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# Fate and transport of particles in estuaries

## Volume III: Laboratory experiments, enterococci decay rates and association with sediments

Science report: SC000002/SR3

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Steve Killeen

**Head of Science**

# Report Context

The overall aim of this project is to assess the impact of distant sources of faecal indicator organisms, specifically enterococci species of bacteria, on bathing beach compliance sites within a highly turbid and high energy estuarine environment. The final experimental design emerged after discussion with Environment Agency personnel and a project steering group that included water company and Scottish Environment Protection Agency (SEPA) personnel, as well as Environment Agency staff. The total project effort can be split into three principal tasks that almost form stand alone studies.

1. Estimating bacterial inputs to the estuary from rivers (*n*=29) and marine sources (*n*=34) using a modelling approach grounded in past Centre for Research into Environment and Health (CREH) empirical studies, which involved 'ground truth' data on enterococci concentrations and land use from 100 subcatchments;

2. Defining decay rates of enterococci under highly turbid conditions by conducting some 40 microcosm experiments on water derived from characteristic sampling points in the Seven Estuary. These experiments were done under both simulated daylight and in the dark. In addition, investigating sediment characteristics by conducting settlement experiments.

3. Developing a hydrodynamic water quality model using data derived from 1 and 2 as inputs. The model incorporates both variable inputs and real-time decay rates for enterococci at key locations in the estuary and was validated with empirical field data that was acquired in the summer of 2001.

Overall, the study presents the first attempt to estimate bacterial 'inputs' to a major estuary at a regional level, the first attempt to define and quantify the environmental controls on enterococci survival in estuarine waters and the first attempt to develop a coastal hydrodynamic water quality model that incorporates a dynamic 'real-time' $T_{90}$ value.

The overall approach offers the potential for regional scale 'profiling' of recreational waters, as suggested by the World Health Organization (WHO, 2003) and the Council of the European Communities (CEC, 2002). It also allows for 'real time' prediction of water quality as a beach management tool, as suggested by the WHO (2003) and by the Department for Food, Environment and Rural Affairs in recent negotiations regarding the revision of EU Directive 76/160/EEC.

This volume (Volume III) presents in detail the results of the second task (laboratory experiments). Volume I addresses the overall objectives by providing an overview, summary of results and conclusions from the entire project. Volume II presents the results of the first task (estimating the enterococci inputs) and Volume IV presents the results of the third task (numerical modelling).

This was an ambitious project from the outset and many lessons have been learned in its execution. These lessons, as well as identified research gaps, are presented in Volume I (Summary and Conclusions).

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# 1. Introduction

This volume describes experiments that were conducted to determine typical values of the time taken for enterococci levels to decay by 90 per cent ($T_{90}$ (hours)) in samples from the Severn estuary and to investigate the association between enterococci and sediments. Determining enterococci $T_{90}$ values in a tidal estuarine environment was necessary to calculate a dynamic $T_{90}$ value for use within the hydrodynamic model developed as part of this project (see Volume IV). The association, or otherwise, of enterococci with sediments is an important factor in understanding the transport pathways of the organism. The Project Management Group agreed that the project should focus on enterococci due to (i) its increasing importance as a regulatory indicator (CEC,2002; WHO, 2003; Kay *et al*, 2004) and (ii) the relatively small amount of data on this indicator in the literature, especially when compared with faecal coliform organisms.

Experiments to determine the inactivation of enterococci in estuarine waters, as described in Section 2, were conducted using microcosms of water sampled at five locations along the Severn estuary coast and irradiated using artificial light. The experimental protocol was developed in association with the Project Management Group and after conducting a literature review, which is presented below (Section 2.1). The detailed protocol is outlined in Section 2.2, which includes details of the artificial light source and statistical methods that were used. The $T_{90}$ estimates and relationships with physiochemical parameters are then presented in Section 2.3. Experiments investigating the association of enterococci with sediments are considered in Section 3, together with a further review of relevant literature. Two investigations were necessary: first, a study to identify the optimum method for separating attached enterococci from sediments; and, second, experiments designed to probe the relationships between enterococci and suspended solids, turbidity and particle size. Finally, in Section 4, the results from this empirical work are compared with published data sources and the implications of the results for bathing water compliance within the Severn estuary are explored.

# 2. Enterococci inactivation in estuarine water microcosm experiments

## 2.1 Literature Review

Bacterial inactivation in natural waters is driven by a number of complex, interacting processes. The principal driver of inactivation is the intensity of the incident irradiance from sunlight (Gameson and Gould, 1975; Fujioka *et al.*, 1981; Gameson and Gould, 1985; Davies-Colley *et al.*, 1994). Sunlight is a complex mixture of irradiance wavelengths from UV-B (290–320 nm), UV-A (320–400 nm), visible light (400–800 nm) and infra-red (800–several thousands nm), although not all wavelengths contribute to bacterial inactivation (infra-red wavelengths contribute only to heating effects). The contribution of these wavelengths to bacterial inactivation is determined by latitude, the diurnal cycle of solar elevation and cloud cover. The bacterial decay generated by this constantly changing irradiance has traditionally been expressed as $T_{90}$ – the time for bacterial concentration to decrease by 90 per cent – and/or $S_{90}$ – the sunlight irradiation dose required for the bacterial concentration to decrease by 90 per cent.

The design of bacterial inactivation studies needs to take into account the following factors:

i) whether to use natural sunlight or artificial lamps with wavelengths that match natural light;
ii) whether indigenous bacterial concentrations in environmental waters are sufficient to undertake inactivation studies, and whether adding bacteria, usually from sewage effluent, should be considered and, if so, what is an appropriate starting concentration;
iii) whether subsidiary factors affecting mortality, such as turbidity, pH or conductivity/salinity, should be taken into account during an inactivation study.

A review of the literature describing inactivation study methodologies was conducted, considering each of the above factors, before the experimental protocol for this research project was designed.

### 2.1.1 Light source

Most bacterial inactivation studies have employed natural sunlight, often involving diffusion chambers suspended in water bodies, such as Sinton *et al.* (1994) and Burkhardt *et al.* (2000). This approach allows sensors to be placed directly into the

chamber, the water sample to be at an ambient temperature, and for chemicals to diffuse and oxygen to dissolve into the chamber. Alternatives include the use of closed pyrex vessels (Evison, 1988; Davies and Evison, 1991), plastic petri dishes (Gameson and Gould, 1985), microwave dishes (Davies-Colley *et al.*, 1997), universals (McGuigan *et al.*, 1998) or dialysis tubes (Duran *et al.*, 2002). In such studies, however, precise control of irradiance intensity is not possible, and irradiance is directly measured using a solarimeter or obtained from local monitoring stations.

Several studies have sought to control the irradiance input variable through the use of artificial light. Gameson and Gould (1985) used mercury lamps, although they found variations in both the wavelength spectra and irradiance energy delivered by the lamps. Much of the data generated in these studies were so poor that regression lines could not be fitted to the data. Mortality rates were also found to be lower than with sunlight. McGuigan *et al.* (1998) used a 150 W Xenon lamp with a solar bandpass filter to create a spectrum similar to sunlight, but it was only capable of irradiating a very small area.

Gameson and Gould (1985) reported that approximately half of the lethal effect of solar radiation occurs at wavelengths below 370nm, a further quarter between 370nm and 400nm and the remainder between 400nm and 500nm. Similar conclusions were reported by Sinton *et al.* (1994), who suggested that approximately half of the inactivation of enterococci in sunlight occurred below wavelengths of 360nm. Inactivation curves of enterococci at wavelengths beyond 550nm were found to be comparable to inactivation in the dark. Infra-red wavelengths do not play a significant role in the bactericidal affects of both real and simulated sunlight (Mancini, 1978; Acra *et al.*, 1990). Temperature control is also essential since heating of the irradiated sample by either natural light or lamps used to simulate sunlight could also cause pasteurisation (McGuigan *et al.*, 1998).

Little UVB radiation reaches the earth, with the shortest wavelengths quoted in the literature being 293nm. Thus, wavelengths below this threshold produced by an artificial light source would generate inaccurate inactivation rates that are too high. Hence, studies such as that of Kashimada *et al.* (1996), who used a low pressure mercury lamp at 254nm to inactivate bacteria, are of little relevance to environmental waters. Thus, any artificial light source used in bacterial inactivation studies should employ a bandpass filter with a cut-off of wavelength of about 300nm.

### 2.1.2 Bacterial source

The ideal situation would be to have a natural water source with sufficient indigenous bacteria to perform inactivation studies. In practice, most researchers have found insufficient bacteria in environmental samples to perform inactivation studies. The main constraint is the sample volume required for analysis of each extracted sample. Multiple extraction of large volume samples (100ml) could alter the characteristics (such as depth and volume) of the experimental microcosm. Thus, many authors have added bacteria to the microcosm to enhance the initial bacterial concentration, thereby allowing smaller sample volumes to be extracted. McGuigan *et al.* (1998) used overnight broth cultures that were washed in sterile water, but most studies have employed environmental waters seeded with raw sewage at dilution factors of between 0.25 per cent and 10 per cent

sewage. Higher sewage fractions may enhance turbidity (and thus protection from irradiance) and add nutrients or chemicals that are toxic in the presence of sunlight. Evison (1988) and Davies and Evison (1991) used enterococci grown in sterile sewage. Sinton *et al.* (1994) found that enterococci species derived from effluent from a meat works exhibited faster inactivation than enterococci species from sewage effluents. This was possibly due to species differences and the age of the effluent at collection. Most researchers use sewage that is no more than 16 hours old from collection (collected on one day and used either that day or the next day). Organisms harvested from sewage that has been stored for several weeks exhibit increased inactivation rates when compared with organisms derived from fresh sewage.

### 2.1.3 Other factors affecting bacterial mortality

Bacteria can attach to particles and flocs and settle-out of the water column to form a semi-permanent store on the lake, river or sea bed. They therefore have the potential to cause a deterioration in water quality when this sediment is disturbed and re-suspended (Weiss, 1951; Marshall, 1979; Jenkins, 1984; Jenkins *et al.*, 1984). The particles may also coat the organisms (Roper and Marshall, 1978), protecting them from predation and sunlight. In addition, being attached to particles can also alter the settling rate of the organisms. For example, Milne *et al.* (1986) compared the settlement of faecal coliforms associated with particles in estuarine water (muds and silts) and in secondary-treated final effluent. In the final effluent, which has already been settled, there was no discernible settlement after three hours. With estuarine muds and silts, settlement, although slow, was related to the concentration of suspended solids. For samples with a suspended sediment concentration of 100 mg $l^{-1}$, it reportedly takes approximately 30 hours for 90 per cent of the sediment to settle out. For samples with a suspended sediment concentration of 25 mg $l^{-1}$, the time for 90 per cent settlement was 92 hours. Gannon *et al.* (1983) and Auer and Niehaus (1993), who both studied settlement in North American lakes, found that 90 per cent of settled organisms were associated with particles of clay or silt (0.45–10 µm diameter) and that settlement rates were in the order of 1.2 m $d^{-1}$.

Increases in water temperature tend to cause the bacteria to die more rapidly (Wilkinson, 2001). However, several research groups have found that faecal coliform mortality is slower in waters with sewage contamination (Verstraete and Voets, 1976; Flint, 1987; Evison, 1988) and low oxygen status (Zerfas, 1970). Possible causes for this are the suppression of autochthonous bacteria, which compete for nutrients, as a result of the oxygen demand exerted by sewage effluent (Verstraete and Voets, 1976) and the increased availability of nutrients from the sewage (Evison, 1988). Thus, a given change in temperature in sewage contaminated water might have less effect on mortality than it would in clean river water (Wilkinson, 2001).

The effect of pH on bacterial mortality is only dramatic under extreme conditions. Observations of the effect of different pH conditions on faecal coliform mortality have shown that death is minimised at neutral pH and occurs more rapidly as conditions become increasingly acid or alkaline (Wilkinson, 2001).

Although microbial interactions (protozoan predation) in the self-purification of natural waters are of potential importance, they are also highly inter-related and complex, making it difficult to quantify their individual effects (Enzinger and Cooper, 1976; Verstraete and Voets, 1976; Roper and Marshall, 1978; Singleton and Sainsbury, 1981; Flint, 1987; Brettar and Hofle, 1992; Wilkinson, 2001).

## 2.2 Experimental methods

### 2.2.1 Sample collection

Water samples were collected in steam disinfected 10-litre containers from five locations along the Severn estuary coast: Langland Bay; Porthcawl; Beachley Slip; Weston-Super-Mare; and Minehead (Table 2.2; Figure 2.1). These sites were chosen to reflect the range of water qualities experienced in the Severn Estuary. On each occasion, a total volume of 60 litres was collected from each site. At beach sites (Langland Bay, Porthcawl and Minehead), samples were collected from beyond the surf zone to avoid near-shore transport of particulates. Further samples were collected at the same time for physiochemical and suspended sediment particle size analysis. Three sites were sampled each week for a seven-week period in January and February 2002. The water samples were transported overnight to the University of Lancaster and stored at ambient temperature until used. Part of the 60-litre sample was analysed for turbidity, suspended solids, pH, conductivity, salinity and total dissolved solids.

To ensure sufficient enterococci in the samples at the start of the experiments crude sewage was added for seeding purposes. Sewage samples were collected daily from Stodday (Lancaster) wastewater treatment works (WwTW). An aliquot of 200ml of sewage was centrifuged at 3,000g for 30 minutes. The supernatant was removed and replaced with deionised water. This washing procedure was repeated twice and the pellet finally re-suspended in deionised water to its original volume (200ml). The added enterococci achieved an initial concentration of $1 \times 10^4$ colony forming units (cfu) $100ml^{-1}$, which would allowed a 4-log reduction in concentrations to be measured.

### 2.2.2 Irradiation

The artificial light source was designed to reproduce the correct solar spectrum and intensity observed in the period from 10.00am to 2.00pm between the beginning of July and the end of August at a latitude of 52° North. This was provided by an existing bench rig at the University of Lancaster with full calibration facilities.

Experiments were carried out in a controlled environment room with air temperature held at 18±2°C. The light source emitted visible, UV-A and UV-B wavebands (400–700nm, 320–400nm, and 280–320nm respectively). All calibration measurements of photosynthetically-available radiation (PAR) and UV-A and UV-B radiation were carried out using a double monochromator spectroradiometer (Model SR991-PC, Macam Photometrics, Livingston, UK). This was calibrated for spectral irradiance against deuterium (240–350nm) and tungsten (350–800nm) standards traceable to the National

Physics Laboratory (London) and checked for wavelength alignment against a mercury argon lamp (LOT Oriel, Surrey, UK).

Visible light was provided from a mixture of metal halide and high pressure sodium lamps, which together provided an irradiance of 260±14W $m^{-2}$ at the surface of the water bath (Table 2.1). This level of irradiance is slightly below that produced by full summer sunlight in the Severn Estuary, but it is at the top end of the range of irradiance reported in previous controlled environment studies. The level was considered appropriate in the current study because it would ensure that all photo-repair processes were fully light-saturated. To repair DNA damage caused by UV-B, many organisms utilise the enzyme photolyase, which requires low irradiances with UV-A or blue light and is a major factor in moderating UV damage. Thus, excluding adequate irradiances of the longer wavelengths that drive this process would overestimate UV-B damage. Long wavelength UV-A radiation was provided from Philips TLD-30/12 lamps, which emit a UV-A irradiance of 5.2±0.4 W $m^{-2}$ (Table 2.1).

UV-B radiation was supplied by UV-B fluorescent tubes (Philips TL40/12, Starna OK, Romford, Essex), which were filtered using cellulose diacetate held 17cm from the lamps (100µm-thick Clarifoil, Courtaulds Ltd, Derby, UK). Cellulose diacetate removes the short wavelength radiation not present in sunlight but produced by all UV-B lamps and which causes artefactual damage if not filtered-out. With the cellulose diacetate filter, the shortest wavelength reaching the samples is approximately 290nm, which is the same as the shortest wavelengths measurable in sunlight in the UK. However, cellulose diacetate photodegrades, leading to reduced UV-B transmission. UV-B irradiances were therefore monitored daily at the start of the UV-B photoperiod and adjusted using Philips HF BHF232 electronic ballasts to keep the irradiance constant at the surface of the sample during the experiment. This procedure did not change the UV-B spectrum. The cellulose diacetate was replaced when the desired irradiances could no longer be maintained. Through this combination of cellulose diacetate replacement and daily lamp adjustment, the UV-B treatment was maintained at an irradiance of 1.1±0.02 $Wm^{-2}$ (Table 2.1) at the surface of the water bath.

It is worth noting that artificial UV sources are relatively richer in short wavelength UV-B compared with sunlight, and biological responses increase exponentially with wavelength in this waveband. As a result, biological 'response' per unit radiation dose tends to be higher with lamps than in sunlight. It is possible to correct for these effects when quantifying UV-B treatments by taking into account the spectral distribution of the light source using a spectroradiometer, and this was done for this analysis. The biological interpretation of spectral data requires some understanding of how the biological response being measured is affected by different wavelengths of UV-B radiation (the 'action spectrum' of the response). Using an appropriate action spectrum allows treatments to be quantified as 'weighted doses' (taking into account both the action spectrum and the spectral distribution of the source), making it easier to compare directly lamps and sunlight. However, while there is no doubt that this is the optimal approach in theory, the action spectra for many biological processes are poorly understood, which constrains the use of this approach in practice. Thus, in the current study, treatments were designed without assuming any particular action spectrum. Nevertheless, since spectral data are available, it would be possible to re-examine the treatments if an action spectrum appropriate for enterococci survival is defined. As an example, Table 2.1 also includes the irradiance for UV-B weighted using the DNA damage action spectrum.

### 2.2.3 Experiment protocol

Twenty-five litres of water from the same source were placed in each of two cooled water baths (Grant, Cambridge) lined with matt-black plastic, with the temperature maintained at 15˚C. One bath was placed under the irradiance system (Plate 2.1 and Plate 2.2) and a second, identical bath was covered with matt-black plastic and kept in a dark room to eliminate all irradiation (Plate 2.3). Water from each of the five sample sites was used on four separate occasions giving a total of forty water bath microcosm experiments, half of which were irradiated. Thus, for each pair of experiments, physiochemical parameters such as turbidity, suspended solids, pH and conductivity, as well as the initial microbiological flora, were, as far as possible, identical. Thus, the difference in microbial mortality observed between the two baths can be attributed solely to the received irradiance energy. In contrast, differences in microbial mortality between water types can be attributed to the difference in the physiochemical properties and microbial flora of the water samples.

The temperature in the baths was first allowed to equilibrate to 15˚C. Irradiance lamps were switched on 30 minutes before each experiment to allow stabilisation and the lamps were also calibrated on a daily basis to establish the correct irradiance levels. An aliquot of 100ml of washed sewage was added to each bath and samples were taken at the beginning of the experiments and at regular intervals throughout the period of irradiation. The intervals varied depending on the turbidity of the samples. Samples were taken in sterile universal containers and immediately refrigerated prior to analysis. All samples were analysed within 24 hours, as recommended by the Environment Agency (2000). Enough bacteria were added to each bath to ensure that no more than 25ml (approximately 0.5 per cent of the total water bath volume) was required to achieve acceptable plate counts at each sampling event.

### 2.2.4 Microbiological analysis

Enterococci were isolated on Slanetz and Bartley agar by membrane filtration using gridded cellulose ester membranes with a 47mm diameter and a pore size of 0.45μm. The membranes were incubated at 37 ± 1˚C for 4 hours followed by 44 ± 0.5˚C for 44 hours (±4 hours) and colonies of all sizes and shades of pink through to maroon were counted as presumptive enterococci. Plates that were positive for enterococci were tested for aesculin hydrolysis (ISO 7899 – 2) by transferring the entire membrane using kanamycin aesculin azide agar (KAAA) incubated at 44 ± 1.0 ˚C for four hours. Colonies that turned brown to black, as evidence of aesculin hydrolysis, were counted as confirmed enterococci. Full analytical quality control (AQC), to United Kingdom accreditation service (UKAS) standards, was undertaken as part of the microbiological analyses.

### 2.2.5 Physicochemical and particle size analysis

Samples were analysed for pH and conductivity (µS $cm^{-1}$ or mS $cm^{-1}$) using a Denver Instruments model 220 pH/conductivity meter. Conductivity values were also expressed as total dissolved solid (mg $l^{-1}$) and practical salinity (unitless[1]), with the concentration of total dissolved solids in selected samples verified by evaporation and gravimetry (BS 1377-3:1990; Greenberg *et al.*, 1992, pp 2-43 to 2-45). Turbidity (NTU) was measured using a HACH model 2100A Turbidimeter (optical scattering, with formazine primary and gelex secondary standards) (Greenberg *et al.*, 1992, pp 2-8 to 2-11). Suspended solids concentration (mg $l^{-1}$) was determined using evaporation and gravimetry based on Whatman 47mm GFC filters (average pore size 1.2µm) (Greenberg *et al.*, 1992, pp 2-53 to 2-54). Particle size distribution was analysed using a laser diffraction particle size analyser (Beckman-Coulter LS230 with variable speed fluidics module).

### 2.2.6 Statistical methods

Statistical tests were undertaken using Minitab (v10.51Xtra; Minitab Inc.) and SPSS (v10.0.8; SPSS Inc.) statistical packages.

The distribution of microbial concentrations found in samples showed a closer approximation to normality when $\log_{10}$ transformed. All microbial concentration data were, therefore, $\log_{10}$ transformed prior to statistical analysis by parametric tests, such as linear regression analysis, to ensure a better distribution of residual values.

Bacterial decay rates were assumed to follow a first order decay model according to Chick's Law (Chick, 1910):

$$\frac{\delta C}{\delta t} = -k_t C \qquad (2.1)$$

where: $C$ = enterococci concentration (cfu $100ml^{-1}$)
$t$ = time (minutes)
$k_t$ = mortality rate over time ($minutes^{-1}$)

This allows $k_t$ to be represented by the slope of the line of best-fit from least-squares regression of $\log_{10}$ transformed enterococci concentration ($\log_{10}C$) plotted against time ($t$). Thus, $T_{90}$ is calculated as:

$$T_{90} = \frac{1}{k_t} \qquad (2.2)$$

To provide an estimate of the irradiation dose (biologically weighted UV-B, UV-B, UV-A, and total irradiation (UV-B + UV-A + visible light)) required to inactivate 90 per cent of the

[1] practical salinity: a scale relative to a standard KCl solution – a seawater with conductivity at 15°C equal to that of a KCl solution containing a mass of 32.4356g in a mass of 1kg of solution is defined as having a practical salinity of 35

bacterial concentration ($D_{90}$), irradiation (W $m^{-2}$) (*D*) can be substituted for time (*t*) in equation 2.1 to provide an inactivation rate due to irradiation ($k_D$):

$$\frac{\delta C}{\delta D} = -k_D C \tag{2.3}$$

This allows $k_D$ to be obtained by plotting $\log_{10}$-transformed enterococci concentration ($\log_{10}C$) against irradiation dose (W $m^{-2}$). Thus, $D_{90}$ is calculated as:

$$D_{90} = \frac{1}{k_D} \tag{2.4}$$

Since the irradiance provided by the artificial light source was constant throughout the experiments, the irradiance data presented in Table 2.1 can be used together with equations 2.1 to 2.4 to relate $D_{90}$ to $T_{90}$ using the following functions:

Total Irradiance $D_{90}$ (MW $m^{-2}$) = $0.936T_{90}$ (2.5)

UV-A $D_{90}$ (kW $m^{-2}$) = $18.72T_{90}$ (2.6)

UV-B $D_{90}$ (kW $m^{-2}$) = $3.96T_{90}$ (2.7)

Biologically weighted UV-B $D_{90}$ (kW $m^{-2}$) = $1.152T_{90}$ (2.8)

The $r^2$ value (coefficient of determination[1]) provides an indication of the strength of any relationship expressed as a percentage. All values of $r^2$ derived from regression models were adjusted for degrees of freedom.

