergamon Press: Oxford.

GAMESON,A.L.H. (1979) EEC Directive on quality of bathing water. Water Pollut. Control 78(2), 206-214.

GAMESON, A.L.H. (1984) Investigations of Sewage Discharges to Some British Coastal Waters. Chapter 8, Bacterial mortality, Part 1. Water Research Centre, Technical Report TR 201.

GAMESON,A.L.H. (1985) Application of Coastal Pollution Research, Part 5, Microbial mortality. Water Research Centre, Technical Report TR 228.

GAMESON,A.L.H. (1986) Investigations of Sewage Discharges to Some British Coastal Waters. Chapter 8, Bacterial mortality, Part 3. Water Research Centre, Technical Report TR 239.

GAMESON,A.L.H., and GOULD,D.J. (1975) Effects of solar radiation on the mortality of some terrestrial bacteria in sea water. In Discharge Of Sewage From Sea Outfalls (ed. Gameson,A.L.H.) pp 209-219. Pergamon Press: Oxford.

GAMESON,A.L.H., and GOULD,D.J. (1985) Investigations of Sewage Discharges to Some British Coastal Waters. Chapter 8, Bacterial mortality, Part 2. Water Research Centre, Technical Report TR 222.

GAMESON, A.L.H., PIKE, E.B., and DAVEY, K.W. (1973) Mortality of coliform bacteria in sea water samples in daylight. Proceedings of the 5th International Colloquium on Medical Oceanography, Messina. pp 353-362.

GAMESON,A.L.H., and SAXON,J.R. (1967) Field studies on effect of daylight on coliform bacteria. Water Res. 1, 279-295.

GATES,F.L. (1930) A study of the bactericidal action of ultra violet light. III. The absorption of ultra violet light by bacteria. J. Gen. Physiol. 14, 31-42.

GAUTHIER, M.J., MUNRO, P.M., & MOHAJER, S. (1987) Influence of salts and sodium chloride on the recovery of E.coli from sea water. Current Microbiol. 15, 5-10.

GAUTHIER,M.J., MUNRO,P.M., & BREITTMAYER,V.A. (1989) Influence of prior growth conditions on low nutrient response of Escherichia coli in seawater. Can. J. Microbiol. 35, 379-383.

GEESEY,G.G., & COSTERTON,J.W. (1981) Application of epifluorescence microscopy to the enumeration of aquatic bacteria concentrated on membrane filters. In Membrane Filtration: Applications, Techniques, and Problems (ed. Dutka,B.J.) pp 253-264. Marcel Dekker, Inc.: New York.

GELDREICH,E.E. (1972) Water-borne pathogens. In Water Pollution Microbiology (ed. Mitchell,R.) pp 207-241. Wiley: New York.

GELDREICH,E.E. (1978) Bacterial populations and indicator concepts in feces, sewage, stormwater and solid wastes. In Indicators of Viruses in Water and Food (ed. Berg,G.) pp 51-98. Ann Arbor Science: Michigan.

GELDREICH,E.E., & KENNER,B.A. (1969) Concepts of fecal streptococci in stream pollution. J. Water Pollut. Control Fed. 41, 336-352.

GERBA,C.P., & McLEOD,J.S. (1976) Effects of sediments on the survival of Escherichia coli in marine waters. Appl. Environ. Microbiol. 32(1), 114-120.

GOSS,W.A., DEITZ,W.H., & COOK,T.M. (1964) Mechanism of action of nalidixic acid on Escherichia coli. J. Bacteriol. 88(4), 1112-1118.

GOULD,D.J., & MUNRO,D. (1981) Relevance of microbial mortality to outfall design. In Coastal Discharges - Engineering Aspects and Experience pp 45-50. Thomas Telford Ltd: London.

GREENBERG,A.E. (1956) Survival of enteric organisms in sea water. Pub. Hlth Rep., Wash. 71(1), 77-86.

GRIGSBY,P., & CALKINS,G. (1980) The inactivation of a natural population of coliform bacteria by sunlight. Photochem. Photobiol. 31, 291-294.

GRIMES,D.J. (1975) Release of sediment-bound fecal coliforms by dredging. Appl. Microbiol. 29(1), 109-111.

GRIMES,D.J. (1980) Bacteriological water quality effects of hydraulically dredging contaminated upper Mississippi River bottom sediment. Appl. Environ. Microbiol. 39(4), 782-789.

GRIMES,D.J. (1982) Bacteriological water quality effects of clamshell dredging. J. Freshwater Ecol. 1(4), 407-419.

GRIMES,D.J. (1986) Assessment of Ocean Waste Disposal, Final Report: 5. Human health impacts of waste constituents. US Congress OTA Report, Contract No. 533-2685.0, pp 1-132.

GRIMES,D.J., ATTWELL,R.W., BRAYTON,P.R., PALMER,L.M., ROLLINS,D.M., ROSZAK,D.B., SINGLETON,F.L., TAMPLIN, M.L., & COLWELL,R.R. (1986) The fate of enteric pathogenic bacteria in estuarine and marine environments. Microbiol. Sci. 3, 324-329.

GRIMES,D.J. & COLWELL,R.R. (1986) Viability and virulence of Escherichia coli suspended by membrane chamber in semitropical ocean water. FEMS Microbiol. Lett. 34, 161-165.

GROSS,R.J., & ROWE,B. (1985) Escherichia coli diarrhoea. J. Hyg., Camb. 95, 531-550.

GUNNERSON,C.G. (1975) Discharge of sewage from sea outfalls. In Discharge of Sewage From Sea Outfalls (ed. Gameson,A.L.H.) pp 415-425. Pergamon Press: Oxford.

HACKNEY,C.R., RAY,B., & SPECK,M.L. (1979) Repair detection procedure for enumeration of fecal coliforms and enterococci from seafoods and marine environments. Appl. Environ. Microbiol. 37, 947-953.

HANES,N.B., SARLES,W.B., & ROHLICH,G.A. (1964) Dissolved oxygen and survival of coliform organisms and enterococci. J. Am. Water Works Assoc. 56, 441-446.

HARREMOES,P. (1975) In situ methods for determination of microbial disappearance in sea water. In Discharge of Sewage From Sea Outfalls (ed. Gameson,A.L.H.) pp 181-189. Pergamon Press: London.

HARRISON,A.P. Jr. (1967) Harmful effects of light, with some comparisons with other adverse physical agents. Ann. Rev. Microbiol. 21, 143-156.

HARVEY,G.R., & BORAN,D.A. (1985) Geochemistry of humic substances in seawater. In Humic Substances In Soil, Sediment, and Water (ed. Aiken,G.R., McKnight,D.M., Wershaw,R.L., & Macarthy,P.) pp 233-247. Wiley: New York.

HEAD,P.C., & JAMES,A. (1970) Influence of the River Tyne on bacteriological and chemical quality of inshore waters of the north-east coast of England. Revue Int. Oceanogr. Med. 17, 147-158.

HEATHMAN,L.S., PIERCE,S.O., & KABLER,P.W. (1936) Resistance of various strains of S.typhi and coli-aerogenes to chlorine and chloramine. Pub. Hlth. Rep. 51, 1367-1372.

HENDERSON,S.T. (1970) Daylight and Its Spectrum. Adam Hilger Ltd.: Bristol.

HENDRICKS,C.W. (1972) Enteric bacterial growth rates in river water. Appl. Microbiol. 24(2), 168-174.

HENDRICKS,C.W., & MORRISON,S.M. (1967) Multiplication and growth of selected enteric bacteria in clear mountain stream water. Water Res. 1, 567-576.

HOADLEY,A.W. (1981) Effect of injury on the recovery of bacteria on membrane filters. In Membrane Filtration: Applications, Techniques, and Problems (ed. Dutka, B.J.) pp 413-450. Marcel Dekker, Inc.: New York.

HOADLEY,A.W. & CHENG,C.M. (1974) The recovery of indicator bacteria on selective media. J. Appl. Bacteriol. 37, 45-57.

HOADLEY,A.W., & DUTKA,B.J. (eds.) (1977) Bacterial Indicators/Health Hazards Associated With Water. ASTM STP 635. ASTM: Philadelphia.

HOBBIE,J.E., DALEY,R.J., & JASPER,S. (1977) Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. 33(5), 1225-1228.

HOLLAENDER,A. (1943) Effect of long ultraviolet and short visible radiation (3500 to 4900A) on Escherichia coli. J. Bacteriol. 46, 531-541.

HOOD,M.A., & NESS,G.E. (1982) Survival of Vibrio cholerae and Escherichia coli in estuarine waters and sediments. Appl. Environ. Microbiol. 43(3), 578-584.

HOPPE,H.G. (1978) Relations between active bacteria and heterotrophic potential in the sea. Neth. J. Sea Res. 12(1), 78-98.

HURST,A. (1977) Bacterial injury: a review. Can. J. Microbiol. 23, 936-944.

HUSSONG,D., COLWELL,R.R., O'BRIEN,M., WEISS,E., PEARSON, A.D., WEINER,R.M., & BURGE,W.D. (1987) Viable Legionella pneumophila not detectable by culture on agar media. Bio/Technology 5, 947-950.

ICE (1981) Coastal Discharges- Engineering Aspects and Experience. Thomas Telford Ltd.: London.

JAGGER,J., STAFFORD,R.S., & SNOW,J.M. (1969) Thymidine-dimer and action-spectrum evidence for indirect photoreactivation in Escherichia coli. Photochem. Photobiol. 10, 383-395.

JAMIESON,W., MADRI,P., & CLAUS,G. (1976) Survival of certain pathogenic microorganisms in seawater. Hydrobiologia 50, 117-121.

JANNASCH,H.W. (1967) Growth of marine bacteria at limiting concentrations of organic carbon an seawater. Limnol. Oceanogr. 12, 264-271.

JOHANNESSON,J.K. (1957) Nature of the bactericidal agent in seawater. Nature 180, 285-286.

JONES,F. & WATKINS,J. (1985) The water cycle as a source of pathogens. J. Appl. Bacteriol. Symp. Suppl. 63, 27S-36S.

JONES,G.E. (1964) Effect of chelating agents on the growth of Escherichia coli in seawater. J. Bacteriol. 87(3), 483-499.

JONES,G.E. (1971) The fate of freshwater bacteria in the sea. Dev. Ind. Microbiol. 12, 141-151.

JONES,J.G. (1979) A Guide to Methods for Estimating Microbial Numbers and Biomass in Fresh Water. Freshwater Biological Association No. 39. Wilson & Son Ltd.: Kendal.

JØRGENSON,E.G. (1962) Antibiotic substances from cells and culture solutions of unicellular algae with special references to some chorophyll derivatives. Physiologia Plantarium 15, 530-545.

KAPUSCINSKI,R.B., & MITCHELL,R. (1981) Solar radiation induces sublethal injury in Escherichia coli in seawater. Appl. Environ. Microbiol. 41(3), 670-674.

KAPUSCINSKI, R.B., & MITCHELL, R. (1983) Sunlight-induced mortality of viruses and Escherichia coli in coastal seawater. Environ. Sci. Technol. 17(1), 1-6.

KAY,D., & McDONALD,A. (1986) Bathing water quality. European Water and Sewage 90(1085), 321-328.

KELNER, A. (1949) Photoreactivation of ultraviolet-irradiated Escherichia coli, with special reference to the dose-reduction principle and to ultraviolet-induced mutation. J. Bacteriol. 58, 511-522.

KING,A., & PHILLIPS,I. (1986) The comparative in-vitro activity of eight newer quinolones and nalidixic acid. J. Antimicrob. Chemother. 18, Suppl. D, 1-20.

KLEIN,D.A., & CASIDA,L.E. JR. (1967) Escherichia coli die-out from normal soil as related to nutrient availability and the indigenous microflora. Can. J. Microbiol. 13, 1461-1470.

KNILL,M.J., SUCKLING,W.G., & PEARSON,A.D. (1982) Campylobacters from water. In Campylobacter: Epidemiology, Pathogenesis and Biochemistry (ed. Newell,D.G.) pp 281-284. MTP Press: Lancaster.

KOGURE,K., SIMIDU,U., & TAGA,N. (1979) A tentative direct microscopic method for counting living marine bacteria. Can. J. Microbiol. 25, 415-420.

KOGURE,K., SIMUDU,U., & TAGA,N. (1984) An improved direct viable count method for aquatic bacteria. Arch. Hydrobiol. 102(1), 117-122.

KOGURE,K., SIMIDU,U., TAGA,N., & COLWELL,R.R. (1987) Correlation of direct viable counts with heterotrophic activity for marine bacteria. Appl. Environ. Microbiol. 53(10), 2332-2337.

KOTT,Y., BEN-ARI,H., & VINOKUR,L. (1978) Coliphages survival as viral indicator in various wastewater quality effluents. Prog. Water Technol. 10(1/2), 337-346.

KOUL,K., & PANHOTRA,B.R. (1988) Isolation of enteropathogenic and enterotoxigenic Escherichia coli from the Dal Lake water of Kashmir. Lett. Appl. Microbiol. 7, 109-111.

KRINSKY,N.I. (1977) Cellular damage initiated by visible light. Symp. Soc. Gen. Microbiol. 26, 209-239.

LALIBERTE,P., & GRIMES,D.J. (1982) Survival of Escherichia coli in lake bottom sediment. Appl. Environ. Microbiol. 43(3), 623-628.

LIM,C.-H., & FLINT,K.P. (1989) The effects of nutrients on the survival of Escherichia coli in lake water. J. Appl. Bacteriol. 66, 559-569.

LUCENA,F., FINANCE,C., JOFRE,J., SANCHO,J., & SCHWARZBROD,L. (1982) Viral pollution determination of the superficial waters (river water and sea water) from the urban area of Barcelona. Water Res. 16, 173-177.

MACDONELL,M.T., & HOOD,M.A. (1982) Isolation and characterisation of ultramicrobacteria from a Gulf Coast estuary. Appl. Environ. Microbiol. 43, 566-571.

MALCOLM,R.L. (1985) Geochemistry of stream fulvic and humic substances. In Humic Substances in Soil, Sediment, and Water (ed. Aiken,G.R., McKnight,D.M., Wershaw,R.L., & Macarthy,P.) pp 203-210. Wiley: New York.

MANCINI,J.L. (1978) Numerical estimates of coliform mortality rates under various conditions. J. Water Pollut. Control Fed. 50(11), 2477-2484.

MATHEWS,M.M., & SISTROM,W.R. (1960) The function of the carotenoid pigments of Sarcina lutea. Arch. Mikrobiol. 35, 139-146.

McCAMBRIDGE,J., & McMEEKIN,T.A. (1979) Protozoan predation of Escherichia coli in estuarine waters. Water Res. 13, 659-663.

McCAMBRIDGE,J., & McMEEKIN,T.A. (1981) Effect of solar radiation and predacious microorganisms on survival of faecal and other bacteria. Appl. Environ. Microbiol. 41(5), 1083-1087.

McCOY,J.H. (1964) Salmonellae in crude sewage, sewage effluent and sewage-polluted natural waters. In Advances in Water Pollution Research (ed. Southgate,B.A.) pp 205-226. Pergamon Press : Oxford.

McFETERS,G.A., BISSONETTE,G.K., JEZESKI,J.J., THOMSON, C.A., & STUART,D.G. (1974) Comparative survival of indicator bacteria and enteric pathogens in well water. Appl. Microbiol. 27(5), 823-829.

McFETERS,G.A., CAMERON,S.A., & LE CHEVALLIER,M.W. (1982) Influence of diluents, media and membrane filters on detection of injured water-borne coliform bacteria. Appl. Environ. Microbiol. 43, 97-103.

McFETERS,G.A., & STUART,D.G. (1972) Survival of coliform bacteria in natural waters: Field and laboratory studies with membrane-filter chambers. Appl. Microbiol. 24(5), 805-811.

McFETERS,G.A., & STUART,D.G. (1981) In situ studies using membrane filter chambers. In Membrane Filtration : Applications, Techniques, and Problems (ed. Dutka, B.J.) pp 481-512. Marcel Dekker Inc.: New York.

MCS & CAPL (1987) The Golden List of British Beaches. MSC & CAPL: Ross-on-Wye.

MESQUITA,M.M.F. (1988) Bacterial and bacteriophage investigations using the mussel Mytilis edulis. Ph.D Thesis. University of Newcastle upon Tyne.

MITCHELL,R. (1968) Factors affecting the decline of non-marine micro-organisms in seawater. Water Res. 2, 535-543.

MITCHELL, R., & CHAMBERLIN, C. (1975) Factors influencing the survival of enteric microorganisms in the sea: An Overview. In Discharge of Sewage From Sea Outfalls (ed. Gameson,A.L.H.) pp 237-248. Pergamon Press: Oxford.

MITCHELL,R. & CHAMBERLIN,C. (1978) Survival of indicator organisms. In Indicators of Viruses in Water and Food (ed. Berg,G.) pp 15-37. Ann Arbor Science: Michigan.

MITCHELL,R., & MORRIS,J.C. (1969) The fate of intestinal bacteria in the sea. In Advances In Water Pollution Research (ed. Jenkins,S.H.) pp 811-817. Pergamon Press: London.

MOORE,B. (1975) The case against microbial standards for bathing beaches. In Discharge of Sewage From Sea Outfalls (ed. Gameson,A.L.H.) pp 103-109. Pergamon Press: Oxford.

MOORE,B. (1977) The EEC Bathing Water directive. Mar. Pollut. Bull. 8(12), 269-272.

MORGAN,S. (1981) Survival of intestinal bacteria and bacteriophages in sea water. WRC Project No. EP 1338RX. University of Newcastle upon Tyne.

MORGAN,S. (1984) Resistance of pathogens to environmental conditions. MRC Report.

MORITA,R.Y. (1985) Starvation and miniaturisation of heterotrophs with special emphasis on maintenance of the starved viable state. In Bacteria In Their Natural Environments (ed. Fletcher,M. & Floodgate,G.D.) pp 111-130. Academic Press: Orlando.

MORRIS,J.G. (1975) The physiology of obligate anaerobiosis. Adv. Microb. Physiol. 12, 169-246.

MOSS,S.H., & SMITH,K.C. (1981) Membrane damage can be a significant factor in the inactivation of Escherichia coli by near-ultraviolet radiation. Photochem. Photobiol. 33, 203-210.

MUNRO,P.M., GAUTHIER,M.J., BREITTMAYER,V.A., & BONGIOVANNI,J. (1989) Influence of osmoregulation processes on starvation survival of Escherichia coli in seawater. Appl. Environ. Microbiol. 55(8), 2017-2024.

MUNRO,P.M., GAUTHIER,M.J., & LAUMOND,F.M. (1987a) Changes in Escherichia coli cells starved in seawater-wastewater mixtures. Appl. Environ. Microbiol. 53(7), 1476-1481.

MUNRO,P.M., LAUMOND,F.M., & GAUTHIER,M.J. (1987b) A previous growth of enteric bacteria on a salted medium increases their survival in seawater. Lett. Appl. Microbiol. 4, 121-124.

NABBUT,N.H., & KURAYIYYAH,F. (1972) Survival of Salmonella typhi in sea-water. J. Hyg., Camb. 70, 223-228.

NOVITSKY,J.A., & MORITA,R.Y. (1978) Possible strategy for the survival of marine bacteria under starvation conditions. Mar. Biol. 48, 289-295.

OCCHIPINTI,A.G. (1975) Discussion to Mitchell & Chamberlin. In Discharge of Sewage From Sea Outfalls (ed. Gameson,A.L.H.) pp 248-249. Pergamon Press: Oxford.

OKUOFU,C.A. (1985) The water pollution indicator potential of bifidobacteria. Ph.D Thesis. University of Newcastle upon Tyne.

ORLOB,G.T. (1956) Viability of sewage bacteria in sea water. Sew. Ind. Wastes 28, 1147-1167.

PALMER,L.M., BAYA,A.M., GRIMES,D.J., & COLWELL,R.R. (1984) Molecular genetic and phenotypic alteration of Escherichia coli in natural water microcosms containing toxic chemicals. FEMS Microbiol. Lett. 21, 169-173.

PEARSON,A.D., LIOR,H., HOOD,A.M., & HAWTIN,P. (1985) Longitudinal study of the occurrence, biotype and serogroup of Campylobacter jejuni and C.coli isolations from environmental sources in southern England between 1977-1985. In Campylobacter III (ed. Pearson,A.D., Skirrow,M.B., Lior,H., & Rowe,B.) pp 279-280. PHLS: London.

PEELE,E.R., & COLWELL,R.R. (1981) Application of a direct microscopic method for enumeration of substrate-responsive marine bacteria. Can. J. Microbiol. 27, 1071-1075.

PHILIPP,R., EVANS,E.J., HUGHES,A.O., GRISDALE,S.K., ENTICOTT,R.G., & JEPHCOTT,A.E. (1985) Health risks of snorkel swimming in untreated water. Int. J. Epidemiol. 14 (4), 624-627.

PHLS (1959) Sewage contamination of coastal bathing waters in England and Wales. J. Hyg., Camb. 57, 435-472.

PIKE,E.B. (1987) Sewage disposal to the sea and the survival of pathogens. Water Research Centre, Report PRU 1542-M.

PIKE,E.B. & GAMESON,A.L.H. (1970) Effects of marine sewage disposal. Water Pollut. Control 69(4), 355-382.

PIKE,E.B., GAMESON,A.L.H., & GOULD,D.J. (1970) Mortality of coliform bacteria in sea water samples in the dark. Rev. Intern. Oceanogr. Med. 18-19, 97-106.

PORTER,K.G., & FEIG,Y.S. (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. 25(5), 943-948.

POSTGATE,J.R. (1977) Death in macrobes and microbes. Symp. Soc. Gen. Microbiol. 26, 1-19.

PRAKASH,A., & MacGREGOR,D.J. (1983) Environmental and human health significance of humic materials: an overview. In Aquatic and Terrestrial Humic Materials (ed. Christman,R.F., & Gjessing,E.T.) Ann Arbor Science: Michigan.

PUGSLEY,A.P., & EVISON,L.M. (1974a) A membrane filtration staining technique for detection of viable bacteria in water. Water Treat. Exam. 20, 1457-1463.

PUGSLEY,A.P., & EVISON,L.M. (1974b) Immunofluorescence as a method for the detection of Escherichia coli in water. Can. J. Microbiol. 20, 1457-1463.

PUGSLEY,A.P., & EVISON,L.M. (1974c) Immunofluorescence identification of faecal streptococci using commercially-available antisera. Water Res. 8, 725-728.

PUGSLEY,A.P., & EVISON,L.M. (1975) A fluorescent antibody technique for the enumeration of faecal streptococci in water. J. Appl. Bacteriol. 38, 63-65.

PURDY,W.C., & BUTTERFIELD,C.T. (1918) The effect of plankton animals upon bacterial death rates. Am. J. Publ. Hlth. 8, 499-505.

RAO,V.C., & MELNICK,J.L. (1986) Environmental Virology. Van Nostrand Reinhold (UK) Co. Ltd.:

RAY,B. (1979) Methods to detect stressed microorganisms. J. Food Protect. 42(4), 346-355.

RAY,B., & SPECK,M.L. (1973) Freeze-injury in bacteria. CRC Crit. Rev. Clin. Lab. Sci. 4, 161-213.

RESTAINO,L., KOMATSU,K.K., & SYRACUSE,M.J. (1982) A note on novobiocin in XLD and HE agars: the optimum levels required in two commercial sources of media to improve isolation of salmonellas. J. Appl. Bacteriol. 53, 285-288.

REYNOLDS,N. (1964) The effect of light on the mortality of E.coli in seawater. Comm. Int. Explor. Sci. Mer. Medit., Pollut. Mar. Prod. Petrol., Monaco.

RHEINHEIMER,G. (1985) Aquatic Microbiology pp 95-117. Wiley: Chichester.

RHODES,M.W., & KATOR,H. (1988) Survival of Escherichia coli and Salmonella spp. in estuarine environments. Appl. Environ. Microbiol. 54(12), 2902-2907.

RIS,H. & PLAUT,W. (1962) Ultrastructure of DNA-containing areas in the chloroplast of Chlamydomonas. J. Cell Biol. 13, 383-391.

RITTENBERG,S.C., MITTWER,T., & IVLER,D. (1958) Coliform bacteria in sediments around three marine sewage outfalls. Limnol. Oceanogr. 3, 101-108.

ROLLINS,D.M., & COLWELL,R.R. (1986) Viable but nonculturable stage of Campylobacter jejuni and its role in survival in the natural aquatic environment. Appl. Environ. Microbiol. 52(3), 531-538.

ROPER,M.M., & MARSHALL,K.C. (1974) Modification of the interaction between Escherichia coli and bacteriophage in saline sediment. Microb. Ecol. 1, 1-13.

ROPER,M.M., & MARSHALL,K.C. (1979) Effects of salinity on sedimentation and of particulates on the survival of bacteria in estuarine habitats. Geomicrobiol. J. 1(2), 103-116.

ROSE,R.E., GELDREICH,E.E., & LITSKY,W. (1975) Improved membrane filter method for fecal coliform analysis. Appl. Microbiol. 29, 532-536.

ROSENBERG,M.L., HAZLET,K.K. SCHAEFFER,J., WELLS,J.G., & PRUNEDA,R.C. (1976) Shigellosis from swimming. J. Am. Med. Assoc. 236, 1849-1852.

ROSENBERG,M.L., KOPLAN,J.P., WACHSMUTH,I.K., WELLS,J.G., GANGAROSA,E.J., GUERRANTI,R.L., & SACK,D.A. (1977) Epidemic diarrhea at Crater Lake from enterotoxigenic Escherichia coli: a large waterborne outbreak. Ann. Intern. Med. 86, 714-718.

ROSENFELD,W.D., & ZOBELL,C.E. (1947) Antibiotic production by marine microorganisms. J. Bacteriol. 54, 393-398.

ROSZAK,D.B. (1986) Estimation of viable, but non-culturable bacteria in aquatic systems. Ph.D Thesis, University of Maryland, College Park, USA.

ROSZAK,D.B., & COLWELL,R.R. (1987a) Survival strategies of bacteria in the natural environment. Microbiol. Rev. 51(3), 365-379.

ROSZAK,D.B., & COLWELL,R.R. (1987b) Metabolic activity of bacterial cells enumerated by direct viable count. Appl. Environ. Microbiol. 53(12), 2889-2983.

ROSZAK,D.B., GRIMES,D.J., & COLWELL,R.R. (1984) Viable but nonrecoverable stage of Salmonella enteritidis in aquatic systems. Can. J. Microbiol. 30, 334-338.

RUSSELL,H.L. (1972) Bacterial investigation of the sea and its floor. Botan. Gaz. 17, 312-321.

SCARPINO,P.V. (1975) Human enteric viruses and bacteriophages as indicators of sewage pollution. In Discharge of Sewage From Sea Outfalls (ed. Gameson,A.L.H.) pp 49-61. Pergamon Press: Oxford.

SCARPINO,P.V., BERG,G., CHANG,S.L., DAHLING,D., & LUCAS,M. (1972) A comparative study of the inactivation of viruses in water by chlorine. Water Res. 6, 959-965.

SCHEUERMAN,P.R., SCHMIDT,J.P., & ALEXANDER,M. (1988) Factors affecting the survival and growth of bacteria introduced into lake water. Arch. Microbiol. 150, 320-325.

SCHLEIFER,K.H., & KILPPER-BALZ,R. (1984) Transfer of Streptococcus faecalis and Streptococcus faecium to the genus Enterococcus nom. rev. as Enterococcus faecalis comb. nov. and Enterococcus faecium comb. nov. Int. J. Syst. Bacteriol. 34, 31-34.

SETLOW,R.B. (1967) Repair of DNA. In Regulation of Nucleic Acid and Protein Biosynthesis (ed. Koningsberger,V.V., and Bosh,L.) pp 51-62. Elsevier: Amsterdam.

SETLOW,R.B. (1968) The photochemistry, photobiology, and repair of polynucleotides. Prog. Nucleic Acid Res. Mol. Biol. 8, 257-295.

SETLOW,R.B. & POLLARD,E.C. (1962) Molecular Biophysics. Pergamon Press: London.

SETLOW,J.K., & SETLOW,R.B. (1963) The nature of the photoreactivable lesion in DNA. Nature 197, 560-562.

SHAPIRO,J. (1957) Chemical and biological studies on the yellow organic acids of lake water. Limnol. Oceanogr. 2(3), 161-179.

SHUVAL,H.I. (1975) The case for microbial standards for bathing beaches. In Discharge of Sewage From Sea Outfalls (ed. Gameson,A.H.L.) pp 95-101. Pergamon Press: London.

SIEBURTH,J.McN. (1959) Antibacterial activity of antarctic marine phytoplankton. Limnol. Oceanogr. 4, 419-424.

SIEBURTH,J.McN. (1960) Acrylic acid, an 'antibiotic' principle in phaeocystis blooms in antarctic waters. Science 132, 676-677.

SIERACKI,M.E., (1980) The effects of short exposures of natural sunlight on the decay rates of enteric bacteria and a coliphage in a simulated sewage outfall microcosm. MS Thesis, University of Rhode Island, Rhode Island.

SINCLAIR,J.L., & ALEXANDER,M. (1984) Role of resistance to starvation in bacterial survival in sewage and lake water. Appl. Environ. Microbiol. 48, 410-415.

