Some Factors Affecting the Survival of Faecal Bacteria in Estuarine Water

by

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SUMMARY

A seasonal variation in the numbers of faecal indicator bacteria in the Derwent Estuary was not observed, although a decrease in numbers along the Estuary associated with a decrease in human population was noted. There were no consistent significant correlations between the numbers of indicator bacteria and predacious microorganisms, temperature, salinity, solar radiation or rainfall. The survival of *E. coli* in estuarine water samples, however, exhibited a marked seasonal variation. This variation was not correlated with changes in salinity or microbial predators, but appeared to be associated with changes in water temperature with greater survival during the colder, winter months (April-July) than in the warmer, summer months (December-February). There was no significant variation in *E. coli* survival or the growth of predators from sites subject to previous sewage pollution to sites free from previous sewage pollution.

The introduction of faecal bacteria into estuarine water samples produced a homeostatic response from a sequence of the indigenous microbial predators. These organisms increased markedly in numbers, bringing about a marked decrease and often complete destruction of the prey bacteria. Following the exhaustion of food supply, the predacious microorganisms gradually returned to their original level. In pure culture studies involving individual predator and prey species, a similar pattern of predator growth and prey destruction also occurred. Once prey numbers had been reduced to a certain level, predator numbers also declined as the food supply declined, until the predatory pressure was removed from the prey population, resulting in the cryptic growth of the prey species.

Bacterial decline following the inhibition of protozoan predators indicated that bacterial predators also contributed to prey destruction,

but in natural estuarine water samples were maintained at lower levels due to"grazing" by predacious protozoa. The periodic inhibition of protozoan predators revealed that their major effect on the prey population and on bacterial predators was exerted during the first 2 days of a 10 day decline period. The initial concentration of *E. coli* prey present influenced the size of the predator population and the sequence of microbial predators which developed.

The survival of faecal indicator bacteria in separate estuarine water samples varied from one organism to another as follows: *Enterobacter aerogenes, Streptococcus faecium* > *E. coli, Salmonella typhimurium* > *Klebsiella pneumoniae*. When incubated together, prey resistance and prey selection by microbial predators resulted in different prey survival patterns: *S. typhimurium* > *E. coli, E. coli* > *K. pneumoniae* and *E. coli* > *S. faecium*.

E. coli and *S. typhimurium* exhibited similar survival curves and their presence resulted in the growth of comparable numbers of predacious microorganisms at a range of incubation temperatures. Bacterial decline was found to be dependent on the presence of both bacterial and protozoan predators, the latter having a temperature optimum of 15-20^{o}C and the former becoming more important as the incubation temperature increased.

The decline of *E. coli* cells in estuarine water samples was found to be significantly greater in the presence of both naturally-occurring microbial predators and solar radiation than when each of these factors was acting independently. The effect of solar radiation on microbial predators was negligible, while the resistance of bacteria to light-induced decay varied from one organism to another as follows: *S. typhimurium, S. faecium, E. aerogenes, E. herbicola* > *E. coli* > *K. pneumoniae*.

I. Introduction

Introduction

The increased use of estuarine waters for recreational and commercial purposes (particularly shellfish production) is threatened by the discharge of increased levels of domestic and industrial wastes, including sewage, into such waters. It is, therefore, important to understand the factors which affect the survival of sewage bacteria in estuarine ecosystems.

It would appear from the numerous reports, that the bactericidal activity of marine waters results from a combination of both physico-chemical and biological factors. The former include adsorption and sedimentation, solar radiation, temperature, nutrient utilization, salinity and heavy metals, and the latter include bacterial and algal antibiotics, bacteriophage, bacterial parasites and predators, and predacious protozoans. While there is general agreement that several factors may act together, different authors emphasise the role of different factors. Thus, Mitchell and Chamberlin (1975) indicate the major role of solar radiation,while Roper and Marshall (1978) favour factors which show thermal instability,and within the latter group Enzinger and Cooper (1976) cite the importance of protozoan rather than bacterial predators.

The majority of the work on the survival of faecal bacteria in seawater has involved the use of the indicator organism *E. coli*. The reliability of indicator organisms, however, to evaluate the bacteriological quality of water is dependent on the survival of the indicator being comparable to that of pathogenic organisms and the few comparative studies conducted have produced conflicting results.

The purpose of this study was to examine the survival of bacteria of faecal origin in estuarine water, and in particular the role played by predacious microorganisms of bacterial and protozoan origin. The main aims were to obtain data on the bacteriological pollution of the Derwent River and Estuary and to attempt to correlate the seasonal and regional distribution of indicator organisms with such factors as temperature, solar radiation, microbial predators, salinity and rainfall; evaluate the relative roles of bacterial and protozoan predators in *E. coli* survival; and investigate their effect on bacterial prey survival in axenic culture, and determine the effect of certain environmental variables in the survival of various bacterial indicators and investigate any interactions between these variables and naturally-occurring microbial predators.

II. Literature Review

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A. Introduction

It is well known that when enteric bacteria are discharged from an outfall into seawater their numbers are significantly reduced. This is a function of a simple dilution effect including such factors as horizontal advection and lateral and vertical dispersion, and is also dependent on the time rate of change in the bacterial population. A typical survival curve, representing the changes which may occur in a bacterial population when exposed to a seawater environment, is shown in Figure 1. It consists of three distinct phases; an initial lag phase in which bacterial numbers remain essentially constant; a phase of linear decline or logarithmic death; and finally an equilibrium phase in which the curve becomes asymptotic. Orlob (1956) refers to this third phase as the resistant phase, in which resistant bacterial forms develop. This may in fact be caused by other factors such as a reduction in the number of predators or a build up of toxic metabolites. Orlob (1956) suggested that these survival curves were essentially logarithmic and could be described by a modification of Chick's Law.

Thus,

$$k = \frac{\log \frac{N_1}{N_2}}{t_2 - t_1}$$

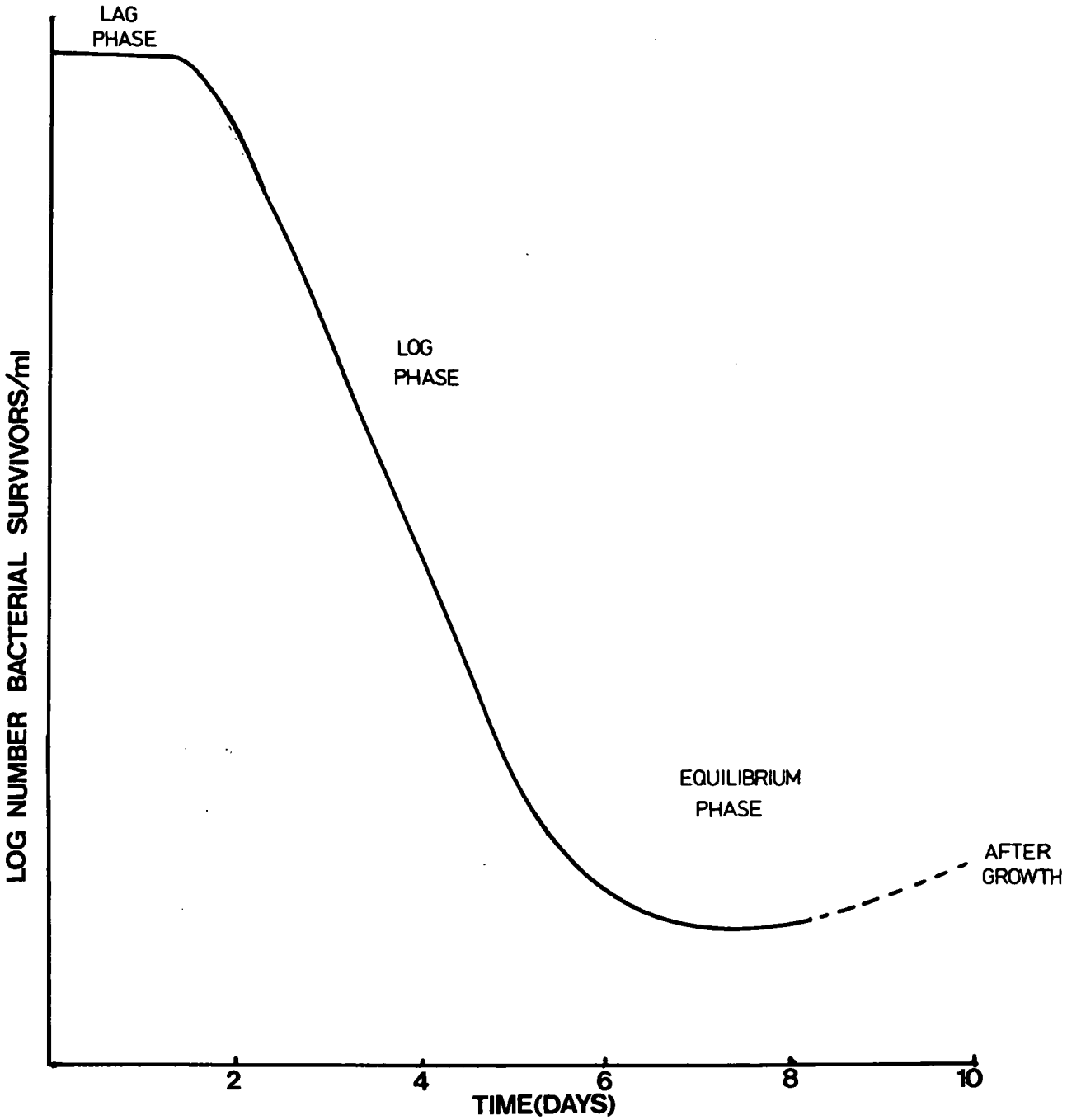
where N_1 = bacterial population at time t_1 (end of lag phase)

N_2 = bacterial population at time t_2

k = rate constant

If the seawater is particularly antagonistic to the bacterial population, such as in the presence of large numbers of predacious microorganisms, the lag phase may be eliminated. Thus N_1 becomes the initial bacterial concentration and $t_1 = 0$. If we then consider

Figure 1: Typical survival curve for bacteria in seawater (adapted from Orlob (1956)).


LAG
PHASE
LOG
PHASE
EQUILIBRIUM
PHASE
AFTER
GROWTH
LOG NUMBER BACTERIAL SURVIVORS/ml
2
4
6
8
10
TIME(DAYS)

the time required for a 90 percent reduction in bacterial numbers (T_{90}), then:

$$k = \frac{1}{T_{90}}$$

The use of complete survival curves, rate constants and T_{90} values are the three most common methods for describing the decline of sewage bacteria in seawater.

Numerous factors have been proposed to influence the survival of sewage bacteria in seawater. These include adsorption and sedimentation, solar radiation, temperature, nutrient availability and utilization, salinity, heavy metals, algal and bacterial antibiotics, bacteriophages, and bacterial and protozoan predators. The effect of each of these factors on bacterial survival may vary, with different factors being more or less important in different localities. In this review the role of each factor on the survival of sewage bacteria has been examined. The use and significance of bacterial indicator organisms has also been considered.

B. Dilution Effect

Dilution has long been recognized as an important factor in the distribution of enteric bacteria in water systems. Jordan (1900) stated that the reduction of the bacterial count in polluted streams occurred due to dilution, sedimentation or the action of sunlight. He concluded that, although not necessarily having an effect on the survival of sewage bacteria, dilution would, by the simple addition of less contaminated water, have an immediate effect in reducing the number of bacteria in a given quantity of water. Similarly, Faust (1976) observed that a decrease in the numbers of total and faecal

coliforms in a river was inversely related to the increasing volume of the river. Other workers have stressed however, that the observed decline in bacterial numbers, particularly from sewage outfalls, could not be accounted for by dilution alone, but that other factors must also be involved (Orlob, 1956; Ketchum *et al.*, 1949). Ketchum *et al.* (1952) calculated that dilution, the bactericidal action of seawater, and predation accounted for 99 percent of the observed decrease in coliform bacteria in a tidal estuary, with bactericidal action the most important, followed by predation and dilution. Similarly, Carter *et al.* (1967) and Zanoni *et al.* (1978) observed that dilution and mortality produced a greater effect on bacterial reduction than dilution alone.

The importance of dilution in providing part of this reduction is incorporated in the design and location of sewage outfalls (Carter *et al.*, 1967; Walters, 1976). Harremos (1970) described two methods using a conservative tracer technique for the field determination of bacterial disappearance in seawater in which he measured both the enteric bacteria and tracer material in the sewage plume. The tracer concentrations are then used to correct bacterial concentrations for effects due solely to dilution. In this way, the lethal effect of seawater on bacterial survival can be estimated. Similarly, Jones and Stewart (1970) in their examinations of the diffusion of sewage from an ocean outfall, concluded that the observed reduction in bacterial numbers was due to the initial dilution of sewage in rising to the surface, subsequent diffusion and natural decline.

C. Factors Affecting Survival

C.1 Adsorption and Sedimentation

As nearly half of the suspended particulate matter in seawater (0.2-2 ppm) is inorganic and clay-like (Harvey, 1957) and since up to 75 percent of coliforms in sewage are already associated with particles of sizeable settling velocities (>0.05cm/sec) before discharge (Mitchell and Chamberlin, 1975), it has been suggested that adsorption and sedimentation may contribute to the removal of bacteria from surface waters.

The role of sedimentation in the reduction of bacterial numbers in water was recognized by Jordan (1900) who suggested that the entanglement of bacteria in slowly subsiding particles and possibly the slow sinking of the bacteria themselves would contribute to this reduction. Several workers have demonstrated the varying adsorptive capacities of different particles and the changes which occur to bacteria in the adsorbed state. Rubentschik *et al.* (1936) found that bacteria isolated from mud showed a higher degree of adsorption than bacteria found in the overlying water. The activities of different species of bacteria were variously modified in the adsorbed state, some having lowered metabolism and others increased. Waksman and Vartiovaara (1938) obtained similar results and noted that following adsorption by the mud, bacterial numbers rapidly increased at the expense of the organic matter in the bottom material. They also demonstrated that there was little or no adsorption on sand. Weiss (1951) showed that the degree of adsorption of *Escherichia coli* to particulate matter in river and estuarine silts was characteristic of the origin and particle sizes of the silt. Further, the adsorption of bacteria to silt particles increased the rate of

sedimentation of the bacterial cells, and in the range of turbidities normally encountered would account for a significant removal of *E. coli* from surface waters. The presence of the excess Na^+ of seawater,however, decreased the adsorptive capacity of the silts, and in some cases desorption of *E. coli* occurred. The effect of sedimentation on the removal of coliforms from seawater was evaluated in a general way by Orlob (1956). Two samples of settled sewage in seawater (1:1000 dilution) were prepared and periodically examined for coliforms. One sample was vigorously shaken prior to examination and the other undisturbed. The agitated sample showed an initial increase in numbers and thereafter decreased, but at all times exhibited greater levels than the undisturbed sample.

Several field studies have been reported which also support the importance of sedimentation. Orlob (1956) observed that bacterial contamination extended to all depths in water adjacent to a sewage outfall. Nusbaum and Garver (1955) and Rittenberg *et al.* (1958) also observed the presence of high numbers of coliform organisms in the vicinity of marine sewage outfalls. The latter authors noted that coliforms were also found in the sediments below the path of movement of the effluent field in the surface water and concluded that they must persist for a reasonable length of time for bacterial numbers to reach the high levels observed, by deposition from the low count waters above. However, no estimate was made of the survival time in sediments and no reasons postulated for their survival.

Hendricks (1970) showed that members of the *Enterobacteraceae* had the ability to utilize nutrients eluted from sediments obtained from a fresh water stream; and Grimes (1975) observed that faecal coliform concentrations increased significantly in the immediate vicinity of a dredging operation in the Mississippi River. Similarly,

E. coli survived for longer periods of time in natural seawater in the presence of sediments than in seawater alone (Gerba and McLeod, 1976; Wait and Sobsey, 1980). Several hypotheses were suggested by the former authors to explain this increased survival and the resultant accumulation of *E. coli* in sediments. An increased level of organic matter in the sediments compared to the overlying water would enable *E. coli* to compete more effectively with the native microflora. Alternatively, *E. coli* may compete more effectively for nutrients against the microflora present in the sediments. A third hypothesis is that the nutrients present in sediments may be different to those in the surface water and more easily utilized by *E. coli*; and finally the sediments may interfere with some bactericidal factor in seawater. Matson *et al.* (1978) suggested that the extended survival of indicator organisms in river sediments depended on nutrient-related factors, as noted by Gerba and McLeod (1976), and also on their ability to withstand predatory pressure.

Roper and Marshall (1979) observed that bacteria and suspended solids were sedimented into the bottom muds of a tidal estuary when a critical salinity was exceeded. The prolonged survival of *E. coli* in the sediments was caused by the protection of *E. coli* cells by the sediments, from the lytic action of bacteriophages and other predators (Roper and Marshall, 1974). A similar protection of the bacteria in laboratory experiments was observed in the presence of a montmorillonitic clay (Roper and Marshall, 1979). The phage and *E. coli* remained firmly sorbed to saline sediments until a critical electrolyte concentration was reached by dilution, when desorption occurred. *E. coli* was protected from phage attack at low electrolyte concentrations by an envelope of sorbed colloidal material around the cell, whereas at high electrolyte concentrations the bacteria were protected by the

sorption of the cells and phages to solid particles, as well as from the colloidal envelope (Roper and Marshall, 1974). In later work (Roper and Marshall, 1978) the effect of crude and colloidal clay on microbial predation by different organisms was examined. The interaction between *E. coli* and *Bdellovibrio* was only partially inhibited by the presence of montmorillonite due to the ability of the latter to penetrate any colloidal envelope which was thin enough. Faust *et al.* (1975) observed an increase in the survival of *E. coli* in estuarine water *in situ* in the presence of montmorillonite, but could not explain the effect. Their use of 0.45 micron filters in membrane chambers would still allow the passage of bdellovibrio into the chambers, and so the clay would offer the same protection as observed by Roper and Marshall (1978). Colloidal clay had little effect on the predation of *E. coli* by the myxobacterium, *Polyangium,* and no effect on the predacious amoeba, *Vexillifera*. The use of crude clay, however, to represent a true sediment situation, slowed the rate of engulfment of *E. coli* by *Vexillifera* and completely inhibited the *E. coli*-*Polyangium* interaction.

It appears,therefore, that adsorption and sedimentation are important factors in the removal of sewage bacteria from surface waters, but their relative significance will depend upon the nature of the released sewage and the extent of the resuspension processes. Orlob (1956) suggested that the effect of sedimentation would not be very pronounced at large distances from the sewage outfall. Further, the effects of wind, wave and current action would tend to prevent deposition, resuspend deposited bacteria and distribute contamination through the entire depth of the water surrounding the outfall, making bacteria more susceptible to other bacteria-reducing factors.

C.2 Solar Radiation

The effect of solar radiation on the survival of bacteria in the sea has been considered by many workers,with conflicting results. Much of the early work summarised by Zobell and McEwen (1935) discounted solar radiation as an important factor in bacterial decline in seawater. Later work also attributed minimal importance to sunlight, largely due to the high attenuation coefficients of ultraviolet and visible light in seawater (Orlob, 1956; Carlucci and Pramer, 1959; Bernard, 1970).

It was not until the work of Gameson and Saxon (1967) that convincing evidence for the importance of solar radiation on coliform mortality was presented. In a series of submerged bottle experiments using mixtures of seawater and macerated sewage they concluded that there was a high mortality of coliforms when exposed to light at depths of up to 4m. The rate of decline at any time of the year was found to be approximately proportional to the intensity of short wave radiation received by the sample. Similarly, Paoletti *et al.* (1978) established that T_{90} for coliforms was greater in seawater in the dark than when exposed to sunlight. Bellair *et al.* (1977) observed a diurnal variation in faecal coliform die-off rates and established an inverse relationship between hourly T_{90} values and hourly solar radiation with a maximum of 40 hours during the night to a minimum of 1.9 hours during the day. This effect has also been observed by Gameson *et al.* (1973) who noted that coliforms exposed to bright sunshine for short periods of time, followed by short periods of darkness, exhibited a step-wise reduction in numbers. The rate of decline during exposure to sunlight was similar to a sample continuously exposed to solar radiation, and the rate of decline during periods of darkness was similar to that of a sample continuously in the dark. Thus the reduction in bacterial numbers was

proportional to the total radiation received over a given time. The surface radiation needed to produce coliform mortality increases with advancing season and with increasing depth (Gameson and Saxon, 1967; Bellair *et al.*, 1977). The latter suggest that it is the radiation of shortest wavelength (ultraviolet and the blue end of the visible spectrum) which is most lethal, as it is this radiation which has very low penetration. It should be noted that due to absorption of ultraviolet radiations in the atmosphere, no solar radiations of wavelengths less than 290nm can be detected at the earth's surface (Jerlov, 1976). The use of gelatin photographic filters by Gameson *et al.* (1973) and later by Gameson and Gould (1975) characterised the wavelengths of lethal radiation for coliform survival. The latter authors concluded that 50 percent of the lethal effect of solar radiation is attributable to wavelengths below 370nm, 25 percent to the near-visible ultraviolet (370-400nm);and 25 percent to the blue-green region of the spectrum (400-500nm) the effect of wavelengths greater than 500nm being negligible. The effect of the visible part of the spectrum was observed by Anson and Ware (1975) who noted coliforms were reduced in numbers in seawater even in the absence of ultraviolet light.

Verstraete and Voets (1976) also observed a correlation between solar radiation and die-off and suggested that the increased decline could be due either to the biocidal action of sunlight itself, the increased photosynthesis of the phytoplankton population, or the increase in temperature. They considered that the low penetration of ultraviolet and the high turbidity of the water system discounted the first hypothesis and that different die-off indices at similar temperatures but different sunlight intensities,discounted the last hypothesis. They concluded, therefore, that compounds of an antibacterial nature,

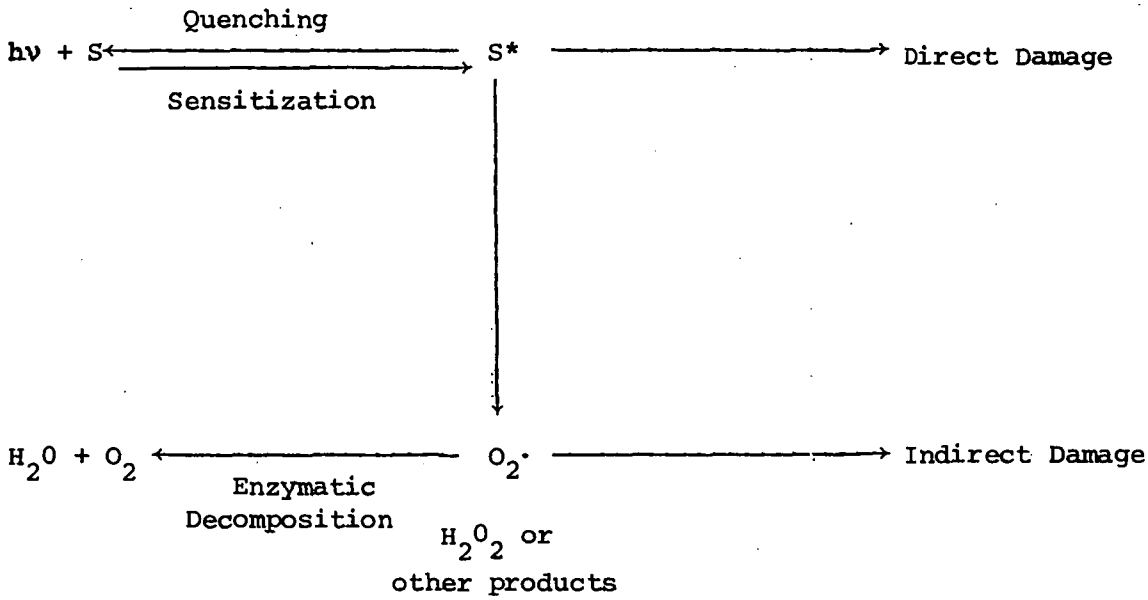
excreted by phytoplankton, were important, although Gameson and Saxon (1967) found no evidence for the release of algal toxins. Similarly, Pike *et al*. (1970) noted that the toxicity of seawater exposed to sunlight before inoculation with *E. coli* was not altered in relation to the degree of exposure, again suggesting that toxin production by phytoplankton is not stimulated by sunlight. The effect of sunlight on coliform mortality would thus appear to be a direct result of light-induced damage.

The general mechanisms involved in light dependent bacterial decay have been discussed in detail by Chamberlin and Mitchell (1978) and are outlined in Figure 2. In this suggested process, light is absorbed in the bacterial cell by an exogenous or endogenous sensitizer (or chromophore) (S) resulting in the raising of the sensitizer to an excited state (S*). A large number of possible endogenous sensitizers have been suggested (Eisenstark, 1971) and these include cytochromes, carotenoids, porphyrins,heme proteins and other pigmented compounds. Exogenous sensitizers include numerous dyes, such as methylene blue, and also certain naturally occurring compounds such as algal pigments and chlorophylls (Clayton, 1971). The excited sensitizer may then transfer the energy via electron transfer to a quencher, and return to the ground state(s). For example, possible chromophores, such as carotenoids, may protect cells from light damage (Harrison, 1967) by absorbing the excitation energy. This may account for the prevalence of pigmented strains such as *Polyangium* and *Caulobacter* among bacteria found in environments subject to high light intensities such as the surface waters of aquatic environments (Singer and Ames, 1970).

The excited sensitizer may also react directly with, and consequently damage, a cell component, or react with oxygen forming

FIGURE 2 : General Processes of Photooxidative Damage

(Taken from Chamberlin and Mitchell (1978))



superoxides ($O_2^{\cdot}$), hydrogen peroxide or organic peroxides, which in turn may react with and damage some cell component. The type of damage occurring in the cell depends on the wavelength of light, UV (less than 300nm) often producing different effects to the near ultraviolet-visible range (greater than 300nm) (Eisenstark, 1971). Instead of causing cell damage, these oxides may be enzymatically decomposed to harmless products such as oxygen and water. Thus it appears that bacterial sensitivity to near ultraviolet and visible light depends on the presence of endogenous or exogenous sensitizing agents, oxygen and possible protection mechanisms.

Kelner (1949) observed that visible light of wavelengths less than 510nm caused the recovery of microbial cells injured by exposure to ultraviolet light. Light-induced recovery or photoreactivation was observed in four diverse species including *E. coli*, but later work by Gameson *et al.* (1973) failed to show any recovery of coliform organisms exposed to ultraviolet light. This is in accordance with the work of Eisenstark (1971), who observed genetic changes produced by light of wavelengths 300 to 500nm which were detrimental to the cell.

Solar radiation, therefore, appears to be a very important factor in the decline of bacteria in seawater, but as noted by Chamberlin and Mitchell (1978), it must not be considered in isolation. For example, turbid water systems may protect bacteria from the lethal effects of sunlight (Verstraete and Voets, 1976) by the specific absorption of clay minerals (Bitton *et al.*, 1972). Gameson *et al.* (1973) added various concentrations of clay to a seawater and sewage mixture and exposed each sample to sunlight. They observed an increase in T_{90} as the clay content increased. Similarly, predation by microorganisms and sunlight may interact in that sunlight may only injure coliforms

making them more susceptible to the activities of microbial predators. The presence of both factors acting together would thus increase the decline of coliforms in seawater, compared to each factor acting alone. The importance of this type of interaction is at present unknown and warrants further investigation.

C.3 Temperature

Early investigators reported conflicting results on the effect of temperature on bacterial survival in seawater. Burke and Baird (1931) found that fresh water bacteria inoculated into seawater survived longer at 20 to 22°C than at 7 to 12°C, while Fraser and Argall (1954) observed greater survival of bacteria in Great Salt Lake water at 6°C than at the warmer summer temperatures. It is now generally accepted, however, that bacteria are more susceptible to the bactericidal effects of water, as the temperature increases (Carlucci and Pramer, 1960a). Nusbaum and Garver (1955) incubated natural seawater at 5, 18 and 30°C. Survival of coliforms in samples incubated at 18 and 30°C were similar, exhibiting an initial lag phase of one to three days followed by a rapid decrease. In the 5°C samples, however, survival was virtually unaltered for periods of up to 9 days. It was suggested that this reduction in mortality was due to either reduced metabolism of the coliform organisms at low temperatures or the reduced effectiveness of the antibiotic activity of seawater. Orlob (1956) conducted a similar series of experiments in which dilutions of settled sewage in seawater were incubated at temperatures ranging from 6 to 25.8°C. He observed a general increase in the rate of decline and a shortening of the lag phase as the temperature increased. In a later study, Hanes *et al.* (1965) using a 1 percent dilution of sewage in "Biochemical Oxygen Demand Water" observed a similar increase in the coliform death rate and a decrease in the lag phase as the temperature

of incubation increased. There have been numerous attempts to correlate the distribution of coliforms with environmental factors such as temperature, but again these have produced conflicting results. Several authors have observed no significant correlation between temperature and coliform numbers in natural water systems (Brasfeild, 1972; Sayler *et al.*, 1975; Goyal *et al.*, 1977), whilst statistically significant negative correlations have been observed by others (Evison and James, 1973; Davenport *et al.*, 1976; Omura and Matsumoto, 1978; Hirn *et al.*, 1980; Yoshikura *et al.*, 1980). Also, numerical models designed to predict coliform mortality rates in water systems generally include temperature as an important factor (Kelch and Lee, 1978; Mancini, 1978; Kay and McDonald, 1980).

Several authors have observed this temperature effect as a seasonal variation in bacterial survival. Vaccaro *et al.* (1950) found that there was a marked seasonal variation in the length of time *E. coli* could survive in seawater (being less viable in summer than in winter), and suggested an antibiotic action from the native microflora. Faust *et al.* (1975) also observed a greater survival in winter than in summer for *E. coli* cells in estuarine water and attributed this variation largely to the differences in temperature, although differences in salinity and dissolved oxygen were also observed. They suggested that the slow metabolic rate of the bacteria at low temperatures was responsible for their increased survival. In a more recent study on the survival and viability of *E. coli* in a thermally altered reservoir, Gorden and Fliermans (1978) observed growth rather than decline of *E. coli* in a eutrophic water system. Orlob (1956) also noted that if nutrients were present in seawater in sufficient amounts, an increase in temperature could result in appreciable growth of bacteria in an environment that would otherwise be unfavourable and cause the decline of bacterial numbers.

Although the effect of temperature on bacterial survival in water has often been observed, as noted above, few explanations have been proposed, except either a reduction in the metabolic activity of the bacteria or an increase in the antibiotic activity of the natural microbial flora. The former is unlikely to account for the large variations in survival which have been observed, while the production of antibiotics by microbes of algal or bacterial origin is open to question (Carlucci and Pramer, 1960c). There have been no reports on the effect of temperature on naturally occurring microbial predators in seawater and their effect, in turn, on coliform survival. Also, some authors have demonstrated a temperature effect in filter-sterilized river and seawater; that is, in the apparent absence of indigenous microorganisms. Mitchell and Starzyk (1975) observed T_{90} for *E. coli* varied inversely with temperature from 5 to 20°C in river water, and Jamieson *et al.* (1976) noted that low temperatures favoured the survival of *E. coli* in seawater. These results indicate a purely physico-chemical effect of temperature on bacterial survival, although filtration of water samples through 0.45µm filters in the former study and 0.5µm in the latter would not exclude organisms of the *Bdellovibrio* group, as originally described by Stolp and Starr (1963) and which are known to be effective in the destruction of *E. coli* in seawater (Mitchell, 1971; Hendricks, 1974). Similarly, workers using membrane filter chambers in the laboratory (McFeters and Stuart, 1972) and *in situ* (Faust *et al.*, 1975; Vasconcelos and Swartz, 1976) have demonstrated an inverse relationship between coliform survival and temperature. In these experiments, also the use of 0.4-0.45µm filters does not eliminate the role of the bdellovibrios.

The work of Verstraete and Voets (1976) has begun to elucidate the temperature-microorganism interaction. In order to evaluate the

relative effects of various biotic components of the microbial community such as algae, protozoa and bacteria, on the survival of *E. coli* in two aquatic ecosystems, a fractionation procedure was used (Table 1). The survival of *E. coli* in each sample showed that the decline was successively reduced with each fractionation step, with little change in the autoclaved sample. The higher die-off indices which occurred in summer with the entire microbial population present did not always correspond to the higher die-off indices observed in the absence of the indigenous microflora (Sample d). This indicates that the temperature effect is not just of a physico-chemical nature, but is also partly due to increased ecological interactions as a result of the higher temperatures. However, there are two major drawbacks with this fractionation procedure. At each filtration step, a number of smaller species, other than the organisms to be removed, are retained on the filter, as are some suspended materials which,as noted earlier,are important for bacterial survival. Also, filtration through 5µm filters may still allow the passage of protozoa such as the flagellates, some of which are as minute as the larger bacteria. The use of antibiotics may be a more suitable method of fractionation, but has received little attention in studies of coliform survival in seawater.

C.4 Nutrient Utilization

Under many circumstances the survival and more particularly the reproduction of heterotrophic bacteria is associated with the availability of nutrient materials. Numerous reports in the past 30 years have shown a reduction in the rate of decline or the stimulated growth of enteric bacteria following the introduction of nutrients into water. Burke and Baird (1931) demonstrated that many fresh water bacteria grow in seawater supplemented with organic matter. Similarly, Vaccaro

TABLE 1 : Fractionation of Water Samples

(Adapted from Verstraete and Voets (1976))

SAMPLE	WATER TREATMENT	ORGANISMS REMOVED	ORGANISMS REMAINING
a.	Nil	Nil	Total microflora
b.	Filtration 5µm	Algae and protozoa	Bacteria and bacteriophages
c.	Filtration 0.45µm	Algae, protozoa and bacteria[a]	Bdellovibrio and bacteriophages[b]
d.	Autoclaved 120°C/10 min	Total microflora[b]	Nil

[a] Except bdellovibrios

[b] Including antimicrobial substances

et al. (1950) showed that the rate of death of *E. coli* in seawater was reduced by the addition of glucose. Orlob (1956) noted that the length of the lag phase, the maximum growth level, and the T_{90} for coliform bacteria were directly proportional to the concentrations of lactose broth added to sewage-seawater dilutions. In later studies, Savage and Hanes (1971) observed that as the nutrient status of seawater increased, as measured by increasing biochemical oxygen demand (BOD), the density of total and faecal coliforms increased. Also, an increase in the concentration of sewage in seawater resulted in an increase in the survival of coliforms in seawater (Strasdine, 1976). Similar results have been observed for inorganic nutrients and Carlucci and Pramer (1960b) noted that the addition of $(NH_4)_2SO_4$ or $(NH_4)_2PO_4$ increased the survival of *E. coli* in seawater and suggested that nitrogen was of greater importance than phosphorous.

The increased survival of foreign bacteria in seawater following the addition of nutrients may be due to the microbial predators, naturally present in seawater, preferentially utilizing the supplemental nutrients instead of the foreign bacteria (Mitchell and Chamberlin, 1975). Alternatively, the increased nutrient levels may result in an increase in the competitive ability of introduced bacteria compared to the native non-predacious microflora. Jannasch (1968), in a series of chemostat studies, showed that when the concentration of carbon and energy sources was limiting at low dilution rates, *E. coli* was competitively displaced by the native microflora. Thus, under the low nutrient conditions normally found in seawater, enteric bacteria cannot compete with the indigenous microorganisms and are eliminated (Moebus, 1972a; Ogawa, 1974). A similar situation occurs in soil, where the low survival of *E. coli* was thought to be due to its inability to step down its metabolic rate to meet the low availability of usable organic carbon

in the soil (Klein and Casida, 1967). A complication which arises here, is that bacteria consume carbon and energy sources for maintenance as well as for growth (McGrew and Mallette, 1962). Thus, at the low growth rates which occur in seawater, this critical level of nutrient required for maintenance may form a major fraction of the energy requirements particularly for *E. coli* and other enterics. Savage and Hanes (1971) observed that below a certain critical level of BOD, no growth occurred for total or faecal coliforms but that once this level was reached growth increased as BOD increased.

The increase in the nutrient level in seawater required for the increased survival of enteric bacteria may come from the sewage plume itself, which may be considered as a dilute culture medium containing some of the most important nutrients in low concentrations. Hendricks and Morrison (1967) found that enteric bacteria were able to grow and multiply in low temperature environments typical of a cold mountain stream, when supplemented with dilute nutrients. Sewage plant effluent added sufficient organic and inorganic nutrients to stimulate this growth to reach levels higher than that in uncontaminated river water. Alternatively, as enteric bacteria decline due to predation, solar radiation and other causes, the protoplasm of these cells may serve as a nutrient source for the surviving organisms. This phenomenon of cryptic growth has been observed in the laboratory (Buck *et al.*, 1952) as a series of decreasing cycles, each cycle consisting of a decrease in numbers of coliform bacteria followed by an increase in numbers. Eventually the protoplasm which consisted in part of carbohydrates, fats and protein is converted by a series of biochemical pathways into carbon dioxide, hydrogen, water, ammonia and other compounds which cannot be utilized by the coliforms as a food supply and so the bacteria disappear completely.

The importance of nutrients in the survival of enteric bacteria in seawater, as with all other factors affecting survival, cannot be considered in isolation. It has been shown by Waksman and Carey (1935a) in their investigation of the decomposition of organic matter in the sea, that seawater contains sufficient organic matter in true solution to support a more extensive bacterial population than is usually observed. In a further study (Waksman and Carey, 1935b), it was demonstrated that this organic matter was not completely resistant to bacterial degradation although the destruction of bacteria in seawater was not accompanied by a decrease in the rate of decomposition of organic matter in the water (Waksman and Hotchkiss, 1937). The latter authors concluded that other organisms such as nannoplankton also affected the decomposition of the organic matter, as well as the destruction of bacteria. Similarly, Hendricks and Morrison (1967) observed that although growth of enteric bacteria in the presence of nutrients occurred in *in vitro* studies, the self-purification mechanisms, such as predation and solar radiation, suppress this growth in the natural environment. Nutrient related effects may become more important in specialised environments such as bottom sediments. The concentration of organic and inorganic nutrients in river sediments (Hendricks and Morrison, 1967) and marine muds (Waksman and Vartiovaara, 1938), for example, is sufficient to support the growth and multiplication of bacteria.

C.5 Salinity

It has been suggested that on the basis of concentration, inorganic salts are the most potentially toxic substances in the sea (Greenberg, 1956). The salinity of surface seawater is 3.3 to 3.8 percent (Harvey, 1957). This may be reduced near river mouths and other areas of

freshwater dilution, while in inland seas, such as the Great Salt Lake, Utah, it may be as high as 27 percent (Zobell *et al.*, 1936). The high salt concentrations may adversely affect fresh water or enteric bacteria by a general osmotic effect or by the presence of specific inhibitory salt concentrations.

Zobell *et al.* (1936) observed that very few bacteria from soil, sewage or the oral cavity, including *E. coli,* could grow on Great Salt Lake water media, and that even 10 percent lake water was inhibitory. Lake water killed 95 percent of sewage bacteria in one minute and apparently halotolerant marine bacteria were killed by a few minutes exposure. Contrary to these results, Fraser and Argall (1954) claimed that *E. coli* was not rapidly killed by Great Salt Lake water if the temperature was low. Fifty percent survival was observed after 24 hours exposure of *E. coli* to lake water at 6°C. They also suggested that the results obtained by Zobell *et al.* (1936) may have been due to a carry-over of the lake water onto the nutrient agar medium used for enumeration of the bacteria, thus further inhibiting their growth. In a recent study Burdyl and Post (1979) examined the survival of *E. coli* in Great Salt Lake water and concluded that although the water had high concentrations of salt, its ionic composition was similar to that of seawater, and did not have a significantly different bactericidal effect on *E. coli* than did seawater itself.

Similarly, other research has indicated that fresh water bacteria survive in seawater nearly as long as in tap water (Burke and Baird, 1931), bacterial persistence being influenced by temperature and the presence of organic matter. Some fresh water bacteria could survive for a considerable time in broth containing 2 to 4 times the concentration of salt in the sea. This suggested that increased salt

tolerance was developed, which eventually allowed growth in concentrations in excess of that found in the sea. Similarly, Zobell (1936) noted that most fresh water bacteria could become acclimatized to and actually grow in seawater media, despite the physiological and often morphological changes which occurred. He also observed, as did Carlucci and Pramer (1960b) and Bernard (1970), that coliform bacteria survived longer in the presence of low concentrations of salt that in its complete absence. In fact, Zobell (1936) noted greater survival in 6 percent NaCl solutions than in natural seawater, although the effect of predators must also be considered in this case.

Carlucci and Pramer (1960b) however, found that the survival of *E. coli* in seawater and NaCl solutions was comparable and inversely related to salt concentration. Similarly, Faust *et al.* (1975) suggested that an inverse relationship existed between the survival of *E. coli* in estuarine water *in situ* and salinity concentrations of the water. This apparent relationship is in doubt however, due to the changing water temperature, a factor which alone had a strong influence on bacterial survival, and also the possible synergistic effect of these factors (Cooper and Morita, 1972). Several studies have found highly significant negative correlations between coliforms and faecal coliforms, and salinity in natural water systems (Goyal *et al.*, 1977; Hirn *et al.*, 1980), but Nusbaum and Garver (1955), Orlob (1956),and more recently Jamieson *et al.* (1976),considered that salinity alone at levels commonly found in ocean water is not particularly detrimental to enteric bacteria.

C.6 Heavy Metals

Heavy metals were implicated as an important factor in bacterial decline in seawater by Jones (1963). He observed,as did Carlucci *et al.*

(1961) that synthetic seawater, like natural seawater, exerted a bactericidal effect, and suggested that this may be due to traces of toxic ions. In later work (Jones, 1964; Jones and Cobet, 1975), a variety of organic chelating agents,when added to seawater in concentrations calculated to react with the concentrations of heavy metal ions in the sea, reversed the toxicity of seawater, thereby improving *E. coli* survival. The addition of metal-complexing agents in the appropriate concentrations also reversed toxicity (Jones, 1964). The often observed beneficial effect of autoclaving on bacterial survival was said to be due to the removal of trace metals by precipitation (Jones, 1967a). It was observed that major fractions of the originally present Al, Ti, Cr, Fe, Cu, Ag, Sn and Pb were precipitated during autoclaving, although there was generally more precipitate from synthetic rather than natural seawater. Graham and Sieburth (1973) examined the effect of temperature, salinity, dissolved organic carbon, trace metals, and diatom metabolites on the growth and death of *Salmonella typhimurium* in artificial and natural seawater and suggested that trace metals were a major influence. The presence of added organic matter caused the partial reduction of trace metals by chelation and promoted better growth, while the removal of trace metals by autoclaving inhibited growth in distilled water. The beneficial effect of small amounts of heavy metals for the vital physiology of living cells was recognised by Jones (1964), who noted that there was a marked specificity of these cations for their particular function in the cell. Too little of certain metals (such as transition elements) resulted in a loss of enzyme function, too much produced toxicity.

In the natural sea environment, the fluctuations in the concentration of heavy metal ions may be affected by various factors (Jones, 1964).

These include shifts in pH, the biological uptake of heavy metal ions, concentration and type of residual organic matter,and the availability of fresh outside sources of these ions. These fluctuations account for the variable bactericidal action observed in different seawater samples. Jones (1967b) examined the growth of *E. coli* in heat and copper-treated synthetic seawater, but concluded that the concentration of copper in natural seawater was at least an order of magnitude too low to inhibit the growth of *E. coli* without other contributing factors. It was considered, however, that the combined toxicity of heavy metal ions in seawater may be the major physiological reason that bacteria are killed in seawater.

C.7 Bacterial and Algal Antibiotics

The rapid death of bacteria in seawater has long been considered to be affected by an indigenous microflora of antibiotic-producing organisms. Rosenfeld and Zobell (1947) tested 58 species of marine microorganisms but found only 9 (16 percent) which were active against non-marine forms, and of these the most actively antagonistic were *Bacillus* and *Micrococcus* species. Similarly, Krasilnikova (1962) isolated 326 bacterial strains from various depths in the world's oceans with only 27 (8 percent) exhibiting antibiotic activity, the greatest percentage of antagonists being sporogenous bacteria. Both groups of authors suggested that the bactericidal activity of seawater was due, at least in part, to antibiotic-producing organisms. In a more recent study (Ituriaga and Garcia-Tello, 1970), 15 percent of the 20 marine bacteria tested were found to be antagonistic towards *Enterobacteriaceae* while Trunova and Izgoreva (1976) noted that 60 percent of 180 strains examined exhibited significant antibiotic activity against pathogenic test bacteria. The antibiotic-producing

strains isolated by the latter authors belonged to the genera *Pseudomonas, Bacterium* and *Micrococcus*. In earlier work, however, the production of antibiotics against *E. coli* or *Bacillus subtilis* was not demonstrated in tests of some 200 marine bacteria (Carlucci and Pramer, 1960c). There was no evidence that antibiotics were produced under natural conditions by marine microorganisms and thereby contribute to the death of *E. coli* cells in seawater. It has been suggested (Aubert *et al.*, 1975) that this failure to isolate antibiotic-producers was a result of the large fluctuations in population numbers exhibited by these organisms and their heterogeneous spatial distribution.

Thus, although bacteria capable of producing antibiotics under laboratory conditions are widespread, there is little evidence to suggest that they are active under natural conditions.

Early research in the field of antibacterial compounds of phytoplankton origin was conducted by Pratt *et al.* (1944) who demonstrated the antibacterial properties of a fresh water green algae. Later Sieburth (1959) observed the presence of an antibacterial substance liberated by certain species of phytoplankton. This compound was capable of retarding bacterial development in surface seawater and also in the gastrointestinal contents of penguins that had fed on phytoplankton-eating crustaceans. He suggested that as most marine animals obtain their food from phytoplankton either directly or indirectly, then this antibacterial compound would be of importance in determining the numbers and types of bacteria in seawater. Sieburth (1960) isolated this substance from *Phaeocystis,* a mucilaginous colonial algae and identified it as acrylic acid. This antibacterial action of marine phytoplankton has also been observed by several other workers (Saz *et al.*, 1963; Aubert *et al.*, 1964; Duff *et al.*, 1966; Aubert *et al.*, 1975). Although

antibacterial activity had been reported in laboratory studies, the ecological importance of these compounds in natural conditions was first observed with the work of Sieburth and Pratt (1962). The anticoliform activity of seawater was associated with the termination of *Skeletonema costatum* blooms, the seasonal changes observed in the anticoliform activity being correlated with the life cycles of phytoplankton communities. Similarly, Moebus (1972a) suggested that the breakdown of phytoplankton flowerings produced the most pronounced influence on the antibacterial activity of seawater and accounted for the observed seasonal changes in the antibacterial activity.

These conclusions have subsequently been questioned. Moebus (1972b) observed that filter-sterilized seawater showed less antibacterial activity than fresh seawater and did not always vary signficantly with season. The antibacterial capacity of seawater depended on the availability of nutrients which, in turn, greatly depended on phytoplankton development. Also, Mitchell and Chamberlin (1975) suggested that the peak of phytoplankton blooms may coincide with a peak in the growth of microbial predators, the predators themselves serving as the antibacterial agent. In fact, Moebus (1972a)observed increased growth of the indigenous bacterial populations following phytoplankton growth and suggested that they may be important in the inactivation of test bacteria due to the competitive consumption of nutrients. The significance of antibacterial toxins produced by planktonic algae in natural conditions is thus unclear.

C.8 Bacteriophages

Guelin (1948) (cited Carlucci and Pramer, 1959) demonstrated that anti-coli bacteriophages were not only present in seawater but that their numbers varied directly with the degree of sewage pollution.

Zobell (1946) however, reported that bacteriophages occurred only sporadically in seawater and there was insufficient evidence for them to be considered of importance in limiting the bacterial population of the open sea. There have been numerous reports on the isolation of marine bacteriophages active against indigenous marine bacteria (Kriss and Roukina, 1947; Spencer, 1955; Hikada, 1971) and foreign bacteria such as *E. coli* (Gabrielli, 1971; Roper and Marshall, 1974). Although Ware and Mellon (1956) examined the coli/coliphage relationship in sewage and found no evidence to suggest that bacteriophage were important in reducing coliform numbers in sewage treatment, the most extensive work in this area was conducted by Carlucci and Pramer (1960d). Bacteriophages could be isolated from seawater when enrichment procedures were used and were able to persist in autoclaved seawater. Their contribution to the death of bacteria in seawater was found to depend on the nutrient status of the water. If the level of organic matter is high enough to support bacterial growth, such as in areas of pollution, then phage multiplication may occur and they may then be of significance in reducing bacterial counts. In seawater free of pollution, however, there will be little dissolved organic matter, and no extensive growth or multiplication of bacteria,and so phages will have little or no effect on bacterial survival. It appears, therefore, that bacteriophages may be of only minor significance in affecting the survival of enteric bacteria in seawater.

C.9 Predators

The increased survival of bacteria in seawater subjected to sterilization by autoclaving, filtering, pasteurizing or chlorinating, compared to their survival in natural seawater, has been reported on numerous occasions (Ketchum *et al.*, 1949; Vaccaro *et al.*, 1950; Moebus,

1972a). Various explanations such as changes in pH and salinity, increases in organic matter, removal of heavy metals by precipitation, destruction of antibiotics and bacteriophages, have been suggested, but most of these have been discounted (Carlucci *et al.*, 1961). An alternative suggestion for the removal of this thermolabile factor from seawater is the destruction of the indigenous microbial population and in particular bacterial and protozoan predators of enteric bacteria.

C.9.1 Bacterial Predators

The role of bacterial predators in the removal of non-marine bacteria from seawater received very little attention until the work of Mitchell and Nevo (1965), who isolated a marine bacterium *Pseudomonas*, capable of killing *E. coli* in artificial seawater by enzymatically degrading the cell walls. In later studies (Mitchell *et al.*, 1967; Mitchell, 1968; Mitchell and Morris, 1969), the decrease in *E. coli* numbers was found to be related to the size of the marine microbial population. As the size of the population increased the death rate of *E. coli* increased, with almost no decrease in autoclaved seawater. Mitchell *et al.* (1967) suggested that a specific lytic microbial population developed in seawater following the inoculation of *E. coli* into seawater. The reinoculation of *E. coli* into seawater 5 days after the initial inoculum resulted in the virtual elimination of the original lag phase and the almost complete destruction of *E. coli* cells. A similar reduction in lag phase and increased destruction of *E. coli* cells has also been observed in subsequent studies (Roper and Marshall, 1978; McCambridge and McMeekin, 1979).

With the use of a double-layer plating technique utilizing prey species as the sole carbon source, two groups of organisms were found

☆Microscopic examination of seawater filtered through 0.45 micron filters (Enzinger and Cooper, 1976) showed the presence of large numbers of *Bdellovibrio*, but these had little effect on *E. coli* survival. Previous studies also indicate the minor role of *Bdellovibrio* in the Derwent Estuary (McCambridge, 1977) which may be the result of water temperatures of less than 15°C (Roper, pers. comm.).

McCambridge, J. (1977). Factors affecting the survival of *Escherichia coli* in the Derwent Estuary. Honours Thesis, University of Tasmania.

to be associated with the decline of *E. coli* (Mitchell *et al.*, 1967). The first was a cell wall lysing bacterium of the *Pseudomonas* group which degraded *E. coli* cell walls by extracellular enzymes exposing the spheroplast to osmotic shock. The second was a group of marine, obligately parasitic bacteria similar to *Bdellovibrio bacteriovorous*. The latter group of organisms are capable of passing through a 0.45µm filter and have a wide host range including a variety of marine and non-marine gram-negative bacteria. They have been repeatedly isolated from seawater (Taylor *et al.*, 1974; Marbach *et al.*, 1976) although their importance in bacterial decline in rivers, waste water and estuarine water is in some doubt (Fry and Staples, 1974; Enzinger and Cooper, 1976; Westergaard and Kramer, 1978). A third group of predacious bacteria, the marine myxobacteria, have also often been isolated (Peterson, 1969; Brockman, 1973), and have been implicated in the decline of *E. coli* in seawater (Roper and Marshall, 1977). The latter authors identified a myxobacter of the genus *Polyangium*, the vegetative bacteria, being 1µm in diameter and 2.5 to 4.5µm in length. This bacterium requires direct contact with prey organisms for the induction of lysis which is believed to be caused by enzymes located on the surface of the myxobacter (Roper and Marshall, 1978). This organism was able to utilize a wide range of hosts including *E. coli*, *Klebsiella aerogenes* and *Salmonella typhimurium*, although not *Streptococcus faecalis*, and could bring about a decline of *E. coli* in natural and autoclaved seawater (Roper and Marshall, 1977).

Enzinger and Cooper (1976) investigated the role of bacteria and protozoa in the removal of *E. coli* from estuarine waters and concluded that the survival of *E. coli* was dependent on the presence of protozoan predators and not on the presence of lytic bacteria. The presence in plaques on double-layer plates of bdellovibrios, non-fruiting myxobacteria

and numerous types of lytic gram-negative bacteria, was demonstrated, but the authors suggested that bacterial competition, antagonism and even bacterial predation were relatively unimportant in removing coliforms from estuarine waters. The importance of bacterial predators, therefore, remains to be clarified.

C.9.2 Protozoan Predators

C.9.2.a Food preferences of protozoa

There have been numerous reports on the effect of different bacterial prey on the growth of protozoa. Burbanck (1942) observed that bacteria in the family *Enterobacteriaceae* supported the highest division rate of the ciliate *Colpidium colpoda,* and the family *Bacillaceae* the lowest. Similarly, Kidder and Stuart (1939), Curds and Vandyke (1966) and Taylor and Berger (1976) have demonstrated that not all bacteria are suitable for the prolonged survival of all protozoa and in fact some bacteria are toxic to certain protozoa. Kidder and Stuart (1939) demonstrated that certain bacterial pigments were toxic to *Colpoda* even in low concentrations while Barna and Weis (1973) observed that some gram-positive cocci were toxic to *Paramecium bursaria*. The latter authors examined the utilization of bacteria as food for *P. bursaria* and observed a great variation in food value. Bacteria yielding the best growth were gram-negative rods, while gram-positive bacilli were poor food organisms. It was also noted that a mixed bacterial flora yielded a growth index (number of organisms at time t/ number of organisms at time 0) intermediate to the growth index on the single bacterial species tested separately.

There are several properties associated with a particular bacteria which play a part in their suitability as food. These include the size and shape of a bacterium (Berk *et al.*,1976), age, spore and capsule production,

chemical composition and extracellular products. Also, certain gram-positive bacilli form long chains or clumps thus limiting their ingestion by protozoans. It has also been suggested (Taylor and Berger, 1976) that wild strains of commonly occurring bacteria isolated from a pond possess mechanisms to discourage predation. These bacteria produced poorer growth from four naturally occurring ciliates than with laboratory bacterial strains. Curds (1977) suggested, however, that the dominant bacteria in the pond and therefore the ones most likely to be isolated would be the bacteria least preferred by the protozoa. The bacterial prey most preferred on the other hand, would be in low numbers due to protozoan predation. Recently Fenchel (1980a) suggested that no qualitative discrimination of food sources occurred among suspension-feeding ciliates, but that a particle size discrimination as a function of the morphological properties of the protozoan mouth apparatus did occur. This particle size selection however, may, under natural conditions, still lead to a selection for certain types of bacteria or other food particles.

C.9.2.b Effects of predation

In a study of the role of ciliated protozoa in an activated-sludge plant, Curds *et al.* (1968) observed a significant drop in the numbers of bacteria in effluents following the introduction of ciliates. Two possible explanations were suggested; either bacteria were food organisms for the protozoa, or certain cilates had the ability to flocculate bacteria. Later work by Curds and Fey (1969) and Curds (1973) enabled predacious protozoa to be implicated as playing the dominant role in the removal of *E. coli* and total viable bacteria in an activated sludge plant. There was, in fact, some death of *E. coli* when the sludge was free from protozoa, this possibly being caused by the lytic action

of bacterial predators. The role of protozoa in the removal of bacteria from sewage treatment plants has thus been firmly established.

One of the earliest studies into the role of protozoa in the purification of fresh water streams was conducted by Jordan (1900) who suggested that plankton may exert an influence on the bacterial population of the stream by actually consuming the bacteria. The effect of protozoa on bacterial death rates, however, was not suggested until later (Purdy and Butterfield, 1918). In the presence of protozoa, bacterial reduction occurred, protozoan predators consuming bacteria in large numbers, resulting in the purification of polluted water. Gray (1951, 1952) observed the relationship between ciliate protozoa and bacteria in a Cambridgeshire chalk stream. He suggested the phagocytic activity of the protozoans was the most important factor in the elimination of bacterial pollution in the brook, and that there was a statistically significant relationship between bacteria-eating ciliates and gram-negative bacteria. Similarly, Small (1973) concluded that ciliate protozoa were a major factor in the "cleanliness" of polluted streams.

Several workers (Lackey, 1936; Zobell, 1941; Orlob, 1956; Greenberg, 1956), suggested that the decrease of bacteria in seawater was due, at least in part, to the predatory activities of marine protozoa. It was not until later, however, that specific marine protozoa were identified and unambiguously implicated in the decline of *E. coli* in seawater (Mitchell and Yankofsky, 1969; Mitchell, 1971). This decline was parallelled by an increase in a specific microbial population capable of causing lysis of the intestinal bacteria. This population was unstable and rapidly declined after the destruction of the immigrant bacteria. One of the largest responses to the intrusion of foreign

bacteria came from a marine amoeba, *Vexillifera telmathalassa*, as identified by Bovee (1956). This organism was also isolated by Roper and Marshall (1978) who found that it produced a significant kill of *E. coli* in static culture but was not an effective predator in shaken culture. This organism would therefore be most effective on surfaces of particulates (including aggregates of microorganisms) or on the surfaces of large solids (Roper and Marshall, 1978).