Calculating an average $T_{90}$ value from the four experiments for each site is inappropriate since this involves averaging of the reciprocals of the slopes of the regression lines. A more appropriate method is to combine normalised data before carrying out the regression analysis (Gameson, 1985). For the present study, the bacterial concentration data were normalised using the value of the constant from each individual experiment's regression equation, which in a perfect log-linear relationship would represent the initial concentration. This was selected in preference to the observed initial concentration, as recommended by Gameson (1985), which is more prone to error in recording levels of bacteria in individual samples.

Microbial mortality in the dark can be attributed to the bactericidal effects of factors such as salinity and pH, starvation, lack of nutrients, osmotic stress and predation (McCambridge and McMeekin, 1981; Davies-Colley *et al.*, 1994; Sinton *et al.*, 1994). Such effects are also present during irradiated experiments. To allow for this and to identify the inactivation effects of radiation alone, irradiated $T_{90}$ values were adjusted using the paired dark $T_{90}$ value from each experiment. It should be noted, however, that these adjusted $T_{90}$ values do not represent solely the bactericidal effects of radiation, since other variables within the water will also be an influence on, or be influenced by, the incoming radiation. For example, the turbidity and particle size distribution will largely

[1] the percentage of the variance in the dependent variable (bacterial concentration) that is explained by the predictor variable (time or irradiance dose)

determine the received irradiance dose, whilst both chemical reactions and biological processes may be influenced by the radiation.

In order to identify which environmental variables influence enterococci $T_{90}$ and $D_{90}$ values, the relationships between environmental variables and estimated $T_{90}$ and $D_{90}$ values were investigated using one-way analysis of variance (Anova) plus multiple range tests, Pearson-product-moment correlation analysis and least squares multiple regression analysis (using the SPSS forward selection stepwise procedure) of the form:

$$T_{90} = a_1x_1 + a_2x_2 \ldots a_i x_i + b \pm u \quad (2.9)$$

$$D_{90} = a_1x_1 + a_2x_2 \ldots a_i x_i + b \pm u \quad (2.10)$$

where:

*a* = slope (change in *y* per unit change in *x*)
*b* = intercept (*y* at *x* = 0)
*u* = stochastic disturbance or random error term.

The entry of variables into an equation was controlled using a tolerance value to limit multi-collinearity between the independent variables. This excluded all predictor variables that have explained variation of ≥50 per cent with predictor variables already entered in the model (strongly inter-correlated predictor variables). Again, all values of $r^2$ derived from regression models were adjusted for degrees of freedom, accounting for the number of variables in the equation. The normality of residual values was assessed using the Ryan-Joiner test for normality and normal probability plots of standardised residuals (Minitab, 1995). The significance (*p*) of all statistical tests were assessed at $\alpha$ = 0.05 ($p<\alpha$, 95 per cent confidence level). Missing values in the predictor variable data were excluded on a pair-wise basis.

Stepwise multiple regressions were carried out on the irradiated and radiation-induced $T_{90}$ data sets. Similar analyses of the dark data sets were also carried out, although these analyses excluded the results of experiments where no bacterial death was observed ($T_{90}$ = infinity). Hence, both long $T_{90}$ values and the higher turbidities and lower salinities associated with most of these experiments would be excluded. Nevertheless, it was necessary to investigate the relationships in the dark for the purposes of developing dynamic $T_{90}$ functions in the hydrodynamic models described in Volume IV.

## 2.3 Microcosm experiment results

### 2.3.1 $T_{90}$ (inactivation over time)

The enterococci concentration data (cfu $100ml^{-1}$) generated from the 40 microcosm experiments (four at each of five sites carried out in both irradiated and dark conditions) are tabulated in Appendix I. The $T_{90}$ values derived from the regressions of enterococci concentrations against time, together with $r^2$ values associated with the regression equation, are presented in Table 2.3 and included on the scattergraphs in Figure 2.2. Inspection of the scattergraphs presented in Figure 2.2 show that, in some cases,

outliers in the data have a detrimental effect on the regression line (see for example Porthcawl, experiment 1). Also, several scattergraphs suggest a decrease in the gradient of the decay relationship at very low concentrations. This may imply a more complex decay process than first order kinetics as suggested by Chick (1910) or, alternatively, it may be a function of the imprecision in enterococci counts at such low concentrations. Thus, additional analyses were completed excluding outlier data or low concentration data where this appeared to be unduly influencing the function of the regression line. These results are presented in Table 2.4 and scattergraphs presented in Figure 2.3. A discussion of the results, including all data, is presented in Section 2.3.1.1, whilst the results of the additional analyses excluding outlying data are presented in Section 2.3.1.2.

Turbidity, suspended solids, pH, conductivity, total dissolved solids (TDS) and practical salinity for each of the experiments are shown in Table 2.8, together with the associated irradiated and dark results. The relationships between $T_{90}$ values and these environmental factors are described in Section 2.3.1.3.

#### *2.3.1.1 $T_{90}$ estimates using all experimental data*

Non-significant ($\alpha$>0.05) regression results were produced for dark conditions in six experiments: three using water from Beachley Slip, two using water from Weston-Super-Mare and one using water from Langland Bay (Table 2.3). All other regression results (all 20 irradiated microcosms and 14 of the dark microcosms) were significant at the 95 per cent confidence level ($p$<0.05).

Shorter $T_{90}$ values were observed in the irradiated microcosms using water from the three outer marine sites in the Bristol Channel – Minehead (3.7–12.8 hours), Porthcawl (4.2–16.8 hours) and Langland Bay (4.8–10.9 hours) – than those using water from the more turbid estuarine sites, namely Weston-Super-Mare (14.2–113.9 hours) and Beachley Slip (27.0–54.2 hours) (Table 2.3). Combining the irradiated data from each sampling site produces the following $T_{90}$ values: Langland Bay – 6.6 hours; Porthcawl – 9.1 hours; and Minehead – 9.7 hours (Table 2.5). These contrast with much longer $T_{90}$ values of 35.1 hours for Beachley Slip and 18.7 hours for Weston-Super-Mare (Table 2.5). One-way Anova indicated that the irradiated $T_{90}$ values for the three marine Bristol Channel sites were significantly different to the two more turbid estuarine sites at the 95 per cent confidence level ($p$<0.05). The three outer marine sites displayed high practical salinities (mean values: Langland Bay – 38.9; Porthcawl – 36.8; Minehead – 34.6), which are typical of marine conditions, and turbidities of less than 100 NTU (mean values: Langland Bay – 30 NTU; Porthcawl – 25 NTU; Minehead – 56 NTU). Suspended solids concentrations were also relatively low at these sites (mean values <200mg $l^{-1}$). These are in contrast to the two estuarine sites, which displayed lower salinities, greater turbidities and greater suspended solids concentrations (Table 2.8). One-way Anova tests for turbidity and salinity indicate no statistically significant differences ($p$>0.05) between the three outer sites but significant differences between the estuarine and marine locations. This was not the case for the salinity data. Figure 2.4 illustrates the mean and range of $T_{90}$ values, turbidity and salinity using water from each of the five sites.

The data from the marine and estuarine sites were combined to calculate a separate $T_{90}$ for each type of environment. For the three 'marine' locations, the combined $T_{90}$ for

irradiated conditions was 8.8 hours, whilst, at the estuarine sites, the combined irradiated $T_{90}$ was 22.5 hours (Table 2.5).

The experiments carried out in dark conditions illustrate the prolonged survival of enterococci in the absence of irradiance. In all cases, irradiated $T_{90}$ was shorter than the corresponding non-irradiated experiment, although, interestingly, irradiated $T_{90}$ values based on water from Beachley Slip and Weston-Super-Mare exceeded dark $T_{90}$ values observed using water from Langland Bay (with the exception of experiment one), Porthcawl and Minehead (Table 2.3). At the outer marine sites, dark $T_{90}$ values ranged between 8.8 hours and 34.6 hours, with one exception (Langland Bay, experiment one, $T_{90}$ = 73.8). Water from the estuarine sites (Weston-Super-Mare and Beachley Slip) displayed $T_{90}$ values of between 52.0 hours and 74.9 hours in the three experiments where significant decay was observed. Five of the non-irradiated experiments using water from the two estuarine sites did not display any significant bacterial decay in the dark. The relatively high dark $T_{90}$ obtained from experiment 1 using Langland Bay water (73.8 hours) is noteworthy. In this experiment, the sample contained an unusually high proportion (81.2 per cent) of sediment greater than 53µm in size. In all the other samples from this site, less than 20% of particles were greater than 53µm (Table 2.9). Coupled with the low coefficient of determination ($r^2$ = 20.1 per cent; Table 2.1), indicating a low level of explained variance (i.e. of change in bacterial concentration explained by time), this experiment may be considered atypical. Despite this, however, the irradiated $T_{90}$ value (10.9 hours) was similar to the results from the other three irradiated experiments for this site, which range between 4.8 hours and 7.5 hours (Table 2.1).

Combining the dark experiment data from each sampling site results in the following $T_{90}$ values: Langland Bay – 24.9 hours; Porthcawl – 29.7 hours; and Minehead – 15.1 hours (Table 2.5). These contrast with a $T_{90}$ value of 54.6 hours for water from Weston-Super-Mare, whilst at Beachley Slip no significant decay was identifiable (Table 2.5). Combining data for the marine and estuarine sites resulted in an estimated $T_{90}$ for dark conditions of 21.5 hours at the marine sites and 66.2 hours at the estuarine sites (Table 2.6).

The radiation-induced $T_{90}$ values (irradiated $T_{90}$ adjusted for decay in the dark) range between 4.9 hours and 113.9 hours (Table 2.7), although some irradiated $T_{90}$ values could not be adjusted because there was no significant decay (slope not significantly different from zero) in the paired dark experiment. Again, the shortest $T_{90}$ values were associated with the three marine sites in the Bristol Channel. A one-way Anova and multiple-range test indicated that only the mean radiation-induced $T_{90}$ value for Langland Bay was significantly different from the two estuarine sites.

#### *2.3.1.2 $T_{90}$ estimates using experimental data excluding outliers*

The results of the additional analyses to estimate $T_{90}$ using the experimental data excluding outliers are presented in Table 2.4, whilst the excluded data are detailed in Figure 2.3. This additional analysis exclusing outliers does not result in a systematic change to the resultant $T_{90}$ values (some increase, whilst others decrease).

Generally, the patterns described above for $T_{90}$ estimates using all experimental data are similar to those excluding outliers. $T_{90}$ values were again shorter at the three outer

marine sites, with $T_{90}$ values ranging between 3.7 hours and 8.7 hours for Minehead, 4.5 hours and 12.8 hours for Porthcawl and between 5.7 hours and 10.9 hours for Langland Bay (Table 2.4). The $T_{90}$ values at the estuarine sites ranged between 18.9 hours and 54.2 hours at Beachley Slip and between 15.5 hours and 113.9 hours at Weston-Super-Mare (Table 2.4). Combining the irradiated data from each sampling site results in $T_{90}$ values of 6.6 hours for Porthcawl, 7.4 hours for Langland Bay and 8.0 hours for Minehead (Table 2.5). These contrast with $T_{90}$ values of 21.6 hours at Weston-Super-Mare and 41.5 hours at Beachley Slip (Table 2.5). Again, Anova and multiple range tests indicated the $T_{90}$ values for water from the three marine sites were significantly different to the $T_{90}$ values of the two estuarine sites. The combined $T_{90}$ values for the marine sites was 6.6 hours (two hours shorter than that estimated including all data (Section 2.3.1.1)), whilst for the estuarine sites the combined $T_{90}$ value was 39.5 hours (17 hours longer than that estimated including all data (Table 2.6)).

Only three dark $T_{90}$ calculations were affected when outliers were excluded (Table 2.4). For Langland Bay water, the $T_{90}$ values from experiment four decreased from 18.2 hours to 17 hours, whilst for Minehead, experiment two, the $T_{90}$ value increased slightly from 12.5 hours to 12.8 hours. However, the $T_{90}$ value from experiment one using water from Beachley Slip decreased by approximately 40 hours from 74.9 hours to 35.2 hours (Table 2.4). Combining the revised dark data from each sampling site results in the following $T_{90}$ values: Langland Bay – 18.5 hours; Porthcawl – 30.1 hours; and Minehead – 16.4 hours (Table 2.5). These represent a decrease in $T_{90}$ estimates of approximately four hours for Langland Bay, whilst for Porthcawl and Minehead the $T_{90}$ values increased by approximately half an hour over those estimated using all experimental data. No outliers were excluded for the Weston-Super-Mare water, which means that this $T_{90}$ value was not adjusted, whilst for Beachley Slip a significant positive regression slope was obtained, indicating possible concentration increase over time, although the $r^2$ value (7.39 per cent) was low (Table 2.5). Combining data for the marine and estuarine sites produced an estimated $T_{90}$ value for dark conditions of 24.8 hours for marine water, whilst for estuarine waters the estimated dark $T_{90}$ value was 65.1 hours (Table 2.6). This represents an increase in the marine $T_{90}$ value of three hours and a decrease in the estuarine $T_{90}$ value of an hour compared with the values obtained using all experimental data.

The radiation-induced $T_{90}$ values range between 5.2 and 22.5 hours for the three marine waters and between 22.1 hours and 113.9 hours for the two estuarine waters (Table 2.7). Again, some irradiated $T_{90}$ values were not adjusted, because there was no discernible mortality in the paired dark experiment. A one-way Anova test of the $T_{90}$ values indicated that the values for all three marine waters were significantly different from the two estuarine water sources.

#### *2.3.1.3 Relationships between $T_{90}$ and environmental parameters*

Relationships between the environmental parameters and the $T_{90}$ data were investigated after appropriate data transformations were performed to ensure normality (Table 2.10). Statistically significant Pearson correlations ($p$<0.05) were evident between all irradiated (irradiated and radiation-induced) $T_{90}$ data sets and practical salinity, TDS, conductivity, turbidity (all $n$=20) and suspended solids ($n$=17) (Table 2.10). Practical salinity, TDS and conductivity were inversely related to $T_{90}$, whilst turbidity and suspended solids displayed positive relationships.

Statistically significant relationships with salinity, TDS, conductivity and turbidity were present for the dark $T_{90}$ experiments using all experimental data, although the relationships were weaker than for the irradiated experiments (indicated by the lower correlation coefficients ($r$)) (Table 2.10). Again salinity was negatively correlated with $T_{90}$ values, while turbidity was positively correlated. No statistically significant correlations with any environmental parameters were present for dark $T_{90}$ estimates when outliers were excluded (Table 2.10).

The results of the regression analyses on irradiated and radiation-induced $T_{90}$ values are summarised in Table 2.11. A statistically significant relationship was identified in each case, but it should be noted that only the regression for radiation-induced $T_{90}$ values excluding outliers produced residuals that were normally distributed. Hence, the remaining models should be treated with caution. Turbidity was the independent variable entered in all cases, with no other variable input to the models.

The results of the regression analyses based on the dark $T_{90}$ values ($n$=15) are shown in Table 2.12. A statistically significant relationship between conductivity and dark $T_{90}$ values was identified, although the coefficient of determination ($r^2$) was relatively low at 28.1 per cent. No significant relationships were identified for the dark $T_{90}$ values using the data set excluding outliers (Table 2.10). The hydrodynamic modelling uses a dynamic $T_{90}$ function based on turbidity and it was therefore necessary to develop relationships between turbidity and dark $T_{90}$ values by including the turbidity variable in the regression equations. A statistically significant relationship was identified for the dark $T_{90}$ values including all data, although the $r^2$ value was only 24.6 per cent. No significant models were identified for dark $T_{90}$ values when outliers were excluded.

The relationships between environmental parameters and dark $T_{90}$ values were also explored, but excluding the atypical results from experiment one at Langland Bay (see Section 2.3.1.1). These results, also shown in Table 2.12, indicate an improved $r^2$ value for the $T_{90}$ data set including outliers (48.9 per cent). On this occasion salinity was the predictor variable entered. A statistically significant relationship between $T_{90}$ and turbidity was discovered when the atypical experiment and outliers were excluded (Table 2.12).

The relationships of irradiated and dark $T_{90}$ values with suspended solids and turbidity are shown in Figure 2.5a for $T_{90}$ estimates based on all experimental data, whilst Figure 2.5b shows the corresponding relationships when outliers are excluded. Regression analysis also indicated a strong, significant relationship between turbidity and suspended solids ($p$<0.05, $r^2$=82.3 per cent). Despite $T_{90}$ values for dark conditions being longer at lower suspended solids concentrations (an artefact of study site characteristics), the curves for irradiated and dark $T_{90}$ values eventually intersect. The convergence of the curves at higher suspended sediment concentrations should be expected, as a greater proportion of the radiation is absorbed by the particles. Indeed, this occurs to such an extent that conditions similar to darkness are approached under irradiation. The intersection of the curves for the $T_{90}$ values using all experimental data and excluding outliers were, respectively, at values of 52.6 hours and 41.4 hours, suspended sediment concentrations of 1,738.5mg $l^{-1}$ and 1,544 mg $l^{-1}$, and turbidities of 207 NTU and 200 NTU. These values of suspended solids and turbidity were exceeded in only one sample from Weston-Super-Mare, which produced an irradiated $T_{90}$ of 113.9 hours (although the $r^2$ value (17.4 per cent) was rather low) with no discernible decay under dark conditions.

Excluding this particular experiment and the atypical dark $T_{90}$ value from experiment one at Langland Bay resulted in only one irradiated $T_{90}$ value (two in the case of the data set excluding outliers) and two dark $T_{90}$ values (one in the case of the data set excluding outliers) that exceeded the intersection threshold $T_{90}$ values (Table 2.3 and Table 2.4). The only 'within site' $T_{90}$ value to exclude the threshold was Weston-Super-Mare in dark conditions (54.6 hours; Table 2.5); the estuarine dark $T_{90}$ value (65–66 hours; Table 2.6) also exceeded this threshold.

### 2.3.2 $D_{90}$ (inactivation over irradiation energy dose)

The irradiation energy dose required to inactivate 90 per cent of enterococci in the irradiated microcosm experiments and adjusted for mortality rates in the dark (radiation-induced) are shown in Table 2.14 in terms of total irradiation received at the surface of each irradiated microcosm (MW $m^{-2}$). The results are also presented for UV-A (kW $m^{-2}$) in Table 2.15, UV-B (kW $m^{-2}$) in Table 2.16 and biologically-weighted UV-B using the DNA damage action spectrum (kW $m^{-2}$) in Table 2.17. Again, the irradiated $D_{90}$ values reflect enterococci inactivation due to direct damage from irradiation and the effects of chemical and biological processes such as salinity and predation, whilst the irradiation-induced data reflect inactivation due solely to direct damage from irradiation.

Since irradiance throughout the experiments was constant, the patterns of $D_{90}$ values for the various wavebands are similar to those described in Section 2.3.1 for $T_{90}$ data (lower $D_{90}$ values for the three outer Bristol Channel sites and higher $D_{90}$ values in experiments using turbid estuarine water from Weston-Super-Mare and Beachley Slip). Similarly, the 'within site' and 'combined site' (marine/estuarine) $D_{90}$ values, shown in Table 2.18 for the various wavebands, are linear functions (see equations 2.5 to 2.8 in Section 2.2.6 above) of the $T_{90}$ values presented in Table 2.5 and Table 2.6. Also shown in Table 2.18 are the $D_{90}$ estimates at each waveband for the two threshold $T_{90}$ values that identify where the suspended solids concentration/turbidity is such that decay is the same under irradiated and dark conditions.

The statistical tests described in Section 2.3.1 will result in the same correlation matrix as shown in Table 2.10 and the same values of *b*, *u*, adjusted $r^2$, *p* and normality statistics as described in Table 2.11. The only factor to vary in the regression analysis will be the constant *a* (*y* at *x*=0), and these are shown in Table 2.19 for the various wavebands.

# 3. Settlement experiments

## 3.1 Literature Review

Aquatic sediments are thought to offer protection to, and act as a reservoir for, faecal indicator organisms. This protection is probably the result of the sedimentary micro-environment providing nutrients and shading from irradiance. Some studies into the presence of enterococci in settled sediments have reported concentrations an order of magnitude greater than in overlying water (Obiri-Danso and Jones, 2000; Alm *et al.*, 2003). However, the concentrations of bacteria in sediment and water are affected by factors such as distance from potential sources of faecal indicator organisms (FIOs), including sewage effluent point sources, the energy of the environment and the degree of re-suspension caused by increased flows, waves and storms. (Alm *et al.*, 2003; Le Fevre and Lewis, 2003). Attachment of bacteria to sediments is dependent on many factors, including temperature (McCaulou *et al.*, 1995), ionic strength (Fontes *et al.*, 1991) and pH (Bales *et al.*, 1995).

Studies into the survival of enterococci in settled sediments have shown highly variable results. This may be due to the different composition and sources of sediments, as well as the presence of nutrients, chemicals, predators and temperature. Reported $T_{90}$ values in sediments range from 25 hours in clay sediments at 10˚C (Craig *et al.*, 2003) to 14–83 days in constructed wetland sediment (Davies and Bavor, 2000). Davies and Bavor (2000) also demonstrated that enterococci $T_{90}$ values in sediments are related to the presence of predators by observing longer $T_{90}$ values (most differences statistically significant ($p$<0.05)) when they inhibited predation with cycloheximide. Ottoson and Stenstrom (2003) compared enterococci mortality in sediments from a grey water treatment system incubated at room temperature and found that, whilst enterococci died off in less than 50 days in the presence of indigenous microflora, in sterilised sediment it was able to grow to a density of $10^7$ cfu $g^{-1}$.

The attachment of bacteria to a mobile or semi-mobile solid phase has important implications for their transport. Bacteria that adhere to particles are no longer buoyant and have the potential to settle out with particulates or to be remobilised if flow velocities increase (Mahler *et al.*, 2000). However, few studies have been conducted to investigate the proportion of enterococci associated with suspended sediments within the water column, or whether attachment to particles is associated with a particular particle size. Mahler *et al.* (2000) demonstrated that up to 100 per cent of enterococci in karst groundwaters were associated with sediments. Alkan (1999) found that adding kaolin clay to a mixed seawater microcosm containing enterococci resulted in 'removal' (adsorption and sedimentation) of up to 60 per cent of the organisms. Both fluid shear rate ($sec^{-1}$) and clay concentration were found to influence the removal of bacteria, although the volume of sewage used to seed the microcosms and, hence, the initial enterococci concentration was found not to affect the removal rate. In general, Alkan found that increasing the level of mixing and particulate concentration resulted in a higher degree of removal, up to an optimal value of 70 mg $l^{-1}$ kaolin, beyond which a decrease in the removal rate occurred (Alkan, 1999). However, these experiments were conducted using sediments of a defined particle size and attachment is likely to vary with particle size in the estuarine environment.

Bacteria are thought preferentially to adsorb to fine particles (Dale, 1974). This is particularly important in the context of bathing water compliance and the affect of potential sources, since particle size determines the flux of attached FIOs. This is because finer particles provide a greater area available for attachment of bacteria per unit mass.