SINGH,A., & McFETERS,G.A. (1986) Recovery, growth, and production of heat-stable enterotoxin by Escherichia coli after copper-induced injury. Appl. Environ. Microbiol. 51, 738-742.

SINGLETON,F.L., ATTWELL,R.W., JANGI,M.S., & COLWELL, R.R. (1982) Influence of salinity and organic nutrient concentration on survival and growth of Vibrio cholerae in aquatic microcosms. Appl. Environ. Microbiol. 43(5), 1080-1085.

SISTROM,W.R., GRIFFITHS,M., & STANIER,R.Y. (1956) The biology of a photosynthetic bacterium which lacks colored carotenoids. J. Cell. Comp. Physiol. 48, 473-515.

SKIRROW,M.B. (1982) Campylobacter enteritis- the first five years. J. Hyg., Camb. 89, 175-184.

SLANETZ,L.W., & BARTLEY,C.H. (1965) Survival of fecal streptococci in seawater. Hlth Lab. Sci. 2(3), 142-148.

SMIGIELSKI,A.J., WALLACE,B.J., & MARSHALL,K.C. (1989) Changes in membrane functions during short-term starvation of Vibrio fluvialis strain NCTC 11328. Arch. Microbiol. 151, 336-347.

SMITH,J.T. (1985) The 4-quinolone antibacterials. Symp. Soc. Gen. Microbiol. 38, 69-94.

SMITH,K.C., & HANAWALT,P.C. (1969) Molecular Photobiology: Inactivation and Recovery. Academic Press: New York.

SPIKES,J.D., & STRAIGHT,R. (1967) Sensitised photochemical processes in biological systems. Ann. Rev. Phys. Chem. 18, 409-436.

SPIRA,L.M., SACK,R.B., & FROELICH,J.L. (1981) Simple adult model for Vibrio cholerae and enterotoxigenic Escherichia coli diarrhea. Infect. Immun. 32(2), 739-747.

SPROTT,G.D., MARTIN,W.G., & SCHNEIDER,H. (1976) Differential effects of near-uv and visible light on active transport and other membrane processes in Escherichia coli. Photochem. Photobiol. 24, 21-27.

STETLER,R.E. (1984) Coliphages as indicators of enteroviruses. Appl. Environ. Microbiol. 48(3), 668-670.

STEVENSON,A.H. (1953) Studies of bathing water quality and health. Am. J. Publ. Hlth 43, 529-538.

STEWART,R.E., PUTMAN,H.D., JONES,R.H., & LEE,T.N. (1971) Diffusion of sewage effluent from ocean outfall. J. San. Eng. Div., Proc. Am. Soc. Civ. Eng. 97(SA4), 485-503.

TABOR,P.S., OHWADA,K. & COLWELL.R.R. (1981) *Filterable* marine bacteria found in the deep sea: Distribution, taxonomy, and response to starvation. Microb. Ecol. 7, 67-83.

TABOR,P.S., & NEIHOF,R.A. (1982) Improved microautoradiographic method to determine individual microorganisms active in substrate uptake in natural waters. Appl. Environ. Microbiol. 44, 945-953.

THOMASON,B.M., COWART,C.S., & CHERRY,W.B. (1965) Current status of immunofluorescence techniques for rapid detection of shigellae in fecal specimens. Appl. Microbiol. 13(4), 605-613.

THOMASON,B.M., & WELLS,J.G. (1971) Preparation and testing of polyvalent conjugates for fluorescent-antibody detection of salmonellae. Appl. Microbiol. 22 (5), 876-884.

TORRELLA,F., & MORITA,R.Y. (1981) Microcultural study of bacterial size changes and microcolony and ultramicrocolony formation by heterotrophic bacteria in seawater. Appl. Environ. Microbiol. 41, 518-527.

VARGO,G.A., HARGRAVES,P.E., & JOHNSON,P. (1975) Scanning electron microscopy of dialysis tubes incubated in flowing sea water. Mar. Biol. 31, 113-120.

VASCONCELOS,G.J., & SWARTZ,R.G. (1976) Survival of bacteria in seawater using a diffusion chamber apparatus in situ. Appl. Environ. Microbiol. 31(6), 913-920.

VAUGHN,J.M., & METCALF,T.G. (1975) Coliphages as indicators of enteric viruses in shellfish and shellfish raising estuarine waters. Water Res. 9, 613-616.

WAIT,D.A., & SOBSEY,M.D. (1980) Dieoff of enteric microorganisms in sea water and marine sediments. Abs. Ann. Meet., Amer. Soc. Microbiol., Miami, Florida.

WAKSMAN,S.A., & HOTCHKISS,M. (1937) Viability of bacteria in sea water. J. Bacteriol. 33, 389-400.

WANG,W.L.L., DUNLOP,S.G., & MUNSON,P.S. (1966) Factors influencing the survival of shigella in wastewater and irrigation water. J. Water Pollut. Control Fed. 38, 1775-1781.

WARD,H.M. (1893a) Experiments on the action of light on Bacillus anthracis. Proc. Roy. Soc., (Lond.) 52, 393-400.

WARD,H.M. (1893b) Further experiments on the action of light on Bacillus anthracis. Proc. Roy. Soc., (Lond.) 53, 23-44.

WEBB,R.B., & LORENZ,J.R. (1970) Oxygen dependance and repair of lethal effects of near ultraviolet and visible light. Photochem. Photobiol. 12, 283-289.

WEBB, R.B., & MALINA, M.M. (1967) Mutagenesis in Escherichia coli by visible light. Science 156, 1104-1105.

WEBB,R.B., & TAI,C.C. (1969) Physiological and genetic implications of selective mutation by light at 320-400nm. Nature 224, 1123-1125.

WEISS,C.M. (1951) Adsorption of E.coli on river and estuarine silts. Sewage Ind. Wastes 23, 227-237.

WESTLAKE,D.F. (1965) The light climate for plants in rivers. In Light as an Ecological Factor (ed. Bainbridge,R., Evans,G.C., & Rackham,O.) pp 99-119. Blackwell: Oxford.

WHITE,J., YEATS,A., & SKIPWORTH,G. (1985) Tables For Statisticians. Stanley Thornes Ltd.: Cheltenham.

WHO (1974) Guides and criteria for recreational quality of beaches and coastal waters. WHO Regional Office for Europe, Copenhagen.

WITTEKIND,D. (1972) Fluorescence properties of the monoaminoacridines and some 2-aminoacridine-derivatives. In Fluorescence Techniques in Cell Biology (ed. Thaer,A.A., & Sernetz,M.) pp 95-105. Springer-Verlag: New York.

WRC. (1977) Bacterial pollution of bathing beaches. Notes on Water Research No. 10.

WRIGHT,R.T., & BURNISON,B.K. (1979) Heterotrophic activity measured with radiolabelled organic substrates. In Native Aquatic Bacteria: Enumeration, Activity, and Ecology (ed. Costerton,J.W., & Colwell,R.R.) pp 140-155. ASTM STP 695. American Society for Testing and Materials: Philadelphia.

XU,H-S., ROBERTS,N., ADAMS,L.B., WEST,P.A., SIEBELING, R.J., HUQ,H-S., HUQ,M.I., RAHMAN,R., & COLWELL,R.R. (1984) An indirect fluorescent antibody staining procedure for detection of Vibrio cholerae serovar 01 cells in aquatic environmental samples. J. Microbiol. Methods 2, 221-231.

XU,H-S., ROBERTS, N., SINGLETON, F.L., ATTWELL, R.W., GRIMES,D.J., & COLWELL,R.R. (1982) Survival and viability of nonculturable Escherichia coli and Vibrio cholerae in the estuarine and marine environment. Microb. Ecol. 8, 313-323.

YANAGITA,T., & TAKAGI,M. (1980) Model experiments on the growth of enteric bacteria in fresh and sea water. J. Gen. Appl. Microbiol., Tokyo 26, 299-305.

YOSHPE-PURER,Y., & SHUVAL,H.I. (1972) Salmonellae and bacterial indicator organisms in polluted coastal water and their hygienic significance. In Marine Pollution and Sea Life (ed. Ruivo,M.) Fishing News (Books) Ltd.,: London.

ZANONI,A.E., & FLEISSNER,J.T. (1982) Indicator bacteria survival under laboratory conditions. J. Water Pollut. Control Fed. 54(5), 500-503.

ZIMMERMAN,R., ITURRIAGA,R., & BECKER-BIRCK,J. (1978) Simultaneous determination of the total number of bacteria and the number thereof involved in respiration. Appl. Environ. Microbiol. 36, 926-935.

ZOBELL,C.E. (1936) Bactericidal action of sea-water. Proceedings of the Society for Experimental Biology and Medicine 34(2), 113-116.

ZOBELL,C.E. (1946) Marine Microbiology. Chronica Botanica Co: Waltham, Mass.

IX. APPENDICES

APPENDIX 1

1) Nutrient Agar (D.B. Roszak, 1986, Ph.D Thesis, University of Maryland, USA.).

10g peptone (L34, Oxoid Ltd.)
2g yeast extract (L21, Oxoid Ltd.)
3mls glycerol (BDH Chemicals Ltd.) (GPR)
15g agar technical (No. 3) (L13, Oxoid Ltd.)
1000mls water (distilled, seawater, freshwater, or treated, unchlorinated water)

The ingredients were dissolved in the water, the pH was adjusted to 8.0 and the medium was autoclaved at 121°C for 15 minutes.

2) Tryptone Water (DHSS, 1984).

20g tryptone (L42, Oxoid Ltd.)
5g NaCl (GPR)
1000mls distilled water

The ingredients were dissolved in the water, the pH adjusted to 7.5 and then autoclaved at 115°C for 10 minutes. Production of indole is indicated by the formation of a oily pink ring on the surface of the tryptone water on addition of 2 drops of Kovac's Reagent.

3) Tyrosine Sorbitol Thallous Acetate Agar (DHSS,1984).

10g peptone (L34, Oxoid Ltd.)
1g yeast extract (L21, Oxoid Ltd.)
2g sorbitol (BDH Chemicals Ltd.) (GPR)
5g L-tyrosine (T-3754, Sigma Chemical Co.)
12g agar technical (No. 3) (L13, Oxoid Ltd.)
10ml (1% (w/v)) tetrazolium salt (TTC) (BDH Chemicals Ltd.) (GPR)
1g thallous acetate (BDH Chemicals Ltd.) (GPR)
1000mls distilled water

The first five ingredients were dissolved in the water and autoclaved at 115°C for 10 minutes. The pH was adjusted to 6.2 and filter-sterilised solutions of TTC and thallous acetate were added. A shallow layer of the agar was poured into a petri dish. An upper layer of agar was poured on top but this layer contained an extra 4g of tyrosine, added whilst the medium was still hot. The presence of *St. faecalis* is shown by maroon colonies encircled by a clear zone.

4) Peptone Water Sugars (Cowan and Steel, 1974).

900mls peptone water (10g peptone (L34, OxoidLtd.); 5g NaCl; 1000mls distilled water) pH 7.1-7.3.
10mls bromocresol purple (BDH Chemicals Ltd.)
10g D-arabinose (A-3131, Sigma Chemical Co.)

Bromocresol purple was added to the peptone water and autoclaved at 115°C for 20 minutes. 10g arabinose was dissolved in 90mls distilled water and steamed for 30 minutes. This was then added to the sterile peptone base, distributed into tubes containing an inverted Durham's tube and steamed for a further 30 minutes. Fermentation of arabinose is indicated by acid and gas prodution.

5) Glucose Phenolphthalein Broth (DHSS, 1984).

10g meat extract (L30, Oxoid Ltd.)
10g peptone (L34, Oxoid Ltd.)
5g NaCl (GPR)
1000mls distilled water

The ingredients were dissolved in the water, the pH adjusted to 7.2 and autoclaved at 115°C for 10

minutes. 50ml of a filter-sterilised 20% (w/v) solution of D-glucose (BDH Chemicals Ltd.) (AR) were added to 950mls of the sterile broth. A buffer consisting of:

0.6g glycine (BDH Chemicals Ltd.) (AR)
0.35g NaCl (GPR)
60mls freshly boiled distilled water
40mls 0.1N sodium hydroxide (GPR).

The glycine and the NaCl were dissolved in the hot water and the sodium hydroxide was then added.

The completed medium consisted of:

900mls glucose broth
100mls glycine buffer
5mls phenolphthalein (0.2% (w/v) aq soln) (BDH Chemicals Ltd.).

Tolerance of pH 9.6 is indicated by heavy growth and decolourisation of medium.

6) Synthetic Sewage (Lim & Flint, 1989)

0.3g	peptone
0.2g	yeast extract
0.05g	urea
1.0g	$(NH_4)_2SO_4$
0.2g	KH_2PO_4
0.001g	$FeSO_4.7H_2O$
5mls	trace element solution
1000mls	distilled water.

The ingredients were dissolved in the water and the pH was adjusted to 8.0. The synthetic sewage was filtered through a membrane of pore size 0.2µm and diameter 47mm and autoclaved at 121°C for 15 minutes. The trace element solution was added.

APPENDIX 2 - OPERATING INSTRUCTIONS FOR ALS

General Operation.

The light is provided by 4 x 400W projector bulbs arranged in a cruciform underneath a floodlight reflector with the facility to adjust the focusing by means of a wheel above the lamp housing.

As the equipment requires a large current to run it must be harnessed to its own electrical supply. The only connections which can be made are those for the magnetic stirrer and for a fan shield for dispelling vapour from the heat/UV filter.

To turn the lamps on the following sequence must be followed:

1) Turn the mains supply switch at the bottom right of the control cabinet on. This should start a fan at the top of the cabinet and a fan on the lamp housing. Also a red warning light (top central on the cabinet) should be illuminated. If any of the above does not happen do not proceed until the fault is rectified.

2) Turn on the Churchill Thermocirculator pump once connected to the cooling water jacket and the magnetic stirrer in the beaker and allow the temperature to reach that desired. Whilst waiting fill the heat/UV filter to the 1cm mark with 0.05% Teepol solution and turn on its circulating pump allowing coolant to flow before continuing to the next step. It is important at this stage to place a piece of opaque material over the beaker and water jacket to ensure that the sample is not exposed

prematurely to the very high amount of low wavelength radiation produced initially by the lamps.

3) On the left-hand side of the cabinet are 4 on/off switches. Each one should be turned on starting at the top. As each one is switched on so a buzzing noise will be heard from within the cabinet and the orange warning lights corresponding to each on/off switch should illuminate. If any of them do not after 2-3 seconds, turn that particular switch off and if desirable the next steps can still be followed.

4) When each orange warning light has been illuminated the righthand column of switches may be turned from START to RUN position. As each switch is turned the buzzing noise from insied for each switch will cease. IT IS IMPORTANT THAT THE TIME INTERVAL BETWEEN TROWING SWITCHES TOP TO BOTTOM IS LESS THAN 10 SECONDS if any longer than this it is likely that the bulb will not strike due to heat produced by the others.

5) Once all of the bulbs are running they should be left for about 1-2 mins until they are at operational intensity (a yellowish bright light) when the experiment or calibration can start.

Adjusting Focus and Plate Positions.

The focusing wheel allows the bulb cluster to be raised or lowered by 1.5mm per revolution to find the optimum position for the experiments/calibrations as desired. Defocusing the cluster will obviously result in reduction of intensity.

The lamp housing and filter holder plates can be lowered or raised when the adjusting clamps are slackened. The base plate may be adjusted by use of the adjusting wheels on either side after slackening the adjusting clamps.

APPENDIX 3. DATA FOR COMPARISON OF DECAY RATES OF SELECTED BACTERIAL SPECIES

E.COLI (9001)		E.COLI (SEW)		S.FAECALIS (775)		S.FAECALIS (SEW)		S.ORANIENBURG		S.VIRCHOW		S.TYPHIMURIUM		S.NEWPORT	
AODVC		AODVC		AODVC*		AODVC*		AOPVC		AODVC		AODVC		AODVC	
0	6.2E08	0	2.6E07	0	2.0E09	0	2.6E08	0	1.6E10	0	2.8E09	0	8.0E09	0	1.3E10
24	4.4E07	24	2.7E07	24	4.7E08	24	3.2E08	24	4.1E07	24	8.1E06	24	3.9E07	24	3.2E07
48	2.6E06	72	7.1E07	48	1.3E07	48	7.8E06	120	8.2E06	120	3.3E06	120	7.4E05	120	7.4E05
96	1.5E08	96	2.5E07	96	1.4E08	96	4.4E07	456	3.6E06	456	3.1E06	456	4.7E06	456	2.1E06
168	3.4E06	168	7.9E07	168	5.6E07	168	2.6E08	648	8.9E05	648	6.7E05	648	2.2E06	648	2.2E06
264	5.7E06	264	6.4E06	264	1.2E07	264	2.4E07								
456	6.8E06	456	5.7E05	456	4.9E06	456	3.6E07								
		648	5.1E06	648	2.1E06	648	9,1E06								
CI		CI		CI		CI		CI		CI		CI		CI	
2.8E08	9.8E08	7.3E06	1.4E08	1.4E09	2.6E09	1.2E08	4.0E08	9.9E09	2.3E10	7.3E08	7.0E09	1.4E09	1.4E10	3.5E09	2.3E10
2.8E07	1.2E08	8.9E06	1.0E08	2.3E07	9.7E08	1.5E07	6.3E08	2.2E07	5.9E08	2.7E06	1.4E07	9.0E06	7.6E07	1.6E07	5.0E08
1.1E06	4.0E06	9.7E06	2.4E08	1.2E05	2.5E08	6.6E05	1.6E07	1.2E05	1.0E07	3.5E05	1.0E07	3.5E05	2.6E06	3.5E05	2.6E06
1.2E07	4.1E08	8.5E06	4.1E07	7.6E06	2.7E08	3.7E06	1.4E08	2.5E05	3.3E07	6.9E05	1.0E07	7.6E05	4.6E07	6.5E05	2.7E07
2.4E06	3.6E06	1.9E07	1.8E08	1.6E07	1.0E08	1.3E08	3.9E08	2.3E05	1.1E06	3.4E05	1.0E06	4.0E05	1.8E07	2.0E06	2.6E06
1.2E06	1.0E07	1.6E06	1.4E07	3.2E06	2.0E07	5.0E06	5.4E07								
1.5E06	5.3E07	1.5E05	4.2E06	1.7E06	3.2E07	1.7E07	7.4E07								
		4.2E05	9.3E06	7.8E05	2.2E06	3.4E06	1.2E07								
SEL.		SEL.		SEL.		SEL.		SEL.		SEL.		SEL.		SEL.	
0	1.3E06	0	1.0E07	0	8.5E05	0	1.4E06	0	9.2E05	0	4.8E05	0	1.0E04	0	1.1E06
24	2.5E03	24	2.0E05	48	2.5E05	48	2.0E05	24	1.0E06	24	3.5E05	24	2.0E03	24	4.0E04
48	6.2E03	72	1.1E06	96	1.4E06	96	1.4E06	120	2.0E04	120	1.1E03	120	1.0E02	120	1.0E02
96	2.0E01	96	6.8E04	168	5.0E04	168	4.5E05	456	1.6E02	456	3.0E00	144	2.5E00	144	0
168	0	168	1.4E02	264	8.4E04	264	9.1E04	504	6.9E01	504	0.5E00	456	0		
816	0	264	2.5E00	456	1.0E03	456	1.0E03	648	5.0E00	648	0				
		288	6.9E00					816	0						
CI		CI		CI		CI		CI		CI		CI		CI	
1.2E06	3.8E06	ALL SAME		5.8E04	7.5E06	7.6E05	2.0E06	5.9E05	1.2E06	4.1E05	5.3E05	ALL SAME		7.0E05	3.0E06
2.9E02	3.4E03	1.7E05	2.3E05	2.2E05	2.7E05	1.1E05	2.9E05	6.5E05	2.1E06	2.2E05	9.1E05	ALL SAME		8.7E03	1.7E05
8.1E02	2.1E04	8.4E05	1.4E06	9.1E05	1.9E06	1.2E06	1.7E06	ALL SAME		1.7E02	2.3E03	ALL SAME		ALL SAME	
ALL SAME		2.2E04	3.5E05	ALL SAME		1.9E05	1.0E06	1.2E02	2.0E02	ALL SAME		3.9E-1	8.9E00	ALL SAME	
ALL SAME		7.6E01	2.0E03	4.0E03	1.6E05	2.1E04	1.6E05	2.4E01	1.1E02	ALL SAME		ALL SAME			
		1.0E00	1.5E01	ALL SAME		ALL SAME		ALL SAME							
		2.4E00	1.1E01												
		ALL SAME													
		NS						NS		NS		NS		NS	
		0	1.0E07					0	9.2E05	0	4.8E05	0	1.0E04	0	1.1E06
		312	1.4E01					456	8.4E02	456	5.0E00	312	4.5E01	456	0.5E00
		456	0.4E00					648	4.4E01	648	3.0E00	456	3.0E00	648	5.0E00
		648	1.5E00					CI		CI		648	4.9E02	CI	
		CI						ALL SAME		ALL SAME		CI		ALL SAME	
		ALL SAME						6.5E02	1.0E03	2.0E00	3.0E01	ALL SAME		5.8E-1	6.8E00
		5.6E00	3.2E02					1.2E00	2.0E02	2.2E00	2.8E01	5.8E00	9.5E01	3.3E00	4.3E01
		ALL SAME										ALL SAME			
		ALL SAME										4.4E02	5.5E02		

APPENDIX **3** CONTINUED.

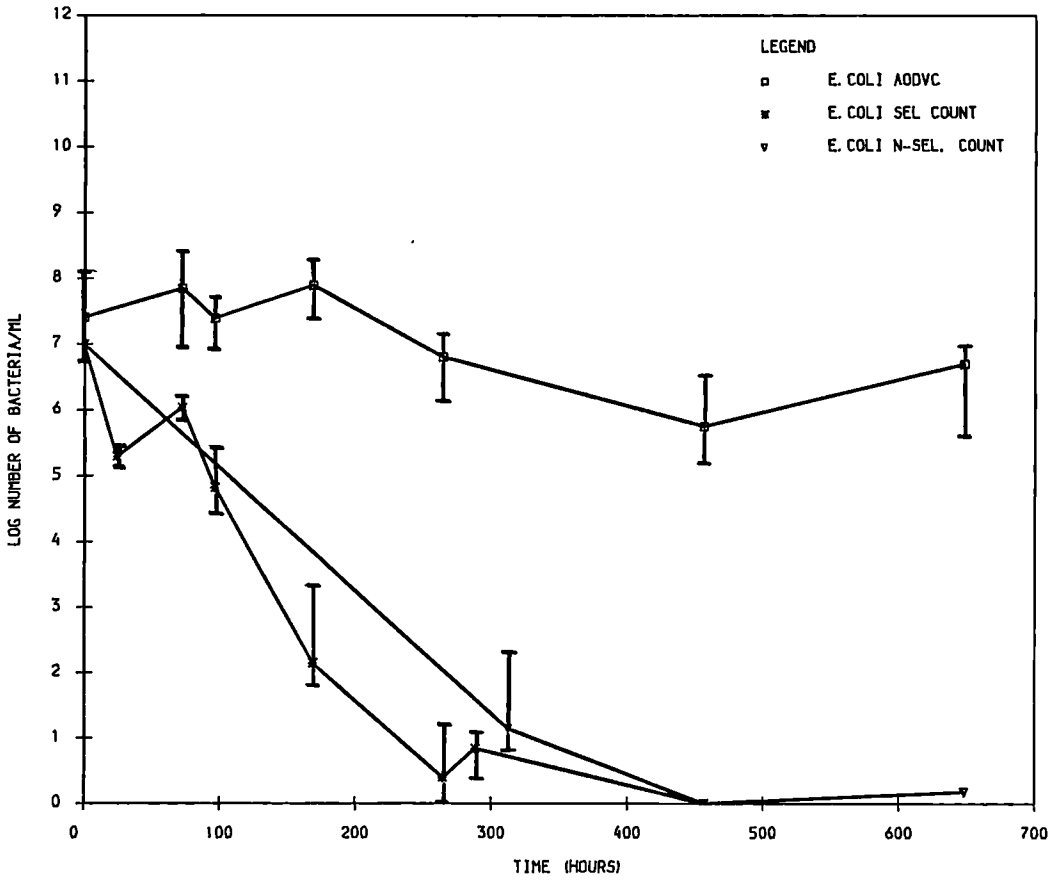


FIGURE 4. SURVIVAL OF AN ISOLATE OF E. COLI IN THE DARK IN SEAWATER AT 15C

APPENDIX 4. DATA FOR DECAY OF E.COLI UNDER DIFFERENT TEMPERATURE AND SALINITY REGIMES IN THE DARK.

EXPERIMENT A.