Enzinger and Cooper (1976) also showed that the decline of *E. coli* in seawater was associated with the presence of protozoa. In the absence of microflagellates or microciliates *E. coli* numbers were not significantly affected regardless of the types or numbers of bacterial predators present. Similarly, McCambridge and McMeekin (1979) demonstrated that the survival of *E. coli* in estuarine waters was mainly dependent on the presence of protozoan predators and that increasing the size of the protozoan population resulted in increased destruction of *E. coli*. The latter authors observed, however, that the activity of bacterial predators was increased in the absence of protozoans and that predacious bacteria were suppressed by protozoa in natural estuarine water, thereby maintaining them at relatively low levels. Roper and Marshall (1978) observed a sequence of microorganisms responsible for *E. coli* decline. These included predacious and parasitic bacteria and small amoebae in the first one to two days, followed by larger ciliates which fed on the smaller microorganisms up to day 7 when *E. coli* disappeared. By day 10, these large protozoans had degenerated,and finally they disappeared altogether due to lack of food. The suggestion that bacterial as well as protozoan predators are important in *E. coli* decline in seawater is in apparent disagreement with the work of Enzinger and Cooper (1976) (noted earlier). Further research is therefore needed to elucidate the relative roles of these two groups of predacious microorganisms.

C.9.3 Predator-Prey Interactions

There is a very considerable body of literature on predator-prey relationships in natural populations and numerous attempts have been made to develop models to describe these interactions (Canale *et al.*, 1973; Curds, 1974; Yoon and Blanch, 1977). The earliest model presented was the "Lotka-Volterra predator-prey equations" and these have been described on numerous occasions (Williams, 1980). When a predator species feeds upon its prey, its population density increases while the number of prey decrease, until a scarcity of prey results in a decline in predators. As the predators decrease in numbers, the prey numbers increase and the cycle is repeated. This model is, however, based on several basic assumptions; the environment, with respect to all factors affecting the organisms, is uniform (or random) in space and time; all organisms,with regard to their effect on each other and the environment, are identical throughout the populations and time; predation loss is the only limitation on the prey population; predation input determines completely the predator growth rate; and the predatory encounter is random in time and space (Williams, 1980). As some of these assumptions are clearly not indicative of natural ecosystems, Williams (1980) presented a general set of equations based on saturation kinetics which he suggested were more applicable to predator-prey systems. He emphasized, however, that a great deal more work was required to fully understand and describe accurately naturally-occurring predator-prey interactions.

A major problem which emerges in naturally-occurring systems is to explain how a prey species can maintain itself in an ecosystem despite the presence of numerous parasites and predators. Data suggest that there may be a critical population density which the prey organism is

able to maintain in the presence of predacious protozoa. For example, *Xanthomonas campestris*, when added to soil, was reduced by protozoans to approximately 10^5 cells per gram and was maintained at this level (Habte and Alexander, 1975), while a *Rhizobium* sp. was not reduced below 10^7 cells per gram by protozoans in soil (Danso, Keya and Alexander, 1975). Similarly, Drake and Tsuchiya (1976) and Berk *et al.* (1976), in chemostat culture and river water respectively, observed that protozoan predator growth rates were inhibited once the bacteria had been reduced to 10^6-10^7 cells per ml. Comparable results have been reported for the *Bdellovibrio*-bacterium relationship (Keya and Alexander, 1975; Varon and Ziegler, 1978). Both groups of authors observed that *Bdellovibrio* appreciably affects its host only when the host is present in abundance. Further, host populations could survive once its numbers were too low to permit maintenance of the parasitic population. There have been several explanations for the inability of predators to eliminate their prey. The presence of physical barriers may prevent a predator from completely destroying a prey population (Habte and Alexander, 1978a). Although large protozoa may have difficulty pursuing prey located in small soil pores, similar bacterial densities remained in solutions inoculated with *Rhizobium meliloti* and amoeba, so it is unlikely that refuge explains the lack of prey elimination (Danso *et al.*, 1975). Alternatively, predators which attack a variety of prey tend to feed on the most abundant species. As this prey declines in numbers, the predator "switches" the great proportion of its attacks to another prey which has become the most abundant. In this way no prey population is totally eliminated (Murdoch, 1969). But the presence of a large population of alternative prey was not necessary to allow the persistence of *Klebsiella pneumoniae* when attacked by *Tetrahymena pyriformis* (Habte

and Alexander, 1978a). A third possibility is the development of intrinsically resistant members of the prey population. Ende (1973) studied the continuous culture of *T. pyriformis* and *K. aerogenes* and observed the development of bacteria in the population capable of adhering to the wall of the culture vessel. This enabled the bacteria to survive and reproduce; these variants having a significant selective advantage in the presence of the predator and eventually "taking over" the bacterial population. Similarly, Gude (1979) suggested that grazing protozoa in activated sludge select for certain "grazing resistant" bacteria which eventually dominate. Danso and Alexander (1975) suggested that resistance in a prey population was due either to the original population containing cells of differing edibility,or because of the presence of a mutant strain intrinsically resistant to amoebas. They did not, however, find evidence that survivors of *R. meliloti* were not eliminated due to their greater resistance to attack by protozoans. In a more recent study of the *Bdellovibrio-Photobacterium leiognathi* relationship (Varon, 1979), the development of a resistant prey population was observed. Varon (1979) noted that a two-membered culture of the bdellovibrio and a fast-growing susceptible prey evolved into a three-membered culture containing bdellovibrios, a prey type similar to the original, and a dark mutant which grows more slowly and is resistant to bdellovibrios.

A fourth explanation for the persistence of bacterial prey in the presence of actively grazing predators is that the cells consumed are replaced by the continued multiplication of the prey; data presented for the *T. pyriformis-E. coli* relationship (Habte and Alexander, 1978a) supports this hypothesis. Another possible explanation is an increase in toxicity of the medium caused by an accumulation of the metabolic

wastes of bacteria and protozoa (Gill, 1972). The growth and activity of *T. pyriformis* in a spent medium containing possible toxic products was, however, comparable with the growth and predatory activity in fresh medium (Habte and Alexander, 1978a), thus suggesting other alternatives. A final explanation for prey persistence is the possible intraspecific competition amongst predators for prey. Further, as the predator density increases there will also be an increase in the competition for space. This will result in an increase in the physical contact between predators and a reduction in the area searched by each protozoan and may lead to cannabalism (Habte and Alexander, 1978b). To summarise, it would appear that prey resistance and multiplication, and predator competition, are the most likely explanations for the maintenance of low levels of prey species in the presence of predators. It should be noted, however, that the majority of the above-mentioned reports are concerned with single predator-single prey interactions, a situation which rarely arises in natural aquatic ecosystems. A more common occurrence is a number of interacting predator and prey species as observed by Roper and Marshall (1978). So, although bacterial prey persist in soil and in saline solution (Habte and Alexander, 1978a, 1978b), this may not occur in natural seawater with a wide range of potential predators of bacterial and protozoan origin. In fact, *E. coli* cells in seawater, in the presence of the natural microflora, are often reduced to population levels approaching zero (Mitchell and Yankofsky, 1969; Mitchell, 1971; Roper and Marshall, 1978). These aquatic ecosystems may be further complicated by the interaction between bacterial and protozoan predators for example, producing complex, interacting food webs.

D. Indicator Organisms

The presence of indicator organisms in water systems in high numbers is assumed to indicate the possible presence of pathogenic microorganisms. There are two important characteristics of these indicator organisms. Firstly, they must have consistently high densities and be exclusively associated with the faecal wastes of man and other warm-blooded animals. Secondly, their survival during sewage treatment and following their release to receiving waters must be comparable to pathogenic microorganisms. Several authors have investigated the relationship between indicator organisms and selected pathogenic bacteria in water systems. Smith *et al.* (1973) reconfirmed the usefulness of the faecal coliform concentration in water quality analysis, while Olivieri *et al.* (1978) conducted a comprehensive study of sewage, urban streams and stormwater run-off. Data was also presented which supported the hypothesis of a positive correlation between bacterial indicators and bacterial pathogens. The latter authors observed that as the levels of total coliform, faecal coliform and faecal streptococci increased, the levels of *Salmonella* sp., *Pseudomonas aeruginosa* and *Staphylococcus aureus* also increased.

Those organisms which have been considered as water quality indicators, together with their significant sources and potential role as indicators are shown in Table 2. It is not the purpose of this review to provide a full discussion of each indicator system mentioned, although several representative indicators will be considered in detail.

TABLE 2 : Water quality indicators, their significant sources and potential uses.

(Taken from Cabelli (1978))

Indicator	Significant Source[a]	Potential Use[b]
Coliforms	F S I R A	S
Escherichia coli	F S	P F S A
Klebsiella sp.	S I R A	P S N
Enterobacter sp.	S I R A	S
Citrobacter sp.	S I R A	S
Faecal coliforms	F S I R A	F[d] S
Enterococci	F S *c*	F S A D
Clostridium perfringens	F S *c*	F S D
Candida albicans	F S	P F S
Bifidobacteria	F S	F S A D
Enteroviruses	F S	P
Salmonella sp.	F S	P
Shigella sp.	F S	P
Coliphage	[d]S *c*	S
Pseudomonas aeruginosa	S I R A	P S N
Aeromonas hydrophila	S I R A	P S N
Vibrio parahaemolyticus	A	P N

[a] Relative to other sources: F, faeces of warm-blooded animals; S, sewage; I, industrial wastes; R, run-off from uncontaminated soils; A, fresh and marine waters.

[b] Potential Use; P, pathogen; F, faecal indicators; S, sewage indicator; A, separation of human from lower animal sources; D, proximity to faecal source; N, indicator of nutrient pollution.

[c] Insufficient information.

[d] Questionable.

D.1 Coliforms

The most widely used bacterial indicators of faecal pollution in water have been the coliform group (A.P.H.A., 1976). These are defined as "all of the aerobic and facultative anaerobic, gram-negative, non-spore-forming, rod-shaped bacteria that ferment lactose with gas formation within 48 hours at 35°C". It was originally considered that these organisms exclusively inhabited the intestinal tracts of warm-blooded animals suggesting that all coliforms were of equal sanitary significance, and that their presence in water resulted from direct faecal contamination. Since coliforms have been isolated subsequently from numerous sources such as plants and soil, their presence does not necessarily infer faecal contamination. More recently,use has been made of the faecal coliform group of organisms as a more precise method of indicating faecal pollution. Faecal coliforms are defined as those members of the *Enterobacteriaceae* which ferment lactose with the production of gas within 24-48 hours at an elevated incubation temperature of 44.5°C (A.P.H.A., 1976). A large percentage of coliforms in faeces and sewage, however, fail to respond positively to the elevated temperature test. Mishra *et al.* (1968) found that 18 percent of coliforms from human faeces, 24 percent from cattle faeces and 43 percent from sewage failed to give faecal coliform positive reactions. Conversely, 55.7 percent of coliform strains from soils gave faecal coliform positive tests, although this was likely as the area from which samples were withdrawn was frequently polluted. The use of faecal coliforms,therefore, as indicators of faecal pollution, must be carefully considered. Many national and international standards now incorporate both these indicators, and tolerate a higher number of total coliforms than previously permitted, provided that the faecal coliforms are

strictly limited. The most commonly used indicator organism of the faecal coliform group is the bacterium *Escherichia coli*. In the following discussion, the survival of other indicator organisms of the coliform group (*Enterobacter aerogenes* and *Klebsiella pneumoniae*), the faecal streptococci (*Streptococcus faecium*), and a potential pathogen (*Salmonella typhimurium*), will be compared with that of *E. coli* in order to determine the usefulness of this organism as an indicator

D.1.1 *Enterobacter aerogenes*

Although *Enterobacter aerogenes* occurs in soil and water, as well as in faeces, it should, when isolated, be considered as being of faecal origin. There have been very few studies comparing the survival of *E. aerogenes* and *E. coli* in water and these have produced conflicting results. Mitchell and Starzyk (1975), using filter-sterilized river water, noted that *E. aerogenes* did not possess great capacity for survival at 5°C, which was the average winter temperature of the river, its survival being only 50 percent that of *E. coli*. At higher temperatures (10 and 20°C) the *E. coli* cells also exhibited greater survival than *E. aerogenes*. Vasconcelos and Swartz (1976), however, using a diffusion chamber *in situ*, found that the survival patterns of *E. coli* and *E. aerogenes* in seawater were similar at the prevailing low temperatures (8.5 ± 0.4°C). The survival in seawater of *E. aerogenes* compared to *E. coli* therefore requires further examination, particularly in the presence of the natural microbial population which was excluded in both of the above-mentioned studies. It should also be noted, however, that *E. coli* appears to survive at least as long, if not longer than *E. aerogenes*, and so the absence of the former would infer the absence of the latter in water systems.

D.1.2 *Klebsiella pneumoniae*

Due to the widespread occurrence of *Klebsiella pneumoniae* in areas apparently free from obvious faecal contamination, it has usually been grouped as a total coliform of no immediate health importance. As noted above, however,the faecal coliform (FC) elevated temperature test is considered indicative of recent faecal contamination and the presence of *E. coli*. Also *K. pneumoniae* is normally carried in the intestinal tract of 30-40 percent of humans and animals (Davis and Matsen, 1974) and environmental isolates from a variety of sources have been reported as FC positive (Dufour and Cabelli, 1976; Bagley and Seidler, 1977). Thus the occurrence of FC positive, environmental *Klebsiella*, particularly in the large numbers found in textile finishing plant effluents (Dufour and Cabelli, 1976),and pulp and paper mill wastes (Duncan and Razzell, 1972), makes *K. pneumoniae* a potential human and animal health hazard. This is particularly suggested by the work of Bagley and Seidler (1978) who examined 97 isolates from pathogenic and environmental origins and found that the environmental isolates were potentially as pathogenic as those from known clinical origins. A knowledge of the survival of this organism in water, whether as an indicator organism or as a pathogen in its own right is thus desirable, but, as for *E. aerogenes*, little information exists. Knittel (1975) found that the occurrence of *K. pneumoniae* was not ubiquitous in surface waters, but when it was isolated *E. coli* could also be found. Vasconcelos and Swartz (1976) observed that, after 2 days in a diffusion apparatus exposed to seawater, viable counts of *E. coli* declined, whereas those of *K. pneumoniae* increased after a slight initial decline, to reach a final concentration similar to the original population level.

D.2 Faecal Streptococci

When the faecal nature of pollution is in doubt, such as when large numbers of coliforms have been isolated, but no *E. coli*, the use of additional intestinal bacteria such as faecal streptococci has been suggested. The faecal streptococci are a heterogeneous group of organisms and, as defined by A.P.H.A. (1976), include the intestinal streptococci from all warm-blooded animal faecal wastes. For the purpose of this review, the term 'faecal streptococci (FS)' will refer to the Lancefield serological group D streptococci, which includes the *Streptococci bovis-equinus* group and the *S. faecalis-faecium-durans* group (enterococci). These groups vary in their distribution in the faeces of warm-blooded animals. Human faeces, for example, contain 74 percent enterococci and zero *S. bovis-equinus* group, whereas livestock faeces contain 30-70 percent enterococci and 19-66 percent *S. bovis-equinus* group (Geldreich and Kenner, 1969). Faecal streptococci, like *E. coli*, do not normally multiply in water; their occurrence therefore indicates direct or indirect faecal contamination.

There have been numerous reports comparing the survival of faecal streptococci and faecal coliforms in fresh water. In early experiments *S. faecalis* was found to survive longer than *E. coli* in dilute buffer (Allen *et al.*, 1952). Similarly, Geldreich and Kenner (1969), using filtered stormwater, observed that the survival of *S. faecalis* was greater than faecal coliforms, which in turn was greater than *S. bovis*. Cohen and Shuval (1973) noted that in sewage treatment plants, a heavily polluted river, a lake and other drinking water sources, the FS were more resistant to the natural water environment and to purification processes than FC. The survival of FC and FS reported by the above-mentioned authors and others (McFeters *et al.*, 1974; Mitchell and Starzyk,

1975; Davenport *et al.*, 1976), may be summarised as *S. faecalis-faecium* group > faecal coliforms > *S. bovis-equinus* group.

Some of the first work examining the survival of faecal streptococci in seawater was conducted by Slanetz and Bartley (1965) using dialysis sacs. It was observed that faecal coliforms in the presence of sufficient nutrient, as in raw sewage, initially increased in numbers and then declined, whereas faecal streptococci showed no growth, but gradually declined. The greater decline of faecal streptococci compared to faecal coliforms may have been due to a high percentage of *S. bovis* and *S. equinus* in the sewage. This pattern was also observed by McFeters *et al.* (1974) who noted that the decline of faecal streptococci exceeded that of coliforms from bovine faecal material in which 25 percent of the faecal streptococci were *S. bovis*. In most cases, however, a similar pattern of decline in seawater to that which occurs in fresh water is observed, that is, faecal streptococci are more resistant than faecal coliforms in the marine environment (Pichot and Barbette, 1978). This has been observed using both pure cultures and diffusion chambers *in situ* (Vasconcelos and Swartz, 1976) and waste water as a source of organisms in laboratory experiments (Hanes and Fragala, 1967). Thus, as faecal streptococci decline more slowly than *E. coli*, they may, after a period of time, become more numerous despite initially being present in lower numbers (Geldreich, 1966). They may, therefore, be more readily detectable in water systems subject to remote or intermittent faecal pollution.

A valuable application of the faecal streptococcus indicator system in water pollution investigations has been through correlation with the faecal coliform group; that is the faecal coliform to faecal streptococci (FC/FS) ratio (Geldreich, 1966). As different animals excrete faecal coliforms and faecal streptococci in different quantities (Table 3),

TABLE 3 : Estimated *per capita* contribution of indicator microorganisms from some animals.

(Taken from Mara (1974))

Animal	Average weight of faeces/24 h wet wt g	Average indicator density per gram of faeces		Average contribution *per capita* per 24 hours		Ratio FC/FS
		Faecal Coliform million	Faecal Streptococci million	Faecal Coliform million	Faecal Streptococci million	
Man	150	13.0	3.0	2000	450	4.4
Duck	336	33.0	54.0	11000	18000	0.6
Sheep	1130	16.0	38.0	18000	43000	0.4
Chicken	182	1.3	3.4	240	620	0.4
Cow	23600	0.23	1.3	5400	31000	0.2
Turkey	448	0.29	2.8	130	1300	0.1
Pig	2700	3.3	84.0	8900	230000	0.04

the FC:FS ratio may show whether pollution derives from human wastes or the wastes of domestic animals. For human wastes and domestic waste waters the FC:FS ratio is greater than 4.0, and for other warm-blooded animals and from farmland drainage and stormwater systems it is less than 0.7 (Geldreich and Kenner, 1969). This difference may be used to differentiate between pollution sources (Table 4).

TABLE 4 :

FC/FS > 4.0	Strong evidence of human waste pollution
2.0 < FC/FS < 4.0	Predominance of human wastes in mixed population
0.7 < FC/FS < 2.0	Predominance of domestic animal wastes in mixed population
FC/FS < 0.7	Strong evidence of domestic animal waste pollution

(Modified from Mara (1974))

A major weakness in the use of this ratio (Geldreich and Kenner, 1969; Feachem, 1974), is that unless the die-off rates of the FC and FS are identical in the water systems sampled, the FC/FS ratio will gradually change and will thus not be indicative of the ratio in the fresh faecal material. It has been suggested therefore (Geldreich and Kenner, 1969), that the FC/FS ratio is valid only during the first 24 hours immediately following the discharge of bacteria into the water system. However, this is often very difficult to estimate, and the differential decline which does occur (Hanes and Fragala, 1967; McFeters *et al.*, 1974) led the latter authors to conclude that the FC/FS ratio was no longer of any significance. It has been claimed, however (Feachem, 1975), that this differential decline (*S. faecalis-faecium* > FC >

S. bovis-equinus) can in fact strengthen the value of the FC/FS ratio if a series of FC and FS concentrations are obtained through time. A predominantly human source should exhibit an initially high (>4) ratio which should then fall, whereas a non-human source should exhibit an initially low ratio (<0.7) which should subsequently rise. This is summarised in Table 5.

TABLE 5 : Faecal source related to FC/FS ratio

(Taken from Feachem (1975))

Initial FC/FS ratio	Change through time of FC/FS ratio	Probable faecal source
>4	Rise	Uncertain
	Fall	Human
<0.7	Rise	Non-human
	Fall	Uncertain

D.3 *Salmonella* sp.

There is considerable evidence as to the fate of human enteric pathogens such as *Salmonella* sp. when introduced into seawater. Much of this research, however, has produced conflicting results. Stryszak (1949) observed the rapid disappearance of bacteria of the *Salmonella* group (including *S. typhi* and *S. typhimurium*) in unsterilized seawater, and suggested this was due to the antagonistic microflora. Similarly, Nabbut and Kurayiyyah (1972) noted that the survival of *S. typhi* was much less in untreated seawater than in autoclaved or filtered seawater due to the removal or destruction of predators or competitors. Buttiaux and Leurs (1953) however, noted that *S. typhi*, although showing an initial decrease in numbers following exposure to seawater, exhibited

an appreciable regrowth. Also, *S. typhimurium* was virtually unaffected by 24 hours exposure to seawater,while these organisms, together with *S. paratyphi* and *S. enteritidis* showed high mortalities in spring water. The first comprehensive examination of the survival of *Salmonella* sp. (*S. typhimurium*) in seawater was conducted by Graham and Sieburth (1973). Although the majority of their experiments involved the use of artificial seawater (ASW), they examined the influence of temperature, salinity, dissolved organic carbon, trace metals and diatom metabolites on the growth and death of *S. typhimurium*. In unsupplemented ASW, salinity had little affect on survival, while above 15^{o}C, the higher the temperature the greater was the antibacterial activity. It was suggested that of the 5 parameters examined, trace metals had the major influence on bacterial survival both in ASW *in vitro* and in natural seawater *in situ*.

If *E. coli* is to be used as an indicator of the presence of pathogens such as *Salmonella* their survival in seawater must be comparable, but, as for the survival of *Salmonella* sp. in seawater, comparisons with *E. coli* survival have produced variable results. Slanetz and Bartley (1965) and Paoletti *et al.* (1978) found that the rate of death of *Salmonella* sp. was not as rapid as that for coliforms, although both organisms had similar shaped survival curves. McFeters *et al.* (1974) however, observed that the die-off rates of coliforms was similar to that of some salmonellae (*S. enteritidis* ser. *paratyphi* A and D, *S. enteritidis* ser. *typhimurium*) but less than the die-off rates of others (*S. typhi, S. enteritidis* ser. *paratyphi* B). The die-off rates for the *Salmonella* sp. should only be considered as preliminary results as they were calculated from the decline of one organism only. The results do, however, emphasize the need for the use of individual species if the survival of pathogenic and indicator organisms is to be compared to obtain meaningful results.

The survival of *E. coli* and *S. enteritidis* was compared in unsterilized river water (Park, 1978) and in diffusion chambers in seawater (Vasconcelos and Swartz, 1976). The former author, using streptomycin-resistant mutants of both organisms, noted a similar survival for the first 4 days. After this time the numbers of both organisms remained virtually unaltered for up to 15 days with *E. coli* showing greater survival. Vasconcelos and Swartz (1976) however, observed a far greater survival of *S. enteritidis* than *E. coli* after 6 days exposure to seawater.

Jamieson *et al.* (1976) and Wait and Sobsey (1980), using filter-sterilized seawater and fresh seawater respectively, examined the survival of *E. coli* and *S. typhi*. Both observed the greater survival of *S. typhi*, the former at a range of incubation temperatures (4, 25 and 37^{o}C) and salinities (5, 20 and 35 percent).

The majority of research comparing the survival of *Salmonella* sp. with coliforms has involved the use of *S. typhimurium*, one of the more common salmonellae, and *E. coli*. Graham and Sieburth (1973) found that *S. typhimurium* was more sensitive to artificial seawater than was *E. coli*, whereas Gallagher and Spino (1968), using filter-sterilized industrial waste water, observed the opposite. In a series of survival experiments, Colwell and Hetrick (1975) seeded dialysis sacs with pure cultures of *E. coli* or *S. typhimurium* and suspended these in tanks of continously-flowing estuarine water with inconsistent results. In one experiment the survival of *S. typhimurium* was greater than that of *E. coli* with an average water temperature of 8.9^{o}C. In two further experiments *E. coli* and *S. typhimurium* survived to a similar degree, the average water temperature being 7.5 and 4.9^{o}C respectively for each experiment. The variability of results may have been due to the attack and degradation

of the dialysis bags by cellulolytic organisms at the higher temperatures. This forced the authors to transfer the test water and organisms to new dialysis bags weekly. Mitchell and Starzyk (1975) examined the survival of *Salmonella* and other indicator organisms in filter-sterilized river water. They observed that *S. typhimurium* had a survival pattern essentially the same as *E. coli* over a range of temperatures from 0 to 20°C.

It is obvious from these results that the comparative survival of salmonellae and coliforms remains unclear. Also, these results are difficult to compare due to differences in the test systems and the organisms used in each case (Table 6), although several important points arise from this summary of the available literature. It can be seen that, there have been very few comparisons of *E. coli* and *Salmonella* survival in natural seawater. Also there is little information regarding the role of naturally occurring microbial predators in the decline of these organisms in water as in most cases their possible effect has been removed by filtering or the use of diffusion chambers or dialysis sacs. Finally, there has been no consideration of the survival of *E. coli* and *Salmonella* in mixed culture in seawater, to investigate possible selection of prey organisms by protozoan predators.

TABLE 6 : Summary of *Salmonella* sp. survival in water.

Test System	Organisms Used	Reference
Natural SW	(i) *Salmonella*	Stryszak (1949)
	(ii) *S. typhi*	Nabbut and Kurayiyyah (1972)
	(iii) *S. typhi* and *E. coli*	Wait and Sobsey (1980)
Natural river water	*E. coli* and *S. enteritidis* - streptomycin-resistant strains	Park (1978)
Dialysis bags in SW	(i) *Salmonella* and *E. coli*	Slanetz and Bartley (1965)
	(ii) *S. typhimurium* and *E. coli*	Colwell and Hetrick (1975)
Diffusion chambers in:		
(i) Well water	*S. enteritidis* ser. *paratyphi* A, D and B, *S. enteritidis* ser. *typhimurium* and faecal coliforms	McFeters *et al.* (1974)
(ii) SW	*S. enteritidis* and *E. coli*	Vasconcelos and Swartz (1976)
Filter-sterilized water:		
(i) Industrial waste	*S. typhimurium* and faecal coliforms	Gallagher and Spino (1968)
(ii) River	*S. typhimurium* and *E. coli*	Mitchell and Starzyk (1975)
(iii) SW	*S. typhi* and *E. coli*	Jamieson *et al.* (1976)
Artificial seawater	*S. typhimurium* and *E. coli*	Graham and Sieburth (1973)

III. Materials and Methods

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A. Seasonal and Site Variations in Bacterial Indicator Organisms

A.1 Sampling

Water samples were taken on 20 occasions during a 2 year period from February 1978 to February 1980, from various sites in the Derwent River and Derwent Estuary. The Derwent water system dominates the topography of the area and has lead to ribbon development of the population. The residential population of the area surrounding the Derwent system was 162,537 in 1976 which is serviced by seventeen small sewage treatment plants releasing sewage effluent (liquid and in some cases sludge also) with varying levels of treatment. The location of the sampling sites and treatment plants is shown in Figure 3, and a brief description of each site is given in Table 7.

Samples were collected in 500ml sterile glass bottles approximately 50m from the shore, 100-200mm below the surface. These were kept on ice for no longer than 3 hours before analysis in the laboratory.

A.2 Bacteriological Examination

Water samples were analysed for total coliforms (TC), faecal coliforms (FC), faecal streptococci (FS) and aerobic heterotrophic bacteria (AHB) using standard membrane filtration (APHA, 1976) and spread-plating techniques.

A.2.1 Total Coliforms

In those sites with less than 30 coliforms per ml, membrane filtration was used. After filtration membrane filters were placed on filter pads soaked in Membrane Enriched Teepol Broth (Oxoid, Code MM369). Following incubation at 30°C for 20 hours, yellow colonies only were counted. In those sites with greater than 30 coliforms per ml 0.1ml of the appropriately diluted sample was spread on the surface

Figure 3: Location of sampling sites, sewage treatment plants and population centres in the Derwent River and Derwent Estuary.

Samples sites: 1-11

Sewage treatment plants: no treatment - n

primary treatment - P

secondary treatment - S

Population centres: 

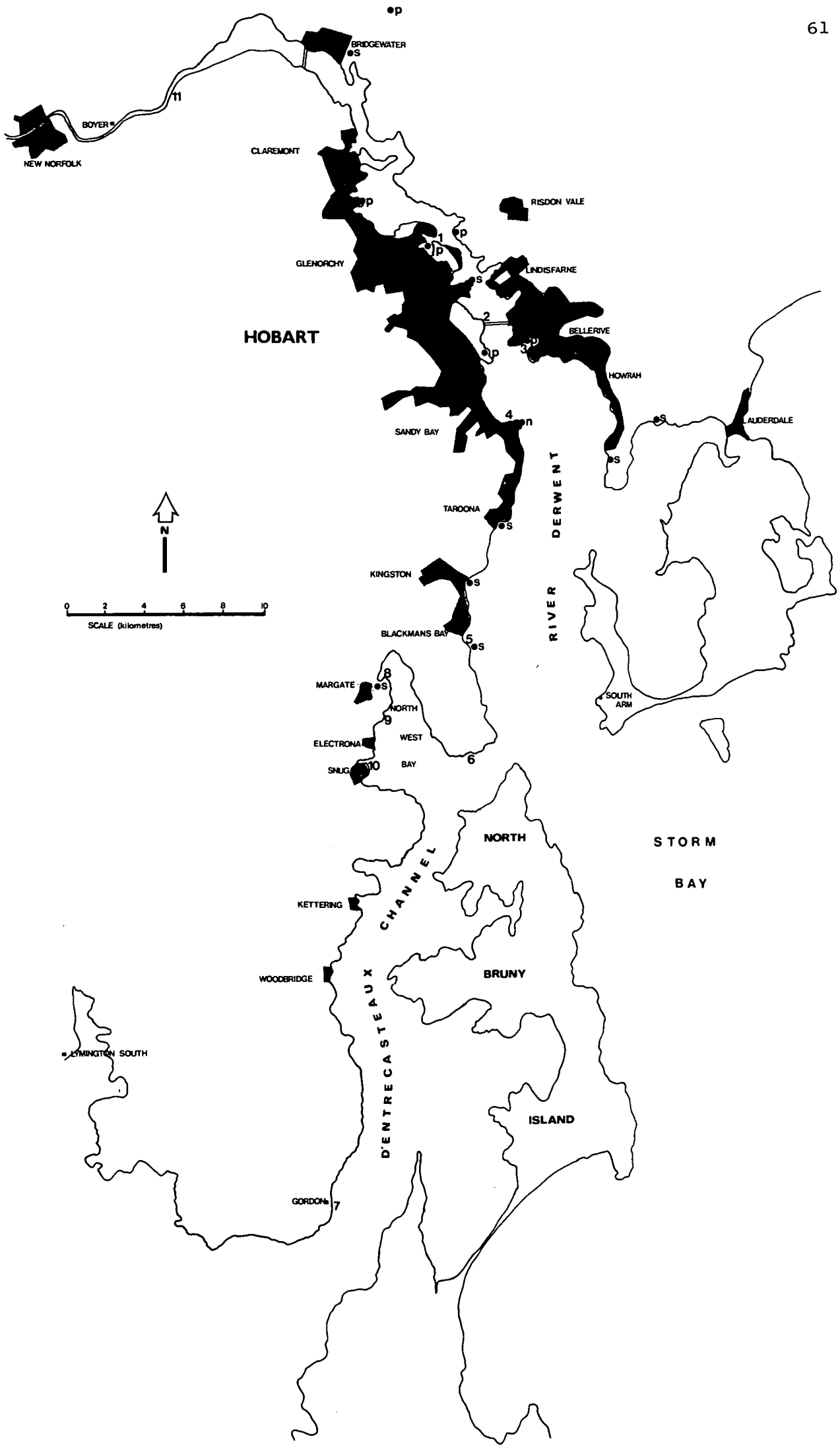

BRIDGEWATER
BOYER
NEW NORFOLK
CLAREMONT
RISDON VALE
GLENORCHY
LINDISFARNE
HOBART
BELLERIVE
HOWRAH
SANDY BAY
LAUDERDALE
TAROONA
RIVER DERWENT
KINGSTON
BLACKMANS BAY
MARGATE
NORTH
WEST
BAY
ELECTRONA
SNUG
SOUTH ARM
NORTH
STORM
BAY
KETTERING
CHANNEL
WOODBRIDGE
BRUNY
D'ENTRECASTEAUX
LYMINGTON SOUTH
ISLAND
GORDON
N
0 2 4 6 8 10
SCALE (kilometres)

TABLE 7: Brief description of sampling sites and year in which each was sampled.

Site No.	Sampling Site	Site Description	Year sampled 1978	Year sampled 1979-80
1	Abbatoirs	High density industrial, 300m from high volume abbatoir (EP4800[a]), high density urban.	+	+
2	Tasman Bridge	High density urban. Adjacent to several primary and secondary sewage treatment plants.	+	+
3	Rosny Point	High density urban, 250m from primary sewage treatment plant (7,100m^3 per day[b]). Disposal pipe 50m offshore.	+	+
4	Blinking Billy Point	Medium density urban, 300m from sewage treatment plant releasing macerated sewage into 30m of water (5,400m^3 per day[b]).	+	+
5	Flowerpot Point	Low to medium density urban, 500m from secondary sewage treatment plant (1,360m^3 per day[b]).	+	+
6	Tinderbox	Rural and recreational area, very low human population.	+	+
7	Gordon	Rural area, very low human population, 100m from oyster bed.	+	+
8	Dru Point	End of sewage outfall pipe, secondary treatment plant (90m^3 per day[b]).		+
9	Marina (North West Bay)	Very low human and animal population.		+
10	Snug River	Site adjacent to entrance of Snug River to North West Bay, low density urban.		+
11	Boyer	Site on Derwent River, 1km south of Boyer (Australian Newsprint Mills). Low animal population. High levels of organic matter.		+

[a] EP = Equivalent Domestic Population.

[b] Southern Metropolitan Sewerage Study (1977).

of Lactose Teepol Agar (Appendix 1). These were incubated at 30°C for 4 hours, followed by 37°C for 20 hours.

A.2.2 Faecal Coliforms

Methods and media used for faecal coliforms were the same as for total coliforms, but incubation temperatures and times varied. In sites with less than 30 coliforms per ml incubation times were 30°C for 4 hours followed by 44°C for 14 hours in a water bath. In sites with greater than 30 coliforms per ml incubation times were 30°C for 4 hours followed by 44°C for 20 hours in a water bath.

A.2.3 Faecal Streptococci

After filtration, membrane filters were placed on KF-Streptococcus Agar (Appendix 1) and incubated at 37°C for 4 hours followed by 44°C for 44 hours in a water bath. Red or maroon colonies were counted.

A.2.4 Aerobic Heterotrophic Bacteria

0.1ml aliquots of water samples were spread on the surface of Salt Nutrient Agar (Appendix 1) and incubated at 22°C for 30 hours.

A.2.5 Bacterial and Protozoan Predators

Bacterial and protozoan predators of *E. coli* were counted as plaque-forming units (PFU) on double-layer plates (bottom layer: Davis agar, 1.5% (wt/vol) in estuarine water; top layer: Davis agar, 0.7% (wt/vol) in estuarine water plus 0.5ml *E. coli* suspension (approximately 10^{10} cells/ml) and 0.5ml of the estuarine water sample to be examined). These were incubated at 22°C for 6 days.

A.3 Salinity Measurements

The electrical conductivity of the water samples was used as a

measure of its salinity. This was determined using a conductivity meter, the specific conductivity being calculated using the formula:

$$Ls = \frac{K}{R}$$

where Ls = specific conductivity (mhos/cm)

K = cell constant = 1.39 (Mullard Conductivity Cell Type E, 7591/A)

R = resistance in ohms as read from the conductivity meter.

This instrument was used to determine the salinity of water samples from February 1978 to January 1979. In the second year of sampling, April 1979 to February 1980, the conductivity was measured directly in millisiemens using a Metrohm Herisau (Model E527) conductivity meter with a conductivity cell (EA608).

The relationship between conductivity and salinity was determined by measuring the conductivity for a series of saline solutions of known strength to obtain a standard curve (Appendix 2).

A.4 Temperature, Solar Radiation and Rainfall

Surface water temperature was measured for each site at the time of sampling using a mercury thermometer. Average daily global radiation for Hobart and total monthly rainfall for several sites were obtained from the Hobart Weather Bureau.

A.5 Analysis of Data

The seasonal and site variations in log numbers of TC, FC, FS, PFU and AHB were examined. To evaluate the possible cause of this variation a statistical analysis of the data was conducted. Correlations between TC, PFU, AHB, temperature, salinity, rainfall and solar radiation were initially derived for each

site separately using the combined data from 1978-79 and 1979-80. The FC and FS counts were excluded from the analysis as neither variate was measured in 1978-79.

Due to the absence of a number of observations scattered randomly throughout the data set, the results for various sites were combined into two groups, although the validity of this grouping could not be statistically tested as a number of sites had insufficient replication. The groups were a "polluted" group consisting of sites 1, 2, 3 and 8 which exhibited high TC counts and an "unpolluted" group consisting of sites 5, 6, 7, 9 and 10 which exhibited low TC counts. Site 4 was intermediate between the two groups and was not considered in comparisons between groups.

The analysis of the structure of the between variate correlations in the two groups was performed on the correlation matrices derived by removing any site effect. A comparison of the two pooled variance-covariance matrices was performed with Box's extension to Bartlett's test (Seal, 1964).

B. Characterization of Experimental Organisms

B.1 Source, Isolation and Growth of Microorganisms

Coliform organisms were isolated from various sites on Lactose Teepol Agar and purified on Nutrient Agar (Appendix 1). Faecal streptococci were isolated and purified from various sites on KF-Streptococcus Agar.

The organisms used in survival experiments in estuarine water, their source and growth media are shown in Table 8. Cells from these cultures were inoculated into the appropriate growth medium and incubated at 37°C on a rotary shaker for 24 hours. The only exception was *S. faecium* which was grown in static culture at 37°C for 24 hours.

TABLE 8: Test organisms, source and growth media.

Test Organism	Source	Growth Medium
Escherichia coli M13	UT[a]	Nutrient Broth (Oxoid Code CM1)
Salmonella typhimurium M48	UT	Nutrient Broth (Oxoid Code CM1)
Enterobacter aerogenes	Site 1	Nutrient Broth (Oxoid Code CM1)
Klebsiella pneumoniae	Site 11	Nutrient Broth (Oxoid Code CM1)
Streptococcus faecium	Site 1	Brain Heart Infusion (BHI) (Oxoid Code CM225)
Erwinia herbicola 851	UQ[b]	Peptone Yeast Extract Broth (Appendix 1)

[a] Culture Collection, Department of Agricultural Science, University of Tasmania.

[b] Culture Collection, Department of Microbiology, University of Queensland.

The cells were then washed by centrifuging at 4000 g for 20 minutes, decanting the supernatant and resuspending the cells in 0.9 percent saline (0.9 percent wt/vol NaCl). This procedure was repeated to obtain a washed cell suspension of approximately 10^{10} cells per ml. To obtain large quantities of bacterial suspensions, 1500ml of the appropriate growth medium was inoculated and incubated as described above for 7 days. Suspensions were generally used immediately after harvest, or otherwise stored at 4°C.

Predacious bacteria of *E. coli* and *S. typhimurium* were isolated from double layer plates (Section A.2.5). A loop of agar was removed from a clearing zone (or plaque) on a double layer plate previously inoculated with an estuarine water sample and placed in 9ml of 0.9 percent saline. After thorough mixing this was then used to inoculate double layer plates which were then incubated at 22°C for 6-8 days. With the appearance of plaques this procedure was repeated until pure cultures were obtained. These were maintained on double layer plates at 4°C.

B.2 Identification of Microorganisms

Coliform organisms were identified using the API20 Enterobacteriaceae (API20E) (Analytab Products Inc., New York) or the Microbact Enterobacteriaceae 12 System (MB12E) (Disposable Products Pty. Ltd., Adelaide).

Typical FS colonies were selected from membrane filters grown on BHI agar slopes at 35 ± 0.5°C for 24 to 48 hours and tested for the absence of catalase by the addition of hydrogen peroxide (3 percent wt/vol). Growth in BHI broth after 48 hours at 45°C and in bile broth medium (Appendix 1) after 3 days at 35°C confirmed isolates as faecal streptococci.

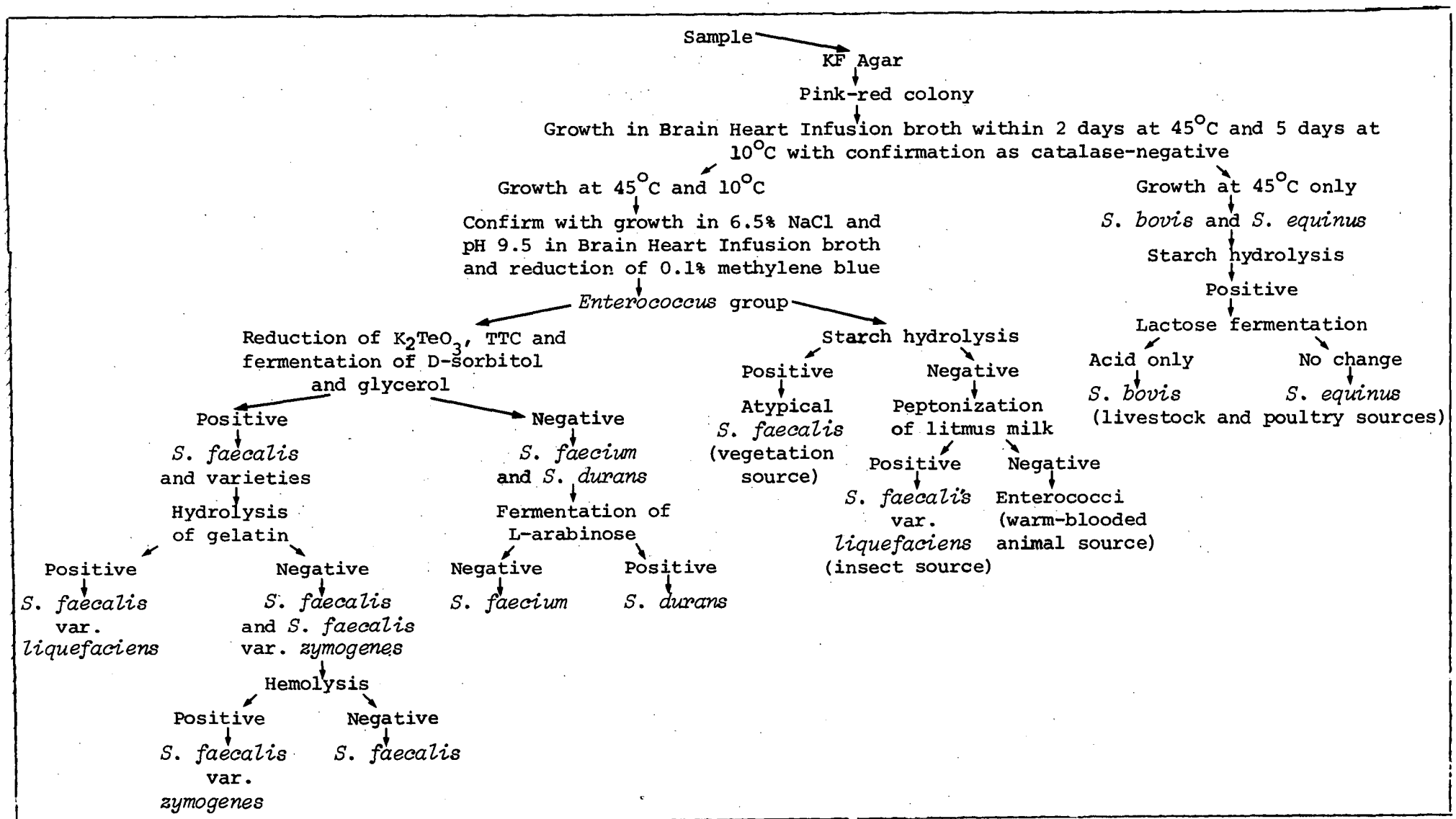
Further identification was achieved by following the schematic outline in Figure 4 (APHA, 1976).

Tellurite reduction was tested on Glucose Yeast Extract Agar (Appendix 1) containing 0.04 percent (wt/vol) potassium tellurite (K_2TeO_3). Reduction to tellurium produces black colonies. Reduction of 2,3,4-triphenyl-tetrazolium chloride (TTC) to red formazan indicated a positive test (Barnes, 1956) and in this case solid medium was used (Appendix 1). Reduction of 0.1 percent methylene blue (or litmus milk) indicated a positive test. Fermentation of D-sorbitol, L-arabinose and glycerol was tested using Hugh and Leifson's medium (Appendix 1). Inoculated tubes were incubated at 37°C for 2 days.

Suspensions of predacious bacteria in 0.9 percent saline selected from plaques were used to assess gram reaction and morphology. Further examination of predacious bacteria was conducted using a transmission electron microscope (TEM). Formvar-coated nickel electron microscope grids were placed on a drop of a bacterial suspension for 15 seconds, removed, and fixed in 1 percent (wt/vol) aqueous uranyl acetate for 10 seconds. Grids were then examined using a Hitachi H-300 TEM operated at 72kV. All micrographs were recorded on Ilford electron microscope film.

The specificity of the predacious bacteria towards various prey organisms was evaluated using three prey organisms - *E. coli*, *K. pneumoniae* and *S. typhimurium*. Heavy suspensions of these prey bacteria were inoculated onto the top layer of double-layer plates, together with a suspension of individual bacterial predators. The appearance of clearing zones after 6 days incubation for *E. coli* predators and 12 days for *S. typhimurium* predators indicated the destruction of that prey by the particular predator. *E. coli* cells were autoclaved at 15 psi for 20 minutes and inoculated onto the top

Figure 4: Schematic outline for identification of faecal streptococci (APHA, 1976).


Sample
KF Agar
Pink-red colony
Growth in Brain Heart Infusion broth within 2 days at 45°C and 5 days at 10°C with confirmation as catalase-negative
Growth at 45°C and 10°C
Confirm with growth in 6.5% NaCl and pH 9.5 in Brain Heart Infusion broth and reduction of 0.1% methylene blue
Enterococcus group
Reduction of K_2TeO_3, TTC and fermentation of D-sorbitol and glycerol
Positive
S. faecalis and varieties
Hydrolysis of gelatin
Positive
S. faecalis var. liquefaciens
Negative
S. faecalis and S. faecalis var. zymogenes
Hemolysis
Positive
S. faecalis var. zymogenes
Negative
S. faecalis
Negative
S. faecium and S. durans
Fermentation of L-arabinose
Negative
S. faecium
Positive
S. durans
Starch hydrolysis
Positive
Atypical S. faecalis (vegetation source)
Negative
Peptonization of litmus milk
Positive
S. faecalis var. liquefaciens (insect source)
Negative
Enterococci (warm-blooded animal source)
Growth at 45°C only
S. bovis and S. equinus
Starch hydrolysis
Positive
Lactose fermentation
Acid only
S. bovis (livestock and poultry sources)
No change
S. equinus

layer of double layer plates to determine if growth of predators occurred on heat-killed cells. The growth of predators on artificial medium was determined by streaking bacteria from plaques onto Nutrient Salt Agar plates (Appendix 1). Plates were incubated at 22°C for 12 days.

B.3 Counting Methods

Viable counts of all bacteria were achieved by making a decimal dilution series in 0.9 percent (wt/vol) saline. The media used, incubation temperatures and times, and where applicable, the colony characteristics of each test organism, is shown in Table 9.

Bacterial and protozoan predators of the test organisms were counted as plaque-forming units (PFU) on double layer plates. In some experiments the addition of the crystalline antibiotic Cycloheximide (The Upjohn Company) to this top layer at a concentration of 500mg per litre enabled the enumeration of predacious bacteria only. This compound is active against eukaryotic organisms such as protozoa, fungi and yeasts, but is tolerated in the concentrations used by most bacteria including *E. coli* (Whiffen, 1948). For all test organisms with the exception of *S. typhimurium*, double layer plates were incubated at 22°C for 6 days. Double layer plates utilising this organism were incubated at 22°C for 12 days.

B.4 Survival of Test Organisms in Estuarine Water Samples

Unless otherwise stated in individual experiments, the effect of various factors on the survival of test organisms in estuarine water samples was determined by inoculation of 1ml of a suspension of the test organism into 49ml of natural estuarine water freshly collected from Site 4.
Bacterial suspensions were also inoculated into 49ml of autoclaved

TABLE 9: Media, incubation temperature and time and colony characteristics of test organisms[a].

Test organism	Enumeration media	Incubation Temperature and time	Colony characteristics
E. coli[b]	MacConkey Agar (Oxoid Code CM7)	37°C/24 hr	Red colour
	C.L.E.D. Medium (Oxoid Code CM301)	37°C/18 hr	Yellow, opaque
	C.L.E.D. Medium (with Andrade indicator (Oxoid Code CM423)	37°C/24 hr	Yellow-orange
S. typhimurium	Brilliant Green Sulphur Agar (Gibco)	37°C/24 hr	
	C.L.E.D. Medium	37°C/18 hr	Flat, blue
	C.L.E.D. Medium (with Andrade indicator)	37°C/24 hr	Blue colour
E. aerogenes	MacConkey Agar	37°C/24 hr	
	C.L.E.D. Medium (with Andrade indicator)	37°C/24 hr	Orange with pink halo
K. pneumoniae	MacConkey Agar	37°C/24 hr	
	C.L.E.D. Medium (with Andrade indicator)	37°C/24 hr	Red-brown colour
S. faecium	K.F.-Streptococcus Agar (Appendix 1)	37°C/24 hr	Small, red
	C.L.E.D. Medium (with Andrade indicator)	37°C/24 hr	Small, orange red
E. herbicola	C.L.E.D. Medium (with Andrade indicator)	37°C/24 hr	Grey-green

[a] The particular medium used is noted for each experiment.

[b] *E. coli* enumerated on MacConkey Agar unless otherwise stated.

estuarine water as a control and all water samples were incubated at 22°C in the dark. At regular intervals test organism survival and predator growth were determined.

C. Seasonal and Site Variations in *E. coli* Survival

Estuarine water was collected from sampling sites 1, 2, 5, 6, and 7 on 9 occasions from March 1978 to February 1979 and the temperature and salinity determined as noted in Section A. *E. coli* cells were inoculated (final concentration approximately 10^8 cells per ml) into natural and autoclaved estuarine water and *E. coli* survival and PFU growth determined every 2 days for a 10 day decline period. The number of *E. coli* cells surviving in the natural estuarine water sample were corrected for the decrease in numbers which occurred in the autoclaved estuarine water samples to evaluate the effect of predators on *E. coli* survival. The log reduction in *E. coli* numbers from day 0 to day 10 was determined for each natural water sample. The sampling times were separated into three periods corresponding to three seasons: season 1 (summer) December, January and February; season 2(spring) September, October and November; season 3(winter) April, June and July. Mean values and standard errors for temperature, salinity, initial PFU and the log reduction in *E. coli* numbers were then compared for each site.

D. Effect of Bacterial and Protozoan Predators on *E. coli* Survival

D.1 Relative Effects of Bacterial and Protozoan Predators

To determine the effect of both protozoan and bacterial predators acting together on the survival of *E. coli* in estuarine water samples, an *E. coli* suspension was added to natural estuarine water to give a

final concentration of approximately 10^8 cells/ml. The effect of bacterial predators alone on the survival of *E. coli* was determined by the inhibition of the protozoan predators. This was achieved by the addition of the antibiotic cycloheximide (final concentration 500mg/litre) to natural estuarine water containing an *E. coli* suspension (approximately 10^8 cells/ml). Microscopic examination of water samples and plaques produced on double layer plates was carried out throughout the duration of the experiment. Two autoclaved estuarine water samples containing a similar *E. coli* suspension were included as control treatments. One of these contained cycloheximide (500mg/litre) to directly determine the effect of this antibiotic on *E. coli*. Three replications of each treatment were incubated at $22^{\circ}C$ for 10 days. Results for *E. coli* survival were analysed by use of analysis of variance techniques (Appendix 6.a.2).

D.2 Effect of Periodic Inhibition of Protozoans

The time interval during which predacious protozoa were active in *E. coli* destruction in a 10 day decline period was determined by the exposure of *E. coli* to these predators for different lengths of time. Following this exposure, protozoan predators were inhibited for the remainder of the experiment.

An *E. coli* suspension (final concentration approximately 10^8 cells/ml) was added to a sample of freshly collected estuarine water (400ml) and incubated at $22^{\circ}C$. At daily intervals for 6 days a 50ml sample was withdrawn, placed in a flask containing cycloheximide (final concentration 500mg/litre) and also incubated at $22^{\circ}C$. The survival of *E. coli* cells and the growth of predators in all flasks was determined immediately following withdrawal of the sample and at 2 day intervals thereafter. An *E. coli* suspension in 400ml of autoclaved estuarine

water was included as a control. This experiment was repeated in more detail with water samples withdrawn at 12 hr intervals for 3 days. Three replications of the complete experiment were conducted.

This was repeated on two further occasions with *E. coli* suspensions of approximately 10^8 and 10^5 cells/ml respectively. In these two experiments total PFU (protozoa and bacteria) were determined as described above. Bacterial predators alone were counted as PFU on double layer plates to which cycloheximide (final concentration 500mg/litre) had been added to the top layer.

D.3 Effect of Initial *E. coli* Concentration

A range of *E. coli* suspensions (10^2 to 10^9 organisms per ml) were inoculated into natural estuarine water and *E. coli* survival and predator growth determined over a 10 day period.

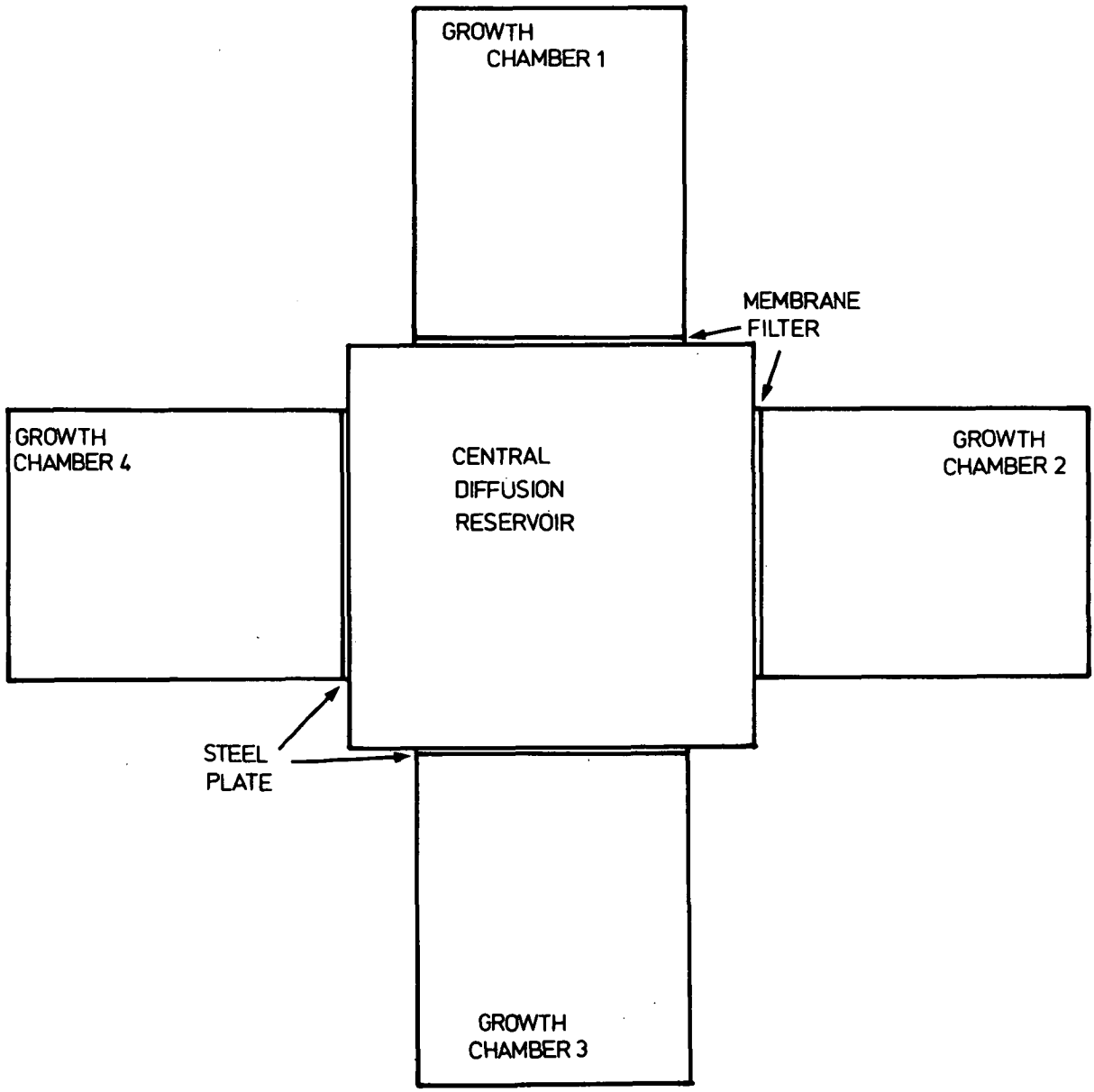
To determine the relative effects of protozoa and bacteria at different initial *E. coli* concentrations, a range of *E. coli* suspensions were prepared as described above, in duplicate. One of the samples was treated with cycloheximide (500mg/litre) to inhibit the protozoan predators and the other was unaltered. Two controls in autoclaved estuarine water were also included.