Working out the amount of enterococci associated with sediments in environmental samples poses many problems (Craig *et al.*, 2002). Viewing samples by microscopy is complicated by the fact that organisms may be obscured by the size of particles, which can increase the working distance of the microscope objective lens, causing a distortion of the image. In addition, observing bacteria through a microscope does not mean that they are still active (McDaniel and Capone, 1985). Counting bacteria using culture techniques such as membrane filtration assumes that micro-organisms attached to sediments are randomly distributed across the surface of the media. However, enterococci commonly form chains of organisms that produce a single colony forming unit, despite being made up of several organisms. Thus, if the enterococci plate count is to reflect sanitary significance, enterococci cells should be separated from particulates and individual organisms should then be counted. Several techniques are available: manual shaking, with or without an 'abrasive' (glass beads); mechanical shaking (for example, using a 'wrist shaker'); sonication using an ultrasonic bath or ultrasonication probe; homogenisation (stomaching/blending); vortexing; and the use of chemical means, such as surfactants or oxidising agents. Diluents commonly used include peptone water, Ringer's solution, Camper's solution (Camper *et al.*, 1985) and maximum recovery diluent (Environment Agency, 2000). However, many of these methods have drawbacks and no 'standard methods' exist. For example: it is difficult to define a replicable manual shaking procedure whilst the use of 'abrasives' may physically damage organisms; sonication may result in an increase in temperature and/or physical damage to organisms; the presence of sediments may physically damage organisms during homogenisation through abrasion; it is difficult to identify suitable vortexing speeds to achieve separation of sediments, and bacteria and different chemical/biological agents have varying recovery rates.

Several studies have sought to compare different separation techniques in potable waters, municipal wastewaters, storm effluents and environmental samples as well as to optimise any one particular technique (Dale, 1974; McDaniel and Capone, 1985; Craig *et al.*, 2002; Borst and Selvakumar, 2003). However, the general consensus from these studies is that the degree of separation associated with any technique is largely dependent on the particle size distribution in the samples and the power outputs/frequencies of treatment methods. Thus, a range of treatments were assessed as a preliminary step in order to determine an optimum method for separating organisms from sediments, prior to starting the experimental programme proper.

## 3.2 Experimental methods

### 3.2.1 Sample site selection and collection

To maintain consistency with the $T_{90}$ experiments described in Section 2, samples for the association experiments were collected from the two inner estuarine locations of

Beachley Slip (three experiments) and Weston-Super-Mare (one experiment) (Table 2.2). Experiments were conducted using natural enterococci concentrations within each sample, as preliminary tests indicated that sufficient numbers were present. The three outer estuary sites of Langland Bay, Porthcawl and Minehead were excluded due to the low levels of suspended solids and background enterococci concentrations observed at these sites.

Inter-tidal cohesive sediment samples, for use in the experiments investigating the optimum method for separating enterococci from sediments (Section 3.2.1), were collected close to the waterline from the uppermost 1cm of sediment using a sterile spatula. The samples were then stored in sterile containers. Simultaneous samples of water adjacent to the sediment sample point were also collected in sterile containers.

Samples of estuarine water for the 'settlement' experiments (Section 3.2.3) were collected in steam-disinfected 10-litre containers. Both the sediment and water samples were kept cool and in the dark, and immediately despatched to the laboratory for analysis.

### 3.2.2 Separation of enterococci attached to sediments

To identify the optimum method for separating enterococci attached to sediments, four methods were applied over seven time intervals. These methods were: (i) stomaching (Seward Model No.3500, normal speed setting) (Plate 3.1); (ii) vortexing (Fisons Whirlimixer, max setting) (Plate 3.2); (iii) wrist shaking (Pall Gelman labs, Model No.4822, at 600 oscillations per minute) (Plate 3.1); and (iv) sonicating (Ultrawave Model No.M12140, operating Load 0.08kVa) (Plate 3.3). The time intervals tested were 30, 60, 120, 180, 300 and 600 seconds, whilst a control with no treatment was also included. All enterococci counts were conducted in triplicate to improve measurement precision.

The sediment, which was collected aseptically from the surface of intertidal areas at Beachley Slip and Weston-Super-Mare, was stirred thoroughly before 1g aliquots were aseptically measured into sterile 50ml centrifuge tubes (Sterilin). Then, 9ml of sterile maximum recovery diluent (MRD, Oxoid) was added to the sample, which was gently agitated to suspend the sediment. The samples were subjected to the various treatments described above, after which they were allowed to settle for five minutes before each aliquot was filtered through 0.45µm sterile, gridded 47mm cellulose ester membranes (Pall). Counts were determined as described in Section 2.2.4.

### 3.2.3 Association of enterococci with sediments

These experiments investigated the hypothesis that enterococci are associated with suspended solids and explored whether they attach specifically to particular particle size fractions. The experiments were based on a modified version of the pipette method for determining particle size through sedimentation. This method uses Stoke's Law to calculate settlement velocity, which is then used to infer particle size at specific depths during a period of settlement. The full protocol for determining particle size requires the use of dispersive agents and oxidation to remove the organic fraction. These steps might affect bacterial survival and were therefore not used. Davies and Bavor (2000)

applied a similar approach in investigating bacterial concentrations in settled bed sediments from a constructed wetland and a water pollution control pond system. However, to the best knowledge of the authors, this approach has not previously been applied to suspended particulates from an estuarine system.

Each experiment comprised two incubated microcosms containing 4.5-litre aliquots of the sample, one of which is allowed to settle whilst the other is continuously mixed to ensure complete suspension of the sediments (Plate 3.4). The results from the settled sample can then be compared with the mixed sample, which acts as a control. A maximum duration of six hours settlement was selected on the basis that estuarine tidal dynamics are unlikely to result in slack water for longer periods.

Each 10-litre sample was agitated to suspend all particles and two 4.5-litre aliquots were poured into sterile 5-litre beakers that were placed within an incubator at 15˚C. The temperature of the stirred samples was taken at the start of the experiment and was found to be 14 °C. The temperature of the samples did not therefore need to be equilibrated prior to starting the tests. The experimental start time for each pair of microcosms was staggered by 15 minutes, to allow for the time needed to take one aliquot (approximately two minutes). Using a sterile syringe and hypodermic needle, aliquots of 210ml were taken from each microcosm for bacterial analysis, at intervals and depths as defined in Table 3.1. The needle and syringe were sterilised between samples by immersing and flushing in boiling water. Sub-samples of the initial aliquots were retained to conduct analyses of suspended solids, turbidity and particle size. At the end of the experiment (six hours), the settled sediment from the unstirred microcosm was transferred to a sterile sample pot using a 10ml pipette.

Samples for enterococci counts were tested as described in Section 3.2.2 after being agitated using the wrist shaker for three minutes (this being the most reliable separation method (see Section 3.3.1 below)). One ml of the settled sediment collected from the unstirred microcosm was added to 9ml of sterile MRD and similarly treated before bacteriological analysis. Laser diffraction was used to determine the particle size of the suspended sediment samples and the final settled sediment. Turbidity and suspended solids concentration were determined as outlined in Section 2.2.5.

## 3.3 Results

### 3.3.1 Separation of enterococci attached to sediments

Enterococci concentrations in sediment samples from Beachley Slip and Weston-Super-Mare intertidal areas, subjected to the various separation treatments, are shown in Table 3.2 and Figure 3.1, together with data for the control samples (no treatment). Table 3.2 also shows the enterococci concentration of the estuarine water samples, which were taken simultaneously, adjacent to the sediment sample points.

The concentration of enterococci in the Beachley Slip inter-tidal sediment varied between the limit of detection (<3,333 cfu $100g^{-1}$) and 10,000 cfu $100g^{-1}$ (Figure 3.1a). The various treatments had little effect on the enterococci concentration and concentrations

were generally similar to the control sample (Table 3.2; Figure 3.1a). However, these data should be treated with caution because the results were derived from a maximum of three colonies on any one membrane. The estuarine water sample yielded an enterococci concentration of 144 cfu $100ml^{-1}$.

Enterococci concentrations in the inter-tidal sediment from Weston-Super-Mare were generally greater than at Beachley Slip, ranging between 10,000 cfu $100g^{-1}$ and 63,333 cfu $100g^{-1}$ (Figure 3.1b). The wrist-shaker consistently produced the greatest enterococci concentration at any given time-interval and was the only treatment method to exceed the concentration in the control sample for all treatment times (Table 3.2; Figure 3.1b). The results suggested that a mixing time of 180 to 600 seconds on the wrist shaker gave the most reliable results and a time of 180 seconds (three minutes) was used for further tests. The water sample taken simultaneously yielded a much lower concentration of 82 cfu $100ml^{-1}$ (Table 3.2).

### 3.3.2 Association of enterococci with sediments

The results from the four settlement experiments together with the corresponding control data are shown in Table 3.3 and Figure 3.2. Summary statistics from the particle size analysis are shown in Table 3.4, whist the particle size distribution of the suspended and settled sediments are summarised in Figure 3.3. For the Weston-Super-Mare and the first Beachley Slip experiments, 3 x 20ml aliquots were taken at each time interval for bacteriological testing, whilst a further 150ml aliquot was collected for particle size, suspended solids and turbidity analysis. Due to high enterococci counts in these initial experiments and concerns that the volume of sample extracted at each time step would affect the settlement times, for subsequent experiments 1 x 35ml aliquot from the stirred/unstirred microcosms was collected for bacterial analysis whilst 150ml aliquots for sediment analysis were taken at intervals described in Table 3.1.

The results from the four experiments all show the same general trend. Confirmed enterococci, turbidity and suspended solids concentrations in the stirred control samples varied little over the duration of the experiments (Table 3.3). Enterococci concentrations in the Weston-Super-Mare sample varied between 185 and 317 cfu $100ml^{-1}$ with an initial concentration of 243 cfu $100ml^{-1}$. Turbidity ranged between 77.5 NTU and 81.5 NTU, whilst suspended solids ranged between 192mg $l^{-1}$ and 245mg $l^{-1}$. The sample from Beachley Slip used for experiment one displayed an initial enterococci concentration of 185 cfu $100ml^{-1}$, which ranged between 163 cfu $100ml^{-1}$ and 222 cfu $100ml^{-1}$ during the experiment. Turbidity ranged between 136 NTU and 165 NTU and suspended solids between 415mg $l^{-1}$ and 485mg $l^{-1}$. Suspended solids (888–916mg $l^{-1}$ and 774–809mg $l^{-1}$) and turbidity (240–249 NTU and 180–230 NTU) in the remaining two samples from Beachley Slip were higher than in the first sample and this was accompanied by greater enterococci concentrations, which ranged between 213 cfu $100ml^{-1}$ and 470 cfu $100ml^{-1}$ in experiment two and between 243 cfu $100ml^{-1}$ and 307 cfu $100ml^{-1}$ in experiment three (Table 3.3; Figure 3.2a–d).

In the microcosms that were allowed to settle, after an initial period of relative stasis, enterococci, turbidity and suspended solids concentrations displayed noticeable decreases. In all experiments, enterococci levels decreased from concentrations similar

to the stirred control samples to concentrations of less than 100 cfu $100ml^{-1}$ (Table 3.3; Figure 3.2e-h). Enterococci and turbidity concentrations in the Weston-Super-Mare sample displayed significant decreases after 34.14 minutes (predicted particle diameter <7.8µm), whilst suspended solids decreased after 8.33 minutes (predicted particle diameter <15.6µm) (Figure 3.2e). The first experiment, using water taken from Beachley Slip, displayed a distinct drop in enterococci concentrations after 68.29 minutes (predicted particle diameter <3.9µm), although turbidity and suspended solids began to decrease after 8.33 minutes (predicted particle diameter <15.6µm) (Figure 3.2f). In the remaining two Beachley Slip experiments, enterococci concentrations decreased from the start of the experiments, although the initial decline during experiment two was slight (Figure 3.2g and h). However, in both experiments there was a marked break point in the decline in enterococci concentrations: at 2.08 minutes (predicted particle diameter <31.2µm) in experiment two and 8.33 minutes (predicted particle diameter <15.6µm) in experiment three. It is difficult to identify accurately any 'break point' in the decline of turbidity and suspended solids concentrations, due to the infrequency of the sampling for these parameters in the two experiments. However, the decrease was initiated between 2.08 minutes (predicted particle diameter <31.2µm) and 34.14 minutes (predicted particle diameter <7.8µm).

Concentrations of enterococci in the settled sediments sampled at the end of the experiments were all over an order of magnitude greater than the initial concentrations in the overlying water (Table 3.3), which is consistent with the findings of other authors (Section 3.1). Thus, it is clear that the decrease in enterococci associated with the decline in suspended sediment and turbidity, which was observed during the 'bench scale' settlement experiments, results in a concentration of enterococci in settled sediments. This indicates that the enterococci are attached to sediments rather than 'free-swimming'.

Ascertaining whether enterococci are preferentially attached to particles of a particular size is more difficult. The results of the particle size analysis indicate median particle size in the range 8–14µm in the Weston-Super-Mare sample and 5–10µm in the Beachley Slip samples (Table 3.3). Although there is a slight decrease in median particle size over time in Beachley Slip, experiments two and three, the median particle size remained fairly constant during Beachley Slip, experiment one, and increased during the Weston-Super-Mare experiment. The particle size data (Table 3.4a-d) also indicate that, contrary to the prediction of Stoke's Law (Table 3.1), the maximum particle size in suspension does not decrease considerably in the Weston-Super-Mare and Beachley Slip, experiment one, settled microcosms. Indeed, in some cases, the particle size is considerably greater than the maximum observed in the settled sediment (Table 3.4a and b). This may be caused by the fact that organic material could not be removed from the samples. However, experiments two and three, using Beachley Slip samples, did show an increase in the proportions of finer material over time and a shift away from the distribution of the settled sediment (Figure 3.3). The maximum particle size also dropped in the samples taken at and after 34.14 minutes – from greater than 75µm to between 30 and 40 µm (Table 3.4c and d). This is the same period during which the enterococci concentrations began to decrease significantly. The samples collected from the stirred control microcosms varied very little over the duration of the experiments and were similar to the settled sediment from the settlement experiments.

The deviation of settled particle size away from that predicted by Stoke's Law may be due to several factors. The pipette method for estimating particle size normally involves drying, removing organic material and using a chemical dispersant to reduce the potential for flocculation. However, since this process would destroy the enterococci organisms, it was not followed. Thus, the particle size distribution may be affected by the presence of organic material in suspension with the sediments, and biofilms on particles (including the presence of attached enterococci) are likely to affect their density and buoyancy. It is also possible that particles formed flocs that would increase particle size, although these would not settle in accordance with Stoke's Law.

# 4. Discussion

The results from the enterococci mortality microcosm experiments (Section 2.3) identified a range of enterococci $T_{90}$ values for irradiated conditions (between 3.7 hours and 113.9 hours) and in the dark (between 8.8 hours and infinity). In general, bacterial decay in the dark was slower than under irradiated conditions for each experiment, implicating radiation as a major driver of bacterial inactivation. There was a degree of variability in the results from each sampling site, both in terms of bacterial mortality and the physiochemical composition of the water.

$T_{90}$ values were lowest (bacterial inactivation was quicker) in experiments using water from the three outer marine sites of Langland Bay, Porthcawl and Minehead, than at the two more turbid estuarine sites of Beachley Slip and Weston-Super-Mare. This was the case for both irradiated conditions (range: 3.3 hours to 16.8 hours) and in the dark (range 8.8 hours to 73.8 hours). The three outer sites had salinities typical of open marine areas, whilst turbidity and suspended solids concentrations were low. Anova tests indicated similarities between the $T_{90}$ values, turbidity and salinity conditions at these three 'outer' sites. Combining the data from all microcosms for these sites produced $T_{90}$ values of between 6.6 hours and 8.8 hours under irradiated conditions and between 21.5 hours and 24.8 hours in the dark. In contrast, combining the data from the two estuarine sites (which displayed similar individual $T_{90}$ values and turbidity, but not salinity) produces an irradiated $T_{90}$ value of between 22.5 hours and 39.5 hours and a dark $T_{90}$ of between 35 hours and 66 hours. Interestingly, the marine dark $T_{90}$ values are shorter than the irradiated $T_{90}$ values in the turbid estuarine waters, which may be due to the enhanced salinity of the outer sites.

The significant relationships between the environmental variables described in Section 2.3.1.3 suggests that, in the context of the Severn estuary and the Bristol Channel, enterococci are more resistant to inactivation at lower salinities and at higher turbidity and suspended solids concentrations. In the case of the irradiated experiments, the association with turbidity and suspended solid concentrations is indicative of the particulate attenuation of light and the shading effects of particles. These particles might also offer attachment sites and provide protection from protozoan grazing (Mahler *et al.*, 2000; Alm *et al.*, 2003). The significant negative relationships of the dark $T_{90}$ values with salinity, conductivity and total dissolved solids indicates that the effects of salinity on bacterial inactivation are also present in the dark. Furthermore, the positive relationships of dark $T_{90}$ values with turbidity and suspended solids suggest that suspended sediments continue to offer a degree of protection from inactivation in the dark. In the case of the relationships developed between the dark $T_{90}$ vales and physiochemical parameters, including conductivity rather than turbidity as the principle predictor variable is plausible in that increased turbidity provides shading during the irradiated experiments. However, in the dark it is the effects of the chemical constituents of the water column that are the primary factors in inducing enterococci decay.

Turbidity was identified as the dominant positive predictor variable for $T_{90}$ values, such that higher turbidities resulted in longer $T_{90}$ values. Functions relating $T_{90}$ and turbidity were generated for irradiated and dark conditions for use in the hydrodynamic models of the Severn estuary developed as part of this research project. These functions

intersected at a point that was interpreted as the point at which turbidity (and/or suspended solids concentrations) absorbed irradiation to such an extent that dark conditions (with the same turbidity/suspended sediment concentration) were effectively replicated. This point was identified from the empirical data at a $T_{90}$ values between 41 hours and 52 hours, turbidity between 200 NTU and 207 NTU and suspended solid concentration of between 1544 mg $l^{-1}$ and 1739 mg $l^{-1}$. However, no identifiable decay occurred during some of the microcosm experiments at turbidity and suspended sediment concentrations below the above threshold limits at the two estuarine sites (Beachley Slip and Weston-Super-Mare). The low $r^2$ values for these regressions possibly indicate that variables such as salinity, which did not appear as a predictor variable, may be confounding the functional relationship. Thus, these functions can be used with some confidence in the deterministic hydrodynamic models for the Severn estuary. However, due to the limited empirical data, care should be taken the functions are utilised beyond this threshold. A cautious approach would be to utilise a constant $T_{90}$ value of between 41.4 hours and 52.6 hours for suspended sediment/turbidity concentrations beyond the threshold values. At turbidities above 200 NTU, approximately 99 per cent of the incident radiation is absorbed in the first centimetre of the optical path through the water column (Joyce *et al.*, 1996). This would support the implication from the empirical data that radiation effects are likely to be minimal above this turbidity threshold.

These results are broadly in line with those from other studies (Table 4.1 (irradiated) and Table 4.2 (dark), Figure 4.1). Studies undertaken by WRc in the UK (Gameson, 1986), which were carried out *in situ*, identified irradiated faecal streptococci $T_{90}$ values of between 2.6 hours and 2,420 hours from the results of five of their experiments, although the upper $T_{90}$ value here is essentially indicative of zero decay. Excluding this result, the range was between 2.6 hours and 23 hours, which encompass the ranges identified from the marine water experiments carried out in the current study. However, other experiments carried out by Gameson (1986) indicated no significant decay, or a even slight increase, in numbers over time. The results of Gameson (1986) were incorporated into the National Rivers Authority (now the Environment Agency) *Interim guidelines on the ranges of enteric organism decay rates ($T_{90}$s) under different marine conditions* (Gale, 1990) for daylight faecal streptococci $T_{90}$ values (Table 4.3).

The majority of studies in Table 4.1 (irradiated conditions) were undertaken using microcosms under natural sunlight. The range of $T_{90}$ values from the studies with seawater was between 1.3 hours and 12 hours, which is similar to the $T_{90}$ range for the three marine waters investigated in the current study. McCambridge and McMeekin (1981) reported a $T_{90}$ value of 16.3 hours for *Streptococcus faecium* using estuarine waters, which is towards the lower end of the range of $T_{90}$ values from the estuarine water experiments in the current study (14.2 hours to 113.9 hours). However, no details of factors such as turbidity and salinity, which are likely to influence $T_{90}$ values, were reported in McCambridge and McMeekin (1981). Freshwater $T_{90}$ values from other studies are also included in Table 4.1 for comparison, and although $T_{90}$ values similar to saline and brackish waters were observed by Sinton *et al.* (1999), those obtained by Duran *et al.* (2002) were considerably longer.

Dark $T_{90}$ values from other studies (Table 4.2) are much longer than those for irradiated conditions, ranging between 82.3 hours and 446 hours in seawater, 32.2 hours and 104 hours in estuarine water, and between 103 hours and 311 hours in freshwater. All

results for dark experiments involving seawater carried out in the current study (Langland Bay, Porthcawl and Minehead) are below the minimum seawater values quoted in Table 4.2. The NRA interim guidelines for $T_{90}$ values in seawater (Table 4.3; (Gale, 1990)) cite the research of Fujioka *et al.* (1981) for dark $T_{90}$ values in the range 36–183 hours. In contrast, the $T_{90}$ values from the estuarine sites in the dark were greater than that found by McCambridge and McMeekin (1981) (32.2 hours; Table 4.2). The study of Gabutti *et al.* (2000) was carried out using sterilised seawater, thus removing the effects of predation, which are likely to be more prominent in the absence of radiation-induced effects.

McCambridge and McMeekin (1981) investigated the impact of predacious microorganisms on faecal bacteria in estuarine waters in both dark and irradiated conditions. They found that bacterial decay was greater ($T_{90}$ times were shorter) in the presence of predacious organisms and irradiation, with the greatest decay being in the presence of both factors. Medema *et al.* (1997) conducted similar studies in the dark using freshwater and observed $T_{90}$ values over ten times greater in the absence of predacious microorganisms.

Of the studies in Table 4.1 and Table 4.2, only Alkan *et al.* (1995) considered the effect of factors such as temperature, turbidity and vertical mixing on enterococci mortality. These authors found that mortality rates did not alter between temperatures of 10˚C and 30˚C. However, they found that increased turbidity resulted in longer $T_{90}$ values, up to a threshold of 0.6 absorbance at 288nm,[1] after which it remained constant. Similarly, vertical mixing up to a rate of 1cm $s^{-1}$ was found to increase the mortality rate, thereafter decay was constant. In separate experiments, Alkan (1999) added kaolin clay to seawater containing sewage to determine the effect of particles on enteric bacterial concentrations. The rate of removal of enterococci from the mixed water column increased up to a suspended sediment concentration of 70mg $l^{-1}$ clay and shear rate of 22 $s^{-1}$, after which it decreased in line with both suspended sediment concentration and shear rate. The NRA interim guidelines for seawater $T_{90}$ values cite the results of an experiment undertaken by Irving (1977) for seawater with a suspended solids concentration of 600 mg $l^{-1}$, which gave an irradiated $T_{90}$ value of 43 hours. There were no directly comparable suspended solids concentrations from marine locations included within this study, although a sample from Weston-Super-Mare containing a suspended solids concentration of 536mg $l^{-1}$ produced a $T_{90}$ value of 14–15 hours. Using the empirical functions described in Section 2.3.1, which relate $T_{90}$ values to turbidity and suspended solids concentrations (Figure 2.5), an irradiated $T_{90}$ value of approximately 22 hours and a dark $T_{90}$ value of approximately 32 hours are obtained for a suspended solids concentration of 600 mg $l^{-1}$. The experiment of Irving (1977) was continued overnight and the resultant $T_{90}$ may be an overestimate of the actual $T_{90}$ if the experiment was performed under continuous light (Gale, 1990). However, longer irradiated $T_{90}$ values were obtained in the current study, which used estuarine waters with suspended solids concentrations lower than that used by Irving (1977), demonstrating the complex interaction between environmental variables and $T_{90}$ values.