0.05% 4oC	0.05% 20oC	0.05% 37oC	1.75% 4oC	1.75% 20oC	1.75% 20oC	1.75% 37oC	3.5% 4oC	3.5% 20oC	3.5% 37oC
AODVC	AODVC	AODVC	AODVC	AODVC	AODVC	AODVC	AODVC	AODVC	AODVC
68 3.7E09	24 4.1E10	24 6.0E10	0 9.8E09	24 9.8E09	0 2.1E10	24 1.4E10	24 1.9E10	0 4.4E10	68 1.3E10
188 5.8E09	68 8.1E09	68 1.8E10	24 3.4E10	68 5.8E09	24 1.0E10	68 8.9E09	68 1.0E10	24 1.4E10	260 2.3E08
260 1.5E08	188 9.8E08	188 7.4E09	68 1.7E10	188 1.3E10	68 2.3E10	188 1.8E10	188 1.2E10	68 1.9E10	500 8.9E03
380 1.3E08	260 1.3E08	260 1.3E08	188 3.2E09	260 2.0E08	188 2.5E10	260 8.9E07	260 3.3E08	188 2.3E10	596 0
500 3.0E06	380 5.8E07	380 2.4E07	260 1.9E08	380 5.2E07	260 1.4E08	380 2.0E04	380 1.3E08	260 6.3E07	740 0
596 1.4E06	500 6.3E06	500 1.1E07	380 1.1E08	596 9.8E05	380 3.9E07	500 2.2E03	500 3.8E06	380 6.5E07	
740 1.6E05	596 8.9E05		500 1.5E07	740 3.9E05	500 1.9E07	596 1.6E03	596 4.9E05	500 7.6E06	
1172 1.8E04	740 3.3E05		596 9.8E05	1172 6.5E03	596 1.8E05	740 0	740 6.9E05	596 4.4E05	
	1172 9.5E03		740 3.5E05		740 4.9E05		1172 2.0E03	740 7.1E05	
			1172 5.2E05		1172 7.9E03			1172 2.7E03	
CI	CI	CI	CI	CI	CI	CI	CI	CI	CI
7.1E08-7.5E09	6.0E09-1.4E11	8.4E09-1.1E11	5.2E09-2.5E10	1.3E09-1.6E10	2.4E09-3.6E10	4.6E09-2.3E10	1.3E10-3.9E10	4.9E09-8.4E10	4.3E11-1.7E12
8.3E08-1.0E10	2.2E09-1.4E10	7.0E09-2.9E10	1.3E10-5.2E10	8.3E08-1.0E10	1.4E09-1.6E10	4.6E09-2.3E10	8.0E09-2.1E10	1.7E09-2.6E10	1.2E10-2.4E10
2.3E07-3.2E08	1.3E09-2.3E10	2.7E09-1.2E10	5.6E09-2.8E10	4.7E09-2.2E10	1.6E10-4.5E10	3.8E09-3.9E10	9.3E09-2.5E10	1.8E10-3.6E10	1.1E08-3.5E08
4.4E07-2.1E08	4.2E07-2.2E08	4.7E07-2.2E08	2.3E09-8.7E09	3.8E07-3.6E08	4.2E09-4.5E10	1.5E07-1.8E08	8.9E07-6.7E08	8.7E09-3.8E10	ALL SAME
1.9E06-7.8E06	4.8E06-1.2E08	1.6E06-7.0E07	1.0E08-4.9E08	8.9E06-1.6E08	1.5E07-2.9E08	3.7E03-8.7E04	4.7E07-2.6E08	1.3E07-1.9E08	8.0E01-4.0E00
5.4E05-2.2E06	1.3E06-1.2E07	6.4E06-2.3E07	2.1E07-2.0E08	3.4E05-1.6E06	7.7E06-8.2E07	2.7E02-7.1E03	2.7E06-1.0E07	2.8E07-1.6E08	
8.0E04-4.0E05	4.5E05-1.3E06		3.0E06-5.2E07	1.8E05-8.9E05	1.3E07-7.1E07	7.2E02-3.7E03	1.1E05-1.1E06	2.2E06-1.5E07	
1.0E04-2.5E04	2.2E05-8.9E05		1.2E05-1.9E06	6.5E02-1.4E04	3.2E04-6.7E05		6.1E05-9.0E05	3.4E05-1.5E06	
	4.4E03-1.7E04		7.2E04-6.6E05		1.1E05-1.1E06		2.0E02-5.2E03	1.3E05-1.5E06	
			2.7E03-1.3E04		3.8E03-1.6E04			4.1E02-5.7E03	
SEL	SEL	SEL	SEL	SEL	SEL	SEL	SEL	SEL	SEL
0 7.2E06	0 7.2E06	0 8.3E06	0 5.5E06	0 7.8E06	0 7.4E06	0 8.1E06	0 8.7E06	0 7.9E06	0 7.9E06
24 8.7E06	24 8.3E06	24 7.9E06	24 9.3E06	24 6.8E06	24 8.1E06	24 6.9E06	24 8.3E06	24 7.6E06	24 1.8E06
68 1.0E07	68 5.4E06	68 1.1E07	68 4.9E06	68 1.1E07	68 9.3E06	68 1.2E06	68 9.3E06	68 6.5E06	68 4.5E05
116 1.0E07	116 1.9E06	116 8.9E06	116 1.0E07	116 4.5E05	116 1.0E05	116 1.1E06	116 9.5E06	116 4.7E06	116 6.0E05
212 1.9E05	212 2.1E04	188 1.5E05	212 2.0E05	212 1.2E05	212 1.7E04	212 8.7E04	212 2.0E05	212 1.8E05	212 5.6E04
380 5.5E03	260 3.0E04	260 2.2E05	260 1.0E06	260 4.9E04	260 1.7E04	260 8.9E02	260 1.0E06	260 2.5E05	260 1.0E01
500 1.3E03	332 1.4E04	332 5.8E04	380 1.0E05	332 2.5E04	332 3.1E04	332 1.0E02	380 1.0E05	332 2.1E05	380 0
596 2.5E03	380 6.3E03	380 1.2E03	500 1.0E04	380 1.9E04	380 1.8E04	380 0	500 1.0E04	380 1.2E05	500 0
740 1.4E03	500 1.1E03	500 5.5E02	596 1.0E04	500 8.9E03	500 1.0E04	500 0	596 1.0E04	500 1.9E04	740 0
1172 1.0E02	596 6.3E02	596 1.2E02	740 1.0E03	596 6.2E03	596 8.7E03	596 0	740 1.0E02	596 1.8E04	
	740 6.2E02	740 3.9E01	1172 1.0E02	740 1.0E03	740 1.0E03	740 0	1172 1.0E02	740 1.0E02	
	1172 1.0E02	1172 1.0E01		1172 1.0E02	1172 1.0E02			1172 1.0E02	
CI	CI	CI	CI	CI	CI	CI	CI	CI	CI
2.9E06-1.2E07	3.1E06-1.3E07	6.8E06-9.9E06	7.8E05-1.9E07	4.8E06-1.1E07	2.3E06-1.2E07	1.7E06-1.4E07	2.4E06-1.5E07	6.7E06-9.2E06	4.2E06-5.1E07
4.9E06-1.3E07	5.1E06-1.1E07	ALL SAME	4.5E06-4.8E07	3.6E06-9.9E06	3.5E06-1.3E06	4.3E06-9.4E06	1.1E06-1.6E07	5.6E06-1.5E07	4.5E05-3.3E06
7.8E06-1.3E07	3.1E06-1.0E07	2.4E06-1.9E07	8.4E05-9.7E06	5.4E06-2.2E07	2.3E06-1.6E07	7.6E05-3.1E06	2.4E06-1.6E07	6.4E05-1.3E07	3.9E04-4.9E06
9.3E06-1.1E07	7.7E05-1.1E07	1.9E06-1.6E07	1.8E06-2.2E07	1.8E05-1.0E06	1.1E04-1.4E06	1.4E05-3.6E06	3.1E06-1.6E07	1.1E06-1.0E07	4.4E04-5.6E06
6.4E04-4.4E05	3.0E03-4.4E04	ALL SAME	1.8E05-5.5E05	7.1E04-2.5E05	3.8E03-2.9E04	1.0E04-2.8E05	ALL SAME	7.4E04-4.3E05	7.5E03-1.2E05
1.4E03-2.5E04	3.4E03-9.3E04	3.4E04-4.7E05	5.1E05-1.8E06	5.3E03-1.5E05	2.1E03-5.5E04	ALL SAME	2.7E05-2.3E06	ALL SAME	5.8E00-6.9E01
2.9E02-2.6E03	7.1E03-2.0E04	4.4E03-1.6E05	ALL SAME	1.3E03-6.3E04	5.6E03-5.6E04	ALL SAME	ALL SAME	1.5E05-2.6E05	ALL SAME
ALL SAME	3.8E03-8.8E03	7.1E02-2.5E03	ALL SAME	6.3E03-3.2E04	1.1E04-2.4E04	ALL SAME	ALL SAME	ALL SAME	ALL SAME
ALL SAME	ALL SAME	2.7E01-1.1E03	ALL SAME	3.7E03-1.4E04	ALL SAME	ALL SAME	ALL SAME	5.8E03-3.1E04	ALL SAME
ALL SAME	2.5E02-1.0E03	7.1E01-2.5E02	ALL SAME	ALL SAME	1.1E03-1.6E04	ALL SAME	ALL SAME	5.2E03-3.1E04	
	4.9E02-7.4E02	1.2E01-8.9E01		ALL SAME	ALL SAME	ALL SAME	ALL SAME	ALL SAME	
	ALL SAME	ALL SAME		ALL SAME	ALL SAME			ALL SAME	

APPENDIX 4 CONTINUED.

NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
24 3.9E07	24 5.0E08	24 2.3E08	24 3.0E08	24 1.4E07	24 1.6E07	24 1.7E07	24 1.1E07	24 8.5E06	24 1.0E06
188 1.2E07	188 2.0E06	188 1.0E06	188 5.0E06	188 2.0E06	188 7.9E06	188 1.0E06	188 6.0E06	188 5.0E06	332 1.0E02
1172 1.5E02	1172 1.6E02	1172 0	1172 1.0E03	212 4.5E04	1172 1.0E03	572 0	1172 4.2E02	212 7.9E05	428 0
				1172 4.2E02				1172 4.1E02	527 0
CI	CI	CI	CI	CI	CI	CI	CI	CI	CI
1.5E07-6.5E07	ALL SAME	1.8E08-2.8E08	3.9E07-9.8E08	9.1E06-2.2E07	4.1E06-1.7E07	ALL SAME	ALL SAME	2.3E06-4.0E07	ALL SAME
1.3E06-3.7E07	ALL SAME	ALL SAME	1.4E06-1.3E07	2.3E05-2.7E07	ALL SAME	ALL SAME	6.7E05-1.9E07	2.0E05-3.0E07	ALL SAME
1.1E02-1.9E02	1.3E02-1.8E02	ALL SAME	ALL SAME	ALL SAME	ALL SAME	ALL SAME	3.6E02-4.6E02	7.5E05-8.6E05	ALL SAME
				3.7E03-4.7E02				3.7E02-4.3E02	ALL SAME
									ALL SAME

EXPERIMENT B.

AODVC	AODVC	AODVC	AODVC	AODVC	AODVC	AODVC	AODVC	AODVC
0 4.9E09	0 5.0E09	0 2.2E09	0 7.6E09	0 6.8E09	0 2.2E09	0 5.6E09	0 9.8E09	0 2.0E09
144 2.5E07	144 1.4E07	144 8.1E06	144 4.4E06	144 1.1E07	144 3.6E06	144 8.9E08	24 9.8E06	24 1.7E09
216 1.8E06	216 1.1E07	216 4.5E06	216 1.0E07	216 4.4E06	216 1.0E06	216 1.0E06	216 3.8E06	144 8.9E06
312 8.1E05	312 2.0E06	312 5.2E05	312 1.0E06	312 1.0E06	312 4.9E05	312 1.1E06	312 5.8E05	216 0
408 4.3E06	408 2.2E06	408 3.7E06	408 3.5E06	408 3.6E06	408 0	408 9.3E06	408 4.7E06	312 0
554 8.9E05	554 2.4E06	554 0	554 3.7E05	554 7.1E05	554 0	554 3.0E05	554 0	408 0
986 7.2E04	986 6.2E03	986 0	986 5.9E03	986 0		986 5.2E03	986 5.2E03	554 0
CI	CI	CI	CI	CI	CI	CI	CI	CI
2.7E08-9.9E09	1.3E09-8.7E09	1.7E09-3.7E09	2.4E09-1.8E10	ALL SAME	2.4E08-4.2E09	2.3E09-8.8E09	1.6E09-9.0E10	2.2E08-5.6E09
1.4E06-6.3E07	8.2E06-1.8E07	2.7E06-1.2E07	1.1E06-1.0E07	2.0E06-2.0E08	2.7E06-2.0E07	2.4E08-2.1E09	1.3E06-3.3E07	ALL SAME
9.1E05-4.4E06	3.4E06-1.8E07	1.1E06-1.0E07	1.4E06-2.2E07	4.7E05-8.4E06	ALL SAME	ALL SAME	2.7E06-1.0E07	1.9E06-1.6E07
2.0E05-1.4E06	9.2E05-3.2E06	8.1E04-1.1E06	9.4E05-2.0E06	2.1E05-1.9E06	1.1E05-1.1E06	2.4E05-1.9E06	1.1E05-1.1E06	ALL SAME
6.2E05-8.6E06	3.9E05-3.9E06	1.2E06-6.1E06	3.7E05-6.5E06	4.8E05-6.6E06	ALL SAME	7.6E06-2.6E07	7.8E05-8.5E06	ALL SAME
4.2E06-1.4E07	6.8E05-4.1E06	ALL SAME	1.1E05-1.1E06	5.4E05-1.4E06	ALL SAME	1.9E05-7.8E05	ALL SAME	ALL SAME
4.4E04-1.0E05	1.9E03-1.2E04	ALL SAME	8.9E02-1.2E04	ALL SAME		1.5E03-1.2E04	1.5E03-1.2E04	ALL SAME
SEL	*SEL*	*SEL*	*SEL*	*SEL*	*SEL*	*SEL*	*SEL*	SEL
0 2.0E03	0 4.0E03	0 3.9E03	0 2.0E03	0 3.0E03	0 1.0E03	0 2.0E03	0 4.0E03	0 5.0E03
24 3.0E03	24 1.6E04	24 1.5E04	24 2.5E03	24 4.1E03	24 3.1E04	24 2.2E03	24 7.1E02	24 1.3E03
144 1.9E03	144 8.3E03	144 7.1E02	144 8.9E02	144 8.1E03	144 1.4E04	144 4.5E02	144 5.0E03	144 3.0E02
216 3.0E02	216 2.4E03	216 2.3E02	216 4.8E02	216 4.8E03	216 1.6E04	216 2.7E02	216 7.4E02	216 0
312 3.9E02	312 2.2E02	312 3.3E02	312 2.3E02	312 5.2E03	312 1.0E01	312 1.5E01	312 4.0E02	312 2.7E00
408 3.2E02	408 1.3E02	408 2.5E01	408 1.1E02	554 1.0E02	408 0	408 2.0E01	408 2.2E02	408 0
554 8.5E01	554 4.9E01	554 0	554 2.3E02		554 0	554 5.0E00	554 7.9E01	554 0
986 3.4E01	986 3.5E01	986 0	986 4.0E01			986 0	986 0	
CI	CI	CI	CI	CI	CI	CI	CI	CI
1.1E02-1.5E04	ALL SAME	8.7E02-1.7E04	1.1E02-1.5E04	ALL SAME	ALL SAME	1.1E02-1.5E04	2.1E03-2.9E04	ALL SAME
ALL SAME	9.4E03-4.1E04	2.1E03-2.8E04	3.9E02-8.9E03	1.6E03-6.6E03	7.1E03-6.9E04	ALL SAME	ALL SAME	1.2E02-3.8E03
1.6E03-2.2E03	3.3E03-1.3E04	6.4E02-7.6E02	ALL SAME	4.4E03-1.2E04	2.8E03-2.6E04	1.8E01-1.1E03	ALL SAME	2.2E01-2.8E03
ALL SAME	2.7E02-7.5E03	1.6E02-2.9E02	1.6E02-7.9E02	2.8E03-1.2E04	9.1E02-2.2E03	3.7E01-9.1E02	2.9E02-1.2E03	ALL SAME
2.6E02-5.2E02	1.0E02-5.3E02	ALL SAME	9.3E01-5.4E02	2.2E03-1.0E04	ALL SAME	4.8E00-7.8E01	7.7E01-7.1E02	1.3E00-1.9E01
1.3E02-7.5E02	1.9E01-4.4E02	1.0E01-1.5E02	2.2E01-2.3E02	ALL SAME	ALL SAME	1.1E01-1.5E02	2.5E01-7.0E02	1.2E-1-1.4E00
2.1E01-1.5E02	5.3E00-1.5E01	ALL SAME	ALL SAME		ALL SAME	ALL SAME	6.0E01-2.2E02	ALL SAME
4.6E00-6.7E01	ALL SAME	ALL SAME	ALL SAME			ALL SAME	ALL SAME	

APPENDIX 4 CONTINUED.

NS	NS	NS	NS	NS
24 1.0E03	24 1.8E04	24 2.0E04	24 1.6E05	24 1.0E03
408 4.0E02	986 4.7E01	986 0	986 5.0E00	986 4.2E02
986 3.0E00	1034 0.4E00		1034 0	
1034 0				
CI	CI	CI	CI	CI
ALL SAME	7.4E03-4.3E04	7.3E03-3.2E04	2.2E04-5.4E05	ALL SAME
ALL SAME	8.9E00-8.5E01	ALL SAME	ALL SAME	3.0E02-5.5E02
9.7E-1-1.6E01	ALL SAME		ALL SAME	
ALL SAME				

NS	NS	NS	NS
24 5.4E06	24 1.0E03	24 1.0E03	24 1.0E03
384 0	986 0.2E03	986 0	384 2.5E00
986 0	1034 0		986 0
CI	CI	CI	CI
2.7E05-1.1E07	ALL SAME	1.2E02-1.4E03	ALL SAME
ALL SAME	ALL SAME	ALL SAME	ALL SAME
ALL SAME	ALL SAME		ALL SAME

APPENDIX 5 - RAW DATA FOR ALS EXPERIMENTS

LEGEND:
K decay rate constant
A AODVC
S selective count
N non-selective count

EC25SW 1.15MJm⁻²

T(m)	0	60	120	210	300	360	420	510
A	1.2E07	1.6E07	2.2E07	2.9E07	9.3E06	8.1E06	1.2E06	1.5E06
S	6.0E05	-	6.0E05	4.6E05	4.4E05	4.0E05	-	-
N	1.8E06	7.9E05	8.9E05	1.0E05	7.1E05	-	5.2E05	-

A K=-0.00222 ±0.00042
S K=-0.00053 ±0.00025
N K=-0.00093 ±0.00668

EC25SW 1.31MJm⁻²

T(m)	0	60	105	240	300	360	420	480
A	6.8E06	1.7E07	1.7E07	3.0E06	-	2.7E06	3.9E06	2.5E06
S	3.8E05	4.0E05	3.6E05	3.7E05	3.0E05	3.0E05	2.9E05	2.3E05
N	4.2E05	4.9E05	5.2E05	4.1E05	4.6E05	3.1E05	3.3E05	-

A K=-0.00152 ±0.00054
S K=-0.00042 ±0.00012
N K=-0.00038 ±0.00037

EC25FW 1.37MJm⁻²

T(m)	0	60	120	180	210	300	360	480
A	2.2E06	1.1E06	6.3E05	7.8E05	2.6E05	4.4E05	8.9E04	-
S	2.1E03	-	1.1E03	-	6.2E02	-	-	1.7E02
N	7.9E02	-	-	1.0E03	-	-	-	3.0E02

A K=-0.00319 ±0.00036
S K=-0.00227 ±0.00069
N K=-0.00097 ±0.00100

EC25FW 1.12MJm⁻²

T(m)	0	60	120	210	300	420	480
A	6.2E06	2.5E06	3.2E06	2.2E06	2.0E06	-	8.9E05
S	2.7E04	7.6E04	3.5E04	2.4E04	1.1E04	6.0E03	7.9E03
N	6.5E04	8.5E04	3.0E04	1.3E04	-	1.5E04	-

A K=-0.00145 ±0.00056
S K=-0.00190 ±0.00086
N K=-0.00182 ±0.00081

EC15SW 1.37MJm⁻²

T(m)	0	30	60	120	180	240	300	360
A	-	3.2E07	2.7E07	1.9E07	6.2E07	4.2E06	1.3E06	3.4E06
S	1.3E05	1.3E05	1.2E05	4.9E05	9.3E04	1.0E05	4.0E04	3.1E04
N	5.6E05	2.3E05	2.7E05	5.0E05	3.0E03	2.2E03	2.2E03	1.7E03

A K=-0.00387 ±0.00576
S K=-0.00432 ±0.00147
N K=-0.00628 ±0.00280

EC15SW 1.16MJm⁻²

T(m)	0	15	30	60	120	180	240
A	-	-	-	2.7E07	9.8E06	3.7E06	6.3E06
S	1.3E02	6.0E01	6.0E01	3.0E01	1.0E01	1.0E01	1.2E01
N	5.8E03	8.9E02	1.2E03	1.4E03	1.2E03	7.2E02	3.0E01

A K=-0.00395 ±0.01610
S K=-0.00188 ±0.00046
N K=-0.00783 ±0.00227

EC15FW 1.17MJm⁻²

T(m)	0	60	120	180	240	360	420	480
A	2.8E07	2.2E07	1.2E05	6.8E04	7.2E04	8.9E03	8.9E03	6.6E03
S	8.0E03	7.1E03	1.1E04	7.1E03	8.0E03	5.0E03	4.5E03	4.5E03
N	-	-	-	-	-	-	-	-

A K=-0.00726 ±0.00105
S K=-0.00057 ±0.00043

EC15FW 1.19MJm⁻²

T(m)	0	60	150	210	270	390	450	510
A	5.9E06	6.3E06	1.5E07	8.9E06	-	7.2E07	4.7E06	3.3E06
S	5.0E00	5.0E00	5.0E00	5.0E00	4.0E00	-	-	-
N	7.4E05	-	6.2E05	-	-	1.5E05	-	-

A K=+0.00153 ±0.00140
S K=-0.00027 ±0.00046
N K=-0.00185 ±0.00078

EC05SW 1.25MJm⁻²

T(m)	0	30	55	150	240	300	360	420
A	-	-	5.5E06	3.7E06	8.9E06	4.9E06	4.9E04	2.4E06
S	1.0E03	4.7E03	1.0E03	7.1E02	4.5E02	4.7E01	8.9E01	1.3E02
N	1.0E04	1.0E04	1.0E04	1.1E03	7.1E02	4.1E02	4.0E02	4.0E02

A K=-0.00288 ±0.00037
S K=-0.00264 ±0.00478
N K=-0.00395 ±0.00096

EC05SW 1.02MJm⁻²

T(m)	0	30	60	150	210	270	360	420
A	1.5E07	1.4E07	1.3E05	3.4E05	3.7E04	2.0E04	8.9E04	5.0E03
S	2.0E04	1.0E04	2.2E04	1.3E04	8.1E03	1.2E04	-	2.0E03
N	7.1E05	6.6E05	1.3E05	1.0E05	2.3E05	2.4E05	-	-

A K=-0.00707 ±0.00107
S K=-0.00291 ±0.00080
N K=-0.00162 ±0.00184

EC05FW 1.19MJm⁻²

T(m)	0	30	60	120	180	240	300	360
A	-	-	3.4E04	8.9E04	-	1.1E05	4.4E03	3.6E03
S	-	-	-	-	-	-	-	-
N	1.0E04	1.0E04	-	6.9E03	6.2E03	7.6E03	9.8E03	2.0E03

A K=-0.00392 ±0.00285
N K=-0.00119 ±0.00119

EC05FW 1.35MJm⁻²

T(m)	0	30	60	180	300	360	420	540
A	-	3.3E07	2.2E07	-	8.9E04	1.2E05	-	2.5E05
S	9.8E02	9.5E02	9.5E02	1.1E03	1.0E03	9.8E02	1.0E03	5.0E01
N	4.0E04	2.0E04	1.3E05	4.0E04	-	1.0E03	-	-

A K=-0.00503 ±0.01200
S K=-0.00117 ±0.00117
N K=-0.00436 ±0.00239

EC25SW 2.97MJm⁻²

T(m)	0	60	120	210	300	
A	4.5E05	-	-	3.6E05	3.2E05	-0.00049 ±0.00091
S	1.2E04	8.5E03	7.5E03	4.0E03	3.5E03	-0.00186 ±0.00072
N	8.1E03	5.7E03	4.8E03	1.6E03	2.0E03	-0.00234 ±0.00089

EC25SW 2.52MJm⁻²

T(m)	0	60	120	240	300	
A	2.6E05	-	1.3E05	1.6E05	1.2E05	-0.00090 ±0.00075
S	2.1E04	1.7E04	1.8E04	1.4E04	8.0E03	-0.00411 ±0.00090
N	3.5E04	5.0E04	2.5E04	3.0E04	3.0E04	-0.00043 ±0.00170

EC25FW 2.70MJm⁻¹

T(m)	0	60	120	240	300	
A	1.4E05	-	1.2E05	1.1E05	7.8E04	-0.00074 ±0.00059
S	3.5E04	2.6E04	2.7E04	2.2E04	8.0E03	-0.00171 ±0.00101
N	1.5E04	4.0E04	3.0E04	3.0E04	2.5E04	+0.00033 ±0.00188

EC25FW 2.52MJm⁻¹

T(m)	0	60	120	220	300	
A	1.4E05	-	1.4E05	7.2E04	4.8E04	-0.00164 ±0.00111
S	3.1E04	3.6E04	2.6E04	2.8E04	2.6E04	-0.00033 ±0.00033
N	2.0E04	1.5E04	1.5E04	1.0E04	9.0E03	-0.00115 ±0.00244

EC15SW 2.52MJm⁻¹

T(m)	0	90	180	240	300	
A	4.6E05	4.5E05	-	2.8E05	1.6E05	-0.00146 ±0.00076
S	7.5E04	5.9E04	6.4E04	5.8E04	-	-0.00037 ±0.00074
N	8.5E04	7.1E04	6.8E04	6.8E04	4.8E04	-0.00067 ±0.00042

EC15SW 2.68MJm⁻¹

T(m)	0	60	120	240	300	
A	1.1E05	-	4.4E04	3.6E04	3.5E04	-0.00079 ±0.00096
S	1.3E04	3.5E03	6.0E03	5.0E03	5.0E03	-0.00460 ±0.00150
N	1.5E04	1.5R04	5.0E03	1.0E03	1.0E03	-0.00120 ±0.00190

EC15FW 2.47MJm⁻¹

T(m)	0	60	120	240	300	
A	6.3E05	-	3.4E05	3.3E05	1.6E05	-0.00168 ±0.00067
S	4.0E03	4.5E03	4.0E03	4.0E03	2.0E03	-0.00083 ±0.00130
N	1.0E04	1.5E04	1.0E04	1.5E04	1.0E04	+0.00003 ±0.00148

EC15FW 2.63MJm⁻¹

T(m)	0	60	120	240	300	
A	4.4E04	-	3.4E04	2.2E04	2.0E04	-0.00120 ±0.00033
S	1.4E04	1.7E04	1.6E04	9.5E03	7.5E03	-0.00108 ±0.00105
N	1.5E04	5.0E03	4.5E03	5.0E03	5.0E03	-0.00109 ±0.00284

EC05SW 2.61MJm⁻¹

T(m)	0	60	120	210	300	
A	1.7E05	-	8.3E04	8.1E04	1.5E05	-0.00026 ±0.00072
S	2.5E04	1.4E04	-	1.9E04	1.5E04	-0.00047 ±0.00094
N	2.0E04	1.0E04	1.0E04	1.5E04	2.0E04	+0.00035 ±0.00410

EC05SW 2.70MJm⁻¹

T(m)	0	60	120	240	300	
A	3.2E04	-	4.7E04	-	8.1E03	-0.00217 ±0.00199
S	1.2E04	9.0E03	9.5E03	3.0E03	3.5E03	-0.00207 ±0.00100
N	1.5E04	1.5E04	5.0E03	5.0E03	5.0E03	-0.00176 ±0.00211

EC05FW 2.63MJm⁻¹

T(m)	0	60	120	240	360	
A	1.6E05	-	1.5E05	1.8E05	2.2E05	+0.00041 ±0.00057
S	6.0E04	1.3E04	5.1E04	3.7E04	4.6E04	-0.00038 ±0.00188
N	5.0E04	1.5E04	6.5E04	3.5E04	8.5E04	-0.00091 ±0.00108

EC05FW 2.54MJm⁻¹

T(m)	0	60	120	240	300	
A	4.4E04	-	2.7E04	-	1.5E04	-0.00155 ±0.00076
S	4.7E04	3.3E04	-	3.2E04	3.3E04	-0.00183 ±0.00042
N	4.0E04	-	-	3.0E04	2.5E04	-0.00040 ±0.00270

FM25SW 1.20MJm⁻¹

T(m)	0	60	120	210	270	330
A	1.9E05	-	2.8E05	-	-	2.2E05
S	1.0E04	8.8E03	9.5E03	8.6E03	-	2.7E02
N	1.1E03	7.0E02	5.0E02	8.0E02	2.0E02	-

A K=+0.00012 ±0.00084
S K=-0.00432 ±0.00246
N K=-0.00198 ±0.00146

FM25FW 1.15MJm⁻¹

T(m)	0	60	120	210	270	
A	1.4E05	-	3.4E05	-	2.6E05	+0.00092 ±0.00107
S	-	2.4E03	2.3E03	2.1E03	-	-0.00039 ±0.00020
N	-	2.3E03	-	2.2E03	2.1E03	-0.00068 ±0.00040

FM15SW 1.17MJm⁻¹

T(m)	0	60	120	210	270	360
A	1.4E07	-	1.1E07	8.6E06	-	-
S	3.6E02	4.8E02	4.2E02	4.2E02	4.1E02	4.4E02
N	3.0E02	4.0E02	2.0E02	2.0E02	6.0E02	2.0E02

A K=-0.00100 ±0.00095
S K=-0.00024 ±0.00025
N K=-0.00068 ±0.00125

FM15FW 1.18MJm⁻¹

T(m)	0	60	120	180	270	360
A	8.9E05	-	1.3E06	-	9.2E05	-
S	-	1.0E02	9.0E01	2.0E01	-	9.0E01
N	1.0E02	-	1.0E02	2.8E01	-	4.5E01

A K=+0.000001 ±0.00088
S K=-0.00103 ±0.00312
N K=-0.00026 ±0.00146

FM05SW 1.13MJm⁻¹

T(m)	0	60	120	180	270	360
A	1.5E06	-	1.2E06	-	-	1.3E06
S	1.1E02	7.0E01	5.0E01	8.0E01	2.0E01	6.0E01
N	5.6E01	7.0E01	4.5E01	6.2E01	5.2E01	5.4E01

A K=-0.00013 ±0.00068
S K=-0.00106 ±0.00140
N K=-0.00013 ±0.00037

FM05FW 1.21MJm⁻¹

T(m)	0	60	120	180	270	360
A	7.9E05	-	8.2E05	-	-	1.0E06
S	2.0E01	1.7E01	1.7E01	-	-	-
N	3.0E03	3.6E03	3.6E03	3.6E03	4.1E03	4.2E03

A K=+0.00030 ±0.00061
S K=-0.00059 ±0.00117
N K=-0.00036 ±0.00010

FM25SW 2.56MJm⁻¹

T(m)	0	60	120	240	300	
A	2.3E04	-	1.7E04	1.8E04	1.9E04	-0.00025 ±0.00085
S	1.3E03	9.4E02	7.0E02	4.5E02	4.2E02	-0.00166 ±0.00046
N	1.2E03	1.6E03	9.9E02	4.9E02	5.0E02	-0.00170 ±0.00106

FM25SW 2.77MJm⁻¹

T(m)	0	60	120	240	300	
A	6.5E03	-	6.4E03	3.6E03	1.8E03	-0.00177 ±0.00073
S	3.6E02	-	3.7E02	1.8E02	1.5E02	-0.00140 ±0.00070
N	6.0E02	5.0E02	2.0E02	1.0E02	1.0E02	-0.00287 ±0.00130

FM25FW 2.74MJm⁻¹

T(m)	0	60	120	180	300	
A	3.3E04	-	2.1E04	1.3E04	1.6E04	-0.00114 ±0.00011
S	1.5E02	1.4E02	1.5E02	1.2E02	1.2E02	-0.00035 ±0.00066
N	8.9E02	5.0E02	-	-	3.0E02	-0.00287 ±0.00342

FM25FW 2.48MJm⁻¹

T(m)	0	60	120	240	300	
A	7.0E03	-	7.9E03	4.9E03	3.5E03	-0.00103 ±0.00140
S	8.9E02	-	8.5E02	-	8.0E02	-0.00015 ±0.00060
N	9.0E02	8.4E02	8.1E02	7.5E02	2.3E02	-0.00156 ±0.00140

FM15SW 2.74MJm⁻¹

T(m)	0	60	120	240	300	
A	-	-	-	-	-	
S	8.8E02	8.1E02	1.9E02	3.0E01	8.1E01	-0.00458 ±0.00209
N	7.5E02	4.5E02	6.0E02	2.6E02	6.5E02	-0.00053 ±0.00229

FM15SW 2.66MJm⁻¹

T(m)	0	60	120	240	300	
A	1.3E04	-	1.4E04	1.2E04	1.6E04	+0.00015 ±0.00088
S	3.3E02	2.5E02	2.0E02	9.9E01	1.0E02	-0.00263 ±0.00075
N	8.0E02	1.0E02	2.0E02	1.5E02	5.0E01	-0.00270 ±0.00320