D.4 Effect of Diffusible Substances

To determine whether diffusible lytic enzymes or metabolic by-products produced from the destruction of *E. coli* cells in estuarine water affect the survival of other *E. coli* cells, an Ecologen E40 (New Brunswick Scientific Co. Inc.) was used (Figure 5).

The Ecologen was autoclaved at 15 psi for 45 minutes and 300ml of autoclaved estuarine water added aseptically to each of the growth chambers 1, 2 and 3. The central diffusion reservoir contained 300ml of natural estuarine water. Growth chambers 1 and 2 were

Figure 5: Ecologen E40.


GROWTH
CHAMBER 1
MEMBRANE
FILTER
GROWTH
CHAMBER 4
CENTRAL
DIFFUSION
RESERVOIR
GROWTH
CHAMBER 2
STEEL
PLATE
GROWTH
CHAMBER 3

separated from the central diffusion reservoir by 0.1 micron filters (Millipore Corporation) allowing the free passage of liquid and growth chamber 3 by a steel plate allowing no passage of liquid. The latter growth chamber was used as a control. An *E. coli* suspension was added to all compartments to give a final cell concentration of approximately 10^8 organisms/ml. The Ecologen was then placed on a rotary shaker R2 (New Brunswick Scientific Co. Inc.) with a shaker stroke of ½" at 150 strokes per minute, in an incubator at 22°C. Samples were withdrawn aseptically at regular intervals and the *E. coli* numbers enumerated.

A similar experiment was conducted with the addition of cycloheximide (500mg/litre) to the natural estuarine water in the central diffusion reservoir, to inhibit growth and activity of protozoan predators.

E. Effect of Individual Predators on the Survival of *E. coli* and *S. typhimurium*

Individual predators were inoculated into 9ml of saline (0.9 percent wt/vol) and 1ml of the resultant predator suspension inoculated into 100ml of autoclaved estuarine water containing a prey bacterium (*E. coli* or *S. typhimurium*) at a concentration of approximately 10^8 organisms per ml. Two autoclaved estuarine water samples, one containing the predator only and the other the prey only, were included as controls. All water samples were incubated in the dark at 22°C, and predator counts on double layer plates and the survival of *E. coli* and *S. typhimurium* enumerated on Brilliant Green Sulphur Agar (Table 9) were conducted at regular intervals. The following experiments were conducted:

1. *E. coli* predators EP3 and EP7 and *E. coli* prey.
2. Combination of *E. coli* predators EP3 and EP7.

   E. coli predators EP3 and EP7 were both inoculated into 100ml of autoclaved estuarine water and the survival of *E. coli* prey determined.
3. *E. coli* predators EP3 and EP7 and *S. typhimurium* prey.
4. *S. typhimurium* predators SP1 and SP6 and *S. typhimurium* prey.
5. *S. typhimurium* predators SP1 and SP6 and *E. coli* prey.

F. Survival of Test Bacteria Compared to *E. coli*

The survival of *S. typhimurium, S. faecium, E. aerogenes* and *K. pneumoniae* were compared to *E. coli* in estuarine water samples. Bacterial suspensions of approximately 10^8 organisms per ml were used and three replications of each treatment were conducted. *E. coli, K. pneumoniae* and *E. aerogenes* were enumerated on MacConkey Agar, *S. faecium* on KF-Streptococcus Agar, and *S. typhimurium* on Brilliant Green Sulphur Agar.

To determine if prey selection by microbial predators occurred in estuarine water samples two alternative prey species were inoculated (final concentration of each species approximately 10^8 cells/ml) into 100ml of natural estuarine water and 100ml natural estuarine water containing cycloheximide (500mg/litre). Control samples in autoclaved estuarine water were also included. The combination of species used were: *E. coli* and *S. faecium; E. coli* and *K. pneumoniae; E. coli* and *S. typhimurium*. Two replications of each treatment were conducted and all organisms were enumerated on C.L.E.D. Medium with Andrade Indicator (Table 9).

G. Effect of Temperature on Bacterial Survival

The survival of *E. coli* at different temperatures was determined using a gradient temperature incubator (Toyo Kaguku Sangyo Co. Ltd., Tokyo, Japan). Cell suspensions (final concentration approximately 10^8 cells/ml) were inoculated into replicate tubes containing 25ml of natural estuarine water and incubated at 5, 10.2, 14.5, 19.8 and 24.1°C.

The survival of *E. coli* and *S. typhimurium* at different temperatures was also determined using a gradient temperature incubator. Cell suspensions were incubated at 6.2, 10.3, 15.3, 20.6 and 26.9°C in natural estuarine water and at 5, 9.5, 14, 18.5 and 24°C in autoclaved estuarine water. *E. coli* survival was determined on MacConkey Agar, *S. typhimurium* survival on Brilliant Green Sulphur Agar, and predator counts on double layer plates. The addition of cycloheximide (500mg/litre) to the top layer of the double layer plates enabled the enumeration of bacterial predators only.

H. Effect of Solar Radiation on Bacterial Survival

To determine the effect of natural solar radiation and predacious microorganisms on *E. coli* survival in estuarine water samples the test organism was inoculated into natural estuarine water to give a final concentration of approximately 10^8 cells/ml. To exclude the effect of solar radiation on *E. coli* survival, flasks containing inoculated natural estuarine water were completely covered in aluminium foil. The effect of predators on *E. coli* survival was removed by autoclaving natural estuarine water at 15 psi for 20 minutes. *E. coli* inoculated into an aluminium foil covered flask containing autoclaved estuarine water was used as a control. Unless otherwise stated, three replications of each treatment were conducted and these were incubated at 22°C in a water bath exposed to direct

solar radiation.

A similar experiment was conducted to determine the survival of *E. coli* in estuarine water exposed to artificial light. The lighting consisted of 4 x 150W Lugon bulbs, 4 x 250W Osram bulbs and 1 x 700W Philips HPLP lamp. These lights produce radiation over a range of wavelengths from 350-750 nanometres (Bickford and Dunn, 1973). The estuarine water samples were exposed to artificial light for 3 hours daily and kept in the dark for 21 hours to simulate the natural radiant exposures of approximately 8 hours natural solar radiation. The temperature was maintained at 22°C throughout the experiment. Bacterial survival of test organisms in all experiments was determined on C.L.E.D. medium (with Andrade Indicator) (Table 9) at regular intervals.

In all experiments the daily radiant exposure was measured using a LI-550 Printing Integrator with a 200M module (LI-COR Ltd.) and a LI-200S Pyranometer Sensor (LI-COR Ltd.).

The survival of the test organisms (Table 8) was compared to *E. coli* in natural estuarine water samples. For each test bacterium one ml of a suspension was inoculated into two replicate samples of natural estuarine water. One of the replicate samples was incubated in the dark at 22°C and the other exposed to natural solar radiation. Survival of the test organisms was determined in two groups: group 1 exposed to solar radiation 1 consisted of *S. typhimurium, S. faecium* and *K. pneumoniae*; group 2 exposed to solar radiation 2 consisted of *E. aerogenes* and *E. herbicola*.

To determine the effect of different radiant exposures on bacterial survival natural estuarine water samples were inoculated with either *E. coli* or *S. typhimurium* and subjected to three radiant

exposures: 0, 50 or 100 percent of natural solar radiation. Solar radiation was reduced by the use of aluminium foil (0 percent) or Sarlon shadecloth (50 percent). Bacterial counts of *E. coli* and *S. typhimurium* were made at the commencement of the experiment and after 2 days incubation.

The effect of radiant exposure on the survival of *E. coli* and *S. typhimurium* in the absence of predacious microorganisms was determined in artificial light described above. Three replicate suspensions of *E. coli* and three of *S. typhimurium* in autoclaved estuarine water were continuously exposed to artificial light. Bacterial survival on C.L.E.D. medium (with Andrade Indicator (Table 9)) and cumulative radiant exposure were determined at regular intervals.

IV. Results and Discussion

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A. Seasonal and Site Variations in Bacterial Indicator Organisms

Results

The seasonal variation in the log number of total coliforms (TC), faecal coliforms (FC), faecal streptococci (FS), PFU and aerobic heterotrophic bacteria (AHB) for 1979-80 for each site is shown in Figures 6-15. The numbers of TC, FC, FS and AHB appear to vary randomly with no distinct overall seasonal pattern emerging for all sites. The numbers of PFU remain fairly constant at each sampling time for all sites.

The site variation in the mean numbers of organisms during the 1979-80 sampling period and the standard errors are shown in Table 10. The polluted sites 1, 2, 3 and 8 generally exhibit significantly higher numbers of TC, FC and FS than the remaining sites. The numbers of PFU and AHB vary from one site to another with the highest levels occurring at both polluted (site 1) and unpolluted (site 7) sites.

Similar observations in the seasonal and site variations in sampling data obtained in the 1978-79 sampling period were also noted (Appendix 3.a.).

The variance-covariance matrix and the correlation matrix pooled over all sites within each group are given in Appendix 3.c. There is no significant difference (F value 1.32, df = 28, 34,483, $p \simeq 0.12$), between the variance-covariance matrices for each group corrected for site effects. A variance-covariance matrix obtained by combining data from all sites (polluted and unpolluted) is given in Appendix 3.c.3. and the correlation matrix in Table 11.

Total coliforms are positively correlated with AHB at the 0.1% level of significance and PFU are negatively correlated with salinity at the 1% level of significance. Solar radiation is positively

Figures 6-15: Seasonal variation in log numbers of:

Total coliforms (●)

(Nos./100ml)

Faecal coliforms (○)

(Nos./100ml)

Faecal streptococci (■)

(Nos./100ml)

PFU (□)

(Nos./ml)

Aerobic heterotrophic bacteria (▲)

(Nos./ml)

for sites 1-10. (Data from Appendix 3.b.)

Figure 6

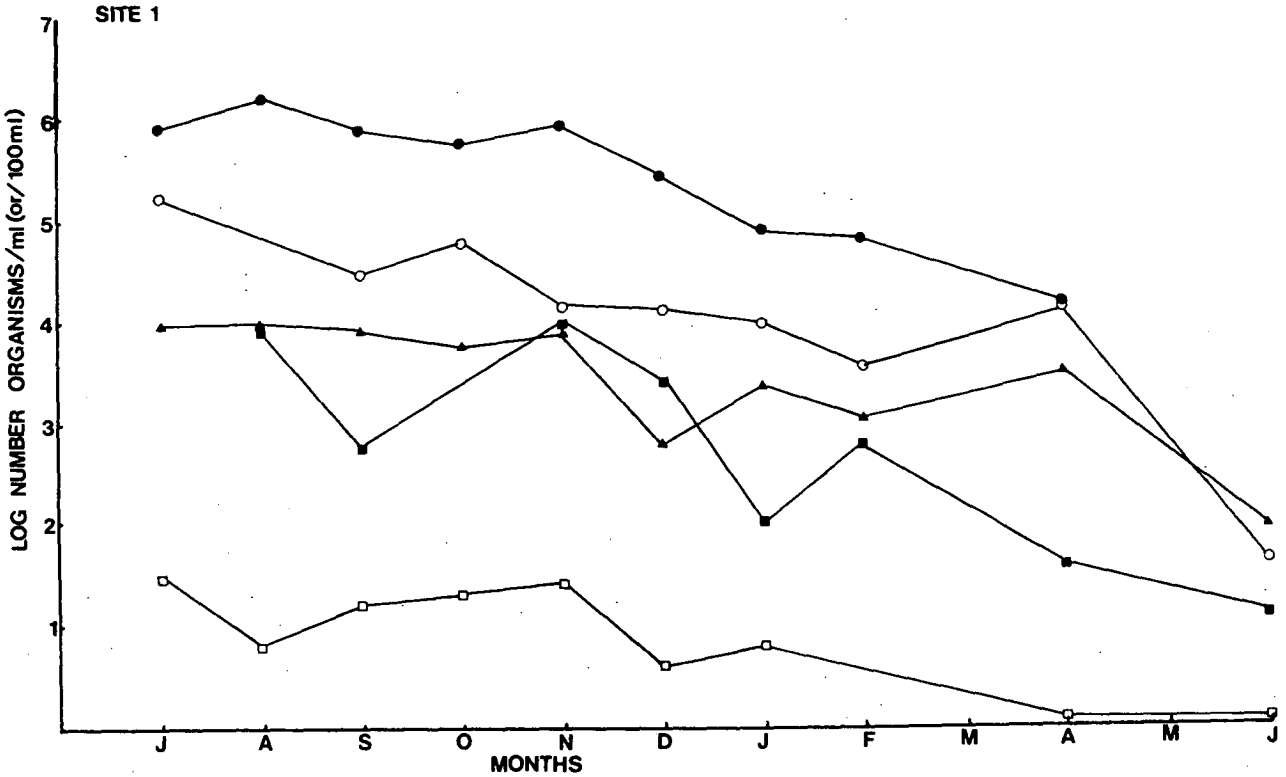


Figure 7

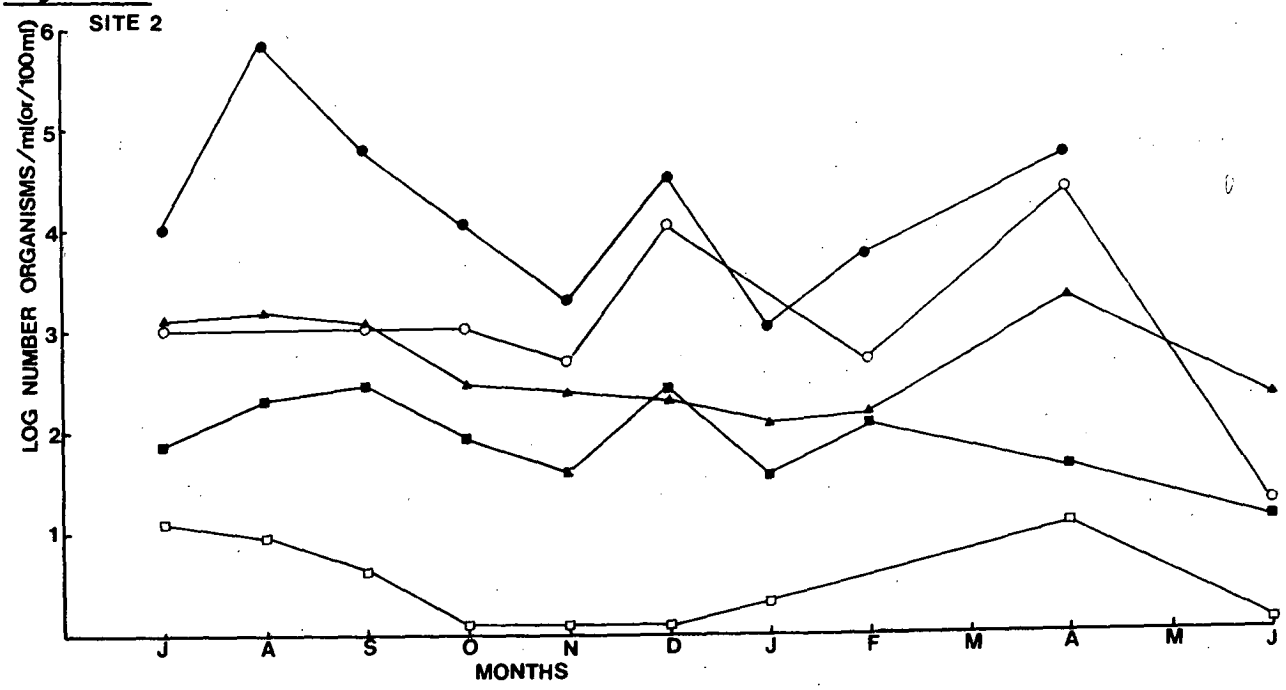


Figure 8

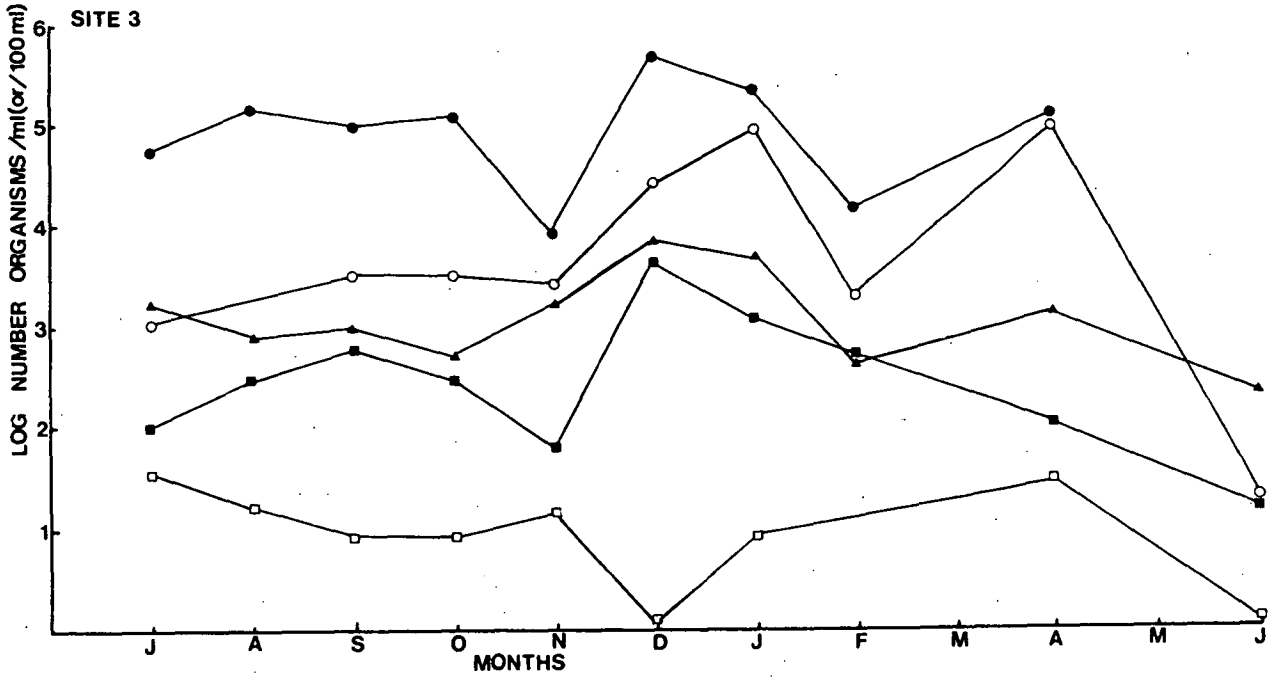


Figure 9

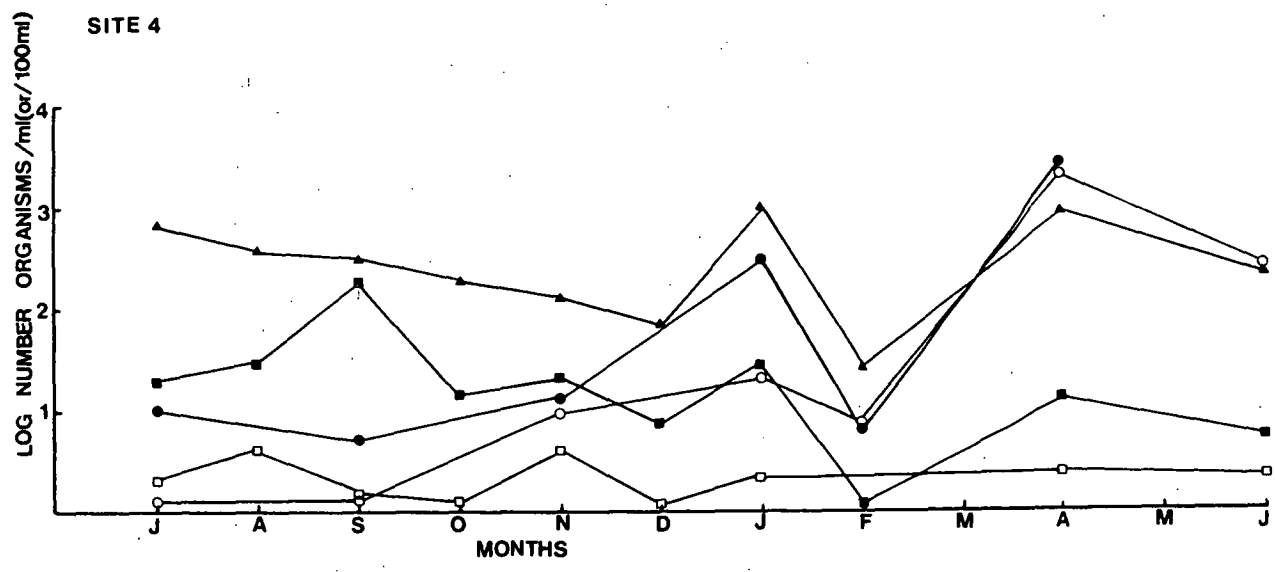


Figure 10

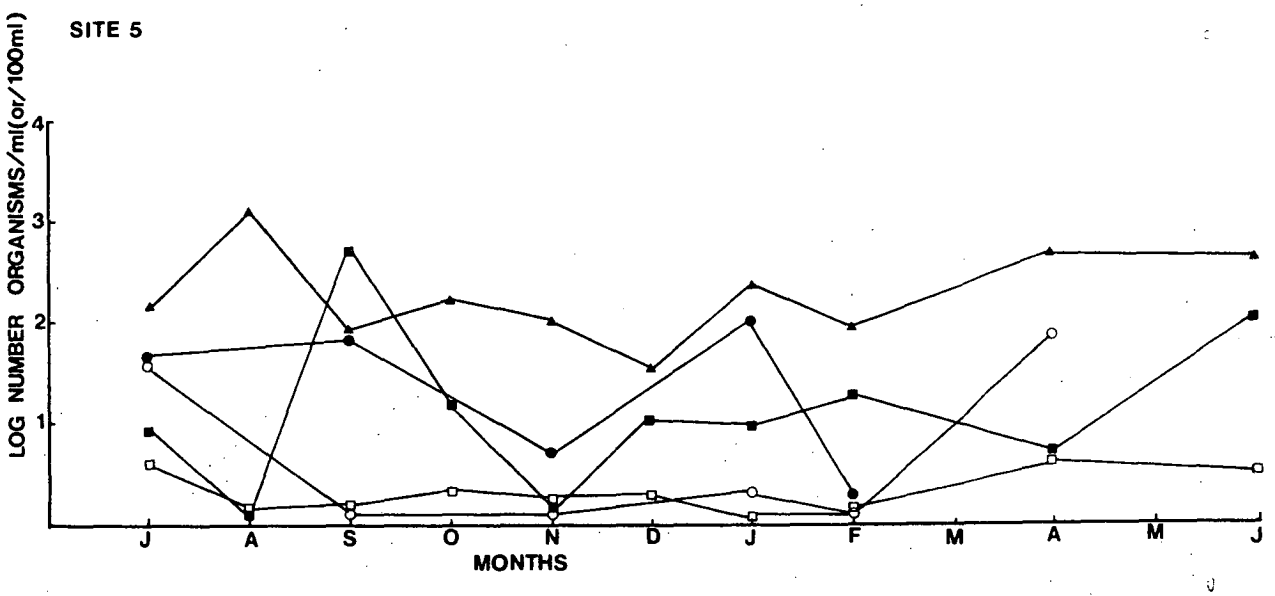


Figure 11

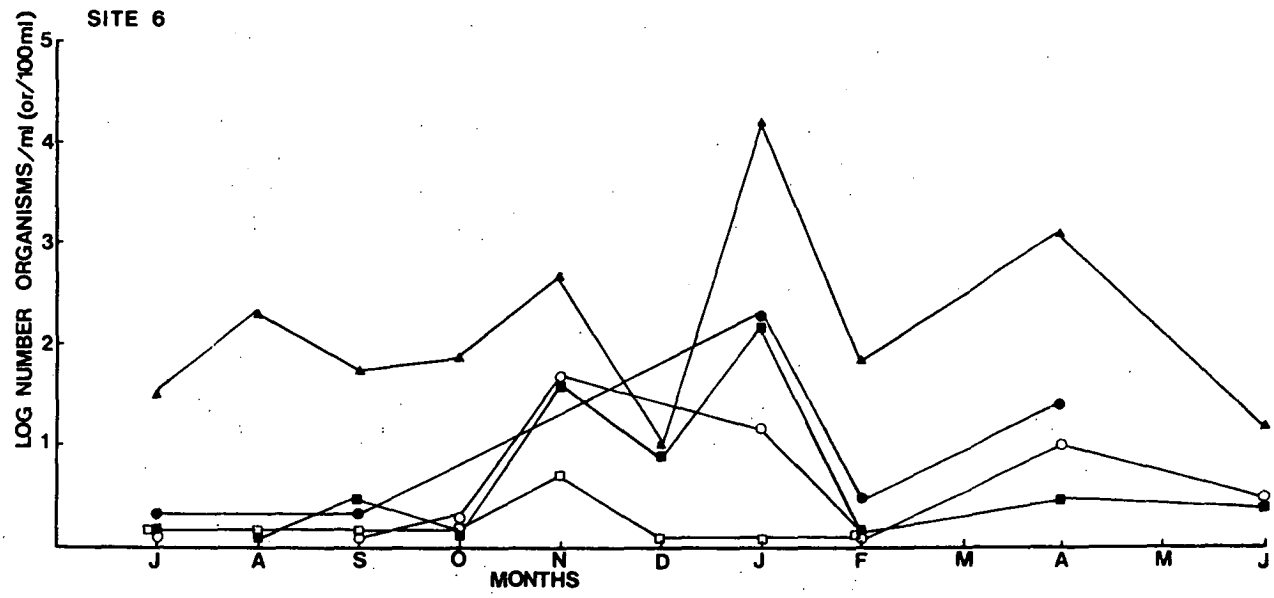


Figure 12

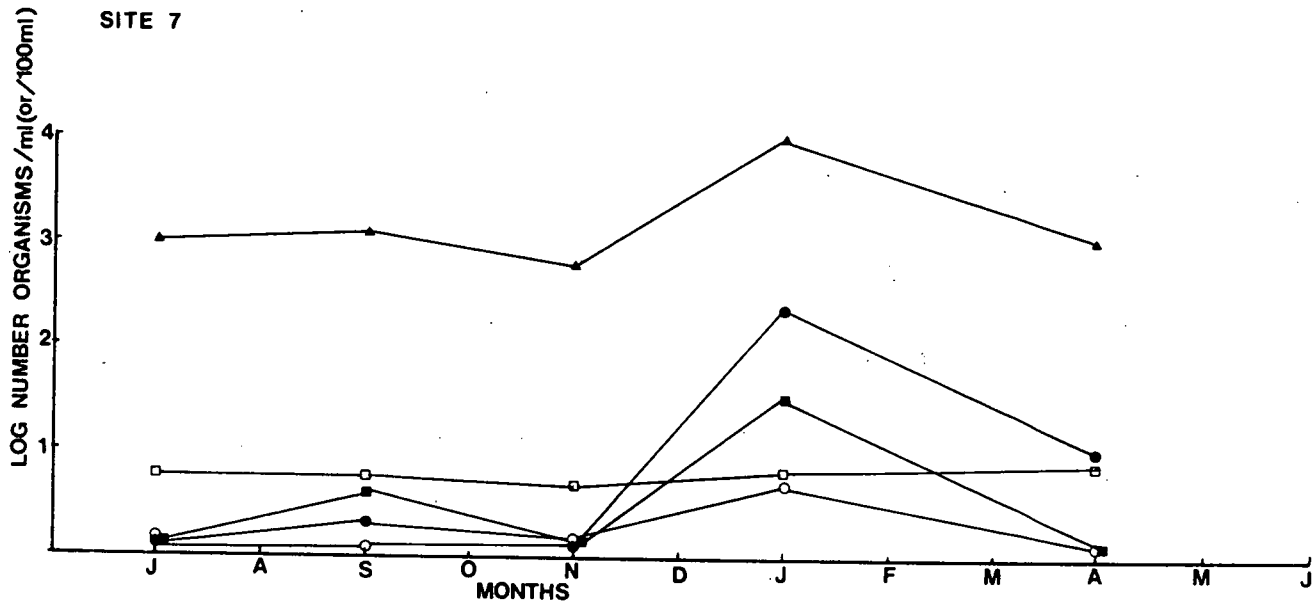


Figure 13

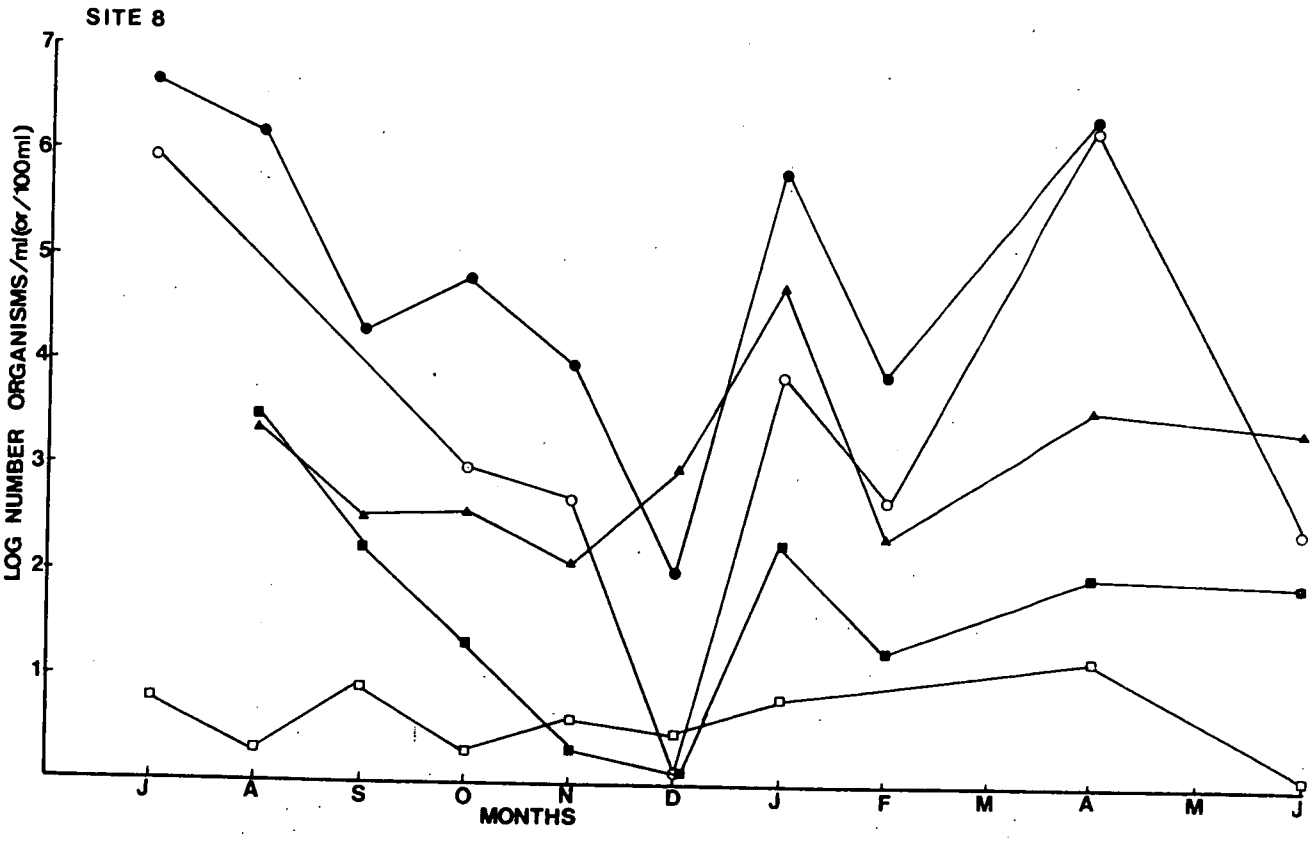


Figure 14

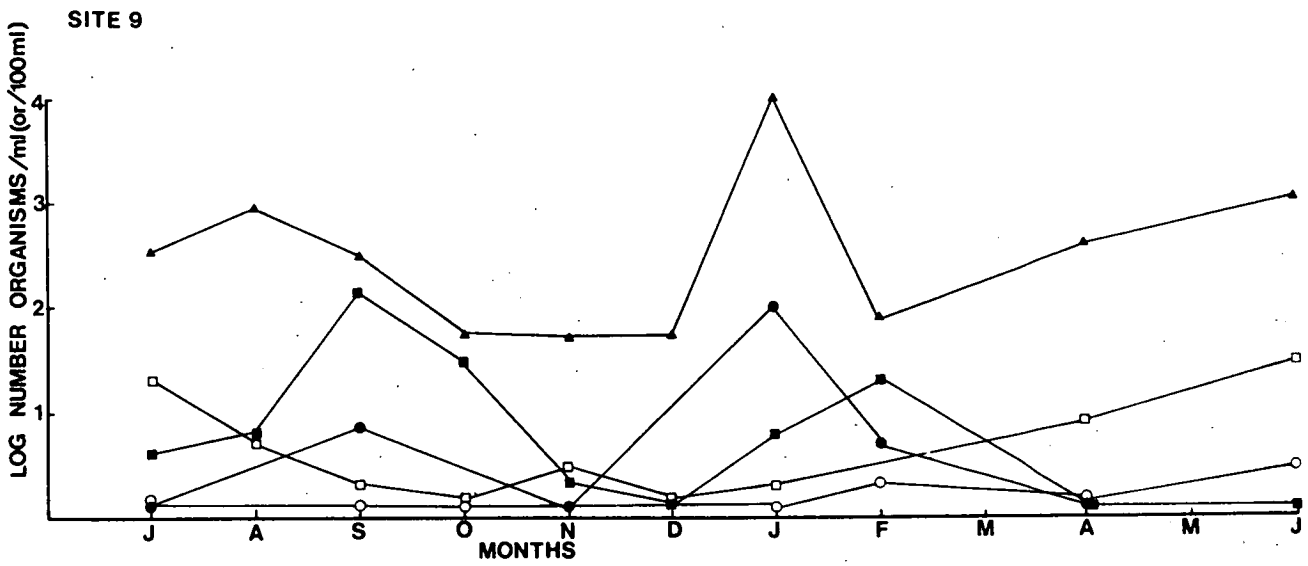


Figure 15

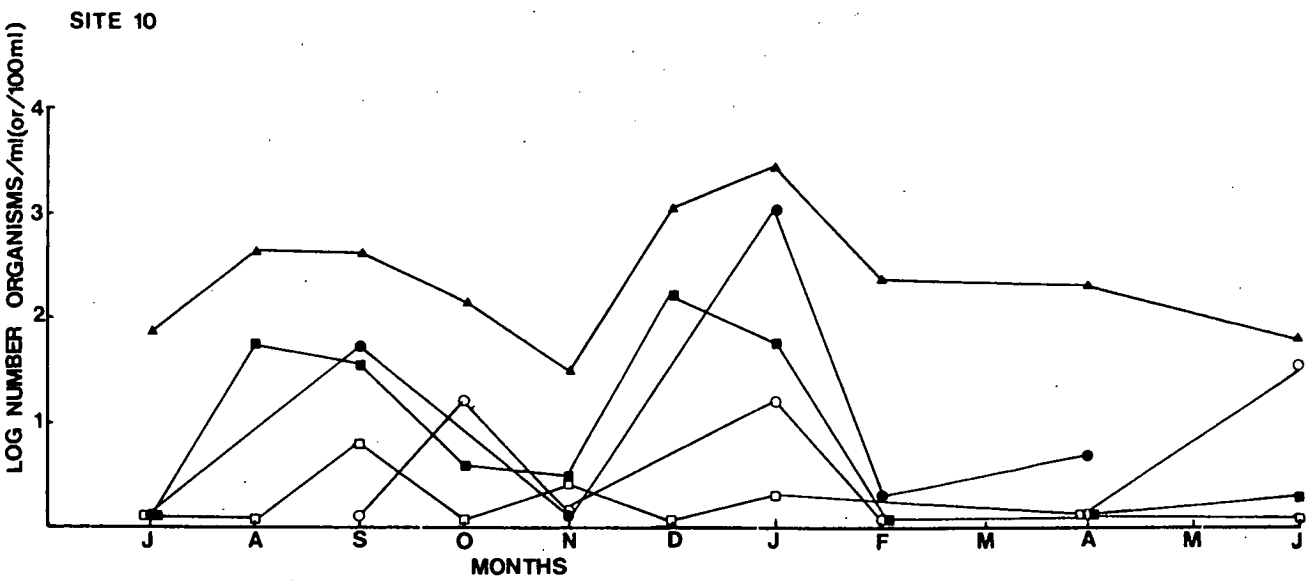


TABLE 10: Site variation in the number of organisms for 1979-80 sampling period (data from Appendix 3.b.).

SITE	LOG NO. ORGANISMS PER ML (OR PER 100ML)[a] TOTAL COLIFORMS (NOS/100ML)	FAECAL COLIFORMS (NOS./100ML)	FAECAL STREPTOCOCCI (NOS./100ML)	PFU (NOS./ML)	AEROBIC HETEROTROPHIC BACTERIA (NOS./ML)
1	5.46 (0.22)	4.00 (0.34)	2.68 (0.38)	0.83 (0.19)	3.40 (0.21)
2	4.21 (0.28)	3.01 (0.33)	1.90 (0.14)	0.45 (0.16)	2.63 (0.15)
3	4.89 (0.19)	3.64 (0.34)	2.41 (0.22)	0.89 (0.19)	3.07 (0.14)
4	1.41 (0.52)	1.24 (0.46)	1.16 (0.19)	0.27 (0.08)	2.37 (0.16)
5	1.30 (0.33)	0.72 (0.32)	1.07 (0.26)	0.25 (0.09)	2.24 (0.14)
6	0.96 (0.39)	0.58 (0.23)	0.62 (0.24)	0.19 (0.13)	2.14 (0.31)
7	0.74 (0.45)	0.14 (0.14)	0.42 (0.30)	0.79 (0.03)	3.17 (0.21)
8	4.90 (0.51)	3.38 (0.72)	1.65 (0.36)	0.59 (0.12)	3.07 (0.27)
9	0.59 (0.32)	0.25 (0.11)	0.74 (0.23)	0.61 (0.18)	2.46 (0.24)
10	0.97 (0.50)	0.60 (0.22)	0.86 (0.27)	0.17 (0.10)	2.37 (0.19)

[a] Values represent mean of five to ten observations; standard error is shown within parentheses.

TABLE 11: Correlation matrix for sampling data, pooled over polluted and unpolluted sites (data from Appendix 3.a. and 3.b.).

PARAMETER	TOTAL COLIFORMS	PFU	AEROBIC HETEROTROPHIC BACTERIA	TEMPERATURE	SALINITY	RAINFALL	SOLAR RADIATION
Total Coliforms	1.0000					df = 102	
PFU	0.1292	1.0000					
Aerobic Heterotrophic Bacteria	0.5498***	0.1218	1.0000				
Temperature	-0.1050	0.0366	-0.0876	1.0000			
Salinity	-0.0827	-0.3133**	-0.0349	0.2226*	1.0000		
Rainfall	0.1498	0.0238	0.1602	0.0432	-0.0320	1.0000	
Solar Radiation	-0.0560	-0.2300*	-0.1221	0.6596***	0.4379***	0.2122*	1.0000

* Significant at 5%

** Significant at 1%

*** Significant at 0.1%

correlated with temperature and salinity at the 0.1% significance level.

The FC/FS ratios for each site calculated from the mean values of the 1979-80 sampling data are shown in Table 12. At the more polluted sites 1, 2, 3, 4 and 8 the FC/FS ratios are greater than 4.0, whereas at the less polluted sites 5, 6, 7, 9 and 10 the FC/FS ratios are less than 0.7.

Discussion

There have been several investigations into the occurrence and distribution of faecal indicator bacteria in aquatic ecosystems. Coliform bacteria and heterotrophic bacteria appear to be strongly positively correlated (Table 11) (Sayler *et al.*, 1975) and seasonal fluctuations of faecal indicator bacteria (Hirn *et al.*, 1980; Yoshikura *et al.*, 1980) and heterotrophic bacteria (Rheinheimer, 1977; Yoshikura *et al.*, 1980) have been observed and found to be partly dependent on the temperature, pH and the nutrient status of the water. Earlier studies, however (Brasfeild, 1972; Sayler *et al.*, 1975; Goyal *et al.*, 1977), found little or no seasonal variation in bacterial numbers and no statistically significant correlations between indicator organisms and such factors as temperature and pH as also observed in Table 11. Similarly, Carney *et al.* (1975) observed no seasonal trend in coliform levels but rather a low background level of coliforms with sporadic increases throughout the year. These increases may be caused by changes in local site conditions such as tidal, current and winds movements (Anson and Ware, 1974), point sources of pollution, or run-off from surrounding land areas (Faust, 1976). It is difficult, therefore, to predict the seasonal distribution of indicator bacteria and the factors affecting this distribution without experimental verification (Walsh, 1971). Further, Sayler *et al.* (1975) noted that

TABLE 12: FC/FS ratios for each sampling site (data from Appendix 3.b.).

SITE	FC/FS RATIO
1	12.9
2	41.6
3	32.4
4	9.82
5	0.36
6	0.43
7	0.19
8	1,046
9	0.09
10	0.29

large water sample sizes were needed before valid conclusions regarding functional relationships between numbers of bacterial indicator organisms and selected physical and chemical indices could be made.

The lower numbers of TC, FC and FS at sites 4, 5, 6, 7, 9 and 10 compared to the remaining sites (Table 10) is a result of the decrease in human population in the areas adjacent to the former sites and the subsequent decrease in the sewage outfalls (Figure 3), and an increase in estuarine volume resulting in greater dilution of the sewage effluent. Similarly, Owens (1978) observed low TC and *E. coli* counts in seawater adjacent to land with low human (and/or domestic animal) populations and high TC and *E. coli* counts in seawater adjacent to land with high human (and/or domestic animal) populations.

The FC/FS ratio provides an indication of the type or source of faecal pollution (Geldreich and Kenner, 1969). Contamination by human domestic sewage results in a FC/FS ratio of greater than 4 and ratio of less than 0.7 indicates that the pollution is derived from warm-blooded animals other than humans. The low FC/FS ratio at sites 5, 6, 7, 9 and 10 (Table 12) indicates the reduction in human population and the increase in domestic animal population in the adjacent land areas.

B. Isolation of Faecal Bacterial Strains

Results

Bacteria capable of growth on Lactose Teepol Agar after incubation at 30°C for 4 hours followed by 44°C for 20 hours, were considered to be faecal coliforms. Bacteria isolated from various sites were identified and appear in Appendix 4. The relative percentage of *Klebsiella* sp.

isolated from the Boyer sampling site compared to the Abbatoirs, Tasman Bridge and Rosny Point sampling sites is shown in Table 13.

Discussion

There are two major assumptions of the faecal coliform elevated temperature test (Geldreich, 1966). Firstly, faecal coliforms occur only in faeces and appear in natural water systems only when faeces of man or warm-blooded animals have entered. Secondly, the presence of faecal coliforms, of which *E. coli* is the most common, is indicative of the probable presence of pathogenic microorganisms.

It has been reported, however, that in carbohydrate-rich effluents such as from pulp and paper mills and textile-finishing plants (Dufour and Cabelli, 1976), that coliforms, other than *E. coli*, are faecal coliform positive. The most significant of these is *K. pneumoniae*, an opportunistic pathogen causing pneumonia and urinary tract infections. This organism is widely distributed in nature (Duncan and Razzell, 1972), has been detected in eutrophic waters (Campbell *et al.*, 1976), and grows in carbohydrate-rich waters from a variety of sources (Dufour and Cabelli, 1976).

Spratt and Felgenhaur (1977) reported that samples of pulp and paper mill effluent from Boyer contained greater than 24,000 faecal coliforms per 100ml. If it is assumed that most are *E. coli*, then the effluent should contain large numbers of enteric pathogens such as *Salmonella* and due to the large volume of effluent produced (100Ml per day, Department of Environment, Housing and Community Development, 1977), would create a major health hazard. But, as suggested above, and as can be seen in Table 13, a large proportion of these faecal coliform positive organisms are in fact *Klebsiella* sp. In those

TABLE 13: Faecal coliform positive bacteria isolated from different sampling sites (data from Appendix 4).

ISOLATION SITE	PERCENTAGE OF ORGANISM (NUMBERS WITHIN PARENTHESES)	
	KLEBSIELLA SPP.	OTHER FAECAL COLIFORMS
Boyer	68.6 (24)	31.4 (11)
Abbatoirs Tasman Bridge Rosny Point	28.6 (12)	71.4 (30)

sampling sites not exposed to high carbohydrate effluents such as at Rosny Point, *Klebsiella* spp. do occur but in considerably lower numbers than at Boyer.

C. Seasonal and Site Variations in *E. coli* Survival

Results

The seasonal and site variations in *E. coli* survival are shown in Figure 16. For all sites with the exception of site 2, there was a significantly greater reduction in *E. coli* numbers during summer (season 1) than during winter (season 3).

The log reduction in *E. coli* numbers for each season does not, in general, vary significantly from site to site (Figure 16).

The variation in the initial number of PFU for each site and season is shown in Table 14.a. In generaly, there is no significant difference in initial PFU numbers between sites or between seasons.

Seasonal and site variations in temperature are shown in Table 14.b. The water temperature was greater in season 1 compared to season 3 for all sites with the exception of site 7 where there was no significant difference. If seasons 1 and 2 are compared the water temperature is significantly greater in season 1 at sites 1, 2, 5 and 6. There is no significant difference in water temperature between seasons 2 and 3. In general, there is no significant difference in water temperature from site to site for each season.

Variations in salinity for each site and season are seen in Table 14.c. Salinity increased from season 3 to season 1 for sites 1, 6 and 7, but was not significantly different from season to season at sites 2 and 5. Site 1, in general, has a significantly lower salinity than all other sites.

Figure 16: Seasonal and site variations in *E. coli* survival.

Season 1 (●) summer

Season 2 (O) spring

Season 3 (■) Winter

(Data from Appendix 5.)

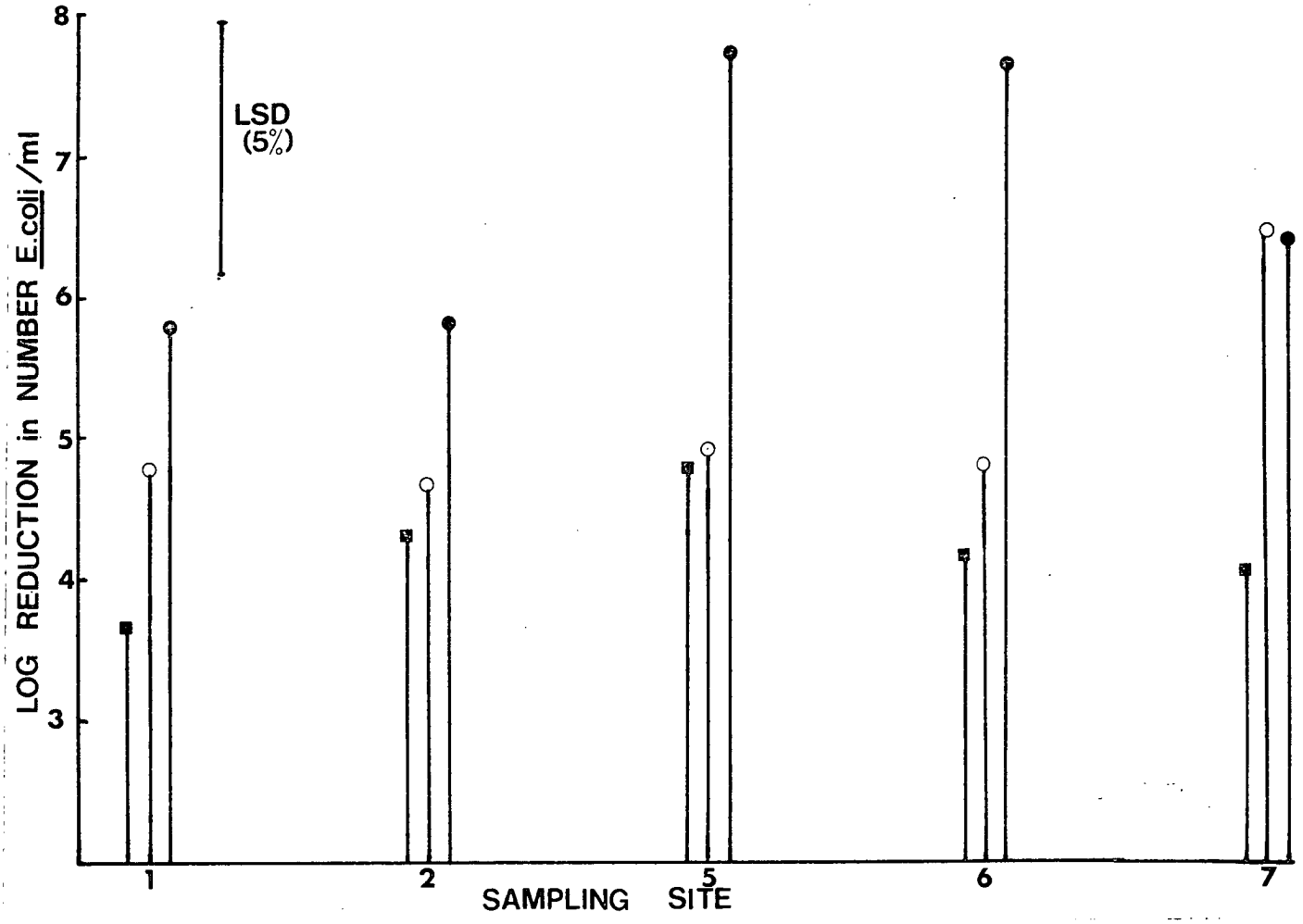

LOG REDUCTION in NUMBER E.coli /ml
8
7
6
5
4
3
LSD
(5%)
1
2
5
6
7
SAMPLING SITE

TABLE 14.a: Seasonal and site variation in initial number of PFU.

(Data from Appendix 3.a.)

LOG INITIAL NUMBER PFU PER ML[a]

SEASON	SITE 1	2	5	6	7
1	0.63 (0.32)	0.00 (0.00)	0.00 (0.00)	0.30 (0.17)	0.48 (0.34)
2	1.63 (0.88)	0.46 (0.46)	0.10 (0.10)	0.10 (0.10)	0.60 (0.60)
3	0.97 (0.19)	1.11 (0.83)[b]	0.98 (0.50)[b]	0.80 (0.42)[b]	0.45 (0.31)[b]

[a] Results represent mean of three values; standard error is shown within parentheses

[b] Mean of two values.

TABLE 14.b: Seasonal and site variation in water temperature.

(Data from Appendix 3.a.)

TEMPERATURE (^{o}C)[a]

SEASON	SITE 1	2	5	6	7
1	17.2 (0.61)	16.8 (0.81)	17.0 (0.84)	17.0 (1.06)	13.1 (1.40)
2	13.1 (1.40)	12.5 (0.97)	12.8 (1.10)	12.9 (1.05)	14.6 (1.39)
3	11.5 (1.98)	12.0 (1.50)	12.5 (1.57)	12.5 (1.49)	11.7 (1.99)

[a] Results represent mean of three values; standard error is shown within parentheses.

TABLE 14.c. Seasonal and site variation in salinity.

(Data from Appendix 3.a.)

SALINITY (% NaCl)[a]

SEASON	SITE 1	2	5	6	7
1	1.86 (0.17)	2.33 (0.23)	2.89 (0.22)	3.02 (0.17)	3.05 (0.19)
2	1.56 (0.55)	2.20 (0.43)	2.83 (0.28)	2.87 (0.29)	2.80 (0.31)
3	0.94 (0.32)	1.80 (0.64)	2.71 (0.23)	2.45 (0.30)	2.37 (0.20)

[a] Results represent mean of three values; standard error is shown within parentheses.

Discussion

Vaccaro *et al.* (1950) observed that the bactericidal properties of fresh seawater exhibited a seasonal variation,being most active in the summer, and suggested that antibiotic production by the normal marine population were responsible for this increased activity. Similarly, Faust *et al.* (1975) and Verstraete and Voets (1976) noted that bacterial survival was highest in winter and lowest in the summer months. The former authors observed that the seasonal variation in temperature of 5 to 30^{o}C was the most marked fluctuation to which the bacteria were subjected while the latter authors also noted a strong positive correlation between seasonal fluctuation in survival and insolation as well as with temperature. This pattern has also been observed in estuarine water samples in this study (Figure 16), with greater survival of *E. coli* in the winter (season 3) than in the summer (season 1). Variations in the initial numbers of predacious microorganisms (Table 14.a.) and salinity (Table 14.c.) do not seem sufficient to explain the seasonal change in *E. coli* survival. Fluctuations in temperature (Table 14.b.) are more marked, however, and may account for these seasonal fluctuations. The effect of temperature on *E. coli* survival will be further considered in Section IV.G.

Ketchum *et al.* (1949), noted that a second or subsequent inoculum of *E. coli* in seawater declined more rapidly than the first, and concluded that the bactericidal activity of seawater was increased by previous "pollution" of the water with *E. coli*. Similarly, Mitchell (1968),comparing seawater exposed to constant sewage inflows and unpolluted seawater, and Roper and Marshall (1978), examining seawater samples at different distances from a sewage outfall, concluded

that a previous history of pollution resulted in the rapid kill of coliforms in seawater. Continuous exposure of seawater to sewage resulted in a build up of a microbial population specifically antagonistic towards sewage bacteria and so produced a rapid decline in the sewage bacterial population (Mitchell and Morris, 1969). Discharge of sewage to unpolluted seawater required time for a build up of predacious microorganisms and thus produced a lag in the decline of sewage bacteria.

The constant exposure to sewage pollution at sites 1 and 2 has not, however, resulted in a decreased survival of *E. coli* compared to the unpolluted sites 6 and 7 (Figure 16). Similarly, there has not been a significant increase in naturally-occurring microbial predators at these polluted sites (Table 14.a.). Earlier work (McCambridge and McMeekin, 1979) using estuarine water samples from these polluted sites, showed the development of antagonistic microbial predators following a second inoculation of *E. coli* into estuarine water as noted by other workers (Roper and Marshall, 1978). In the natural water system, however, wind, current and tidal movements may cause substantial mixing at these sites preventing a build up of microbial predators despite constant enrichment from sewage outfalls.

D. Effect of Bacterial and Protozoan Predators on *E. coli* Survival

Results

D.1 Relative Effects of Bacterial and Protozoan Predators

E. coli cells inoculated into natural estuarine water containing both protozoa and bacteria were reduced from approximately 10^8 to less than 10 organisms/ml in 10 days (Figure 17.a.), compared to 10^4 organisms/ml in the presence of bacteria alone. Microscopic examination of all

Figure 17:

(a) The effect of protozoa and bacteria on *E. coli* survival.

E. coli survival in:

autoclaved estuarine water (▲);

autoclaved estuarine water + cycloheximide (●);

natural estuarine water (□);

natural estuarine water + cycloheximide (○).

(Data from Appendix 6.a.1.)