Some studies (Davies-Colley *et al.*, 1994; Sinton *et al.*, 1994; Sinton *et al.*, 2002) also sought to identify the response of enterococci to irradiation at different wavelengths or wavebands and with depth. These indicated that UV-B contributed little to the overall

[1] turbidity measured as absorbance at 288nm is not easily compared to measurements made in NTU, as used in this and most other studies cited here, due to the effects of factors such as colour

inactivation of enterococci in effluent-seawater mixtures, with inactivation concentrated around two spectral bands (330nm (UV-A) and 400nm (short visible)). The difference between these results and published action spectra on the effect of wavelength on bacterial inactivation was attributed to the latter normally being obtained with pure cultures in buffer solutions rather than in effluent-seawater mixtures (Sinton *et al.*, 1994). However, it may also be related to the different levels of irradiance between UV-A and UV-B wavelengths. Inactivation of enterococci also decreased with increasing seawater depth, which corresponded with the attenuation of UV-A wavelengths (Davies-Colley *et al.*, 1994; Sinton *et al.*, 1994; Sinton *et al.*, 1999).

A summary of $S_{90}$ (sunlight inactivation of 90 per cent of organisms (W $m^{-2}$)) from various studies are presented in Table 4.4 for comparison with the $D_{90}$ data for total irradiation from the microcosm experiments carried out for this study (Table 2.14). The various studies of Sinton *et al.* (1994; 1999; 2002) produced similar $S_{90}$ values, which were within the range of values obtained by Gameson (1986). $S_{90}$ values estimated by Davies-Colley *et al.* (1994) were lower, although this may be related to the use of activated sludge-treated effluent rather than raw sewage as the source of faecal indicator organisms in the microcosms. The range of $D_{90}$ values for the marine water experiments from the current study (3.5–15.7 MW $m^{-2}$) were within the range of the $S_{90}$ values for seawater in Table 4.4. However, only one $D_{90}$ value from the experiments using water from the estuarine sites (13.3 MW $m^{-2}$) was similar to the estuarine $S_{90}$ values quoted by Sinton *et al.* (2002) (Table 4.4), the remaining values (18.7–106.7 MW $m^{-2}$) being greater. However, this may be due to the fact that the estuarine data presented by Sinton *et al.* (2002) were obtained from an artificial estuarine water, produced from a mixture of fresh and saline waters, which displayed lower turbidity and suspended solids concentrations than the water used for the microcosm experiments in this research project.

The association of enterococci with suspended sediments seen in the experiments detailed in Section 3, and also described by Dale (1974), Alkan (1999), Davies and Bavor (2000), Mahler *et al.* (2000) and Craig *et al.* (2002), has important implications for bathing water compliance within the Severn estuary/Bristol Channel. The periodicity of suspension, settlement and re-suspension will be influenced by wave generation, tidal density and residual currents, as well as by the impacts of meteorological events such as storm surges or high flow discharges from riverine and point inputs at a range of scales across the estuary. Thus, the prediction of sediment transport pathways, sources and sinks become an important tool for investigating bathing water compliance failures. High flow riverine and point source inputs close to the bathing water compliance points are likely to affect pollution levels through the increased delivery of faecal indicator organisms. Sediment transport and the re-suspension of transient sinks of settled sediment, resuspended during periods of increased flow velocities or wave action, may also have an impact.

The results of the enterococci settlement and association experiments indicated that the bacteria are associated with sediments with a median particle size of between 5 µm and 14µm. This is in broad accord with the results of Gannon *et al.* (1983) and Auer and Niehaus (1993), who found that 90 per cent of settled organisms were associated with particles of clay or silt (0.45µm to 10µm diameter) in the North American lakes. However, Davies and Bavor (2000) found that bacteria were almost exclusively associated with particles of less than 2µm in a water pollution control pond and a

constructed wetland. Given that transport velocities for such particles is low when compared to the velocities experienced in the Severn estuary, sediments with attached bacteria are likely to remain in suspension for almost the entire tidal cycle. This may be of particular importance in the context of rainfall-mobilising sediments in river channels and over the diurnal and spring-neap tidal cycles.

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# Tables

**Table 2.1: Irradiance for the different wavebands produced by the light rig used in the microcosm experiments**

| Waveband | Wavelength range (nm) | Irradiance (W $m^{-2}$) |
|---|---|---|
| Biologically weighted[1] UV-B | 280–320 | 0.32 |
| UV-B | 280–320 | 1.10 |
| UV-A | 320–400 | 5.20 |
| Total irradiance | 280–800 | 260.00 |

[1] weighted using the DNA damage action spectrum

**Table 2.2 Details of locations from which estuarine water samples were collected for use in microcosm experiments**

| Location | Comment | National Grid Reference |
|---|---|---|
| Langland Bay | Beach | SS 608 872 |
| Porthcawl | Harbour | SS 819 765 |
| Beachley Slip | Slipway | ST 553 908 |
| Weston-Super-Mare | Pier | ST 314 613 |
| Weston-Super-Mare | Knightstone slipway | ST 312 618 |
| Minehead | Terminus | SS 972 468 |

**Table 2.3: $T_{90}$ values (hours) for enterococci in irradiated and non-irradiated environmental waters estimated using unadjusted data (the table also shows the adjusted $r^2$ for the regression equation used to determine the $T_{90}$)**

| Location | | Experiment 1 | | Experiment 2 | | Experiment 3 | | Experiment 4 | |
|---|---|---|---|---|---|---|---|---|---|
| | | $T_{90}$ | $r^2$ | $T_{90}$ | $r^2$ | $T_{90}$ | $r^2$ | $T_{90}$ | $r^2$ |
| Langland Bay | Irradiated | 10.9 | 64.7 | 4.8 | 76.5 | 7.5 | 93.4 | 5.4 | 91.3 |
| | Dark | 73.8 | 20.1 | 19.9 | 71.8 | 20.3 | 85.2 | 18.7 | 85.5 |
| Porthcawl | Irradiated | 16.8 | 69.5 | 5.1 | 92.9 | 4.2 | 92.5 | 8.8 | 61.2 |
| | Dark | 25.8 | 81.1 | 34.6 | 83.9 | 31.2 | 71.6 | 30.8 | 80.5 |
| Beachley Slip | Irradiated | 27.0 | 74.4 | 54.2 | 51.0 | 44.1 | 36.5 | 32.0 | 76.0 |
| | Dark | 74.9 | 25.1 | n.s. | — | n.s. | — | n.s. | — |
| Weston-s-Mare | Irradiated | 113.9 | 17.4 | 46.5 | 27.2 | 26.6 | 89.2 | 14.2 | 86.8 |
| | Dark | n.s. | — | n.s. | — | 68.5 | 43.4 | 52.0 | 77.4 |
| Minehead | Irradiated | 5.0 | 67.8 | 8.2 | 93.4 | 12.8 | 64.8 | 3.7 | 96.1 |
| | Dark | 18.0 | 88.4 | 12.5 | 84.6 | 31.9 | 79.2 | 8.8 | 95.5 |

n.s. not significant – slope of regression line not significantly different to zero (no significant mortality)

**Table 2.4: $T_{90}$ values (hours) for enterococci in irradiated and non-irradiated environmental waters estimated using data adjusted to remove outliers and/or low concentration data[1] (the table also shows the adjusted $r^2$ for the regression equation used to determine the $T_{90}$)**

| Location | | Experiment 1 | | Experiment 2 | | Experiment 3 | | Experiment 4 | |
|---|---|---|---|---|---|---|---|---|---|
| | | $T_{90}$ | $r^2$ | $T_{90}$ | $r^2$ | $T_{90}$ | $r^2$ | $T_{90}$ | $r^2$ |
| Langland Bay | Irradiated | — | — | 5.7 | 76.9 | — | — | 6.5 | 96.0 |
| | Dark | — | — | — | — | — | — | 17.0 | 96.2 |
| Porthcawl | Irradiated | 9.2 | 71.2 | — | — | 4.5 | 94.5 | 12.8 | 68.2 |
| | Dark | — | — | — | — | — | — | — | — |
| Beachley Slip | Irradiated | 18.9 | 92.1 | — | — | — | — | — | — |
| | Dark | 35.2 | 70.4 | — | — | — | — | — | — |
| Weston-s-Mare | Irradiated | — | — | 20.0 | 86.2 | — | — | 15.5 | 92.3 |
| | Dark | — | — | — | — | — | — | — | — |
| Minehead | Irradiated | 4.2 | 75.6 | — | — | 8.7 | 78.6 | — | — |
| | Dark | — | — | 12.8 | 95.7 | — | — | — | — |

[1] Only relationships producing $r^2$ values greater than those for unadjusted data (see Table 2.3) are included – where more than one relationship was investigated, the relationship producing the greatest $r^2$ is shown

**Table 2.5: Estimated $T_{90}$ values (hours) for enterococci in irradiated and non-irradiated environmental waters using all data from experiments for each sampling location (the table also shows the adjusted $r^2$ for the regression equation used to determine the $T_{90}$)**

| Location | All Data | | | | Excluding outlying data | | | |
|---|---|---|---|---|---|---|---|---|
| | Irradiated | | Dark | | Irradiated | | Dark | |
| | $T_{90}$ | $r^2$ | $T_{90}$ | $r^2$ | $T_{90}$ | $r^2$ | $T_{90}$ | $r^2$ |
| Langland Bay | 6.8 | 63.8 | 24.9 | 57.5 | 7.5 | 72.2 | 18.5 | 80.8 |
| Porthcawl | 9.4 | 41.5 | 29.7 | 60.1 | 6.5 | 57.2 | 30.1 | 78.3 |
| Beachley Slip | 35.1 | 58.2 | n.s. | — | 40.1 | 56.3 | [1] | 7.9 |
| Weston-s-Mare | 18.7 | 57.2 | 54.6 | 62.7 | 21.6 | 60.7 | 54.6 | 62.7 |
| Minehead | 9.5 | 46.7 | 15.1 | 60.3 | 7.9 | 61.8 | 16.4 | 55.5 |

n.s. not significant – slope of regression line not significantly different to zero (no significant mortality)
[1] Positive regression slope indicating possible regrowth (see text)

**Table 2.6: Estimated $T_{90}$ values (hours) for enterococci in irradiated and non-irradiated environmental waters using all data from experiments grouped by 'marine' or 'estuarine' location[1] (the table also shows the adjusted $r^2$ for the regression equation used to determine the $T_{90}$)**

| Location* | All Data | | | | Excluding outlying data | | | |
|---|---|---|---|---|---|---|---|---|
| | Irradiated | | Dark | | Irradiated | | Dark | |
| | $T_{90}$ | $r^2$ | $T_{90}$ | $r^2$ | $T_{90}$ | $r^2$ | $T_{90}$ | $r^2$ |
| Marine | 8.8 | 48.8 | 21.5 | 50.6 | 6.6 | 57.6 | 24.8 | 71.6 |
| Estuarine | 22.5 | 53.7 | 66.2 | 33.6 | 39.5 | 47.4 | 65.1 | 36.7 |

[1] Marine = Langland Bay, Porthcawl and Minehead Estuarine = Beachley Slip and Weston-Super-Mare

**Table 2.7: $T_{90}$ values (hours) for enterococci attributable to the effects of radiation[1] ($T_{90}$ values quoted are adjusted using the data presented in Table 2.3 whilst those in brackets use data from Table 2.4 where relevant)**

| Location | Experiment 1 | Experiment 2 | Experiment 3 | Experiment 4 |
|---|---|---|---|---|
| Langland Bay | 12.9 | 6.4 (8.0) | 11.9 | 7.5 (10.6) |
| Porthcawl | 47.9 (14.3) | 5.9 | 4.9 (5.2) | 12.3 (21.9) |
| Beachley Slip | 42.2 (61.3) | 54.2[2] | 44.1[2] | 32.0[2] |
| Weston-s-Mare | 113.9[2] | 46.5 (20.0)[2] | 43.5 | 19.5 (22.1) |
| Minehead | 6.9 (5.5) | 23.5 (22.5) | 21.5 (12.0) | 6.6 |

[1] Note that the $T_{90}$ attributable to the effects of radiation is not just the bactericidal effect of radiation – it also includes factors such as shielding by particles, and increased chemical reactions and biological activity stimulated by radiation
[2] $T_{90}$ values not adjusted due to no mortality during dark experiments

**Table 2.8: Turbidity (NTU), suspended solids (mg $l^{-1}$), pH, conductivity (mS $cm^{-1}$), total dissolved solids (mg $l^{-1}$) and practical salinity for the $T_{90}$ experiments ($T_{90}$ values quoted for irradiated and dark conditions are those included in Table 2.3 whilst those in brackets are the adjusted values from Table 2.4 whilst those for radiation induced $T_{90}$ values are taken from Table 2.7)**

| Location | Experiment | Irradiated $T_{90}$ (hours) | Rad. induced $T_{90}$ (hours) | Dark $T_{90}$ (hours) | Turbidity (NTU) | Suspended solids (mg $l^{-1}$) | pH | Conductivity mS $cm^{-1}$ | TDS (mg $l^{-1}$) | Salinity |
|---|---|---|---|---|---|---|---|---|---|---|
| Langland Bay | 1 | 10.9 | 12.9 | 73.8 | 16 | — | 7.7 | 46.5 | 32550 | 38.8 |
| | 2 | 4.8 (5.7) | 6.4 (8.0) | 19.9 | 42 | 331.5 | 7.8 | 46.1 | 32270 | 38.4 |
| | 3 | 7.5 | 11.9 | 21.9 | 24 | 71.4 | 7.1 | 48.1 | 33670 | 40.3 |
| | 4 | 5.4 (6.5) | 7.5 (10.6) | 18.7 (17.0) | 38 | 109.8 | 7.8 | 45.7 | 31990 | 38.0 |
| Porthcawl | 1 | 16.8 (9.2) | 47.9 (14.3) | 25.8 | 14 | — | 7.7 | 46.2 | 32340 | 38.5 |
| | 2 | 5.1 | 5.9 | 34.6 | 29 | 88.1 | 7.8 | 45.1 | 31570 | 37.5 |
| | 3 | 4.2 (4.5) | 4.9 (5.2) | 31.2 | 25 | 76.7 | 7.7 | 43.7 | 30590 | 36.1 |
| | 4 | 8.8 (12.8 | 12.3 (21.9) | 30.8 | 32.5 | 78.1 | 7.2 | 42.7 | 29890 | 35.2 |
| Beachley Slip | 1 | 27.0 (18.9) | 42.2 (61.3) | 74.9 (35.2) | 180 | — | 7.7 | 14.9 | 10430 | 11.0 |
| | 2 | 54.2 | 54.2† | n.s. | 145 | 316.2 | 7.7 | 12.0 | 8400 | 8.7 |
| | 3 | 44.1 | 44.1† | n.s. | 245 | 1268.4 | 7.9 | 13.6 | 9520 | 10.0 |
| | 4 | 32.0 | 32.0† | n.s. | 145 | 261.7 | 7.3 | 8.5 | 5950 | 6.0 |
| Weston-s-Mare | 1 | 113.9 | 113.9† | n.s. | 220 | 2931.0 | 7.6 | 26.2 | 18340 | 20.4 |
| | 2 | 46.5 (20.0) | 46.5 (20.0)† | n.s. | 145 | 365.6 | 7.9 | 30.5 | 21350 | 24.2 |
| | 3 | 26.6 | 43.5 | 68.5 | 170 | 1372.9 | 7.8 | 29.5 | 20650 | 23.3 |
| | 4 | 14.2 (15.5) | 19.5 (22.1) | 52.0 | 120 | 536.3 | 7.4 | 27.5 | 19250 | 21.6 |
| Minehead | 1 | 5.0 (4.2) | 6.9 (5.5) | 18.0 | 25 | 156.4 | 7.8 | 44.8 | 31360 | 37.2 |
| | 2 | 8.2 | 23.5 (22.5) | 12.5 (12.8) | 90 | 241.3 | 7.9 | 40.5 | 28350 | 33.2 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 3 | 12.8 (8.7) | 21.5 (12.0) | 31.9 | 95 | 244.2 | 7.9 | 39.6 | 27720 | 32.4 |
| 4 | 3.7 | 6.6 | 8.8 | 14 | 74.1 | 7.4 | 43.2 | 30240 | 35.7 |

**Table 2.9: Results of the suspended sediment particle size analysis.**

| | | Particle Size (μm) | | % less than (by volume) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Location | Experiment | Mean | Median | 0.04μm | 2μm | 3.9μm | 7.8μm | 15.6μm | 53μm |
| Langland Bay | 1 | 835.90 | 472.70 | 0 | 1.2 | 2.2 | 5.4 | 12.1 | 18.8 |
| | 2 | 11.92 | 9.85 | <0.01 | 7.5 | 15.5 | 38.6 | 74.3 | 99.8 |
| | 3 | 15.41 | 12.42 | <0.01 | 5.3 | 11.1 | 28.9 | 62.1 | 98.7 |
| | 4 | 77.04 | 15.42 | <0.01 | 4.6 | 10.2 | 25.2 | 50.7 | 80.4 |
| Porthcawl | 1 | 0.30 | 0.24 | 0 | 3.6 | 6.7 | 15.1 | 35.8 | 80.9 |
| | 2 | 14.83 | 12.22 | 0 | 5.6 | 11.9 | 29.1 | 63.2 | 99.1 |
| | 3 | 14.80 | 12.69 | 0 | 5.6 | 11.2 | 27.9 | 61.5 | 100.0 |
| | 4 | 21.74 | 11.68 | <0.01 | 6.2 | 12.8 | 31.8 | 65.1 | 92.6 |
| Beachley Slip | 1 | 12.81 | 10.60 | <0.01 | 6.1 | 13.7 | 35.2 | 70.9 | 99.4 |
| | 2 | 10.90 | 9.33 | <0.01 | 7.9 | 16.9 | 41.1 | 77.2 | 100.0 |
| | 3 | 11.70 | 9.88 | <0.01 | 6.9 | 14.9 | 38.2 | 74.2 | 100.0 |
| | 4 | 10.65 | 8.67 | <0.01 | 8.9 | 19.6 | 44.9 | 79.2 | 99.9 |
| Weston-s-Mare | 1 | 15.48 | 12.28 | <0.01 | 5.9 | 11.9 | 30.0 | 61.8 | 99.1 |
| | 2 | 11.78 | 10.11 | <0.01 | 6.5 | 14.6 | 37.3 | 73.3 | 100.0 |
| | 3 | 12.65 | 10.19 | <0.01 | 6.7 | 14.7 | 37.1 | 72.0 | 99.8 |
| | 4 | 12.87 | 10.28 | <0.01 | 7.1 | 15.2 | 37.2 | 70.7 | 99.8 |
| Minehead | 1 | 15.85 | 11.86 | 0 | 5.9 | 12.0 | 30.5 | 64.6 | 96.3 |
| | 2 | 15.66 | 12.48 | <0.01 | 6.2 | 12.6 | 30.3 | 60.7 | 99.1 |
| | 3 | 17.04 | 11.96 | <0.01 | 6.2 | 13.1 | 32.0 | 62.1 | 95.0 |
| | 4 | 14.84 | 8.56 | 0 | 6.6 | 19.3 | 45.1 | 74.0 | 94.9 |

**Table 2.10: Results from Pearson product-moment correlation analysis for enterococci $T_{90}$ values and environmental parameters (only environmental parameters which display a statistically significant relationship with at least one of the $T_{90}$ data sets at the 95% level ($\alpha$=0.05) are shown)**

| Parameter | Coefficient[1] | $\log_{10}$ Light $T_{90}$ including outliers | $\log_{10}$ Light $T_{90}$ excluding outliers | $\log_{10}$ Irradiation effect $T_{90}$ including outliers | $\log_{10}$ Irradiation effect $T_{90}$ excluding outliers | $\log_{10}$ Dark $T_{90}$ including outliers | $\log_{10}$ Dark $T_{90}$ excluding outliers |
|---|---|---|---|---|---|---|---|
| Salinity | *r* | -0.790 | -0.806 | -0.699 | -0.774 | -0.575 | -0.383 |
| | *p* | <0.001 | <0.001 | 0.001 | <0.001 | 0.025 | n.s. |
| | *n* | 20 | 20 | 20 | 20 | 15 | 15 |
| Conductivity | *r* | -0.783 | -0.801 | -0.692 | -0.767 | -0.576 | -0.381 |
| | *p* | <0.001 | <0.001 | 0.001 | <0.001 | 0.024 | n.s. |
| | *n* | 20 | 20 | 20 | 20 | 15 | 15 |
| Total dissolved solids | *r* | -0.783 | -0.801 | -0.692 | -0.767 | -0.576 | -0.381 |
| | *p* | <0.001 | <0.001 | 0.001 | <0.001 | 0.024 | n.s. |
| | *n* | 20 | 20 | 20 | 20 | 15 | 15 |
| Turbidity | *r* | 0.864 | 0.869 | 0.801 | 0.860 | 0.547 | 0.408 |
| | *p* | <0.001 | <0.001 | <0.001 | <0.001 | 0.035 | n.s. |
| | *n* | 20 | 20 | 20 | 20 | 15 | 15 |
| $\text{Log}_{10}$ suspended solids | *r* | 0.799 | 0.799 | 0.814 | 0.793 | 0.551 | 0.558 |
| | *p* | <0.001 | <0.001 | <0.001 | <0.001 | n.s. | n.s. |
| | *n* | 17 | 17 | 17 | 17 | 12 | 12 |

[1] *r* – Pearson product-moment correlation coefficient
*p* – probability (two-tailed)
*n* – number of cases included in correlation
n.s. not significant

**Table 2.11: Results of the multivariate regression analysis investigating the relationships between water property parameters and irradiated/radiation induced $T_{90}$ values**

| Dependant variable | Variables entered in the regression equation | *a* | *b* | *u* | Adjusted $r^2$ (%) | *p* | Ryan-Joiner test for normality *p*[1] |
|---|---|---|---|---|---|---|---|
| $Log_{10}$ Irradiated $T_{90}$ (including outliers) | Turbidity | 0.0050 | 0.685 | 0.224 | 73.2 | <0.001 | n.s. |
| $Log_{10}$ Irradiated $T_{90}$ (excluding outliers) | Turbidity | 0.0047 | 0.677 | 0.207 | 74.1 | <0.001 | n.s. |
| $Log_{10}$ Radiation induced $T_{90}$ (including outliers) | Turbidity† | 0.0043 | 0.892 | 0.247 | 62.2 | <0.001 | n.s. |
| $Log_{10}$ Radiation induced $T_{90}$ (excluding outliers) | Turbidity | 0.0043 | 0.860 | 0.193 | 73.1 | <0.001 | >0.10 |
| Models excluding data with large standard residuals (see [2]) (NB NOT NOTED BY SPSS) | | | | | | | |
| $Log_{10}$ Radiation induced $T_{90}$ (including outliers) | Turbidity | 0.0049 | 0.819 | 0.173 | 81.5 | <0.001 | >0.10 |