FM15FW 2.75MJm⁻¹

T(m)	0	60	120	240	300	
A	1.5E04	-	1.4E04	1.7E04	1.9E04	+0.00028 ±0.00101
S	5.3E02	4.8E02	3.2E02	1.4E02	1.3E02	-0.00229 ±0.00056
N	6.9E02	7.4E02	3.2E02	5.0E02	3.0E02	-0.00104 ±0.00150

FM15FW 2.77MJm⁻¹

T(m)	0	60	120	240	300	
A	1.6E04	-	9.6E03	7.2E03	-	-0.00144 ±0.00140
S	9.6E02	8.6E02	6.2E02	8.0E02	5.9E02	-0.00052 ±0.00054
N	1.3E03	1.1E03	8.0E02	1.1E03	1.0E03	-0.00022 ±0.00410

FM05SW 2.73MJm⁻¹

T(m)	0	60	120	240	300	
A	1.9E04	-	1.9E04	1.6E04	-	-0.00031 ±0.00120
S	3.3E02	2.5E02	1.9E02	1.6E02	6.0E01	-0.00210 ±0.00097
N	2.2E02	4.2E02	2.0E02	1.5E02	5.0E01	-0.00113 ±0.00290

FM05SW 2.48MJm⁻¹

T(m)	0	60	120	240	300	
A	1.9E04	-	2.3E04	2.4E04	1.5E04	-0.00019 ±0.00085
S	8.5E02	6.6E02	6.6E02	3.7E02	3.4E02	-0.00137 ±0.00040
N	7.0E02	3.0E02	2.5E02	1.0E01	1.0E02	-0.00277 ±0.00100

FM05FW 2.63MJm⁻¹

T(m)	0	60	120	240	300	
A	1.5E04	-	1.3E04	-	3.6E03	-0.00215 ±0.00112
S	8.0E01	-	5.3E01	-	3.0E01	-0.00142 ±0.00102
N	2.0E02	1.5E02	2.0E02	1.0E02	-	-0.00111 ±0.00280

FM05FW 2.63MJm⁻¹

T(m)	0	60	120	240	300	
A	4.1E03	-	2.8E03	5.3E03	6.4E03	+0.00059 ±0.00087
S	1.0E01	1.0E01	2.0E01	1.0E00	1.0E00	-0.00419 ±0.00480
N	1.0E01	1.0E01	2.0E01	1.0E00	1.0E00	-0.00419 ±0.00480

FS25SW 1.21MJm⁻¹

T(m)	0	60	120	240	300	360
A	4.8E05	-	-	3.1E06	-	1.2E06
S	1.9E01	1.6E01	9.0E00	4.4E00	4.5E00	1.1E00
N	1.5E01	-	4.0E00	-	3.5E00	0.00

A K=+0.00143 ±0.00096
S K=-0.00307 ±0.00087
N K=-0.00197 ±0.00107

FS25FW 1.17MJm⁻¹

T(m)	0	60	120	210	270	360
A	1.1E06	-	8.4E05	-	-	4.8E05
S	1.5E01	1.4E01	2.0E01	1.4E01	7.2E00	1.1E00
N	3.2E01	3.2E01	3.2E01	-	2.2E01	-

A K=-0.00100 ±0.00159
S K=-0.00276 ±0.00145
N K=-0.00063 ±0.00038

FS15SW 1.16MJm⁻¹

T(m)	0	60	120	240	300	360
A	1.1E05	-	-	7.3E04	-	7.0E04
S	2.6E01	2.2E01	2.0E01	8.8E00	7.0E00	5.6E00
N	3.5E01	3.8E01	2.8E01	1.2E01	5.0E00	4.0E00

A K=-0.00057 ±0.00596
S K=-0.00199 ±0.00025
N K=-0.00295 ±0.00059

FS15FW 1.07MJm⁻¹

T(m)	0	60	120	210	270	360
A	4.6E04	-	-	1.5E05	-	2.6E04
S	3.0E02	1.0E02	1.5E02	1.6E02	1.1E02	1.3E02
N	1.8E02	1.3E02	8.7E01	-	-	-

A K=-0.00049 ±0.00049
S K=-0.00064 ±0.00081
N K=-0.00263 ±0.00056

FS05SW 1.06MJm⁻¹

T(m)	0	60	120	240	300	360
A	1.4E05	-	-	5.3E04	-	1.1E05
S	2.3E02	-	2.5E02	1.3E02	6.8E01	1.1E02
N	1.8E02	2.0E02	1.4E02	5.6E01	2.2E01	1.3E01

A K=-0.00040 ±0.00050
S K=-0.00133 ±0.00089
N K=-0.00360 ±0.00068

FS05FW 1.22MJm^{-1}

T(m)	0	90	180	240	330	420
A	3.3E04	-	1.8E04	-	-	2.0E04
S	1.2E03	8.4E02	1.2E03	7.3E02	8.6E02	8.2E02
N	9.0E02	5.0E02	1.3E03	2.0E02	6.0E02	7.0E02

A K=-0.00039 ±0.00111
S K=-0.00034 ±0.00036
N K=-0.00032 ±0.00127

FS25SW 2.83MJm^{-1}

T(m)	0	60	120	300	
A	-	-	-	-	
S	1.0E02	8.0E01	4.5E01	1.0E00	-0.00703 ±0.00170
N	7.3E02	3.1E02	6.0E01	3.0E00	-0.00808 ±0.00101

FS25SW 2.70MJm^{-1}

T(m)	0	60	120	240	300	
A	8.3E04	-	7.1E04	7.0E04	4.5E04	-0.00072 ±0.00038
S	1.3E03	1.2E03	5.5E02	1.3E02	1.0E02	-0.00416 ±0.00106
N	8.8E02	6.6E02	2.5E02	0.00	0.00	-0.01570 ±0.00620

FS25FW 2.65MJm^{-1}

T(m)	0	60	120	180	300	
A	2.2E03	-	2.0E03	3.2E03	5.9E03	+0.00135 ±0.00111
S	1.0E02	1.0E02	9.7E01	9.9E01	3.4E01	-0.00118 ±0.00350
N	2.4E02	1.9E02	1.3E02	1.8E02	1.9E02	-0.00253 ±0.00069

FS25FW 2.66MJm^{-1}

T(m)	0	60	120	240	300	
A	-	-	-	-	-	
S	2.4E01	6.7E02	5.5E02	2.6E02	-	-0.00234 ±0.00610
N	7.0E02	1.1E03	8.0E02	4.0E02	4.7E02	-0.00173 ±0.00110

FS15SW 2.75MJm^{-1}

T(m)	0	60	120	180	300	
A	2.1E04	-	1.1E04	1.4E04	2.6E04	+0.00036 ±0.00086
S	2.0E02	9.8E01	1.7E00	1.5E00	1.0E00	-0.00923 ±0.00420
N	4.5E02	-	-	5.0E00	5.0E00	-0.00686 ±0.01130

FS15SW 2.81MJm^{-1}

T(m)	0	60	120	240	300	
A	6.8E04	-	1.2E05	1.2E05	7.3E04	-0.00173 ±0.00106
S	3.8E02	1.8E02	1.0E02	1.5E01	5.0E01	-0.00373 ±0.00207
N	2.0E02	1.5E02	1.5E02	1.0E02	5.0E01	-0.00177 ±0.00206

FS15FW 2.67MJm^{-1}

T(m)	0	60	120	180	300	
A	1.8E05	-	4.1E05	6.8E05	4.7E05	+0.00148 ±0.00106
S	2.0E01	1.6E01	1.4E01	9.5E00	7.3E00	-0.00151 ±0.00087
N	4.5E02	3.0E02	3.0E02	1.5E02	1.0E02	-0.00221 ±0.00127

FS15FW 2.68MJm^{-1}

T(m)	0	60	120	240	300	
A	8.9E03	-	1.3E04	1.2E04	2.0E04	+0.00094 ±0.00120
S	6.0E02	9.8E02	7.1E02	3.2E02	3.4E02	-0.00136 ±0.00082
N	4.0E02	8.4E02	5.0E02	7.4E02	-	-0.00139 ±0.00380

FS05SW 2.77MJm^{-1}

T(m)	0	60	120	240	300	
A	2.2E04	-	2.9E04	2.5E04	3.4E04	+0.00046 ±0.00084
S	8.7E02	2.9E02	1.1E02	5.0E01	5.0E01	-0.00405 ±0.00113
N	1.5E03	3.2E02	2.0E02	1.0E02	1.0E02	-0.00340 ±0.00332

FS05SW 2.70MJm^{-1}

T(m)	0	60	120	240	300	
A	8.9E03	-	1.1E04	6.1E03	3.3E03	-0.00141 ±0.00093
S	2.2E03	1.9E03	1.9E03	1.3E02	2.4E01	-0.00674 ±0.00230
N	2.0E03	1.2E03	1.7E03	2.0E02	1.0E01	-0.00702 ±0.00430

FS05FW 2.60MJm^{-1}

T(m)	0	60	120	240	300	
A	2.1E04	-	1.6E04	1.3E04	1.4E04	-0.00064 ±0.00088
S	2.9E02	2.4E02	2.3E02	4.9E01	4.2E01	-0.00316 ±0.00115
N	4.6E02	2.0E02	2.5E02	-	2.0E02	-0.00861 ±0.00135

FS05FW 2.72MJm⁻¹
T(m) 0 60 120 240 300
A 1.2E04 - 4.0E03 4.8E03 6.3E03 -0.00087 ±0.00107
S 6.6E02 5.0E02 2.0E02 1.3E02 5.0E01 -0.00355 ±0.00081
N 2.5E02 4.0E02 1.0E02 3.5E02 1.0E02 -0.00090 ±0.00220

SO25SW 1.12MJm⁻¹
T(m) 0 60 120 180 300 360
A 4.3E06 - 2.4E06 - - 8.6E05
-0.00193 ±0.00074
S 9.0E04 - 5.0E04 5.2E04 4.0E04 -
-0.00112 ±0.00082
N 2.8E05 6.4E04 6.0E04 7.1E04 4.9E04 -
-0.00190 ±0.00168

SO25FW 1.13MJm⁻¹
T(m) 0 60 120 180 300 360
A 2.7E06 - 9.3E05 - - 1.0E06
-0.00101±0.00067
S 2.2E03 2.0E03 2.5E03 2.5E03 2.5E03 1.1E03
-0.00020±0.00109
N 3.8E04 3.0E04 5.0E04 4.8E04 4.2E04 -
-0.00034±0.00072

SO15SW 1.06MJm⁻¹
T(m) 0 60 120 180 300 360
A 1.2E06 - - 8.3E05 - 7.2E05
-0.00062±0.00059
S 2.0E03 2.0E03 2.0E03 2.0E03 2.0E03 3.2E02
-0.00189±0.00230
N 1.8E04 - 1.0E04 1.8E04 1.5E04 1.6E04
+0.00019±0.00068

SO15FW 1.12MJm⁻¹
T(m) 0 60 150 270 330 390
A 1.7E05 - 1.2E05 - - 5.1E04
-0.00136±0.00053
S 5.0E01 3.7E01 3.2E01 2.1E01 8.0E00 2.0E00
+0.00119±0.00220
N 7.8E03 9.1E03 - 8.8E03 9.0E03 9.5E03
+0.00014±0.00209

SO05SW 0.97MJm⁻¹
T(m) 0 60 120 240 300 360
A 3.1E04 1.7E04 - 1.9E04 - 1.9E04
-0.00045±0.00049
S 1.3E01 7.0E00 1.5E01 1.6E01 1.3E01 2.1E01
+0.00071±0.00069
N 9.0E02 1.0E03 6.0E02 8.0E02 4.0E02 1.1E02
-0.00203±0.00069

SO05FW 1.13MJm⁻¹
T(m) 0 60 120 180 270 360
A 2.5E06 1.6E06 - 1.0E06 - 6.2E05
-0.00161±0.00062
S 1.8E02 5.0E02 1.0E02 - 6.0E02 3.0E02
-0.00202±0.00160
N 1.1E04 - 1.1E04 1.0E04 1.0E04 9.1E03
-0.00023±0.00196

SO25SW 2.52MJm⁻¹
T(m) 0 60 120 240 300
A 5.6E05 4.9E05 4.9E05 - 2.6E05 -0.00112 ±0.00087
S 1.5E04 1.4E04 1.3E04 - 1.2E04 -0.00031 ±0.00024
N 2.2E04 2.3E04 2.1E04 1.8E04 1.8E04 -0.00037 ±0.00015

SO25SW 2.95MJm⁻¹
T(m) 0 60 120 240 300
A 1.8E06 - 7.9E05 5.7E05 5.7E05 -0.00167 ±0.00114
S 2.0E03 1.0E03 1.9E03 1.7E03 8.0E02 -0.00070 ±0.00108
N 1.6E04 1.5E04 1.4E04 1.4E04 1.4E04 -0.00048 ±0.00012

SO25FW 2.30MJm⁻¹
T(m) 0 60 120 240 300
A 7.6E05 - 5.6E05 1.1E05 4.4E05 -0.00026 ±0.00073
S - 3.2E03 3.4E03 8.5E02 1.6E03 -0.00018 ±0.00201
N - 1.1E04 1.1E04 1.1E04 9.7E03 -0.00025 ±0.00013

SO25FW 2.73MJm⁻¹
T(m) 0 60 120 240 300
A 1.2E06 - 1.6E06 5.8E05 9.4E05 -0.00082 ±0.00106
S 5.0E03 4.0E03 1.9E03 1.7E03 2.6E03 -0.00115 ±0.00106
N 3.2E04 2.1E04 2.1E04 1.8E04 - -0.00083 ±0.00091

SO15SW 2.88MJm⁻¹
T(m) 0 60 120 240 300
A 1.5E06 - - 1.2E06 5.6E05 -0.00113 ±0.00076
S 1.3E03 2.2E03 2.2E03 1.3E03 1.0E03 -0.00069 ±0.00289
N 1.1E04 1.2E04 7.9E03 7.5E03 1.0E02 -0.00524 ±0.00559

SO15SW 2.54MJm⁻¹

T(m)	0	60	120	240	300	
A	1.8E05	-	1.3E05	1.3E05	1.2E05	-0.00053 ±0.00056
S	4.5E03	2.9E03	2.5E03	6.0E02	1.2E03	-0.00245 ±0.00138
N	7.8E03	7.7E03	-	-	7.2E03	-0.00012 ±0.00032

SO15FW 2.48MJm⁻¹

T(m)	0	60	120	240	300	
A	5.1E06	-	3.6E06	3.9E06	2.0E06	-0.00107 ±0.00068
S	7.5E03	4.6E03	5.5E03	6.6E03	3.7E03	-0.00052 ±0.00120
N	1.6E04	1.3E04	1.4E04	1.3E03	-	-0.00029 ±0.00026

SO15FW 2.20MJm⁻¹

T(m)	0	60	120	240	300	
A	2.2E06	-	-	9.2E05	2.4E05	-0.00274 ±0.00071
S	2.4E03	1.9E03	1.2E03	1.3E03	9.0E02	-0.00123 ±0.00053
N	1.1E04	1.1E04	1.3E04	7.6E03	6.1E03	-0.00092 ±0.00053

SO05SW 2.61MJm⁻¹

T(m)	0	60	120	240	300	
A	7.4E05	-	3.6E05	2.2E05	1.7E05	-0.00210 ±0.00117
S	1.5E04	1.5E04	1.4E04	1.1E04	1.1E04	-0.00054 ±0.00014
N	1.2E04	1.5E04	1.1E04	1.1E04	1.5E04	-0.00032 ±0.00197

SO05SW 2.32MJm⁻¹

T(m)	0	60	120	240	300	
A	5.8E06	-	5.0E06	-	3.5E06	-0.00074 ±0.00077
S	1.8E03	2.1E03	9.0E02	8.0E02	9.0E02	-0.00121 ±0.00111
N	5.7E04	4.7E04	4.8E04	4.5E04	3.8E04	-0.00046 ±0.00018

SO05FW 2.84MJm⁻¹

T(m)	0	60	120	240	300	
A	-	-	-	-	-	
S	4.1E03	3.2E03	3.6E03	3.9E03	-	+0.00003 ±0.00064
N	1.8E04	1.8E04	1.5E04	1.3E04	1.6E04	-0.00064 ±0.00026

SO05FW 2.21MJm⁻¹

T(m)	0	60	120	240	300	
A	3.2E06	-	2.9E06	1.6E06	9.9E05	-0.00395 ±0.00078
S	3.7E03	3.2E03	3.0E03	-	1.7E03	-0.00104 ±0.00056
N	1.9E04	1.7E04	1.6E04	1.5E04	1.4E04	-0.00040 ±0.00016

SM25SW 1.03MJm⁻¹

T(m)	0	60	150	240	300	360
A	1.7E06	3.1E06	-	1.5E06	-	1.8E04
-0.00033±0.00056						
S	1.7E03	1.0E03	2.0E03	2.0E03	1.6E03	1.1E03
-0.00006±0.00065						
N	1.3E04	2.1E04	1.4E04	2.6E04	9.0E03	-
-0.00026±0.00120						

SM25FW 1.12MJm⁻¹

T(m)	0	60	120	180	270	360
A	5.7E06	4.6E06	-	4.4E06	-	3.3E06
-0.00072±0.00072						
S	4.0E03	5.0E03	-	3.0E03	3.0E03	4.0E03
-0.00027±0.00083						
N	1.5E04	1.3E04	1.8E04	2.0E04	1.5E04	2.9E04
+0.00068±0.00077						

SM15SW 1.12MJm⁻¹

T(m)	0	60	120	180	300	360
A	7.8E04	4.3E04	-	4.1E04	-	5.1E04
-0.00032±0.00073						
S	1.4E02	5.3E01	2.0E02	5.4E01	9.0E01	5.3E01
-0.00063±0.00104						
N	7.1E02	1.0E03	8.8E02	-	8.1E02	5.0E02
-0.00044±0.00186						

SM15FW 1.17MJm⁻¹

T(m)	0	60	120	240	360	420
A	4.8E04	-	-	5.0E04	5.4E04	-
+0.00013±0.00076						
S	2.0E02	5.0E02	3.0E02	9.0E02	7.0E02	6.0E02
+0.00105±0.00070						
N	5.4E03	1.2E04	7.8E03	8.8E03	1.0E04	8.4E03
+0.00023±0.00046						

SM05SW 1.04MJm⁻¹

T(m)	0	60	120	210	270	360
A	4.3E04	3.4E04	-	3.4E04	-	2.1E04
-0.00075±0.00049						
S	2.0E02	1.0E02	1.0E02	3.0E02	4.0E02	4.0E02
+0.00066±0.00098						
N	3.8E03	3.4E03	3.5E03	3.4E03	3.5E03	3.8E03
+0.00002±0.00032						

SM05FW 1.08MJm⁻²

T(m)	0	60	150	270	330	360
A	3.6E04	-	-	2.2E04	-	2.0E04
	-0.00068±0.00055					
S	4.0E02	6.0E02	1.0E03	9.0E02	6.0E02	1.1E03
	+0.00072±0.00089					
N	4.8E03	5.7E03	4.3E03	3.7E03	4.8E03	4.8E03
	-0.00013±0.00034					

SM25SW 2.81MJm⁻²

T(m)	0	60	120	240	300	
A	-	-	-	-	-	
S	1.6E03	1.3E03	7.0E02	1.9E03	7.5E02	-0.00107 ±0.00318
N	2.5E04	2.1E04	2.5E04	2.5E04	1.9E04	-0.00025 ±0.00028

SM25SW 2.52MJm⁻²

T(m)	0	60	120	240	300	
A	2.9E05	-	1.3E05	1.5E05	1.8E05	-0.00064 ±0.00091
S	5.8E03	-	5.2E03	5.4E03	3.8E03	-0.00047 ±0.00043
N	1.2E04	1.5E04	1.1E04	-	1.1E04	-0.00043 ±0.00059

SM25FW 2.77MJm⁻²

T(m)	0	60	120	240	300	
A	8.9E05	-	3.9E05	3.3E05	1.0E05	-0.00272 ±0.00101
S	7.2E03	7.1E03	6.8E03	6.2E03	4.4E03	-0.00062 ±0.00057
N	1.3E04	1.2E04	1.0E04	-	1.1E04	-0.00022 ±0.00035

SM25FW 2.81MJm⁻²

T(m)	0	60	120	240	300	
A	-	-	-	-	-	
S	1.5E03	3.0E03	1.8E03	1.2E03	-	-0.00063 ±0.00157
N	6.3E04	5.3E04	6.1E04	3.2E04	4.5E04	-0.00072 ±0.00057

SM15SW 2.83MJm⁻²

T(m)	0	60	120	240		
A	8.3E05	-	6.7E05	4.0E05		-0.00132 ±0.00131
S	7.1E03	7.1E03	6.6E03	6.2E03		-0.00027 ±0.00037
N	1.5E04	1.3E04	1.2E04	1.1E04		-0.00054 ±0.00035

SM15SW 3.02MJm⁻²

T(m)	0	60	120	180	240	300
A	4.9E05	-	5.1E05	4.4E05	2.2E05	-
	-0.00097±0.00107					
S	1.5E03	1.2E03	1.4E03	-	-	1.1E03
	-0.00037±0.00120					
N	3.6E04	4.7E04	2.8E04	3.0E04	-	2.6E04
	-0.00059±0.00076					

SM15FW 2.81MJm⁻²

T(m)	0	60	120	240	300	
A	1.5E05	-	1.5E05	1.7E05	9.4E04	-0.00044 ±0.00079
S	5.0E02	4.0E02	4.0E02	-	1.0E02	-0.00081 ±0.00219
N	9.0E03	1.4E04	5.8E03	6.5E03	5.2E03	-0.00089 ±0.00077

SM15FW 2.82MJm⁻²

T(m)	0	60	120	240	300	
A	7.4E05	-	2.0E05	3.2E05	-	-0.00152 ±0.00149
S	1.9E03	2.1E03	1.7E03	1.4E03	1.7E03	-0.00037 ±0.00107
N	3.3E04	4.5E04	3.7E04	3.6E04	2.7E04	-0.00036 ±0.00075

SM05SW 2.75MJm⁻²

T(m)	0	60	120	240	300	
A	6.1E05	-	1.6E05	2.5E05	1.4E05	-0.00192 ±0.00100
S	3.3E03	-	3.3E03	-	1.8E03	-0.00092 ±0.00095
N	5.0E04	5.1E04	4.8E04	-	3.7E04	-0.00048 ±0.00030

SM05SW 2.86MJm⁻²

T(m)	0	60	120	150	300	
A	3.0E05	-	2.6E05	1.7E05	1.8E05	-0.00086 ±0.00089
S	2.3E03	2.3E03	2.2E03	2.5E03	1.2E03	-0.00065 ±0.00088
N	2.7E04	2.5E04	-	2.0E04	2.3E04	-0.00025 ±0.00049

SM05FW 2.73MJm⁻²

T(m)	0	60	120	240	300	
A	1.6E05	-	6.9E04	1.1E05	6.9E04	-0.00085 ±0.00102
S	1.0E02	5.0E01	1.0E02	1.0E02	1.5E02	+0.00085 ±0.00118
N	7.5E04	-	6.9E04	7.3E04	7.1E04	-0.00014 ±0.00029

SM05FW 2.58MJm^{-1}

T(m)	0	60	120	240	300	
A	1.3E05	-	7.5E04	5.4E04	3.6E04	-0.00188 ±0.00072
S	2.8E03	2.1E03	2.5E03	-	2.0E02	-0.00037 ±0.00067
N	3.5E04	2.9E04	2.6E04	-	3.5E04	-0.00108 ±0.00105

ALS DATA (DARK CONTROLS)

EC25SW (1.15)

T(h)	0	168	336	432	648	1056
A	1.2EO7	1.5E05	1.6E06	-	9.1E05	1.6E06
S	6.0E05	3.4E04	-	0	0	0
N	1.8E06	5.2E04	-	0	0	0

A K=-0.00022 ±0.00032
S K=-0.01620 ±0.00018
N K=-0.01720 ±0.00313

EC25SW (1.31)

T(h)	0	120	144	312	408	624	1032
A	6.8E06	-	5.2E06	1.6E06	-	2.0E06	2.2E06
S	3.8E05	2.3E04	-	-	9.8E03	0	0
N	4.2E05	2.8E04	-	-	7.9E03	0	0

A K=-0.00045 ±0.00045
S K=-0.00991 ±0.00991
N K=-0.01010 ±0.01010

EC25FW (1.37)

T(h)	0	168	192	240	288	552	960
A	2.2E06	-	-	1.3E06	-	2.5E05	9.5E05

K=-0.00049 ±0.00043

EC25FW (1.12)

T(h)	0	168	192	240	288	552	960
S	2.7E04	1.4E03	-	-	8.9E02	-	-
N	6.5E04	-	5.0E02	-	4.7E02	-	-

K=-0.00531 ±0.00364
K=-0.00794 ±0.00375

EC15SW (1.37)

T(h)	0	264	432	504	624	960
A	-	1.8E06	9.5E05	9.5E05	6.2E05	1.8E05

K=-0.00016 ±0.00016

T(h)	0	72	144	240	432	504	768
S	1.3E02	1.8E01	8.3E00	8.9E00	3.7E00	2.4E00	0

K=-0.00272 ±0.00272

EC15SW (1.16)

T(h)	0	432	624	888	936	1392
N	5.6E05	-	-	2.0E05	7.9E04	1.5E00

K=-0.00427 ±0.00427

EC15FW (1.17)

N	9.5E03	-	-	-	2.0E01	4.6E01	-

K=-0.00315 ±0.00062

EC15FW (1.19)

T(h)	0	96	216	600	1128	1176	1224
A	5.9E04	5.8E05	7.1E04	1.1E05	-	-	3.1E06
S	3.9E00	1.3E00	-	0	0	0	0

A K=+0.00107 ±0.00302
S K=-0.00438 ±0.00089

EC05SW (1.25)

T(h)	0	120	144	312	384	648	840	960
A	-	-	3.2E03	-	1.0E05	-	1.9E04	-
S	1.0E03	1.0E02	-	5.0E01	-	1.3E01	-	4.7E00
N	1.0E04	-	-	-	6.0E03	-	2.0E03	-

A K=+0.00076 ±0.00978
S K=-0.00213 ±0.00575
N K=-0.00160 ±0.00191

EC05SW (1.02) NO DARK CONTROL

EC05FW (1.19)

T(h)	0	120	192	360	816	840	888	1320
A	-	1.3E05	-	5.9E05	1.8E06	-	-	1.6E06
S	9.8E02	-	-	-	-	6.0E01	4.5E01	-
N	1.0E04	-	1.2E03	-	-	-	-	1.5E02

K=+0.00121 ±0.00468
K=-0.00148 ±0.00097
K=-0.00118 ±0.00057

EC05FW (1.35) NO DARK CONTROL

EC25SW (2.97)

T(h)	0	432	864	1200	
A	4.5E05	-	5.4E05	4.8E04	-0.00088 ±0.00011
S	1.2E04	6.1E01	1.0E00	0	-0.00412 ±0.01150
N	8.1E03	4.6E02	2.3E01	0	-0.00405 ±0.00084

EC25SW (2.52)

T(h)	0	240	528	900	1080	
A	2.6E05	-	4.6E05	-	2.7E04	-0.00092 ±0.00022
S	2.1E04	1.2E04	2.0E02	0	0	-0.00578 ±0.00125
N	3.5E04	1.9E04	1.7E02	0	0	-0.00602 ±0.00166

EC25FW (2.70)

T(h)	0	336	864	1200	
A	1.4E05	3.3E05	-	3.5E04	-0.00063 ±0.00026
S	3.5E04	2.6E02	1.3E01	1.0E00	-0.00357 ±0.00180
N	1.5E04	8.7E02	4.4E01	6.0E00	-0.00277 ±0.00156

EC25FW (2.52)

T(h)	0	240	504	900	1056	
A	1.4E05	-	4.2E05	-	9.6E03	-0.00113 ±0.00018
S	3.1E04	1.6E04	1.0E02	0	0	-0.00606 ±0.00104
N	2.0E04	1.1E04	3.6E02	0	0	-0.00578 ±0.00230

EC15SW (2.52)

T(h)	0	384	768	1400		
A	4.6E05	-	1.7E06	3.6E04		-0.00032 ±0.00018
S	7.5E04	3.8E04	1.0E02	-		-0.00447 ±0.00085
N	8.5E04	4.3E02	1.0E01	3.5E00		-0.00306 ±0.00240

EC15SW (2.68)

T(h)	0	288	600	900		
A	1.1E05	-	1.9E05	2.6E03		-0.00162 ±0.00043
S	1.3E04	6.6E03	1.0E02	4.0E01		-0.00290 ±0.00096
N	1.5E04	7.6E03	1.0E02	5.0E01		-0.00288 ±0.00100

EC15FW (2.47)

T(h)	0	312	588	1176		
A	6.3E05	2.9E05	-	6.2E04		-0.00084 ±0.00019
S	4.0E03	1.0E02	9.0E01	1.0E01		-0.00199 ±0.00038
N	1.0E04	1.0E03	1.5E02	1.0E01		-0.00252 ±0.00083

EC15FW (2.63)