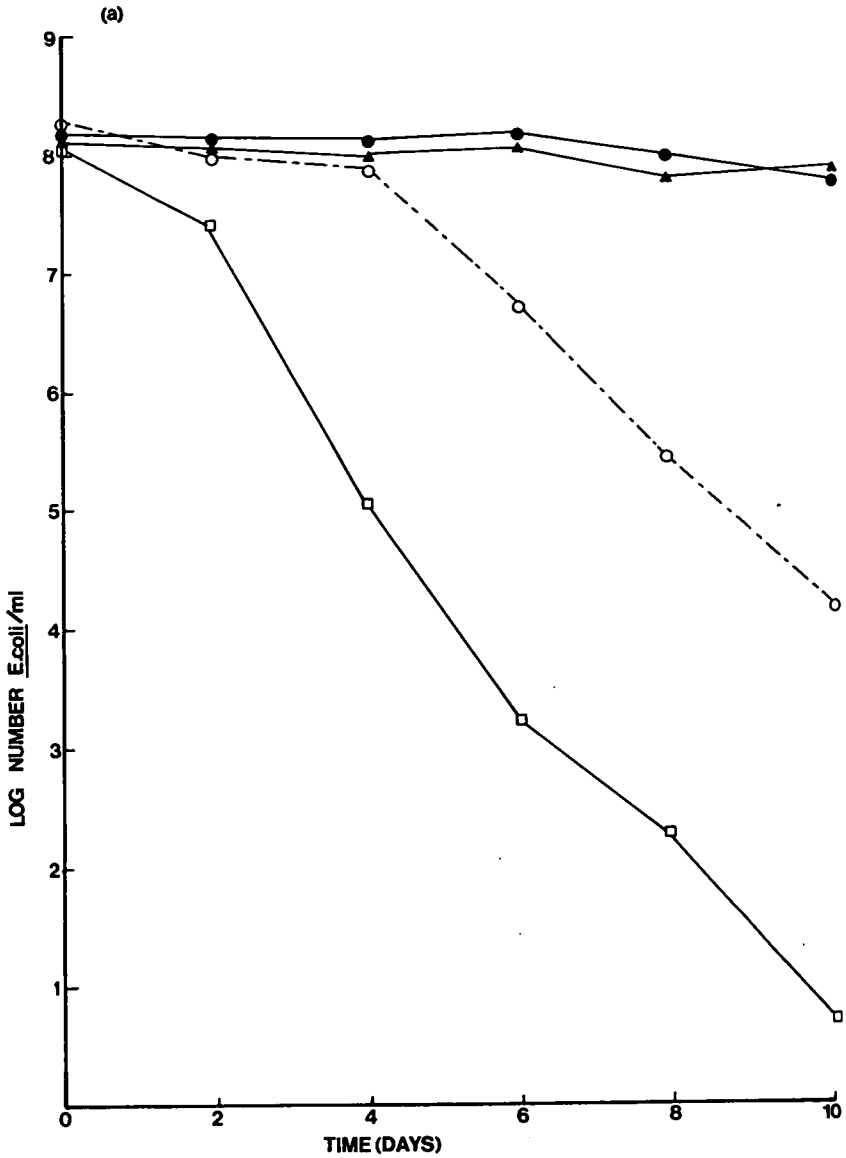
(b) Growth of bacterial and protozoan predators in:

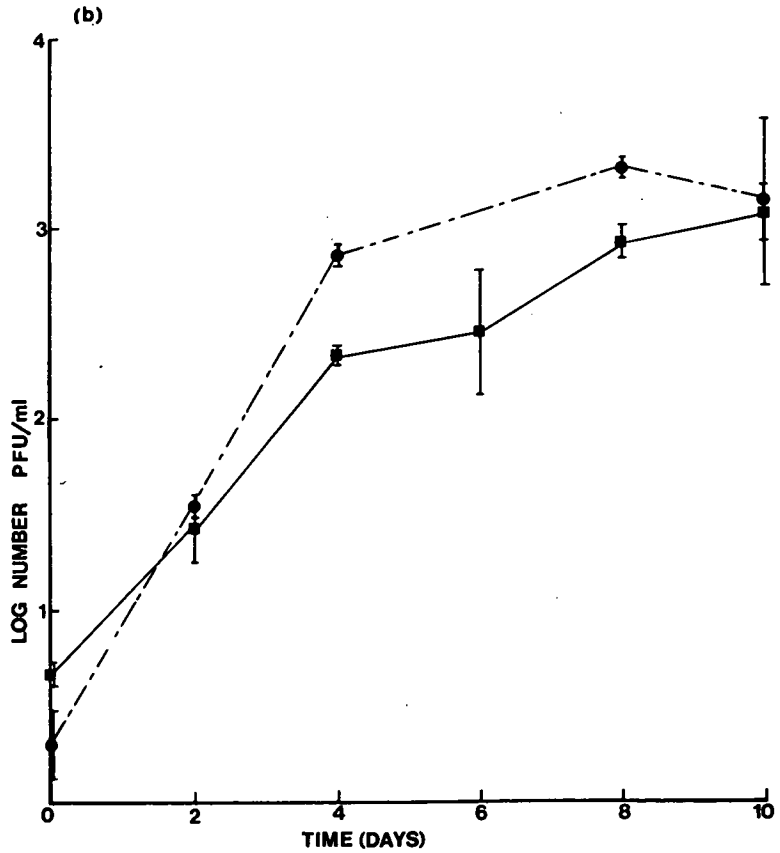
natural estuarine water (■);

natural estuarine water + cycloheximide (●).

Each point represents the mean ± 1 standard error of the mean.

(Data from Appendix 6.a.3.)


(a)
LOG NUMBER E.coli/ml
TIME (DAYS)
0
2
4
6
8
10
1
2
3
4
5
6
7
8
9


(b)
LOG NUMBER PFU/ml
TIME (DAYS)
0
2
4
6
8
10
1
2
3
4

water samples containing cycloheximide and plaques produced on the corresponding double-layer plates revealed the complete inhibition of protozoan predators. The destruction of all microbial predators by autoclaving resulted in *E. coli* numbers remaining virtually unaltered after 10 days. Similarly, the effect of cycloheximide on *E. coli* survival directly, in autoclaved estuarine water, was negligible (Figure 17.a.). Numbers of predacious microorganisms increased in both unautoclaved water samples (Figure 17.b.). On double-layer plates inoculated with natural estuarine water the number of PFU (protozoa and bacteria) increased from less than 10 to a maximum of 10^3/ml in 10 days. Similarly, on plates incubated with estuarine water samples treated with cycloheximide, the number of PFU (bacteria alone) increased from less than 10 to 10^3 per ml in 10 days. A preliminary experiment without replication yielded similar results (Appendix 6.b.).

D.2. Effect of Periodic Inhibition of Protozoans

The effect of periodic inhibition of protozoa by cycloheximide on the survival of *E. coli* in estuarine water samples is shown in Table 15. In natural estuarine water *E. coli* cells are reduced from 3.7×10^7 to 17 organisms per ml in the 10 day decline period. If protozoa are inhibited at the commencement of the experiment (day 0), *E.coli* cells are only reduced to 3.2×10^3 organisms per ml. But, inhibition of the protozoa at day 2, 3, 4, 5 and 6 does not increase *E. coli* survival compared to the natural sample. The number of predators developing in various treatments, determined as PFU, are shown in Table 16. The Log_{10} number of PFU in natural estuarine water represent both bacterial and protozoan predators, while those in the remaining samples are bacterial predators only. The number of PFU in

TABLE 15: *E. coli* Survival in Estuarine Water after Periodic Inhibition of Protozoa.

TIME (DAYS)	LOG. NO. OF *E. COLI* PER ML		LOG NO. OF *E. COLI* PER ML AT FOLLOWING TIME OF PROTOZOAN INHIBITION (DAYS)[a]					
	AUTOCLAVED ESTUARINE WATER	NATURAL ESTUARINE WATER	0	2	3	4	5	6
0	7.57	7.62	7.58					
2	7.63	7.41	7.46	7.30				
3	7.23	6.62	6.80	6.78	5.15			
4	7.20	4.60	6.27	4.08	4.65	4.12		
5	6.96	3.54	5.52	3.75	2.26	1.85	3.32	
6	6.67	2.81	4.44	1.60	1.65	1.15	2.78	2.46
7	6.63	2.66	3.76	0.90	1.36	0.00	2.19	1.53
10	5.93	1.23	3.50	0.00	0.00		0.00	0.00

[a] Time of cycloheximide addition to sample.

TABLE 16: Growth of PFU in Estuarine Water after Periodic Inhibition of Protozoa.

TIME (DAYS)	LOG NO. PFU PER ML IN NATURAL ESTUARINE WATER	LOG NO. OF PFU PER ML AT FOLLOWING TIME OF PROTOZOAN INHIBITION (DAYS)[a]					
		0	2	3	4	5	6
0	0.00	0.30					
2	3.75	2.26	4.01				
3	3.39	3.76	4.08	1.58			
4	3.76	3.90		2.38	1.70		
5	2.66	4.66	3.45	3.21	1.78	2.08	
6	2.51	4.30	3.75	3.51	2.42	1.30	1.30
7	2.15	4.23	3.94	3.25	2.38	2.45	1.78
10	1.53	3.60	3.12	2.45	2.34	2.08	1.90

[a]Time of cycloheximide addition to sample.

samples in which protozoa were inhibited after 0, 2 and 3 days were higher throughout the experiment than the number of PFU present in natural estuarine water. Inhibition of protozoa after 4, 5 and 6 days, however, resulted, in general, in little or no difference between the number of PFU in these samples compared to natural estuarine water.

To examine this effect in more detail, this experiment was repeated, protozoa being inhibited at 12 hourly intervals over a 3 day period. The effect of protozoan inhibition on *E. coli* survival is shown in Table 17. In natural estuarine water *E. coli* cells are reduced from 3.8×10^7 to less than 10 organisms per ml in 10 days. When protozoa are inhibited at the commencement of the experiment, *E.coli* cells are only reduced to 1.7×10^2 organisms per ml. Similarly, inhibition of protozoa after 0.5, 1 and 1.5 days results in a significantly increased survival of *E. coli* cells compared to natural estuarine water (Table 17). However, subsequent inhibition of the protozoa at 2, 2.5 and 3 days does not increase *E. coli* survival compared to the natural sample. The Log_{10} number of *E. coli* cells surviving at day 10 after exposure to protozoa for various times is shown in Figure 18. As the time of protozoan action increased from 0 to 2 days, the Log_{10} number of *E. coli* surviving at day 10 gradually declined. Inhibition of predacious protozoa after day 2, however, had no further effect on *E. coli* survival. The number of PFU in each treatment are shown in Table 18. The number of PFU at day 10 in samples in which protozoa were inhibited after 0, 0.5, 1, 1.5 and 2 days were significantly higher than the number of PFU present in natural estuarine water. Inhibition of protozoa after 2.5 and 3 days however, resulted in no significant difference between the number of PFU in these samples compared to natural estuarine water.

TABLE 17: *E. coli* survival in estuarine water after periodic inhibition of protozoa (data from Appendix 7.a.1.).

TIME (DAYS)	LOG NO. *E. COLI* PER ML[a] IN NATURAL ESTUARINE WATER	LOG NO. OF *E. COLI* PER ML[a] AT FOLLOWING TIME OF PROTOZOAN INHIBITION[b] (DAYS):						
		0	0.5	1.0	1.5	2.0	2.5	3.0
0	7.58 (0.02)	7.56 (0.02)						
0.5			7.55 (0.01)					
1.0	7.62 (0.01)	7.63 (0.01)	7.58 (0.01)	7.49 (0.02)				
1.5					7.55 (0.04)			
2.0	7.30 (0.04)	7.42 (0.01)	7.35 (0.05)	7.41 (0.01)	7.38 (0.01)	7.28 (0.10)		
2.5							6.72 (0.11)	
3.0	5.33 (0.2)	7.26 (0.04)	7.00 (0.19)	7.47 (0.06)	7.30 (0.05)	7.34 (0.07)	6.84 (0.32)	5.49 (0.25)
4.0	4.50 (0.11)	6.53 (0.07)	5.93 (0.21)	6.63 (0.26)	6.68 (0.10)	6.12 (0.48)	5.87 (0.85)	4.34 (0.15)
6.0	3.20 (0.15)	4.76 (0.45)	4.16 (0.20)	4.88 (0.37)	4.81 (0.08)	3.35 (0.26)	3.18 (0.49)	3.30 (0.07)
8.0	1.63 (0.11)	2.94 (0.36)	2.17 (0.09)	3.46 (0.28)	2.17 (0.09)	1.43 (0.22)	1.05 (0.55)	1.28 (0.16)
10.0	0.46 (0.24)	2.23 (0.13)	1.91 (0.03)	1.92 (0.33)	1.46 (0.19)	0.36 (0.36)	0.23 (0.23)	0.20 (0.20)

[a] Values represent mean of three observations; standard error is shown within parentheses.

[b] Time of cycloheximide addition to sample.

Figure 18: Effect of time of protozoan action on the survival of *E. coli* after 10 days.
Each point represents the mean ± 1 standard error of the mean.
(Data from Appendix 7.a.1.)

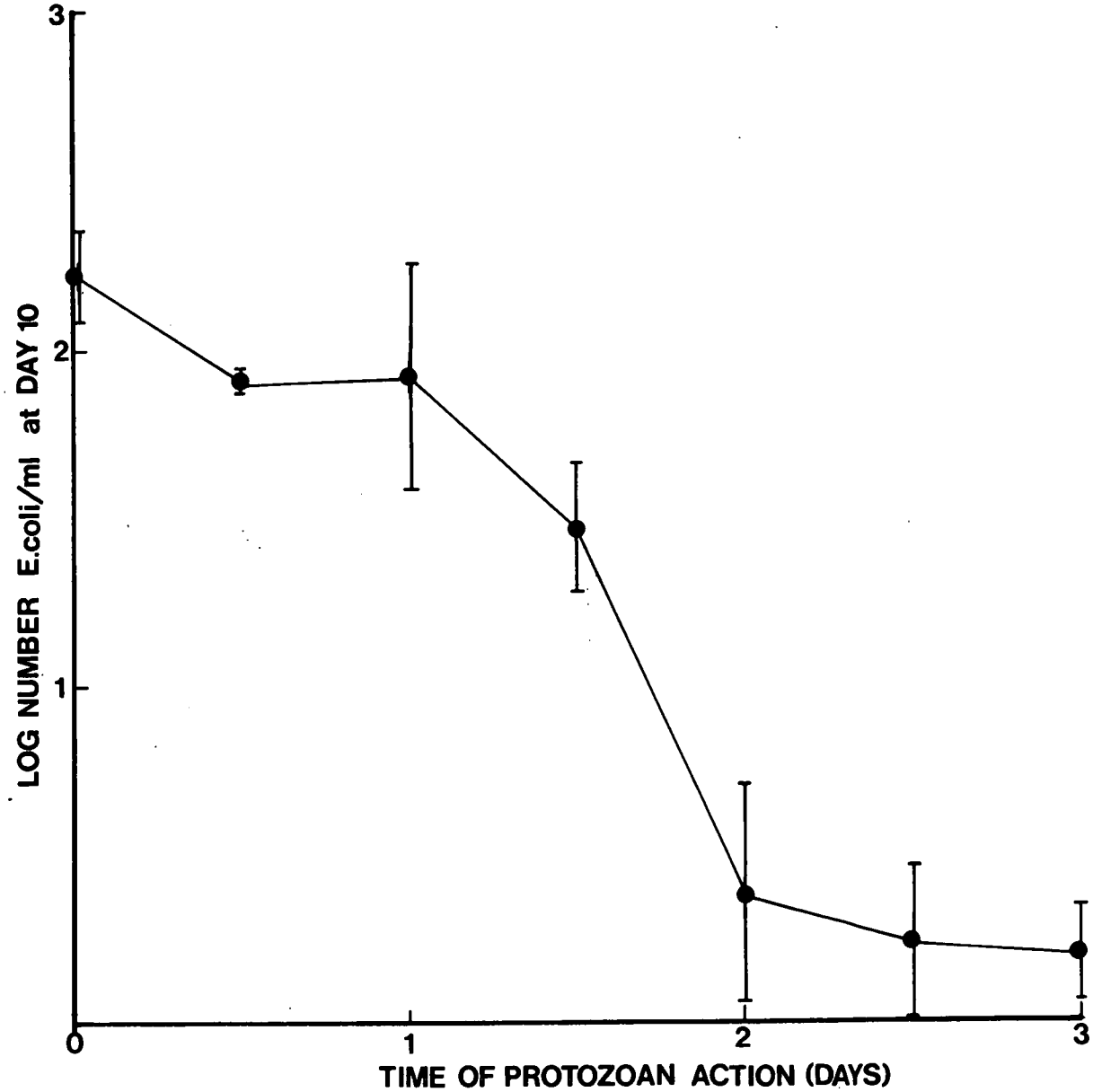

3
2
1
0
1
2
3
LOG NUMBER E.coli/ml at DAY 10
TIME OF PROTOZOAN ACTION (DAYS)

TABLE 18: Growth of PFU in estuarine water after periodic inhibition of protozoa (data from Appendix 7.a.2.).

TIME (DAYS)	LOG NO. OF PFU PER ML[a] IN NATURAL ESTUARINE WATER	LOG NO. OF PFU PER ML[a] AT FOLLOWING TIME OF PROTOZOAN INHIBITION[b] (DAYS):						
		0	0.5	1.0	1.5	2.0	2.5	3.0
0	0.79 (0.40)	1.04 (0.09)						
0.5			1.03 (0.03)					
1.0				1.79 (0.14)				
1.5					1.86 (0.06)			
2.0	1.73 (0.17)	1.91 (0.22)	2.04 (0.20)	2.10 (0.19)	2.05 (0.14)	1.75 (0.13)		
2.5							1.74 (0.06)	
3.0								2.44 (0.05)
4.0	2.50 (0.09)	2.70 (0.30)	3.12 (0.26)	3.00 (0.14)	3.01 (0.08)	2.72 (0.26)	2.71 (0.15)	2.62 (0.09)
6.0	2.70 (0.05)	3.53 (0.10)	3.51 (0.15)	2.71 (0.35)	2.77 (0.02)	2.76 (0.23)	2.79 (0.15)	2.71 (0.03)
8.0	2.96 (0.13)	3.63 (0.07)	3.30 (0.18)	3.25 (0.07)	3.42 (0.15)	3.12 (0.13)	3.09 (0.09)	2.98 (0.09)
10.0	2.61 (0.18)	3.26 (0.06)	3.20 (0.13)	3.31 (0.14)	3.38 (0.07)	3.42 (0.08)	2.81 (0.10)	2.43 (0.13)

[a] Values represent mean of three observations; standard error is shown within parentheses.

[b] Time of cycloheximide addition to sample.

When this experiment was again repeated using an initial *E. coli* concentration of 10^8 and 10^5 cells per ml, the pattern of *E. coli* decline and predator growth (Appendix 7.b and 7.c) was similar to that shown in Tables 17 and 18 respectively. With an initial *E. coli* concentration of 10^8 cells per ml the total PFU (protozoa and bacteria) in natural estuarine water increased from 10 to a maximum of 2×10^4 PFU per ml after 2-3 days and gradually declined to the end of the incubation period (Figure 19). Similarly, bacterial predators increased to reach a maximum of 8×10^3 PFU per ml at day 2 and then gradually declined. Inhibition of protozoa, however, resulted in a marked increase in the number of bacterial predators compared to those present in natural estuarine water reaching a maximum level of 5×10^4 PFU per ml after 5 days. When an initial *E. coli* concentration of 10^5 cells per ml was used, the total number of PFU which developed was reduced to 35 PFU per ml after 2-3 days and bacterial PFU to 25 PFU per ml (Figure 20). Inhibition of protozoa again resulted in a marked increase in bacterial PFU to 2.5×10^3 PFU per ml in 5-6 days. Microscopic examination of all plaques produced on plates containing cycloheximide revealed the presence of bacterial predators only.

D.3. Effect of Initial *E. coli* Concentration

The effect of the initial *E. coli* concentration on the survival of *E. coli* cells determined as the log reduction in *E. coli* numbers after 2 days incubation, is shown in Figure 21. As the initial concentration of *E. coli* is increased the reduction in *E. coli* numbers is increased until an optimum initial concentration is reached where *E. coli* reduction is at its maximum value. Increasing the initial concentration beyond this optimum value of 10^6 to 10^7 organisms per ml resulted in a decrease in the reduction of *E. coli* cells enumerated.

Figure 19: Growth of PFU in estuarine water after periodic inhibition of protozoa. (Initial *E. coli* concentration 10^8 cells/ml.)
Natural estuarine water;
protozoa and bacteria (O);
bacteria only (●).
Protozoa inhibited after 0 days (▽).
(Data from Appendix 7.b.2.)

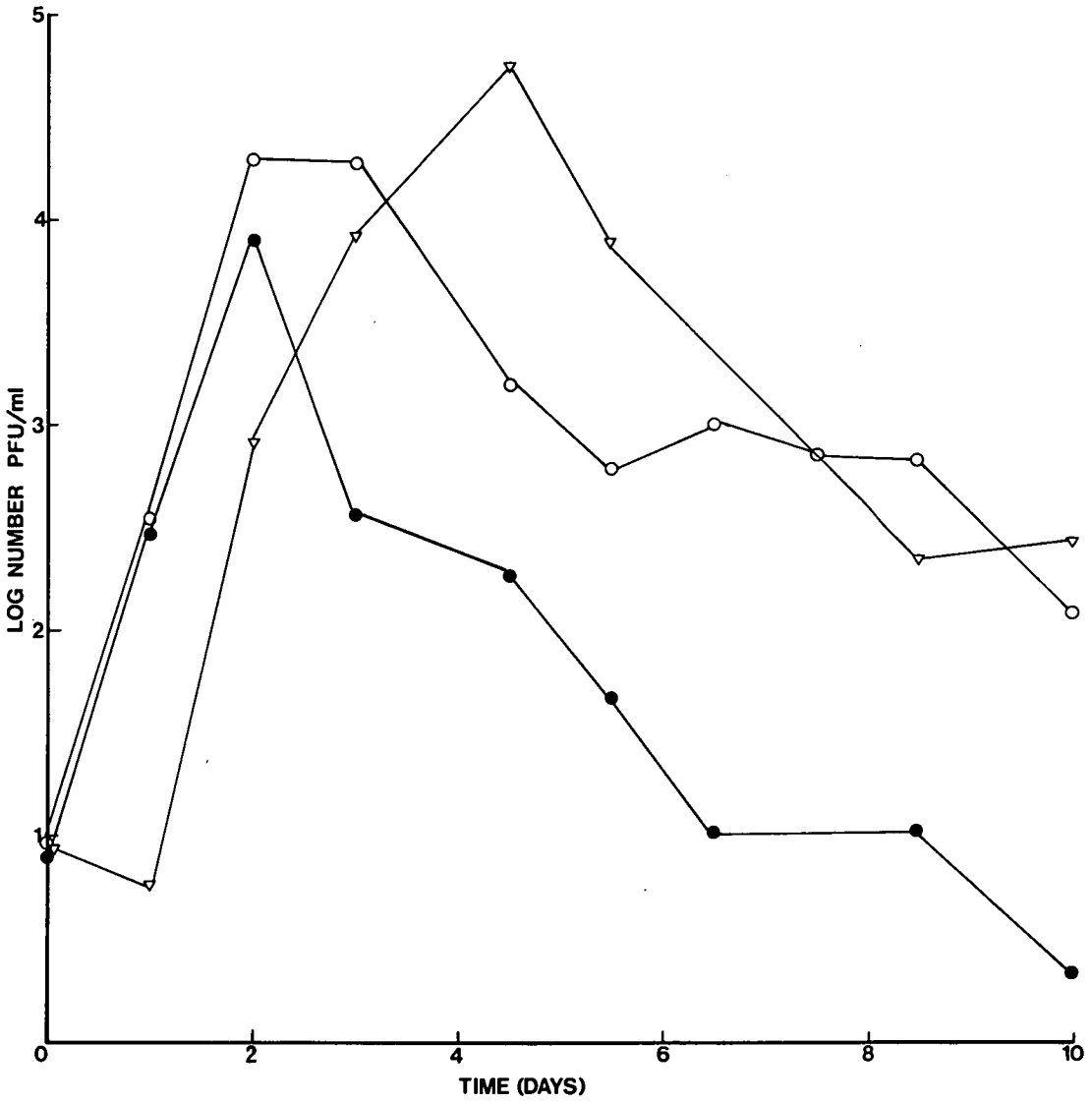

LOG NUMBER PFU/ml
5
4
3
2
1
0
2
4
6
8
10
TIME (DAYS)

Figure 20: Growth of PFU in estuarine water after periodic inhibition of protozoa. (Initial *E. coli* concentration 10^5 cells/ml).
Natural estuarine water;
protozoa and bacteria (O);
bacteria only (●).
Protozoa inhibited after 0 days (▽).
(Data from Appendix 7.c.2.)

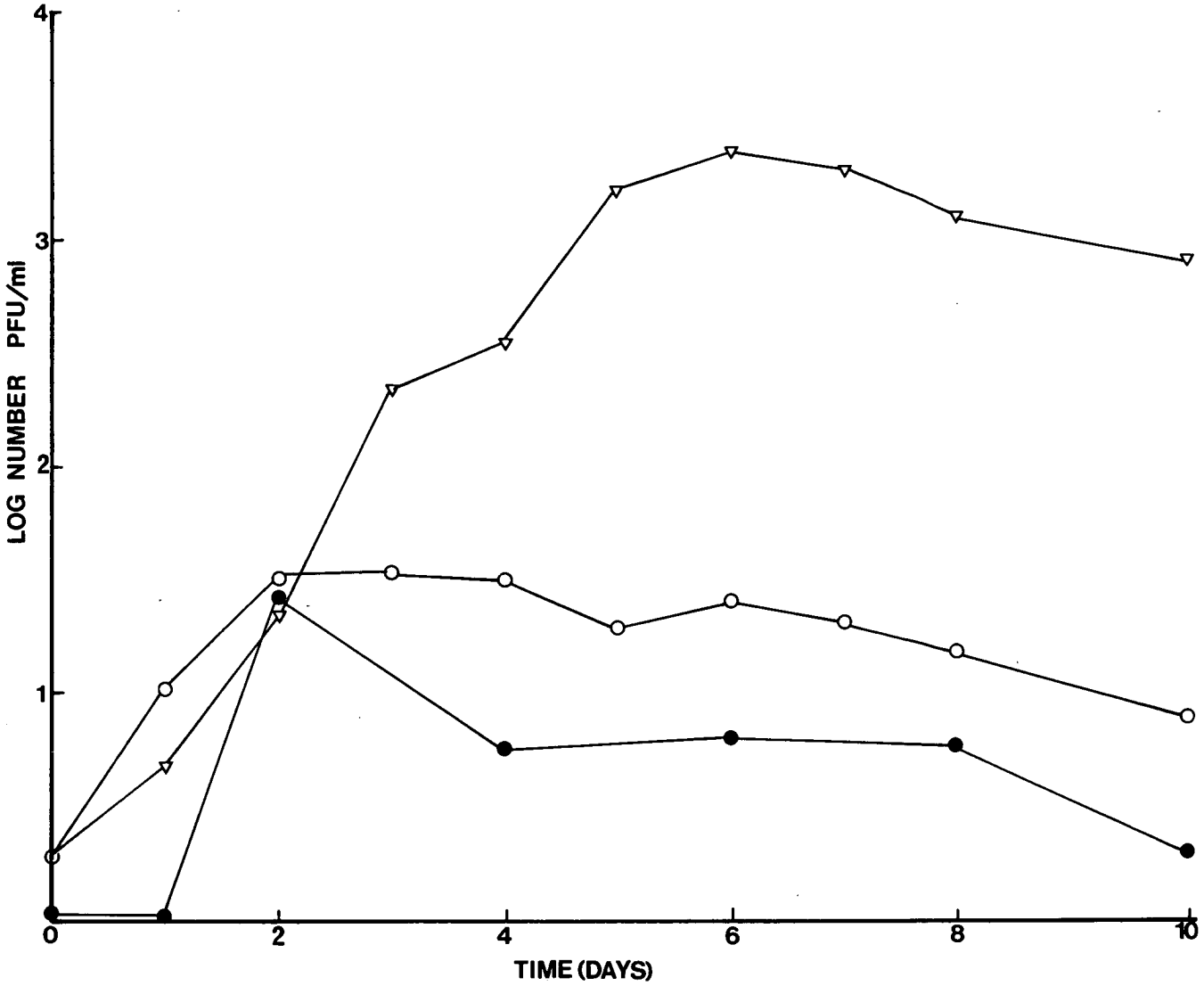
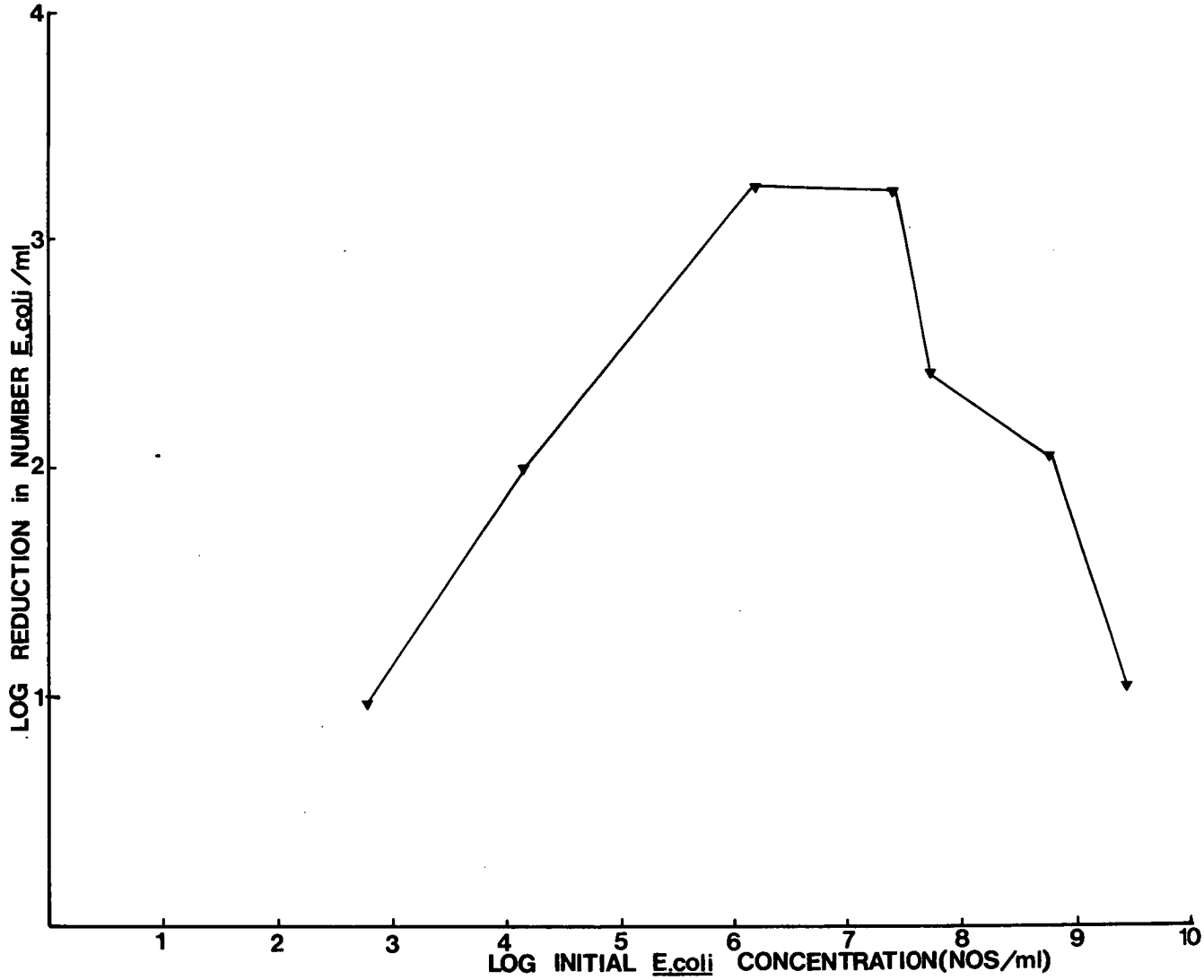

LOG NUMBER PFU/ml
4
3
2
1
0
2
4
6
8
10
TIME (DAYS)

Figure 21: Effect of initial *E. coli* concentration on *E. coli* survival.
(Data from Appendix 8.a.)


LOG REDUCTION in NUMBER E.coli /ml
4
3
2
1
1
2
3
4
5
6
7
8
9
10
LOG INITIAL E.coli CONCENTRATION(NOS/ml)

To investigate the relative role of protozoan and bacterial predators at different initial *E. coli* concentrations, the above experiment was repeated with the addition of cycloheximide to estuarine water samples. The value for *E. coli* reduction (Figure 22.a.) in the presence of bacteria and protozoa again reached a maximum level at an optimum initial *E. coli* concentration of 10^6 to 10^7 organisms per ml as in Figure 21. In the presence of bacteria only, the reduction in *E. coli* numbers is lower than in the presence of bacteria and protozoa together for most initial *E. coli* concentrations (Figure 22.a.). There was a negligible decline in *E. coli* numbers in the two control samples (Appendix 8.b.3.). The effect of the initial *E. coli* concentration on the maximum number of PFU per ml is shown in Figure 22.b. In natural estuarine water the maximum number of PFU (bacteria and protozoa) remain fairly constant as the initial *E. coli* concentration is increased until a concentration of approximately 10^7 *E. coli* cells per ml is reached after which the number of PFU per ml increased as the initial *E. coli* concentration is increased. In the presence of bacterial predators only, however, the number of PFU per ml increases as the initial *E. coli* concentration increases from 10^2 to 10^9 organisms per ml. A similar pattern of *E. coli* decline and predator growth was obtained when this experiment was repeated (Appendix 8.c.).

Microscopic examination of the natural estuarine water samples and plaques from double-layer plates revealed the development of a number of predacious microorganisms following the inoculation of *E. coli*. At all initial *E. coli* concentrations amoeboid protozoans were not observed. The number of microflagellates, microciliates and rod-shaped bacterial predators, however, increased markedly from day 0 to day 2. These organisms persisted in all samples throughout the duration of the experiment, gradually declining in numbers to day 10.

Figure 22: Effect of initial *E. coli* concentration on *E. coli* survival.

(a) *E. coli* survival in:

Natural estuarine water (▼);

Natural estuarine water + cycloheximide (▽).

(Data from Appendix 8.b.1.).

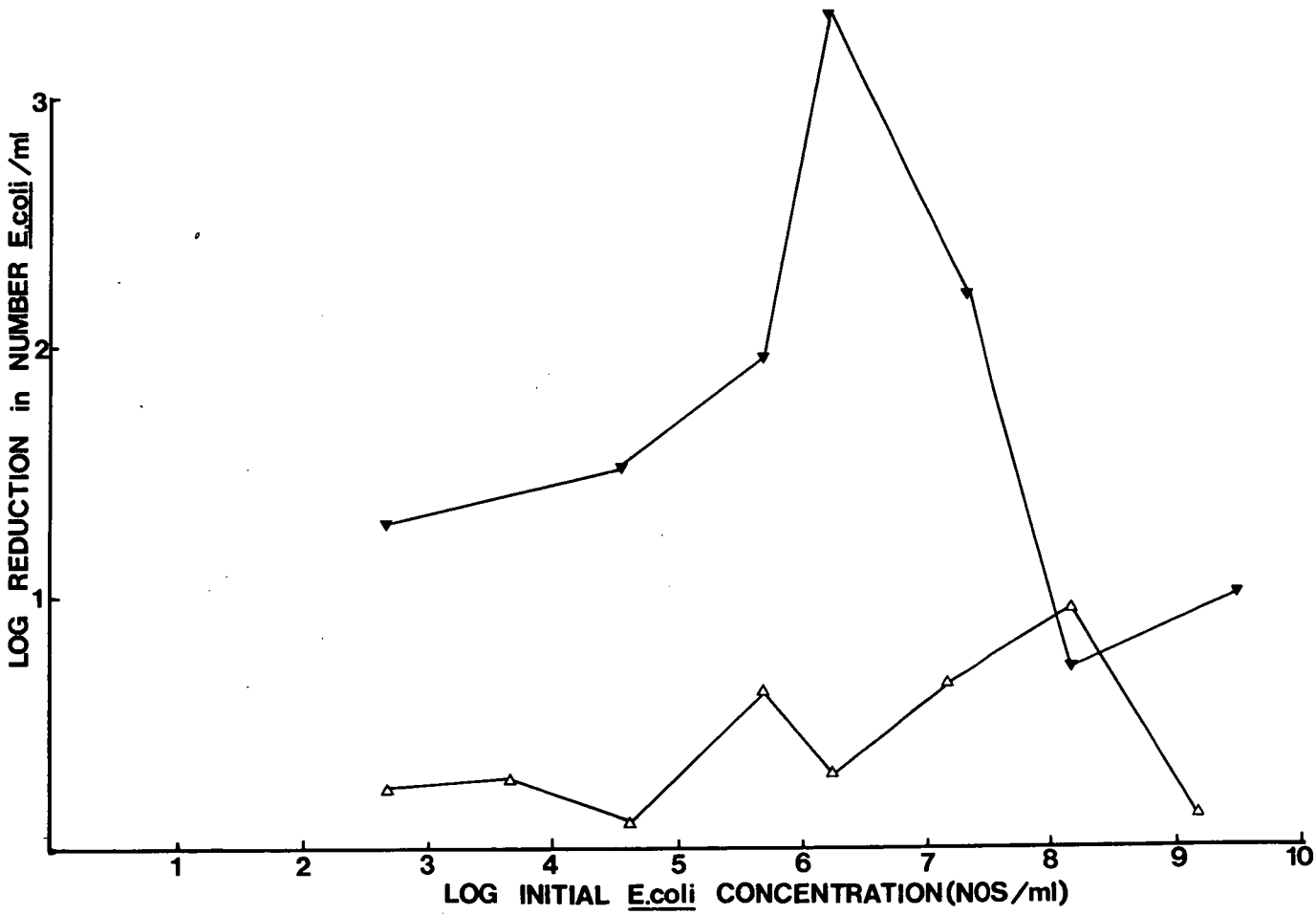

LOG REDUCTION in NUMBER E.coli /ml
3
2
1
1
2
3
4
5
6
7
8
9
10
LOG INITIAL E.coli CONCENTRATION(NOS/ml)

Figure 22: Effect of initial *E. coli* concentration on *E. coli* survival.

(b) Growth of PFU in:

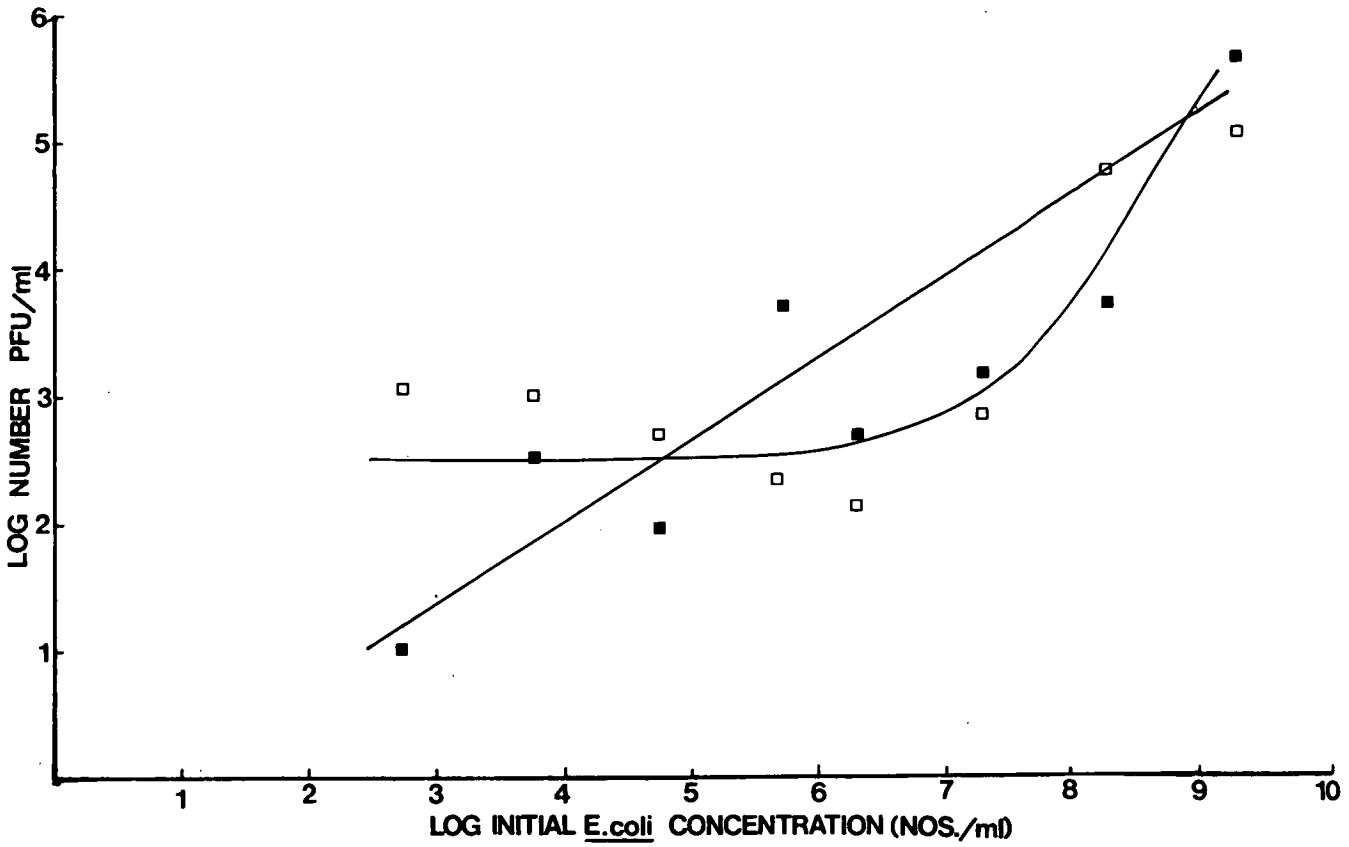
Natural estuarine water (□);

Natural estuarine water + cycloheximide (■).

Regression data: $y = 0.55x - 0.24$;

Correlation coefficient: 0.88.

(Data from Appendix 8.b.2.)


LOG NUMBER PFU/ml
1
2
3
4
5
6
1
2
3
4
5
6
7
8
9
10
LOG INITIAL E.coli CONCENTRATION (NOS./ml)

Large ciliates appeared at day 5 to day 6 in those samples containing high initial *E. coli* concentrations (>10^7-10^8 cells per ml) and also gradually declined to day 10.

D.4. Effect of Diffusible Substances

The decline of *E. coli* in natural estuarine water in the central diffusion reservoir of the Ecologen is shown in Figure 23. *E. coli* numbers are reduced from 1.5 x 10^8 to 88 organisms per ml in 16 days. The decline of *E. coli* cells in the replicate autoclaved estuarine water samples exposed to diffusible substances from the central diffusion reservoir is, however, not significantly different to the decline of *E. coli* cells in the control sample. *E. coli* cells in the three growth chambers exhibited only a gradual decline in numbers from approximately 10^8 to 10^6 organisms per ml in 16 days.

To determine the effect of bacterial predators only, in this experiment cycloheximide was added to the central diffusion reservoir. *E. coli* cells were reduced from 1.4 x 10^8 to 28 organisms per ml in 16 days (Figure 24). The *E. coli* cells in the control chamber also declined in numbers from 1.6 x 10^8 to 1.3 x 10^5 organisms per ml. In the replicate growth chambers exposed to diffusible substances from the central diffusion reservoir, however, *E. coli* cells were reduced to only 1 x 10^7 organisms per ml after 16 days incubation.

Discussion

D.1 Relative Effects of Bacterial and Protozoan Predators

Enzinger and Cooper (1976) examined the effect of predacious protozoans on the survival of *E. coli* in estuarine water using an antibiotic-resistant *E. coli* mutant and subsequently destroying the bacterial predators present in the water sample. They found that

Figure 23: Effect of diffusible substances on *E. coli* survival in natural estuarine water.

Central diffusion reservoir (▽);
Growth chambers[a] (O);
Control chamber (●).

[a]Each point represents the mean ± 1 standard error of the mean.

(Data from Appendix 9.a.)

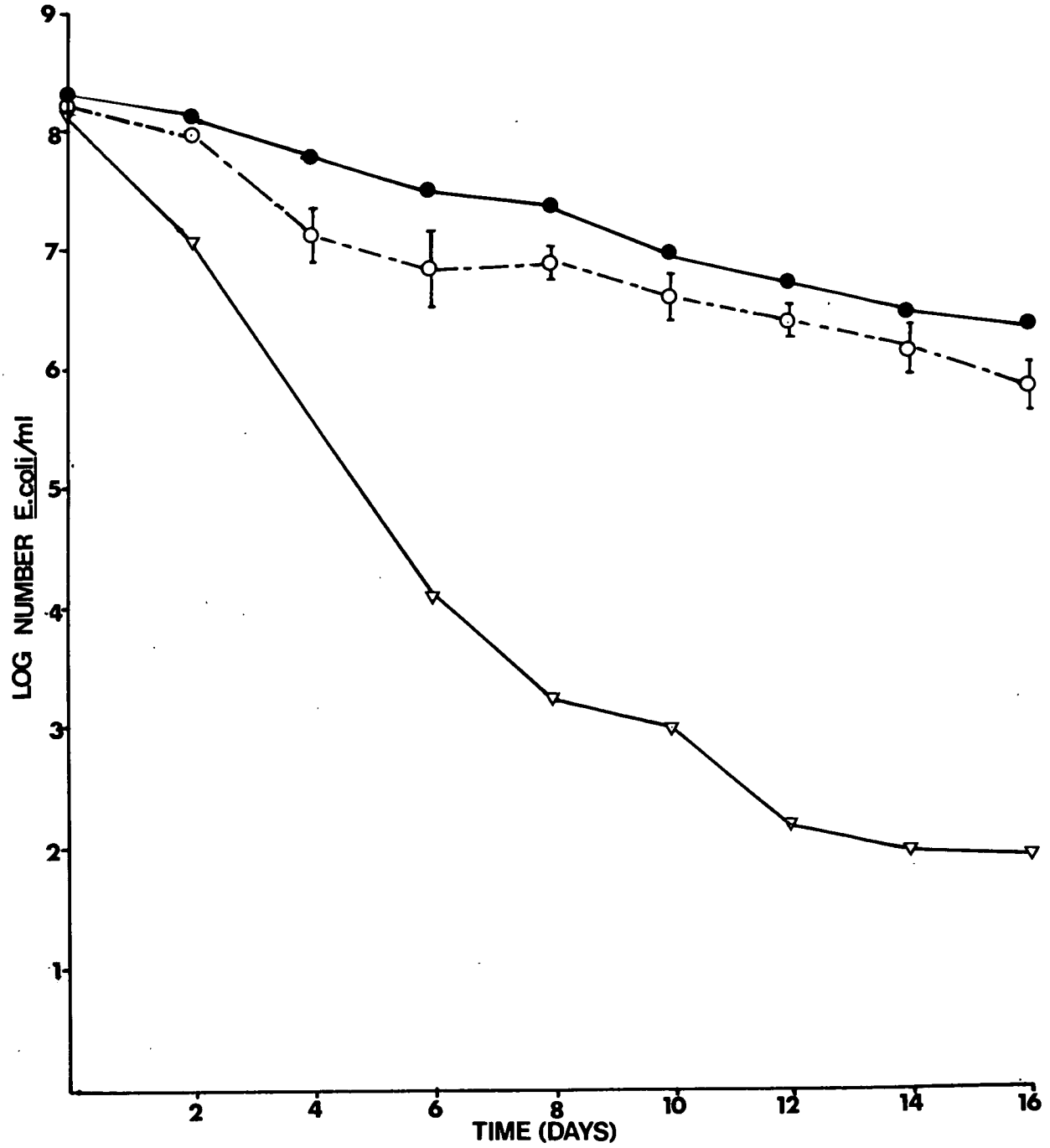
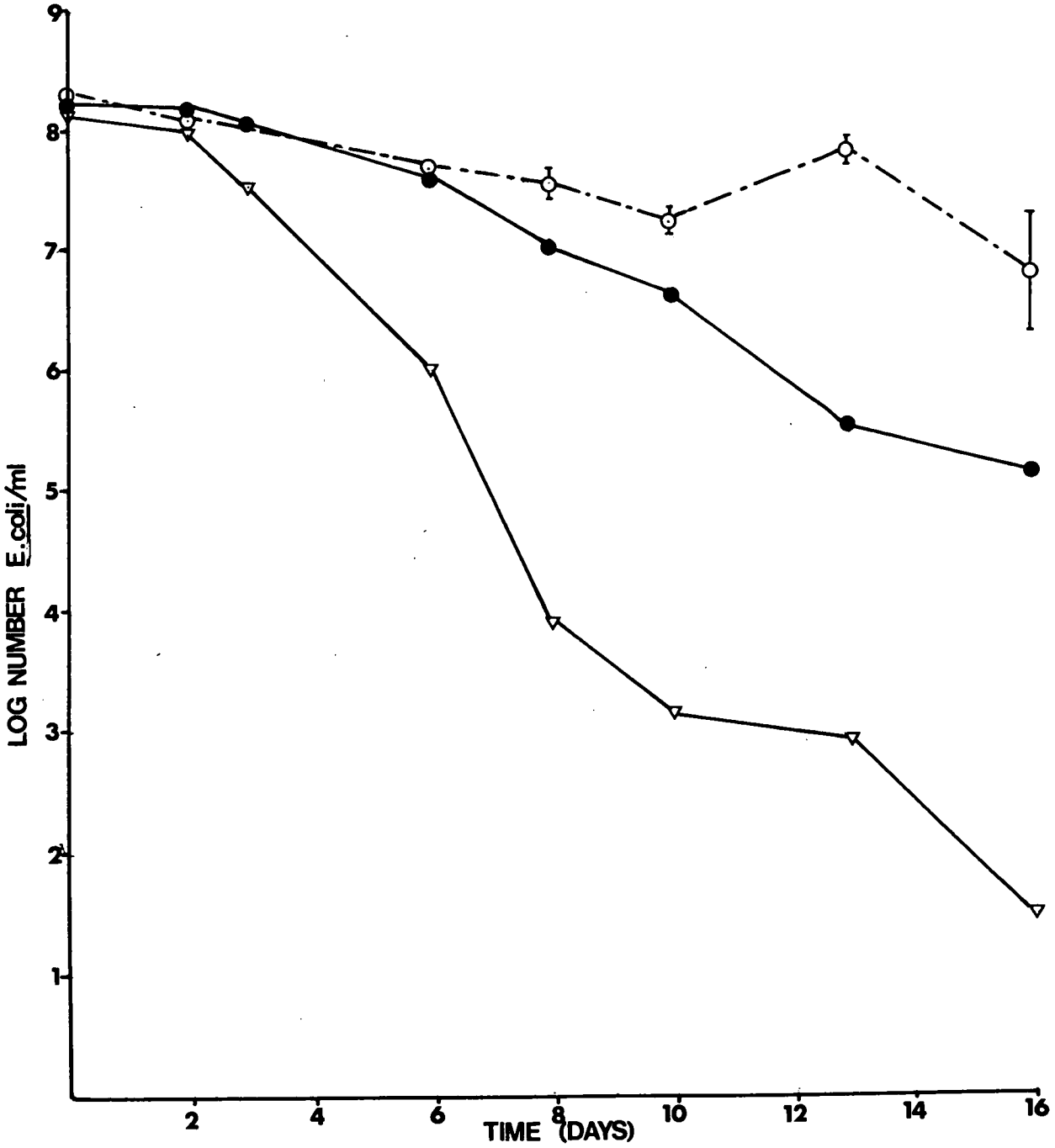

9
8
7
6
5
4
3
2
1
LOG NUMBER E.coli/ml
2
4
6
8
10
12
14
16
TIME (DAYS)

Figure 24: Effect of diffusible substances on *E. coli* survival in natural estuarine water + cycloheximide.

Central diffusion reservoir (▽);
Growth chambers[a] (O);
Control chamber (●).

[a]Each point represents the mean ± 1 standard error of the mean.

(Data from Appendix 9.b.)


LOG NUMBER E.coli/ml
9
8
7
6
5
4
3
2
1
2
4
6
8
10
12
14
16
TIME (DAYS)

survival of *E. coli* was dependent on the presence of protozoan predators and not on the presence of lytic bacteria. However, if protozoa alone were responsible for the destruction of the *E. coli* prey, then inhibition of this predacious group should result in prey survival similar to that which occurs in autoclaved estuarine water. In Figure 17.a. the survival of *E. coli* in estuarine water was increased when the predacious protozoan population was inhibited by the use of cycloheximide, but the survival was less than that in the autoclaved sample. This suggests that bacterial predators are also important in the decline of *E. coli* in estuarine water samples.

Concurrent with this destruction of *E. coli*, there was an increase in the natural microbial population antagonistic towards these bacteria (Figure 17.b.). This pattern of prey destruction and predator growth has been repeatedly observed following the introduction of bacteria of non-marine origin and in particular *E. coli*, into seawater (Mitchell and Morris, 1969; Enzinger and Cooper, 1976; Roper and Marshall, 1978; McCambridge and McMeekin, 1979). When the protozoan predators were inhibited, the bacterial predators reached, and were maintained, at similar levels to when protozoan and bacterial predators were present together (Figure 17.b.). This indicated that the protozoan predators"grazed" not only on *E. coli* cells, but also on bacterial predators, thereby maintaining them at relatively low levels in the natural estuarine water sample.

D.2 Effect of Periodic Inhibition of Protozoans

When protozoan predators were inhibited after 2 days incubation, survival of *E. coli* was similar to that in the natural sample (Table 17), suggesting that these predators exerted their major influence on the

E. coli prey population during this period. Roper and Marshall (1978) observed the development of a sequence of microbial predators following the introduction of *E. coli* into seawater. An initial population of bacterial and smaller protozoan predators was replaced after 5 to 7 days by larger carnivorous ciliates which destroyed the smaller predators and the remaining *E. coli* population. In this study, the addition of cycloheximide to water samples before day 2 resulted in the inhibition of these smaller protozoan predators and so produced the observed increase in *E. coli* survival. In the first 2 days in natural estuarine water samples these protozoan predators exerted their major influence on the *E. coli* population and thus inhibition after this time had no effect on *E. coli* survival. The inhibition of the larger ciliate protozoans which developed in the population similarly had little or no effect on the *E. coli* population.

When protozoa were inhibited, bacterial predators increased in numbers to a higher level than that which occurred in natural estuarine water samples (Figures 19 and 20), further indicating the destruction of bacterial predators by protozoa. Periodic inhibition of the protozoan predators showed that their major effect on bacterial predators (as for *E. coli*) was also during the first 2 days of the 10 day decline period.

If protozoan predators were only able to reduce a bacterial prey population to a level of 10^6 to 10^7 cells/ml (Berk *et al.*, 1976), the inoculation of an *E. coli* prey population of 10^5 cells/ml would not be expected to invoke a response from protozoan predators present in natural estuarine water samples. However, an *E. coli* population of this concentration produced a similar response from the protozoan population as that produced by 10^8 prey cells/ml, as noted above, again exerting their major effect in the first 2 days. This again

suggests that it is the smaller ciliates and microflagellates which are responsible for much of the *E. coli* decline during the first 2 days and not the larger ciliates similar to those observed by Berk *et al.* (1976).

D.3 Effect of Initial *E. coli* Concentration

In an examination of the effect of different experimental procedures on *E. coli* survival in seawater, Carlucci and Pramer (1960a) concluded that there was no significant difference in the survival of cells inoculated at concentrations of 10^3 to 10^6 organisms per ml after 48 hours incubation. Further, Pike *et al.* (1970) found no conclusive relationship existed between T_{90} for coliform survival and initial cell counts, although the initial counts were low,ranging from only 10 to 10^6 organisms per 100ml. In Figures 21 and 22a., however, the maximum reduction in *E. coli* cells occurred at an optimum initial prey concentration of 10^6 to 10^7 cells per ml.

The introduction of prey cells into estuarine water resulted in an increase in the naturally-occurring microbial predators to a certain level. As the concentration of prey was increased up to a level of 10^6 to 10^7 organisms per ml, the microbial predators (bacteria and protozoa) did not increase in numbers (Figure 22.b.), but still caused an increased reduction of prey cells (Figure 22.a.). Hamilton and Preslan (1969) examined the growth of the marine ciliate, *Uronema* sp., and observed that increasing the prey concentration resulted in increases in the individual cell size of the protozoan rather than causing an increase in cell numbers by reproduction. This, it was suggested, would enable the organism to survive under conditions of low food supply as it would maintain its numbers if not its size.

Fenchel (1980a) noted that the uptake of food particles by ciliates at increasing prey concentrations followed a hyperbolic function. The maximum uptake level was dependent upon the mouth apparatus of the ciliate and the particle size of the prey. Similarly, at a prey concentration of 10^6 to 10^7 organisms per ml (Figures 21 and 22.a.), the predators became saturated with prey and brought about the maximum level of prey destruction. Increasing prey concentrations above this level resulted in reproduction and so an increase in the number of predators. Due to the high prey concentrations, however, these predators required a greater length of time to cause a reduction in prey numbers. Thus, there was a reduction in the destruction of prey numbers in a 2 day decline period as the prey concentration increased. Enzinger and Cooper (1976) observed a 2 to 4 day lag period in *E. coli* survival in estuarine water and suggested that this was the time required for protozoa to reach a sufficient density to effect *E. coli*. This lag period could be reduced by the addition of a less concentrated suspension of *E. coli* at the start of the experiment.

Rogerson (1980) observed that optimum generation times for an *Amoeba* sp. were found at prey concentrations less than the maximum prey level investigated. In fact, the highest prey concentrations used caused the complete destruction of the *Amoeba* sp. population due to the inability of the cells to feed at this prey density. Similarly, it has been observed (Proper and Garver, 1966; Curds and Cockburn, 1968; Laybourn and Stewart, 1975), that there is a reduction in the growth rates of predacious protozoa at high prey concentrations. The increase in predators therefore, above a prey concentration of 10^6 to 10^7 organisms per ml, may largely be due to an increase in bacterial predators. These organisms are not apparently subject to the feeding restrictions of protozoan predators, but increase in a linear fashion as

prey concentration is increased (Figure 22.b.).

D.4 Effect of Diffusible Substances

When examining the role of diffusible substances on the survival of non-marine bacteria in seawater, there are three main groups of substances to be considered. These are antibiotics and extracellular enzymes which cause destruction of bacterial cells, and nutrients released from cellular breakdown, resulting in the growth of bacterial cells. Although antibiotic-producing bacteria have been isolated from seawater (Trunova and Izgoreva, 1976), their importance in the natural environment is considered minimal (Carlucci and Pramer, 1960c).

Mitchell and Nevo (1965) demonstrated that a marine *Pseudomonas* sp. released polysaccharidases active against *Flavobacterium* capsular material, extracellularly, while the enzyme system active against *E. coli* 'B' walls was intracellular. Roper and Marshall (1978) suggested that the marine myxobacter *Polyangium* required direct contact with *E. coli* to cause lysis of the prey cells, as *E. coli* was not lysed by a filtrate of the myxobacter growth medium and no diffusible enzymes were detected. It was concluded that enzymes located on the surface of the myxobacter were responsible for lysis of *E. coli* cells.

The rumen protozoan *Epidinium* sp. was found to release enzymes *in vivo* which lysed *Bacillus* and *Micrococcus* sp. (Coleman and Laurie, 1974), although no evidence was presented that lytic enzymes were actually present in the rumen. Berk *et al.* (1976) showed that filtrates of estuarine ciliate *Uronema* sp. had no effect on bacterial populations and concluded that engulfment of bacteria by the protozoan was most important. It would appear, therefore, that both protozoan and bacterial

predators require contact with their prey and that diffusible extracellular enzymes are not produced.