*a* = slope (change in *y* per unit change in *x*)
*b* = intercept (*y* at *x* = 0)
*u* = stochastic disturbance or random error term

[1] Normality of standardised residuals tested using Ryan-Joiner test for normality. The hypothesis of normality is rejected if the $\alpha$-value is larger than the *p* value. Significance level $\alpha$=0.05 (95% confidence level). n.s. – not significant (residuals are not normally distributed)

[2] Models identified to contain data with large standard residuals (in these cases, the data producing the large standard residuals were omitted and the analysis repeated)

## Table 2.12: Results of the multivariate regression analysis investigating the relationships between water property parameters and dark $T_{90}$ values

| Dependant Variable | Variables entered in the regression equation | *a* | *b* | *u* | Adjusted $r^2$ (%) | *p* | Ryan-Joiner test for normality *p*[1] |
|---|---|---|---|---|---|---|---|
| $Log_{10}$ Dark $T_{90}$ (including outliers) | Conductivity | 0.012 | 1.877 | 0.236 | 28.1 | 0.024 | >0.10 |
| $Log_{10}$ Dark $T_{90}$ (excluding outliers) | No statistically significant models developed | | | | | | |
| Models forcing turbidity into the equations (see [2]) | | | | | | | |
| $Log_{10}$ Dark $T_{90}$ (including outliers) | Turbidity | 0.0020 | 1.276 | 0.242 | 24.6 | 0.035 | >0.10 |
| $Log_{10}$ Dark $T_{90}$ (excluding outliers) | No statistically significant models developed | | | | | | |

*a* = slope (change in *y* per unit change in *x*)
*b* = intercept (*y* at *x* = 0)
*u* = stochastic disturbance or random error term
[1] Normality of standardised residuals tested using Ryan-Joiner test for normality. The hypothesis of normality is rejected if the $\alpha$-value is larger than the *p* value. Significance level $\alpha$=0.05 (95% confidence level). n.s. – not significant (residuals are not normally distributed)
[2] The dynamic $T_{90}$ functions utilised in the hydrodynamic model of the Severn estuary developed as part of this project (Volume IV) required a relationship to be identified with turbidity. To achieve this, turbidity was forced into the regression equation

## Table 2.13: Results of the multivariate regression analysis investigating the relationships between water property parameters and dark $T_{90}$ values excluding the atypical result from Langland Bay experiment one

| Dependant variable | Variables entered in the regression equation | *a* | *b* | *u* | Adjusted $r^2$ (%) | *p* | Ryan-Joiner test for normality *p*[1] |
|---|---|---|---|---|---|---|---|
| $Log_{10}$ Dark $T_{90}$ (including outliers) | Salinity | -0.0165 | 1.897 | 0.189 | 48.9 | 0.00 | 0.06 |
| $Log_{10}$ Dark $T_{90}$ (excluding outliers) | Turbidity | 0.0019 | 1.237 | 0.199 | 29.5 | 0.02 | >0.10 |
| Model forcing turbidity into the equations (see [2]) | | | | | | | |
| $Log_{10}$ Dark $T_{90}$ (including outliers) | Turbidity | 0.0025 | 1.203 | 0.192 | 46.9 | 0.00 | n.s. |

*a* = slope (change in *y* per unit change in *x*)
*b* = intercept (*y* at *x* = 0)
*u* = stochastic disturbance or random error term
[1] Normality of standardised residuals tested using Ryan-Joiner test for normality. The hypothesis of normality is rejected if the $\alpha$-value is larger than the *p* value. Significance level $\alpha$=0.05 (95% confidence level). n.s. – not significant (residuals are not normally distributed)
[2] The dynamic $T_{90}$ functions utilised in the hydrodynamic model of the Severn estuary developed as part of this project (Volume IV) required a relationship to be identified with turbidity. To achieve this, turbidity was forced into the regression equation

**Table 2.14: Irradiated and radiation-induced (adjusted for mortality in the dark) total irradiation $D_{90}$ values (MW $m^{-2}$) for enterococci in environmental waters estimated using unadjusted data (data in brackets are adjusted to remove outliers and/or low concentration data)[1]**

| Location | | Experiment 1 | Experiment 2 | Experiment 3 | Experiment 4 |
|---|---|---|---|---|---|
| Langland Bay | Irradiated | 10.2 | 4.5 (5.4) | 7.0 | 5.0 (6.1) |
| | Rad. ind. | 12.0 | 5.9 (7.5) | 11.1 | 7.0 (9.9) |
| Porthcawl | Irradiated | 15.7 (8.6) | 4.7 | 3.9 (4.2) | 8.2 (12.0) |
| | Rad. ind. | 44.8 (13.4) | 5.5 | 4.5 (4.9) | 11.5 (20.5) |
| Beachley Slip | Irradiated | 25.3 (17.7) | 50.7 | 41.2 | 30.0 |
| | Rad. ind. | 39.5 (38.4) | 50.7[2] | 41.2[2] | 30.0[2] |
| Weston-s-Mare | Irradiated | 106.7 | 43.5 (18.7) | 24.9 | 13.3 (14.5) |
| | Rad. ind. | 106.7[2] | 43.5 (18.7)[2] | 40.7 | 18.3 (20.7) |
| Minehead | Irradiated | 4.7 (4.0) | 7.6 | 12.0 (8.2) | 3.5 |
| | Rad. ind. | 6.5 (5.2) | 22.0 (21.1) | 20.1 (11.2) | 6.1 |

[1] Only relationships producing $r^2$ values greater than those for unadjusted data are included. Where more than one relationship was investigated, the relationship producing the greatest $r^2$ is shown. $r^2$ values are the same as those presented in Table 2.3 (including outliers) and Table 2.4 (excluding outliers)

[2] $D_{90}$ values not adjusted due to no mortality during dark experiments

**Table 2.15: Irradiated and radiation-induced (adjusted for mortality in the dark) UV-A $D_{90}$ values (kW $m^{-2}$) for enterococci in environmental waters estimated using unadjusted data (data in brackets are adjusted to remove outliers and/or low concentration data)[1]**

| Location | | Experiment 1 | Experiment 2 | Experiment 3 | Experiment 4 |
|---|---|---|---|---|---|
| Langland Bay | Irradiated | 204.9 | 90.2 (107.1) | 140.0 | 100.5 (122.1) |
| | Rad. ind. | 240.6 | 119.0 (150.3) | 221.2 | 141.0 (198.0) |
| Porthcawl | Irradiated | 313.8 (172.5) | 94.6 | 78.7 (83.2) | 164.1 (239.6) |
| | Rad. ind. | 895.8 (268.3) | 110.8 | 90.9 (97.1) | 229.4 (409.6) |
| Beachley Slip | Irradiated | 505.3 (354.5) | 1013.9 | 824.6 | 599.4 |
| | Rad. ind. | 790.0 (767.8) | 1013.9[2] | 824.6[2] | 599.4[2] |
| Weston-s-Mare | Irradiated | 2133.16 | 869.9 (374.6) | 497.9 | 265.0 (290.8) |
| | Rad. ind. | 2133.16[2] | 869.9 (374.6)[2] | 813.9 | 365.9 (414.6) |
| Minehead | Irradiated | 93.5 (79.3) | 152.7 | 240.3 (163.2) | 70.2 |
| | Rad. ind. | 129.5 (103.8) | 440.2 (421.2) | 402.2 (224.7) | 122.7 |

[1] Only relationships producing $r^2$ values greater than those for unadjusted data are included. Where more than one relationship was investigated, the relationship producing the greatest $r^2$ is shown. $r^2$ values are the same as those presented in Table 2.3 (including outliers) and Table 2.4 (excluding outliers)

[2] $D_{90}$ values not adjusted due to no mortality during dark experiments

**Table 2.16: Irradiated and radiation-induced (adjusted for mortality in the dark) UV-B $D_{90}$ values (kW $m^{-2}$) for enterococci in environmental waters estimated using unadjusted data (data in brackets are adjusted to remove outliers and/or low concentration data)[1]**

| Location | | Experiment 1 | Experiment 2 | Experiment 3 | Experiment 4 |
|---|---|---|---|---|---|
| Langland Bay | Irradiated | 43.3 | 19.1 (22.7) | 29.6 | 21.3 (25.8) |
| | Rad. ind. | 50.9 | 25.2 (31.8) | 47.0 | 29.8 (41.9) |
| Porthcawl | Irradiated | 66.4 (36.5) | 20.0 | 16.6 (17.6) | 34.7 (50.7) |
| | Rad. ind. | 189.5 (56.8) | 23.4 | 19.2 (20.5) | 48.5 (86.6) |
| Beachley Slip | Irradiated | 106.9 (75.0) | 214.5 | 174.4 | 126.8 |
| | Rad. ind. | 167.1 (162.4) | 214.5[2] | 174.4[2] | 126.8[2] |
| Weston-s-Mare | Irradiated | 451.2 | 184.0 (79.2) | 105.3 | 56.3 (61.5) |
| | Rad. ind. | 451.2[2] | 184.0 (79.2)[2] | 172.2 | 77.4 (87.7) |
| Minehead | Irradiated | 19.8 (16.8) | 32.3 | 50.8 (34.5) | 14.8 |
| | Rad. ind. | 27.4 (22.0) | 93.1 (89.1) | 85.1 (47.5) | 25.9 |

[1] Only relationships producing $r^2$ values greater than those for unadjusted data are included. Where more than one relationship was investigated, the relationship producing the greatest $r^2$ is shown. $r^2$ values are the same as those presented in Table 2.3 (including outliers) and Table 2.4 (excluding outliers).

[2] $D_{90}$ values not adjusted due to no mortality during dark experiments

**Table 2.17: Irradiated and radiation-induced (adjusted for mortality in the dark) biologically-weighted[3] UV-B $D_{90}$ values (kW $m^{-2}$) for enterococci in environmental waters estimated using unadjusted data (data in brackets are adjusted to remove outliers and/or low concentration data)[1]**

| Location | | Experiment 1 | Experiment 2 | Experiment 3 | Experiment 4 |
|---|---|---|---|---|---|
| Langland Bay | Irradiated | 12.6 | 5.5 (6.6) | 8.6 | 6.2 (7.5) |
| | Rad. ind. | 14.8 | 7.3 (9.3) | 13.7 | 8.7 (12.2) |
| Porthcawl | Irradiated | 19.3 (10.6) | 5.8 | 4.8 (5.1) | 10.1 (14.7) |
| | Rad. ind. | 55.1 (16.5) | 6.8 | 5.6 (6.0) | 14.1 (25.2) |
| Beachley Slip | Irradiated | 31.1 (21.8) | 62.4 | 50.7 | 36.9 |
| | Rad. ind. | 48.6 (47.3) | 62.4[2] | 50.7[2] | 36.9[2] |
| Weston-s-Mare | Irradiated | 131.3 | 53.5 (23.1) | 30.6 | 16.4 (17.9) |
| | Rad. ind. | 131.3[2] | 53.5 (23.1)[2] | 50.1 | 22.5 (25.5) |
| Minehead | Irradiated | 5.8 (4.9) | 9.4 | 14.8 (10.0) | 4.3 |
| | Rad. ind. | 8.0 (6.4) | 27.1 (25.9) | 24.8 (13.8) | 7.5 |

[1] Only relationships producing $r^2$ values greater than those for unadjusted data are included. Where more than one relationship was investigated, the relationship producing the greatest $r^2$ is shown. $r^2$ values are the same as those presented in Table 2.3 (including outliers) and Table 2.4 (excluding outliers)

[2] $D_{90}$ values not adjusted due to no mortality during dark experiments

[3] Weighted using the DNA damage action spectrum

**Table 2.18: Estimated $D_{90}$ values for enterococci in irradiated environmental waters using all data from experiments for each sampling location and for marine/estuarine sites[1]**

| | All data $D_{90}$s | | | | Excluding outlying data $D_{90}$s | | | |
|---|---|---|---|---|---|---|---|---|
| Waveband | Total | UV-A | UV-B | Weighted UV-B | Total | UV-A | UV-B | Weighted UV-B |
| Location | (MW $m^{-2}$) | (kW $m^{-2}$) | (kW $m^{-2}$) | (kW $m^{-2}$) | (MW $m^{-2}$) | (kW $m^{-2}$) | (kW $m^{-2}$) | (kW $m^{-2}$) |
| Langland Bay | 6.4 | 127.6 | 27.0 | 7.8 | 7.0 | 139.8 | 29.6 | 8.6 |
| Porthcawl | 8.8 | 176.2 | 37.3 | 10.8 | 6.1 | 122.1 | 25.8 | 7.5 |
| Beachley Slip | 32.9 | 657.2 | 139.0 | 40.4 | 37.6 | 751.1 | 158.9 | 46.2 |
| Models excluding data with large standard residuals (see †) (NB NOT NOTED BY SPSS) | | | | | | | | |
| Weston-s-Mare | 17.5 | 350.0 | 74.0 | 21.5 | 20.2 | 403.4 | 85.3 | 24.8 |
| Minehead | 8.9 | 177.4 | 37.5 | 10.9 | 7.3 | 146.9 | 31.1 | 9.0 |
| Coastal[1] | 8.2 | 164.7 | 34.8 | 10.1 | 6.1 | 123.0 | 26.0 | 7.6 |
| Estuarine[1] | 21.1 | 421.6 | 89.2 | 25.9 | 37.0 | 739.2 | 156.4 | 45.5 |
| Threshold[2] | 49.2 | 984.7 | 208.3 | 60.6 | 38.8 | 775.0 | 163.9 | 47.7 |

n.s. not significant –slope of regression line not significantly different to zero (no significant mortality)

† positive regression slope indicating possible regrowth (see text).

[1] Coastal = Langland Bay, Porthcawl and Minehead Estuarine = Beachley Slip and Weston-Super-Mare

[2] Threshold – this is the estimated $D_{90}$ at the point where suspended solids concentration/turbidity is replicating dark conditions

**Table 2.19: Results of the multivariate regression analysis investigating the relationships between water property parameters and $D_{90}$ values**

| Dependant variable | Variables entered in the regression equation | Total Irradiation ($MW\ m^{-2}$) | UV-A ($kW\ m^{-2}$) | UV-B ($kW\ m^{-2}$) | Bio. weighted[1] UV-B ($kW\ m^{-2}$) |
|---|---|---|---|---|---|
| $Log_{10}$ Irradiated $D_{90}$ (including outliers) | Turbidity | 0.656 | 1.957 | 1.283 | 0.746 |
| $Log_{10}$ Irradiated $D_{90}$ (excluding outliers) | Turbidity | 0.648 | 1.949 | 1.75 | 0.738 |
| $Log_{10}$ Radiation-induced $D_{90}$ (including outliers) | Turbidity[2] | 0.862 | 2.163 | 1.489 | 0.952 |
| $Log_{10}$ Radiation-induced $D_{90}$ (excluding outliers) | Turbidity | 0.831 | 2.132 | 1.457 | 0.922 |
| Models excluding data with large standard residuals (see [2]) (NB NOT NOTED BY SPSS) | | | | | |
| $Log_{10}$ Radiation-induced $D_{90}$ (including outliers) | Turbidity | 0.790 | 2.091 | 1.416 | 0.880 |

[1] Weighted using the DNA damage action spectrum

[2] Models identified to contain data with large standard residuals (in these cases, the data producing the large standard residuals were omitted and the analysis repeated)

**Table 3.1: Pipette withdrawal times and depths calculated using Stoke's Law[3] and sampling protocol for the settlement experiments**

| Diameter finer than: | | Withdrawal depth | Elapsed time | Sample collected for:[1,2] | | | |
|---|---|---|---|---|---|---|---|
| Φ (phi) | µm | cm | | Unstirred | | Stirred | |
| All suspended sediments | | 20 | 0 | E | S | E | S |
| 5.0 (silt) | 31.2 | 10 | 2 min 8 sec | E | S | E | – |
| 6.0 (silt) | 15.6 | 10 | 8 min 33sec | E | – | E | – |
| 7.0 (silt) | 7.8 | 10 | 34min 14 sec | E | S | E | S |
| 8.0 (silt) | 3.9 | 5 | 1 hour 8min 29sec | E | – | E | – |
| 9.0 (clay) | 1.95 | 5 | 4 hour 33 min 59 sec | E | S | E | – |
| 9.2 | 1.70 | 5 | 6 hours | E | S | E | S |

[1] E = sample for enterococci enumeration S = sample for particle size analysis

[2] This protocol applies to Beachley Slip experiments 2 and 3 only – enterococci and sediment samples were collected at each time step from both beakers during Beachley Slip experiment one and Weston-Super-Mare experiment one

[3] Stoke's Law:

$$w = \frac{(\rho_s - \rho)g}{18\mu} d^2$$

where: $w$ = settling velocity (cm $s^{-1}$) $\rho_s$ = particle density (g $cm^{-3}$)
$\rho$ = water density (g $cm^{-3}$) $\mu$ = water viscosity (poise)
$g$ = acceleration due to gravity (cm $s^{-2}$) $d$ = equivalent spherical diameter (cm)

**Table 3.2: Results from the experiments conducted to investigate the optimum method for separating enterococci from Severn estuary sediments**

| | Confirmed enterococci concentration (cfu $100g^{-1}$) after treatment for specified time | | | | | | |
|---|---|---|---|---|---|---|---|
| Method | 30 sec | 60 sec | 90 sec | 120 sec | 150 sec | 300 sec | 600 sec |
| Beachley Slip | | | | | | | |
| Stomaching[1] | 3333 | 3333 | <3333 | 3333 | <3333 | 13333 | 6667 |
| Sonicating[2] | 3333 | 3333 | 6667 | 10000 | <3333 | <3333 | <3333 |
| Vortexing[3] | <3333 | 3333 | 3333 | 6667 | 6667 | 3333 | 6667 |
| Shaking[4] | 6667 | 13333 | 10000 | 6667 | <3333 | 3333 | 3333 |
| No treatment | 3333 | | | | | | |
| Water[5] | 144 cfu $100ml^{-1}$ | | | | | | |
| Weston-Super-Mare | | | | | | | |
| Stomaching[1] | 46667 | 40000 | 50000 | 20000 | 23333 | 26667 | 33333 |
| Sonicating[2] | 26667 | 36667 | 23333 | 20000 | 30000 | 13333 | 23333 |
| Vortexing[3] | 23333 | 16667 | 23333 | 23333 | 10000 | 53333 | 20000 |
| Shaking[4,6] | 53333 | 43333 | 63333 | 33333 | 56667 | 63333 | 56667 |
| No treatment | 20000 | | | | | | |
| Water[5] | 82 cfu $100ml^{-1}$ | | | | | | |

1 Stomaching: Seward Model No.3500, normal speed setting
2 Sonicating: Ultrawave Model No.M12140, operating load 0.08kVa
3 Vortexing: Fisons Whirlimixer, maximum setting
4 Shaking: Wrist shaker, Pall Gelman labs, Model No.4822, at 600 oscillations per minute
5 Enterococci concentration in estuarine water sample collected simultaneously adjacent to the sediment sample location
6 A further time step of 900 seconds was also tested; this yielded an enterococci concentration of 26667 cfu $100ml^{-1}$

## Table 3.3: Results of the experiments conducted into the association of enterococci with particles

| Time and predicted particle diameter finer than[1] | | Confirmed enterococci (cfu 100ml$^{-1}$) | | Median particle diameter (μm)[3] | | Turbidity (NTU) | | Suspended solids (mg l$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|
| (min:secs) | (μm) | Control | Settled | Control | Settled | Control | Settled | Control | Settled |
| Weston-Super-Mare | | | | | | | | | |
| 0 | 62.5 | 233 | 243 | 8.68 | 9.28 | 79.0 | 78.0 | 207.14 | 198.32 |
| 2:08 | 31.2 | 170 | 268 | 8.93 | 9.63 | 77.5 | 76.5 | 212.20 | 192.97 |
| 8:33 | 15.6 | 170 | 288 | 8.77 | 10.00 | 82.0 | 75.5 | 197.17 | 184.03 |
| 34:14 | 7.8 | 312 | 273 | 9.70 | 8.52 | 81.5 | 77.0 | 245.07 | 146.56 |
| 68:29 | 3.9 | 317 | 150 | 13.23 | 14.04 | 81.0 | 36.5 | 202.44 | 133.71 |
| 270:59 | 1.95 | 185 | 88 | 13.26 | 15.81 | 79.5 | 19.0 | 216.00 | 86.79 |
| 360:00 | 1.7 | 185 | 83 | 9.08 | 13.32 | 80.0 | 15.5 | 192.74 | 60.24 |
| Settled sediment[2] | | — | 10879 | — | 11.74 | — | — | — | — |
| Beachley Slip Experiment 1 | | | | | | | | | |
| 0 | 62.5 | 185 | 187 | 10.27 | 8.41 | 160.0 | 190.0 | 468.00 | 540.41 |
| 2:08 | 31.2 | 187 | 210 | 8.26 | 8.45 | 160.0 | 190.0 | 418.88 | 425.69 |
| 8:33 | 15.6 | 163 | 205 | 8.50 | 9.41 | 165.0 | 190.0 | 426.62 | 502.96 |
| 34:14 | 7.8 | 163 | 205 | 7.90 | 8.22 | 165.0 | 150.5 | 484.62 | 359.71 |
| 68:29 | 3.9 | 178 | 193 | 8.15 | 6.62 | 136.0 | 99.0 | 438.73 | 202.48 |
| 270:59 | 1.95 | 222 | 67 | 8.60 | 10.13 | 165.0 | 33.5 | 415.22 | 62.20 |
| 360:00 | 1.7 | 193 | 77 | 8.56 | 8.28 | 160.0 | 28.0 | 427.40 | 89.00 |
| Settled sediment[2] | | — | 11000 | — | 8.12 | — | — | — | — |
| Beachley Slip Experiment 2 | | | | | | | | | |
| 0 | 62.5 | 470 | 510 | 8.68 | 8.53 | 242.5 | 250.0 | 911.43 | 900.00 |
| 2:08 | 31.2 | 470 | 497 | — | 8.54 | — | 250.0 | — | 827.04 |
| 8:33 | 15.6 | 333 | 383 | — | — | — | — | — | — |
| 34:14 | 7.8 | 420 | 250 | 9.51 | 7.53 | 249.0 | 180.0 | 888.02 | 401.20 |
| 68:29 | 3.9 | 313 | 80 | — | — | — | — | — | — |
| 270:59 | 1.95 | 353 | 50 | — | 5.57 | — | 35.0 | — | 94.59 |
| 360:00 | 1.7 | 213 | 37 | 9.74 | 5.65 | 240.0 | 28.0 | 915.85 | 92.21 |
| Settled sediment[2] | | — | 16000 | — | 9.99 | — | — | — | — |
| Beachley Slip Experiment 3 | | | | | | | | | |
| 0 | 62.5 | 260 | 313 | 9.37 | 8.49 | 180.0 | 240.0 | 774.86 | 753.53 |
| 2:08 | 31.2 | 243 | 243 | — | 10.33 | — | 240.0 | — | 761.82 |
| 8:33 | 15.6 | 307 | 220 | — | — | — | — | — | — |
| 34:14 | 7.8 | 293 | 127 | 8.75 | 7.62 | 230.0 | 180.0 | 809.41 | 348.41 |
| 68:29 | 3.9 | 283 | 77 | — | — | — | — | — | — |
| 270:59 | 1.95 | 257 | 43 | — | 5.97 | — | 36.0 | — | 60.48 |
| 360:00 | 1.7 | 257 | 40 | 8.39 | 4.95 | 230.0 | 27.0 | 781.70 | 76.19 |
| Settled sediment[2] | | — | 33333 | — | 9.41 | — | — | — | — |