T(h)	0	288	600	840	1200	
A	4.4E04	-	9.9E04	-	3.5E03	-0.00114 ±0.00038
S	1.4E04	7.5E03	1.0E03	6.0E02	1.0E01	-0.00262 ±0.00054
N	1.5E04	9.0E03	3.0E03	1.8E03	3.0E01	-0.00225 ±0.00067

EC05SW (2.61)

T(h)	0	312	432	627	864	
A	1.7E05	-	2.1E05	-	8.8E04	-0.00033 ±0.00028
S	2.5E04	6.5E03	1.0E04	6.3E02	1.0E02	-0.00278 ±0.00056
N	2.0E04	1.4E04	1.3E03	4.5E03	1.0E02	-0.00266 ±0.00150

EC05SW (2.70)

T(h)	0	240	432	600	900	
A	3.2E04	-	1.2E05	-	4.4E03	-0.00095 ±0.00054
S	1.2E04	5.9E03	1.0E03	6.8E02	1.0E02	-0.00231 ±0.00028
N	1.5E04	7.2E03	1.0E03	7.6E02	3.3E02	-0.00183 ±0.00069

EC05FW (2.63)

T(h)	0	288	480	864	1152	
A	1.6E05	2.6E05	-	-	3.9E04	-0.00063 ±0.00026
S	6.0E04	1.4E04	1.2E04	7.2E03	4.4E02	-0.00158 ±0.00026
N	5.0E04	5.4E04	4.5E04	2.7E04	4.0E02	-0.00156 ±0.00072

EC05FW (2.54)

T(h)	0	432	816	1200		
A	4.4E04	1.3E05	-	3.6E03		-0.00133 ±0.00050
S	4.7E04	1.0E03	8.0E02	6.0E02		-0.00143 ±0.00091
N	4.0E04	1.0E03	8.7E02	7.3E02		-0.00130 ±0.00090

FM25SW (1.20)

T(h)	0	168	572	984	1104	1680	3384
A	1.9E05	-	1.3E05	-	5.6E04	-	3.1E04
S	1.0E04	8.5E02	1.2E02	7.4E01	1.0E01	0	0
N	1.1E03	6.6E02	-	3.3E02	2.8E00	0	0

A K=-0.00028 ±0.00016
S K=-0.00220 ±0.00063
N K=-0.00241 ±0.00091

FM25FW (1.15)

T(h)	0	168	552	960	984	1656	3336
A	1.4E05	-	1.4E05	-	1.4E05	-	2.3E05
S	-	8.8E01	4.6E01	3.6E00	-	0	0
N	-	6.2E01	3.2E01	-	1.5E00	0	0

A K=-0.00045 ±0.00022
S K=-0.00284 ±0.00099
N K=-0.00273 ±0.00107

FM15SW (1.17)

T(h)	0	168	552	960	1056	1632	3408
A	1.4E07	-	7.0E06	-	1.0E05	-	8.6E06
S	3.6E02	4.0E01	2.0E01	1.0E00	0	-	-
N	3.0E02	3.0E01	1.7E01	3.6E00	-	-	-

A K=-0.00006 ±0.00023
S K=-0.00133 ±0.00066
N K=-0.00174 ±0.00069

FM15FW (1.18)

T(h)	0	144	528	936	1032	1608	3384
A	8.9E05	-	4.6E05	-	2.9E04	-	7.8E04
S	-	8.0E00	5.7E00	3.4E00	-	0	0
N	1.0E02	0.1E00	0	0	-	-	-

A K=-0.00023 ±0.00024
S K=-0.00176 ±0.00094
N K=-0.00300 ±0.00650

FM05SW (1.13)

T(h)	0	288	504	912	1008	1584	3384
A	1.5E06	-	4.6E06	-	7.6E06	-	4.8E04
S	1.1E02	1.0E01	-	5.0E00	-	3.1E00	-
N	5.6E01	2.5E01	-	5.0E00	-	-	-

A K=-0.00021 ±0.00023
S K=-0.00083 ±0.00053
N K=-0.00115 ±0.00013

FM05FW (1.21) NO DARK CONTROL

FM25SW (2.56)

T(h)	0	672	744	1344	1488	1968
A	2.3E04	-	-	7.7E03	-	6.9E03
S	1.3E03	6.6E02	-	2.0E01	1.1E01	1.6E00
N	1.2E03	-	6.1E02	-	1.5E01	7.3E00

A K=-0.00028 ±0.00012
S K=-0.00145 ±0.00012
N K=-0.00116 ±0.00037

FM25SW (2.77)

T(h)	0	240	312	504	864	
A	6.5E03	-	-	6.5E03	4.9E03	-0.00013 ±0.00024
S	3.6E02	2.4E02	1.5E02	1.9E01	0	-0.00402 ±0.00402
N	6.0E02	3.4E02	2.6E02	5.0E01	8.0E00	-0.00217 ±0.00217

FM25FW (2.74)

T(h)	0	312	648	1320		
A	3.3E04	-	4.9E03	5.5E03		-0.00062 ±0.00023
S	1.5E02	7.7E01	0	0		-0.00495 ±0.00272
N	8.9E02	1.0E02	0	0		-0.00614 ±0.00247

FM25FW (2.48)

T(h)	0	240	528	600	
A	7.0E03	4.9E03	5.5E03	-	-0.00015 ±0.00040
S	8.9E02	4.5E02	0	-	-0.00763 ±0.00454
N	9.0E02	9.8E02	-	0	-0.00694 ±0.00427

FM15SW (2.74)

T(h)	0	624	1200	1320	
A	-	-	-	-	
S	8.8E02	4.5E02	2.5E01	2.5E01	-0.00122 ±0.00040
N	-	-	-	-	

FM15SW (2.66)

T(h)	0	576	672	1176	
A	1.3E04	9.8E03	-	9.1E03	-0.00022 ±0.00020
S	3.3E02	7.0E01	4.0E01	-	-0.00130 ±0.00028
N	8.0E02	1.6E02	1.8E01	-	-0.00140 ±0.00036

FM15FW (2.75)

T(h)	0	1224	1344	1824	
A	1.5E04	3.5E03	1.2E03	-	-0.00069 ±0.00137
S	5.3E02	1.1E01	8.2E00	3.0E00	-0.00127 ±0.00122
N	6.9E02	-	1.0E01	4.0E00	-0.00126 ±0.00029

FM15FW (2.77)

T(h)	0	504	600	1104	
A	1.6E04	2.0E04	-	8.9E03	-0.00024 ±0.00024
S	9.6E02	7.5E01	1.5E02	3.4E01	-0.00128 ±0.00313
N	1.3E03	-	1.0E02	5.0E01	-0.00130 ±0.00049

FM05SW (2.73)

T(h)	0	575	1176	1800	
A	1.9E04	-	7.9E03	8.1E03	-0.00022 ±0.00015
S	3.3E02	1.8E02	2.6E01	2.3E01	-0.00068 ±0.00026
N	2.2E02	1.2E02	2.6E01	2.3E01	-0.00060 ±0.00033

FM05SW (2.48)

T(h)	0	384	768	1272	
A	1.9E04	8.9E03	-	9.5E03	-0.00024 ±0.00024
S	8.5E02	5.2E02	1.9E02	1.0E02	-0.00074 ±0.00009
N	7.0E02	5.3E02	3.5E02	1.9E02	-0.00044 ±0.00105

FM05FW (2.63)

T(h)	0	624	1272	1776	
A	1.5E04	-	8.3E03	7.8E03	-0.00017 ±0.00012
S	8.0E01	6.3E01	4.5E01	2.5E00	-0.00072 ±0.00052
N	2.0E02	1.6E02	1.0E02	0	-0.00154 ±0.00019

FM05FW (2.63)

T(h)	0	360	504	984	
A	4.1E03	3.9E03	-	4.0E03	-0.00008 ±0.00024
S	1.0E01	7.4E00	2.2E00	2.0E00	-0.00040 ±0.00055
N	1.0E01	-	1.0E01	4.0E00	-0.00076 ±0.00036

FS25SW (1.21)

T(h)	0	312	456	912	984	3192	
A	4.8E05	-	6.9E05	8.9E05	-	8.9E05	
S	1.9E01	4.8E00	-	-	0	-	
N	1.5E01	-	-	-	-	-	
A	K=+0.00006 ±0.00029						
S	K=-0.00235 ±0.00022						

FS25FW (1.17)

T(h)	0	96	408	816	3168	
A	1.1E06	-	6.0E05	9.9E04	4.4E04	-0.00038 ±0.00021
S	1.5E01	0	-	-	0	-0.01124 ±0.00016
N	3.2E01	0	-	-	0	-0.01299 ±0.00016

FS15SW (1.16)

T(h)	0	312	432	504	768	864	3168	
A	1.1E05	-	8.2E04	-	5.4E04	-	1.3E05	
S	2.6E01	1.7E01	-	1.5E01	-	0	0	
N	3.5E01	2.7E01	-	2.2E01	1.5E01	-	-	
A	K=+0.00006 ±0.00008							
S	K=-0.00178 ±0.00070							
N	K=-0.00137 ±0.00031							

FS15FW (1.07)

T(h)	0	240	648	744	1320	
A	4.6E04	-	2.0E05	-	1.3E05	+0.00034 ±0.00029
S	3.0E02	6.4E01	1.0E00	0	0	-0.00390 ±0.00212
N	1.8E02	5.3E01	1.0E01	0	0	-0.00192 ±0.00022

FS05SW (1.06)

T(h)	0	360	720	792	1296	3048
A	1.4E05	8.9E04	3.7E04	-	-	1.5E05
S	2.3E02	5.0E01	-	1.3E02	6.0E01	3.0E00
N	1.8E02	7.4E01	-	2.4E01	-	1.5E00
A	K=+0.00007 ±0.00028					
S	K=-0.00057 ±0.00017					
N	K=-0.00065 ±0.00034					

FS05FW (1.22)

T(h)	0	168	300	600	1248	3000
A	3.3E04	-	2.0E04	6.7E03	-	1.4E05
S	1.2E03	6.1E02	-	2.5E01	3.6E00	-
N	9.0E02	7.6E02	-	1.1E02	0	-
A	K=+0.00030 ±0.00012					
S	K=-0.00209 ±0.00043					
N	K=-0.00159 ±0.00059					

FS25SW (2.83)

T(h)	0	360	600	1032	1560	
A	-	-	-	-	-	
S	1.0E02	6.8E01	4.6E01	7.7E00	0	-0.00180 ±0.00073
N	7.3E02	4.8E02	3.1E02	9.5E00	0	-0.00240 ±0.00112

FS25SW (2.70)

T(h)	0	504	1032	1176		
A	8.3E04	-	9.7E03	7.5E03		-0.00089 ±0.00007
S	1.3E03	6.6E02	1.0E01	0		-0.00296 ±0.00156
N	8.8E02	4.4E02	9.1E00	0		-0.00282 ±0.00163

FS25FW (2.65)

T(h)	0	312	480	1200	1536	
A	2.2E03	-	1.9E03	1.9E03	-	-0.00005 ±0.00027
S	1.0E02	6.8E01	5.0E01	0	0	-0.00259 ±0.00069
N	2.4E02	1.3E02	7.2E01	0	0	-0.00291 ±0.00109

FS25FW (2.66)

T(h)	0	312	480	768	1008	
A	-	-	-	-	-	
S	2.4E01	1.6E01	1.2E01	0	0	-0.00269 ±0.00138
N	7.0E02	2.3E02	3.4E01	1.6E01	1.0E00	-0.00291 ±0.00014

FS15SW (2.75)

T(h)	0	720	816	1200	2136	
A	2.1E04	1.3E04	-	8.3E03	-	-0.00033 ±0.00017
S	2.0E02	8.1E01	5.5E01	0	0	-0.00240 ±0.00185
N	4.5E02	9.1E01	4.3E01	0	0	-0.00276 ±0.00770

FS15SW (2.81)

T(h)	0	312	720	816	1320	
A	6.8E04	-	2.8E04	-	9.8E03	-0.00063 ±0.00022
S	3.8E02	2.2E02	2.0E01	5.0E00	0	-0.00211 ±0.00059
N	2.0E02	1.4E02	3.0E01	5.0E00	0	-0.00119 ±0.00022

FS15FW (2.67)

T(h)	0	312	696	792	1200	
A	1.8E05	-	1.7E05	-	8.9E04	-0.00024 ±0.00022
S	2.0E01	1.5E01	7.8E00	5.5E00	0	-0.00174 ±0.00108
N	4.5E02	3.0E02	1.1E02	5.8E01	0	-0.00278 ±0.00197

FS15FW (2.68)

T(h)	0	312	696	792	1296	
A	8.9E03	3.4E03	-	3.1E03	-	-0.00036 ±0.00031
S	6.0E02	3.4E02	3.0E01	5.0E00	0	-0.00289 ±0.00064
N	4.0E02	3.2E02	-	1.9E02	1.9E00	-0.00167 ±0.00142

FS05SW (2.77)

T(h)	0	744	1392	1512	2016	
A	2.2E04	-	4.8E03	-	4.3E03	-0.00037 ±0.00014
S	8.7E02	4.7E02	-	6.5E01	2.5E01	-0.00075 ±0.00075
N	1.5E03	7.6E02	-	2.0E01	3.3E01	-0.00092 ±0.00042

FS05SW (2.70)

T(h)	0	384	504	1008		
A	8.9E03	-	5.0E03	2.5E03		-0.00055 ±0.00023
S	2.2E03	1.5E03	1.3E03	5.0E01		-0.00168 ±0.00297
N	2.0E03	1.2E03	1.0E03	7.9E02		-0.00040 ±0.00005

FS05FW (2.60)

T(h)	0	1368	1488	1992		
A	2.1E04	6.8E03	-	4.9E03		-0.00032 ±0.00013
S	2.9E02	2.5E01	6.5E01	2.0E01		-0.00058 ±0.00024
N	4.6E02	1.3E02	1.0E02	4.9E01		-0.00048 ±0.00019

FS05FW (2.72)

T(h)	0	360	744	1248		
A	1.2E04	4.3E03	2.2E03	-		-0.00099 ±0.00036
S	6.6E02	3.7E02	8.0E01	1.7E01		-0.00127 ±0.00046
N	2.5E02	2.1E02	1.6E02	4.4E01		-0.00058 ±0.00019

SO25SW (1.12)

T(h)	0	300	432	600	1032	1176	2016
A	4.3E06	-	-	2.9E06	-	4.3E06	1.2E06
S	9.0E04	2.9E04	1.5E03	-	0	-	-
N	2.8E05	2.0E03	1.1E03	1.8E02	-	-	-
A	K=-0.00022 ±0.00014						
S	K=-0.00549 ±0.00050						
N	K=-0.00532 ±0.00180						

SO25FW (1.13)

T(h)	0	216	480	576	1152	1869
A	2.7E06	-	-	4.4E05	1.2E06	3.6E05
S	2.2E03	-	2.2E03	-	1.2E03	4.9E01
N	3.8E04	2.3E03	1.2E03	8.0E02	-	-
A	K=-0.00035 ±0.00015					
S	K=-0.00430 ±0.00020					
N	K=-0.00274 ±0.00153					

SO15SW (1.06)

T(h)	0	456	552	1128	1968	
A	1.2E06	-	4.1E05	1.1E06	8.9E05	+0.00001 ±0.00012
S	2.0E03	2.0E01	-	0	0	-0.00377 ±0.00022
N	1.8E04	2.3E02	-	0	0	-0.00467 ±0.00058

SO15FW (1.12)

T(h)	0	408	504	936	1080	1440
A	1.7E05	-	1.5E04	-	4.1E03	-
S	5.0E01	1.0E00	-	0	-	-
N	7.8E03	3.9E03	3.2E03	1.2E01	-	1.0E01
A	K=-0.00058 ±0.00019					
S	K=-0.00284 ±0.00090					
N	K=-0.00303 ±0.00091					

SO05SW (0.97)

T(h)	0	336	432	1008	1368	1848
A	3.1E04	-	1.3E03	8.9E03	-	1.2E04
S	1.3E01	1.2E01	1.1E01	-	0	-
N	9.0E02	1.5E01	1.4E01	-	1.0E00	-
A	K=+0.000007 ±0.00023					
S	K=-0.00170 ±0.00026					
N	K=-0.00192 ±0.00114					

SO05FW (1.13)

T(h)	0	144	288	384	960	1320	1800	
A	2.5E06	-	-	4.1E05	9.2E05	-	1.0E06	
S	1.8E03	-	1.3E02	1.2E02	-	2.0E00	-	
N	1.1E04	5.6E03	2.0E03	-	-	-	-	
A	K=-0.00009 ±0.00009							
S	K=-0.00218 ±0.00063							
N	K=-0.00257 ±0.00054							

SO25SW (2.52)

T(h)	0	168	360	720	1200	
A	5.6E05	-	-	3.9E05	1.0E05	-0.00054 ±0.00018
S	1.5E04	1.2E02	7.6E03	1.8E02	0	-0.00418 ±0.00150
N	2.2E04	1.7E04	1.1E04	8.9E02	0	-0.00425 ±0.00088

SO25SW (2.95)

T(h)	0	168	504	720	1128	
A	1.8E06	-	2.0E06	-	1.5E06	-0.00006 ±0.00025
S	2.0E03	1.5E03	4.5E02	2.9E02	0.4E00	-0.00334 ±0.00140
N	1.6E04	1.1E04	8.0E02	5.2E02	1.0E00	-0.00425 ±0.00082

SO25FW (2.30)

T(h)	0	360	504	720	1296	
A	7.6E05	-	-	7.7E05	7.3E05	-0.00001 ±0.00011
S	-	1.6E03	9.6E02	1.0E01	0	-0.00348 ±0.00084
N	-	5.7E03	3.5E03	3.2E02	0	-0.00382 ±0.00124

SO25FW (2.73)

T(h)	0	240	504	816	1104	
A	1.2E06	-	5.4E06	-	1.9E06	-0.00633 ±0.00031
S	5.0E03	3.0E03	7.2E02	3.5E02	0.5E00	-0.00368 ±0.00110
N	3.2E04	1.7E04	1.2E03	5.8E02	5.3E00	-0.00344 ±0.00046

SO15SW (2.88)

T(h)	0	504	816	1272	
A	1.5E06	-	2.8E06	2.3E06	+0.00017 ±0.00016
S	1.3E03	5.1E02	2.0E01	3.0E01	-0.00311 ±0.00095
N	1.1E04	4.3E03	1.1E02	8.6E01	-0.00175 ±0.00080

SO15SW (2.54)

T(h)	0	312	576	720	1080	
A	1.8E05	-	4.1E04	-	4.5E04	-0.00057 ±0.00015
S	4.5E03	2.1E03	2.0E01	1.7E01	7.9E00	-0.00259 ±0.00134
N	7.8E03	3.5E03	1.4E02	1.2E02	7.5E01	-0.00190 ±0.00422

SO15FW (2.48)

T(h)	0	312	504	792	1248	
A	5.1E06	-	-	2.4E06	2.0E06	-0.00034 ±0.00017
S	7.5E03	4.7E03	3.0E03	4.0E02	1.0E01	-0.00223 ±0.00212
N	1.6E04	1.2E04	9.3E03	5.5E03	1.0E01	-0.00235 ±0.00198

SO15FW (2.20)

T(h)	0	312	552	720	1056	
A	2.2E06	-	7.5E06	-	5.8E06	+0.00041 ±0.00010
S	2.4E03	1.2E03	3.3E02	2.3E02	1.9E01	-0.00198 ±0.00037
N	1.1E04	6.4E03	2.8E03	1.9E03	2.0E02	-0.00164 ±0.00051

SO05FW (2.84)

T(h)	0	360	720	1200	
A	-	-	1.2E06	6.0E05	
S	4.1E03	2.3E03	4.5E02	4.2E01	-0.00163 ±0.00034
N	1.8E04	1.3E04	8.4E03	1.2E02	-0.00171 ±0.00092

SO05FW (2.21)

T(h)	0	240	480	720	984	
A	3.2E06	-	3.4E06	-	3.0E06	-0.00003 ±0.00019
S	3.7E03	2.0E03	3.0E02	1.6E02	1.8E01	-0.00235 ±0.00021
N	1.9E04	1.0E04	1.5E03	1.1E03	6.2E02	-0.00150 ±0.00062

SM25SW (1.03)

T(h)	0	240	768	936	1296	1680
A	1.7E06	-	8.6E06	4.5E06	-	2.5E06
S	1.7E03	4.6E02	-	3.4E01	0	-
N	1.3E04	1.0E03	-	-	0	-
A	K=+0.00009 ±0.00017					
S	K=-0.00333 ±0.00020					
N	K=-0.00391 ±0.00080					

SM25FW (1.12)

T(h)	0	240	744	912	1272	1656
A	5.7E06	-	4.1E06	6.3E05	-	7.7E05
S	4.0E03	5.1E02	-	-	1.4E02	5.2E00
N	1.5E04	-	1.0E03	6.7E02	5.0E02	-
A	K=-0.00057 ±0.00021					
S	K=-0.00163 ±0.00163					
N	K=-0.00180 ±0.00099					

SM15SW (1.12)

T(h)	0	432	672	720	1008	
A	7.8E04	-	5.6E04	-	2.1E03	-0.00137 ±0.00060
S	1.4E02	1.0E01	-	6.0E00	2.0E00	-0.00180 ±0.00058
N	7.1E02	1.8E02	-	1.0E02	2.0E01	-0.00154 ±0.00044

SM15FW (1.17)

T(h)	0	312	408	744	1500	1704
A	4.8E04	-	-	7.4E04	2.5E04	-
S	2.0E02	1.6E02	-	1.0E02	-	0
N	5.4E03	-	5.0E04	2.8E02	-	0
A	K=-0.00019 ±0.00027					
S	K=-0.00199 ±0.00025					
N	K=-0.00158 ±0.00130					

SM05SW (1.04)

T(h)	0	168	384	600	624	960
A	4.3E04	-	-	-	1.1E05	1.1E05
S	2.0E02	1.2E02	2.0E01	1.4E01	-	5.0E00
N	3.8E03	3.1E03	2.1E03	1.4E03	-	1.5E02
A	K=+0.00045 ±0.00023					
S	K=-0.00162 ±0.00055					
N	K=-0.00149 ±0.00058					

SM05FW (1.08)

T(h)	0	336	672	792	984	
A	3.6E04	-	2.2E04	1.9E03	-	-0.00206 ±0.00039
S	4.0E02	1.0E02	5.0E00	0	0	-0.01190 ±0.00115
N	4.8E03	4.8E02	1.0E02	-	4.5E01	-0.00189 ±0.00042

SM25SW (2.81)

T(h)	0	432	864	1200		
A	-	1.8E05	4.9E04	6.8E04		-0.00054 ±0.00038
S	1.6E03	3.3E02	1.7E00	0		-0.00496 ±0.00157
N	2.5E04	7.2E03	2.4E01	0		-0.00529 ±0.00132

SM25SW (2.52)

T(h)	0	120	312	480	960	
A	2.9E05	-	-	3.9E05	8.8E04	-0.00059 ±0.00024
S	5.8E03	4.4E03	2.3E03	3.7E02	0	-0.00368 ±0.00200
N	1.2E04	9.5E03	5.4E03	1.8E03	0	-0.00457 ±0.00290

SM25FW (2.77)

T(h)	0	312	408	600	816	
A	8.9E05	-	9.3E04	-	3.2E04	-0.00022 ±0.00021
S	7.2E03	2.7E03	5.0E02	2.7E02	3.4E00	-0.00336 ±0.00101
N	1.3E04	9.1E03	7.2E03	3.8E03	7.5E01	-0.00325 ±0.00170

SM25FW (2.81)

T(h)	0	456	720	912	1152	
A	-	4.2E04	-	4.5E04	1.8E04	-0.00054 ±0.00034
S	1.5E03	1.2E02	5.1E01	1.3E00	-	-0.00408 ±0.00408
N	6.3E04	1.4E03	6.2E02	4.8E01	-	-0.00425 ±0.00325

SM15SW (2.83)

T(h)	0	168	360	504	720	
A	8.3E05	-	3.9E05	-	2.0E05	-0.00086 ±0.00086
S	7.1E03	5.0E03	2.6E03	1.6E03	1.5E02	-0.00233 ±0.00233
N	1.5E04	1.1E04	5.6E03	3.5E03	4.3E02	-0.00264 ±0.00092

SM15SW (3.02) NO DARK CONTROL

SM15FW (2.81)

T(h)	0	336	672	1200		
A	1.5E05	7.9E05	8.4E04	-		
S	5.0E02	1.0E02	5.5E00	0		-0.00020 ±0.00050
N	9.0E03	4.8E03	1.3E02	1.0E01		-0.00205 ±0.00062

SM15FW (2.82)

T(h)	0	120	312	624		
A	7.4E05	-	4.6E04	3.8E04		-0.00037 ±0.00033
S	1.9E03	1.7E03	1.4E03	1.0E02		-0.00316 ±0.00085
N	3.3E04	2.4E04	8.9E03	9.0E02		-0.00264 ±0.00056

SM05SW (2.75)

T(h)	0	264	504	792	1056	
A	6.1E05	1.1E05	-	4.5E05	-	-0.00025 ±0.00025
S	3.3E03	2.5E03	1.6E03	4.8E02	1.0E01	-0.00222 ±0.00222
N	5.0E04	4.3E04	2.3E04	5.2E01	2.3E00	-0.00202 ±0.00202

SM05SW (2.86)

T(h)	0	288	504	864		
A	3.0E05	3.0E05	-	1.9E05		+0.00002 ±0.00021
S	2.3E03	8.0E02	5.1E02	3.0E01		-0.00218 ±0.00037
N	2.7E04	1.8E04	1.1E04	5.9E02		-0.00160 ±0.00067

SM05FW (2.73)

T(h)	0	240	504	720	1200	
A	1.6E05	5.4E05	-	1.3E05	-	-0.00024 ±0.00054
S	1.0E02	8.0E01	6.0E01	2.9E01	5.0E00	-0.00102 ±0.00049
N	7.5E04	4.0E04	8.5E03	5.0E03	2.0E03	-0.00133 ±0.00005

SM05FW (2.58)

T(h)	0	264	504	792		
A	1.3E05	9.9E04	-	8.2E04		-0.00029 ±0.00015
S	2.8E03	7.2E02	4.8E02	2.0E02		-0.00139 ±0.00048
N	3.5E04	8.0E03	6.2E03	4.1E03		-0.00109 ±0.00079

APPENDIX 6 - RAW DATA FOR NATURAL SUNLIGHT EXPERIMENTS
1988
LEGEND see Appendix 5

ECSW1

T(m)	0	60	120	240	360	420
A	1.1E06	7.4E05	4.4E05	4.4E05	4.0E05	1.7E05
K=-0.00147 ±0.00077						
S	6.5E04	-	6.1E04	1.3E03	-	1.0E02
K=-0.00735 ±0.00218						
N	4.0E04	3.1E04	2.9E04	-	-	2.0E02
K=-0.00582 ±0.00128						

ECFW1

T(m)	0	60	120	240	360	420
A	1.5E06	7.7E05	3.3E05	-	5.6E05	-
K=-0.00087 ±0.001158						
S	4.1E04	-	2.8E04	-	6.0E03	1.4E03
K=-0.00323±0.00108						
N	2.4E04	1.8E04	1.8E04	1.8E04	1.8E04	-
K=-0.000233 ±0.00025						

LI 0.482, 0.536, 0.777, 1.026 Mean=0.705MJm^{-1}
Temp.(°C) 17, 18, 19, 20 400nm=0.198MJm^{-1}
350nm=0.197MJm^{-1}
315nm=0.036MJm^{-1}

ECSW2

T(m)	0	60	180	300	420	480	600
A	6.9E05	-	5.8E05	1.2E06	-	-	4.6E05
K=-0.00369 ±0.00057							
S	2.5E05	-	1.7E05	-	6.0E01	1.5E01	2.5E01
K=-0.00827 ±0.00240							
N	3.0E05	2.4E05	4.0E04	1.0E02	1.0E02	-	-
K=-0.00970 ±0.00220							

ECFW2

T(m)	0	60	180	300	420	480	600
A	5.8E05	9.3E05	8.1E05	7.3E05	-	-	8.1E05
K=+0.00009 ±0.00042							
S	2.0E05	-	1.6E05	1.6E05	1.6E05	-	1.2E05
K=-0.00032 ±0.00015							
N	3.0E05	-	2.9E05	-	2.5E05	-	1.9E05
K=-0.00032 ±0.00025							

LI 0.773, 1.044, 0.87, 0.998 Mean=0.921MJ^{-1}
Temp. (°C) 16.5, 21, 22, 19 400nm=0.44MJm^{-1}
350nm=0.38MJm^{-1}
315nm=0.03MJm^{-1}

ECSW3

T(m)	0	60	180	300	420	480	600
A	4.4E05	2.2E05	9.9E05	2.8E05	4.6E05	4.4E05	-
K=-0.00115 ±0.00066							
S	3.3E05	2.9E05	1.9E05	3.2E04	1.0E00	1.0E00	-
K=-0.00327 ±0.00610							
N	6.1E05	-	1.2E05	-	1.0E02	-	1.0E02
K=-0.00881 ±0.00350							

ECFW3

T(m)	0	60	180	300	420	480	600
A	5.1E05	6.7E05	2.9E05	2.7E05	2.6E05	2.4E05	-
K=-0.00083 ±0.00039							
S	2.4E05	2.2E05	2.2E05	2.2E05	8.3E04	1.6E05	-
K=-0.00063 ±0.00052							
N	3.2E05	-	5.2E05	-	2.6E05	-	5.3E05
K=+0.00008 ±0.00059							