Buck *et al.* (1952) noted that when coliform bacteria were destroyed in estuarine water, the protoplasm of their cells acted as a source of nutrients for the surviving organisms which subsequently increased in numbers. Similarly, in this study, the absence of protozoan predators enabled the build up of large numbers of bacterial predators which caused the lysis of *E. coli* prey and the release of nutrients. These diffused through the membrane filter and caused growth of *E. coli* cells (Figure 24). Protozoan predators, however, maintained bacterial predators at low levels, and engulf *E. coli* cells in food vacuoles, thereby minimizing the release of nutrients to the aqueous system and resulting in negligible growth of the remaining *E. coli* cells (Figure 23).

E. Effect of Individual Predators on Survival of *E. coli* and *S. typhimurium*

Results

Morphology, gram reaction and prey specificity of *E. coli* and *S. typhimurium* predators isolated from estuarine water samples is shown in Tables 19 and 20. All bacterial predators isolated were long, gram negative rods. *E. coli* and *S. typhimurium* predators were capable of forming clearing zones or plaques on the two alternative prey strains used. Bacterial predators used in survival experiments were further examined using a transmission electron microscope (TEM). These are uniformly cylindrical rod-shaped bacteria with blunt, rounded ends. *E. coli* predators EP3 and EP7 (Plates 1 and 2) are 4.0-8.0μm in length and 0.8 to 1.0μm in diameter. *S. typhimurium* predators SP1 and SP6

TABLE 19: *E. coli* predators.

	EP1	EP3	EP4	EP5	EP6	EP7	EP8	EP9	EP10
Morphology[a]	LR	LR	LR	LR	LR	LR	LR	LR	LR
Gram reaction	-	-	-	-	-	-	-	-	-
Growth on NAS[b]	-	-	-	-	-	-	-	-	-
Plaque formation[c]: *S. typhimurium*	+	+	+	+	+	+	+	+	+
K. pneumoniae	+	+	+	+	+	+	+	+	+
Autoclaved *E. coli*	-	-	-	-	-	-	-	-	-

[a] LR - long bacterial rod

[b] Incubation at 5 days/22°C

[c] Formation of plaques on double-layer plates after 6 days/22°C (results of three observations).

TABLE 20: *S. typhimurium* predators.

	SP1	SP2	SP3	SP6	SP7	SP8
Morphology[a]	LR	LR	LR	LR	LR	LR
Gram reaction	-	-	-	-	-	-
Growth on NAS[b]	-	-	-	-	-	-
Plaque formation[c]: *E. coli*	+	+	+	+	+	+
K. pneumoniae	+	+	+	+	+	+
Autoclaved *E. coli*	-	-	-	-	-	-

[a] LR - long bacterial rods.

[b] Incubation at 5 days/22°C.

[c] Formation of plaques on double-layer plates after 12 days/22°C.

(results of three observations).

Plate 1 *E. coli* predator EP3.

Bar = 1µm.

Plate 2 *E. coli* predator EP7.

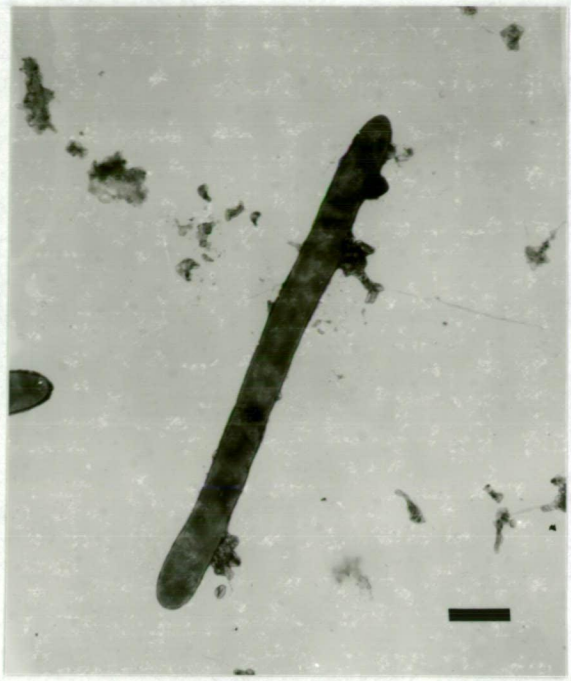
Bar = 1µm.

Plate 3 *S. typhimurium* predator SP1.

Bar = 1µm.

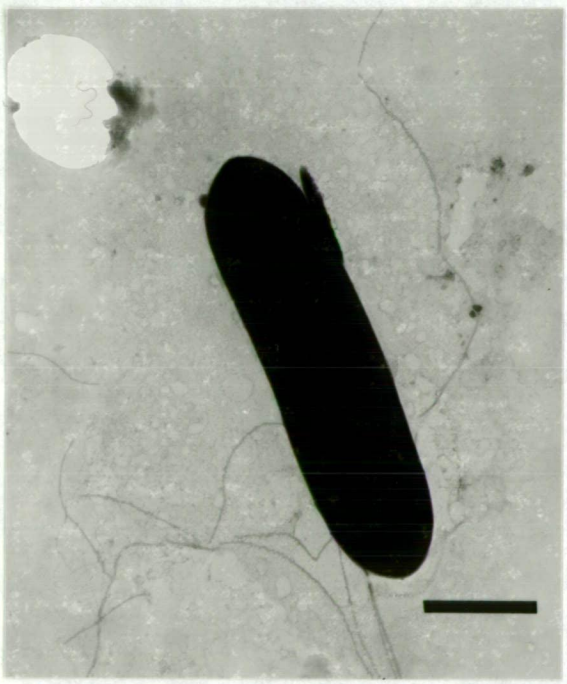
Plate 4 *S. typhimurium* predator SP6.

Bar = 1µm.

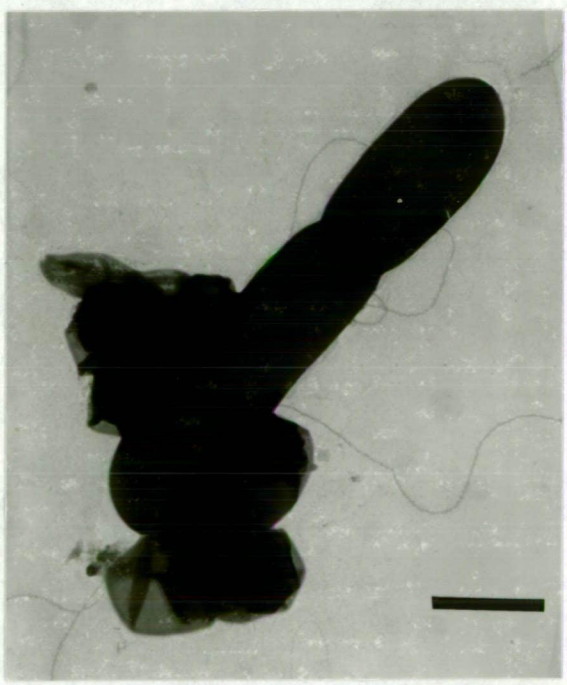


1

2



3



4

(Plates 3 and 4) are 4.0μm in length and 1.0μm in diameter.

E.1. *E. coli* predators EP3 and EP7 and *E. coli*

The survival of *E. coli* in the presence of EP3 and EP7 is shown in Figure 25.a. The latter predator reduced *E. coli* from approximately 10^8 to 3.5 x 10^2 organisms per ml after 30 days. Predator EP3 reduced *E. coli* to 1.2 x 10^4 organisms per ml after 20 days incubation, after which *E. coli* numbers increased to 7 x 10^4 organisms per ml. The growth of predators is seen in Figure 25.b. The number of PFU per ml increased for both predators in the first 4 days to reach a maximum level of approximately 5 x 10^5 PFU per ml and then gradually declined to approximately their original level after 30 days. The inoculation of predator suspensions into control autoclaved estuarine water samples in the absence of *E. coli* in fact resulted in the transfer of *E. coli* prey from the double-layer plates (Table 21) and the subsequent small increase in PFU numbers (Table 21).

E.2. Combination of *E. coli* Predators EP3 and EP7

The effect of two *E. coli* predators, inoculated together, on *E. coli* survival is seen in Figure 26.a. *E. coli* numbers were reduced to a similar level of approximately 10^5 organisms per ml, by both predators acting individually or when acting together. *E. coli* cells were reduced in numbers reaching a minimum level at day 16. The number of *E. coli* cells subsequently increased to day 24 and then declined to day 30. Conversely, predator numbers increased markedly reaching a maximum level of approximately 5 x 10^5 PFU per ml after 12 days, decreased to day 24 to 28, and then increased slightly to day 32 (Figure 26.b.).

Figure 25: (a) Survival of *E. coli* prey in the presence of *E. coli* predators:

EP3 (●)

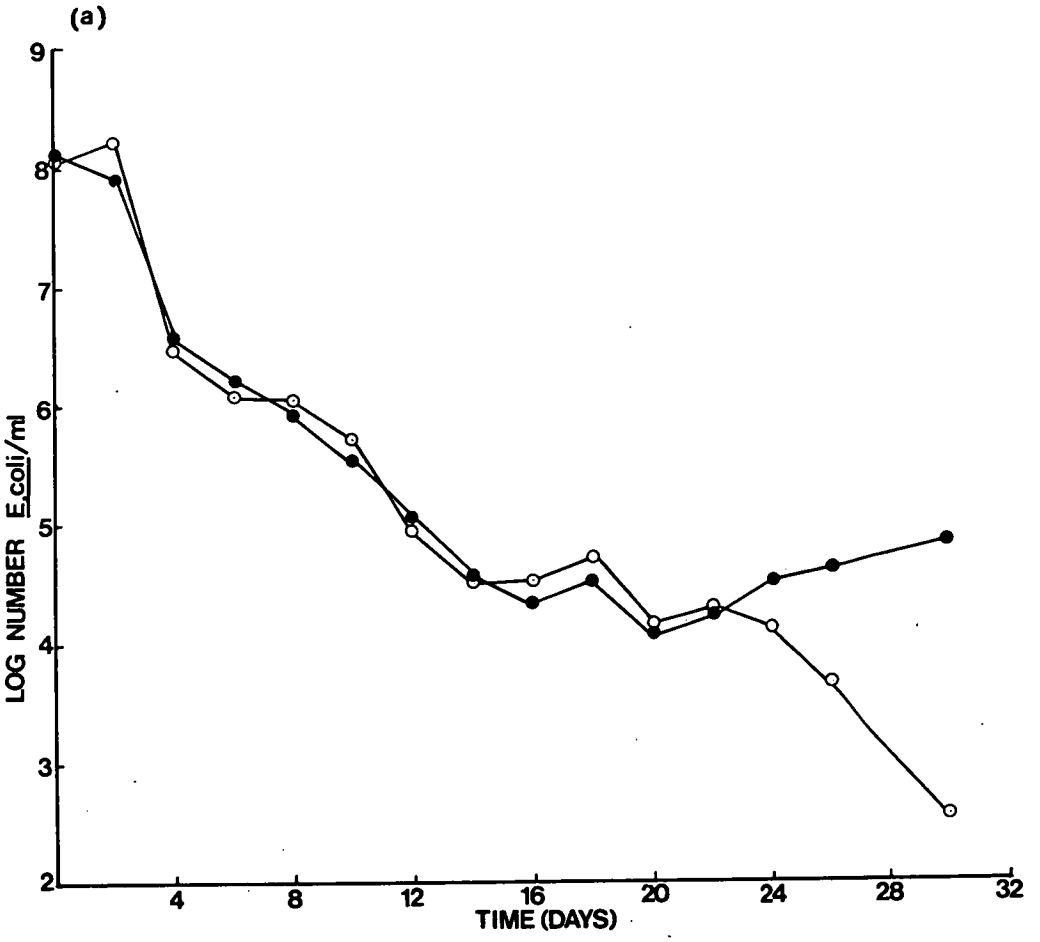
EP7 (O)

(b) Growth of *E. coli* predators:

EP3 (●)

EP7 (O)

(Data from Appendix 10.a.)


(a)
LOG NUMBER E.coli/ml
9
8
7
6
5
4
3
2
4
8
12
16
20
24
28
32
TIME (DAYS)

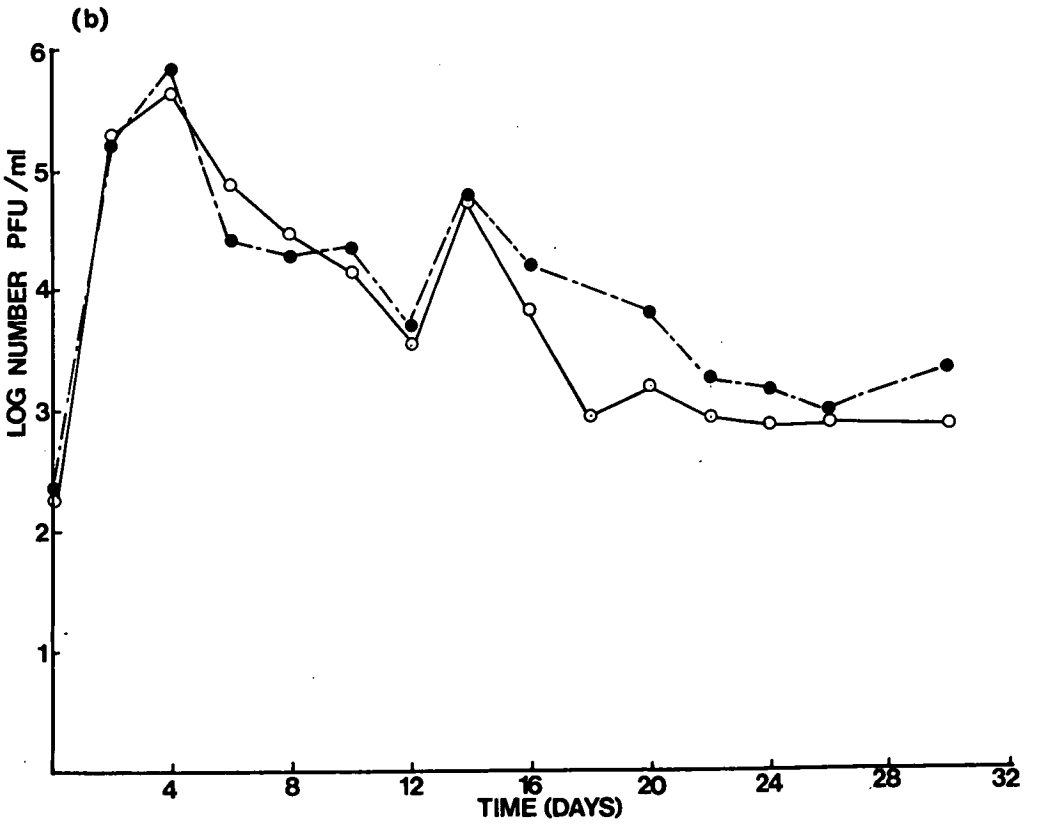

(b)
LOG NUMBER PFU /ml
6
5
4
3
2
1
4
8
12
16
20
24
28
32
TIME (DAYS)

TABLE 21: *E. coli* survival and PFU growth in predator controls.

LOG NO. ORGANISMS PER ML

TIME (DAYS)	*E. COLI* SURVIVAL		PFU GROWTH	
	EP3	EP7	EP3	EP7
0	2.66	1.42	2.26	2.38
2	2.76	0.60	2.26	2.68
4	-	2.57	3.14	2.60
6	-	-	2.99	2.08
8	4.59	4.28	2.30	-
10	4.05	4.05	-	1.70
12	4.26	4.27	1.81	-
14	3.94	4.02	2.73	3.06
16	4.00	3.78	2.90	3.27
18	3.54	3.46	2.83	3.16
20	3.40	3.49	3.24	3.26
22	3.00	3.18	3.01	2.89
24	3.35	3.31	3.54	3.26
26	3.17	2.92	3.71	3.78
30	2.30	2.32	3.65	3.00

Figure 26:

(a) Survival of *E. coli* prey in the presence of *E. coli* predators:

EP3 (●)

EP7 (O)

EP3 + EP7 (▼)

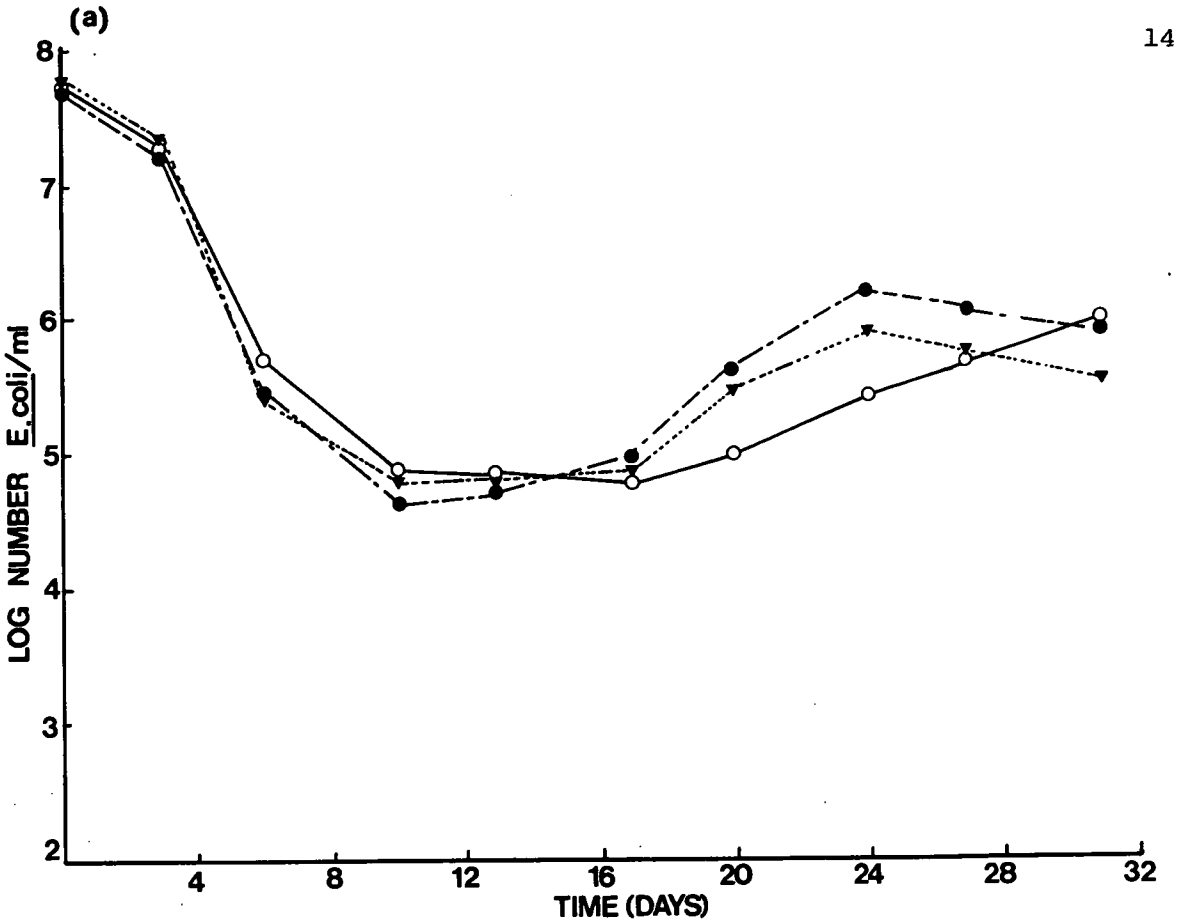
(b) Growth of *E. coli* predators:

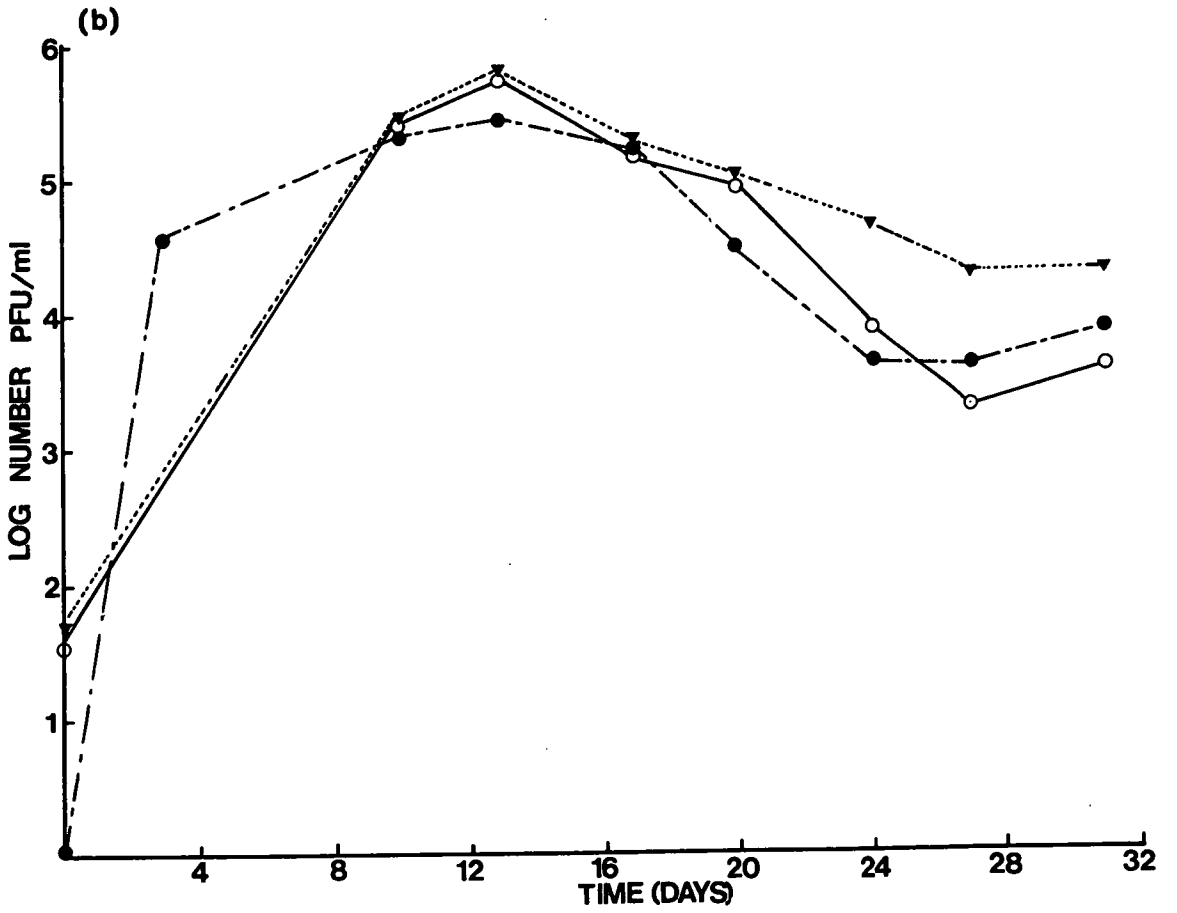
EP3 (●)

EP7 (O)

EP3 + EP7 (▼)

(Data from Appendix 10.b.)


(a)
LOG NUMBER E.coli/ml
8
7
6
5
4
3
2
4
8
12
16
20
24
28
32
TIME (DAYS)


(b)
LOG NUMBER PFU/ml
6
5
4
3
2
1
4
8
12
16
20
24
28
32
TIME (DAYS)

E.3. *E. coli* Predators EP3 and EP7 and *S. typhimurium* Prey

The survival of *S. typhimurium* in the presence of *E. coli* predators EP3 and EP7 is shown in Figure 27.a. There is a lag phase of 12 to 16 days before prey numbers are reduced for both predators in comparison to a 0 to 4 day lag phase with *E. coli* as the prey organism (Figures 25.a. and 26.a.). After this lag phase prey numbers are reduced to similar levels (approximately 10^5 organisms per ml) for both *E. coli* predators. The growth of predators is shown in Figure 27.b. The number of PFU increased markedly in the first 4 to 8 days, reaching a maximum value of approximately 10^6 PFU per ml on day 13 followed by a gradual decline in numbers till the completion of the experiment (day 32). This experiment was repeated and a similar pattern of predator growth and prey destruction was obtained (Appendix 10.c.2.).

E.4. *S. typhimurium* Predators SP1 and SP6 and *S. typhimurium* Prey

The effect of *S. typhimurium* predators on *S. typhimurium* prey is shown in Figure 28.a. Bacterial predators SP1 and SP6 reduced prey numbers from 10^8 to 5×10^5 organisms per ml in 20 days, with prey numbers remaining fairly constant for the following 18 days of incubation. Predator numbers (Figure 28.b.) again increased markedly in the first 8 days to reach a maximum of approximately 10^5 PFU per ml and then gradually declined. A similar pattern of predator growth and prey destruction was obtained when this experiment was repeated (Appendix 10.d.2.). Prey cells were again transferred with the bacterial predators to the control estuarine water samples, although with *S. typhimurium* as the prey organism little or no growth of predators occurred (Table 22).

Figure 27: (a) Survival of *S. typhimurium* prey in the presence of *E. coli* predators:

EP3 (●)

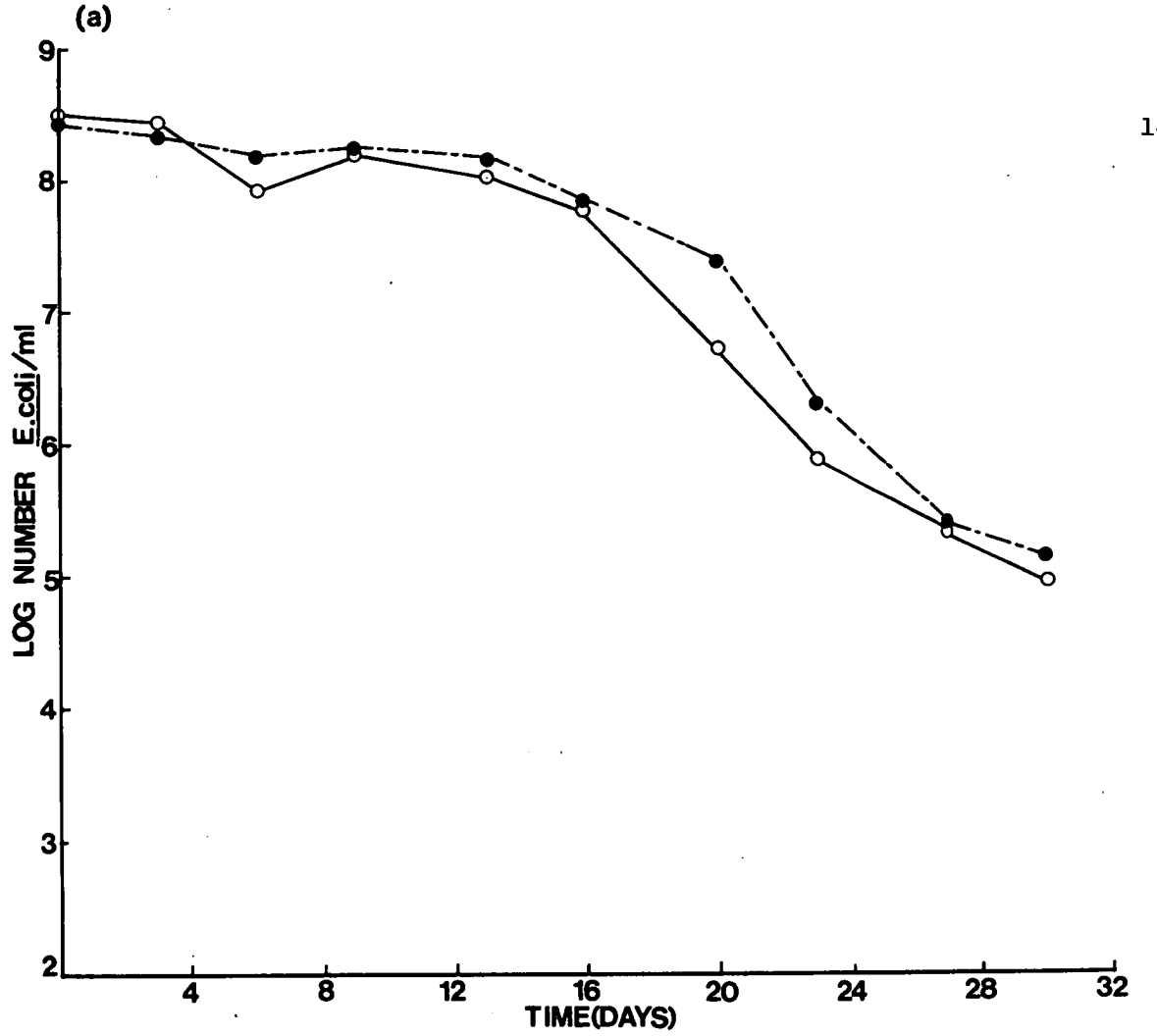
EP7 (O)

(b) Growth of *E. coli* predators:

EP3 (●)

EP7 (O)

(Data from Appendix 10.c.1.)


(a)
LOG NUMBER E.coli/ml
9
8
7
6
5
4
3
2
4
8
12
16
20
24
28
32
TIME(DAYS)

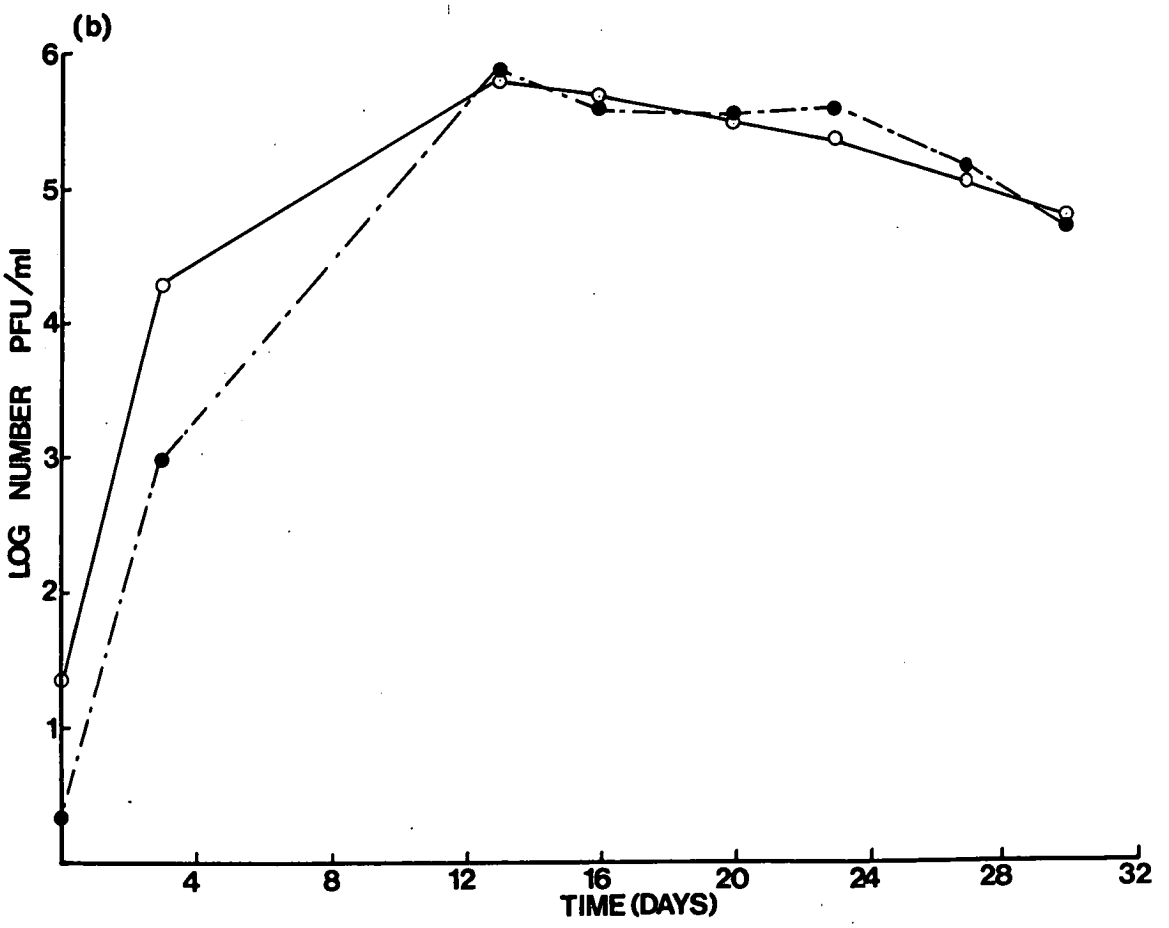

(b)
LOG NUMBER PFU/ml
6
5
4
3
2
1
4
8
12
16
20
24
28
32
TIME (DAYS)

Figure 28: (a) Survival of *S. typhimurium* prey in the presence of *S. typhimurium* predators:

SP1 (■)

SP6 (□)

(b) Growth of *S. typhimurium* predators:

SP1 (■)

SP6 (□)

(Data from Appendix 10.d.1.)

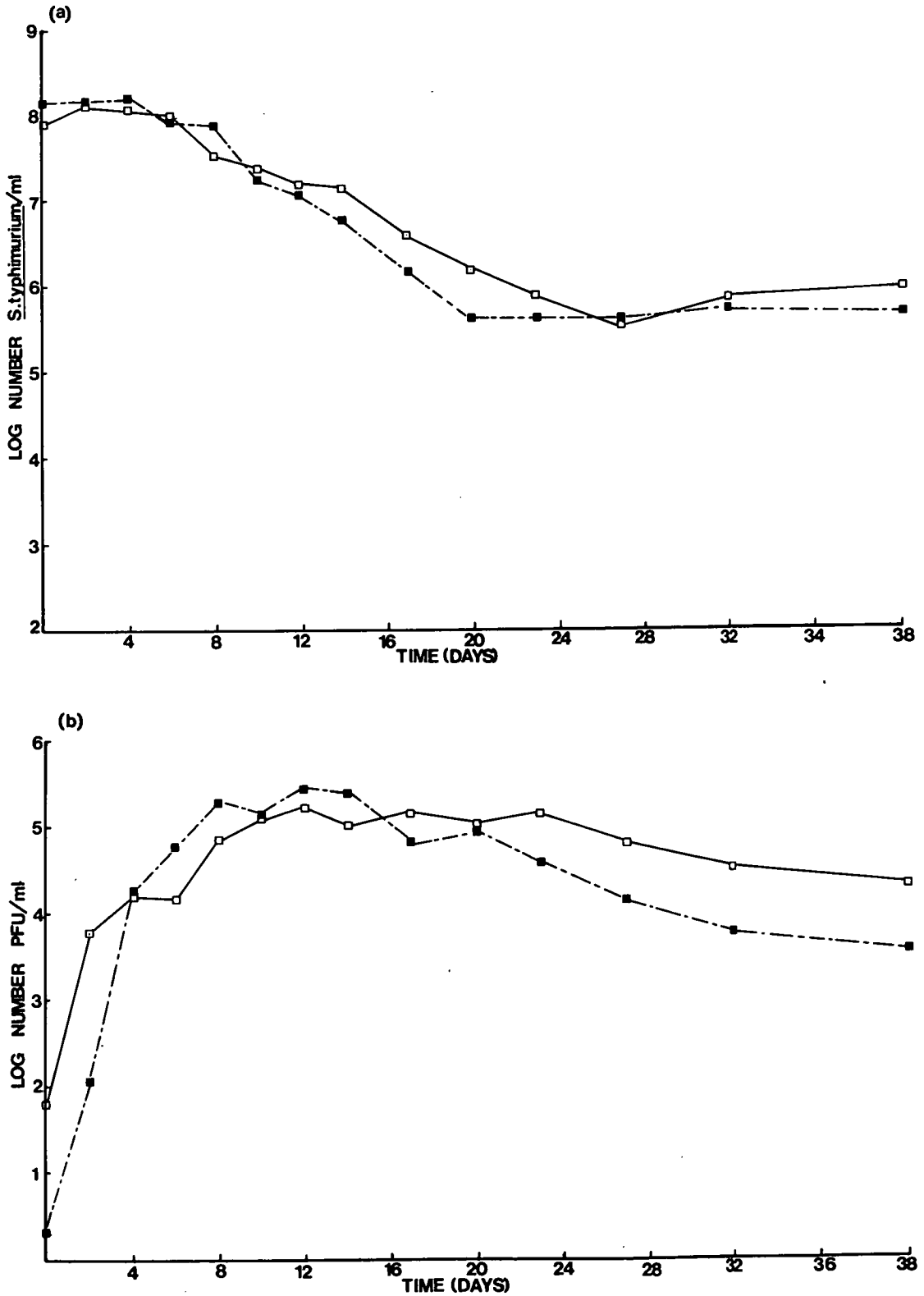

(a)
LOG NUMBER S.typhimurium/ml
9
8
7
6
5
4
3
2
4
8
12
16
20
24
28
32
34
38
TIME (DAYS)
(b)
LOG NUMBER PFU/ml
6
5
4
3
2
1
4
8
12
16
20
24
28
32
36
38
TIME (DAYS)

TABLE 22: *S. typhimurium* survival and PFU growth in predator controls.

LOG NO. ORGANISMS PER ML

TIME (DAYS)	*S. TYPHIMURIUM* SURVIVAL		PFU GROWTH	
	SP1	SP6	SP1	SP6
0	2.57	3.62	1.26	2.30
2	2.35	3.31	0.00	1.60
4	2.31	2.90	0.00	0.00
6	2.46	1.33	0.00	0.00
8	2.39	1.53	1.15	0.00
10	2.27	0.00	1.08	0.00
12	2.62	0.00	-	0.00
14	2.83	0.00	0.78	0.00
17	2.84	0.00	-	0.00
20	3.13	0.00	-	0.00
23	3.32	0.00	0.00	0.00
27	3.55	0.00	-	0.00
32	3.42	0.00	-	0.00
38	3.33	0.00	0.78	0.00

E.5. *S. typhimurium* Predators SP1 and SP6 and *E. coli* Prey

The survival of *E. coli* in the presence of *S. typhimurium* predators SP1 and SP6 is shown in Figure 29.a. Prey numbers were reduced from 1.5×10^8 to 1.7×10^6 organisms per ml after 16 days in the presence of predator SP1, followed by a period of growth with prey numbers almost returning to their original level. Similarly, predator SP6 reduced *E. coli* numbers from 1.5×10^8 to 4×10^6 organisms per ml after 16 days, followed by a gradual increase in numbers. Predator numbers (Figure 29.b.) increased during the initial 8 days incubation, reaching maximum numbers of 10^5 to 10^6 PFU per ml and then gradually declined in numbers during the remainder of the experiment.

Discussion

The bacterial predators isolated in this study were similar to myxobacters described by McCurdy (1974). The vegetative stage of one of these strains, *Polyangium*, has been consistently isolated from water near sewage outfalls (Roper and Marshall, 1977) and was similar to *S. typhimurium* predators (Plates 3 and 4). These bacteria were capable of growth on a wide host range including *E. coli*, *Klebsiella* sp., and *S. typhimurium*, as observed in Table 20.

The lysis of *E. coli* in sterile seawater by a marine myxobacter was examined by Roper and Marshall (1977). The growth of the myxobacter from approximately 10^2 to 10^6 organisms per ml in 3 days resulted in the decline of *E. coli* cells from approximately 10^9 to 10^3 organisms per ml. Similar patterns of bacterial predator growth and *E. coli* and *S. typhimurium* prey decline have been observed in Figures 25 to 29 In three of these experiments (Figures 25.a., 26.a. and 29.a.) the initial period of *E. coli* decline was followed by a period of *E. coli*

Figure 29: (a) Survival of *E. coli* prey in the presence of *S. typhimurium* predators:

SP1 (■)

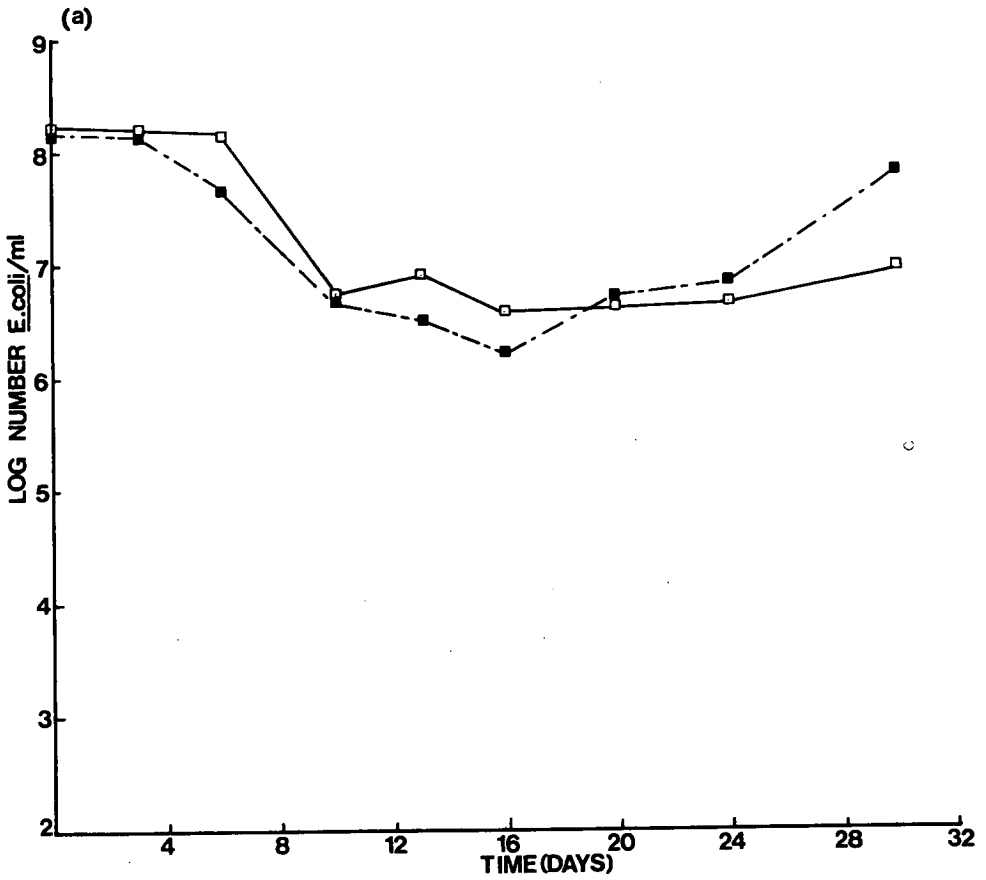
SP6 (□)

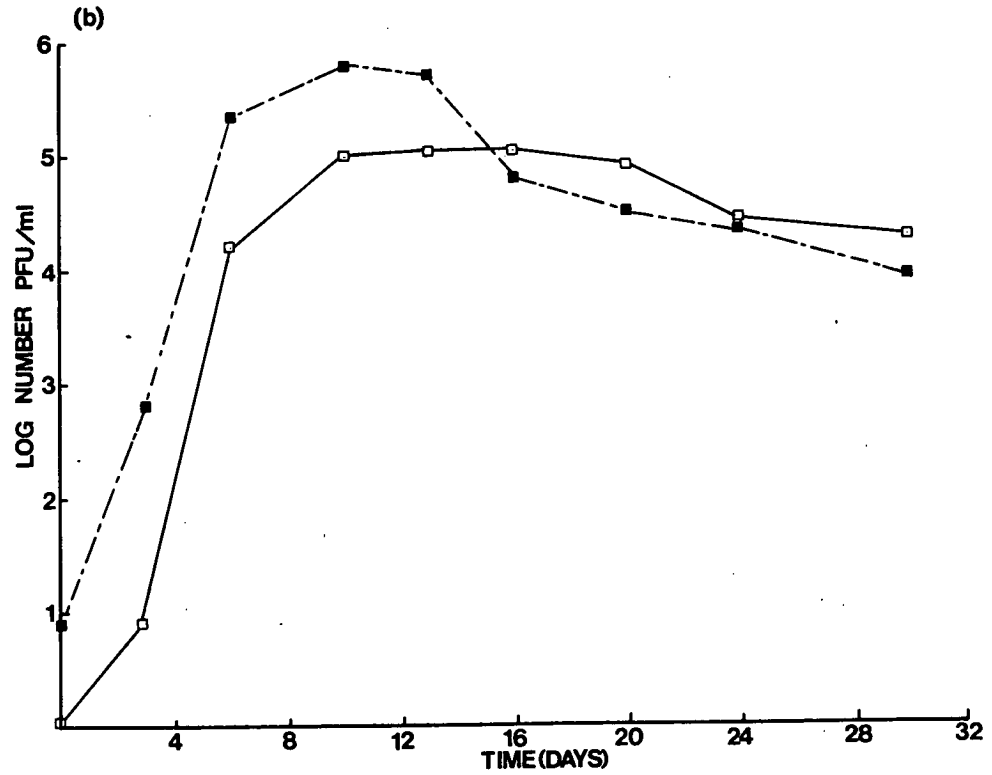
(b) Growth of *S. typhimurium* predators:

SP1 (■)

SP6 (□)

(Data from Appendix 10.e.)


(a)
LOG NUMBER E.coli/ml
9
8
7
6
5
4
3
2
4
8
12
16
20
24
28
32
TIME (DAYS)


(b)
LOG NUMBER PFU/ml
6
5
4
3
2
1
4
8
12
16
20
24
28
32
TIME (DAYS)

growth. This phenomenon of cryptic growth, also observed by Buck *et al.* (1952), resulted from the growth of the *E. coli* prey on the breakdown products of other *E. coli* cells.

It appears from Figures 25 to 29 that there are certain differences between *E. coli* and *S. typhimurium* predators in the presence of *E. coli* and *S. typhimurium* as prey species. *E. coli* predators have a short lag phase (0-2 days) with *E. coli* as the prey organism (Figure 26.a.) compared to a lag phase of 12-16 days with *S. typhimurium* as they prey organism (Figure 27.a.), although both prey species were eventually reduced in numbers to a similar degree (Table 23). For both prey species the maximum number of predators reached was the same (Table 23). *S. typhimurium* predators reached similar maximum numbers as the *E. coli* predators for both prey species. *E. coli* and *S. typhimurium* prey organisms were reduced to the same level by the *S. typhimurium* predators, but survived to a significantly greater degree than in the presence of *E. coli* predators. Thus, although for a prey concentration of approximately 10^8 organisms per ml (irrespective of the prey species), a similar maximum level of predators was reached for all predators (Table 23), *E. coli* predators were more effective in reducing the prey population than were *S. typhimurium* predators.

F. Survival of Test Bacteria Compared to *E. coli*

Results

The survival of *S. typhimurium* in estuarine water samples is compared to that of *E. coli* in Figure 30.a. Both organisms were reduced in numbers from approximately 10^8 to 10^2 organisms per ml in the 10 day decline period with no significant differences between the two species at each sampling time. The growth of predators is shown in Figure 30.b.

TABLE 23: Survival of prey species and growth of predators.

(Data from Figures 25 to 29)

LOG NO. ORGANISMS PER ML[a]

PREY SPECIES	PREDATOR SPECIES			
	E. COLI		*S. TYPHIMURIUM*	
	LOG REDUCTION	MAXIMUM PFU	LOG REDUCTION	MAXIMUM PFU
E. coli	3.50 (0.50)	5.65 (0.16)	1.80 (0.10)	5.50 (0.40)
S. typhimurium	2.90 (0.50)	5.85 (0.05)	2.20 (0.30)	5.30 (0.20)

[a] Values represent mean of two to four observations; standard error is shown within parentheses.

Figure 30: (a) Bacterial survival in estuarine water samples.

E. coli (■)

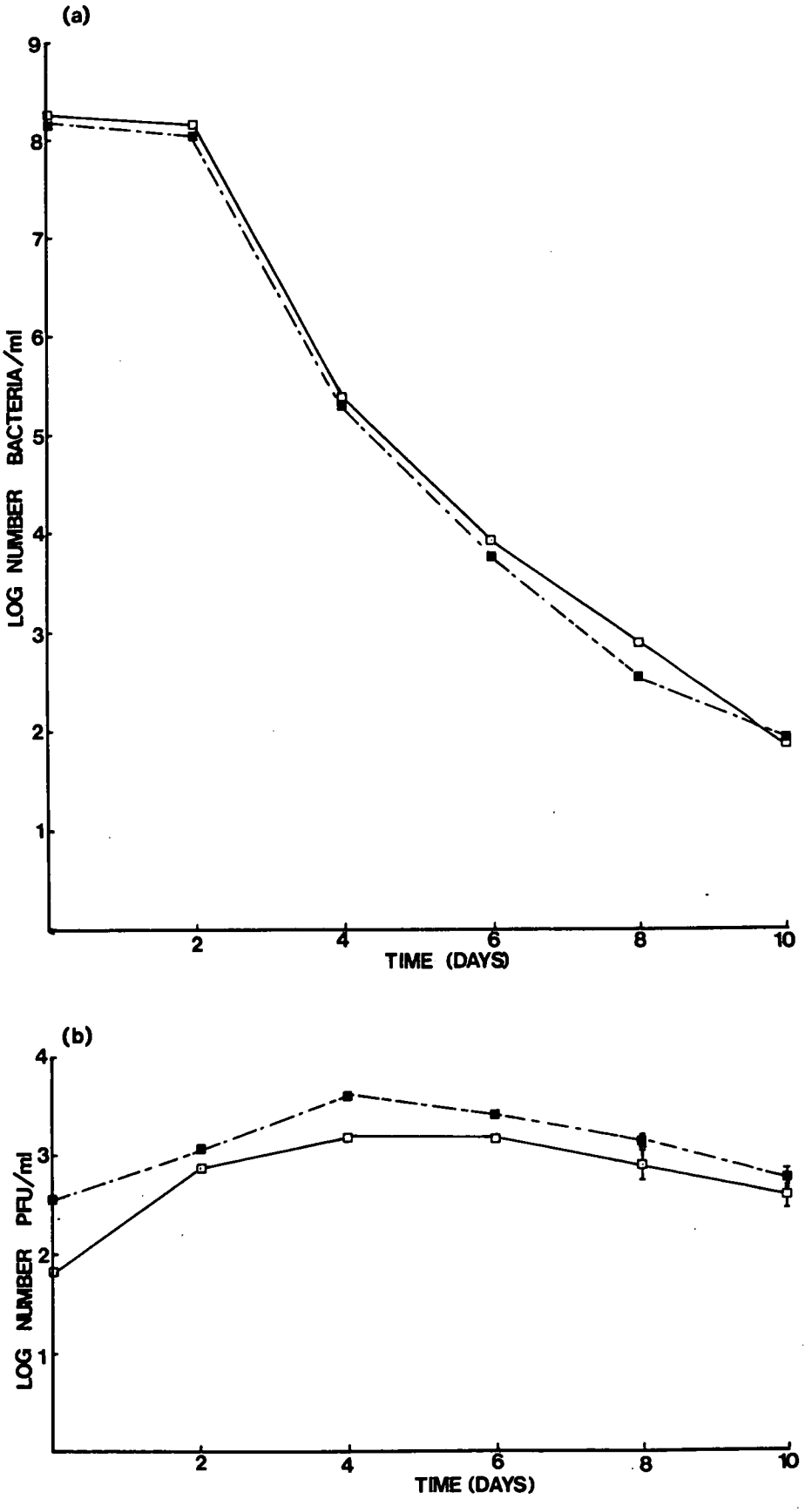
S. typhimurium (□)

(b) Growth of PFU in estuarine water samples.

E. coli (■)

S. typhimurium (□)

(Data from Appendix 11.a.)


(a)
LOG NUMBER BACTERIA/ml
9
8
7
6
5
4
3
2
1
2
4
6
8
10
TIME (DAYS)
(b)
LOG NUMBER PFU/ml
4
3
2
1
2
4
6
8
10
TIME (DAYS)

The number of PFU for *E. coli* and *S. typhimurium* increased from day 0 to reach maximum values of 3.9×10^3 and 7×10^3 PFU per ml respectively after 4 days.

The survival of *S. faecium*, *E. aerogenes* and *K. pneumoniae* are compared to that of *E. coli* in natural estuarine water samples in Table 24. *E. coli* cells were reduced from approximately 10^8 to 2.5×10^2 organisms per ml in 10 days. *E. aerogenes* survived to a significantly greater degree than *E. coli* after 6 and 8 days incubation, but was not significantly different to *E. coli* after 10 days. *S. faecium*, however, survived to a similar extent as *E. coli* for the first 8 days of incubation, but survived to a significantly greater degree than *E. coli* after 10 days. The survival of *K. pneumoniae* was significantly lower than that of all test bacteria, including *E. coli*, throughout the duration of the experiment, the numbers of *K. pneumoniae* being reduced from 5×10^7 to less than 10 organisms per ml in 8 days.

The growth of predators of the test bacteria is shown in Table 25. Bacterial and protozoan predators of *S. faecium* failed to produce plaques on double-layer plates. The number of PFU for all test bacteria increased in a similar fashion, from approximately 3×10^2 PFU per ml to a maximum of approximately 5×10^3 PFU per ml after 4 to 6 days, and then gradually declined. Similar results for test bacterial survival and PFU growth were obtained for *E. aerogenes*, *S. faecium* and *K. pneumoniae* in preliminary experiments (Appendix 11.b.3.).

The effect of prey selection by microbial predators on the survival of *E. coli* and *S. typhimurium* is shown in Table 26. In natural estuarine water in the presence of protozoan and bacterial predators, *S. typhimurium* survived to a significantly greater extent than *E. coli* after 6 days incubation when these organisms were introduced as alternative prey species.

TABLE 24: Survival of Test Bacteria Compared to *E. coli.*

(Data from Appendix 11.b.1.).

TIME (DAYS)	LOG NO. BACTERIA PER ML[a]			
	E. COLI	*E. AEROGENES*	*S. FAECIUM*	*K. PNEUMONIAE*
0	8.13 (0.02)	8.32 (0.01)	7.93 (0.05)	7.71 (0.01)
2	8.01 (0.05)	8.35 (0.01)	7.73 (0.05)	7.32 (0.04)
4	5.32 (0.16)	5.49 (0.12)	5.23 (0.13)	4.06 (0.06)
6	3.77 (0.22)	4.50 (0.10)	3.46 (0.16)	1.94 (0.52)
8	2.56 (0.78)	3.74 (0.26)	2.61 (0.31)	0.33 (0.33)
10	1.90 (0.55)	2.79 (0.40)	2.67 (0.07)	0.00

[a]Values represent mean of three observations; standard error is shown within parentheses.

TABLE 25: Growth of PFU for Test Bacteria(data from Appendix 11.b.2.).

TIME (DAYS)	LOG NO. PFU PER ML[a]		
	E. COLI	*E. AEROGENES*	*K. PNEUMONIAE*
0	2.53 (0.01)	2.36 (0.06)	2.48 (0.06)
2	3.06 (0.01)	2.76[b] (0.10)	2.76 (0.05)
4	3.58 (0.10)	3.39 (0.08)	3.53 (0.06)
6	3.37 (0.03)	3.50[b] (0.08)	2.93[b] (0.06)
8	3.14 (0.02)	2.39[b] (0.14)	3.16 (0.12)
10	2.77 (0.09)	2.90[b] (0.11)	3.03[b] (0.05)

[a]Values represent mean of three observations; standard error is shown within parentheses.

[b]Mean of two observations only.

TABLE 26: Bacterial Survival in the Presence of Alternative Prey Species *E. coli* and *S. typhimurium*.

(Data from Appendix 12.a.1.)

TIME (DAYS)	LOG NO. BACTERIA PER ML[a]			
	NATURAL ESTUARINE WATER		NATURAL ESTUARINE WATER + CYCLOHEXIMIDE	
	E. COLI	*S. TYPHIMURIUM*	*E. COLI*	*S. TYPHIMURIUM*
0	7.52 (0.05)	7.88 (0.01)	7.53 (0.04)	7.87 (0.01)
2	7.49 (0.12)	7.80 (0.02)	7.45 (0.05)	7.87 (0.01)
4	5.20[b]	5.17[b]	7.22 (0.12)	7.32 (0.15)
6	4.47 (0.35)	4.92 (0.02)	6.92 (0.02)	7.40 (0.03)
8	4.03 (0.40)	4.59 (0.10)	6.43 (0.07)	6.25 (0.35)
10	3.29 (0.08)	4.07 (0.21)	5.92 (0.05)	5.88 (0.09)
13	2.08 (0.03)	3.38 (0.22)	4.25 (0.03)	3.90 (0.37)
15	<1.00	3.05 (0.28)	3.56 (0.32)	4.01 (0.18)

[a] Values represent mean of two observations; standard error is shown within parentheses.

[b] One observation only.

In the presence of bacterial predators only, however, the survival of the prey species was not significantly different after 15 days. The growth of predators is shown in Table 27. Bacterial predators reached a maximum level of 3.9×10^3 PFU per ml after 8 days. Similarly, total PFU (bacteria and protozoa) reached a maximum of 2.5×10^3 PFU per ml after 13 days incubation. After 6 days incubation the number of PFU per ml in both treatments were not significantly different.

The survival of *E. coli* and *K. pneumoniae* in the presence and absence of protozoan predators is shown in Table 28. In the presence of protozoan predators the survival of *E. coli* was greater than that of *K. pneumoniae* after 10 days incubation. Similarly, in the absence of protozoan predators, the survival of *E. coli* was greater than *K. pneumoniae* after 8 days incubation.

The growth of predacious microorganisms is seen in Table 29. In the presence of protozoans, total PFU (protozoa and bacteria) reached a maximum level of 4×10^2 PFU per ml after 8 days compared to 8.8×10^3 PFU/ml (bacteria only) after 4 days in the absence of protozoans.

The survival of *E. coli* and *S. faecium* when inoculated together into estuarine water samples is shown in Table 30. *E. coli* cells survived to a significantly greater extent than *S. faecium* cells in the presence and absence of protozoan predators.