[1] Particle diameter finer than (μm) is that predicted using Stoke's Law (see Table 3.1)
[2] Settled sediment: sediment which had settled on the bottom of the beaker after six hours
[3] Further details of the particle size analysis are provided in Table 3.4 and Figure 3.3

**Table 3.4a: Summary statistics from the particle size analysis for the stirred control and settled microcosms (µm) – Weston-Super-Mare**

| | Time (mins:secs) 0 | 2:08 | 8:33 | 34:14 | 68:29 | 270:59 | 360:00 | Settled Sediment[2] |
|---|---|---|---|---|---|---|---|---|
| Stirred control | | | | | | | | |
| Mean | 9.81 | 12.40 | 10.27 | 11.02 | 14.92 | 13.40 | 11.22 | — |
| Median | 8.68 | 8.93 | 8.77 | 9.24 | 13.23 | 10.81 | 9.08 | — |
| Mode | 10.29 | 10.29 | 10.29 | 11.29 | 16.40 | 13.61 | 10.29 | — |
| Upper 95% CL[1] | 21.85 | 40.32 | 24.11 | 27.07 | 34.10 | 35.28 | 28.57 | — |
| Std deviation | 6.14 | 14.24 | 7.06 | 8.19 | 9.79 | 11.16 | 8.86 | — |
| Maximum | 39.76 | 69.62 | 52.60 | 57.77 | 63.41 | 69.62 | 69.61 | — |
| Settled | | | | | | | | |
| Mean | 10.75 | 12.88 | 11.75 | 10.11 | 15.77 | 16.12 | 14.57 | 13.43 |
| Median | 9.28 | 9.63 | 9.13 | 8.52 | 14.04 | 15.81 | 12.48 | 11.74 |
| Mode | 11.29 | 11.29 | 10.29 | 10.29 | 18.00 | 28.69 | 16.40 | 14.94 |
| Upper 95% CL[1] | 25.10 | 38.43 | 32.05 | 25.63 | 36.32 | 33.93 | 34.68 | 30.92 |
| Std deviation | 7.32 | 13.04 | 10.36 | 7.92 | 10.49 | 9.09 | 10.26 | 8.93 |
| Maximum | 57.56 | 146.80 | 69.62 | 131.70 | 76.42 | 57.77 | 63.41 | 57.77 |

[1] Upper 95% confidence limit; the lower 95% confidence limit was zero in all cases
[2] Settled sediment: sediment which had settled on the bottom of the beaker after six hours

**Table 3.4b: Summary statistics from the particle size analysis for the stirred control and settled microcosms (µm) – Beachley Slip, experiment 1.**

| | Time (mins:secs) 0 | 2:08 | 8:33 | 34:14 | 68:29 | 270:59 | 360:00 | Settled Sediment[2] |
|---|---|---|---|---|---|---|---|---|
| Stirred control | | | | | | | | |
| Mean | 12.46 | 10.43 | 10.69 | 9.44 | 10.29 | 11.12 | 10.73 | — |
| Median | 9.74 | 8.26 | 8.50 | 7.90 | 8.15 | 8.60 | 8.56 | — |
| Mode | 11.29 | 10.29 | 10.29 | 9.37 | 9.37 | 10.29 | 10.29 | — |
| Upper 95% CL[1] | 34.04 | 28.43 | 28.24 | 22.79 | 27.38 | 32.41 | 28.38 | — |
| Std deviation | 11.01 | 9.19 | 8.95 | 6.81 | 8.72 | 10.86 | 9.00 | — |
| Maximum | 76.43 | 161.00 | 83.53 | 57.75 | 76.41 | 76.43 | 76.42 | — |
| Settled | | | | | | | | |
| Mean | 10.52 | 10.28 | 12.77 | 10.00 | 7.45 | 10.74 | 11.32 | 9.89 |
| Median | 8.41 | 8.45 | 9.41 | 8.22 | 6.62 | 10.13 | 8.28 | 8.12 |
| Mode | 10.29 | 10.29 | 11.29 | 10.29 | 8.54 | 21.69 | 7.78 | 9.37 |
| Upper 95% CL[1] | 27.73 | 25.88 | 41.71 | 26.34 | 16.82 | 23.90 | 32.26 | 24.92 |
| Std deviation | 8.78 | 7.96 | 14.76 | 8.34 | 4.78 | 6.71 | 10.69 | 7.67 |
| Maximum | 92.03 | 63.40 | 213.10 | 92.03 | 47.86 | 57.77 | 63.41 | 82.26 |

[1] Upper 95% confidence limit; the lower 95% confidence limit was zero in all cases
[2] Settled sediment: sediment which had settled on the bottom of the beaker after six hours

**Table 3.4c: Summary statistics from the particle size analysis for the stirred control and settled microcosms (µm); Beachley Slip experiment 2.**

| | Time (mins:secs) 0 | 2:08 | 34:14 | 270:59 | 360:00 | Settled Sediment† |
|---|---|---|---|---|---|---|
| Stirred Control | | | | | | |
| Mean | 11.30 | — | 11.66 | — | 14.64 | — |
| Median | 8.68 | — | 9.51 | — | 9.74 | — |
| Mode | 10.29 | — | 11.29 | — | 10.29 | — |
| Upper 95% CL* | 30.31 | — | 29.19 | — | 49.57 | — |
| Std Deviation | 9.70 | — | 8.95 | — | 17.82 | — |
| Maximum | 76.42 | — | 69.59 | — | 194.10 | — |
| Settled | | | | | | |
| Mean | 11.07 | 10.77 | 8.35 | 6.42 | 7.24 | 11.99 |
| Median | 8.53 | 8.54 | 7.53 | 5.57 | 5.65 | 9.99 |
| Mode | 9.37 | 9.68 | 9.37 | 6.45 | 6.45 | 11.29 |
| Upper 95% CL* | 29.45 | 28.43 | 18.35 | 15.10 | 18.97 | 29.23 |
| Std Deviation | 9.38 | 9.01 | 5.10 | 4.43 | 5.98 | 8.80 |
| Maximum | 76.40 | 77.91 | 36.22 | 33.00 | 36.24 | 63.40 |

* Upper 95% confidence limit. The lower 95% confidence limit was 0 in all cases.
† Settled Sediment: sediment which had settled on the bottom of the beaker after 6 hours.

**Table 3.4d: Summary statistics from the particle size analysis for the stirred control and settled microcosms (µm); Beachley Slip experiment 3.**

| | Time (mins:secs) 0 | 2:08 | 34:14 | 270:59 | 360:00 | Settled Sediment† |
|---|---|---|---|---|---|---|
| Stirred Control | | | | | | |
| Mean | 11.54 | — | 11.14 | — | 10.38 | — |
| Median | 9.37 | — | 8.75 | — | 8.39 | — |
| Mode | 11.29 | — | 10.29 | — | 10.29 | — |
| Upper 95% CL* | 29.09 | — | 29.24 | — | 26.37 | — |
| Std Deviation | 8.95 | — | 9.24 | — | 8.16 | — |
| Maximum | 69.56 | — | 76.42 | — | 63.40 | — |
| Settled | | | | | | |
| Mean | 10.69 | 12.95 | 8.26 | 7.10 | 5.66 | 10.89 |
| Median | 8.49 | 10.33 | 7.62 | 5.97 | 4.95 | 9.41 |
| Mode | 10.29 | 12.40 | 9.37 | 7.08 | 5.88 | 11.29 |
| Upper 95% CL* | 27.94 | 33.53 | 17.69 | 17.33 | 12.96 | 25.39 |
| Std Deviation | 8.80 | 10.50 | 4.81 | 5.22 | 3.73 | 7.40 |
| Maximum | 92.03 | 83.87 | 30.06 | 36.24 | 39.69 | 52.62 |

* Upper 95% confidence limit. The lower 95% confidence limit was 0 in all cases.
† Settled Sediment: sediment which had settled on the bottom of the beaker after 6 hours.

**Table 4.1: Summary of irradiated $T_{90}$ values for enterococci[1] obtained from other studies**

| Reference | $T_{90}$ (hours)* | Salinity | Irradiation (W $m^{-2}$) | Temperature (˚C) | Bacterial source | Study type & location[2] | Notes |
|---|---|---|---|---|---|---|---|
| (Alkan *et al.*, 1995) | 0.5–9.8 | Seawater[4] | Artificial 100–900 | 10–30˚C | Synthetic sewage 0.25–3.25% v/v | Microcosm UK | Microcosms seeded at different concentrations of synthetic sewage and sediment (kaolin) Turbidity =0.044–0.0864 absorbance at 288nm |
| (Davies-Colley *et al.*, 1994) | 1.3[3] | Seawater[4] | Natural | summer | AS-treated sewage 2% v/v | Microcosm NZ | $T_{90}$ values quoted for average midday radiation (1.2 kW $m^{-2}$) Bacterial source – activated sludge-treated sewage used |
| (Gameson, 1986) | 2.6–23 (see notes) | Seawater | Natural | | Sewage outfalls | *In-situ* UK | Faecal streptococci. Seven experiments reported – two resulted in no mortality and one in a $T_{90}$ value of 2420 hours |
| (Sinton *et al.*, 1994) | 6.4[3]<br>12[3] | Seawater | Natural | 12.5–17.2<br>9.1 | Raw sewage 2% v/v | Microcosm NZ | Summer – total insolation: 25 MJ $m^{-2}$<br>Winter – total insolation: <4 MJ $m^{-2}$ |
| (Sinton *et al.*, 1999) | 6.9[3] | Seawater | Natural | 19 | Raw sewage 2% v/v | Microcosm NZ | Total insolation: 30 MJ $m^{-2}$ |
| (McCambridge and McMeekin, 1981) | 16.3 | Estuarine | Natural | 22 | Laboratory cultures | Microcosm Australia | *Streptococcus faecium* |
| (Duran *et al.*, 2002) | 88<br>303 | Freshwater | Natural | 13.4–26.0<br>4.7–10.0 | Raw sewage 2% v/v | *In-situ* Spain | Summer – total insolation: 17.7–25.9 MJ $m^{-2}$ Turbidity: 11.7 – 20.7 NTU<br>Winter - total insolation: 7.5–8.6 MJ $m^{-2}$, Turbidity: 6.7–35.0 NTU |
| (Sinton *et al.*, 2002) | 6.4[3]<br>12.6[3] | Freshwater | Natural | 16<br>12 | Raw sewage 3% v/v | Microcosm NZ | Summer – total insolation: 25 MJ $m^{-2}$,<br>Winter – total insolation: 14 MJ $m^{-2}$ |

1 $T_{90}$ is for enterococci unless otherwise stated in the notes column
2 Location refers to where samples were collected in the case of microcosm experiments (NZ = New Zealand).
3 Corrected for mortality in the dark
4 Sterilised or subjected to filtering at 0.22µm pore size prior to addition of faecal indicator organisms

**Table 4.2: Summary of dark $T_{90}$ values for enterococci[1] obtained from other studies.**

| Reference | $T_{90}$ (hours)[1] | Salinity | Irradiation (W $m^{-2}$) | Temperature (˚C) | Bacterial source | Study Type & Location[2] | Notes |
|---|---|---|---|---|---|---|---|
| (Gabutti *et al.*, 2000) | 88 | Seawater[3] 35 ‰ | Dark | 22 | Raw Sewage $10^6$ cfu $ml^{-1}$ | Microcosm Italy | Faecal streptococci |
| (Sinton *et al.*, 1994) | 82.3<br>115 | Seawater | Dark | 12.5 - 17.2<br>9.1 | Raw sewage 2% v/v | Microcosm NZ | Summer<br>Winter |
| (Sinton *et al.*, 1999) | 446 | Seawater | Dark | 19 | Raw sewage 2% v/v | Microcosm NZ | |
| (Gabutti *et al.*, 2000) | 104 | Estuarine[3] 27‰ | Dark | 22 | Raw Sewage $10^6$ cfu $ml^{-1}$ | Microcosm Italy | Faecal streptococci |
| (McCambridge and McMeekin, 1981) | 32.2 | Estuarine | Dark | 22 | Laboratory cultures | Microcosm Australia | *Streptococcus faecium* |
| (Medema *et al.*, 1997) | 103<br>311 | Freshwater | Dark | 15<br>5 | Primary Sewage | Microcosm Netherlands | |
| | 1714<br>4800 | Freshwater[3] | | 15<br>5 | | | |
| (Sinton *et al.*, 2002) | 192 | Freshwater | Dark | 14 | Raw sewage 3% v/v | Microcosm NZ | |

[1] $T_{90}$ is for enterococci unless otherwise stated in the notes column
[2] Location refers to where samples were collected in the case of microcosm experiments (NZ = New Zealand).
[3] Sterilised or subjected to filtering at 0.22µm pore size prior to addition of faecal indicator organisms.

**Table 4.3: Interim guidelines for range of faecal streptococci $T_{90}$ values (hours) under differing marine water conditions (Gale, 1990)**

| Daylight | Dark or no sunlight | Seawater with suspended solids (600mg $l^{-1}$) |
|---|---|---|
| 2.6– 23.0[1] | 36–183[2] | 43[3] |

[1] *In situ* experiments performed by Gameson (1986); ome experiments demonstrated either negligible mortality ($T_{90}$ = 2420 hours) or even growth

[2] *In vitro* experiments performed at 24˚C (Fujioka *et al.*, 1981) in the absence of sunlight but under overhead fluorescent lighting for 10–12h $day^{-1}$ and in complete darkness at 25˚C

[3] $T_{90}$ value determined from paddling pool experiment performed outside (Irving, 1977). Since the $T_{90}$ value is longer than the day length, the experiment was continued overnight, during which little mortality occurs. Thus, the $T_{90}$ presented is longer than if the experiments were performed under continuous light

**Table 4.4: Summary of irradiated $S_{90}$ values for enterococci[1] obtained from other studies**

| Reference | $S_{90}$ (MW $m^{-2}$) | Salinity | Irradiation (W $m^{-2}$) | Temperature (˚C) | Bacterial source | Study type & location[2] | Notes |
|---|---|---|---|---|---|---|---|
| (Davies-Colley *et al.*, 1994) | 3.0–7.3 | Seawater[4] | Natural | summer | AS-treated sewage 2% v/v | Microcosm & *in situ* NZ | Bacterial source – activated sludge-treated sewage used<br>Experiments in microcosms produced lower $S_{90}$ values than *in situ* experiments |
| (Gameson, 1986) | 4.7–28.8 (see notes) | Seawater | Natural | | Sewage outfalls | *In-situ* UK | Faecal streptococci. Seven experiments reported – two resulted in no mortality and one in a $D_{90}$ of 154.8 MW $m^{-2}$ |
| (Sinton *et al.*, 1994) | 11.8[3]<br>6.0[3]<br>10.7[3] | Seawater | Natural | 12.5–17.2<br>9.1<br>9.1–17.2 | Raw sewage 2% v/v | Microcosm NZ | Summer – total insolation: 25 MW $m^{-2}$<br>Winter – total insolation: <4 MW $m^{-2}$<br>All data |
| (Sinton *et al.*, 1999) | 15.8[3] | Seawater | Natural | 14-20 | Raw sewage 2% v/v | Microcosm NZ | Total insolation: 20-35 MW $m^{-2}$ |
| (Sinton *et al.*, 2002) | 11.69[3]<br>6.81[3] | Seawater | Natural | summer | Raw sewage 3% v/v | Microcosm NZ | Heavy cloud cover<br>Clear Skies |
| (Sinton *et al.*, 2002) | 14.9[3]<br>11.0[3] | Estuarine (see notes) | Natural | summer | Raw sewage 3% v/v | Microcosm NZ | Heavy cloud cover<br>Clear skies<br>'Estuarine' water comprised 50:50 mix of seawater and freshwater |
| (Sinton *et al.*, 2002) | 16.8[3]<br>17.6[3]<br>12.2[3]<br>16.7[3] | Freshwater | Natural | 16<br><br>12 | Raw sewage 3% v/v | Microcosm NZ | Summer Mean – total insolation: 25 MW $m^{-2}$<br>Summer – heavy cloud<br>Summer – clear skies<br>Winter Mean – total insolation: 14 MW $m^{-2}$ |

1 $S_{90}$ is for enterococci unless otherwise stated in the notes column
2 Location refers to where samples were collected in the case of microcosm experiments (NZ = New Zealand)
3 Corrected for mortality in the dark
4 Sterilised or subjected to filtering at 0.22µm pore size prior to addition of faecal indicator organisms

# Figures

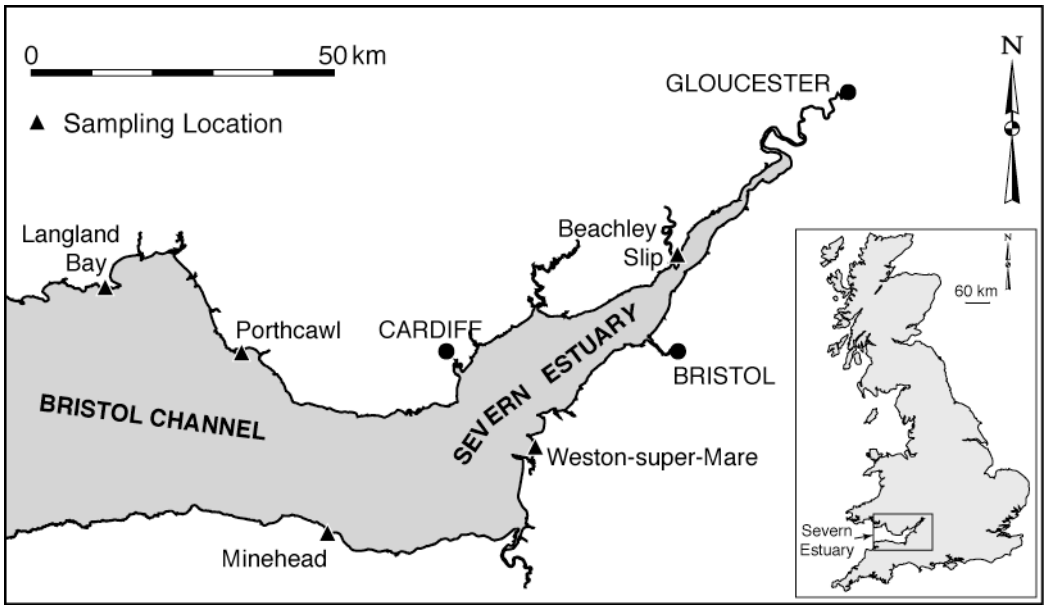



**Figure 2.1: Sampling locations in the Severn estuary and Bristol Channel.**

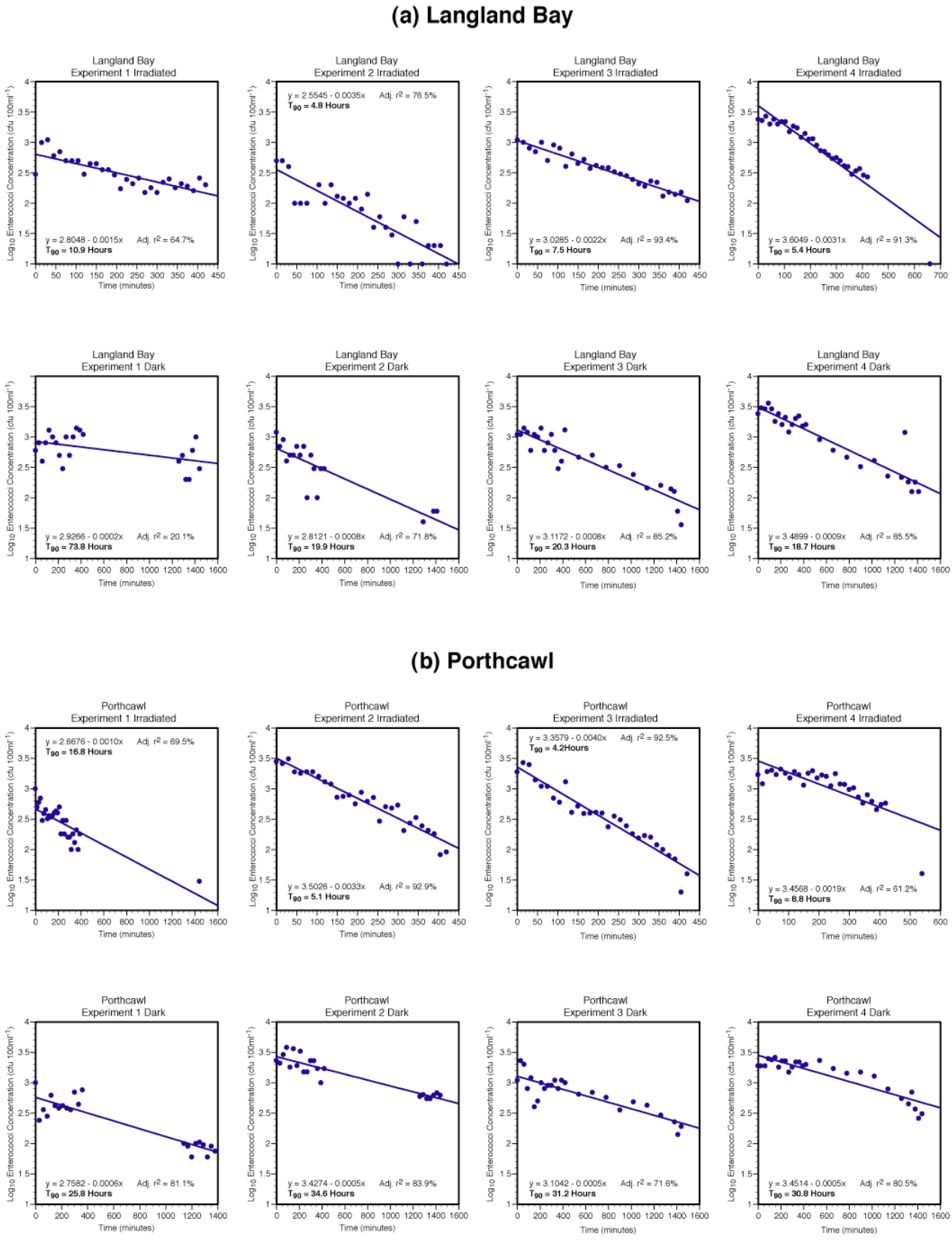
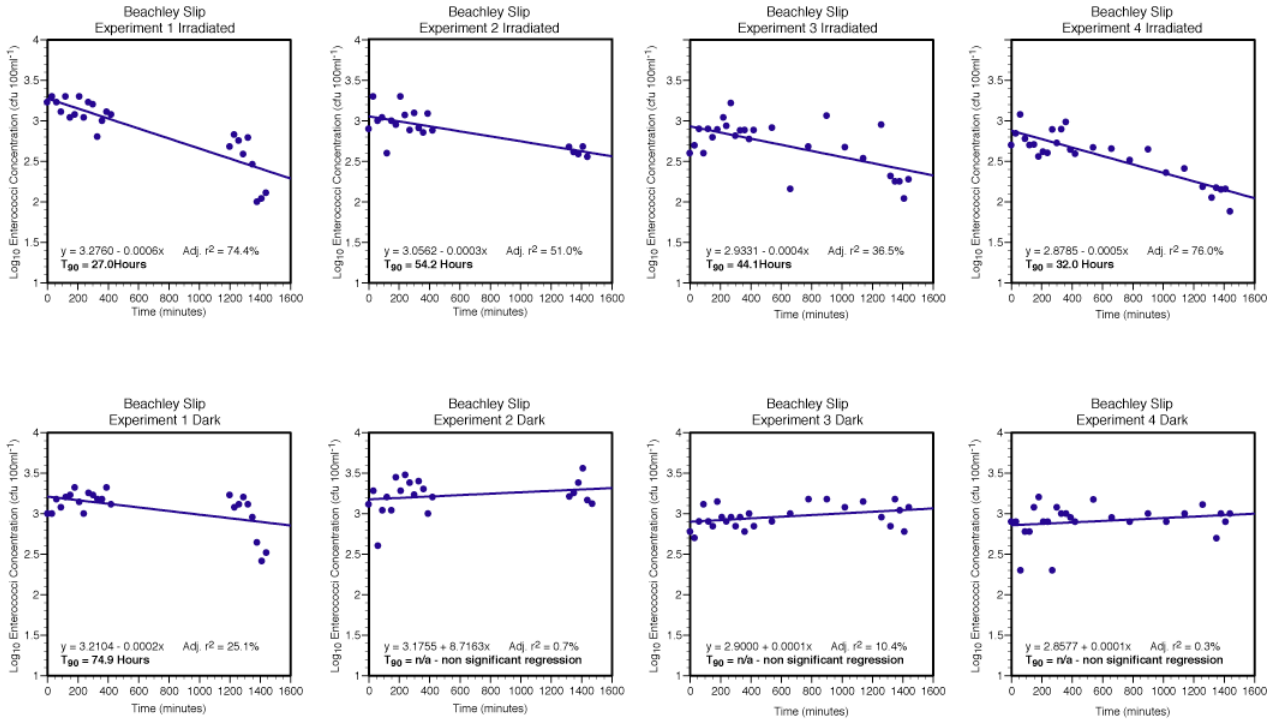