LI 0.723, 0.875, 0.768, 0.987, 1.043 Mean=0.879MJm^{-1}
Temp. (°C) 17, 18, 19.5, 18, 18.5 400nm=0.323MJm^{-1}
350nm=0.305MJm^{-1}
315nm=0.021MJm^{-1}

ECSW4

T(m)	0	60	120	240	360	480
A	5.1E05	1.7E06	1.0E06	1.0E06	-	5.4E05
K=-0.00042 ±0.00056						
S	3.4E05	-	2.4E05	-	8.4E04	-
K=-0.000173 ±0.00173						
N	3.5E05	-	-	-	1.0E04	1.5E02
K=-0.00639 ±0.00319						

ECFW4

T(m)	0	60	120	240	360	480
A	1.1E06	1.2E06	-	1.0E06	-	1.7E06
K=+0.00034 ±0.00040						
S	4.0E05	-	3.5E05	-	2.5E05	-
K=-0.00057 ±0.00007						
N	5.4E05	-	-	-	3.3E05	2.2E05
K=-0.00076 ±0.00010						

LI 0.54, 0.656, 0.684, 0.557 Mean=0.609MJm^{-1}
Temp. (°C) 16, 17, 17, 18.5, 17, 15 *400nm=0.282MJm^{-1}*
350nm=0.261MJm^{-1}
315nm=0.042MJm^{-1}

ECSW5

T(m)	0	48	120	240	360	480
A	1.1E06	7.1E05	2.2E05	2.5E05	2.0E05	8.9E04
K=-0.00190 ±0.00053						
S	2.2E05	2.0E05	6.8E04	1.4E03	2.0E01	1.5E01
K=-0.01010 ±0.00161						
N	2.7E05	-	2.3E05	4.0E02	-	2.0E02
K=-0.00733 ±0.00390						

ECFW5

T(m)	0	48	120	240	360	480
A	5.2E05	1.9E05	-	1.5E05	1.7E05	7.4E04
K=-0.00127 ±0.00099						
S	2.4E05	2.2E05	2.3E05	1.8E05	1.7E05	-
K=-0.00043 ±0.00017						
N	2.6E05	-	2.4E05	-	4.3E05	-
K=+0.00067 ±0.00065						

LI 0.884, 0.909, 0.954, 1.064 — Mean=0.951MJm⁻²
Temp. (°C) 17.5, 22.5, 26.5, 21.5 — 400nm=0.588MJm⁻²
350nm=0.389MJm⁻²
315nm=0.083MJm⁻²

FMSW1

T(m)	0	60	120	180	270	
A	3.1E04	4.4E04	-	5.0E04	-	+0.00094 ±0.00161
S	1.2E04	1.0E04	9.2E03	2.1E02	-	-0.00885 ±0.00650
N	2.4E04	1.9E04	1.3E04	9.5E01	0	-0;01230 ±0.00680

FMFW1

T(m)	0	60	120	180	270	
A	8.6E04	-	8.1E04	5.8E04	-	-0.00082 ±0.00180
S	1.2E04	9.5E03	8.5E03	8.0E03	-	-0.00100 ±0.00260
N	1.7E04	2.0E04	1.2E04	1.7E04	-	-0.00037 ±0.00140

LI 0.482, 0.418, 0.628, 0.600, 0.378 — Mean=0.501MJm⁻²
Temp. (°C) 20, 20, 20, 22, 23 — 400nm=0.291MJm⁻²
350nm=0.324MJm⁻²
315nm=0.053MJm⁻²

FMSW2

T(m)	0	60	120	300	
S	1.6E02	4.0E01	0.1E00	0.1E00	-0.01070 ±0.00750
N	7.8E01	7.0E00	0	0	-0.00868 ±0.00630

FMFW2

T(m)	0	60	120	300	
S	5.8E03	5.6E03	4.0E03	5.9E02	-0.00353 ±0.00094
N	8.1E03	6.0E03	6.0E03	1.2E03	-0.00281 ±0.00084

LI 0.185, 0.445, 0.229, 0.252 — Mean=0.378MJm⁻²
Temp. (°C) 17, 18, 19, 18 — 400nm=0.165MJm⁻²
350nm=0.018MJm⁻²
315nm=0.021MJm⁻²

FMSW3

T(m)	0	60	120	255	
S	7.7E03	5.3E03	2.2E03	0	-0.00439 ±0.00780
N	9.2E03	7.3E03	3.1E01	0	-0.02060 ±0.01520

FMFW3

T(m)	0	60	120	255	
S	1.0E04	8.3E03	3.3E03	1.0E02	-0.00823 ±0.00223
N	9.9E03	1.0E04	7.0E03	1.0E02	-0.00821 ±0.00360

LI 0.725, 0.493, 0.562, 0.753, 0.633 — Mean=0.633MJm⁻²
Temp. (°C) 21, 21, 23, 23, 27 — 400nm=0.318MJm⁻²
350nm=0.335MJm⁻²
315nm=0.042MJm⁻²

FSSW1

T(m)	0	60	120	180	270	
S	9.2E03	8.0E01	4.1E01	2.4E00	0	-0.01730 ±0.00297
N	2.0E03	3.9E02	3.7E02	2.0E00	-	-0.00611 ±0.01090

FSFW1

T(m)	0	60	120	180	270	
S	2.0E04	1.7E04	1.4E04	8.0E01	5.4E01	-0.00129 ±0.00600
N	2.0E03	-	1.0E04	4.0E03	-	+0.00227 ±0.00440

LI 0.72, 0.948, 0.599, 0.650 — Mean=0.729MJm⁻²
Temp. (°C) 19, 20, 20, 21

FSSW2

T(m)	0	60	120	180	240	
S	2.0E04	1.5E04	1.0E04	2.1E02	4.0E01	-0.00208 ±0.00230
N	5.0E04	5.0E02	1.6E01	-	0	-0.01250 ±0.00340

FSFW2

T(m)	0	60	120	180	240	
S	2.0E04	1.8E04	1.5E04	1.2E04	1.0E04	-0.00104 ±0.00190
N	1.2E05	1.9E04	1.5E04	6.2E03	5.0E03	-0.00753 ±0.00094

LI 0.605, 0.468, 0.447, 0.45 — Mean=0.492MJm⁻²
Temp. (°C) 18, 17, 20, 21

FSSW3

T(m)	0	60	120	180	240	
S	5.0E04	4.8E03	4.6E03	5.3E00	6.9E00	-0.01810 ±0.00762
N	6.0E04	4.2E04	1.3E01	7.0E00	1.0E00	-0.02220 ±0.00910

FSFW3

T(m)	0	60	120	180	240	
S	2.0E04	1.8E04	1.1E04	1.0E01	0	-0.02000 ±0.02020
N	4.2E04	5.5E04	4.0E04	1.1E03	4.0E01	-0.01350 ±0.00506

LI 0.893, 0.918, 0.938, 0.959, 0.959 — Mean=0.933MJm⁻²
Temp. (°C) 22, 24, 25, 26, 28

STSW1

T(m)	0	60	120	180	240	
A	5.9E05	-	3.9E05	-	9.4E04	-0.00287 ±0.00060
S	4.8E03	1.9E03	1.1E03	4.5E00	1.0E00	-0.01320 ±0.00490
N	5.2E04	5.0E04	4.0E04	7.4E01	0	-0.01870 ±0.00310

STFW1

T(m)	0	60	120	180	240	
A	3.2E05	-	2.0E05	-	8.9E04	-0.00189 ±0.00057
S	1.2E03	6.3E03	1.8E03	7.0E02	3.9E01	-0.00514 ±0.00458
N	7.8E04	7.3E04	3.9E04	1.8E04	1.3E03	-0.00531 ±0.00962

LI 0.800, 1.03, 1.27, 0.865 — Mean=0.99MJm⁻²
Temp. (°C) 19, 20, 22, 23 — 400nm=0.48MJm⁻²
350nm=0.35MJm⁻²
315nm=0.72MJm⁻²

STSW2

T(m)	0	60	180	270	
A	1.3E06	-	8.0E05	3.1E05	-0.00214 ±0.00204
S	2.5E04	4.2E03	2.3E01	0	-0.02000 ±0.00240
N	6.2E05	4.1E05	2.0E01	0	-0.02710 ±0.00170

STFW2

T(m)	0	60	180	270	
A	5.7E06	-	6.5E06	2.7E06	-0.00098 ±0.00085
S	3.0E04	1.8E04	9.5E03	2.7E04	-0.00040 ±0.00187
N	1.8E05	1.7E05	1.4E05	1.2E05	-0.00066 ±0.00017

LI 1.168, 0.999, 0.835, 1.216 Mean=1.055MJm^{-1}
Temp.(°C) 19, 20, 22, 23 400nm=0.471MJm^{-1}
350nm=0.396MJm^{-1}
315nm=0.084MJm^{-1}

ECSW6

T(m)	0	60	120	240	
A	5.4E05	4.4E05	-	2.8E05	-0.00120 ±0.00053
S	1.4E05	2.0E04	1.8E04	1.0E04	-0.00413 ±0.00350
N	2.0E04	1.2E04	1.3E04	-	-0.00106 ±0.00196

SNSW1

T(m)	0	60	120	240	
A	3.4E05	2.2E05	-	1.0E05	-0.00106 ±0.00196
S	9.2E04	1.8E04	1.3E04	4.0E04	-0.00071 ±0.00197
N	1.2E05	1.2E05	1.2E05	8.7E04	-0.00059 ±0.00069

LI 0.185, 0.427, 0.489, 0.160 Mean=0.252MJm^{-1}
Temp. (°C) 20, 19, 20, 21 400nm=0.144MJm^{-1}
350nm=0.125MJm^{-1}
315nm=0.0036MJm^{-1}

ECSW7

T(m)	0	60	120	180	300	
A	1.6E05	-	1.6E05	-	1.6E05	-0.00065 ±0.00010
S	2.0E03	2.0E03	1.5E03	1.5E03	1.2E01	-0.00709 ±0.00368
N	2.3E03	3.0E02	7.0E02	1.2E03	2.0E01	-0.00544 ±0.00370

FMSW4

T(m)	0	60	120	180	300	
A	1.3E05	-	3.6E05	-	2.1E05	+0.00054 ±0.00082
S	4.0E03	9.7E02	9.3E02	1.5E01	0	-0.00528 ±0.00603
N	3.0E03	2.5E03	1.8E02	1.0E01	0	-0.00132 ±0.00460

FSSW4

T(m)	0	60	120	180	300	
A	1.1E06	-	1.3E05	-	2.4E05	-0.00191 ±0.00113
S	2.4E01	1.3E00	0	0	0	-0.02110 ±0.00510
N	6.1E01	0	0	0	0	-0.02320 ±0.00670

LI 0.936, 0.997, 0.323, 0.841 Mean=0.774MJm^{-1}
Temp. (°C) 13, 15, 13, 17

ECSW8

T(m)	0	75	120	210	
A	5.7E05	4.8E05	-	4.6E05	-0.00044 ±0.00059
S	4.9E04	4.3E04	3.0E04	2.5E04	-0.00148 ±0.00070
N	6.1E03	3.4E03	-	3.2E03	-0.00120 ±0.00128

FSSW5

T(m)	0	75	120	210	
S	5.0E03	1.1E03	6.7E02	1.0E00	-0.00877 ±0.00493
N	3.9E03	0	0	0	-0.01930 ±0.00130

FSSW6*

T (m)	0	75	120	210	
S	1.3E04	2.6E04	7.8E02	0	-0.02430 ±0.00840
N	5.8E04	0	0	0	-0.02520 ±0.02750

LI 1.025, 0.562, 0.481, 0.775, 0.758 Mean=0.72MJm^{-1}
Temp. (°C) 13, 19, 14, 17, 17

APPENDIX 7 - RAW DATA FOR NATURAL SUNLIGHT EXPERIMENT 1989

LEGEND see Appendix 5

ECSW1 unreg.

T(m)	0	60	150	240	300	
A	2.0E05	-	1.8E05	-	3.9E04	-0.00237 ±0.00045
S	5.4E04	3.9E04	2.5E04	1.8E02	1.0E01	-0.01260 ±0.00179
N	5.6E04	7.6E04	1.4E04	1.0E02	1.0E01	-0.01340 ±0.00272

FMSW1 unreg.

T(m)	0	60	150	240	300	
A	2.1E04	-	8.9E03	-	8.9E02	-0.00458 ±0.00111
S	3.8E03	1.6E03	8.8E02	2.5E01	1.3E01	-0.00870 ±0.00110

FSSW1 unreg.

T(m)	0	60	150	240	300	
A	1.6E04	-	7.0E03	-	9.9E03	-0.00070 ±0.00125
S	1.5E02	1.5E01	5.0E00	0	0	-0.01100 ±0.00292
N	4.0E03	4.4E02	-	0	0	-0.01670 ±0.00119

SOSW1 unreg.

T(m)	0	60	150	240	300	
A	7.7E04	-	1.0E04	-	4.4E03	-0.00414 ±0.00114
S	5.0E03	3.0E03	5.0E03	9.6E02	1.0E02	-0.00489 ±0.00260
N	4.8E03	7.9E03	6.4E03	3.2E03	2.8E02	-0.00360 ±0.00106

SMSW1 unreg.

T(m)	0	60	150	240	300	
A	2.7E04	-	1.7E04	-	1.0E04	-0.00144 ±0.00144
S	2.0E03	2.0E03	1.3E03	1.8E02	1.3E02	-0.00445 ±0.00164
N	5.7E03	5.4E03	5.0E03	4.4E03	7.5E02	-0.00229 ±0.00163

SW1 unreg. Total LI 10.79MJm^{-1} Average temperature 24.7°C
Light intensity readings 0.81, 1.023, 1.14, 1.113, 0.981, 1.671, 1.023, 1.002, 2.022
Temps. 22, 21, 25, 25, 26, 26, 26, 25, 25, 26

ECSW2 unreg.

T(m)	0	60	150	240	300	
A	1.3E05	-	1.3E05	-	5.6E03	-0.00455 ±0.00182
S	3.4E04	2.3E04	1.5E04	1.0E02	0	-0.00989 ±0.00713
N	2.2E04	2.6E04	1.7E04	1.0E03	0	-0.00564 ±0.00088

FMSW2 unreg.

T(m)	0	60	150	240	300	
A	2.6E04	-	1.2E04	-	1.3E04	-0.00100 ±0.00130
S	9.0E02	1.0E02	3.7E01	8.4E00	3.7E00	-0.00743 ±0.00135
N	2.1E03	9.0E02	6.0E01	1.0E00	0	-0.01490 ±0.00184

FSSW2 unreg.

T(m)	0	60	150	240	300	
A	1.5E04	-	6.2E03	-	5.6E03	-0.00143 ±0.00107
S	5.9E02	6.6E01	1.5E01	1.6E00	1.0E00	-0.00917 ±0.00153
N	8.6E03	8.2E03	2.7E03	-	1.5E01	-0.00915 ±0.00484

SOSW2 unreg.

T(m)	0	60	150	240	300	
A	1.7E04	-	6.2E03	-	3.5E03	-0.00229 ±0.00132
S	3.2E02	2.5E02	4.6E01	3.7E01	3.7E01	-0.00352 ±0.00135
N	8.8E03	7.8E03	5.1E03	1.5E03	1.9E02	-0.00514 ±0.00514

SMSW2 unreg.

T(m)	0	60	150	240	300	
A	2.4E04	-	1.9E04	-	1.3E04	-0.00088 ±0.00097
S	2.9E02	2.2E02	6.2E01	5.4E01	5.2E01	-0.00273 ±0.00092
N	1.6E03	1.5E03	1.4E03	7.7E02	7.8E02	-0.00119 ±0.00043

SW2 unreg. Total LI 9.363MJm^{-1} Average temperature 23.8°C
Light intensity readings 0.705, 0.609, 1.716, 1.014, 1.005, 0.726, 1.431, 1.11, 1.047
Temps. 23, 22, 22, 24, 25, 25, 24, 24, 25

ECSW1 15°C

T(m)	0	60	150	210	
A	7.8E04	-	8.1E04	4.1E04	-0.00093 ±0.00068
S	5.9E03	5.3E03	3.3E03	1.2E03	-0.00314 ±0.00208
N	1.0E04	1.3E04	1.1E04	9.0E03	-0.00031 ±0.00040

FMSW1 15°C

T(m)	0	60	150	210	
A	1.0E04	-	4.4E03	4.0E03	-0.00133 ±0.01250
S	9.8E02	1.0E03	6.3E02	1.1E02	-0.00417 ±0.00270
N	1.3E03	9.0E02	3.2E02	2.2E02	-0.00388 ±0.00140

FSSW1 15°C

T(m)	0	60	150	210	
A	3.2E04	-	3.1E04	4.4E04	+0.00051 ±0.00172
S	2.1E03	8.5E02	1.0E02	5.0E01	-0.00813 ±0.00161
N	5.2E03	4.0E03	5.0E02	1.5E02	-0.00775 ±0.00161

SOSW1 15°C

T(m)	0	60	150	210	
A	3.8E04	-	4.4E04	6.7E04	+0.00082 ±0.00098
S	1.6E02	1.5E02	8.0E01	9.1E01	-0.00146 ±0.00079
N	5.1E03	2.0E03	3.3E03	7.8E02	-0.00291 ±0.00240

SMSW1 15°C

T(m)	0	60	150	210	
A	9.3E04	-	6.7E04	5.3E04	-0.00112 ±0.00089
S	2.1E02	1.9E02	1.4E02	1.1E02	-0.00136 ±0.00333
N	4.1E03	4.0E03	3.9E03	7.8E02	-0.00292 ±0.00248

SW1 15°C Total LI 4.64MJm^{-1}
Light intensity readings 0.465, 0.981, 0.966, 0.909, 0.843, 0.405, 0.107
Temp. 20, 17, 18, 20, 20, 17, 18

ECSW2 15°C

T(m)	0	60	150	240	300	
A	2.7E04	-	5.1E04	-	3.5E04	+0.00038 ±0.00087
S	6.4E03	5.9E03	4.2E03	7.0E02	4.0E02	-0.00431 ±0.00131
N	4.5E03	4.0E02	2.0E02	-	5.2E01	-0.00707 ±0.00190

FMSW2 15°C

T(m)	0	60	150	240	300	
A	6.4E03	-	4.9E03	-	3.8E03	-0.00076 ±0.00156
S	7.5E02	4.3E02	8.5E01	3.0E00	9.5E00	-0.00782 ±0.00225
N	4.0E02	1.5E02	1.5E02	5.0E01	0	-0.00953 ±0.00693

FSSW2 15°C

T(m)	0	60	150	240	300	
A	4.4E03	-	2.8E03	-	2.5E03	-0.00082 ±0.00139
S	2.8E02	1.0E01	-	1.0E0	0	-0.00992 ±0.00345
N	7.4E02	2.3E02	7.1E01	1.1E01	4.0E00	-0.00750 ±0.00560

SOSW2 15°C

T(m)	0	60	150	240	300	
A	1.0E04	-	1.3E04	-	6.3E03	-0.00067 ±0.00138
S	2.0E02	3.0E02	1.0E02	1.0E01	0	-0.01030 ±0.00439
N	2.4E03	4.3E03	2.3E03	3.5E02	2.8E02	-0.00389 ±0.00161

SMSW2 15°C

T(m)	0	60	150	240	300	
A	9.4E03	-	7.9E03	-	7.9E03	-0.00025 ±0.00106
S	8.4E01	8.0E01	6.3E01	2.9E01	1.2E01	-0.00272 ±0.00096
N	2.4E02	2.3E02	2.2E02	3.8E01	3.5E01	-0.00320 ±0.00310

SW2 15°C Total LI 7.1MJm^{-1}
Light intensity readings 0.240, 0.975, 1.215, 1.11, 1.095, 0.522, 0.894, 0.504, 0.543
Temps. 18, 17, 20, 19, 20, 20, 20, 19, 19

ECFW1 unreg.

T(m)	0	60	150	240	300	
A	3.3E04	-	2.0E04	-	1.4E04	-0.00124 ±0.00105
S	1.0E03	1.2E03	1.6E03	2.9E02	-	-0.00197 ±0.00073
N	3.7E03	1.0E03	2.5E03	1.2E03	-	-0.00118 ±0.00248

FMFW1 unreg.

T(m)	0	60	150	240	300	
A	1.4E04	-	7.7E03	-	6.2E03	-0.00118 ±0.00105
S	1.0E01	-	-	1.6E00	0.2E00	-0.00499 ±0.00262
N	3.0E02	1.4E02	1.0E02	1.1E02	6.0E01	-0.00187 ±0.00086

FSFW1 unreg.

T(m) 0	60	150	240	300	
A 8.5E03	-	6.7E03	-	5.3E03	-0.00068 ±0.00093
S 5.0E00	1.0E00	-	0	0	-0.00607 ±0.00141
N 2.4E02	5.2E01	1.2E01	1.1E01	8.8E00	-0.00450 ±0.00195

SOFW1 unreg.

T(m) 0	60	150	240	300	
A 1.9E04	-	1.1E04	-	6.7E03	-0.00151 ±0.00091
S 1.0E02	1.8E02	6.5E01	5.1E01	2.0E01	-0.00252 ±0.00108
N 1.3E03	1.5E03	1.2E03	-	-	-0.00030 ±0.00067

SMFW1 unreg.

T(m) 0	60	150	240	300	
A 1.6E04	-	8.5E03	-	4.0E03	-0.00210 ±0.00106
S 2.7E02	2.3E02	1.2E02	7.9E01	6.2E01	-0.00225 ±0.00035
N 2.3E03	2.1W03	1.8E03	1.6E03	1.2E03	-0.00087 ±0.00019

FW1 unreg. Total LI 12.86MJm^{-1} Average temperature 23.1°C
Light intensity readings 1.08, 1.014, 1.269, 1.428, 1.272, 1.401, 1.914, 0.921, 1.389, 1.176
Temps. 19, 21, 22, 23, 24, 24, 24, 24, 24, 25, 24

ECFW2 unreg.

T(m) 0	60	150	240	300	
A 3.0E04	-	8.3E03	-	6.2E03	-0.00228 ±0.00079
S 1.9E03	1.1E03	9.2E02	6.5E02	7.6E02	-0.00131 ±0.00060
N 6.0E03	4.1E03	3.2E03	2.2E03	-	-0.00174 ±0.00038

FMFW2 unreg.

T(m) 0	60	150	240	300	
A 1.0E04	-	7.0E03	-	1.9E03	-0.00240 ±0.00143
S 5.1E02	1.0E02	1.4E02	4.4E01	1.9E01	-0.00403 ±0.00191
N 2.2E03	7.4E02	5.7E02	1.7E02	1.3E02	-0.00397 ±0.00103

FSFW2 unreg.

T(m) 0	60	150	240	300	
A 6.7E03	-	4.4E03	-	1.8E03	-0.00190 ±0.00112
S 8.1E01	1.0E01	1.0E01	0	0	-0.01010 ±0.00281
N 5.2E03	-	5.2E02	3.4E02	2.9E02	-0.00427 ±0.00138

SOFW2 unreg.

T(m) 0	60	150	240	300	
A 2.0E04	-	9.6E03	-	5.7E03	-0.00182 ±0.00113
S 7.7E01	8.5E01	8.5E01	1.9E01	4.1E00	-0.00408 ±0.00196
N 2.2E03	1.9E03	1.3E03	-	-	-0.00154 ±0.00038

SMFW2 unreg.

T(m) 0	60	150	240	300	
A 1.4E04	-	1.0E04	-	4.4E03	-0.00168 ±0.00101
S 1.6E02	1.1E02	9.5E01	4.6E01	7.2E00	-0.00386 ±0.00480
N 1.0E03	9.3E02	7.6E02	3.1E02	8.0E01	-0.00340 ±0.00150

FW2 unreg. Total LI 13.2MJm^{-1} Average temperature 23.8°C
Light intensity readings 1.014, 1.182, 1.272, 1.473, 1.206, 1.473, 2.904, 1.371, 1.305
Temps. 22, 23, 23, 23, 24, 24, 24, 24, 25, 26

ECFW1 15°C

T(m) 0	60	150	240	300	
A 1.1E05	-	5.6E04	-	4.4E04	-0.00133 ±0.00088
S 9.1E03	1.2E04	1.1E04	2.6E03	-	-0.00219 ±0.00201
N 2.5E04	1.5E04	1.3E04	7.9E03	1.7E03	-0.00327 ±0.00127

FMFW1 15°C

T(m) 0	60	150	240	300	
A 1.5E04	-	2.8E04	-	1.3E04	-0.00021 ±0.00112
S 2.9E03	2.4E03	1.9E03	1.7E03	5.9E02	-0.00192 ±0.00205
N 3.7E03	3.9E03	3.5E03	3.3E03	2.7E03	-0.00044 ±0.00039

FSFW1 15°C

T(m) 0	60	150	240	300	
A 7.2E03	-	4.4E03	-	6.6E03	-0.00013 ±0.00125
S 1.4E02	1.0E02	6.0E01	-	3.9E01	-0.00184 ±0.00043
N 8.5E03	7.5E03	6.6E02	-	-	-0.00774 ±0.00015

SOFW1 15°C

T(m) 0	60	150	240	300	
A 1.4E04	-	4.4E03	-	3.2E03	-0.00214 ±0.00078
S 5.0E01	1.0E02	4.0E01	3.0E00	0.5E00	-0.00714 ±0.00319
N 3.3E03	5.3E03	3.0E03	-	-	-0.00047 ±0.00233

SMFW1 15°C

T(m) 0	60	150	240	300	
A 1.6E04	-	1.5E04	-	8.8E03	-0.00087 ±0.00085
S 2.1E02	1.8E02	1.2E02	4.9E01	4.9E01	-0.00238 ±0.00055
N 3.2E03	3.2E03	3.2E03	3.1E03	-	-0.00007 ±0.00016

FW1 15°C Total LI 13.2MJm^{-1}
Light intensity readings 1.083, 1.263, 1.068, 1.281, 1.167, 3.501, 0.993, 1.341, 1.314
Temps. 19, 20, 21, 24, 22, 22, 26, 25, 25, 26

ECFW2 15°C

T(m) 0	60	150	240	300	
A 7.2E04	-	4.1E04	-	7.6E04	+0.00008 ±0.00082
S 8.7E03	6.7E03	5.1E03	2.9E03	1.6E03	-0.00234 ±0.00056
N 4.1E04	1.4E04	1.0E04	-	8.0E03	-0.00205 ±0.00336

FMFW2 15°C

T(m) 0	60	150	240	300	
A 1.7E04	-	3.0E04	-	3.7E03	-0.00221 ±0.00304
S 3.9E02	-	5.1E01	-	2.8E01	-0.00381 ±0.00168
N 2.3E03	2.0E02	-	-	2.7E02	-0.00214 ±0.00338

FSFW2 15°C

T(m) 0	60	150	240	300	
A 3.6E04	-	4.9E04	-	8.1E04	+0.00117 ±0.00102
S 2.9E03	1.0E01	1.0E00	1.0E00	0	-0.02210 ±0.00810
N 2.5E03	1.9E01	3.0E00	-	1.3E00	-0.00642 ±0.00642

SOFW2 15°C

T(m) 0	60	150	240	300	
A 8.6E04	-	3.0E04	-	2.0E04	-0.00211 ±0.00081
S 8.1E02	1.0E03	2.4E02	2.0E02	9.8E01	-0.00328 ±0.00138
N 6.5E03	3.8E03	4.6E03	4.6E03	-	-0.00260 ±0.00038

SMFW2 15°C

T(m) 0	60	150	240	300	
A 3.1E04	-	2.3E04	-	2.1E04	-0.00056 ±0.00109
S 1.9E03	1.5E03	1.0E03	3.2E02	1.7E02	-0.00356 ±0.00104
N 8.1E03	6.0E03	4.5E03	3.6E03	6.6E02	-0.00300 ±0.00157

FW2 15°C Total LI 13.0MJm⁻²
Light intensity readings as above (FW1 15°C)
Temperature readings as above

EXPERIMENTS WITH SETTLED SEWAGE 15°C
FMSW+0.25%SS1

T(m) 0	60	150	240	300	
A 2.3E04	-	8.9E03	-	8.9E03	-0.00137 ±0.00083
S 8.1E02	1.2E03	4.5E02	1.7E02	6.0E01	-0.00402 ±0.00130
N 2.5E03	1.3E03	4.5E02	3.0E02	5.0E00	-0.00755 ±0.00337

FSSW+0.25%SS1

T(m) 0	60	150	240	300	
A 4.1E04	-	3.1E04	-	2.8E04	-0.00055 ±0.00109
S 3.2E03	3.2E03	3.4E02	1.7E01	8.0E00	-0.00972 ±0.00062
N 5.3E03	2.6E03	3.0E02	1.0E01	7.3E00	-0.01060 ±0.00165

FMSW1

T(m) 0	60	150	240	300	
A 6.1E03	-	1.3E04	-	1.5E04	+0.00130 ±0.00104
S 1.6E03	7.5E02	3.7E02	8.3E01	3.1E01	-0.00560 ±0.00062
N 1.9E03	8.5E02	1.7E02	7.7E01	1.4E02	-0.00431 ±0.00184