Discussion

The comparative survival of indicator organisms and enteric pathogens in water has been examined by several authors (McFeters *et al.*, 1974; Mitchell and Starzyk, 1975; Vasconcelos and Swartz, 1976), although the role played by predacious microorganisms in survival has not been considered.

TABLE 27: Growth of PFU in the Presence of Alternative Prey Species; *E. coli* and *S. typhimurium*.

(Data from Appendix 12.a.2.).

TIME (DAYS)	LOG NO. PFU PER ML[a] NATURAL ESTUARINE WATER	NATURAL ESTUARINE WATER + CYCLOHEXIMIDE
0	0.45 (0.15)	0.15 (0.15)
2	2.95 (0.15)	1.87 (0.27)
4	3.16 (0.16)	3.01[b]
6	2.89 (0.08)	2.50 (0.50)
8	2.93 (0.12)	3.48 (0.31)
10	3.20 (0.18)	3.16 (0.29)
13	3.38 (0.12)	2.81 (0.23)
15	2.76 (0.32)	2.78 (0.09)

[a] Values represent mean of two observations; standard error is shown within parentheses.

[b] One observation only.

TABLE 28: Bacterial Survival in the Presence of Alternative Prey Species; *E. coli* and *K. pneumoniae*.

(Data from Appendix 12.b.1.)

LOG NO. BACTERIA PER ML[a]

TIME (DAYS)	NATURAL ESTUARINE WATER		NATURAL ESTUARINE WATER + CYCLOHEXIMIDE	
	E. COLI	*K. PNEUMONIAE*	*E. COLI*	*K. PNEUMONIAE*
0	8.15 (0.02)	7.92 (0.03)	8.09 (0.05)	7.87 (0.06)
2	8.06 (0.06)	7.77 (0.03)	8.09 (0.01)	7.96 (0.02)
4	<5.00	5.00[b]	7.80 (0.13)	7.71 (0.11)
6	3.78 (0.08)	4.03 (0.03)	5.96 (0.66)	5.30[b]
8	3.21 (0.12)	3.18 (0.16)	4.37 (0.32)	3.00[b]
10	2.42 (0.21)	1.84 (0.24)	3.68 (0.06)	2.40[b]

[a]Values represent mean of two observations; standard error is shown within parentheses.

[b]One observation only.

TABLE 29: Growth of PFU in Presence of Alternative Prey Species; *E. coli* and *K. pneumoniae*.

(Data from Appendix 12.b.2.).

TIME (DAYS)	LOG NO. PFU PER ML[a]	
	NATURAL ESTUARINE WATER	NATURAL ESTUARINE WATER + CYCLOHEXIMIDE
0	0.87 (0.27)	0.45 (0.15)
2	2.09 (0.15)	1.94 (0.02)
4	2.30 (0.00)	3.93 (0.12)
6	2.52 (0.04)	3.60 (0.52)
8	2.59 (0.11)	3.37 (0.02)
10	2.56 (0.26)	3.53 (0.35)

[a]Values represent mean of two observations; standard error is shown within parentheses.

TABLE 30: Bacterial Survival in the Presence of Alternative Prey Species; *E. coli* and *S. faecium*.

(Data from Appendix 12.c.).

TIME (DAYS)	LOG NO. BACTERIA PER ML[a]			
	NATURAL ESTUARINE WATER		NATURAL ESTUARINE WATER + CYCLOHEXIMIDE	
	E. COLI	*S. FAECIUM*	*E. COLI*	*S. FAECIUM*
0	8.10 (0.01)	8.00 (0.04)	8.10 (0.04)	7.94 (0.06)
2	7.23 (0.12)	6.35 (0.05)	7.58 (0.13)	7.02 (1.02)
4	5.35 (0.01)	5.00[b]	6.26 (0.05)	5.39 (0.09)
6	4.16 (0.07)	2.94 (0.01)	6.01 (0.02)	5.15 (0.15)
8	3.17 (0.20)	2.65 (0.35)	6.09 (0.11)	4.57 (0.17)
10	2.57 (0.15)	2.30 (0.00)	4.60 (0.28)	4.00 (0.00)

[a] Values represent mean of two observations; standard error is shown within parentheses.

[b] One observation only.

A comparison of the survival of coliforms and salmonellae in artificial and sterilised seawater by various workers has produced a series of conflicting results. Graham and Sieburth (1973) suggested that *S. typhimurium* was more sensitive to artifical seawater than *E. coli*, while Mitchell and Starzyk (1975) claimed that survival of these organisms was essentially the same. Conversely, Jamieson *et al.* (1976) and Paoletti *et al.* (1978) showed that the survival of salmonellae was greater than that of coliforms, the latter authors determining T_{90} values of 92 and 66 minutes respectively.

In the present study, *S. typhimurium* and *E. coli* when introduced into natural estuarine water were reduced in numbers to a similar degree after an initial lag phase of 2 days (Figure 30.a.). The survival of these organisms, virtually unaltered after 10 days incubation in autoclaved estuarine water, suggests that factors of biological origin may be important in this decline. Concurrent with this destruction of sewage microorganisms, there was an increase in the natural microbial population antagonistic towards these bacteria. Stryszak (1949) noted that 3 days after the inoculation of *Salmonella* spp. (including *S. typhimurium*) into seawater, their numbers declined, while the indigenous bacteria and protozoa increased significantly. The latter group of organisms in particular, consumed large numbers of prey cells.

Vasconcelos and Swartz (1976), using a diffusion chamber in seawater, found that at low temperatures (8.5°C) *S. faecalis* was more persistent than *E. coli* or *E. aerogenes*, both of which exhibited similar survival patterns. *S. faecium* survives to a greater extent than *S. faecalis*, which in turn is more persistent than *E. coli* in filter-sterilized river water (Mitchell and Starzyk, 1975). Similarly,

faecal streptococci show a greater survival than faecal coliforms in diffusion chambers in well water (McFeters *et al.*, 1974) and in sewage discharged directly into the sea (Pichot and Barbette, 1978). The latter authors calculated first order mortality rates of 14.9 day^{-1} for faecal coliforms and 9.7 day^{-1} for faecal streptococci, indicating the greater resistance of the latter in the marine environment. From the above-mentioned studies and Table 24, test bacterial survival appears to vary from one organism to another.

The similar numbers of microbial predators reached in response to each of the test bacteria (Table 25) and the differential survival of these bacteria, indicate a variation in the ability of the predators to destroy their prey. This may be a function of the predator itself, or a greater resistance to predation of the prey bacteria, both of which may be involved in the selection of prey by the predators.

It has been well established that the growth of different protozoan species is significantly affected by the strains of bacteria offered as food (Taylor and Berger, 1976). There are several properties of the bacterial prey species which may be important in determining its suitability as a food organism (Barna and Weis, 1973). These include the size and shape of the bacteria, the production of a capsule or spore reducing the digestibility of the cells, chemical composition of the cell walls, and the production of extracellular products. Berk *et al.* (1976), for example, observed that 32 percent of *Vibrio* sp. and only 13.8 percent of a *Bacillus* sp. were consumed per hour by an estuarine ciliate. This difference, they suggested, may have been due to the larger size of the *Bacillus* sp. compared to *Vibrio* sp., and the different cell wall chemical structure of the former bacterium.

Fenchel (1980a) found no evidence for the qualitative discrimination of food particles retained and ingested by ciliates, but suggested that particle size selection as a function of the morphological properties of the mouth apparatus did occur. However, Rapport *et al.* (1972) fed four protistan species to *Stentor coeruleus* and showed consistent food preferences which were not correlated with differences in the prey size.

E. coli and *S. typhimurium* exhibited similar survival in estuarine water samples when incubated separately (Figure 30.a.), and when incubated together in the presence of bacterial predators only (Table 26). The greater survival of *S. typhimurium* in the presence of protozoan and bacterial predators (Table 26) indicated a selection of *E. coli* prey organisms by protozoan predators.

The greater survival of *E. coli* compared to *K. pneumoniae* when these organisms were incubated separately (Table 24) and the increased survival of *K. pneumoniae* when incubated together, in the presence of protozoan predators (Table 28), indicates that 2 mechanisms are involved in this predator-prey interaction, prey resistance and predator selection. *K. pneumoniae* was less resistant to predation than *E. coli*, and so when inoculated into separate estuarine water samples was reduced in numbers to a greater degree. When incubated together, however, *E. coli* was preferred as a prey species, and *K. pneumoniae* exhibited greater survival than when it was present individually. Inhibition of protozoan predators removed the preferential selection from the prey population and due to the greater resistance of *E. coli* cells, these organisms exhibited greater survival than *K. pneumoniae*.

When *E. coli* and *S. faecium* are incubated in separate estuarine water samples, the greater resistance of the latter to predation results

in the greater survival of *S. faecium* (Table 24). When these organisms are incubated together, *S. faecium* is preferentially selected by protozoan predators and so *E. coli* exhibits greater survival (Table 30). The inhibition of protozoans does not in this case remove the preferential selection of prey from the population. This may be caused by clumping of *S. faecium*, enabling greater utilization by the bacterial predators.

G. Effect of Temperature on Bacterial Survival

Results

The log reduction in the number of *E. coli* per ml after 10 days incubation at various temperatures and the regression data are shown in Figure 31. As the temperature is increased from 5 to 24°C, the log reduction of *E. coli* is increased.

The effect of temperature on the survival of *E. coli* and *S. typhimurium* in autoclaved estuarine water is shown in Table 31. Bacterial numbers remained virtually unaltered for both organisms after 10 days incubation at all temperatures.

The effect of natural estuarine water on the survival of these organisms at different temperatures is shown in Figures 32.a. and 33.a. *E. coli* cells (Figure 32.a.) incubated at 6.2°C were reduced from 4.5×10^8 to 1.3×10^6 organisms per ml compared to 1.4×10^2 organisms per ml when incubated at 26.9°C. As the temperature of incubation was increased from 6.2 to 26.9°C, the lag phase for *E. coli* decline was reduced from 4 days to less than 2 days. *S. typhimurium* (Figure 33.a.) followed a similar pattern with numbers reduced from 4×10^8 to 3.5×10^5 organisms per ml at 6.2°C and to 2.2×10^2 organisms per ml at 26.9°C. The length of the lag phase is also reduced as the temperature

Figure 31: Effect of temperature on the survival of *E. coli*.

Regression data: $y = 0.17x + 3.52$

Correlation coefficient: 0.90

(Data from Appendix 13.a.)

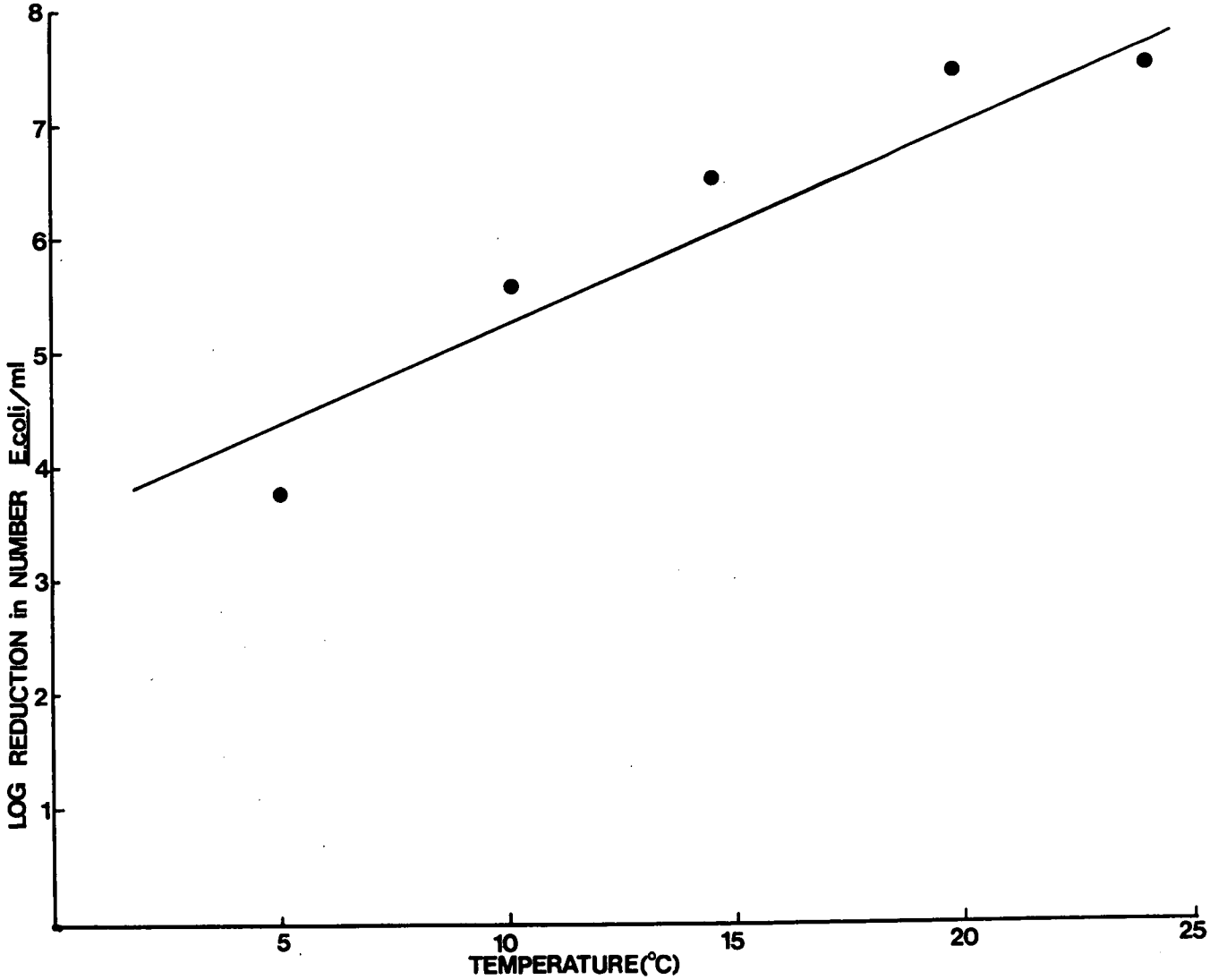

LOG REDUCTION in NUMBER Ecoli/ml
8
7
6
5
4
3
2
1
5
10
15
20
25
TEMPERATURE(°C)

TABLE 31: Survival of *E. coli* and *S. typhimurium* at Different Temperatures in Autoclaved Estuarine Water Samples.

(Data from Appendix 13.b.).

TEMPERATURE (°C)	LOG NO. BACTERIA PER ML			
	E. COLI		*S. TYPHIMURIUM*	
	DAY 0	DAY 10	DAY 0	DAY 10
5.0	8.59	8.41	8.54	8.42
9.5	8.46	8.41	8.56	8.38
14.0	8.57	8.53	8.48	8.30
18.5	8.55	8.37	8.51	8.07
24.0	8.52	8.27	8.52	7.89

Figure 32: (a) Survival of *E. coli* in autoclaved estuarine water (O) and natural estuarine water at 6.2°C (□), 10.3°C (▼), 15.3°C (△); 20.6°C (■), and 26.9°C (●).

(Data from Appendix 13.c.1.)

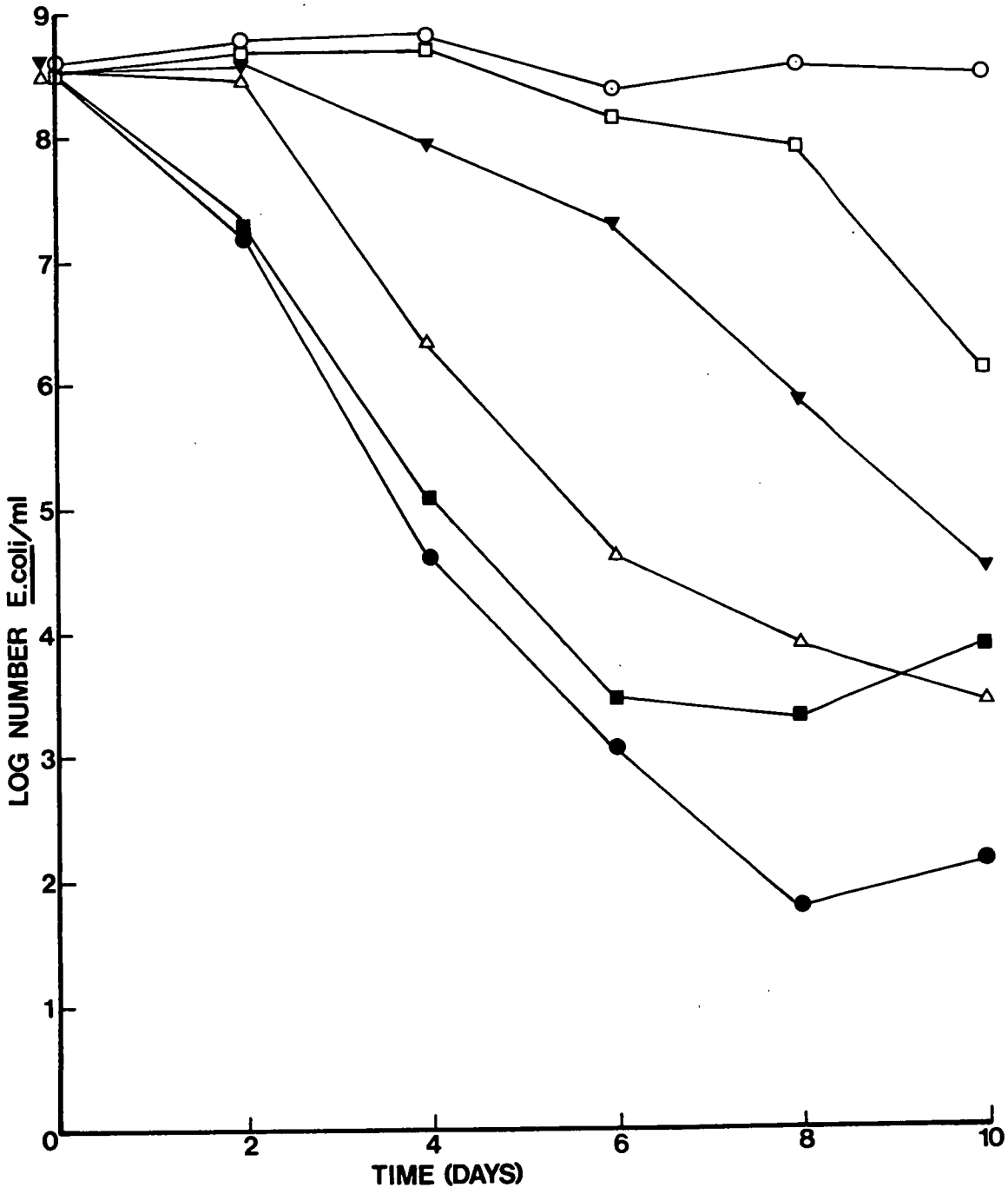

LOG NUMBER E.coli/ml
9
8
7
6
5
4
3
2
1
0
2
4
6
8
10
TIME (DAYS)

Figure 32: (b) Growth of PFU in natural estuarine water containing *E. coli* incubated at 6.2°C (□), 10.3°C (▼), 15.3°C (△), 20.6°C (■), and 26.9°C (●).

(Data from Appendix 13.c.2.)

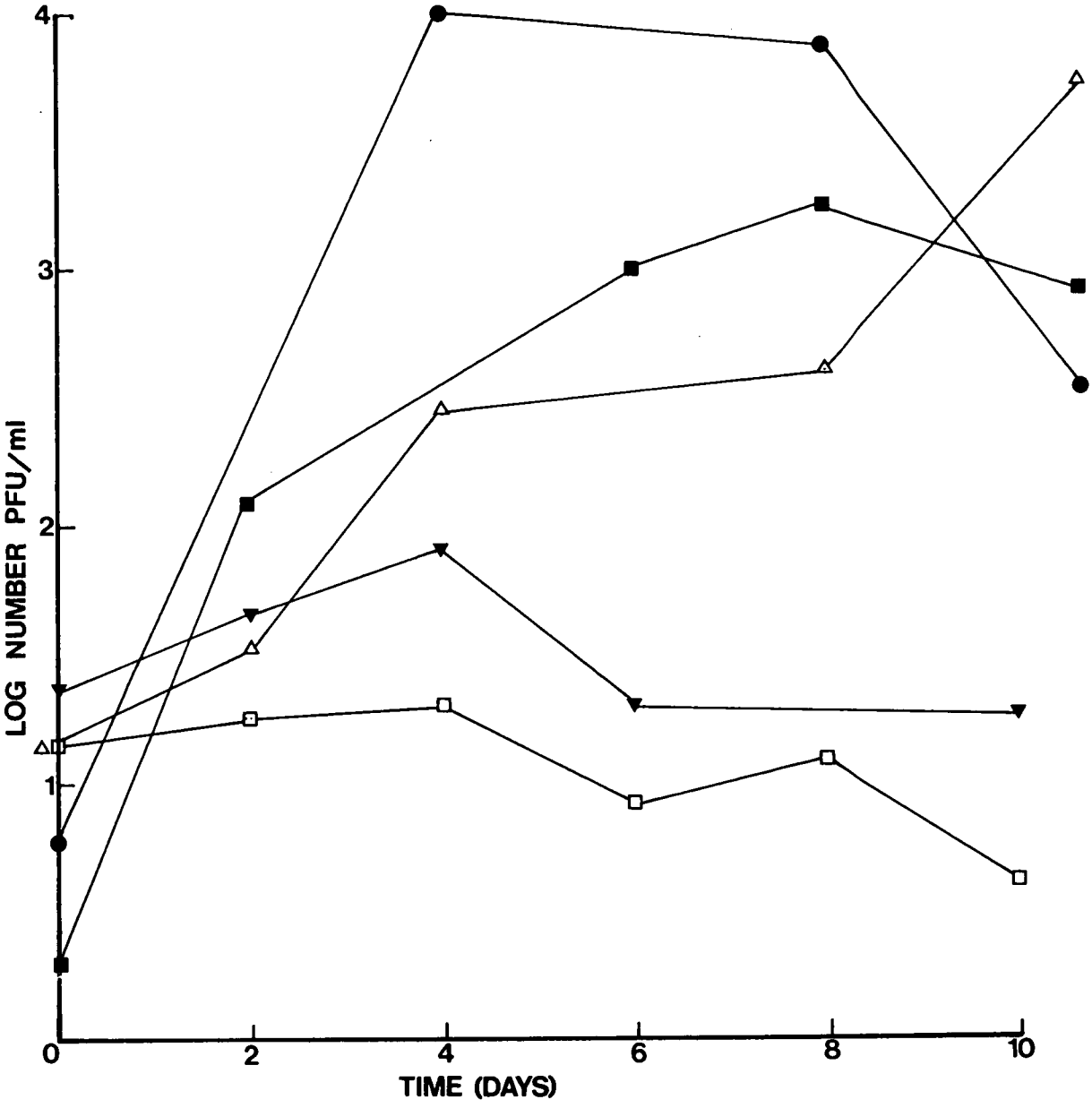

LOG NUMBER PFU/ml
4
3
2
1
0
2
4
6
8
10
TIME (DAYS)

Figure 33: (a) Survival of *S. typhimurium* in autoclaved estuarine water (●) and natural estuarine water at 6.2°C (□), 10.3°C (▼), 15.3°C (△), 20.6°C (■), and 26.9°C (○).

(Data from Appendix 13.c.1.)

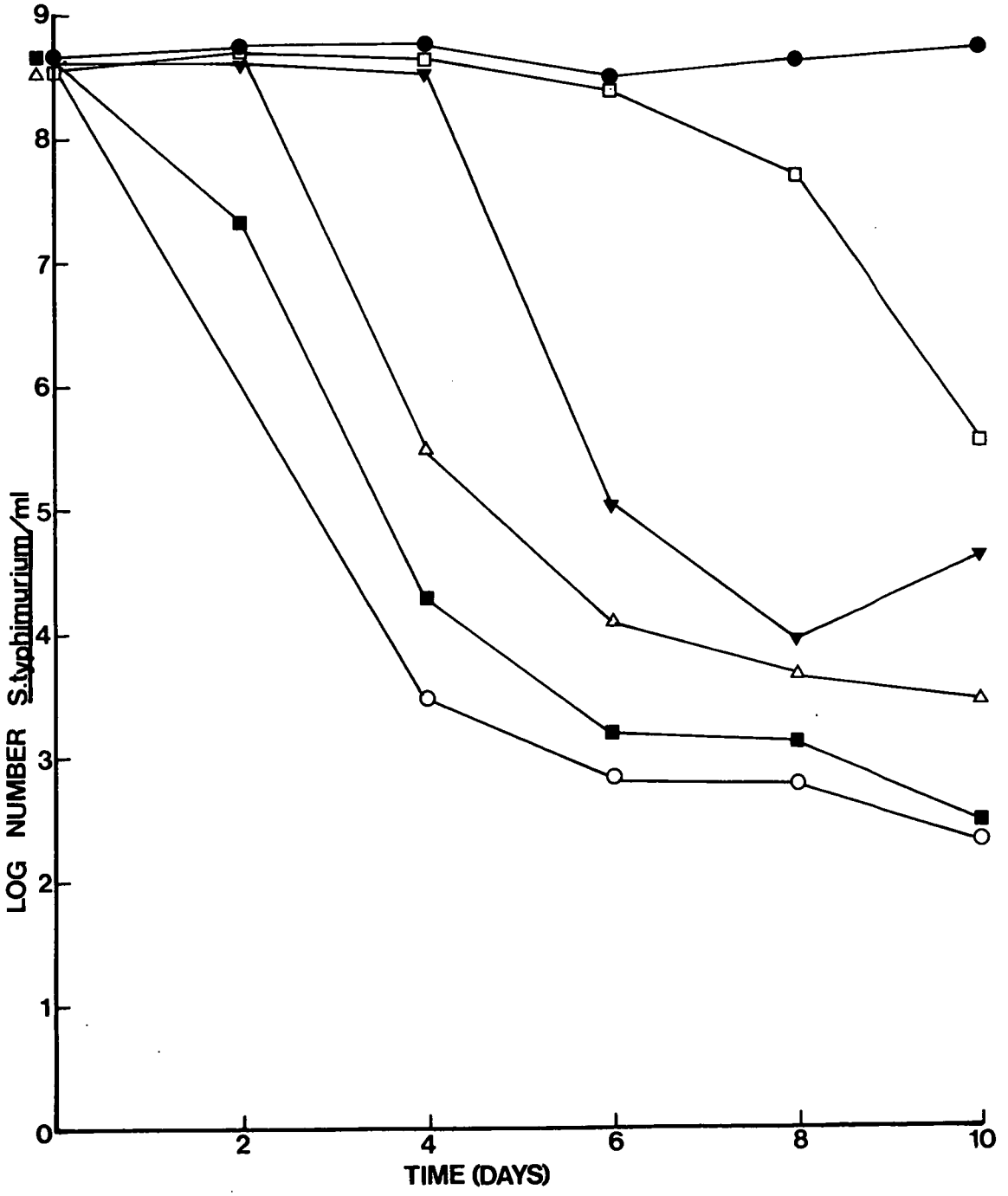
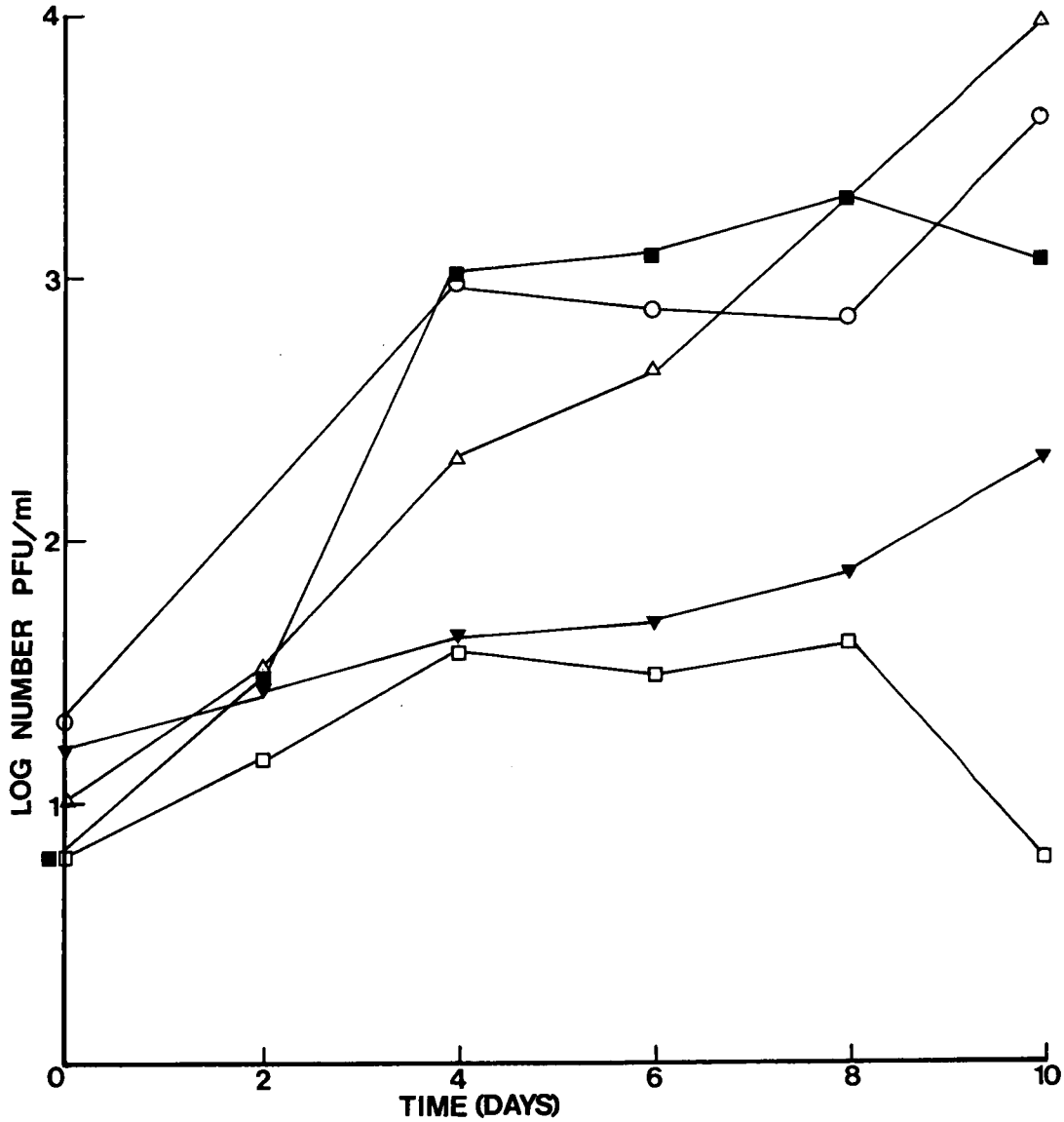

LOG NUMBER S.typhimurium/ml
TIME (DAYS)
0
1
2
3
4
5
6
7
8
9
2
4
6
8
10

Figure 33: (b) Growth of PFU in natural estuarine water containing *S. typhimurium* incubated at 6.2°C (□), 10.3°C (▼), 15.3°C (△), 20.6°C (■), and 26.9°C (○).

(Data from Appendix 13.c.2.)


LOG NUMBER PFU/ml
4
3
2
1
0
2
4
6
8
10
TIME (DAYS)

was increased from 6.2 to 26.9°C from 6 days to less than 2 days respectively.

The growth of PFU for *E. coli* and *S. typhimurium* again followed a similar pattern as shown in Figures 32.b. and 33.b. The number of PFU remain fairly constant for both organisms at 6.2°C throughout the duration of the experiment. When the organisms were incubated at 15.3°C predator numbers increased from 14 to 5.4 x 10^3 PFU per ml for *E. coli* with similar increases for *S. typhimurium*. At 20.6°C the number of PFU per ml for both organisms increased during the first 6 days of incubation and then PFU numbers remained relatively stable. When the temperature was increased to 26.9°C a similar pattern was observed during the first 8 days of incubation. These experiments have been repeated and similar results for *E. coli* and *S. typhimurium* survival (Appendix 13.d.1.) and PFU growth (Appendix 13.d.2.) were obtained.

The log reduction in the number of *E. coli* and *S. typhimurium* cells per ml after 10 days incubation at various temperatures and the regression data are shown in Figure 34. As the temperature was increased from 5 to 25°C the log reduction of these organisms was increased at a similar rate.

The effect of temperature on the maximum number of bacterial and protozoan predators reached during the 10 day experiment is shown in Table 32. Bacterial predators exhibited a marked increase in numbers above 20°C. Predacious bacteria of *E. coli* increased from 18 PFU per ml at 6.2°C to 60 PFU per ml at 20.6°C and 1.76 x 10^3 PFU per ml at 26.9°C. Similarly, bacterial predators of *S. typhimurium* increased from 12 PFU per ml at 6.2°C to 2 x 10^2 PFU per ml at 20°C and 3.2 x 10^3 PFU per ml at 26.9°C. For both organisms the maximum number of protozoan predators was reached at 15.3°C. with numbers decreasing markedly above and below this temperature.

Figure 34: Effect of temperature on the survival of *S. typhimurium* (O) and *E. coli* (□).

Regression data: *S. typhimurium* $y = 0.16x + 2.04$

Correlation coefficient: 0.85.

Regression data: *E. coli* $y = 0.16x + 2.26$;

Correlation coefficient: 0.93.

(Data from Appendix 13.c.1.)

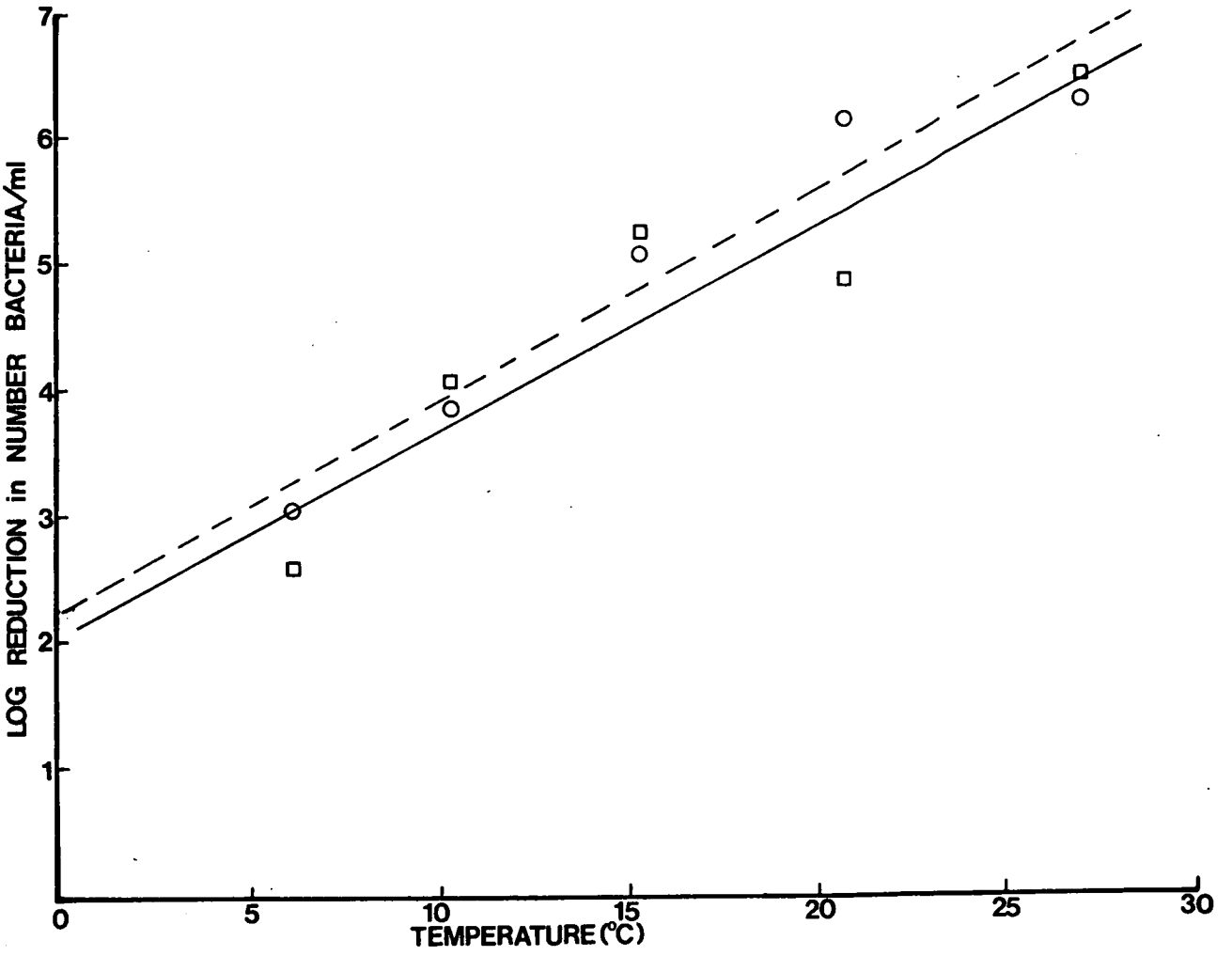

LOG REDUCTION in NUMBER BACTERIA/ml
7
6
5
4
3
2
1
0
5
10
15
20
25
30
TEMPERATURE (°C)

TABLE 32: Effect of temperature on the number of bacterial and protozoan predators.

(Data from Appendix 13.c.2.).

LOG NO. PREDATORS PER ML

TEMPERATURE (°C)	*E. COLI*		*S. TYPHIMURIUM*	
	BACTERIA	PROTOZOA	BACTERIA	PROTOZOA
6.2	1.26	0.05	1.09	0.48
10.3	1.30	0.60	1.64	0.66
15.3	1.56	2.18	2.15	1.84
20.6	1.78	1.48	2.30	1.00
26.9	3.25	0.76	3.51	0.08

Discussion

As the temperature of incubation of bacteria was increased, a reduction in the length of the lag phase in a normal bacterial survival curve was observed (Orlob, 1956; Hanes *et al.*, 1965). The latter authors noted lag periods for coliform survival of 0.3 days at 25.8°C and 2.3 days at 6°C, while the former observed periods of 1 day at 30°C and 33 days at 10°C. These values compare well with those observed in Figure 32.a., that is, less than 2 days at 26.8°C and 4 days at 6.2°C.

Orlob (1956) also noted an increase in the rate of decline of coliform organisms as the temperature increased from 6 to 25.8°C. Carlucci and Pramer (1960a) and Faust *et al.* (1975) stated that survival of *E. coli* in seawater varied inversely with temperature from 5 to 40°C and 5 to 30° C respectively, similar to the pattern observed in Figures 31 and 34. This increased survival at lower temperatures,often observed as increased survival during the colder, winter months (Bernard, 1970), is not just of a physico-chemical nature, but is also due to increased ecological interactions as a result of higher temperatures (Verstraete and Voets, 1976). However, this study has also indicated a subtle change in the composition of the antagonistic microflora as the temperature increases. Protozoan predators for both *E. coli* and *S. typhimurium* have a temperature optimum of 15 to 20°C which is consistent with that observed for other protozoans (Hamilton and Preslan, 1969; Laybourn and Stewart, 1975). As the temperature increased from 5 to 15°C, the number of bacterial and, in particular, protozoan predators, increased causing a rapid increase in the destruction of prey cells. Above 15°C protozoan predators fall in numbers as their temperature optimum for growth was reached. This allowed a build up of bacterial predators resulting in

no alteration to the rate of destruction of *E. coli* and *S. typhimurium* cells. Bacterial predators, therefore, although not producing an increased destruction of prey cells as the temperature was increased from 20 to 30°C, maintain prey reduction at a constant level even with protozoan predators in low numbers. It appears, therefore, that the combined action of a number of bacterial and protozoan predators and the effect of temperature on these predators, rather than the effect of temperature alone, are important in the decline of *E. coli* and *S. typhimurium* in estuarine water.

H. Effect of Solar Radiation on Bacterial Survival

Results

E. coli cells inoculated into natural estuarine water in the presence of bacterial and protozoan predators, were reduced from 5 x 10^8 to 6 x 10^2 organisms per ml after 10 days incubation in the dark (Figure 35.a.). In the absence of predators in the autoclaved estuarine water samples, *E. coli* numbers remained virtually unaltered when incubated in the dark, but when exposed to solar radiation were reduced to similar levels to those samples in the presence of predators in the dark. When *E. coli* cells were exposed to solar radiation in estuarine water samples in the presence of bacterial and protozoan predators, their numbers were reduced from 6 x 10^8 to zero organisms per ml in 8 days (Figure 35.a.). A similar pattern of *E. coli* survival was observed using artificial lighting (Table 33). The growth of predacious microorganisms, measured as PFU, in the unautoclaved estuarine water samples increased from approximately 15 per ml to reach maximum numbers of approximately 7 x 10^3 per ml after 6 days and then gradually declined.

Figure 35: Effect of sunlight on *E. coli* survival.

(a) *E. coli* survival in:

autoclaved estuarine water (●)

autoclaved estuarine water + sunlight (○)

natural estuarine water (■)

natural estuarine water + sunlight (□).

(Data from Appendix 14.a.1.)

(b) Growth of predators in:

natural estuarine water (■)

natural estuarine water + sunlight (□)

Each point represents mean of 3 replicates.

(Data from Appendix 14.a.2.)

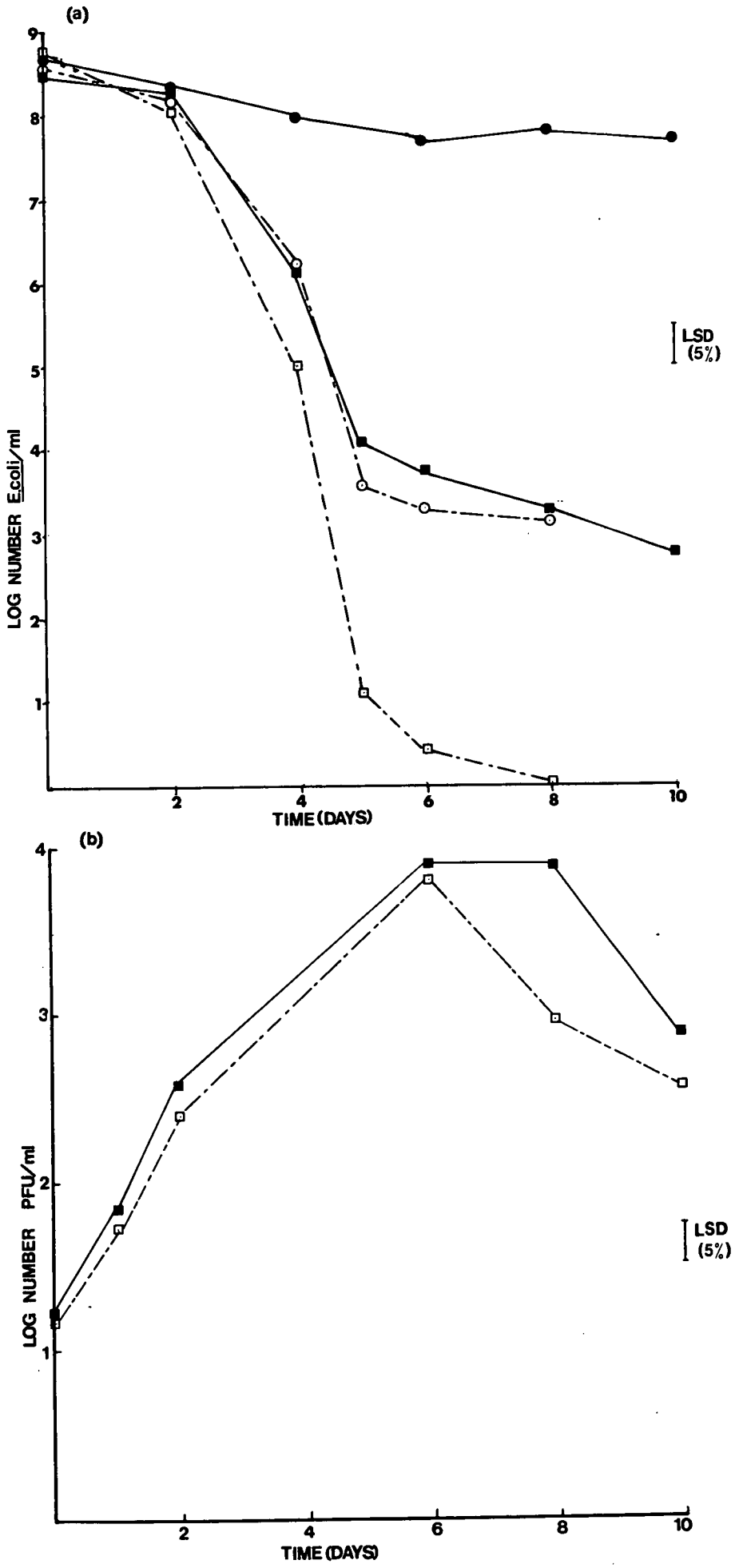

(a)
LOG NUMBER E.coli/ml
TIME (DAYS)
LSD (5%)
(b)
LOG NUMBER PFU/ml
TIME (DAYS)
LSD (5%)

TABLE 33: Survival of *E. coli* in Artificial Light in Estuarine Water Samples (Data from Appendix 14.b.).

LOG NO. BACTERIA PER ML[a]

TIME (DAYS)	AUTOCLAVED ESTUARINE WATER		NATURAL ESTUARINE WATER
	DARK	LIGHT[b]	LIGHT
0	8.84 (0.03)	8.81 (0.01)	8.78 (0.02)
1		8.64 (0.01)	8.67 (0.00)
2	8.77 (0.02)	8.49 (0.01)	8.20 (0.01)
3		8.03 (0.05)	7.30 (0.48)
4	8.52 (0.04)	6.77 (0.16)	5.22 (0.72)
5		6.54 (0.13)	4.30 (1.17)
6	8.47 (0.02)	5.27 (0.26)	2.78 (0.91)
7	8.18 (0.05)	3.55 (0.09)	<1.00

[a] Values represent mean of three observations; standard error is shown within parentheses.

[b] Total radiant exposure for duration of experiment, 627 cal cm^{-2}.

The survival of the test organisms in estuarine water samples exposed to solar radiation is shown in Table 34. *E. coli* cells exposed to 298 cal cm^{-2} (solar radiation 1) were reduced in numbers from approximately 10^8 to 25 organisms per ml after 3 days. *S. typhimurium* and *S. faecium*, however, showed a significantly greater survival than *E. coli* when exposed to solar radiation, these organisms being reduced from approximately 10^8 to 8.5×10^4 and 3.1×10^4 organisms per ml respectively. The survival of *K. pneumoniae* is significantly lower than all test organisms including *E. coli*. The exposure of *E. coli* cells to total radiant exposure of 1,510 cal cm^{-2} (solar radiation 2) resulted in the complete elimination of the population after 2 days. Both *E. aerogenes* and *E. herbicola* exhibited significantly greater survival than *E. coli* after 2 days incubation. The growth of predators is shown in Table 35. When exposed to solar radiation 1 (298 cal cm^{-2}), the *E. coli* predators reached a maximum level of 3×10^2 PFU per ml compared to 1.5×10^2 PFU per ml for *S. typhimurium* predators. When exposed to solar radiation 2 (1,510 cal cm^{-2}), *E. coli* predators again reached a maximum level of 3×10^2 PFU per ml compared to 1.3×10^2 PFU per ml for *E. aerogenes* predators.

The effect of different radiant exposures on the survival of *E. coli* and *S. typhimurium* in estuarine water in the presence of microbial predators is seen in Table 36. When *E. coli* and *S. typhimurium* were incubated in the absence of solar radiation (0% radiant exposure) they exhibited comparable survival after 2 days. As the radiant exposure was increased the survival of both organisms after 2 days incubation was decreased. *S. typhimurium*, however, showed a significantly greater survival than did *E. coli* at radiant exposures of 50 and 100 percent of natural solar radiation.

TABLE 34: Comparative survival of test bacteria with *E. coli* in estuarine water exposed to solar radiation.

(Data from Appendix 14.c.1.).

TIME (DAYS)	LOG NO. OF BACTERIA PER ML[a]						
	E. COLI		*S. TYPHIMURIUM*	*S. FAECIUM*	*K. PNEUMONIAE*	*E. AEROGENES*	*E. HERBICOLA*
	SOLAR RADIATION 1[b]	SOLAR RADIATION 2[c]	SOLAR RADIATION 1	SOLAR RADIATION 1	SOLAR RADIATION 1	SOLAR RADIATION 2	SOLAR RADIATION 2
0	8.23 (0.05)	8.42 (0.02)	8.39 (0.03)	7.82 (0.03)	8.03 (0.05)	8.47 (0.03)	8.31 (0.02)
0.5	7.97 (0.01)	6.56 (0.13)	8.31 (0.02)	7.90 (0.02)	7.34 (0.03)		
1.0	7.09 (0.10)	2.63 (0.32)	8.22 (0.06)	7.46 (0.13)	5.86 (0.38)	7.04 (0.05)	6.52 (0.16)
2.0	5.99 (0.05)	0.00	7.02 (0.19)	5.94 (0.12)	3.00 (0.20)	3.43 (0.15)	4.39 (0.07)
3.0	1.39 (0.39)		4.93 (0.22)	4.49 (0.26)	<1.00	2.20 (0.06)	2.69 (0.02)
4.0			2.80 (0.08)	2.05 (0.44)			

[a] Values represent mean of three observations; standard error is shown within parentheses.

[b] Total radiant exposure for duration of experiment 298 cal cm^{-2}.

[c] Total radiant exposure for duration of experiment 1,510 cal cm^{-2}.

TABLE 35: Growth of PFU for test bacteria exposed to solar radiation.

(Data from Appendix 14.c.2.).

TIME (DAYS)	LOG NO. ORGANISMS PER ML[a]			
	E. COLI		*S. TYPHIMURIUM*	*E. AEROGENES*
	SOLAR RADIATION 1[c]	SOLAR RADIATION 2[d]	SOLAR RADIATION 1	SOLAR RADIATION 2
0	1.25 (0.05)	0.60 (0.00)	1.46 (0.09)	0.40 (0.20)
1	1.66 (0.07)		1.70 (0.05)	
2	2.03 (0.04)	1.93 (0.10)	1.89 (0.06)	2.06 (0.05)
3	2.16[b]	2.30 (0.04)	2.18 (0.07)	2.12 (0.06)
4	2.30 (0.03)		2.04 (0.04)	

[a] Values represent mean of three observations; standard error is shown within parentheses.

[b] One observation only.

[c] Total radiant exposure for duration of experiment 298 cal cm^{-2}.

[d] Total radiant exposure for duration of experiment 1,510 cal cm^{-2}.

TABLE 36: Effect of radiant exposure on the survival of *E. coli* and *S. typhimurium* in natural estuarine water samples. (Data from Appendix 14.d.).

RADIANT EXPOSURE (%)	LOG NO. BACTERIA PER ML[a]			
	E. COLI		*S. TYPHIMURIUM*	
	DAY 0	DAY 2	DAY 0	DAY 2
0	8.28 (0.01)	7.67 (0.03)	8.13 (0.03)	7.13 (0.03)
50	8.26 (0.01)	2.93 (0.51)	8.05 (0.02)	4.30[b] (0.00)
100	8.17 (0.02)	1.50[b] (0.50)	8.09 (0.02)	3.60[b] (0.00)

[a]Values represent mean of three observations; standard error is shown within parentheses.

[b]Mean of two observations.

The effect of different radiant exposures on *E. coli* and *S. typhimurium* survival in the absence of predacious microorganisms is shown in Table 37. As the cumulative radiation increased the number of *E. coli* and *S. typhimurium* cells decreased in a linear fashion. But the slope of the regression line for *E. coli* is significantly greater than the slope of the regression line for *S. typhimurium*.

Discussion

It was not until the work of Gameson and Saxon (1967) that convincing evidence for the importance of solar radiation on coliform mortality in seawater was presented. In a series of submerged bottle experiments, they concluded that there was a high mortality of coliforms when exposed to light and that the rate of die-off at any time of the year was approximately proportional to the intensity of the short-wave radiation received by the sample. Later workers (Pike *et al.*, 1970; Gameson and Gould, 1975), suggested that the effect of sunlight on coliform mortality was a direct result of light-induced damage, but Chamberlin and Mitchell (1978) suggested that solar radiation may only injure coliforms making them more susceptible to the activities of microbial predators. From Figure 35.a. it would appear that both factors are important, as the combined action of predators and solar radiation produced a significantly greater reduction in *E. coli* numbers than each factor acting independently.

Bellair *et al.* (1977) observed a diurnal variation in faecal coliform die-off rates and established an inverse relationship between hourly T_{90} values and solar radiation. This effect has also been observed by Gameson *et al.* (1973) who noted that coliforms exposed to bright sunshine for short periods of time, followed by short periods of darkness, exhibited a stepwise reduction in numbers. The rate of decline during exposure to sunlight was similar to a sample continuously

TABLE 37: Effect of radiant exposure on survival of *E. coli* and *S. typhimurium* in autoclaved estuarine water samples.

(Data from Appendix 14.e.).

CUMULATIVE RADIATION (cal cm^{-2})	NO. *E. COLI* AND *S. TYPHIMURIUM* PER ML[a]	
	E. COLI	*S. TYPHIMURIUM*
0	1.56×10^8 (0.14)	1.07×10^8 (0.10)
55.4	1.48×10^8 (0.04)	1.21×10^8 (0.02)
114.5	1.34×10^8 (0.04)	1.3×10^8 (0.03)
174.8	7.55×10^7 (0.10)	1.32×10^8 (0.02)
244.3	5.68×10^6 (1.41)	1.08×10^8 (0.06)
310.4	1.51×10^6 (0.12)	4.78×10^7 (0.13)
573.0	1.61×10^5 (0.04)	2.94×10^6 (0.10)
726.8	9.16×10^4 (1.43)	6.17×10^5 (0.44)

[a] Values represent mean of three observations; standard error is shown within parentheses.

Regression data: *E. coli* slope - 2.30×10^5, y intercept 1.28×10^8, correlation coefficient 0.82; *S. typhimurium* slope - 1.99×10^5, y intercept 1.36×10^8, correlation coefficient 0.91.

exposed to solar radiation, and the rate of decline during periods of darkness was similar to that of a sample continuously in the dark. Thus, the reduction in bacterial numbers was proportional to the total radiation received over a given time. Similarly, when *E. coli* and *S. typhimurium* were exposed to continuous light in the absence of microbial predators (Table 37) their survival was directly related to the total radiation received by the water sample. The slope of the regression line for *E. coli* is significantly greater than that for *S. typhimurium*, however, indicating a greater susceptibility of the former organism to solar radiation. Similarly, *E. coli* was more sensitive to solar radiation than *S. faecium*, *E. aerogenes* and *E. herbicola* (Table 34). Chamberlin and Mitchell (1978) have examined the only work conducted in this field and stated that faecal streptococci, *Serratia marcescens* and *Bacillus subtilis* var. *niger* were, to varying degrees, less sensitive to light than were coliforms, although no explanations for these differences were proposed.

The mechanism of light-induced bacterial decay depends on the presence of endogenous sensitizers or chromophores (Eisenstark, 1971) which absorb light energy and cause cell damage directly or by reaction with oxides to form superoxides, which in turn may cause damage to the cells (Chamberlin and Mitchell, 1978). Alternatively, some bacteria such as the bacterial predator *Polyangium* (Roper and Marshall, 1977) may possess pigments such as carotenoids which can absorb the excitation energy and thus prevent cell damage. The presence of protozoan predators and bacterial predators such as *Polyangium*, both of which are not greatly affected by light-induced decay, would explain why there was no significant difference between total predator numbers in the presence or absence of solar radiation (Figure 35.b.).

Thus, sensitivity to near ultraviolet and visible light depends jointly on the presence of a sensitizing agent, oxygen, and on protection mechanisms. The differences in sensitivity of the test organisms to solar radiation may therefore be explained in these terms, although no confirmatory data exist.

Note Added in Proof

A recent study (Fujioka *et al.*, 1981) also demonstrated the inactivation of FC and FS in seawater exposed to solar radiation, the former organisms being more susceptible than the latter. The greater resistance of bacteria to inactivation, observed in fresh mountain stream waters compared to seawater, when exposed to similar solar radiation levels, may be a result of lower predator numbers in the former aquatic environment, or, as noted above, the absence of exogenous sensitizers.

The actual mechanism of light-induced bacterial decline in seawater was examined by Kapuscinski and Mitchell (1981). It was concluded that solar radiation induced sublethal injury in *E. coli* cells through damage to the catalase system which was repaired by the addition of catalase or pyruvate to minimal medium.

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V. General Discussion

General Discussion

The reduced survival of bacteria of faecal origin in seawater has been attributed to many factors, each of which have been reviewed in detail in Section II. The removal of the bactericidal action of seawater by sterilization of the water sample, suggested that factors of biological origin, such as naturally-occurring predacious micro-organisms, were involved in bacterial destruction. In this study, the role of predacious microorganisms in bacterial decline in estuarine water samples and the interaction of these predators with other bactericidal factors has been investigated.

Microbial predators of bacterial (Mitchell 1967) and protozoan (Mitchell and Yankofsky, 1969) origin have been implicated in the decline of faecal bacteria in seawater. Although various models have been developed in an attempt to describe the complex predator-prey interactions involved (Curds, 1974), the relative roles of these two groups of microorganisms is unclear.