**Figure 2.2a & b: Regression analysis of unadjusted microcosm $T_{90}$ experiment results using water taken from (a) Langland Bay and (b) Porthcawl**

## (c) Beachley Slip




## (d) Weston-Super-Mare

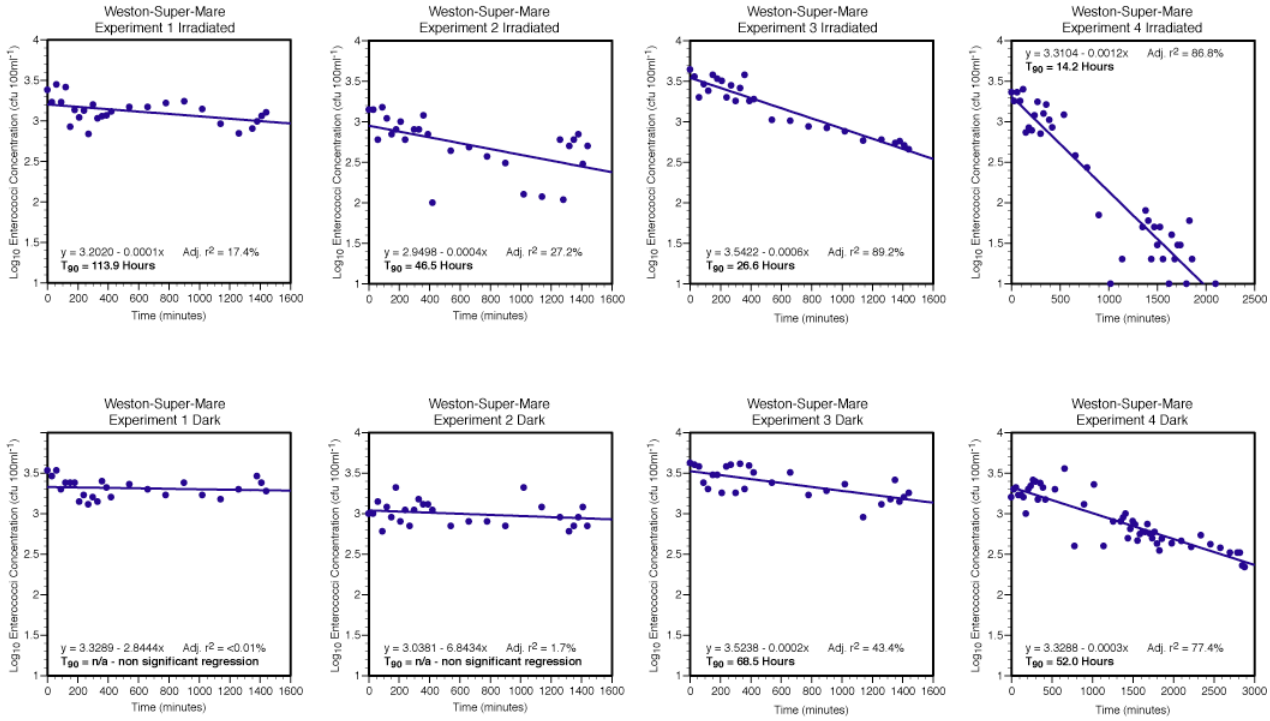



**Figure 2.1c & d: Regression analysis of unadjusted microcosm $T_{90}$ experiment results using water taken from (c) Beachley Slip and (d) Weston-Super-Mare**

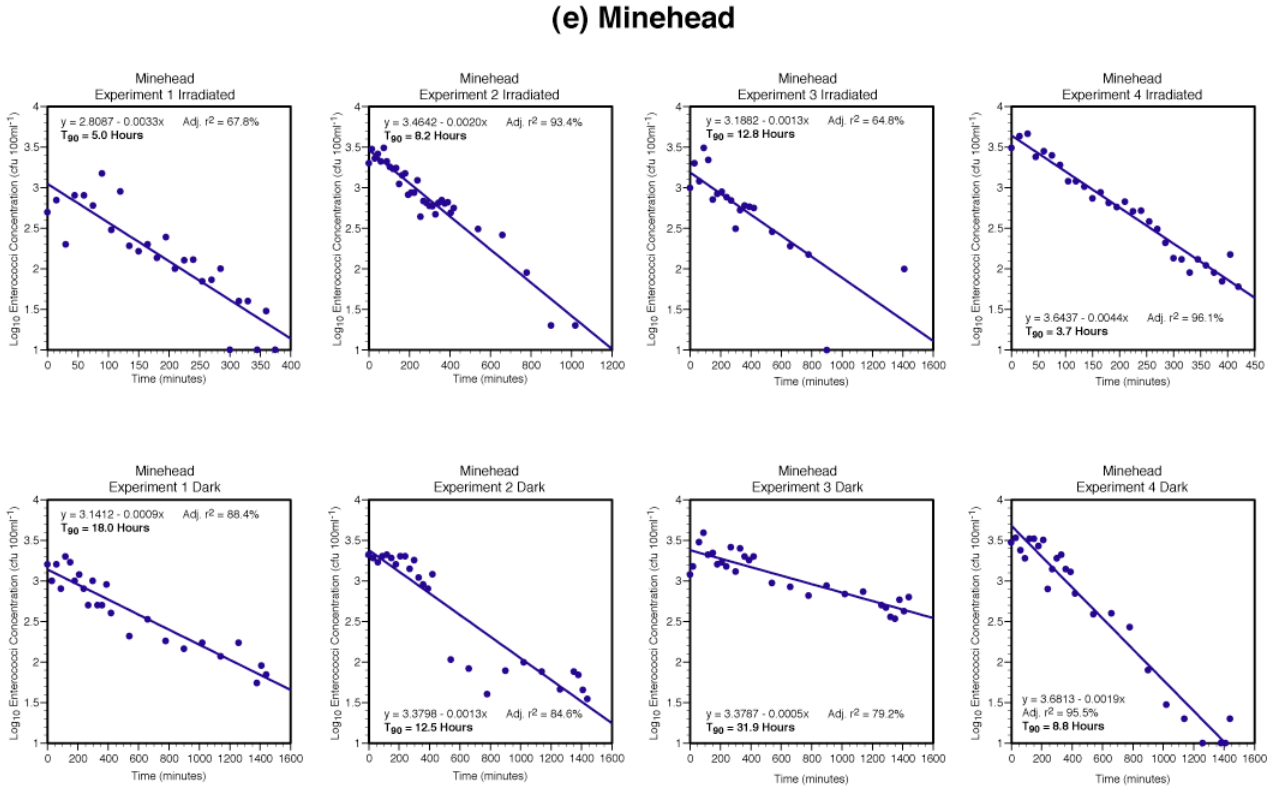



**Figure 2.2e: Regression analysis of unadjusted microcosm $T_{90}$ experiment results using water taken from (e) Minehead.**

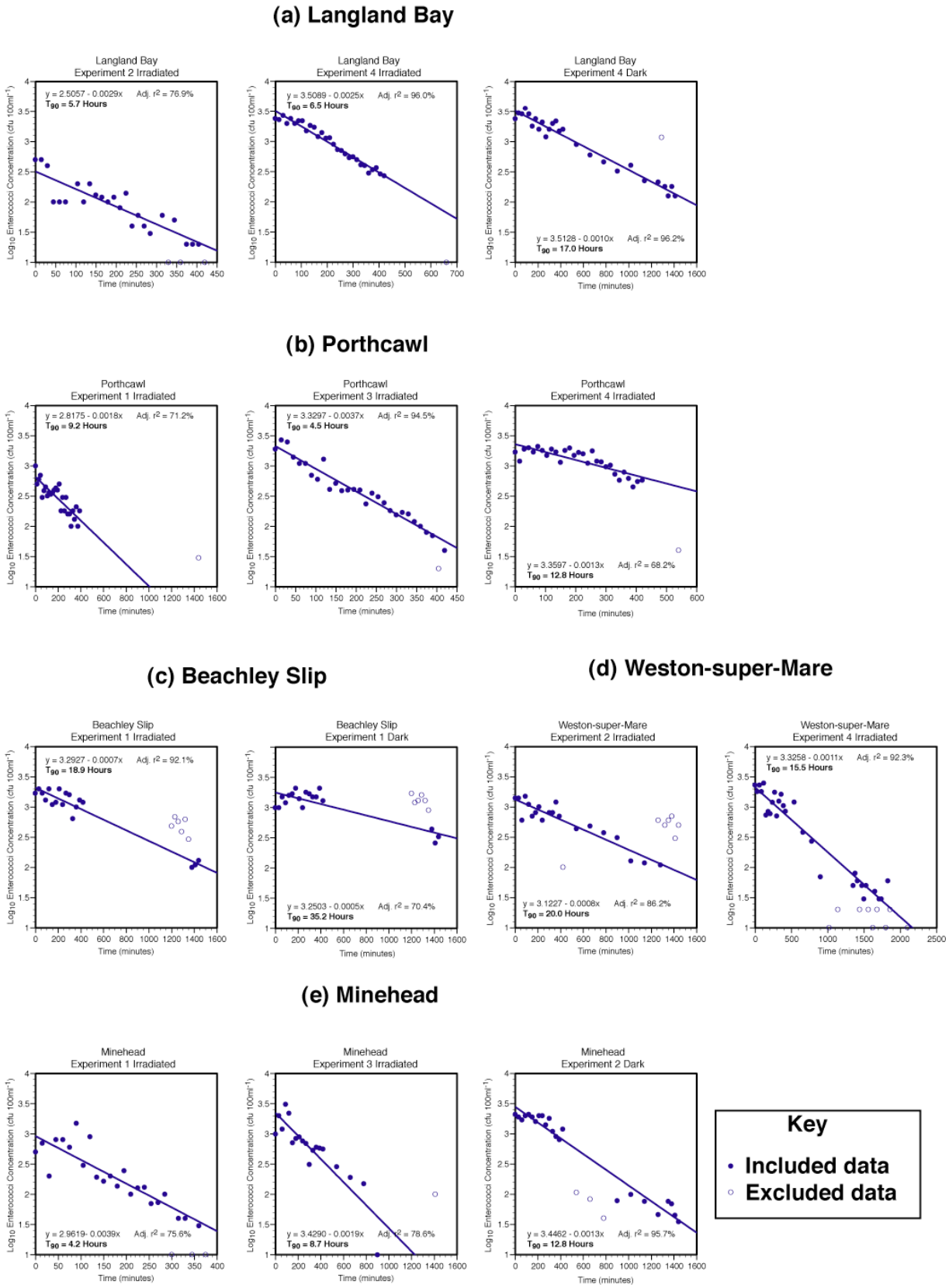



**Figure 2.3a–e:** **Regression analysis of microcosm $T_{90}$ experiment results adjusted to remove outliers and/or low concentration data: (a) Langland Bay; (b) Porthcawl; (c) Beachley Slip; (d) Weston-super-Mare; (e) Minehead**

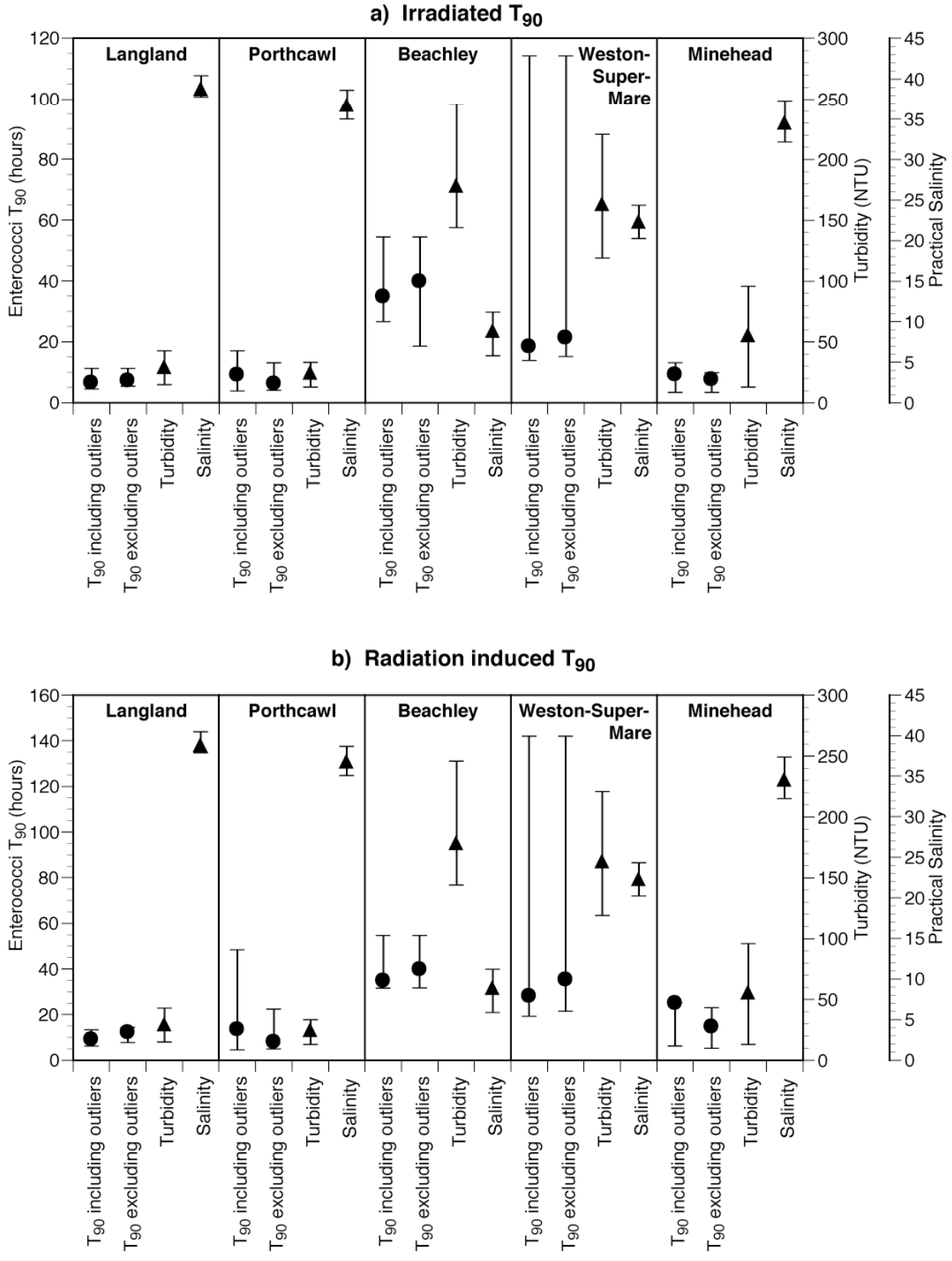



**Figure 2.4a & b:** **Range of $T_{90}$ values for enterococci (hours), turbidity (NTU) and salinity using water taken from the five sites: (a) irradiated and (b) radiation-induced (circles indicate the $T_{90}$ calculated using data from all four experiments at each site; triangles represent the mean turbidity and salinity)**

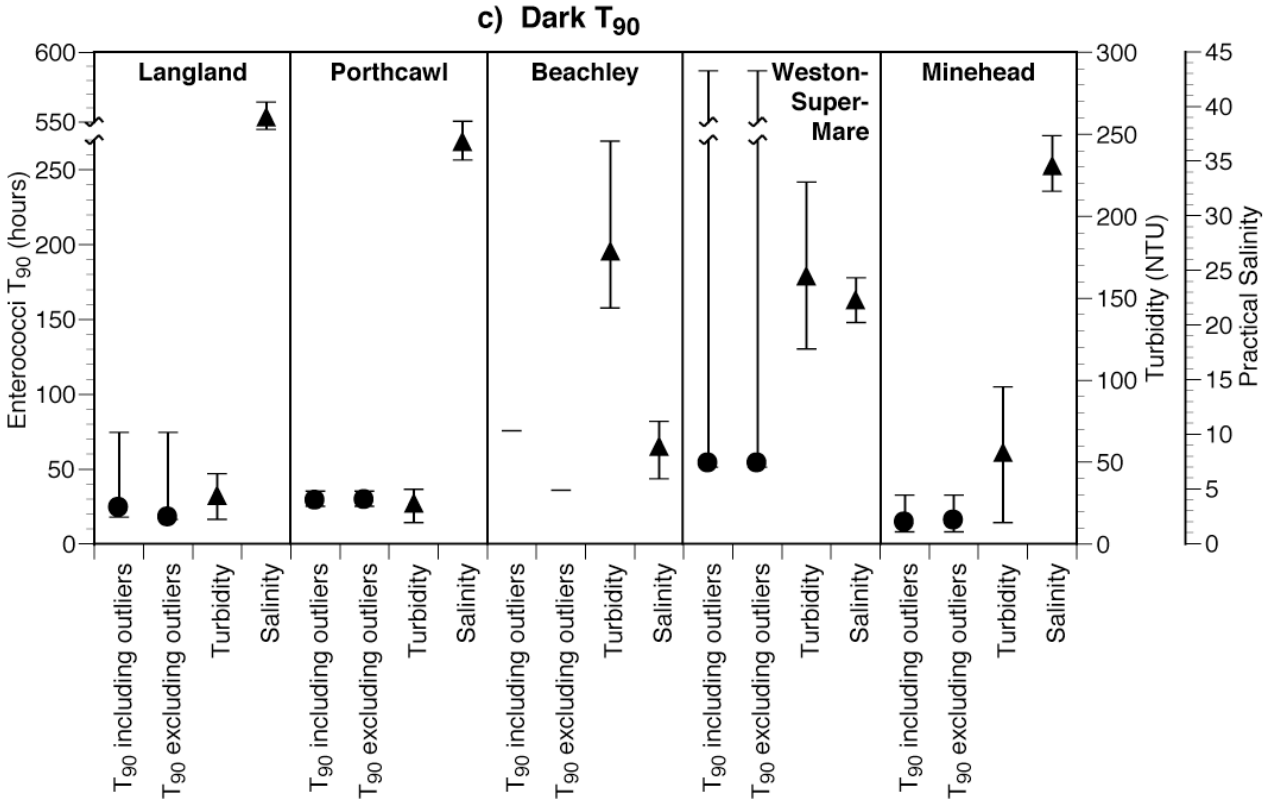
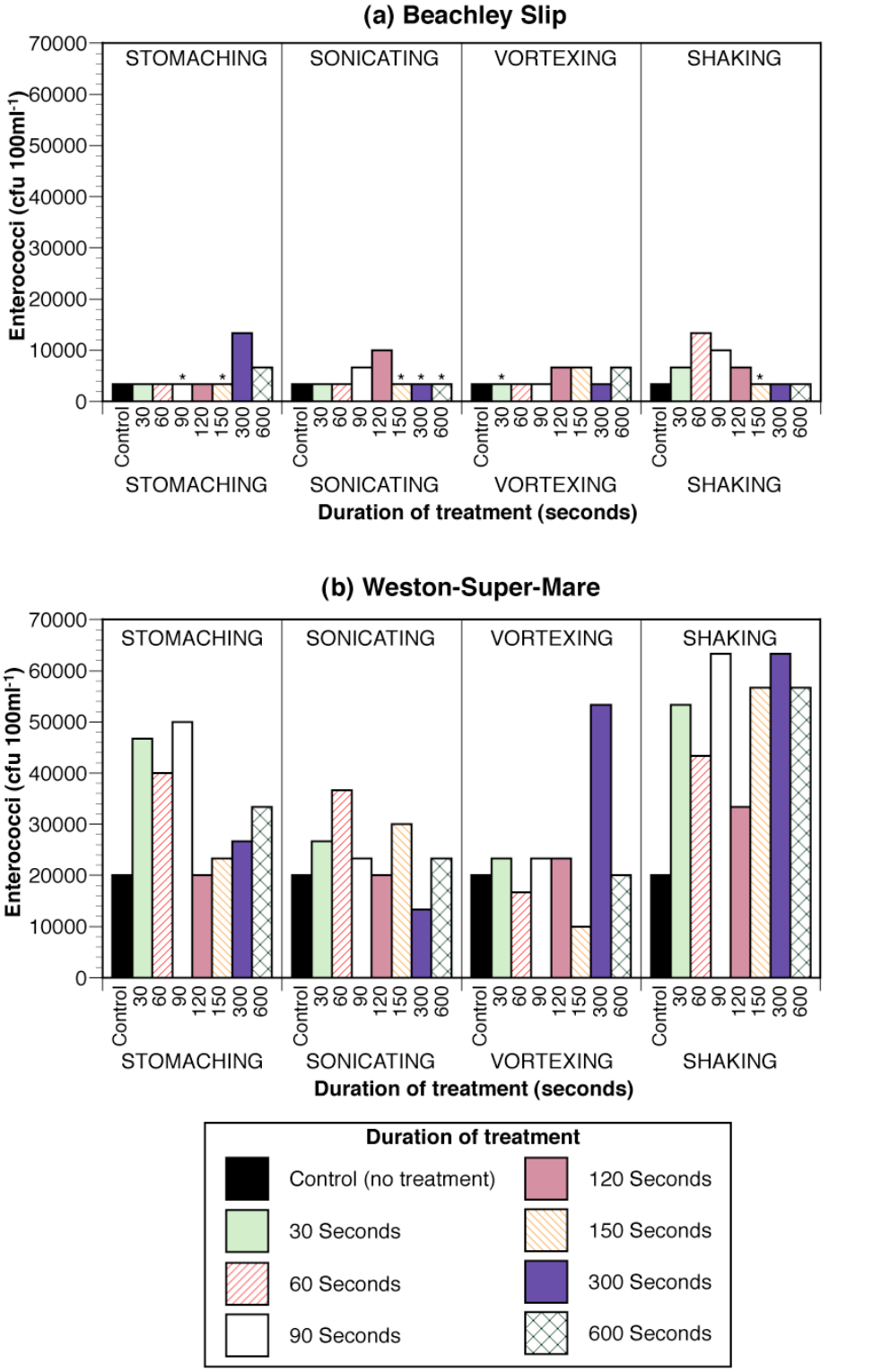