FSSW1

T(m) 0	60	150	240	300	
A 1.1E04	-	1.4E04	-	1.7E04	-0.00063 ±0.00101
S 2.5E03	1.0E03	7.5E01	0	0	-0.01670 ±0.00326
N 3.2E03	1.3E03	5.0E01	0	0	-0.01710 ±0.00330

SW1(ss) Total LI 12.081 MJm⁻²
Light intensity readings 0.888, 1.002, 1.146, 0.918, 1.35, 1.524, 2.328, 1.275, 1.65

FMSW+0.25%SS2

T(m) 0	60	150	240	300	
A 3.5E03	-	1.2E04	-	1.8E03	-0.00096 ±0.00313
S 9.0E02	4.2E02	1.7E02	5.0E01	4.9E01	-0.00446 ±0.00103
N 8.1E02	7.3E02	-	6.7E01	2.7E01	-0.00122 ±0.00118

FSSW+0.25%SS2

T(m) 0	60	150	240	300	
A 1.1E04	-	6.2E03	-	5.9E03	-0.00173 ±0.00107
S 1.0E01	3.0E01	1.0E01	1.0E01	-	-0.00076 ±0.00357
N 5.0E01	5.0E01	1.0E01	3.5E01	3.5E01	-0.00061 ±0.00281

FMSW2

T(m) 0	60	150	240	300	
A 7.3E03	-	7.6E03	-	6.1E03	-0.00026 ±0.00114
S 9.4E02	2.5E02	1.8E02	2.0E01	1.5E01	-0.00602 ±0.00184
N 5.0E03	1.0E02	5.0E01	6.0E01	1.5E01	-0.00651 ±0.00373

FSSW2

T(m) 0	60	150	240	300	
A 9.9E03	-	2.7E03	-	5.4E03	-0.00088 ±0.00108
S 2.5E01	1.0E01	-	9.9E00	1.3E00	-0.00315 ±0.00268
N 5.0E01	5.0E01	-	2.0E01	2.0E00	-0.00401 ±0.00493

SW2(ss) Total LI 5.256MJm⁻²
Light intensity readings 0.276, 0.291, 0.576, 0.408, 0.936, 1.044, 0.432, 0.579, 0.687

FMFW+0.25%SS1

T(m) 0	60	150	240	300	
A 7.0E03	-	1.0E04	-	6.0E03	-0.00022 ±0.00213
S 2.0E03	1.6E03	7.3E02	6.2E02	5.9E02	-0.00190 ±0.00057
N 2.9E03	1.5E03	6.0E02	8.0E02	4.6E02	-0.00236 ±0.00111

FSFW+0.25%SS1

T(m) 0	60	150	240	300	
A 1.1E04	-	6.5E03	-	1.0E04	-0.00014 ±0.00128
S 2.1E03	2.0E03	1.5E03	1.4E03	1.3E03	-0.00074 ±0.00020
N 3.6E03	2.4E03	2.0E03	2.6E03	2.1E03	-0.00052 ±0.00072

FMFW1

T(m) 0	60	150	240	300	
A 1.0E04	-	7.7E03	-	6.7E03	-0.00058 ±0.00087
S 1.6E03	1.7E03	1.1E03	4.2E02	2.0E02	-0.00311 ±0.00085
N 2.5E03	2.5E03	1.7E03	9.2E02	2.0E02	-0.00333 ±0.00136

FSFW1

T(m) 0	60	150	240	300	
A 5.7E03	-	7.8E03	-	1.0E04	-0.00081 ±0.00102
S 1.8E03	1.4E03	6.2E02	2.3E02	2.1E02	-0.00344 ±0.00043
N 2.8E03	1.6E03	1.0E03	7.5E02	3.6E02	-0.00267 ±0.00047

FW1(ss) Total LI 8.021MJm⁻²
Light intensity readings 0.702, 0.555, 0.708, 0.300, 0.159, 0.795, 0.864, 2.718, 1.22

FMFW+0.25%SS2

T(m) 0	60	150	240	300	
A 4.9E03	-	8.8E03	-	5.7E03	+0.00022 ±0.00075
S 2.2E02	1.7E02	8.0E01	4.0E01	-	-0.00317 ±0.00163
N 2.4E02	1.3E02	9.3E01	6.7E01	5.0E01	-0.00209 ±0.00080

FSFW+0.25%SS2

T(m)0	60	150	240	300	
A 1.3E04	-	9.8E03	-	8.9E03	-0.00055 ±0.00085
S 1.5E01	1.5E01	6.6E00	1.0E00	1.0E00	-0.00461 ±0.00133
N 2.0E02	1.0E02	5.0E01	-	5.3E01	-0.00182 ±0.00167

FMFW2

T(m)	0		60		150		240		300	
A	5.8E03	-			7.7E03	-			6.1E03	+0.00007 ±0.00114
S	2.3E02	1.1E02	2.0E02	2.0E01	1.3E01					-0.00415 ±0.00100
N	9.9E02	4.5E02	2.0E02	1.5E02	1.2E02					-0.00295 ±0.00680

FSFW2

T(m)	0		60		150		240		300	
A	2.8E03	-			4.9E03	-			8.4E03	+0.00159 ±0.00104
S	1.0E02	3.0E01	1.0E01	1.0E00		0				-0.00953 ±0.00161
N	4.7E02	7.0E02	3.2E02	2.0E02	9.2E01					-0.00254 ±0.00163

FW2(ss) Total LI 9.43MJm^{-1}
Light intensity readings 0.729, 0.537, 0.918, 1.089, 0.75, 1.092, 1.149, 1.00, 0.861, 1.305

APPENDIX 8 - RAW DATA FOR GROWTH CABINET EXPERIMENT

LEGEND see Appendix 5
LIGHT

E.coli

T(h)	0	19	43	66	138	186	282	354
AC	4.5E06	8.2E06	1.6E06	6.7E06	9.9E06	1.5E07	9.8E06	2.3E06
A	-	9.6E05	2.2E05	-	5.9E05	2.2E06	6.1E05	1.1E05
S	5.5E03	1.3E03	1.1E03	1.1E03	2.5E02	7.3E01	1.2E01	5.6E00

T(h)	498	570
S	3.2E00	0.2E00

AC K=+0.00010 ±0.00270
A K=-0.00116 ±0.00233
S K=-0.00689 ±0.00211

S.faecalis NCTC 775

T(h)	0	19	43	66	138	186	282	354
AC	1.9E06	6.8E06	1.7E06	-	5.8E06	2.5E06	2.8E06	9.8E05
A	-	3.0E05	3.1E05	-	8.9E04	4.1E05	5.9E05	7.1E04
S	7.0E02	6.8E02	3.9E02	1.1E02	3.1E00	6.0E00	1.0E00	0

AC K=-0.00910 ±0.00322
A K=-0.00075 ±0.00278
S K=-0.00868 ±0.00191

S.typhimurium

T(h)	0	19	43	66	138	186	282	354
AC	4.3E06	1.0E07	5.8E05	5.1E06	3.1E06	4.5E06	2.8E06	7.1E05
A	-	1.6E05	1.1E05	-	2.1E05	8.9E04	4.1E05	1.7E05
S	8.0E01	7.5E01	5.0E01	1.0E01	1.0E01	1.0E01	5.0E00	0

AC K=-0.00140 ±0.00428
A K=+0.00073 ±0.00228
S K=-0.00608 ±0.00156

DARK

E.coli

T(h)	0	19	43	66	138	186	282
AC	4.5E06	4.2E06	9.8E05	8.2E06	6.2E06	3.8E06	8.0E06
	354						
	5.7E06						
A	-	6.3E04	8.4E04	-	7.9E04	8.9E05	8.9E05
	354						
	6.2E05						
S	-	1.9E03	1.8E03	9.4E03	1.0E04	-	-
	354						
	1.0E05						

AC K=+0.00091 ±0.00370
A K=+0.00358 ±0.00154
S K=+0.00496 ±0.00164

S.faecalis

T(h)	0	19	43	66	138	186	282
AC	1.9E06	4.8E06	7.8E05	-	6.5E06	1.8E06	3.2E06
	354						
	3.8E06						
A	-	5.1E04	5.4E05	-	5.6E05	2.5E05	1.2E05
	354						
	4.7E06						
S	7.0E02	7.8E02	1.4E03	5.9E02	4.4E03	4.6E03	4.0E03
	354						
	1.6E04						
T(h)	498						
S	2.7E04						

AC K=+0.00069 ±0.00158
A K=+0.00287 ±0.00160
S K=+0.00330 ±0.00179

S.typhimurium

T(h)	0	19	43	66	138	186	282
AC	4.3E06	9.6E06	1.3E06	6.4E06	5.1E06	5.6E06	2.5E06
	354						
	3.4E06						
A	-	1.1E05	4.4E04	4.3E04	2.8E05	8.9E05	1.1E06
	354						
	-						
S	8.0E01	5.5E01	2.3E03	4.0E03	-	-	-
	354						
	5.3E05						

AC K=-0.00047 ±0.00319
A K=+0.00410 ±0.00120
S K=+0.01200 ±0.00352

APPENDIX 9 - RAW DATA FOR ALS HUMIC ACID EXPERIMENTS

EC25TW (1.032MJm^{-1})

T(m)	0	60	120	180	300	360
A	7.2E05	5.2E05	-	4.4E05	-	3.1E05
S	1.5E05	-	1.3E05	8.5E04	-	7.8E04
N	9.2E04	7.0E04	4.9E04	7.2E04	1.0E04	7.4E03

A K=-0.00093 ±0.00063
S K=-0.00085 ±0.00045
N K=-0.00318 ±0.00091

EC25FW (1.044MJm^{-1})

T(m)	0	60	120	180	300	360
A	1.9E05	1.8E05	-	-	1.2E05	1.2E05
S	2.5E04	2.4E04	2.3E04	2.1E04	1.8E04	-
N	4.0E04	2.9E04	3.0E04	3.0E04	2.8E04	-

A K=-0.00061 ±0.00056
S K=-0.00049 ±0.00030
N K=-0.00318 ±0.00243

EC15SW+HA (1.080MJm^{-1})

T(m)	0	60	150	240	330	390
A	3.0E05	4.8E03	1.0E05	8.9E04	4.9E04	2.2E05
S	3.3E03	3.3E03	1.2E04	3.3E03	3.0E03	3.0E03
N	5.4E03	2.7E03	8.1E03	1.0E04	1.1E04	7.8E03

A K=+0.00092 ±0.00569
S K=-0.00023 ±0.00152
N K=-0.00245 ±0.00146

EC15TW (1.008MJm^{-1})

T(m)	0	60	120	240	300	360
A	1.4E05	1.3E05	-	1.4E05	-	5.7E04
S	4.0E03	6.0E03	6.5E03	6.0E03	5.2E03	7.5E03
N	1.5E04	6.5E03	6.2E03	5.7E03	5.5E03	-

A K=-0.00089 ±0.00060
S K=+0.00056 ±0.00072
N K=-0.00112 ±0.00087

EC15FW (1.062MJm^{-1})

T(m)	0	60	120	270	330	390
A	5.9E05	2.7E05	-	-	2.3E05	1.6E05
S	4.7E04	-	4.6E04	-	4.5E04	2.9E04
N	4.0E04	3.6E04	3.0E04	-	1.5E04	1.5E04

A K=-0.00107 ±0.00049
S K=-0.00040 ±0.00056
N K=-0.00121 ±0.00056

EC05SW+HA (1.021MJm^{-1})

T(m)	0	30	60	120	210	300	360	420
A	1.6E05	6.8E05	1.9E05	-	3.4E05	-	-	-
S	1.3E04	1.3E04	5.0E03	1.4E04	1.5E04	1.2E04	1.2E04	9.8E03
N	1.7E04	-	1.9E04	4.5E03	1.4E04	1.5E04	1.1E04	7.2E03

A K=+0.00057 ±0.00911
S K=-0.00034 ±0.00188
N K=-0.00269 ±0.00135

EC05TW (1.053MJm^{-1})

T(m)	0	60	120	180	270	360
A	2.6E05	1.9E05	-	1.6E05	-	1.3E05
S	2.1E04	1.8E04	2.1E04	2.5E04	1.2E04	-
N	1.5E04	-	1.0E04	-	9.3E03	9.0E03

A K=-0.00076 ±0.00054
S K=-0.00060 ±0.00079
N K=-0.00057 ±0.00060

EC05FW (0.981MJm^{-2})

T(m)	0	60	150	180	270	360
A	3.0E05	1.5E05	-	1.9E05	-	1.9E05
S	5.3E04	4.3E04	-	2.4E04	4.7E04	4.1E04
N	7.0E04	5.0E04	2.6E04	4.5E04	4.5E04	5.5E04

A K=-0.00027 ±0.00050
S K=-0.00020 ±0.00073
N K=-0.00019 ±0.00092

DARK CONTROLS FOR ALS HUMIC ACID EXPERIMENTS

EC25TW

T(h)	0	600	888	1176	1344	
A	7.2E05	2.0E05	-	7.1E03	-	-0.00170 ±0.00025
S	1.5E05	7.6E03	3.8E03	1.0E00	0	-0.00439 ±0.00217
N	9.2E04	4.9E03	2.5E03	3.2E01	0	-0.00294 ±0.00094

EC25FW

T(h)	0	600	864	1152	2004	
A	1.9E05	-	-	2.1E05	7.3E03	-0.00121 ±0.00020
S	2.5E04	3.5E02	3.3E02	3,0E02	4.1E01	-0.00169 ±0.00048
N	4.0E04	1.8E03	9.6E02	1.1E02	-	-0.00222 ±0.00034

EC15SW+HA

T(h)	0	72	216	720	1032	1224	1248
A	6.2E05	1.6E05	1.5E05	1.1E05	8.2E05	-	6.3E04
S	5.2E04	4.8E04	4.0E04	-	1.1E03	4.3E02	-
N	3.8E04	2.8E04	7.9E03	2.0E00	4.0E00	-	-

A K=-0.00055 ±0.00051
S K=-0.00175 ±0.00032
N K=-0.00454 ±0.00263

EC15TW

T(h)	0	564	960	1128	1800	
A	1.4E05	-	-	1.4E05	2.2E03	-0.00164 ±0.00027
S	4.0E03	1.0E02	3.5E01	7.0E00	0	-0.00244 ±0.00551
N	1.5E04	7.6E03	2.3E03	1.0E02	0	-0.00295 ±0.00103

EC15FW

T(h)	0	516	888	1032	1200	
A	5.9E05	-	-	1.2E05	2.5E03	-0.00230 ±0.00036
S	4.7E04	2.4E04	6.8E03	1.0E02	1.0E01	-0.00312 ±0.00112
N	4.0E04	2.0E04	5.6E03	6.0E00	1.0E00	-0.00411 ±0.00148

EC05SW+HA

T(h)	0	240	696	1008	1224	
A	6.3E05	4.9E05	7.3E05	-	1.0E06	+0.00021 ±0.00037
S	3.0E01	1.0E01	-	0.3E00	-	-0.00190 ±0.00024
N	1.0E02	1.0E01	0	0	0	-0.00432 ±0.00070

EC05TW

T(h)	0	492	984	1200	
A	2.6E05	-	1.7E05	1.4E04	-0.00129 ±0.00029
S	2.1E04	1.5E04	8.1E03	1.0E01	-0.00217 ±0.00199
N	1.5E04	7.6E03	1.5E02	2.4E00	-0.00302 ±0.00130

EC05FW

T(h)	0	480	960	1200	
A	3.0E05	-	3.5E05	5.9E03	-0.00178 ±0.00047
S	5.3E04	2.7E04	1.0E02	0	-0.00284 ±0.00208
N	7.0E04	3.5E04	1.0E02	1.0E00	-0.00402 ±0.00155

APPENDIX 10 - RAW DATA FOR GROWTH CABINET HUMIC ACID EXPERIMENTS (*E.coli* at 15°C)

LEGEND see Appendix 5

SW1

T(h)	0	24	72	96	120	144
A	3.4E05	3.7E05	-	6.7E04	1.5E04	1.2E04
S	7.0E03	1.9E03	2.0E00	4.8E00	0	0
N	1.0E04	1.0E03	7.0E01	1.8E01	0	0

A K=-0.00962 ±0.00176
S K=-0.03050 ±0.00683
N K=-0.03090 ±0.00612

SW2

T(h)	0	24	72	96	120	144
A	2.8E05	1.2E05	4.4E05	8.9E04	1.5E04	1.1E04
S	3.7E03	2.7E03	4.0E01	6.7E00	0	0
N	1.0E04	3.0E03	5.0E01	2.3E01	0	0

A K=-0.00833 ±0.00145
S K=-0.03050 ±0.00551
N K=-0.03220 ±0.00618

FW1

T(h)	0	48	96	144	268	
A	3.3E05	5.5E05	4.2E05	1.3E05	1.9E05	-0.00173 ±0.00107
S	9.1E03	4.0E03	7.6E03	9.5E03	2.5E03	-0.00132 ±0.00247
N	1.0E04	1.4E04	1.5E04	1.4E04	2.0E02	-0.00600 ±0.00372

FW2

T(h)	0	48	96	144	268	
A	1.3E05	4.1E05	1.9E05	1.2E05	1.4E05	-0.00078 ±0.00087
S	8.6E03	4.0E03	2.8E03	6.2E03	2.3E03	-0.00142 ±0.00141
N	9.0E03	8.4E03	8.6E03	7.1E03	3.0E03	-0.00172 ±0.00061

FW3

T(h)	0	24	96	120	
A	4.0E05	1.5E05	1.4E05	-	+0.00426 ±0.00370
S	1.6E03	3.9E02	1.5E02	1.4E02	-0.00801 ±0.00329
N	1.9E03	5.0E02	1.5E02	-	-0.01050 ±0.00293

FW4

T(h)	0	24	72	120	144	
A	1.6E04	1.4E04	2.9E03	-	-	-0.01090 ±0.00713
S	9.8E02	7.3E02	1.0E01	1.0E01	-	-0.01920 ±0.02581
N	1.6E03	2.0E02	-	1.0E03	-	+0.00087 ±0.00074

TW1

T(h)	0	24	48	96	120	
A	2.5E06	1.1E05	-	1.9E05	-	-0.00820 ±0.00383
S	1.6E03	1.0E01	0	0	0	-0.03310 ±0.01774
N	1.4E03	1.0E02	-	1.0E00	0	-0.03640 ±0.00554

TW2

T(h)	0	24	48	96	120	
A	1.9E06	1.6E05	-	3.0E05	-	-0.00555 ±0.00335
S	1.6E03	1.0E01	0	0	0	-0.08760 ±0.02467
N	2.2E03	1.0E02	-	2.0E00	0	-0.03290 ±0.00555

TW3

T(h)	0	24	72	120	144	
A	7.0E03	4.8E03	4.8E03	-	3.8E02	-0.00846 ±0.00902
S	9.6E02	2.7E02	1.0E01	0	-	-0.00485 ±0.01364
N	9.0E02	3.0E02	9.0E01	1.8E02	-	-0.00586 ±0.00309

TW+HA1

T(h)	0	24	96	120	
A	9.2E05	4.0E05	1.9E05	-	-0.00065 ±0.00228
S	1.3E03	7.0E02	4.0E03	-	+0.00634 ±0.00613
N	2.4E03	2.1E03	2.0E04	-	+0.01050 ±0.00444

TW+HA2

T(h)	0	24	96	120	
A	6.1E05	3.5E05	1.1E06	-	+0.00361 ±0.01210
S	1.5E03	5.7E02	4.0E03	2.0E03	+0.00613 ±0.00477
N	3.2E03	1.5E03	3.3E03	-	+0.00120 ±0.00451

TW+HA3

T(h)	0	24	72	120	144	
A	4.4E03	2,8E03	-	-	4.6E02	-0.00613 ±0.00175
S	9.1E02	3.0E02	-	2.2E00	0	-0.02190 ±0.00300
N	1.3E03	6.0E02	1.4E02	1.1E02	-	-0.00920 ±0.00028

TW+HA4

T(h)	0	24	72	120	144	
A	3.7E03	4.4E02	1.8E03	-	8.9E02	-0.00186 ±0.00466
S	8.8E02	4.8E02	0	0	0	-0.02170 ±0.02257
N	1.1E03	1.2E03	4.0E02	1.5E02	-	-0.00779 ±0.00178

SW+HA1

T(h)	0	24	96	120	
A	9.4E05	9.9E04	1.3E06	-	+0.00470 ±0.01780
S	2.4E03	2.1E02	0	0	-0.03910 ±0.00153
N	2.4E03	1.0E03	0	0	-0.04150 ±0.00971

SW+HA2

T(h)	0	24	96	120	
A	6.5E05	2.0E05	2.1E05	-	-0.00386 ±0.00131
S	1.5E03	2.5E02	0	0	-0.03810 ±0.00771
N	7.0E02	3.0E02	2.0E00	0	-0.03160 ±0.00478

SW+HA3

T(h)	0	24	72	120	144	
A	9.6E03	4.1E03	2.0E03	-	2.4E03	-0.00376 ±0.00187
S	8.4E02	1.4E02	0	0	0	-0.03270 ±0.01105
N	8.0E02	1.1E03	-	0	0	-0.03530 ±0.03090

SW+HA4

T(h)	0	24	72	120	144	
A	3.4E04	6.9E03	4.4E03	-	8.9E02	-0.00981 ±0.00785
S	9.1E02	7.4E02	0	0	-	-0.05860 ±0.00300
N	3.0E02	1.0E02	0	0	-	-0.03270 ±0.01464

DARK CONTROLS FOR GROWTH CABINET HUMIC ACID EXPERIMENTS

FW4

T(h)	0	48	144	672	
A	1.6E04	5.2E03	3.0E03	5.5E03	+0.00001 ±0.00135
S	9.8E02	7.4E02	4.0E02	1.0E02	-0.00140 ±0.00026
N	1.6E03	2.0E02	1.2E03	-	+0.00041 ±0.00235

TW3

T(h)	0	48	144	672	
A	7.0E03	3.7E03	7.0E03	3.2E03	-0.00038 ±0.00044
S	9.6E02	6.1E02	5.0E02	-	-0.00212 ±0.00015
N	9.0E02	1.5E03	2.0E03	-	+0.00225 ±0.00114

TW+HA3

T(h)	0	48	144	672	
A	4.4E03	2.4E03	5.7E03	3.9E03	+0.00004 ±0.00041
S	9.1E02	2.0E02	3.8E01	1.5E00	-0.00368 ±0.00115
N	1.3E03	7.0E02	3.0E02	4.0E00	-0.00366 ±0.00015

SW+HA3

T(h)	0	48	144	672	
A	9.6E03	3.2E03	3.3E03	5.1E03	-0.00004 ±0.00033
S	8.4E02	2.1E02	2.9E01	0	-0.00550 ±0.00123
N	8.0E02	8.0E02	1.0E01	0	-0.00570 ±0.00191

APPENDIX 11 - RAW DATA FOR S.ANATUM EXPERIMENTS 15°C

LEGEND see Appendix 5

SASW GH1 2.63MJm⁻¹

T(m)	0	60	120	180	240	300
A	1.2E04	-	1.6E04	-	7.4E03	4.4E03
S	1.8E03	-	1.4E03	2.0E02	9.3E00	0
N	2.0E03	6.5E03	4.2E03	3.0E02	1.5E01	1.0E00

A K=-0.00152 ±0.00136
S K=-0.01389 ±0.00572
N K=-0.01190 ±0.00422

SASW GH2 2.63MJm⁻¹

T(m)	0	60	120	180	240	300
A	5.3E04	-	1.4E04	7.4E03	-	2.8E03
S	2.0E03	1.6E03	7.0E02	4.1E02	4.7E01	0
N	2.9E03	2.1E03	1.4E03	4.4E02	6.3E01	2.3E01

A K=-0.00427 ±0.00111
S K=-0.01389 ±0.00516
N K=-0.00758 ±0.00160

SAFW GH1 2.66MJm⁻²

T(m)	0	60	120	180	240	300
A	5.9E04	-	8.1E03	8.1E03	-	3.6E03
S	1.4E03	9.3E02	-	2.0E02	2.0E02	1.5E01
N	6.6E03	5.6E03	4.9E03	5.7E02	3.0E02	7.6E01

A K=-0.00388 ±0.00100
S K=-0.00575 ±0.00257
N K=-0.00694 ±0.00694

SAFW GH2 2.59MJm⁻²

T(m)	0	60	120	180	240	300
A	3.2E04	-	3.2E04	1.1E04	-	1.1E04
S	1.4E03	9.0E02	8.0E02	7.5E02	3.5E02	2.8E02
N	7.8E03	5.6E03	1.3E03	5.3E02	5.3E02	4.6E02

A K=-0.00177 ±0.00093
S K=-0.00225 ±0.00055
N K=-0.00463 ±0.00129

SASW NCTC 3072 1 2.66MJm⁻²

T(m)	0	60	120	180	240	300
A	3.4E04	-	2.2E04	-	-	2.2E04
S	1.1E03	1.0E03	8.5E02	3.0E02	4.0E02	3.0E02
N	7.5E03	5.2E03	7.0E03	7.5E02	7.0E02	7.4E02

A K=-0.00057 ±0.00062
S K=-0.00214 ±0.00121
N K=-0.00407 ±0.00163

SASW NCTC 3072 2 2.81MJm⁻²

T(m)	0	60	120	180	240	300
A	5.7E04	-	5.2E04	4.8E04	-	5.7E04
S	6.3E02	4.5E02	2.5E02	2.0E02	1.5E02	5.0E01
N	1.7E03	1.3E03	1.2E03	1.1E03	3.2E02	1.0E02

A K=-0.00002 ±0.00078
S K=-0.00333 ±0.00333
N K=-0.00379 ±0.00379

SAFW NCTC 3072 1 2.59MJm⁻²

T(m)	0	60	120	180	240	300
A	7.4E03	-	3.8E03	5.2E03	-	1.2E04
S	1.2E03	6.3E02	3.2E02	2.5E02	2.5E02	1.0E02
N	3.2E03	2.8E03	2.2E03	1.7E03	1.2E03	9.0E02

A K=+0.00076 ±0.00087
S K=-0.00321 ±0.00125
N K=-0.00189 ±0.00025

SAFW NCTC 3072 2 2.70MJm⁻²

T(m)	0	60	120	180	240	300
A	4.8E03	-	1.6E03	4.8E03	-	2.7E03
S	4.8E02	3.6E02	2.8E02	2.8E02	2.7E02	4.6E01
N	1.7E03	1.0E03	8.7E02	5.6E02	3.5E02	1.1E02

A K=-0.00051 ±0.00048
S K=-0.00260 ±0.00133
N K=-0.00355 ±0.00078

SASW NCTC 5779 1 2.66MJm⁻²

T(m)	0	60	120	180	240	300
A	1.7E04	-	1.3E04	-	8.1E03	6.1E03
S	7.3E02	3.9E02	2.4E02	2.6E01	6.3E00	4.8E00
N	2.9E03	3.7E03	3.5E03	4.4E02	-	6.8E01

A K=-0.00150 ±0.00065
S K=-0.00397 ±0.00147
N K=-0.00595 ±0.00276

SASW NCTC 5779 2 2.81MJm⁻²

T(m)	0	60	120	180	240	300
A	9.8E03	1.4E04	-	2.EE03	1.9E03	1.8E03
S	1.9E03	1.6E03	6.7E02	7.4E02	5.6E01	3.5E01
N	3.8E03	2.7E03	2.8E03	2.6E03	8.1E02	8.4E01

A K=-0.00265 ±0.00067
S K=-0.00617 ±0.00183
N K=-0.00476 ±0.00228

SAFW NCTC 5779 1 2.70MJm⁻²

T(m)	0	60	120	180	240	300
A	2.9E04	2.4E04	1.7E04	-	1.7E04	2.4E04
S	1.2E03	7.5E02	7.7E02	5.5E02	4.0E02	3.1E02
N	2.6E03	2.4E03	2.1E03	1.6E03	9.2E02	7.1E02

A K=-0.00036 ±0.00516
S K=-0.00187 ±0.00053
N K=-0.00198 ±0.00450

SAFW NCTC 5779 2 2.70MJm⁻²

T(m)	0	60	120	180	240	300
A	1.7E04	-	8.4E03	5.7E03	-	4.4E03
S	1.2E03	1.0E03	6.3E02	-	4.7E02	3.3E02
N	1.2E04	1.1E04	7.7E03	6.7E03	3.0E03	1.4E03

A K=-0.00198 ±0.00078
S K=-0.00183 ±0.00043
N K=-0.00309 ±0.00086

DARK CONTROLS

SASW GH1

T(h)	0	24	72	120	
A	1.2E04	1.0E04	7.0E03	7.2E03	-0.00194 ±0.00134
S	1.8E03	8.0E02	7.4E02	0	-0.03230 ±0.01923
N	2.0E03	9.5E02	1.0E01	0	-0.03700 ±0.00472

SASW GH2

T(h)	0	24	72	120	144	
A	5.3E04	4.7E04	3.3E04	7.9E03	-	-0.00667 ±0.00104
S	2.0E03	8.0E01	1.0E01	0	0	-0.03330 ±0.00633
N	2.9E03	2.5E02	2.0E01	0	0	-0.03570 ±0.00534