It has been suggested by several workers (Danso and Alexander, 1975; Berk *et al.*, 1976; Habte and Alexander, 1978b),for example, that protozoan multiplication and thus the consequent bacterial decline, stop when the prey density falls to about 10^6 to 10^7 cells per ml. At this level the energy used by the protozoan predator in searching for the prey equals that obtained from feeding (Danso *et al.*, 1975). In open waters, bacterial levels of 7×10^5 bacteria per ml are too low to sustain bacterivorous ciliates (Fenchel, 1980b). Bacterial concentrations high enough to maintain a ciliate population are found in highly polluted waters, sewage treatment plants, and in sediments. Thus, large ciliate predators, acting in natural estuarine water samples, would be expected to only reduce an influx of sewage bacteria to this

critical level of 10^6 to 10^7 cells per ml. In fact, bacterial numbers may be reduced to less than 10 cells per ml in 10 days (Figure 17a) and to zero in 8 days (Mitchell and Yankofsky, 1969), indicating that predators other than ciliate protozoans may also be exerting an influence on the *E. coli* prey population. These include heterotrophic microflagellates (Fenchel, 1980b), and bacterial predators (Roper and Marshall, 1977).

Enzinger and Cooper (1976) considered that bacterial competition, antagonism, and even bacterial predation were unimportant in the decline of *E. coli* in estuarine water, and that protozoan predators alone were responsible. If this were the case, the inhibition of predacious protozoans should result in the increased survival of the bacterial prey. Although this does occur, the survival of *E. coli* prey is less than that observed in the complete absence of microbial predators (Section IV.D.1.), suggesting that bacterial predators are also important. Pure cultures of these predators are capable of destroying *E. coli* cells in sterile seawater (Roper and Marshall, 1977) and in autoclaved estuarine water (Section IV.E.).

When *E. coli* cells were introduced into natural estuarine water samples, bacterial and protozoan predators increased rapidly in numbers (Section IV.D.2.). When the protozoan predators were inhibited, however, the bacterial predators increased in numbers to an even greater degree, indicating that the former graze not only on *E. coli* prey, but also on bacterial predators. The periodic inhibition of protozoan predators led to the conclusion that their major effect on *E. coli* prey and also on bacterial predators was exerted during the first 2 days of a 10 day decline period. Inhibition after this time had no effect on the destruction of *E. coli* prey or the growth of bacterial predators.

There appears, therefore, to be a sequence of microbial predators which develop following the introduction of *E. coli* into estuarine water.

The initial population of predacious microorganisms consists of bacterial predators and parasites and small predacious protozoans such as flagellates and ciliates, which bring about most of the destruction of the *E. coli* population in a 10 day decline period. These protozoan predators also graze on the bacterial predators in the first 1 to 2 days following *E. coli* introduction. After 5 to 6 days, large ciliate protozoa capable of consuming smaller ciliates and flagellates develop in the population. These organisms destroy some of the smaller protozoans and predacious bacteria and together with this latter group of predators destroy the remaining *E. coli* population. Following the exhaustion of the available food supply the larger ciliates encyst.

The concentration of the prey organisms present in the estuarine water samples appears to have an effect on the sequence of microbial predators developing and also on the size of the predator population which is produced. A prey concentration of only 10^2 cells per ml resulted in a marked increase in the numbers of the bacterial and smaller protozoan predators (Section IV.D.3.). Increasing the prey concentration to 10^6 to 10^7 cells per ml resulted in the development of a similar number of microbial predators. It was not until a prey population of 10^8 cells per ml was present that an increase in the number of bacterial predators and smaller protozoans, and the development of larger predacious ciliates occurred. These larger ciliates develop rapidly, exploiting localised areas of high bacterial concentrations such as in sediments and sewage (Fenchel, 1980b).

Roper and Marshall (1978) observed a similar sequence of predacious microorganisms, although no attempt was made to determine their period of action. The initial population of predacious and parasitic bacteria and small amoebae which developed destroyed the

E. coli population. Larger, predacious ciliate protozoans then developed feeding on *E. coli* and the smaller predators and following the reduction in food supply declined to their original level.

It has been suggested (Mitchell, 1968), that the constant exposure of seawater to sewage pollution results in a build up of microbial predators which then cause a more rapid decline in prey species introduced at a later time. This has been observed in the laboratory (McCambridge and McMeekin, 1979), and in seawater samples at varying distances from an ocean sewage outfall (Roper and Marshall, 1978). In this study, of an estuarine ecosystem, however, no such enrichment of microbial predators was observed at sites constantly exposed to sewage contamination (Section IV.A.), and no change noted in the survival of *E. coli* prey in water samples from these sites compared to *E. coli* survival in water samples from uncontaminated sites (Section IV.C.). In estuaries such as the Derwent Estuary, which is tidal above site 1 (Guiler, 1955), the circulation of the water masses is important and so any localised increase in microbial predators will be over-ridden by changes in conditions caused by tidal and current movements. These movements, which include an outward movement of surface water, an inner movement of more saline seawater and vertical mixing (Ketchum *et al.*, 1949) result in a mixing of the water system and dilution of highly polluted areas.

The introduction of foreign bacteria such as *E. coli* into estuarine water samples thus produces a homeostatic response which results in the rapid build up of a number of interacting microbial predators antagonistic towards the introduced species. These bacterial and protozoan predators then bring about a reduction in prey numbers, until the numbers of prey species are too low to support the predator population which then declines in numbers. This effect has also been

observed using a single bacterial predator and prey in pure culture (Section IV.E.). The inoculation of *E. coli* prey was followed by an increase in the numbers of the bacterial predator which caused a reduction in prey numbers. But, as there is only a single predator rather than a succession of predators as occurs in natural estuarine water samples, the prey organisms are not completely eliminated. Instead, prey numbers are reduced to a level which cannot support predator growth and so the latter organisms decline in numbers. This decline removes the predatory pressure from the prey organisms which then increase in numbers apparently growing on the breakdown products of other prey organisms (Buck *et al.*, 1952) (Section IV.D.4.).

The coliforms are the most common group of organisms utilized in the bacteriological examination of water systems, but due to certain anomalies associated with the use of this group (Dutka, 1979) it has become important to examine other indicator organisms. One such anomaly is the presence of elevated faecal coliform counts, particularly in water systems exposed to carbohydrate-rich effluents. In such systems, coliforms other than *E. coli*, such as *K. pneumoniae*, may be present in large numbers (Section IV.B.) and give rise to an overestimate of the potential health hazard from enteric pathogens. A factor which has often been overlooked in an investigation of alternative indicator organisms has been the difference in survival between these organisms.

There are three major reasons for the differences in the survival of various indicator organisms in estuarine water. The effect of predacious microorganisms on indicator prey species may vary due to a property of the predator or the prey organism. Alternatively, different prey species may be differentially affected by certain environmental factors to which they are exposed or a different

interaction may occur between predators and the environmental factors for each prey species. These alternatives may be more closely examined by a comparison of the survival in estuarine water samples of the common indicator organism, *E. coli*, and the remaining test organisms (Table 8).

When *E. coli* or *S. typhimurium* prey are exposed to *E. coli* predators in pure culture the survival of both organisms is similar (Section IV.E.). Similarly, when these prey organisms are exposed to *S. typhimurium* predators, their survival is similar. Prey survival for both species, is, however, less in the presence of *E. coli* predators than *S. typhimurium* predators, indicating that the former predators are more effective than the latter. When the survival of the two prey species is compared in natural estuarine water samples (Section IV.F.), both organisms again exhibit similar survival. If, however, the two prey species are incubated together in natural estuarine water in the presence of protozoan and bacterial predators, *S. typhimurium* survives to a greater degree than *E. coli*, whereas in the presence of bacterial predators only, their survival is the same. Thus, when the two bacterial strains are offered as alternative prey species, protozoan predators preferentially select *E. coli*, resulting in the increased survival of *S. typhimurium*. The properties of a bacterium which may affect their suitability as a prey organism include its size and shape, chemical composition and extracellular products, spore and capsule production (Barna and Weiss, 1973). When *E. coli* and *S. faecium* are incubated together, for example, the latter is preferentially selected by both bacterial and protozoan predators and thus exhibits lower survival than *E. coli*.

When the survival of *E. coli* and *K. pneumoniae* is compared, two mechanisms are involved, that of predator selection as noted above, and

also prey resistance. *K. pneumoniae* is less resistant to attack by predators than is *E. coli* and so exhibits lower survival in natural estuarine water samples when the two organisms are inoculated separately. Varon (1979) observed the development of a *Photobacterium leiognathi* mutant which grew more slowly and was more resistant to *Bdellovibrio* attack, than the original strain. When *E. coli* and *K. pneumoniae* were incubated together, however, preferential selection of *E. coli* by the protozoan predators resulted in the increased survival of *K. pneumoniae*.

The effect of temperature on bacterial survival in seawater is not merely of a physico-chemical nature, but is rather associated with an increase in predator-prey interactions (Verstraete and Voets, 1976). The survival of *E. coli* and *S. typhimurium* in the absence of predacious microorganisms was unaffected by a change in temperature (Section IV.G.). In the presence of microbial predators, however, the effect of temperature on prey survival was quite marked. As the temperature of incubation is increased, the survival of prey organisms is decreased, with both prey bacteria exhibiting similar survival curves at each temperature. This effect of temperature is associated with the predator population which undergoes a subtle change in composition as the temperature of incubation alters. The bacterial predators increase in numbers as the temperature is increased from 5 to 30^{o}C, while the protozoan predators exhibit an optimum temperature for growth of 15^{o}C. The protozoan predators which are initially present in lower numbers than the bacterial predators, increase markedly as the temperature is increased from 5 to 15^{o}C. Above 15^{o}C the protozoan predators decline in numbers and the bacterial predators again dominate the population. The combined action of both groups of predators, each having varying importance at different temperatures, thus produces the observed effect

of temperature on prey survival.

Solar radiation is an important factor in bacterial decline in seawater (Gameson and Saxon, 1967), although its effect on various prey species, other than coliforms, is not well documented. *Serratia marcescens*, *Bacillus subtilis* var. *niger* and faecal streptococci are less sensitive to solar radiation than are the coliforms (Chamberlin and Mitchell, 1978). Similarly, *S. faecium* and also *S. typhimurium*, *E. aerogenes* and *E. herbicola* were less sensitive to solar radiation than *E. coli* (Section IV.H.). As distinct from temperature, however, solar radiation had no effect on naturally-occurring microbial predators, but rather the prey species were affected directly. The variation in sensitivity of the various test bacteria to solar radiation is probably associated with the mechanism of light-induced decay which depends upon the presence of protection mechanisms such as bacterial pigments, sensitizing agents such as cytochromes, and the presence of oxygen.

The overall greater survival of *S. typhimurium* compared to *E. coli* observed in this study, therefore, cast doubts on the usefulness of the latter as an indicator of the presence of *S. typhimurium* in aquatic systems. Similarly, variations in the survival of the other test organisms used, compared to *E. coli*, may reduce the importance of *E. coli* as a useful indicator. It should be stressed, however, that further information regarding the effect of such factors as temperature, and the possibility of strain differences, is required before definitive conclusions regarding bacterial indicators are produced.

Of the bactericidal factors reviewed in Section II, solar radiation is considered to be the most important factor in bacterial

decline in seawater (Chamberlin and Mitchell, 1978). The importance of this factor may, however, vary from one site to another depending on other variables. In turbid waters, for example, the penetration of near ultraviolet and visible light will be greatly reduced, and so the effect of solar radiation on bacterial survival may be minimal. Further, the presence of large quantities of suspended material, such as clay particles, in water systems, may severely reduce or completely inhibit the role of predacious microorganisms in bacterial decline (Roper and Marshall, 1978). In clear waters, free of suspended material, where the penetration of solar radiation is highest, it has been suggested (Chamberlin and Mitchell, 1978), that solar radiation may only injure coliforms making them more susceptible to scavenging by predators. In fact, the combined action of predators and solar radiation produces a greater reduction of *E. coli* in estuarine water samples than either factor acting independently (Section IV.H.). Solar radiation may also affect bacterial survival indirectly, through an increase in the temperature of the water system, as these factors exhibit a strong positive correlation in natural estuarine water (Section IV.A.).

It is thus apparent that there are many factors to be considered when examining the survival of faecal bacteria in estuarine water samples and in particular the various interactions between these factors. It is also clear that predacious microorganisms, whether acting directly or in association with other bactericidal factors, are important in this bacterial decline. There are, however, many factors which warrent further investigation. These include an examination of the effects of such environmental factors as temperature, and particularly solar radiation, on the survival of various faecal bacteria, including different strains of each bacterium, and to assess more closely the

interrelationships between these factors. It is of particular importance that the mechanism of light-induced decay is investigated and understood, in order to more carefully ascertain the role of solar radiation in bacterial decline. The isolation of predators of bacterial and protozoan origin is also required and their individual and combined effects on different prey species and on each other determined to establish the nature of the complex food webs involved in bacterial decline. The establishment of the effect of the various bactericidal factors and their interactions in the laboratory, although essential, does not necessarily infer their effectiveness *in situ*. Thus, accurate field determinations of bacterial survival and the factors affecting this survival are needed if quantitative predictions regarding the extent and fate of bacterial pollution in aquatic ecosystems are to be made.

VI. Bibliography

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VII. Appendices

APPENDIX 1

Media

a. Lactose Teepol Agar

Oxoid Peptone Bacteriological (Code L37)	20g
Lactose	10g
NaCl	5g
Teepol 610	1ml
0.1% alc. Bromocresol Purple	10ml
Davis Agar	15g
Distilled Water	990ml

b. KF-Streptococcus Agar

Oxoid Proteose Peptone (Code L46)	10g
Oxoid Yeast Extract Powder (Code L21)	10g
NaCl	5g
Na glycerophosphate	10g
Maltose	20g
Lactose	1g
Na azide	0.4g
Bromocresol Purple	15mg
Davis Agar	20g
Distilled Water	1*l*

pH 7.2

Autoclave, at 121 psi/20 mins, cool to 50°C and add 1ml of sterile 10% aq. 2,3,5-triphenyl-tetrazolium chloride per *litre*.

APPENDIX 1 (continued)

c. Nutrient Agar (NA)

Oxoid Peptone Bacteriological (Code L37)	5g
Kraft Bonox	3g
Davis Agar	15g
Distilled Water	1ℓ

Salt Nutrient Agar (NAS) is NA plus 2.5g NaCl per litre.

d. Peptone Yeast Extract Broth

Oxoid Peptone Bacteriological (Code L37)	10g
Oxoid Yeast Extract Powder (Code L21)	5g
NaCl	5g
Distilled water	1ℓ

Dissolve and adjust pH to 7.2. Sterilise by autoclaving at 15 psi for 20 min.

e. Bile Broth Medium

Oxoid Ox-Bile Dessicated (10 percent solution) (Code L50)	40ml
Oxoid Brain Heart Infusion (Code CM225)	60ml

f. Glucose Yeast Extract Agar

Oxoid Yeast Extract Powder (Code L21)	3g
Oxoid Peptone Bacteriological (Code L37)	5g
Davis Agar	15g
Glucose	1g
Distilled Water	1ℓ

APPENDIX 1 (Continued)

g. Tetrazolium Medium

Oxoid Peptone Bacteriological (Code L37)	1g
Oxoid 'Lab-Lemco' Agar (Code CM17)	1g
Davis Agar	1.2g
NaCl	0.5g
TTC	0.01g
Glucose	1g
Distilled Water	100ml

h. Hugh and Leifson's Medium

Oxoid Peptone Bacteriological (Code L37)	2g
NaCl	5g
K_2HPO_4	0.3g
Davis Agar	30g
Bromothymol Blue (1 percent aqueous solution)	3ml
Distilled Water	1l

APPENDIX 2

Salinity Standard Curves

a. Sampling Period February 1978 to January 1979

NaCl (% w/v)	$\frac{1}{C}$	Log $\frac{1}{C}$	R	Ls = $(\frac{1.39}{R})$	$\frac{1}{Ls}$	Log $\frac{1}{Ls}$
0.0005	2000	3.3010	164,000	0.0000085	118,063	5.0721
0.005	200	2.3010	7,825	0.00018	5,629	3.7505
0.01	100	2.0000	6,755	0.00021	4,860	3.6866
0.025	40	1.6021	2,750	0.00051	1,980	3.2967
0.05	20	1.3010	1,585	0.00088	1,140	3.0570
0.1	10	1.0000	800	0.0017	574.7	2.7595
0.3	3.33	0.5229	292	0.0048	210.1	2.3223
0.5	2.0	0.3010	172.5	0.0081	124.1	2.0938
0.9	1.11	0.0458	117	0.0119	84.18	1.9252

Regression data: Slope 1.91; y intercept 1.06; correlation coefficient 0.99.

b. Sampling Period April 1979 to February 1980

NaCl C (% w/v)	$\frac{1}{C}$	Log $\frac{1}{C}$	Conductivity Ls (ms)	$\frac{1}{Ls}$	Log $\frac{1}{Ls}$
0.05	20	1.3010	1.09	0.9174	-0.0374
0.1	10	1.0000	1.95	0.5128	-0.2900
0.2	5	0.6990	3.87	0.2584	-0.3278
0.5	2	0.3010	8.9	0.1124	-0.9486
1.0	1	0.0000	16.6	0.0602	-1.2206
2.0	0.5	-0.3010	30.4	0.0329	-1.4831
3.0	0.33	-0.4771	43.0	0.0233	-1.6327
4.0	0.25	-0.6021	54.5	0.0183	-1.7372
5.0	0.20	-0.6990	64.5	0.0155	-1.8099

Regression data: Slope 1.25; y intercept 1.06; correlation coefficient 0.99.

APPENDIX 3.a Sampling Data 1978-79 (data for Tables 11, 14a, 14b, 14c).

Appendix 3.a.1. Site 1

SAMPLING DATE	TOTAL COLIFORMS (Nos./100ml)	PFU (Nos./ml)	AEROBIC HETERO-TROPHIC BACTERIA (Nos./ml)	TEMPERATURE (^{o}C)	SALINITY (% NaCl)	RAINFALL (mm)	SOLAR RADIATION (cal cm^{2})
14/3/78	5.08	1.30	3.56	18.0	2.01	87	341
17/4/78	1.70	0.70	1.30	15.4	0.92	14	224
7/6/78	4.99	1.53	3.86	10.0	0.40	49	124
3/7/78	6.01	0.00	4.18	9.0	1.51	51	139
5/9/78	6.50	2.27	4.59	11.2	0.50	109	309
10/10/78	5.35	1.88	3.70	12.2	1.87	14	425
7/11/78	4.34	0.78	3.21	15.8	2.31	26	476
4/12/78	5.05	1.66	3.72	16.0	1.74	94	501
10/1/79	4.77	1.38	3.52	17.6	2.20	52	547
13/2/79	3.65	0.00	2.65	18.0	1.64	39	442

APPENDIX 3.a Sampling Data 1978-79 (data for Tables 11, 14a, 14b, 14c).

Appendix 3.a.2. Site 2

SAMPLING DATE	TOTAL COLIFORMS (Nos./100ml)	PFU (Nos./ml)	AEROBIC HETERO-TROPHIC BACTERIA (Nos./ml)	TEMPERATURE (°C)	SALINITY (% NaCl)	RAINFALL (mm)	SOLAR RADIATION (cal cm^2)
14/3/78	3.48	1.52	3.54	18.0	1.44	87	341
17/4/78	3.77	2.60	3.00	15.0	1.73	14	224
7/6/78	4.32	0.85	3.04	10.8	0.73	49	124
3/7/78	3.85	0.00	3.42	10.2	2.94	51	139
5/9/78	3.70	0.00	3.18	11.4	1.47	109	309
10/10/78	3.22	0.00	1.85	11.6	2.17	14	425
7/11/78	3.30	1.00	2.68	14.4	2.97	26	476
4/12/78	4.20	1.00	2.89	15.2	2.14	94	501
10/1/79	3.24	0.78	1.60	17.4	2.79	52	547
13/2/79	3.88	0.00	2.72	17.8	2.06	39	442

APPENDIX 3.a Sampling Data 1978-79 (data for Tables 11, 14a, 14b, 14c).

Appendix 3.a.3. Site 3

SAMPLING DATE	TOTAL COLIFORMS (Nos./100ml)	PFU (Nos./ml)	AEROBIC HETERO-TROPHIC BACTERIA (Nos./ml)	TEMPERATURE (^{o}C)	SALINITY (% NaCl)	RAINFALL (mm)	SOLAR RADIATION (cal cm^{2})
14/3/78	3.00	1.23	3.29	18.0	2.32	87	341
17/4/78	4.63	2.53	2.23	15.4	2.85	14	224
7/6/78		1.86	3.59	11.2	2.15	49	124
3/7/78	3.60	0.00	3.75	9.4	2.68	51	139
5/9/78	4.16	1.30	3.00	11.5	2.52	1 09	309
10/10/78	3.49	1.15	2.45	12.8	2.31	14	425
7/11/78	1.85	0.78	2.27	13.8	3.27	26	476
4/12/78	4.12	0.90	3.30	14.8	2.46	94	501
10/1/79	3.65	0.90	2.10	17.8	3.05	52	547
13/2/79	4.91	0.30	3.04	18.2	2.33	39	442

APPENDIX 3.a Sampling Data 1978-79 (data for Tables 11, 14a, 14b, 14c).

Appendix 3.a.4. Site 4

SAMPLING DATE	TOTAL COLIFORMS (Nos./100ml)	PFU (Nos./ml)	AEROBIC HETERO-TROPHIC BACTERIA (Nos./ml)	TEMPERATURE (^{o}C)	SALINITY (% NaCl)	RAINFALL (mm)	SOLAR RADIATION (cal cm^2)
14/3/78	0.00	1.76	2.98	18.0	2.15	87	341
17/4/78	2.18	2.26	2.48	15.5	2.45	14	224
7/6/78	3.71	0.60	2.66	11.0	2.50	49	124
3/7/78	3.08	0.00	2.13	10.6	2.05	51	139
5/9/78	1.18	0.30	2.56	11.8	2.39	109	309
10/10/78	2.40	0.00	1.85	11.6	2.44	14	425
7/11/78	0.78	0.00	1.54	14.6	3.39	26	476
4/12/78	3.48	0.30	2.92	14.5	2.75	94	501
10/1/79	1.40	0.00	1.18	17.4	3.31	52	547
13/2/79	3.23	0.00	1.93	18.2	2.64	39	442

APPENDIX 3.a Sampling Data 1978-79 (data for Tables 11, 14a, 14b, 14c).

Appendix 3.a.5. Site 5

SAMPLING DATE	TOTAL COLIFORMS (Nos./100ml)	PFU (Nos./ml)	AEROBIC HETERO-TROPHIC BACTERIA (Nos./ml)	TEMPERATURE (^{o}C)	SALINITY (% NaCl)	RAINFALL (mm)	SOLAR RADIATION (cal cm^{2})
14/3/78	2.65	1.69	2.26	18.0	1.94	91	341
17/4/78	2.00	1.78	2.74	15.5	2.26	19	224
7/6/78	2.30	1.72	2.18	11.8	2.94	72	124
3/7/78	1.48	0.00	3.98	10.2	2.94	66	139
5/9/78	1.48	0.78	2.44	11.6	2.68	121	309
10/10/78	0.85	0.30	1.95	11.8	2.44	13	425
7/11/78	0.93	0.00	1.93	15.0	3.38	57	476
4/12/78	2.08	0.00	2.63	15.5	2.70	90	501
10/1/79	1.59	0.60	0.70	17.2	3.34	64	547
13/2/79	1.81	0.00	0.00	18.4	2.64	32	442

APPENDIX 3.a Sampling Data 1978-79 (data for Tables 11, 14a, 14b, 14c).

Appendix 3.a.6. Site 6

SAMPLING DATE	TOTAL COLIFORMS (Nos./100ml)	PFU (Nos./ml)	AEROBIC HETERO-TROPHIC BACTERIA (Nos./ml)	TEMPERATURE (^{o}C)	SALINITY (% NaCl)	RAINFALL (mm)	SOLAR RADIATION (cal cm^{2})
14/3/78	0.00	1.64	1.90	18.0	1.65	91	341
17/4/78	0.78	0.78	2.13	15.4	2.26	19	224
7/6/78	1.02	1.45	2.81	11.8	2.05	72	124
3/7/78	1.32	0.00	2.40	10.4	3.04	66	139
5/9/78	2.28	0.30	2.54	11.6	2.76	121	309
10/10/78	0.70	0.00	1.74	12.2	2.42	13	425
7/11/78	0.78	0.78	2.51	15.0	3.42	57	476
4/12/78	2.56	1.15	3.58	15.0	2.84	90	501
10/1/79	0.30	0.30	1.00	17.4	3.36	64	547
13/2/79	0.90	0.00	2.33	18.6	2.87	32	442

APPENDIX 3.a Sampling Data 1978-79 (data for Tables 11, 14a, 14b, 14c).

Appendix 3.a.7.; Site 7.

SAMPLING DATE	TOTAL COLIFORMS (Nos./100ml)	PFU (Nos./ml)	AEROBIC HETERO-TROPHIC BACTERIA (Nos./ml)	TEMPERATURE (^{o}C)	SALINITY (% NaCl)	RAINFALL (mm)	SOLAR RADIATION (cal cm^{2})
14/3/78	0.00	1.68	2.34	18.0	2.49	25	341
17/4/78	0.48	1.00	2.44	15.4	2.26	53	224
7/6/78	1.18	1.28	2.53	11.0	2.10	62	124
3/7/78	1.70	0.00	3.05	8.6	2.76	54	139
5/9/78	1.74	0.30	3.05	12.2	2.60	112	309
10/10/78	0.48	0.48	3.88	14.6	2.39	27	425
7/11/78	0.00	0.00	2.02	17.0	3.42	61	476
4/12/78	2.60	0.60	2.61	15.5	2.92	75	501
10/1/79	0.00	0.60	2.96	21.2	3.42	73	547
13/2/79	0.70	0.00	3.70	18.8	2.81	34	442

APPENDIX 3.b Sampling Data 1979-80 (data for Figures 6 to 15 and Tables 10 to 12).

Appendix 3.b.1; Site 1.

SAMPLING DATE	TOTAL COLIFORMS (Nos./100ml)	FAECAL COLIFORMS (FC) (Nos./100ml)	FAECAL STREPTOCOCCI (FS) (Nos./100ml)	PFU (Nos./ml)	AEROBIC HETEROTROPHIC BACTERIA (AHB) (Nos./ml)	TEMPERATURE (^{o}C)	SALINITY (% NaCl)	RAINFALL (mm)	SOLAR RADIATION (cal cm^{-2})
6/4/79	4.18	4.18	1.57	0.00	3.46	15.4	2.29	27	224
19/6/79		1.60	1.08	0.00	1.93	10.8	2.31	19	124
24/7/79	5.95	5.22	*a*	1.45	3.96	9.0	1.97	2	139
23/8/79	6.20		3.90	0.78	3.95	8.6	1.09	12	206
25/9/79	5.90	4.49	2.75	1.20	3.90	11.0	0.78	71	309
16/10/79	5.79	4.79	*a*	1.30	3.75	12.6	1.01	66	425
6/11/79	5.93	4.15	3.98	1.40	3.89	14.5	2.46	38	476
11/12/79	5.45	4.11	3.40	0.60	2.77	14.8	2.06	18	501
15/1/80	4.90	3.98	2.00	0.78	3.36	18.2	2.58	42	547
12/2/80	4.81	3.54	2.78		3.03	16.2	2.46	8	442

a Too numerous to count.

APPENDIX 3.b Sampling Data 1979-80 (data for Figures 6 to 15 and Tables 10 to 12).

Appendix 3.b.2; Site 2.

SAMPLING DATE	TOTAL COLIFORMS (Nos./100ml)	FAECAL COLIFORMS (FC) (Nos./100ml)	FAECAL STREPTOCOCCI (FS) (Nos./100ml)	PFU (Nos./ml)	AEROBIC HETEROTROPHIC BACTERIA (AHB) (Nos./ml)	TEMPERATURE (^{o}C)	SALINITY (% NaCl)	RAINFALL (mm)	SOLAR RADIATION (cal cm^{-2})
6/4/79	4.71	4.38	1.62	1.08	3.31	15.6	2.56	27	224
19/6/79		1.30	1.11	0.00	2.30	11.0	3.16	19	124
24/7/79	4.04	3.00	1.86	1.08	3.08	9.6	2.35	2	139
23/8/79	5.81		2.30	0.95	3.18	8.6	1.62	12	206
25/9/79	4.77	3.00	2.44	0.60	3.00	11.0	2.46	71	309
16/10/79	4.04	3.00	1.92	0.00	2.45	12.4	2.01	66	425
6/11/79	3.30	2.70	1.60	0.00	2.37	14.2	2.94	38	476
11/12/79	4.52	4.02	2.43	0.00	2.30	15.2	2.70	18	501
15/1/80	3.04		1.58	0.30	2.08	18.4	3.05	42	547
12/2/80	3.70	2.70	2.09		2.19	16.0	2.32	8	442

APPENDIX 3.b Sampling Data 1979-80 (data for Figures 6 to 15 and Tables 10 to 12).

Appendix 3.b.3; Site 3.

SAMPLING DATE	TOTAL COLIFORMS (Nos./100ml)	FAECAL COLIFORMS (FC) (Nos./100ml)	FAECAL STREPTOCOCCI (FS) (Nos./100ml)	PFU (Nos./ml)	AEROBIC HETEROTROPHIC BACTERIA (AHB) (Nos./ml)	TEMPERATURE (^{o}C)	SALINITY (% NaCl)	RAINFALL (mm)	SOLAR RADIATION (cal cm^{-2})
6/4/79	5.08	4.95	2.02	1.48	3.12	15.4	2.75	27	224
19/6/79		1.78	1.23	0.00	2.37	11.3	3.31	19	124
24/7/79	4.72	3.00	2.00	1.51	3.23	9.0	2.39	2	139
23/8/79	5.19		2.47	1.20	2.90	8.6	2.06	12	206
25/9/79	4.96	3.48	2.75	0.90	2.97	11.8	3.50	71	309
16/10/79	5.05	3.48	2.43	0.90	2.70	12.5	2.47	66	425
6/11/79	3.88	3.40	1.79	1.15	3.22	14.8	2.94	38	476
11/12/79	5.65	4.40	3.64	0.00	3.82	14.8	2.90	18	501
15/1/80	5.34	4.95	3.05	0.90	3.69	18.2	3.45	42	547
12/2/80	4.15	3.28	2.72		2.64	16.0	3.35	8	442

APPENDIX 3.b Sampling Data 1979-80 (data for Figures 6 to 15 and Tables 10 to 12).

Appendix 3.b.4; Site 4.

SAMPLING DATE	TOTAL COLIFORMS (Nos./100ml)	FAECAL COLIFORMS (FC) (Nos./100ml)	FAECAL STREPTOCOCCI (FS) (Nos./100ml)	PFU (Nos./ml)	AEROBIC HETEROTROPHIC BACTERIA (AHB) (Nos./ml)	TEMPERATURE (^{o}C)	SALINITY (% NaCl)	RAINFALL (mm)	SOLAR RADIATION (cal cm^{-2})
6/4/79	3.40	3.30	1.11	0.30	2.93	16.4	3.01	27	224
19/6/79		2.38	0.70	0.30	2.32	11.2	3.43	19	124
24/7/79	0.00	0.00	1.26	0.30	2.79	10.8	3.19	2	139
23/8/79			1.46	0.60	2.57	9.4	2.46	12	206
25/9/79	0.70	0.00	2.29	0.00	2.47	11.3	3.46	71	309
16/10/79			1.15	0.00	2.26	12.6	2.83	66	425
6/11/79	1.08	0.95	1.34	0.60	2.10	14.0	3.02	38	476
11/12/79			0.85	0.00	1.85	15.2	3.35	18	501
15/1/80	2.48	1.30	1.43	0.30	2.97	17.2	3.60	42	547
12/2/80	0.78	0.78	0.00		1.40	15.8	3.70	8	442

APPENDIX 3.b Sampling Data 1979-80 (data for Figures 6 to 15 and Tables 10 to 12).

Appendix 3.b.5; Site 5.

SAMPLING DATE	TOTAL COLIFORMS (Nos./100ml)	FAECAL COLIFORMS (FC) (Nos./100ml)	FAECAL STREPTOCOCCI (FS) (Nos./100ml)	PFU (Nos./ml)	AEROBIC HETEROTROPHIC BACTERIA (AHB) (Nos./ml)	TEMPERATURE (^{o}C)	SALINITY (% NaCl)	RAINFALL (mm)	SOLAR RADIATION (cal cm^{-2})
6/4/79		1.86	0.70	0.60	2.67	16.2	3.24	24	224
19/6/79			2.00	0.48	2.59	11.2	3.35	36	124
24/7/79	1.64	1.68	0.90	0.60	2.16	10.2	3.35	4	139
23/8/79			0.00	0.00	3.08	9.8	2.68	28	206
25/9/79	1.81	0.00	2.68	0.00	1.90	10.8	3.46	103	309
16/10/79		1.20	1.18	0.30	2.20	12.8	3.50	99	425
6/11/79	0.70	0.00	0.00	0.30	2.00	14.2	3.11	55	476
11/12/79			1.04	0.00	1.54	15.2	3.30	43	501
15/1/80	2.00	0.30	0.95	0.00	2.34	17.0	3.69	61	547
12/2/80	0.30	0.00	1.26		1.95	16.8	3.95	25	442

APPENDIX 3.b Sampling Data 1979-80 (data for Figures 6 to 15 and Tables 10 to 12).

Appendix 3.b.6; Site 6.

SAMPLING DATE	TOTAL COLIFORMS (Nos./100ml)	FAECAL COLIFORMS (FC) (Nos./100ml)	FAECAL STREPTOCOCCI (FS) (Nos./100ml)	PFU (Nos./ml)	AEROBIC HETEROTROPHIC BACTERIA (AHB) (Nos./ml)	TEMPERATURE (^{o}C)	SALINITY (% NaCl)	RAINFALL (mm)	SOLAR RADIATION (cal cm^{-2})
6/4/79	1.40	1.00	0.48	1.00	3.13	16.0	3.20	24	224
19/6/79		0.48	0.48	0.00	1.18	11.0	3.54	36	124
24/7/79	0.30	0.00	0.00	0.00	1.48	9.6	3.23	4	139
23/8/79			0.00	0.00	2.30	9.6	2.84	28	206
25/9/79	0.30	0.00	0.48	0.00	1.74	11.2	3.51	103	309
16/10/79		0.30	0.00	0.00	1.85	12.5	3.35	99	425
6/11/79		1.69	1.64	0.70	2.67	14.7	3.19	55	476
11/12/79			0.90	0.00	1.00	14.8	3.28	43	501
15/1/80	2.30	1.18	2.24	0.00	4.20	17.0	3.69	61	547
12/2/80	0.48	0.00	0.00		1.83	16.8	3.84	25	442

APPENDIX 3.b Sampling Data 1979-80 (data for Figures 6 to 15 and Tables 10 to 12).

Appendix 3.b.7; Site 7.

SAMPLING DATE	TOTAL COLIFORMS (Nos./100ml)	FAECAL COLIFORMS (FC) (Nos./100ml)	FAECAL STREPTOCOCCI (FS) (Nos./100ml)	PFU (Nos./ml)	AEROBIC HETEROTROPHIC BACTERIA (AHB) (Nos./ml)	TEMPERATURE (^{o}C)	SALINITY (% NaCl)	RAINFALL (mm)	SOLAR RADIATION (cal cm^{-2})
6/4/79	1.00	0.00	0.00	0.90	3.03	16.0	3.16	47	224
24/7/79	0.00	0.00	0.00	0.78	3.00	9.0	3.16	8	139
25/9/79	0.30	0.00	0.60	0.78	3.07	10.8	3.44	111	309
6/11/79	0.00	0.00	0.00	0.70	2.77	12.0	3.16	58	476
15/1/80	2.38	0.70	1.51	0.78	4.00	17.0	3.76	71	547

APPENDIX 3.b Sampling Data 1979-80 (data for Figures 6 to 15 and Tables 10 to 12).

Appendix 3.b.8; Site 8.

SAMPLING DATE	TOTAL COLIFORMS (Nos./100ml)	FAECAL COLIFORMS (FC) (Nos./100ml)	FAECAL STREPTOCOCCI (FS) (Nos./100ml)	PFU (Nos./ml)	AEROBIC HETEROTROPHIC BACTERIA (AHB) (Nos./ml)	TEMPERATURE (^{o}C)	SALINITY (% NaCl)	RAINFALL (mm)	SOLAR RADIATION (cal cm^{-2})
6/4/79	6.38	6.37	2.00	1.20	3.58	16.2	2.24	24	224
19/6/79		2.45	1.95	0.00	3.40	9.0	2.76	36	124
24/7/79	6.70	5.95	*a*	0.78	*a*	9.0	2.72	4	139
23/8/79	6.17		3.46	0.30	3.35	8.4	1.84	28	206
25/9/79	4.32		2.25	0.90	2.52	10.8	3.06	103	309
16/10/79	4.82	3.00	1.32	0.30	2.56	11.4	2.53	99	425
6/11/79	4.00	2.70	0.30	0.60	2.10	14.2	3.02	55	476
11/12/79	2.00	0.00	0.00	0.48	3.00	13.6	3.31	43	501
15/1/80	5.85	3.90	2.30	0.78	4.75	18.0	3.13	61	547
12/2/80	3.90	2.70	1.26		2.38	19.0	3.65	25	442

a Too numerous to count.

APPENDIX 3.b Sampling Data 1979-80 (data for Figures 6 to 15 and Tables 10 to 12).

Appendix 3.b.9; Site 9.

SAMPLING DATE	TOTAL COLIFORMS (Nos./100ml)	FAECAL COLIFORMS (FC) (Nos./100ml)	FAECAL STREPTOCOCCI (FS) (Nos./100ml)	PFU (Nos./ml)	AEROBIC HETEROTROPHIC BACTERIA (AHB) (Nos./ml)	TEMPERATURE (^{o}C)	SALINITY (% NaCl)	RAINFALL (mm)	SOLAR RADIATION (cal cm^{-2})
6/4/79	0.00	0.00	0.00	0.90	2.59	16.2	3.24	24	224
19/6/79		0.48	0.00	1.48	3.02	10.8	3.50	36	124
24/7/79	0.00	0.30	0.60	1.30	2.56	9.2	3.20	4	139
23/8/79			0.78	0.70	2.94	9.2	2.76	28	206
25/9/79	0.85	0.00	2.16	0.30	2.44	11.2	3.50	103	309
16/10/79		0.00	1.48	0.00	1.74	11.2	3.38	99	425
6/11/79	0.00	0.90	0.30	0.48	1.65	13.6	3.02	55	476
11/12/79			0.00	0.00	1.74	14.5	3.31	43	501
15/1/80	2.00	0.00	0.78	0.30	4.00	17.6	3.76	61	547
12/2/80	0.70	0.30	1.30		1.88	17.0	3.75	25	442

APPENDIX 3.b Sampling Data 1979-80 (data for Figures 6 to 15 and Tables 10 to 12).

Appendix 3.b.10; Site 10.

SAMPLING DATE	TOTAL COLIFORMS (Nos./100ml)	FAECAL COLIFORMS (FC) (Nos./100ml)	FAECAL STREPTOCOCCI (FS) (Nos./100ml)	PFU (Nos./ml)	AEROBIC HETEROTROPHIC BACTERIA (AHB) (Nos./ml)	TEMPERATURE (^{o}C)	SALINITY (% NaCl)	RAINFALL (mm)	SOLAR RADIATION (cal cm^{-2})
6/4/79	0.70	0.00	0.00	0.00	2.30	16.6	1.87	24	224
19/6/79		1.52	0.30	0.00	1.78	9.8	3.49	36	124
24/7/79	0.00	0.60	0.00	0.00	1.88	9.4	3.16	4	139
23/8/79			1.75	0.00	2.64	8.0	0.83	28	206
25/9/79	1.72	0.00	1.52	0.78	2.61	10.8	3.13	103	309
16/10/79		1.20	0.60	0.00	2.15	10.8	2.98	99	425
6/11/79	0.00	0.30	0.48	0.48	1.48	14.0	3.16	55	476
11/12/79			2.20	0.00	3.05	13.4	2.44	43	501
15/1/80	3.08	1.20	1.74	0.30	3.41	19.0	3.56	61	547
12/2/80	0.30	0.00	0.00		2.39	18.0	3.63	25	442

APPENDIX 3.b Sampling Data 1979-80 (data for Figures 6 to 15 and Tables 10 to 12).

Appendix 3.b.11; Site 11.

SAMPLING DATE	TOTAL COLIFORMS (Nos./100ml)	FAECAL COLIFORMS (FC) (Nos./100ml)	FAECAL STREPTOCOCCI (FS) (Nos./100ml)	PFU (Nos./ml)	AEROBIC HETEROTROPHIC BACTERIA (AHB) (Nos./ml)	TEMPERATURE (°C)	SALINITY (% NaCl)	RAINFALL (mm)
19/6/79		1.81	1.48	0.00	2.13	8.4	0.37	40
23/8/79	4.89		1.92	0.00	2.32	6.4	0.01	14
16/10/79	4.30	3.00	1.72	0.00	2.57	11.0	0.02	74
11/12/79	5.03	4.30	2.14	0.00	2.78	15.0	2.47	20
12/2/80	4.86	3.95	1.85		2.33	15.8	5.60	11

APPENDIX 3.c

Appendix 3.c.1. Variance-Covariance and Correlation Matrices; Pooled over Unpolluted Sites.

PARAMETER	TOTAL COLIFORMS	PFU	AEROBIC HETEROTROPHIC BACTERIA	TEMPERATURE	SALINITY	RAINFALL	SOLAR RADIATION
Variance-Covariance Matrix							
Total Coliforms	0.70534						df = 47
PFU	0.00900	0.32124					
Aerobic Heterotrophic Bacteria	0.32758	0.02660	0.63219				
Temperature	0.46472	0.13535	0.04865	11.47445			
Salinity	0.06221	-0.13213	0.05306	-0.00498	0.28291		
Rainfall	10.84286	0.33899	3.89269	-2.78600	2.12786	1178.38247	
Solar Radiation	24.71947	-25.91850	0.44790	349.93757	30.32324	1235.82705	24741.75015
Mean	0.02415	0.01200	0.05302	0.30153	0.06287	1.21638	7.37013
							1.12766
Correlation Matrix							
Total Coliforms	1.0000						df = 47
PFU	0.0189	1.0000					
Aerobic Heterotrophic Bacteria	0.4906***	0.0590	1.0000				
Temperature	0.1634	0.0705	0.0181	1.0000			
Salinity	0.1393	-0.4383***	0.1255	-0.0028	1.0000		
Rainfall	0.3761***	0.0174	0.1426	-0.0240	0.1165	1.0000	
Solar Radiation	0.1871	-0.2907**	0.0036	0.6568***	0.3624***	0.2289*	1.0000

*Significant at 5%; **Significant at 1%; ***Significant at 0.1%.

APPENDIX 3.c

Appendix 3.c.2. Variance-Covariance and Correlation Matrices; Pooled over Polluted Sites.

PARAMETER	TOTAL COLIFORMS	PFU	AEROBIC HETEROTROPHIC BACTERIA	TEMPERATURE	SALINITY	RAINFALL	SOLAR RADIATION
Variance-Covariance Matrix							
Total Coliforms	1.08882						df = 55
PFU	0.13005	0.39777					
Aerobic Heterotrophic Bacteria	0.41982	0.07501	0.41751				
Temperature	-1.01377	0.01978	-0.42867	10.56818			
Salinity	-0.13652	-0.08617	-0.07184	0.78300	0.35903		
Rainfall	-0.59618	0.57952	3.64810	11.06433	-2.92305	973.13381	
Solar Radiation	-36.12613	-16.68033	-24.99911	314.09246	43.98064	879.46322	21262.91135
Mean	0.08174	0.01590	0.05635	0.24955	0.04086	0.84758	6.55424
							1.09091
Correlation Matrix							
Total Coliforms	1.0000						df = 55
PFU	0.1976*	1.0000					
Aerobic Heterotrophic Bacteria	0.6227***	0.1841	1.0000				
Temperature	-0.2989**	0.0096	-0.2041*	1.0000			
Salinity	-0.2183*	-0.2280*	-0.1856	0.4020***	1.0000		
Rainfall	-0.0183	0.0295	0.1810	0.1091	-0.1564	1.0000	
Solar Radiation	-0.2374*	-0.1814	-0.2653**	0.6626***	0.5034***	0.1933*	1.0000

*Significant at 5%; **Significant at 1%; ***Significant at 0.1%.

APPENDIX 3.c

Appendix 3.c.3. Variance-Covariance Matrix; Pooled over Unpolluted and Polluted Sites.

PARAMETER	TOTAL COLIFORMS	PFU	AEROBIC HETEROTROPHIC BACTERIA	TEMPERATURE	SALINITY	RAINFALL	SOLAR RADIATION
Total coliforms	0.91212						df = 103
PFU	0.07427	0.36251					
Aerobic Heterotrophic Bacteria	0.37732	0.05270	0.51643				
Temperature	-0.33250	0.07304	-0.20873	10.98578			
Salinity	-0.04495	-0.10735	-0.01429	0.41991	0.32395		
Rainfall	4.67475	0.46869	3.76080	4.68232	-0.59567	1067.70917	
Solar Radiation	-8.08943	-20.93714	-13.27353	330.60932	37.68752	1043.67008	22865.90570
Mean	0.02862	0.00714	0.02759	0.13661	0.02529	0.50555	3.46937
							1.10784

APPENDIX 4.

"Faecal" Coliforms Isolated from Various Sampling Sites (data for Table 13).

ORGANISM NO.	ORGANISM IDENTIFICATION	ISOLATION SITE
FC1	*Escherichia coli*	Boyer
FC2	*Klebsiella pneumoniae*	"
FC3	*K. pneumoniae*	"
FC4	*K. pneumoniae*	"
FC5	*K. pneumoniae*	"
FC6	*E. coli*	"
FC7	*K. pneumoniae*	"
FC9	*Enterobacter cloacae*	"
FC10	*K. pneumoniae*	"
FC11	*Citrobacter freundii*	Abbatoirs
FC12	*E. coli*	"
FC13	*E. aerogenes*	"
FC14	*C. freundii*	"
FC15	*E. coli*	"
FC16	*E. coli*	"
FC17	*K. pneumoniae*	"
FC19	*K. pneumoniae*	"
FC20	*K. pneumoniae*	"
FC21	*E. coli*	"
FC22	*K. pneumoniae*	Tasman Bridge
FC23	*E. coli*	Rosny Point
FC24	*E. coli*	Abbatoirs
FC25	*E. coli*	"
FC26	*E. coli*	"
FC27	*K. pneumoniae*	"

APPENDIX 4 (continued)

FC28	*E. coli*	Abbatoirs
FC29	*C. freundii*	"
FC30	*E. coli*	"
FC31	*E. coli*	"
FC32	*C. freundii*	"
FC33	*C. freundii*	"
FC34	*C. freundii*	"
FC35	*C. freundii*	"
FC36	*K. pneumoniae*	"
FC37	*K. pneumoniae*	Boyer
FC38	*E. coli*	"
FC39	*E. aerogenes*	"
FC40	*K. ozaenae*	"
FC41	*E. coli*	"
FC42	*K. ozaenae*	"
FC43	*K. pneumoniae*	"
FC44	*E. aerogenes*	"
FC45	*E. aerogenes*	"
FC46	*K. pneumoniae*	"
FC47	*K. pneumoniae*	"
FC48	*E. coli*	"
FC49	*K. ozaenae*	"
FC50	*K. ozaenae*	"
FC51	*K. pneumoniae*	"
FC52	*K. pneumoniae*	"
FC53	*E. aerogenes*	"

APPENDIX 4 (continued)

FC54	*K. pneumoniae*	Boyer
FC55	*K. pneumoniae*	"
FC56	*K. pneumoniae*	"
FC57	*K. pneumoniae*	"
FC58	*Yersinia enterolitica*	"
FC59	*K. ozaenae*	"
FC60	*K. pneumoniae*	"
FC61	*K. pneumoniae*	"
FC62	*K. pneumoniae*	"
FC63	*K. pneumoniae*	Rosny Point
FC64	*E. coli*	"
FC65	*K. pneumoniae*	"
FC66	*K. pneumoniae*	"
FC67	*K. pneumoniae*	"
FC68	*C. freundii*	"
FC69	*E. coli*	"
FC70	*C. freundii*	"
FC71	*K. ozaenae*	"
FC72	*E. coli*	Abbatoirs
FC73	*E. coli*	"
FC74	*E. coli*	"
FC75	*K. ozaenae*	"
FC76	*E. coli*	"
FC77	*E. coli*	"
FC78	*E. coli*	"
FC79	*E. coli*	Tasman Bridge

APPENDIX 5. *E. coli* Survival in Estuarine Water Samples from Various Sites at Different Sampling Times (data for Figure 16).

Appendix 5.a Season 3 (sampling date 17/4/78)

1. *E. coli* Survival — Log Number Organisms per ml

TIME (DAYS)	SITE 1	2	5	6	7
Autoclaved Estuarine Water					
0	*a*	7.25	7.02	7.25	7.09
2		6.35	6.69	6.71	6.85
4		6.20	5.60	6.60	6.11
6		5.15	5.35	5.92	5.75
8		5.55	5.36	5.57	5.54
10		5.51	5.04	5.16	5.23
Natural Estuarine Water					
0	*a*	7.04	7.05	7.09	7.10
2		6.88	6.85	6.85	6.89
4		6.65	6.93	6.76	6.83
6		4.40	4.40	4.90	4.48
8		2.90	2.70	3.51	2.48
10		2.79	2.18	2.32	2.48

2. PFU Growth

TIME (DAYS)	SITE 1	2	5	6	7
0	*a*	2.73	1.48	1.00	1.04
2		3.48	3.90	3.80	3.37
4					
6		2.15	3.70	4.36	3.76
8		<1.00	1.00	<1.00	4.08
10					

[a] No counts recorded.

APPENDIX 5. *E. coli* Survival in Estuarine Water Samples from Various Sites at Different Sampling Times (data for Figure 16).

Appendix 5.b. Season 3 (Sampling date 7/6/78)

1. *E. coli* Survival — Log Number Organisms per ml

TIME (DAYS)	SITE 1	2	5	6	7
Autoclaved Estuarine Water					
0	8.20	8.24	8.21	8.27	8.31
2	7.85	7.74	7.80	7.81	7.83
4	7.80	7.68	7.73	7.65	7.51
6	7.70	7.69	7.60	7.67	7.79
8	7.70	7.42	7.31	7.16	7.52
10	7.49	7.28	6.65	6.81	6.95
Natural Estuarine Water					
0	8.36	8.26	8.23	8.25	8.26
2	7.88	7.76	7.83	7.84	7.94
4	7.81	7.42	7.62	7.74	7.83
6	6.41	5.23	7.35	7.47	7.62
8	4.77	3.80	4.40	5.47	5.00
10	4.54	3.47	2.60	4.11	4.11

2. PFU Growth

TIME (DAYS)	SITE 1	2	5	6	7
0	1.15	0.60	0.48	1.40	0.30
2	2.08	1.70	3.09	4.21	1.60
4	2.70	3.23	3.30	4.76	3.60
6	2.74	3.57	2.62	3.52	4.27
8	3.85				
10	4.15	3.00	3.00	3.00	

APPENDIX 5. *E. coli* Survival in Estuarine Water Samples from Various Sites at Different Sampling Times (data for Figure 16).

Appendix 5.c. Season 3 (sampling date 3/7/78)

1. *E. coli* Survival — Log Number Organisms per ml

TIME (DAYS)	SITE 1	2	5	6	7
Autoclaved Estuarine Water					
0	7.00	7.06	7.00	7.20	7.13
2	6.95	6.99	6.88	6.88	6.89
4	6.87	6.92	6.74	6.80	6.84
6	6.56	6.69	6.72	6.70	6.51
8	6.18	5.74	6.19	6.18	5.81
10	6.28	5.55	5.31	5.33	5.13
Natural Estuarine Water					
0	7.08	7.26	7.15	7.00	7.04
2	6.94	6.85	6.60	6.89	6.51
4	5.90	6.74	5.89	6.68	6.34
6	4.80	5.34	5.11	6.32	5.23
8	4.03	3.56	4.21		4.00
10	3.78	3.09	3.08	3.06	3.42

2. PFU Growth

TIME (DAYS)	SITE 1	2	5	6	7
0	0.78	0.00		0.00	0.00
2	1.20	2.30		0.30	0.00
4	2.43	3.51		1.63	2.62
6	3.71	3.27		2.61	2.70
8	3.04	3.08		2.32	3.02
10	2.70	3.51		2.32	2.78

APPENDIX 5. *E. coli* Survival in Estuarine Water Samples from Various Sites at Different Sampling Times (data for Figure 16).

Appendix 5.d. Season 2 (sampling date 5/9/78).

1. *E. coli* Survival — Log Number Organisms per ml

TIME (DAYS)	SITE 1	2	5	6	7
Autoclaved Estuarine Water					
0	7.09	6.90	6.91	6.87	6.94
2	7.15	6.86	6.97	6.91	6.72
4	7.03	7.01	6.86	6.89	6.80
6	6.00	6.75	6.91	6.82	6.76
8	6.95	6.86	6.64	6.85	6.68
10	6.74	6.51	6.54	6.67	6.35
Natural Estuarine Water					
0	7.02	7.00	6.93	7.05	6.86
2	5.04	6.58	6.57	6.48	6.26
4	3.86	3.08	6.67	6.20	4.51
6	3.48	4.30	4.54	5.08	3.72
8	3.39	3.71	3.26	4.36	1.00
10	2.96	3.46	2.04	2.88	0.00

2. PFU Growth

TIME (DAYS)	SITE 1	2	5	6	7
0	3.00	1.38	0.30	0.30	
2	2.95	2.47	1.60	0.00	
4	2.51	2.34	1.78	0.00	
6	2.03	1.53	1.79	0.30	
8	2.42	1.30	1.79	1.70	
10	2.01	1.51	1.42	1.56	

APPENDIX 5. *E. coli* Survival in Estuarine Water Samples from Various Sites at Different Sampling Times (data for Figure 16).

Appendix 5.e. Season 2 (sampling date 10/10/78)

1. *E. coli* Survival — Log Number Organisms per ml

TIME (DAYS)	SITE 1	2	5	6	7
Autoclaved Estuarine Water					
0	7.95	7.90	7.70	7.74	7.79
2	7.76	7.73	7.70	7.71	7.69
4	7.76	7.78	7.64	7.63	7.66
6	7.69	7.67	7.50	7.48	7.49
8	7.58	7.45	7.42	7.32	7.42
10	7.64	7.57	7.37	7.47	7.33
Natural Estuarine Water					
0	7.83	7.83	7.74	7.69	7.79
2	7.42	7.75	7.84	7.69	7.93
4	5.40	7.60	7.67	7.72	7.59
6	4.60		7.45	7.48	5.30
8	3.72	5.14	7.80	6.47	3.93
10	3.29	3.83	4.00	4.00	2.56

2. PFU Growth

TIME (DAYS)	SITE 1	2	5	6	7
0	1.88	0.00	0.00	0.00	1.20
2	2.93	1.70	0.90	1.00	2.08
4	2.66	1.64	2.56	1.38	2.56
6	1.70	1.75	2.81	1.15	2.03
8	1.90	2.01	3.98	2.58	2.20
10	2.15	2.38	2.48	2.38	2.82

APPENDIX 5. *E. coli* Survival in Estuarine Water Samples from Various Sites at Different Sampling Times (data for Figure 16).

Appendix 5.f. Season 2 (sampling date 7/11/78)

1. *E. coli* Survival — Log Number Organisms per ml

TIME (DAYS)	SITE 1	2	5	6	7
Autoclaved Estuarine Water					
0	8.08	7.99	8.00	8.00	8.07
2	7.79	7.68	7.68	7.74	7.71
4	7.79	7.88	7.57	7.58	7.60
6	7.50	7.38	7.23	7.26	7.13
8	7.66	7.54	7.16	6.95	7.01
10	7.55	7.28	6.82	6.71	6.56
Natural Estuarine Water					
0	8.40	7.98	7.93	7.93	8.01
2	6.06	7.70	7.65	7.57	7.23
4	4.39	6.82		7.44	6.25
6	3.79	5.06	4.48		
8	3.39	3.27	2.00	2.18	1.95
10	2.98	1.71	1.54	1.30	1.54

2. PFU Growth

TIME (DAYS)	SITE 1	2	5	6	7
0	0.00	0.00	0.00	0.00	0.00
2	1.34	1.08	1.15	1.26	1.08
4	1.51	1.62	2.52	2.60	2.20
6	2.15	4.76	4.29	3.90	3.45
8	2.34	5.56	3.92	2.73	3.45
10	2.73	4.00	3.08	2.62	1.48

APPENDIX 5. *E. coli* Survival in Estuarine Water Samples from Various Sites at Different Sampling Times (data for Figure 16).

Appendix 5.g. Season 1 (sampling date 4/12/78)

1. *E. coli* Survival — Log Number Organisms per ml

TIME (DAYS)	SITE 1	2	5	6	7
Autoclaved Estuarine Water					
0	8.16	8.19	8.22	8.29	8.16
2	8.06	8.09	8.06	7.93	7.97
4	8.05	7.99	7.97	7.75	7.84
6					
8	7.76	7.80	7.40	6.98	7.28
10	7.62	7.65	7.08	7.02	6.89
Natural Estuarine Water					
0	8.29	8.14	8.19	8.19	8.20
2	8.37	8.19	8.53	8.24	8.20
4	5.00	5.60	6.31	5.00	7.19
6					
8	3.10	3.10	2.83	2.40	3.07
10	3.01	2.53	1.42	1.97	2.74

2. PFU Growth

TIME (DAYS)	SITE 1	2	5	6	7
0	1.00	0.00	0.00	0.60	0.00
2	2.30	2.11			
4	3.09	3.45	3.80	3.30	4.00
6	3.24	2.34	3.26	2.30	3.37
8	2.81	2.18	2.64	3.30	2.88
10	3.38	2.20	2.15	2.30	3.20

APPENDIX 5. *E. coli* Survival in Estuarine Water Samples from Various Sites at Different Sampling Times (data for Figure 16).