**Figure 2.4c: Range of $T_{90}$ values for enterococci (hours), turbidity (NTU) and salinity using water taken from the five sites: (c) dark (circles indicate the $T_{90}$ calculated using data from all four experiments at each site; triangles represent the mean turbidity and salinity)**




STOMACHING: Seward Model No.3500, normal speed setting.
SONICATING: Ultrawave Model No.M12140, operating Load 0.08kVa.
VORTEXING: Fisons Whirlimixer, max setting.
SHAKING: Pall Gelman labs wrist shaker, Model No.4822, at 600 oscillations per minute).
* indicates enterococci concentration less than the limit of detection (i.e. <3,333 cfu 100ml$^{-1}$)

**Figure 3.1: Effect of different methods for separating enterococci from inter-tidal sediments collected from (a) Beachley Slip and (b) Weston-Super-Mare**

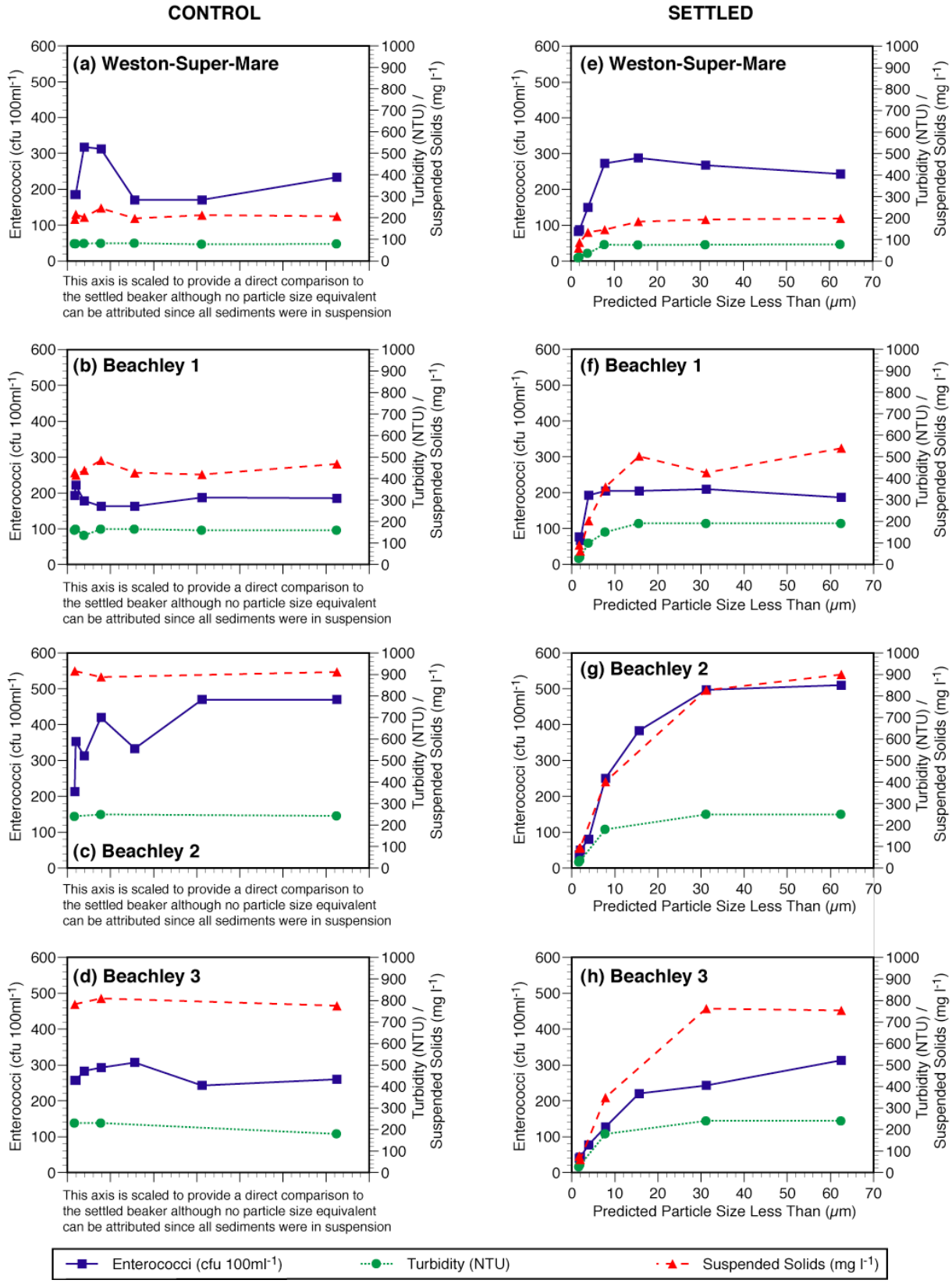



**Figure 3.2: Enterococci (cfu 100ml$^{-1}$), turbidity (NTU) and suspended solid concentrations (mg l$^{-1}$) plotted against predicted particle size less than (estimated using Stoke's Law (see Table 3.1)) from the four sediment settlement experiments: (a–d) stirred controls; (e–h) settled samples**

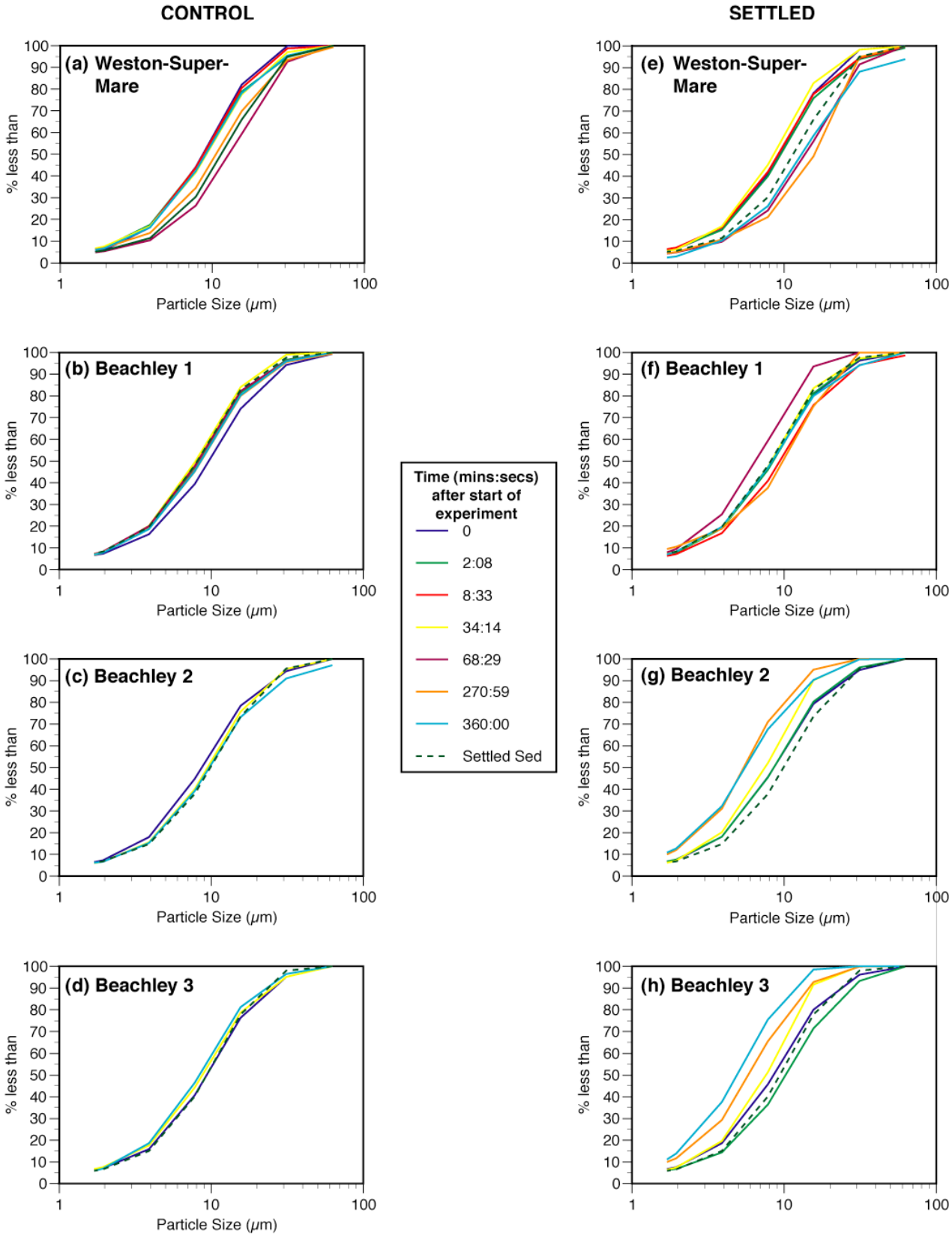



**Figure 3.3: Particle size distribution (% less than) of samples taken during the from the four sediment settlement experiments: (a-d) stirred controls; (e-h) settled samples**

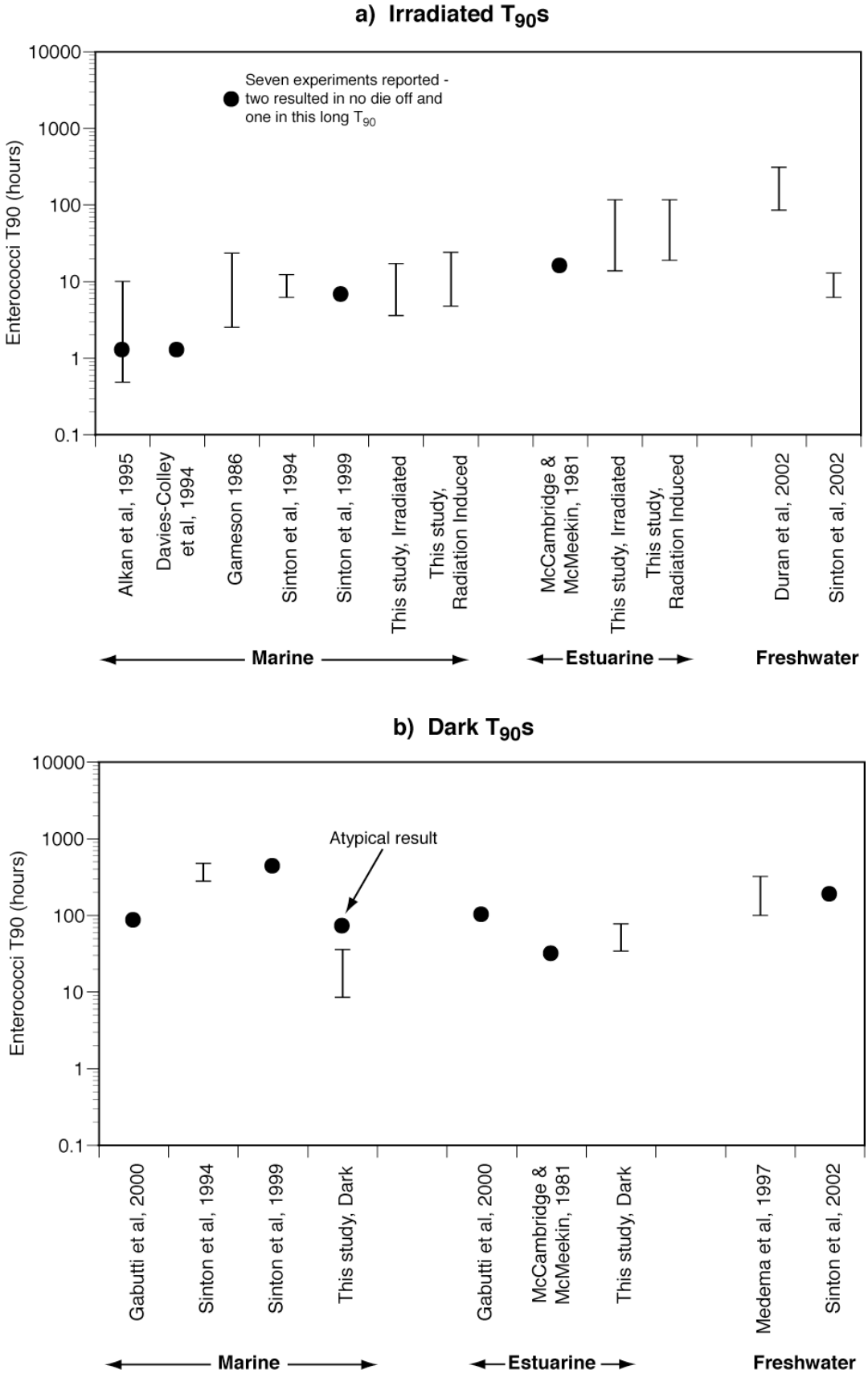



**Figure 4.1: Comparison of the range of enterococci $T_{90}$ values obtained from the laboratory microcosm experiments and other studies: (a) irradiated; (b) dark**

# Plates

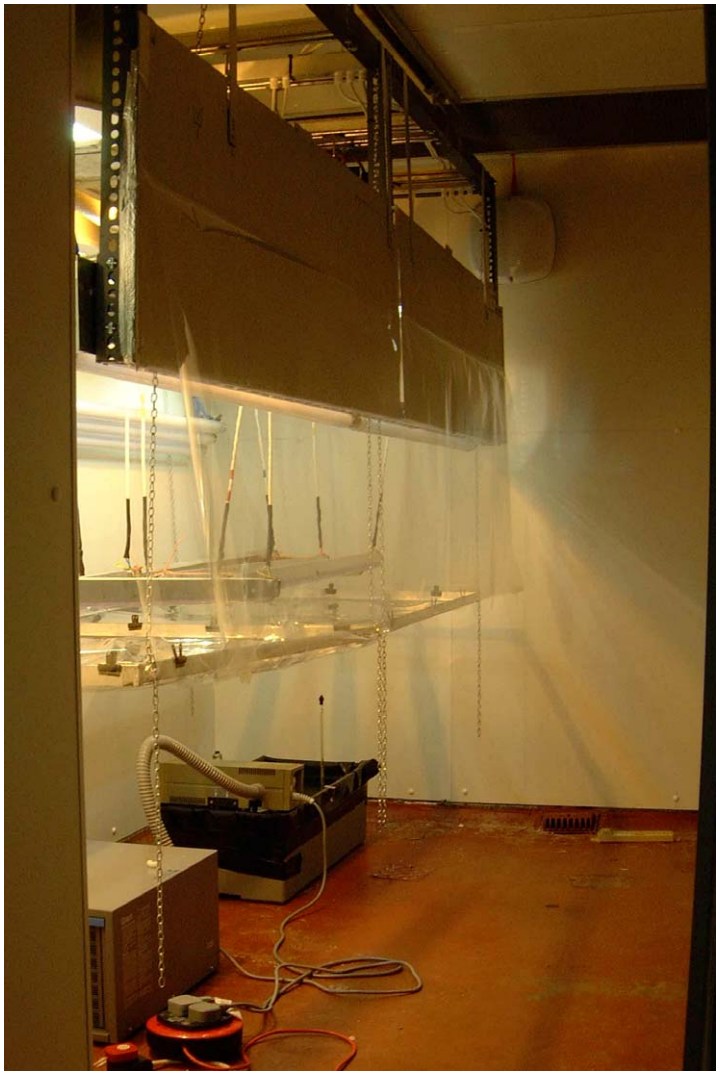
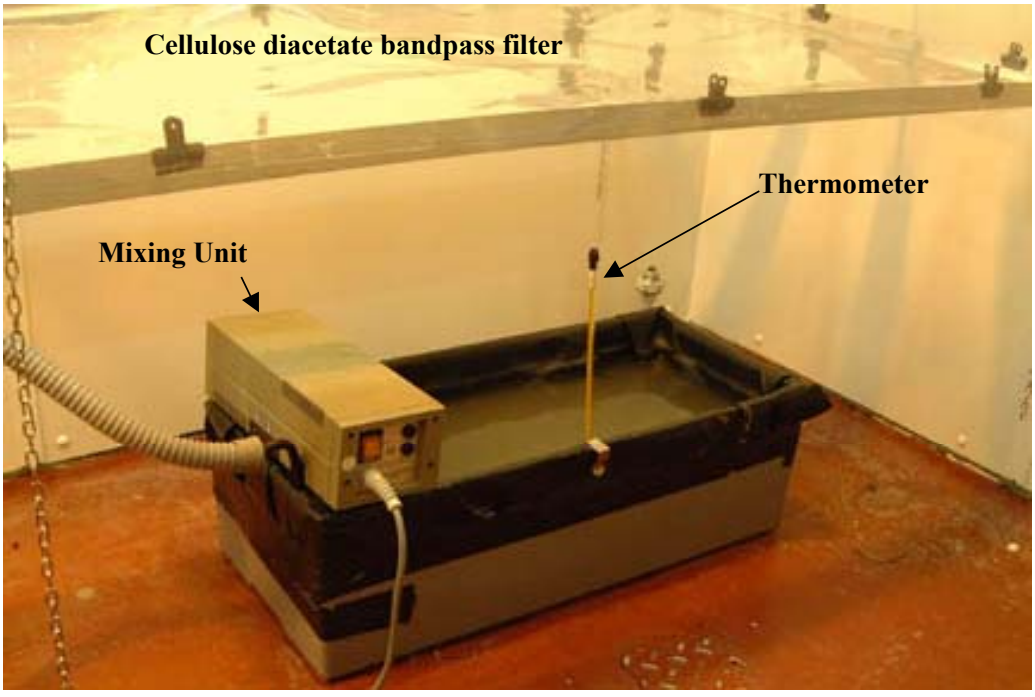


**Plate 2.1 (left):**
**Irradiated microcosm *in-situ* beneath artificial light source**

**Plate 2.2 (below):**
**Detail of irradiated microcosm**



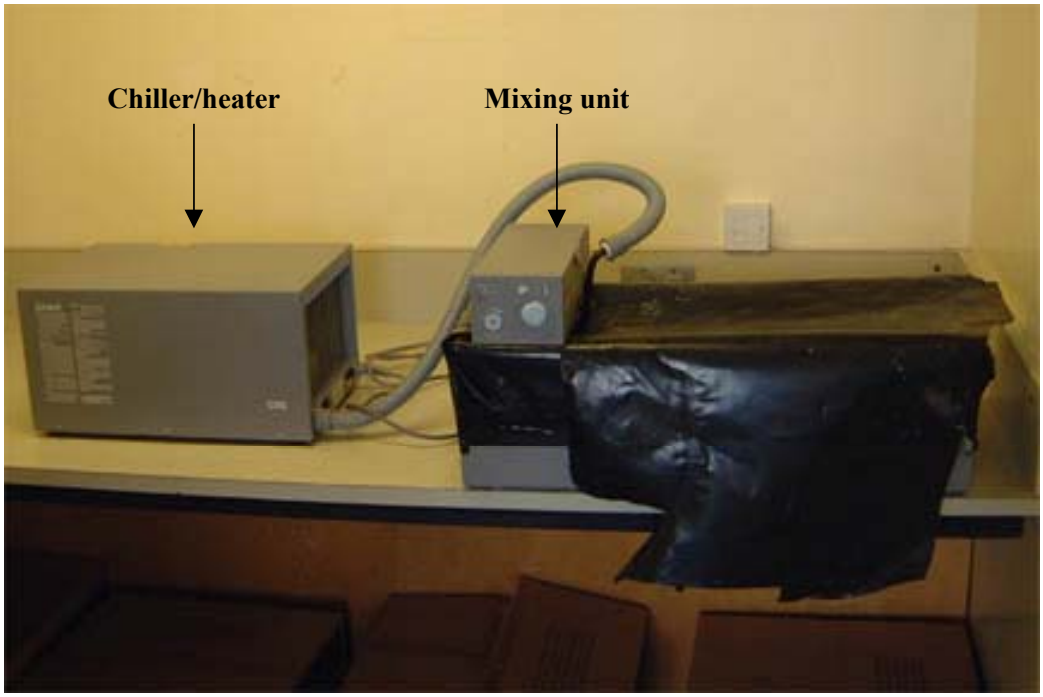



**Plate 2.3:** **Dark microcosm with cover in place (note: lights within the room are extinguished for the duration of the experiment)**

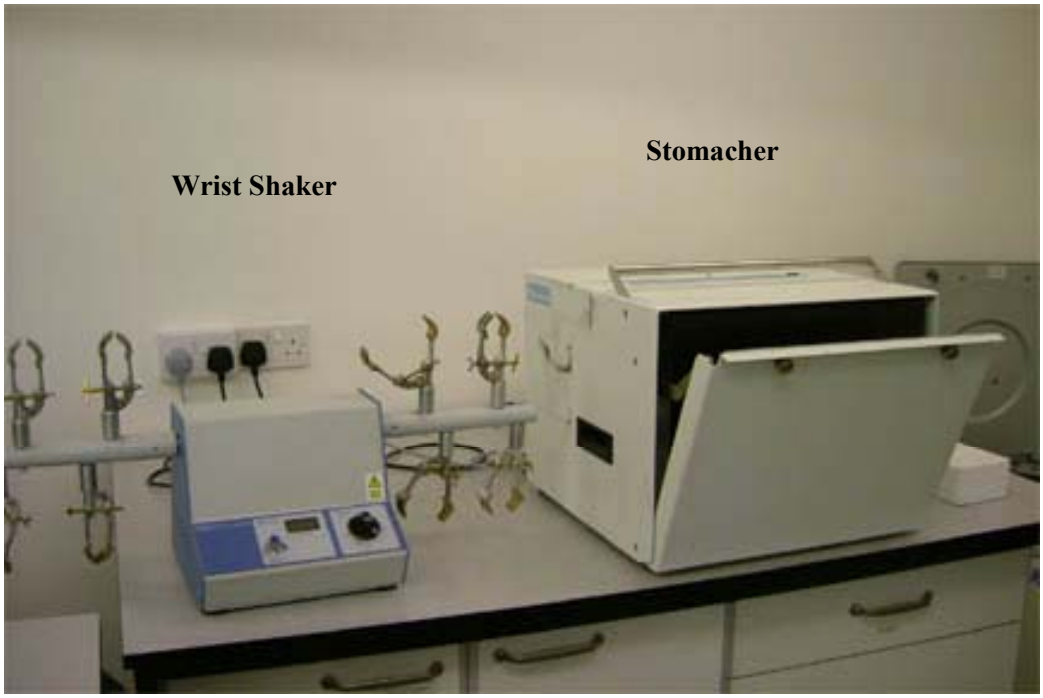



**Plate 3.1:** **Wrist Shaker (Pall Gelman Labs. Model No. 4822) and Stomacher (Seward Model No. 3500) used in the experiments to determine the optimum method for separating enterococci from sediments**


**Plate 3.2: Vortexer (Fisons Whirlimixer) used in the experiments to determine the optimum method for separating enterococci from sediments**

**Plate 3.3: Ultrasonic bath (Ultrawave Model No. M12140) used in the experiments to determine the optimum method for separating enterococci from sediments**


**Plate 3.4: Stirred (left) and settled (right) microcosms within incubator at end of six hour experimental period (note the difference in turbidity between the two microcosms)**

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