SAFW GH1

T(h)	0	24	72	120	
A	5.9E04	4.4E04	2.2E04	1.8E04	-0.00305 ±0.00069
S	1.4E03	3.7E02	5.0E00	0	-0.03570 ±0.00931
N	6.6E03	4.0E02	2.0E01	5.0E00	-0.02500 ±0.00672

SAFW GH2

T(h)	0	24	72	120		
A	3.2E04	1.7E04	9.7E03	1.0E04		-0.00279 ±0.00144
S	1.4E03	1.1E02	-	0		-0.03330 ±0.00338
N	7.8E03	2.8E02	-	7.0E00		-0.02270 ±0.00997

SASW NCTC 5779 1

T(h)	0	24	72	120	192	
A	1.7E04	1.1E04	9.6E03	9.5E03	-	-0.00182 ±0.00146
S	7.3E02	-	1.6E01	1.6E01	3.0E01	-0.00680 ±0.00709
N	2.9E03	-	6.0E00	0	0	-0.02220 ±0.00509

SASW NCTC 5779 2

T(h)	0	24	72	120	192	
A	9.8E03	9.5E03	9.5E03	9.2E03	-	-0.00024 ±0.00113
S	1.9E03	2.6E02	-	6.5E01	1.0E01	-0.01230 ±0.00257
N	3.8E03	-	-	1.0E00	0	-0.02380 ±0.01080

SAFW NCTC 5779 1

T(h)	0	24	72	120	168	
A	2.9E04	1.0E04	1.4E04	9.7E03	-	-0.00165 ±0.00167
S	1.2E03	2.5E02	5.0E01	1.4E01	9.0E00	-0.01230 ±0.00293
N	2.6E03	-	-	4.6E01	1.8E01	-0.01320 ±0.00195

SAFW NCTC 5779 2

T(h)	0	24	72	120	168	
A	1.7E04	9.4E03	9.8E03	9.1E03	-	-0.00109 ±0.00141
S	1.2E03	3.0E01	-	2.0E00	0	-0.01890 ±0.00609
N	1.2E04	6.8E03	-	5.0E01	0	-0.02860 ±0.00283

APPENDIX 12 - RAW DATA FOR CIPROFLOXACIN EXPERIMENTS

TO FIND OPTIMUM CONCENTRATION OF CIPROFLOXACIN

T(h)	0	2	4	6
Control	4.0E08	7.0E08	7.5E08	8.6E08
0.1mg/l	3.1E08	6.0E08	4.8E08	8.2E08
1.0mg/l	6.7E08	4.6E08	6.0E08	5.8E08
10mg/l	3.5E08	2.3E08	3.0E08	2.6E08
25mg/l	4.5E08	2.8E08	8.2E07	4.7E07

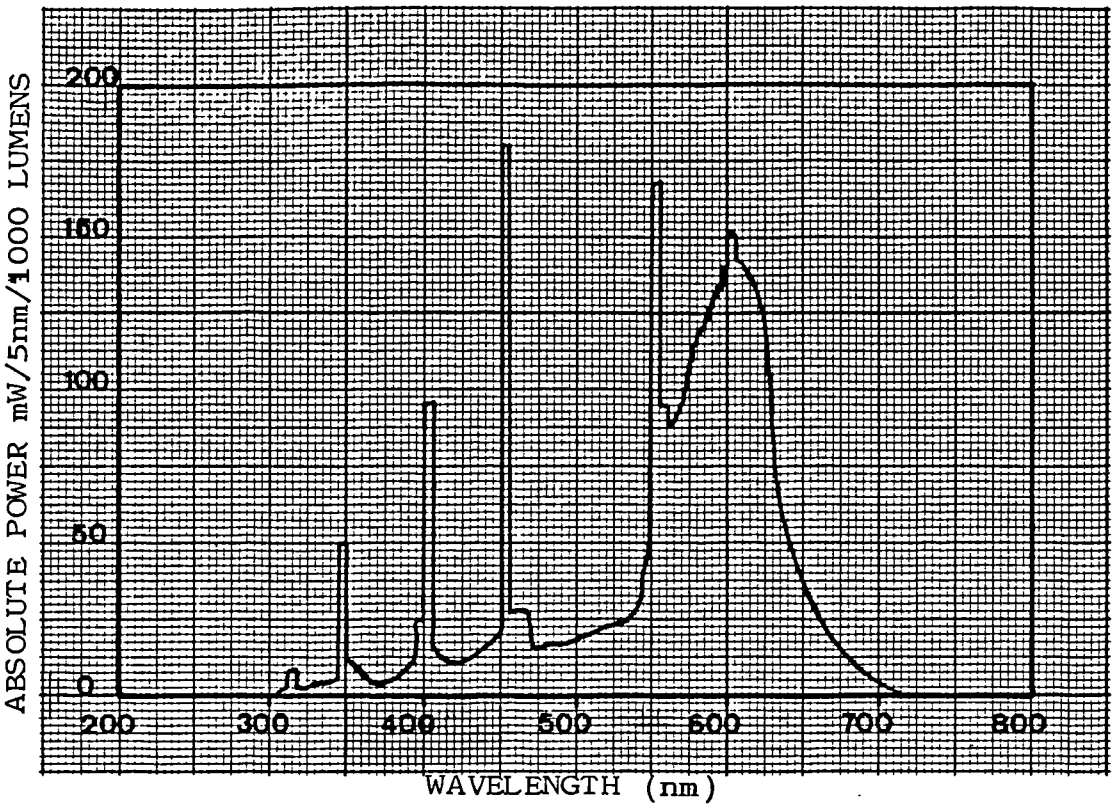
TO FIND OPTIMUM INCUBATION TIME

1) 1.0mg/l Ciprofloxacin

T(h)	TDC	DVC	DVC (%) TDC
1/2	5.5E05	4.1E04	7.5
2	5.0E05	1.2E05	24
4	5.3E05	2.5E05	47
6	5.3E05	3.6E05	68
8	5.0E05	3.3E05	66
10	5.1E05	3.3E05	65

2) Control (YE only)

T(h)	TDC	DVC	DVC (%) TDC
1/2	5.3E05	1.4E05	26
2	6.5E05	2.7E05	42
4	6.0E05	3.0E05	50
6	6.9E05	4.2E05	61
8	6.2E05	4.4E05	71
10	6.9E05	5.4E05	78



Emission spectrum for fluorescent tubes in growth cabinet.

APPENDIX 14.

An Investigation into the Removal of Faecal Bacteria by Preliminary Treatment

Introduction

Following the introduction in 1976 of the European Economic Community (EEC) standards for bathing water quality (Anon., 1976), the Water Authorities have been obliged to ensure that any sewage discharges to sea do not compromise the water quality at designated beaches with respect to the mandatory standards stipulated by the Directive. This has meant that alterations to some of the existing sea outfall systems have had to be made, e.g., interception and redirection of sewage discharges, construction of headworks providing adequate pretreatment, and extension of old sea outfalls. Many short outfalls still do exist, however, which do not allow the discharge to reach the deeper waters and currents necessary for adequate dilution and dispersion of sewage in the marine environment. Samples taken from beaches adjacent to these outfalls thus frequently fail to meet the standards. The EEC Directive has started to influence the design of headworks, and recently constructed sea outfalls have been made longer.

The proposed new outfall at Seaton Carew, Hartlepool has been designed according to dispersion models developed by WRC and ICI to provide efficient assimilation of the combined flows currently being discharged separately from two existing 25 year old, short outfalls of Seaton Carew and Burn Road, Hartlepool.

The aim of this investigation was to determine the effects of various types of preliminary treatment on numbers of faecal indicator bacteria with a view to determining the most efficient means (if any) of removing bacteria from discharges before reaching the sea. This would give an indication of the type of treatment to be installed at the new Seaton Carew headworks to ensure compliance of nearby bathing beaches with the EEC Directive. Preliminary treatments investigated included screening, grit removal, comminution and maceration by pumping.

Methods and Experimental Work

Disruption of Aggregates

A major problem with microbiological analysis of raw sewage is that a large proportion of the faecal bacteria is associated in aggregates with solid faecal material. When cultured on solid media, one colony may arise from one aggregate containing many bacteria, whereas when freely suspended bacteria are cultured, a colony arises from a single bacterium. Aggregation, therefore, lowers the real count of bacteria.

A number of experiments were carried out to determine the effect of disruption by ultrasonic treatment on the release of bacteria and disaggregation of faecal solids to yield individual organisms. Different types of sewage and receiving waters were subjected to sonication for differing periods of time in order to determine the duration of ultrasonic treatment

required to give the highest counts. Various agents were tested for their ability to desorb bacteria from aggregates.

Measurement of Disaggregation

A direct epifluorescent technique was used to observe the effect of sonication on the process of bacterial release and disaggregation under the microscope. A large proportion of microscopically visible bacteria in sewage treatment processes are not recoverable by culturable counting methods. The discrepancy between total direct counts and total culturable counts has, in the past, been attributed to either difficulties in distinguishing bacteria and other particles under the microscope, deficiencies in cultural techniques, or low viability of the bacteria. The direct epifluorescent technique allows detection of viable and non-viable cells.

Culture Techniques

Comparisons of the multiple tube technique, membrane filtration and the pour plate method for the enumeration of faecal coliforms and faecal streptococci were made. Little difference was found between the counts obtained by the multiple tube and membrane filtration techniques, both of which gave higher counts than those obtained using the pour plate method. Both faecal coliforms and faecal streptococci were, therefore, enumerated by membrane filtration as this was the most convenient technique to use for this study.

Media and Diluents

Faecal streptococci were enumerated using the glucose azide medium of Slanetz and Bartley, otherwise known as Membrane Enterococcus Agar (DHSS, 1984). A comparison of Membrane Enriched Teepol Broth and Membrane Faecal Coliform Broth, with and without Rosolic Acid indicated that the former gave higher counts for faecal coliforms. A non-selective plate count was also carried out using Casitone Glycerol Yeast Extract Agar.

In all cases, except for the initial sonication when Tween 80 was used, dilutions were made using quarter strength Ringers Solution.

Summary of Enumeration Procedures

Faecal streptococci were enumerated by membrane filtration on Membrane Enterococcus Agar and incubated at 44.5°C for 48 hours.

Faecal coliforms were enumerated by membrane filtration on Membrane Enriched Teepol Broth and incubated at 44.5°C for 18 hours.

Plate counts were carried using Casitone Glycerol Yeast Extract Agar and were incubated at 20°C for 7 days.

Sampling Sites

Hendon, Langbaurgh and Amble headworks were chosen to provide a comparison of the bacterial removal efficiencies of different size screens, i.e., 4mm, 5mm, and 6mm respectively. In addition, Langbaurgh headworks also provided grit removal by detritor. Howdon Sewage Treatment Works provided treatment by primary settlement.

Settled sewage from Howdon is discharged into the River Tyne which flows into the North Sea a few miles down river, close to the beaches of Prior's Haven and Long Sands. Newbiggin headworks provided the opportunity to assess the effect of pumping sewage along an outfall, on the removal of faecal bacteria. Burn Road and Seaton Carew headworks were sampled to determine whether or not there was any difference in flow, in the morning, and in the evening. These two headworks also provided treatment by comminution.

Results

Because of the large number carried out, individual results for the statistical analyses are not given.

Samples were sonicated for between 2 and 60 seconds to find the duration of treatment giving maximum disaggregation. This was carried out because 2 seconds have successfully been used to disperse bacteria in marine sediment samples (West, 1988) and 90 seconds to disperse bacteria in activated sludge samples (Banks & Walker, 1976). Analysis of variance in counts for different periods of sonication by each enumeration method did, however, indicate that the yield of faecal bacteria from sewage was not significantly increased by sonication.

Each headworks was sampled twice, at roughly the same time of day (except Seaton Carew and Burn Road which were sampled in the morning and in the evening),

and on the same day of the week. Seawater samples were taken at the same stage of the tide. Analysis of variance indicated that in most cases there was no significant difference between the numbers of bacteria measured in the replicate samples.

Analysis of variance in counts before and after individual treatment units indicated that, in general, preliminary treatment did not significantly decrease numbers of bacteria in sewage. This was evident for all enumeration methods, indicating that bacteria are neither removed nor killed by the treatment processes investigated. The numbers of bacteria in samples taken from receiving waters did, however, decrease significantly the further away from the outfall they travelled.

Discussion and Conclusions

Faecal particles are much more cohesive than particles in sediments or activated sludge and therefore bacteria are not disaggregrated to the same degree by sonication. West (1988) found that dispersion of sediment bacteria by hand shaking was more effective.

The results of this investigation indicate that none of the preliminary treatment units investigated gave a significant decrease in the number of faecal bacteria in sewage discharges. In fact, the action of pumping and of comminution has the potential to increase numbers through physical disaggregation, though this was not observed in this particular study.

Bacterial numbers would seem to be reduced by natural dispersion after entering the receiving waters. This is, however, probably due to dilution rather than to actual physical removal. Bacterial decay due to the adverse influence of the environmental conditions encountered, may also account for the reduction in numbers of bacteria on entering the receiving waters.

The above observations on the inefficiency of preliminary treatment units in removing faecal bacteria, indicate that it may be more productive to study the efficiencies of primary and secondary treatment units in the removal of faecal bacteria where this is necessary for compliance with standards.

References

Anon. (1976) Council Directive of 8 December 1975 Concerning the quality of bathing water (76/160/EEC). Off. J. Eur. Communities, L31, 1-7.

Banks,C.J., & Walker,I. (1976) Sonication of activated sludge flocs and the recovery of their bacteria on solid media. J. Gen. Microbiol. 98, 363-368.

DHSS/HMSO (1984) Bacteriological Examination of Drinking Water Supplies 1982. Methods for the Examination of Waters and Associated Materials. Report 71. HMSO.

West,P.A. (1988) An improved procedure for shipboard enumeration of faecal indicator bacteria in marine sediments from sewage sludge disposal areas. J. Appl. Bacteriol. 64, 345-353.

Appendix

Sample	Sonication (sec)	Direct /ml	Plate /ml	Faecal coliforms /ml	Faecal streptococci /ml
River Tyne and estuary I:					
Howdon Raw Sewage	0	1.7E08	3.9E08	5.2E04	2.6E04
	60	4.0E08	6.2E08	5.3E04	7.1E04
Howdon Settled Sewage	0	1.2E08	8.4E05	6.0E03	8.0E02
	30	2.9E08	2.1E07	3.7E04	2.7E04
	60	6.2E07	1.3E07	3.6E04	2.8E04
Howdon Outfall	0	7.6E07	-	2.9E04	2.7E04
	15	1.9E07	-	-	TNTC
	30	1.0E07	-	-	2.0E04
D/S of Outfall	0	5.8E06	1.3E06	1.4E04	1.7E03
	10	8.0E06	-	1.3E04	7.4E03
	20	-	-	1.6E04	8.6E03
	30	1.4E07	-	1.3E04	6.6E03
Fish Quay	0	2.8E07	6.0E05	8.7E02	1.6E02
	10	2.1E07	-	1.1E03	3.8E02
	20	1.4E07	1.5E07	9.5E02	1.8E02
	30	1.4E07	3.0E05	9.1E02	1.4E02
Priors' Haven- Tynemouth	0	1.4E07	4.9E04	1.2E02	1.4E02
	10	1.5E07	8.3E05	1.3E02	5.5E01
	20	1.4E07	1.8E05	1.4E02	-
Long Sands	0	8.5E06	-	1.0E01	1.6E01
	10	1.4E07	3.5E05	1.2E01	7.0E00
	20	9.8E06	5.2E05	1.1E01	1.5E01
River Tyne and estuary II:					
Howdon Raw Sewage	0	4.2E08	7.6E07	5.2E04	1.4E03
	30	9.8E08	1.4E07	4.5E04	7.5E03
	60	7.6E08	TNTC	6.8E04	2.8E04
Howdon Settled Sewage	0	9.3E08	-	3.0E04	9.2E03
	30	1.0E09	-	2.3E04	1.0E04
	60	1.7E09	-	3.3E04	1.5E04
Howdon Outfall	0	5.8E08	9.3E06	8.5E04	7.5E03
	10	5.8E08	5.4E06	7.5E03	5.0E03
	20	1.1E08	1.4E07	3.3E04	1.0E04
	30	4.9E08	8.5E06	3.2E05	5.0E03
D/S of Outfall	0	6.2E08	3.6E06	1.0E04	2.5E03
	10	6.2E08	5.7E06	1.8E04	1.0E03
	20	4.9E08	9.7E06	2.0E04	1.0E03
	30	5.8E08	4.1E07	5.0E03	2.5E03

Fish	0	4.9E08	9.7E04	3.3E02	1.5E02
Quay	10	-	1.3E05	4.5E02	2.5E02
	20	5.8E08	2.4E05	7.8E02	1.5E02
	30	3.1E08	1.8E05	6.5E02	2.5E02
Priors'	0	8.4E06	4.7E04	2.4E02	7.3E01
Haven	10	8.0E06	4.8E04	5.0E02	6.5E01
	20	5.8E06	5.8E04	2.5E02	6.8E01
	30	7.1E06	1.0E05	2.1E02	4.8E01
Long	0	8.0E06	3.6E04	2.5E00	7.5E00
Sands	10	5.8E06	4.2E04	1.0E01	7.5E00
	20	6.2E06	7.5E04	1.8E01	7.5E00
	30	7.1E06	4.5E04	2.0E01	1.3E01
Newbiggin I:					
Pumping	0	7.3E09	6.0E07	4.0E04	2.7E04
Station	30	5.3E09	8.1E07	4.0E04	2.7E04
	60	9.3E09	1.4E08	5.0E04	1.3E04
	90	6.7E09	4.9E07	4.5E04	3.8E04
Outfall	0	6.7E09	1.4E06	4.7E03	2.9E04
	20	6.2E09	1.4E06	4.7E03	1.9E05
	30	5.3E09	8.5E06	4.5E03	2.2E05
	60	5.8E09	2.2E06	4.9E03	2.6E05
100m from	0	4.2E07	1.2E06	5.5E01	7.0E01
Outfall	10	3.6E07	1.7E06	4.0E01	6.0E01
	20	6.2E07	1.4E06	5.5E01	7.5E01
	30	4.0E07	1.3E06	4.0E01	7.0E01
Newbiggin II:					
Pumping	0	8.0E09	4.4E08	3.2E04	3.4E04
Station	30	7.1E09	1.5E09	3.7E04	2.9E04
	60	1.2E10	2.4E09	3.3E04	2.6E04
Outfall	0	5.3E09	5.3E06	5.0E02	8.3E01
	15	5.3E09	2.8E06	5.0E02	8.5E01
	30	5.3E09	2.4E06	5.0E02	8.3E01
	60	5.8E09	1.3E06	2.5E02	7.8E01
100m from	0	4.4E07	5.0E04	7.5E01	2.5E01
Outfall	10	2.0E07	7.5E04	5.0E01	2.5E01
	20	4.0E07	5.0E05	5.0E01	2.5E01
	30	5.8E07	1.5E05	2.5E01	1.0E01
Langbaurgh I:					
Works	0	1.3E10	2.5E07	1.5E05	4.5E04
Inlet	2	8.0E09	1.4E07	1.2E05	7.0E04
	5	1.8E10	1.7E07	1.5E05	3.8E04
	10	1.3E10	1.5E07	1.1E05	6.5E04
	60	2.3E10	5.9E07	1.4E05	6.0E04

Returned	0	8.2E10	3.7E07	1.1E05	1.3E04
Screen	5	3.3E10	2.6E07	1.4E05	5.0E03
Washings	30	5.4E10	2.7E07	9.5E04	1.0E04
	60	7.5E10	4.1E07	8.0E04	1.3E04
Before	0	5.7E10	9.0E06	1.3E05	1.0E04
Screens	5	7.3E10	1.0E07	8.5E04	2.5E03
(5mm)	30	8.9E10	4.4E07	8.5E04	2.0E04
	60	4.3E10	2.1E07	1.1E05	1.3E04
After	0	5.7E10	1.8E08	1.5E05	3.0E04
Screens	5	5.7E10	-	1.4E05	3.3E04
	30	2.6E11	-	1.7E05	2.0E04
	60	1.7E11	-	1.5E05	1.3E04
Before	0	5.7E10	2.6E08	9.5E04	2.3E04
Detritor	5	4.1E10	1.3E08	1.3E05	4.0E04
	30	7.5E10	1.1E08	1.1E05	3.8E04
	60	5.5E10	1.1E08	2.1E05	6.3E04
After	0	6.3E10	6.5E07	1.2E05	4.3E04
Detritor	5	8.3E10	4.3E07	8.0E04	2.8E04
	30	9.4E10	1.3E07	3.0E04	2.5E03
	60	1.0E11	3.8E07	1.4E04	2.0E04

Langbaurgh II:

Works	0	9.7E10	2.5E06	5.0E04	8.0E03
Inlet	2	7.6E10	6.5E06	6.0E04	8.3E03
	5	1.0E11	6.5E06	4.2E04	9.0E03
	60	1.2E11	2.1E07	4.1E04	1.0E04
Returned	0	1.2E10	7.0E06	4.9E04	1.0E04
Screen	2	1.2E10	4.0E06	4.5E04	8.0E03
Washings	5	7.7E09	8.0E06	5.1E04	1.2E03
	60	1.4E10	6.5E06	2.5E04	1.1E03
Before	0	9.8E09	9.6E06	4.3E04	8.3E03
Screens	2	7.1E09	1.5E07	5.2E04	1.1E04
(5mm)	5	4.0E09	6.1E06	3.9E04	6.3E03
	60	8.3E09	1.2E07	4.7E04	5.8E03
After	0	7.5E09	8.4E06	5.4E04	6.8E03
Screens	2	1.3E10	1.3E07	3.5E04	1.0E04
	5	9.7E09	2.3E07	4.5E04	8.8E03
	60	6.9E09	1.6E07	4.2E04	5.8E03
Before	0	8.3E09	9.7E06	5.1E04	2.8E04
Detritor	2	6.5E09	6.5E06	4.6E04	2.5E04
	5	5.2E09	1.0E07	4.6E04	2.8E04
	60	4.7E09	1.1E07	4.9E04	3.4E04
After	0	2.5E09	1.1E07	8.5E04	6.0E04
Detritor	2	3.6E09	1.8E07	7.3E04	6.8E04
	5	4.1E09	1.8E07	7.5E04	8.5E04
	60	5.2E09	1.4E07	4.0E04	6.0E04

Hendon I:

Inlet	0	9.3E10	6.5E07	7.8E04	2.5E03
	5	2.6E11	1.2E08	4.7E05	1.4E04
	60	1.6E11	7.1E07	6.5E04	1.0E04
Returned	0	2.0E11	5.0E06	7.3E04	1.0E03
Screen	5	1.9E11	8.0E06	6.3E04	2.5E03
Washings	60	2.2E11	1.3E07	4.8E04	2.5E03
Before	0	1.1E11	2.1E07	8.8E04	2.3E04
Screens	5	9.9E10	8.0E06	9.0E04	2.5E04
(4mm)	60	9.8E10	1.3E07	7.5E04	3.8E04
After	0	4.8E10	1.2E07	7.0E04	1.0E04
Screens	5	1.1E11	1.1E07	7.8E04	7.5E03
	60	7.9E10	1.4E07	9.0E04	2.3E04

Hendon II:

Inlet	0	1.7E10	6.0E06	2.5E04	7.5E03
	5	1.5E10	8.0E06	2.9E04	7.5E03
	60	1.5E10	2.7E07	2.9E04	1.1E04
Returned	0	7.3E08	3.6E06	2.2E04	1.9E04
Screen	5	8.0E08	3.7E06	2.3E04	2.4E04
Washings	60	1.0E09	6.8E06	2.1E04	4.3E04
Before	0	1.1E10	3.0E06	2.6E04	8.0E03
Screens	5	9.0E09	5.2E06	2.9E04	7.0E03
(4mm)	60	1.2E10	9.0E06	3.6E04	1.1E04
After	0	6.2E09	4.8E06	2.5E04	8.5E03
Screens	5	7.4E09	3.2E06	3.1E04	6.8E03
	60	6.1E09	7.6E06	2.5E04	7.3E03

Amble I:

Inlet	0	8.3E09	1.6E06	1.9E05	2.8E04
	2	4.9E09	1.0E07	2.1E05	8.3E04
	60	1.3E10	1.0E07	1.9E05	1.1E05
Returned	0	1.4E10	1.4E07	1.4E05	1.2E05
Screen	2	1.3E10	2.2E07	1.7E05	5.8E04
Washings	60	1.4E10	2.0E07	1.7E05	1.2E05
Before	0	1.1E10	7.6E06	1.8E05	1.4E05
Screens	2	1.2E10	5.8E06	2.1E05	1.5E05
(6mm)	60	1.8E10	1.2E07	2.2E05	1.4E05
After	0	9.3E09	5.4E06	1.5E05	9.8E04
Screens	2	8.9E09	6.7E06	1.9E05	1.4E05
	60	1.7E10	8.5E06	1.9E05	1.7E05

Amble II:

Inlet	0	1.2E10	1.5E07	1.4E05	4.3E04
	2	9.7E09	1.3E07	1.5E05	2.3E04
	60	-	-	2.1E05	4.0E04
Returned Screen Washings	0	4.0E09	1.8E07	9.8E04	1.5E04
	2	6.1E09	2.0E07	1.5E05	1.3E04
	60	4.1E09	1.9E07	1.2E05	1.5E04
Before Screens (6mm)	0	7.8E09	6.9E06	2.1E05	4.5E04
	2	9.1E09	1.3E07	1.8E05	5.5E04
	60	4.1E09	1.3E07	1.8E05	6.8E04
After Screens	0	5.4E09	1.2E07	1.7E05	7.8E04
	2	3.7E09	9.8E06	1.3E05	3.3E04
	60	2.3E09	1.6E07	1.9E05	2.3E04

Seaton Carew I: (am)

Before Comminutor	0	1.2E10	5.0E06	8.5E04	3.1E04
	2	8.2E09	8.4E06	9.3E04	2.7E04
	60	9.8E09	5.3E06	7.5E04	2.7E04
After Comminutor	0	1.4E10	6.5E06	1.0E05	6.0E04
	2	1.3E10	7.4E06	1.3E05	3.8E04
	60	1.8E10	1.2E07	1.1E05	6.3E04

Burn Road I: (am)

Before Comminutor	0	2.2E10	3.1E06	4.5E04	1.3E04
	2	2.9E10	5.1E06	1.0E05	1.6E04
	60	3.2E10	5.1E06	9.0E04	1.7E04
After Comminutor	0	2.5E10	7.8E06	6.8E04	2.4E04
	2	4.1E10	6.0E06	3.8E04	3.0E04
	60	1.2E10	7.0E06	4.3E04	2.5E04

Seaton Carew II: (am)

Before Comminutor	0	1.1E10	7.3E06	1.4E05	1.8E04
	2	2.6E09	7.2E06	1.4E05	1.8E04
	60	6.1E09	1.1E07	1.5E05	2.1E04
After Comminutor	0	1.9E10	8.3E06	1.4E05	1.1E04
	2	4.2E09	6.6E06	1.4E05	2.4E04
	60	2.7E09	1.0E07	1.4E05	1.2E04

Burn Road II: (am)

Before Comminutor	0	3.6E09	9.8E06	8.5E04	9.0E04
	2	2.1E09	5.8E06	7.3E04	7.3E04
	60	3.4E09	9.9E06	6.5E04	8.0E04
After Comminutor	0	4.5E09	6.1E06	4.8E04	9.3E04
	2	4.0E09	6.2E06	6.3E04	6.0E04
	60	3.0E09	1.5E07	6.3E04	8.5E04

Seaton Carew III: (pm)

Before	0	7.7E09	6.0E06	3.0E04	5.5E03
Comminutor	2	6.7E09	2.6E06	2.5E04	3.0E03
	60	7.7E09	1.5E06	5.3E04	2.3E04
After	0	8.0E09	9.1E06	3.1E04	6.3E03
Comminutor	2	4.8E09	8.5E06	4.3E04	9.5E05
	60	8.4E09	-	-	5.0E03

Burn Road III: (pm)

Before	0	9.3E09	7.1E06	5.0E04	4.3E03
Comminutor	2	6.1E09	9.6E06	3.3E04	6.0E03
	60	4.6E09	2.0E07	2.0E04	2.3E03
After	0	9.9E09	6.5E06	2.9E04	4.3E03
Comminutor	2	8.2E09	1.5E07	3.0E04	3.3E03
	60	7.8E09	1.1E07	2.4E04	3.8E03

Seaton Carew IV: (pm)

Before	0	2.2E09	1.3E07	2.0E04	5.0E03
Comminutor	2	2.5E09	6.7E06	1.8E04	2.8E03
	60	2.4E09	8.1E06	1.7E04	1.3E03
After	0	3.2E09	5.6E06	1.9E04	4.8E03
Comminutor	2	2.7E09	1.1E07	2.1E04	3.8E03
	60	1.7E09	8.6E06	1.9E04	1.8E03

Burn Road IV: (pm)

Before	0	1.3E09	3.5E06	1.3E04	1.0E03
Comminutor	2	1.0E09	3.3E06	1.1E04	2.5E03
	60	1.9E09	3.1E06	1.1E04	3.8E03
After	0	1.6E09	2.2E06	1.5E04	3.0E03
Comminutor	2	1.9E09	2.0E06	1.5E04	7.5E03
	60	2.7E09	2.6E06	1.1E04	3.8E05