Appendix 5.h. Season 1 (sampling date 10/1/79)

1. *E. coli* Survival — Log Number Organisms per ml

TIME (DAYS)	SITE 1	2	5	6	7
Autoclaved Estuarine Water					
0	8.10	8.19	8.06	8.14	8.11
2					
4	7.94	7.85	7.60	7.85	7.82
6	7.81	7.77	7.46	7.64	7.56
8	7.72	7.51	7.10	7.30	7.06
10	7.40	7.17	6.74	6.94	6.83
Natural Estuarine Water					
0	8.45	8.06	8.10	8.08	8.12
2					
4	4.98	7.11	<4.00	7.13	6.46
6	4.18	<4.00	<1.00	6.33	6.05
8	3.84	3.29		0.00	
10	3.41	2.07			1.49

2. PFU Growth

TIME (DAYS)	SITE 1	2	5	6	7
0	0.90	0.00		0.30	1.15
2					
4	2.88			2.94	2.83
6	2.53	3.72		2.92	2.73
8	3.56	3.36		3.56	2.79
10	3.60	3.30		3.59	2.60

APPENDIX 5. *E. coli* Survival in Estuarine Water Samples from Various Sites at Different Sampling Times (data for Figure 16).

Appendix 5.i. Season 1 (sampling date 13/2/79)

1. *E. coli* Survival — Log Number Organisms per ml

TIME (DAYS)	SITE 1	2	5	6	7
Autoclaved Estuarine Water					
0	8.30	8.21	8.18	8.14	8.09
2	7.97	8.03	7.99	8.00	7.93
4	8.04	8.10	8.16	7.89	7.53
6	7.73	7.69	7.50	7.20	7.11
8	7.65	7.51	7.13	6.65	6.72
10	7.52	7.38	6.85	6.61	6.66
Natural Estuarine Water					
0	8.12	8.08	8.13	8.20	8.16
2	7.98		8.35	7.94	7.83
4	7.53	5.40	5.60	5.65	
6	6.24	3.58	3.76	<2.00	4.57
8	<3.00	2.83	2.45	1.04	2.30
10	1.30	2.03	1.63	0.95	1.28

2. PFU Growth

TIME (DAYS)	SITE 1	2	5	6	7
0	0.00	0.00	0.00	0.00	0.30
2	2.41	1.96	3.21	2.00	1.78
4	2.96	2.30		3.03	
6	3.72	2.53	3.39	4.64	3.21
8	3.30	2.48	3.62	2.83	2.64
10	2.96	2.45	3.42	1.41	2.38

APPENDIX 5 (continued)

Appendix 5.j. Analysis of Variance (data for Figure 16)

SOURCE OF VARIATION	df	SS	MS	F
Treatments	14	58.9	4.207	3.825***
Error	29	31.901	1.100	
Total	43	90.801		

*** Significant at 0.1%.

APPENDIX 6. Effect of Bacterial and Protozoan Predators on *E. coli* Survival.

Appendix 6.a.1. *E. coli* Survival in Estuarine Water Samples (data for Figure 17a).

Log Number *E. coli* per ml

TIME (DAYS)	TREATMENT 1 AUTOCLAVED ESTUARINE WATER	2 AUTOCLAVED ESTUARINE WATER + CYCLOHEXIMIDE	3 NATURAL ESTUARINE WATER	4 NATURAL ESTUARINE WATER + CYCLOHEXIMIDE
REP 1				
0	8.06	8.05	8.03	8.15
2	8.01	8.05	7.64	7.92
4	7.93	7.99	5.01	7.72
6	8.01	8.00	3.18	5.70
8	7.60	7.84	2.19	4.95
10	7.63	7.64	0.78	4.04
REP 2				
0	8.05	8.07	8.03	8.15
2	8.03	8.00	7.53	7.90
4	7.85	7.98	5.00	7.78
6	8.00	8.03	3.18	6.85
8	7.63	7.83	2.35	5.30
10	7.64	7.57	1.15	3.98
REP 3				
0	8.05	8.03	7.99	8.04
2	7.96	8.00	6.85	7.83
4	7.89	7.91	5.00	7.74
6	7.99	8.03	3.18	7.37
8	7.64	7.74	2.08	5.81
10	7.60	7.58	0.00	4.33

Appendix 6.a.2. Analysis of Variance (after $\log_{10}$ transformation).

SOURCE OF VARIATION	df	SS	MS	VR
Between treatments	3	3.87257	1.29086	1394.7***
Residual	8	0.00740	0.000926	
Total	11	3.87997		

*** Significant at 0.1%.

The results of an l.s.d. test on the log transformed data can be summarised as follows:

Treatment	2	1	4	3	l.s.d. (.05)
	.4516	.4994	1.4552	1.7329	.9573

Appendix 6.a.3. Growth of PFU in Estuarine Water Samples (data for Figure 17b).

TIME (DAYS)	NATURAL ESTUARINE WATER (EW)	NATURAL ESTUARINE WATER + CYCLOHEXIMIDE
REP 1		
0	0.60	0.00
2	1.58	1.53
4	2.38	2.78
6	2.70	1.60
8	2.81	3.26
10	3.00	2.30
REP 2		
0	0.60	0.60
2	1.62	1.48
4	2.30	2.90
6	2.78	2.60
8	2.92	3.33
10	2.86	3.19
REP 3		
0	0.78	0.30
2	1.08	1.60
4	2.38	2.90
6	1.78	2.60
8	3.06	3.38
10	3.34	3.78

Appendix 6.b. Preliminary Experiment; Survival of *E. coli* and Growth of PFU in Estuarine Water Samples.

	LOG NUMBER *E. COLI* PER ML			
TIME (DAYS)	NATURAL EW	NATURAL EW + CYCLOHEXIMIDE	AUTOCLAVED EW	AUTOCLAVED EW + CYCLOHEXIMIDE
0	7.51	7.47	8.23	8.18
2	7.12	7.10	7.94	8.09
4	6.22	6.88	8.01	7.82
6	3.00	6.54	7.20	7.54
8	1.84	5.61	6.56	7.12
10	0.00	3.60	6.11	7.66

	LOG NUMBER PFU PER ML	
TIME (DAYS)	NATURAL EW	NATURAL EW + CYCLOHEXIMIDE
0	0.00	0.00
2	1.83	0.00
4	1.34	0.00
6	0.70	3.30
8	2.82	5.15
10	2.08	4.38

APPENDIX 7. Effect of Periodic Inhibition of Protozoans

Appendix 7.a.1. *E. coli* survival in estuarine water samples after periodic inhibition of protozoa (data for Figure 18 and Table 17).

TIME (DAYS)	LOG NO. *E. COLI* PER ML		LOG NO. OF *E. COLI* PER ML AT FOLLOWING TIME OF PROTOZOAN INHIBITION[a] (DAYS)						
	AUTOCLAVED ESTUARINE WATER	NATURAL ESTUARINE WATER	0	0.5	1.0	1.5	2.0	2.5	3.0
REP 1									
0	7.66	7.61	7.57						
0.5				7.54					
1.0	7.65	7.63	7.65	7.59	7.55				
1.5						7.61			
2.0	7.62	7.23	7.43	7.43	7.40	7.37	7.16		
2.5								6.98	
3.0	7.49	5.00	7.18	7.15	7.37	7.22	7.22	6.20	5.00
4.0	7.46	4.36	6.66	5.98	6.13	6.58	5.18	4.18	4.20
6.0	7.31	3.36	5.66	4.54	4.18	4.66	3.00	3.62	3.32
8.0	7.16	1.48	3.30	2.32	3.18	2.18	1.00	0.00	1.30
10.0	6.96	0.78	2.45	1.85	1.78	1.70	0.00		0.00
REP 2									
0	7.61	7.55	7.58						
0.5				7.57					
1.0	7.66	7.62	7.61	7.56	7.47				
1.5						7.48			
2.0	7.58	7.38	7.40	7.37	7.43	7.41	7.48		
2.5								7.33	
3.0	7.45	5.70	7.30	7.25	7.55	7.39	7.47	7.19	5.81
4.0	7.39	4.43	6.48	6.28	6.72	6.87	6.72	6.72	4.65
6.0	7.41	3.34	4.30	4.08	5.08	4.92	3.86	3.70	3.18
8.0	7.15	1.85	3.30	2.00	3.18	2.00	1.70	1.30	1.00
10.0	6.86	0.60	2.23	1.95	1.43	1.60	0.00	0.00	0.00
REP 3									
0	7.54	7.57	7.52						
0.5				7.53					
1.0	7.66	7.60	7.62	7.58	7.53				
1.5						7.55			
2.0	7.58	7.31	7.44	7.26	7.39	7.37	7.21		
2.5								7.27	
3.0	7.45	5.30	7.29	6.63	7.50	7.29	7.32	7.18	5.65
4.0	7.41	4.72	6.44	5.54	7.03	6.57	6.48	6.72	4.18
6.0	7.35	2.90	4.32	3.86	5.40	4.86	3.20	2.20	3.40
8.0	7.05	1.58	2.23	2.18	4.02	2.32	1.60	1.85	1.54
10.0	6.82	0.00	2.00	1.92	2.54	1.08	1.08	0.70	0.60

[a] Time of cycloheximide addition to sample.

Appendix 7.a.2. Growth of PFU in estuarine water samples after periodic inhibition of protozoa (data for Table 18).

TIME (DAYS)	LOG NO. OF PFU PER ML IN NATURAL ESTUARINE WATER	LOG NO. OF PFU PER ML AT FOLLOWING TIME OF PROTOZOAN INHIBITION[a] (DAYS)						
		0	0.5	1.0	1.5	2.0	2.5	3.0
REP 1								
0	0.00	1.00						
0.5			1.08					
1.0				1.78				
1.5					1.98			
2.0	1.89	2.35	2.37	2.27	2.22	1.48		
2.5							1.91	
3.0								2.48
4.0	2.34	3.08	2.60	3.27	2.90	2.20	2.53	2.75
6.0	2.60	3.53	3.27	2.08	2.79	2.48	2.60	2.66
8.0	2.72	3.51	3.18	3.39	3.35	3.02	3.01	3.05
10.0	2.60	3.21	3.00	3.43	3.33	3.27	2.76	2.26
REP 2								
0	1.30	1.20						
0.5			1.00					
1.0				1.56				
1.5					1.82			
2.0	1.91	1.72	2.06	2.31	2.14	1.89		
2.5							1.60	
3.0								2.34
4.0	2.64	2.90	3.37	2.85	2.96	2.99	2.58	2.66
6.0	2.73	3.35	3.78	2.75	2.73	3.21	2.70	2.72
8.0	3.16	3.74	3.65	3.14	3.20	2.96	2.99	2.81
10.0	2.92	3.39	3.44	3.03	3.52	3.57	3.01	2.68
REP 3								
0	1.08	0.90						
0.5			1.00					
1.0				2.03				
1.5					1.79			
2.0	1.38	1.66	1.68	1.72	1.78	1.87		
2.5							1.70	
3.0								2.51
4.0	2.51	2.11	3.38	2.88	3.16	2.96	3.02	2.45
6.0	2.78	3.71	3.47	3.30	2.79	2.60	3.08	2.76
8.0	3.00	3.66	3.08	3.23	3.70	3.38	3.26	3.07
10.0	2.30	3.19	3.15	3.48	3.30	3.43	2.66	2.34

[a] Time of cycloheximide addition to sample

APPENDIX 7.b.1. Initial *E. coli* concentration 10^8 organisms per ml - *E. coli* survival.

TIME (DAYS)	LOG NO. *E. COLI* PER ML		LOG NO. OF *E. COLI* PER ML AT FOLLOWING TIME OF PROTOZOAN INHIBITION[a] (DAYS)						
	AUTOCLAVED ESTUARINE WATER	NATURAL ESTUARINE WATER	0	0.5	1.0	1.5	2.0	2.5	3.0
0	7.76	7.88	7.82						
0.5	7.72	7.61		7.78					
1.0	7.66	7.79	7.84		7.76				
1.5	7.62	7.72		7.74		7.59			
2.0	7.30	7.52	7.08		7.18		7.38		
2.5	7.26	7.16		7.11		6.80		7.16	
3.0	6.62	6.02	6.05		5.87		4.54		6.05
3.5	7.04	5.02	5.67	5.45	4.22	4.42	3.08		3.00
4.5	6.51	2.34	5.00	4.79	3.34	2.99	2.54	2.75	2.06
5.5	6.60	2.36	4.42	3.53	2.42	2.37	2.26	2.27	2.06
6.5	6.45	2.29	3.75	2.70	2.27	2.02	1.75	1.72	1.49
7.5	6.46	2.32	3.35	2.34	2.14	2.03	1.93	1.83	1.62
8.5	6.42	2.29	2.98	2.26	1.90	1.90	1.43	1.43	1.46
10.0	6.42	0.00	2.52	1.90	1.30	1.30	0.00	0.00	0.00

[a]Time of cycloheximide addition to sample.

Appendix 7.b.2. Initial *E. coli* concentration 10^8 organisms per ml - PFU growth (data for Figure 19).

TIME (DAYS)	LOG NO. PFU PER ML		LOG NO. OF PFU PER ML AT FOLLOWING TIME OF PROTOZOAN INHIBITION[a] (DAYS)						
	NATURAL ESTUARINE WATER + CYCLOHEXIMIDE	NATURAL ESTUARINE WATER	0	0.5	1.0	1.5	2.0	2.5	3.0
0	0.90	1.00	1.00						
0.5	0.90	1.00	0.78	1.30					
1.0	2.48	2.56	0.78		2.05				
1.5	3.23	3.26		1.96		2.81			
2.0	3.91	4.31	2.92		3.26		4.08		
2.5	3.00	3.20		2.60		3.00		3.48	
3.0	2.60	4.27	3.96		3.03		2.94		3.68
3.5	3.15	3.38	4.36	3.20	4.31	2.82	3.66	3.60	3.19
4.5	2.16	3.20	4.74	4.72	4.67	4.04	2.26	4.30	3.60
5.5	1.68	2.81	2.90	4.69	4.71	3.91	3.91	3.73	3.26
6.5	1.00	2.99	1.34	4.15	4.25	3.60	3.00	2.89	3.11
7.5	2.62	2.85		3.68	2.30		2.08	2.64	2.30
8.5	1.00	2.82	2.34	3.19	2.60	2.08	2.26	3.06	2.64
10.0	0.30	2.08	2.42	3.44	2.30	2.00	2.34	2.20	2.60

[a]Time of cycloheximide addition to sample.

APPENDIX 7.c.1. Initial *E. coli* concentration 10^5 organisms per ml - *E. coli* survival.

TIME (DAYS)	LOG NO. *E. COLI* PER ML		LOG NO. OF *E. COLI* AT FOLLOWING TIME OF PROTOZOAN INHIBITION[a] (DAYS)						
	AUTOCLAVED ESTUARINE WATER	NATURAL ESTUARINE WATER	0	0.5	1.0	1.5	2.0	2.5	3.0
0	5.23	5.30	5.32						
0.5				5.31					
1.0	5.13	5.05	5.15	5.13	5.11				
1.5						5.13			
2.0	5.05	5.10		5.11	5.10	5.14	5.12		
2.5								5.06	
3.0	4.88	4.58	5.06	5.03	4.96	5.19	5.03	5.00	4.70
4.0	4.92	3.51	4.91	4.64	4.71	3.62	3.69	3.45	3.80
5.0	4.58	2.20	4.18	3.00	3.28	0.30	0.00	0.30	0.00
6.0	4.51	0.78	3.51	1.42	1.89	0.00		0.00	
7.0	4.28	0.00	2.27	1.30	0.00				
8.0	4.01		0.90	0.00					

[a]Time of cycloheximide addition to sample.

APPENDIX 7.c.2. Initial *E. coli* concentration 10^5 organisms per ml - PFU growth (data for Figure 20).

TIME (DAYS)	LOG NO. OF PFU PER ML		LOG NO. OF PFU PER ML AT FOLLOWING TIME PROTOZOAN INHIBITION[a] (DAYS)						
	NATURAL ESTUARINE WATER + CYCLOHEXIMIDE	NATURAL ESTUARINE WATER	0	0.5	1.0	1.5	2.0	2.5	3.0
0	0.00	0.30	0.30						
0.5				0.78					
1.0	0.00	1.00	0.60	0.60	0.30				
1.5						1.00			
2.0	1.34	1.48	1.30	0.78	0.78	1.85	1.91		
2.5								1.66	
3.0		1.75	2.34	2.00	1.62	2.23	1.94	2.26	1.00
4.0	0.78	1.72	2.60	2.53	2.56	2.84	2.58	3.03	1.78
5.0		1.26	3.16	3.17	3.10	3.47	3.62	3.62	2.72
6.0	0.78	1.38	3.34	3.43	3.26	3.33	3.64	3.42	3.08
7.0		1.28	3.27	3.16	2.87	3.13	3.45	3.23	2.64
8.0	0.78	1.15	3.11	3.03	2.48	2.68	2.98		2.64
10.0	0.30	0.90	2.91	2.75	3.05	2.53	3.18	2.78	2.34

[a] Time of cycloheximide addition to sample.

APPENDIX 8. Effect of initial *E. coli* concentration on *E. coli* survival

Appendix 8.a. Experiment 1: *E. coli* survival (data for Figure 21)

LOG NO. *E. COLI* PER ML

TIME	INITIAL *E. COLI* CONCENTRATION							
(DAYS)	1	2	3	4	5	6	7	8
0	2.53	2.78	4.12	6.12	7.38	7.72	8.74	9.40
1	1.90	2.18	3.02	4.00	5.22	6.30	7.22	8.66
2		1.76	2.12	2.85	4.11	5.29	6.64	8.32
3	1.64	0.00	0.70	1.53	3.28	3.56	5.38	7.64
4	0.00		0.00	2.15	1.81	2.82	4.35	6.40
6				0.00	0.48	1.36	3.70	4.45
8					0.00	0.48	3.12	
10						0.00	2.11	3.24

APPENDIX 8.b.1. Experiment II: *E. coli* survival (data for Figure 22a)

NATURAL ESTUARINE WATER. LOG NO. *E. COLI* PER ML

TIME	INITIAL *E. COLI* CONCENTRATION							
(DAYS)	1	2	3	4	5	6	7	8
0	2.66	3.68	4.60	5.62	6.17	7.34	8.16	9.48
1	2.22	3.22		5.02				
2	1.65	2.53	3.38	4.15	3.48	5.56	7.60	8.66
3	1.00	1.60	2.75	3.27				
4	0.00	0.00	1.88	2.51	2.40	4.70	5.00	8.05
6			0.78	0.90	1.54	1.81	3.52	7.25
8			0.00	0.00	0.70	0.00	2.15	6.86
10					0.00		0.60	6.68

NATURAL ESTUARINE WATER + CYCLOHEXIMIDE.

0	2.68	3.69	4.62	5.64	6.23	7.19	8.19	9.18
1	2.47	3.63	4.49	5.48				
2	2.51	3.48	4.56	5.15	6.00	6.66	7.43	9.08
3	2.13	3.34	4.27	5.06				
4	2.02	3.26	4.29	5.00	5.16	6.16	7.15	8.60
6	1.46	2.43	3.33	4.69	4.30	5.59	5.76	7.78
8	0.95	1.28	1.78	4.30	3.10	4.70	4.42	7.54
10	0.00	0.00	0.00	3.73	3.32	4.16	3.29	7.28

APPENDIX 8.b.2. Experiment II: Growth of PFU (data for Figure 22b)

TIME (DAYS)	LOG NO. PFU PER ML — INITIAL *E. COLI* CONCENTRATION							
	1	2	3	4	5	6	7	8
NATURAL ESTUARINE WATER								
0	1.00	1.00	1.00	1.00	0.60	0.60	0.60	0.60
1	1.00		1.30	0.60				
2	1.45	1.30	1.30	1.90	0.78	1.57	2.30	2.15
3	2.34	1.72	2.20	2.38				
4	2.76	2.48	2.48	2.08	1.82	2.82	4.74	3.08
6	3.07	2.20	2.75	2.04	2.15	1.85	3.30	4.02
8	2.89	1.94	1.73	1.60	1.77	1.85	3.23	5.04
10	1.95	3.03	2.40	1.60	1.87	2.04	3.00	5.06
NATURAL ESTUARINE WATER + CYCLOHEXIMIDE								
0	1.00	1.00	1.00	1.00	0.60	0.60	0.60	0.60
2							0.48	2.38
4					1.48	1.34	2.20	3.72
6	0.78	2.30	1.64	3.66	2.73	2.73	3.16	5.52
8	0.30	2.53	1.89	3.20	2.72	3.18	3.71	5.68
10	0.30	2.43	0.90	3.33	2.51	2.90	3.48	5.48

APPENDIX 8.b.3. Control Samples: Autoclaved estuarine water.

TIME (DAYS)	LOG NO. *E. COLI* PER ML	
	CONTROL 1	CONTROL 2
0	5.64	9.20
2	5.26	9.15
4	5.00	8.77
6	4.33	8.18
8	4.15	8.02
10	4.00	8.18

APPENDIX 8.c.1. Experiment III: *E. coli* survival.

LOG NO. *E. COLI* PER ML

TIME	INITIAL *E. COLI* CONCENTRATION							
(DAYS)	1	2	3	4	5	6	7	8
NATURAL ESTUARINE WATER								
0	2.40	3.40	4.34	5.37	6.25	7.32	8.32	9.19
2	2.15	3.02	3.89	4.89	5.90	6.47	7.44	8.85
NATURAL ESTUARINE WATER + CYCLOHEXIMIDE								
0	2.41	3.43	4.40	5.38	6.35	7.32	8.27	9.26
2	2.17	2.43	4.21	5.20	6.14	7.08	8.20	8.93

APPENDIX 8.c.2. Experiment III: Growth of PFU.

LOG NUMBER PFU PER ML

TIME	INITIAL *E. COLI* CONCENTRATION							
(DAYS)	1	2	3	4	5	6	7	8
NATURAL ESTUARINE WATER								
0	0.30	0.30	0.60	0.60	0.30	1.00	1.00	0.60
6	1.64	1.50	2.20	1.34	-	1.34	2.73	2.00
8	2.20	1.89	2.50	-	1.48	2.00	3.16	2.66
10	1.48	2.18	2.48	1.48	1.70	1.50	3.48	4.85
NATURAL ESTUARINE WATER + CYCLOHEXIMIDE								
0	1.00	0.60	1.00	1.00	0.60	0.60	0.30	0.30
6	1.45	2.04	1.82	1.50	1.85	1.57	1.85	2.15
8		2.78	1.89	2.78	3.00	2.30	2.00	4.18
10	1.60	2.50	1.77	2.70	3.00	3.08	3.38	2.53

APPENDIX 9. Effect of Diffusible Substances on *E. coli* Survival.

Appendix 9.a. Natural Estuarine Water (data for Figure 23)

LOG NO. *E. COLI* PER ML

TIME (DAYS)	CENTRAL DIFFUSION RESERVOIR	GROWTH CHAMBER 1[a]	GROWTH CHAMBER 2[a]	GROWTH CHAMBER 3[b]
0	8.18	8.29	8.28	8.30
2	7.09	8.04	7.92	8.13
4		7.38	6.87	7.78
6	4.06	6.44	7.18	7.48
8	3.25	7.00	6.72	7.35
10	3.00	6.80	6.40	6.97
12	2.18	6.52	6.26	6.70
14	1.95	6.34	5.93	6.47
16	1.94	6.06	5.59	6.33

[a]Separated from control diffusion reservoir by 0.1μ filter.

[b]Control chamber.

Appendix 9.b. Natural estuarine water + cycloheximide (data for Figure 24).

LOG NO. *E. COLI* PER ML

TIME (DAYS)	CENTRAL DIFFUSION RESERVOIR	GROWTH CHAMBER 1[a]	GROWTH CHAMBER 2[a]	GROWTH CHAMBER 3[b]
0	8.15	8.20	8.23	8.20
2	7.97	8.21	8.03	8.16
3	7.53	8.06	7.93	8.07
6	6.00	7.69	7.67	7.58
8	3.90	7.58	7.44	7.01
10	3.16	7.27	7.16	6.62
13	2.91	7.82	7.80	5.54
16	1.45	7.28	6.30	5.11

[a]Separated from central diffusion reservoir by 0.1μ filter.

[b]Control chamber.

APPENDIX 10. Effect of Individual Predators on the Survival of *E. coli* and *S. typhimurium*.

Appendix 10.a. *E. coli* predators EP3 and EP7 and *E. coli* prey (data for Figures 25a and 25b).

LOG NO. ORGANISMS PER ML

TIME (DAYS)	*E. COLI* SURVIVAL			PFU GROWTH	
	CONTROL	EP3	EP7	EP3	EP7
0	8.35	8.11	8.10	2.38	2.34
2	7.51	7.10	7.38	5.20	5.30
4	7.54	5.79	5.69	5.83	5.65
6	7.46	5.32	5.19	4.42	4.87
8	6.98	4.53	4.68	4.30	4.48
10	6.63	3.80	3.98	4.34	4.15
12	6.86	3.54	3.45	3.72	3.51
14	6.82	3.05	3.00	4.76	4.76
16	6.61	2.60	2.82	4.20	3.78
18	6.37	2.57	2.74	-	2.95
20	6.32	2.06	2.12	3.85	3.21
22	6.13	2.01	2.05	3.26	2.91
24	6.31	2.50	2.06	3.19	2.89
26	6.31	2.60	1.62	3.00	2.99
30	6.28	2.78	0.48	3.37	2.89

APPENDIX 10.b. Combination of *E. coli* predators EP3 and EP7 (data for Figures 26a and 26b).

LOG NO. ORGANISMS PER ML

TIME (DAYS)	*E. COLI* SURVIVAL				PFU GROWTH		
	CONTROL	EP3	EP7	EP3 + EP 7	EP3	EP7	EP3 + EP7
0	7.39	7.74	7.74	7.76	0.00	1.58	1.68
3	7.09	6.91	6.94	6.98	4.60	-	-
6	7.01	5.06	5.32	5.06	-	-	-
10	6.43	3.66	3.93	3.84	5.30	5.39	5.34
13	6.20	3.52	3.65	3.60	5.45	5.72	5.78
17	6.18	3.78	3.55	3.71	5.25	5.15	5.18
20	6.16	4.37	3.75	4.24	4.49	4.93	4.99
24	6.20	4.99	4.23	4.71	3.68	3.88	4.66
27	6.31	4.96	4.62	4.64	3.64	3.30	4.33
31	6.34	4.85	4.93	4.48	3.87	3.60	4.33

APPENDIX 10.c. *E. coli* predators EP3 and EP7 and *S. typhimurium* Prey

Appendix 10.c.1. - Experiment 1. (Data for Figures 27a and 27b.)

LOG NO. ORGANISMS PER ML

TIME (DAYS)	*S. TYPHIMURIUM* SURVIVAL			PFU GROWTH			
				CONTROLS		TREATMENTS	
	CONTROL	EP3	EP7	EP3	EP7	EP3	EP7
0	8.44	8.46	8.47	0.90	1.20	0.30	1.30
3	8.54	8.44	8.48	-	1.42	3.01	4.29
6	8.69	8.45	8.18	1.42	2.26	-	-
9	8.48	8.29	8.24	1.72	2.82	-	-
13	8.12	7.85	7.71	1.30	2.66	5.89	5.77
16	7.96	7.37	7.35	1.75	3.23	5.60	5.66
20	7.26	6.20	5.54	1.68	3.27	5.56	5.50
23	7.10	4.93	4.52	-	3.38	5.60	5.37
27	6.68	3.64	3.58	1.68	3.04	5.19	5.05
30	6.65	3.36	3.21	1.82	2.91	4.72	4.75

Appendix 10.c.2. - Experiment 2.

LOG NO. ORGANISMS PER ML

TIME (DAYS)	*S. TYPHIMURIUM* SURVIVAL			PFU GROWTH	
				TREATMENTS	
	CONTROL	EP3	EP7	EP3	EP7
0	8.15	8.10	8.09	2.26	1.90
4	7.79	7.45	7.54	4.70	4.68
8	7.33	6.76	6.69	5.20	5.20
12	7.11	6.41	6.02	4.78	4.26
18	6.95	5.29	5.29	-	-
22	6.82	4.79	4.85	3.30	4.34
26	6.73	4.44	4.28	4.54	4.13
30	6.71	4.22	4.15	4.52	4.24

APPENDIX 10.d. *S. typhimurium* predators SP1 and SP6 and *S. typhimurium* Prey

10.d.1. - Experiment 1. (Data for Figures 28a and 28b.)

LOG NO. ORGANISMS PER ML

TIME (DAYS)	*S. TYPHIMURIUM* SURVIVAL			PFU GROWTH	
	CONTROL	SP1	SP6	SP1	SP6
0	8.20	8.14	7.94	0.30	1.81
2	8.18	8.15	8.14	2.07	3.78
4	8.18	8.16	8.06	4.26	4.25
6	8.18	7.91	7.93	4.76	4.20
8	8.16	7.81	7.46	5.30	4.87
10	8.08	7.14	7.25	5.16	5.11
12	8.03	6.89	6.99	5.46	5.23
14	7.76	6.31	6.69	5.41	5.02
17	7.64	5.61	6.00	4.87	5.21
20	7.47	4.89	5.42	4.98	5.03
23	7.19	4.61	4.86	4.59	5.19
27	6.95	4.38	4.32	4.16	4.82
32	6.87	4.37	4.48	3.79	4.53
38	6.95	4.41	4.70	3.52	4.33

APPENDIX 10.d.2. - Experiment 2.

LOG NO. ORGANISMS PER ML

TIME (DAYS)	*S. TYPHIMURIUM* SURVIVAL			PFU GROWTH	
	CONTROL	SP1	SP6	SP1	SP6
0	8.11	8.16	8.16	0.00	0.60
4	7.62	7.73	7.85	2.88	3.24
8	7.19	7.22	7.10	4.83	4.56
12	7.08	6.45	6.33	3.30	2.08
18	6.88	5.77	5.89	2.48	3.90
22	6.78	5.34	5.57	3.81	3.78
26	6.77	4.93	5.06	4.16	3.58
30	6.80	4.91	4.94	4.61	4.63

APPENDIX 10e. *S. typhimurium* predators SP1 and SP6 and *E. coli* prey (data for Figures 29a and 29b).

	LOG NO. ORGANISMS PER ML				
TIME (DAYS)	*E. COLI* SURVIVAL			PFU GROWTH	
	CONTROL	SP1	SP6	SP1	SP6
0	8.17	8.16	8.17	0.90	0.00
3	8.11	8.08	8.10	2.82	0.90
6	7.99	7.48	7.97	5.35	4.23
10	7.62	6.12	6.16	5.81	5.01
13	7.30	5.63	6.03	5.72	5.05
16	7.05	5.12	5.46	4.83	5.07
20	6.63	5.18	5.10	4.51	4.91
24	6.31	4.99	4.81	4.35	4.45
30	5.61	5.29	4.34	3.93	4.30

APPENDIX 11. Survival of Test Bacteria Compared to *E. coli*.

APPENDIX 11.a. Survival of *E. coli* compared to *S. typhimurium* (data for Figure 30).

LOG NO. ORGANISMS PER ML

TIME (DAYS)	BACTERIAL SURVIVAL		PFU GROWTH	
	E. coli	*S. typhimurium*	*E. coli*	*S. typhimurium*
REP 1				
0	8.12	8.23	2.52	1.98
2	8.05	8.13	3.05	3.00
4	5.48	5.74	3.70	2.92
6	3.34	4.48	3.42	3.30
8	1.00	3.77	3.15	3.05
10	0.90	2.83	2.75	2.48
REP 2				
0	8.11	8.28	2.52	1.70
2	7.91	8.16	3.08	2.75
4	5.00	5.00	3.65	3.34
6	3.92	3.06	3.37	3.16
8	3.41	1.18	3.10	2.66
10	2.80	0.00	2.62	2.42
REP 3				
0	8.18	8.24	2.56	1.83
2	8.08	8.15	3.06	2.83
4	5.48	5.43	3.38	3.30
6	4.04	4.20	3.32	3.02
8	3.28	3.72	3.16	2.95
10	2.00	2.84	2.94	2.86

APPENDIX 11.b.1. Survival of test bacteria in estuarine water samples (data for Table 24).

TIME (DAYS)	LOG NO. BACTERIA PER ML			
	E. COLI	*E. AEROGENES*	*S. FAECIUM*	*K. PNEUMONIAE*
REP 1				
0	8.12	8.34	7.86	7.71
2	8.05	8.36	7.77	7.31
4	5.48	5.48	5.48	4.00
6	3.34	4.38	3.23	2.04
8	1.00	3.32	2.00	0.00
10	0.90	2.00	2.77	
REP 2				
0	8.11	8.31	7.91	7.71
2	7.91	8.36	7.79	7.25
4	5.00	5.30	5.18	4.18
6	3.92	4.40	3.77	2.79
8	3.41	3.93	2.99	1.00
10	2.80	3.19	2.72	0.00
REP 3				
0	8.18	8.31	8.01	7.70
2	8.08	8.32	7.64	7.39
4	5.48	5.70	5.04	4.00
6	4.04	4.70	3.37	1.00
8	3.28	4.08	2.84	0.00
10	2.00	3.19	2.53	

APPENDIX 11.b.2. Growth of PFU in estuarine water samples (data for Table 25).

TIME (DAYS)	LOG NO. PFU PER ML		
	E. COLI	*E. AEROGENES*	*K. PNEUMONIAE*
REP 1			
0	2.52	2.47	2.52
2	3.05	2.86	2.81
4	3.70	3.40	3.43
6	3.42	3.42	-
8	3.15	-	3.18
10	2.75	2.79	-
REP 2			
0	2.52	2.28	2.56
2	3.08	-	2.82
4	3.65	3.25	3.54
6	3.37	-	2.98
8	3.10	2.26	2.94
10	2.62	-	3.08
REP 3			
0	2.55	2.32	2.35
2	3.06	2.66	2.66
4	3.38	3.53	3.64
6	3.32	3.58	2.87
8	3.16	2.53	3.36
10	2.94	3.02	2.97

APPENDIX 11.b.3. Survival of test bacteria and growth of PFU in estuarine water samples (preliminary experiment).

E. aerogenes. LOG. NO. ORGANISMS PER ML

TIME (DAYS)	AUTOCLAVED ESTUARINE WATER		NATURAL ESTUARINE WATER			
	BACTERIAL SURVIVAL		BACTERIAL SURVIVAL		PFU GROWTH	
	E. COLI	*E. AEROGENES*	*E. COLI*	*E. AEROGENES*	*E. COLI*	*E. AEROGENES*
0	8.41	8.18	8.45	8.15	0.30	0.00
2	8.24	7.91	8.22	7.83	3.51	3.46
5	7.92	7.63	5.65		3.18	3.00
6	7.98	7.30	5.36	3.62	3.00	2.30
8	7.90	7.20	3.33	3.04	2.64	2.45
10	7.79	7.09	1.75	2.73	2.00	2.08

S. faecium. LOG. NO. ORGANISMS PER ML

TIME (DAYS)	AUTOCLAVED ESTUARINE WATER		NATURAL ESTUARINE WATER		
	BACTERIAL SURVIVAL		BACTERIAL SURVIVAL		PFU GROWTH
	E. COLI	*S. FAECIUM*	*E. COLI*	*S. FAECIUM*	*E. COLI*
0	8.05	7.60	8.00	7.76	1.76
2	7.84	7.68	7.84	7.82	2.48
4	7.74	7.50	5.81	7.23	2.51
6	7.64	7.48	3.78	5.76	2.76
8	7.65	7.31	2.41	4.85	2.73
11	7.65	7.46	1.04	3.72	3.19

K. pneumoniae. LOG. NO. ORGANISMS PER ML

TIME (DAYS)	AUTOCLAVED ESTUARINE WATER		NATURAL ESTUARINE WATER			
	BACTERIAL SURVIVAL		BACTERIAL SURVIVAL		PFU GROWTH	
	E. COLI	*K. PNEUMONIAE*	*E. COLI*	*K. PNEUMONIAE*	*E. COLI*	*K. PNEUMONIAE*
0	8.05	7.97	8.15	7.90	1.08	0.78
2	7.20	7.38	7.37	5.74	2.61	2.31
4	7.71	7.26	4.04	2.69	3.68	3.51
6	7.64	7.05	3.64	2.06	2.60	2.43
8	7.16	6.60	2.93	0.85	1.90	1.78
10	6.94	6.12	2.50	0.00	2.20	1.30

APPENDIX 12. Bacterial Survival and Growth of PFU in the Presence of Alternative Prey Species.

Appendix 12.a.1. Bacterial survival of *E. coli* and *S. typhimurium* (data for Table 26).

LOG NO. BACTERIA PER ML

TIME (DAYS)	AUTOCLAVED ESTUARINE WATER		NATURAL ESTUARINE WATER		NATURAL ESTUARINE WATER + CYCLOHEXIMIDE	
	E. coli	*S. typhimurium*	*E. coli*	*S. typhimurium*	*E. coli*	*S. typhimurium*
REP 1						
0	7.62	8.00	7.47	7.88	7.49	7.86
2	7.50	7.94	7.48	7.76	7.28	7.82
4	7.13	7.89	-	-	6.85	7.35
6	7.22	7.37	4.41	4.27	6.54	6.74
8	7.22	7.56	4.02	4.05	6.10	6.16
10	7.15	7.36	2.90	3.62	5.50	5.31
13	6.92	6.95	1.40	2.54	3.52	2.48
15	6.66	6.80	<1.00	2.11	2.91	2.97
REP 2						
0	7.48	7.98	7.57	7.87	7.56	7.87
2	7.45	7.99	7.34	7.78	7.48	7.86
4	7.28	7.81	5.00	5.00	6.90	7.00
6	7.23	7.69	3.88	4.64	6.66	7.13
8	7.18	7.50	3.33	4.21	6.06	5.41
10	7.08	7.46	2.81	3.34	5.48	5.27
13	6.83	7.05	1.40	2.23	3.62	3.32
15	6.75	6.91	<1.00	1.70	2.51	2.76

APPENDIX 12.a.2. Growth of PFU - *E. coli* and *S. typhimurium* (data for Table 27).

TIME (DAYS)	LOG NO. PFU PER ML NATURAL ESTUARINE WATER	NATURAL ESTUARINE WATER + CYCLOHEXIMIDE
REP 1		
0	0.60	0.30
2	2.81	1.60
4	3.31	3.01
6	2.97	3.00
8	2.81	3.18
10	3.02	2.87
13	3.26	2.58
15	3.08	2.87
REP 2		
0	0.30	0.00
2	3.10	2.15
4	3.00	-
6	2.81	2.00
8	3.05	3.79
10	3.38	3.45
13	3.51	3.03
15	2.45	2.70

APPENDIX 12.b.1. Bacterial Survival - *E. coli* and *K. pneumoniae* (data for Table 28).

TIME (DAYS)	LOG NO. BACTERIA PER ML					
	AUTOCLAVED ESTUARINE WATER		NATURAL ESTUARINE WATER		NATURAL ESTUARINE WATER + CYCLOHEXIMIDE	
	E. coli	*K. pneumoniae*	*E. coli*	*K. pneumoniae*	*E. coli*	*K. pneumoniae*
REP 1						
0	8.23	8.04	8.16	7.95	8.04	7.81
2	8.13	7.89	8.12	7.80	8.09	7.99
4	7.98	7.75	<5.00	5.00	7.67	7.60
6	7.88	7.68	3.70	4.00	5.30	<5.00
8	7.30	7.77	3.33	3.34	4.04	3.00
10	7.05	7.74	2.63	2.08	3.62	2.40
REP 2						
0	8.27	7.98	8.13	7.89	8.14	7.92
2	8.13	7.85	8.00	7.74	8.10	7.94
4	7.88	7.76	<5.00	<5.00	7.93	7.82
6	7.84	7.80	3.85	4.06	6.62	5.30
8	7.40	7.57	3.08	3.03	4.69	<3.00
10	7.08	7.29	2.22	1.60	3.74	<1.00

APPENDIX 12.b.2. Growth of PFU - *E. coli* and *K. pneumoniae* (data for Table 29).

TIME (DAYS)	NATURAL ESTUARINE WATER	NATURAL ESTUARINE WATER + CYCLOHEXIMIDE
REP 1		
0	1.15	0.60
2	2.24	1.92
4	2.30	4.05
6	2.48	4.12
8	2.70	3.39
10	2.30	3.18
REP 2		
0	0.30	0.60
2	1.96	1.94
4	3.81	2.30
6	3.09	2.56
8	3.35	2.48
10	3.87	2.82

APPENDIX 12.c. Bacterial Survival - *E. coli* and *S. faecium* (data for Table 30).

LOG NO. BACTERIA PER ML

TIME (DAYS)	AUTOCLAVED ESTUARINE WATER		NATURAL ESTUARINE WATER		NATURAL ESTUARINE WATER + CYCLOHEXIMIDE	
	E. coli	*S. faecium*	*E. coli*	*S. faecium*	*E. coli*	*S. faecium*
REP 1						
0	8.14	8.00	8.11	8.04	8.06	8.00
2	7.98	8.18	7.35	6.30	7.72	8.04
4	7.81	7.92	5.33	-	6.31	5.48
6	7.59	7.40	4.23	3.93	5.99	5.00
8	7.51	7.36	2.97	2.30	5.97	4.40
10	7.16	7.50	2.42	2.30	4.32	4.00
REP 2						
0	8.17	7.85	8.08	7.95	8.14	7.88
2	7.97	8.15	7.11	6.40	7.45	6.00
4	7.88	7.87	5.36	5.00	6.20	5.30
6	7.74	7.36	4.10	3.95	6.03	5.30
8	7.46	7.45	3.36	3.00	6.20	4.74
10	7.17	7.32	2.72	2.30	4.88	4.00

APPENDIX 13. Effect of Temperature on Bacterial Survival.

APPENDIX 13.a. *E. coli* survival (data for Figure 31).

LOG NO. *E. COLI* PER ML

TIME (DAYS)	TEMPERATURE (^{o}C)									
	5		10.2		14.5		19.8		24.1	
	REP 1	REP 2	REP 1	REP 2	REP 1	REP 2	REP 1	REP 2	REP 1	REP 2
0	7.52	7.53	7.45	7.33	7.52	7.41	7.51	7.38	7.47	7.41
2	7.36	7.54	7.10	6.98	6.89	6.54	5.00		5.70	
4	7.00	6.99	5.91	4.48	4.28	4.30	3.08	3.28		3.16
6	6.72	6.27	4.00	4.11	2.30			1.00	0.00	1.76
8	5.89	5.67	3.01	1.65			0.00	0.00		2.29
10	3.82	3.69	2.04	0.60	0.30	1.54				0.70

APPENDIX 13.b. Survival of *E. coli* and *S. typhimurium* at different temperatures in autoclaved estuarine water samples (data for Table 31).

LOG NO. BACTERIA PER ML

TIME (DAYS)	TEMPERATURE (°C)									
	5.0		9.5		14.0		18.5		24.0	
	E. coli	*S. typhimurium*	*E. coli*	*S. typhimurium*	*E. coli*	*S. typhimurium*	*E. coli*	*S. typhimurium*	*E. coli*	*S. typhimurium*
0	8.59	8.54	8.46	8.56	8.57	8.48	8.55	8.51	8.52	8.51
2	8.47	8.52	8.56	8.55	8.58	8.48	8.52	8.48	8.45	8.51
4	8.70	8.62	8.66	8.53	8.81	8.57	8.89	8.51	8.72	8.37
6	8.38	8.39	8.34	8.33	8.41	8.39	8.48	8.21	8.27	8.15
8	8.56	8.36	8.51	8.44	8.59	8.36	8.46	8.14	8.34	7.96
10	8.41	8.42	8.41	8.38	8.53	8.30	8.37	8.07	8.27	7.89

APPENDIX 13.c.1. Survival of *E. coli* and *S. typhimurium* at different temperatures in natural estuarine water samples. Bacterial survival - Experiment 1. (Data for Figures 32a, 33a and 34.)

LOG NO. BACTERIA PER ML

TIME (DAYS)	AUTOCLAVED ESTUARINE WATER		NATURAL ESTUARINE WATER									
	15.3°C		6.2°C		10.3°C		15.3°C		20.6°C		26.9°C	
	E. coli	*S. typhimurium*	*E. coli*	*S. typhimurium*	*E. coli*	*S. typhimurium*	*E. coli*	*S. typhimurium*	*E. coli*	*S. typhimurium*	*E. coli*	*S. typhimurium*
0	8.63	8.64	8.66	8.58	8.62	8.53	8.67	8.54	8.64	8.57	8.63	8.60
2	8.79	8.74	8.79	8.71	8.64	8.72	8.65	8.72	7.28	7.30	7.23	-
4	8.80	8.75	8.81	8.64	7.90	8.55	6.31	5.48	5.10	4.30	4.60	3.46
6	8.38	8.47	8.14	8.37	7.26	5.00	4.60	4.06	3.48	3.19	3.04	2.84
8	8.59	8.63	7.90	7.69	5.85	3.92	3.88	3.66	3.30	3.10	1.71	2.77
10	8.48	8.73	6.10	5.54	4.54	4.64	3.43	3.45	3.86	2.47	2.16	2.34

APPENDIX 13.c.2. Survival of *E. coli* and *S. typhimurium* at different temperatures in natural estuarine water samples. PFU growth - Experiment 1. (Data for Figures 32b and 33b and Table 32.)

LOG NO. ORGANISMS PER ML (TOTAL PFU[a])

TIME (DAYS)	TEMPERATURE (°C)									
	6.2		10.3		15.3		20.6		26.9	
	E. coli	*S. typhimurium*	*E. coli*	*S. typhimurium*	*E. coli*	*S. typhimurium*	*E. coli*	*S. typhimurium*	*E. coli*	*S. typhimurium*
0	1.15	0.78	1.38	1.20	1.15	1.00	0.30	0.78	0.78	1.30
2	1.26	1.15	1.64	1.42	1.53	1.26	2.08	1.45	-	-
4	1.30	1.56	1.90	1.62	2.45	2.30	-	3.02	-	3.00
6	0.90	1.45	1.30	1.68	1.60	2.64	3.00	3.09	-	2.88
8	1.08	1.60	-	1.88	2.60	-	3.26	3.30	3.88	2.84
10	0.60	0.78	1.26	2.30	3.73	3.98	2.87	3.04	2.53	3.58

[a]Bacterial and protozoan predators.

LOG NO. ORGANISMS PER ML (BACTERIAL PREDATORS)

TIME (DAYS)	TEMPERATURE (°C)									
	6.2		10.3		15.3		20.6		26.9	
	E. coli	*S. typhimurium*	*E. coli*	*S. typhimurium*	*E. coli*	*S. typhimurium*	*E. coli*	*S. typhimurium*	*E. coli*	*S. typhimurium*
0	0.30	1.08	0.30	0.60	0.00	0.30	0.00	0.78	0.00	1.08
2	0.00	0.60	0.90	1.08	1.56	0.90	1.60	1.20	3.11	2.85
4	1.26	1.00	1.30	1.45	1.42	1.48	1.30	2.05	3.25	2.83
6	-	0.90	<1.00	1.20	<1.00	0.90	-	0.78		2.08
8	0.78	1.00	<1.00	1.30	-	1.15	1.78	1.45	1.60	2.86
10	0.00	0.90	0.30	1.64	<1.00	2.15	-	2.30	-	3.51

APPENDIX 13.d.1. Survival of *E. coli* and *S. typhimurium* at different temperatures in natural estuarine water samples. Bacterial survival - Experiment 2.

LOG NO. BACTERIA PER ML

TIME (DAYS)	AUTOCLAVED ESTUARINE WATER		NATURAL ESTUARINE WATER									
	TEMPERATURE (°C)											
	15.2		5.5		10.2		15.2		20.5		26.8	
	E. coli	*S. typhimurium*	*E. coli*	*S. typhimurium*	*E. coli*	*S. typhimurium*	*E. coli*	*S. typhimurium*	*E. coli*	*S. typhimurium*	*E. coli*	*S. typhimurium*
0	8.01	8.53	7.96	8.39	7.98	8.44	7.96	8.47	7.88	8.40	8.02	8.43
2	7.85	8.43	7.66	8.41	7.82	8.44	7.88	8.33	7.84	8.32	7.49	7.41
4	7.77	8.17	7.36	8.21	7.81	8.26	7.61	8.03	5.40	5.40	4.65	-
6	7.63	8.22	7.15	8.22	7.62	8.00	5.81	-	4.44	3.02	3.18	3.57
8	7.68	8.61	7.00	8.03	5.72	-	3.88	4.46	3.08	3.26	2.81	2.85
10	7.69	7.98	6.44	8.11	4.72	3.20	3.00	3.06	2.43	3.04	2.28	2.95

APPENDIX 13.d.2. - PFU Growth - Experiment 2.

LOG NO. ORGANISMS PER ML (TOTAL PFU[a])

TIME (DAYS)	TEMPERATURE (°C)									
	5.5		10.2		15.2		20.5		26.8	
	E. coli	*S. typhimurium*	*E. coli*	*S. typhimurium*	*E. coli*	*S. typhimurium*	*E. coli*	*S. typhimurium*	*E. coli*	*S. typhimurium*
0	1.90	1.34	1.76	1.34	1.90	1.38	1.99	1.66	1.91	1.56
2	1.72	0.60	3.02	1.38	3.84	1.45	-	2.05	-	3.20
4	1.68	1.08	4.18	1.34	4.00	1.97	3.48	2.08	4.00	3.20
6	1.60	1.60	5.12	1.30	3.00	2.51	4.20	3.60	3.83	3.16
8	0.60	1.26	4.37	1.51	3.51	2.68	4.21	2.70	3.01	2.91
10	0.30	1.56	2.38	1.81	3.56	2.99	4.15	2.30	3.79	3.03

[a]Bacterial and protozoan predators.

APPENDIX 14. Effect of Solar Radiation and Predacious Microorganisms on Bacterial Survival in Estuarine Water Samples.

APPENDIX 14.a.1. Effect of sunlight on *E. coli* survival.

(Data for Figure 35a)

LOG NO. BACTERIA PER ML

TIME (DAYS)	AUTOCLAVED ESTUARINE WATER		NATURAL ESTUARINE WATER	
	DARK	SOLAR RADIATION[a]	DARK	SOLAR RADIATION
REP 1				
0	8.78	8.58	8.57	8.67
1			8.86	8.86
2	8.40	8.07	8.30	8.13
4	8.04		5.65	5.00
5		3.63	3.70	1.00
6	7.30	3.29	3.40	0.30
8	7.70	3.12	3.03	0.00
10	7.57		2.64	
REP 2				
0	8.67	8.54	8.55	8.80
1			8.77	8.75
2	8.39	8.13	8.27	8.19
4	8.10	6.00	5.30	
5		3.11	3.78	1.00
6	7.95	2.91	3.66	0.30
8	7.90	2.64	3.45	0.00
10	7.77		3.19	
REP 3				
0	8.56	8.61	8.51	8.78
1			8.82	8.60
2	8.27	8.30	8.37	7.86
4	7.82	6.48	7.57	
5		3.95	4.78	1.30
6	7.78	3.65	4.07	0.60
8	7.86	3.64	3.32	
10	7.78		2.48	

[a] Total radiant exposure for duration of experiment, 628 cal cm^{-2}.

APPENDIX 14.a.2. Effect of sunlight on *E. coli* survival. Growth of PFU. (Data for Figure 35b.)

LOG NO. ORGANISMS PER ML

TIME (DAYS)	DARK		SOLAR RADIATION	
	TOTAL PFU[a]	BACTERIAL PFU	TOTAL PFU	BACTERIAL PFU
REP 1				
0	1.42	0.00	1.00	0.00
1	1.87	0.60	1.59	1.00
2	2.68	1.66	2.40	1.48
4		1.82		1.83
6	3.90		3.90	
8	3.89	2.03	2.86	1.53
10	3.26	2.48	2.89	1.60
REP 2				
0	1.08	0.00	1.20	0.30
1	1.88	0.00	1.70	0.30
2	2.60	0.60	2.35	1.73
4		2.22		1.58
6	3.85	1.38	3.82	1.85
8	3.86	2.15	2.68	1.98
10	2.05	2.26	2.20	2.20
REP 3				
0	1.08	0.00	1.26	0.30
1	1.76	0.00	1.91	0.00
2	2.46	1.72	2.45	1.26
4		3.08		1.42
6	3.90	1.42	3.68	2.10
8	3.90	2.11	3.34	2.11
10	3.27		2.58	2.08

[a] Bacterial and protozoan predators.

APPENDIX 14.b. Survival of *E. coli* in artificial light in estuarine water samples. (Data for Table 33.)

LOG NO. BACTERIA PER ML

TIME (DAYS)	AUTOCLAVED ESTUARINE WATER		NATURAL ESTUARINE WATER
	DARK	LIGHT	LIGHT
REP 1			
0	8.79	8.83	8.76
1		8.63	8.67
2	8.80	8.50	8.22
3		7.94	7.70
4	8.51	6.48	5.85
5		6.35	5.47
6	8.45	4.91	3.31
7	8.10	3.57	<1.00
REP 2			
0	8.88	8.80	8.82
1		8.65	8.67
2	8.77	8.51	8.20
3		8.03	7.87
4	8.45	6.79	6.02
5		6.50	5.47
6	8.45	5.12	4.02
7	8.27	3.38	<1.00
REP 3			
0	8.84	8.81	8.76
1		8.64	8.67
2	8.73	8.47	8.18
3		8.13	6.34
4	8.60	7.03	3.78
5		6.78	1.95
6	8.51	5.77	1.00
7	8.18	3.70	<1.00

APPENDIX 14.c.1. Comparative survival of test bacteria with *E. coli* in estuarine water samples exposed to solar radiation. Bacterial survival (Data for Table 34).

LOG NO. BACTERIA PER ML

TIME	*E. coli*		*S. typhimurium*	*S. faecium*	*K. pneumoniae*	*E. aerogenes*	*E. herbicola*
(DAYS)	SOLAR RADIATION 1 [a]	SOLAR RADIATION 2 [b]	SOLAR RADIATION 1	SOLAR RADIATION 1	SOLAR RADIATION 1	SOLAR RADIATION 2	SOLAR RADIATION 2
REP 1							
0	8.20	8.43	8.34	7.76	7.94	8.44	8.27
0.5	7.96		8.26	7.90	7.37		
1	7.27	6.68	8.10	7.69	6.60	6.94	6.20
2	6.07	2.85	6.65	5.78	3.00	3.18	4.53
3	1.00	0.00	4.49	4.49	<1.00	2.16	2.68
4			2.90	2.49			
REP 2							
0	8.28	8.38	8.41	7.87	8.06	8.52	8.34
0.5	7.99		8.34	7.87	7.38		
1	7.09	6.30	8.29	7.24	5.63	7.09	6.62
2	5.99	2.00	7.13	5.88		3.68	4.34
3		0.00	5.18	4.93	<1.00	2.11	2.65
4			2.65	1.60			
REP 3							
0	8.29	8.46	8.42	7.84	8.09	8.45	8.32
0.5	7.97		8.31	7.93	7.27		
1	6.92	6.71	8.26	7.46	5.35	7.07	6.74
2	5.91	3.04	7.27	6.16		3.43	4.30
3	1.78	0.00	5.11	4.04	<1.00	2.31	2.73
4			2.86				

[a] Total radiant exposure for duration of experiment 298 cal cm^{-2}.

[b] Total radiant exposure for duration of experiment 1,510 cal cm^{-2}.

APPENDIX 14.c.2. Comparative survival of test bacteria with *E. coli* in estuarine water samples exposed to solar radiation. PFU Growth (data for Table 35).

LOG NO. ORGANISMS PER ML

TIME (DAYS)	*E. coli*		*S. typhimurium*	*E. aerogenes*
	SOLAR RADIATION 1	SOLAR RADIATION 2	SOLAR RADIATION 1	SOLAR RADIATION 2
REP 1				
0	1.30	0.60	1.48	0.60
1	1.78		1.72	
2	1.94	2.11	2.00	2.15
3		2.33	2.30	2.06
4	2.34		2.12	
REP 2				
0	1.30	0.60	1.30	0.00
1	1.68		1.78	
2	2.09	1.78	1.85	2.02
3		2.35	2.15	2.05
4	2.26		2.00	
REP 3				
0	1.15	0.60	1.60	0.60
1	1.53		1.60	
2	2.06	1.90	1.82	2.00
3	2.16	2.23	2.08	2.25
4	2.30		2.00	

APPENDIX 14.d. Effect of radiant exposure on the survival of *E. coli* and *S. typhimurium* in natural estuarine water samples. (Data for Table 36.)

RADIANT EXPOSURE (PERCENT)	LOG NO. BACTERIA PER ML			
	E. coli		*S. typhimurium*	
	DAY 0	DAY 2	DAY 0	DAY 2
REP 1				
0	8.29	7.74	8.18	7.18
50	8.23	3.00	8.03	
100	8.13	2.00	8.09	
REP 2				
0	8.25	7.64	8.10	7.06
50	8.26	3.78	8.04	4.30
100	8.19	1.00	8.06	3.60
REP 3				
0	8.29	7.63	8.10	7.15
50	8.28	2.00	8.09	4.30
100	8.18		8.11	3.60

APPENDIX 14.e. Effect of radiant exposure on the survival of *E. coli* and *S. typhimurium* in autoclaved estuarine water samples (data for Table 37).

CUMULATIVE RADIATION (CAL CM^{-2})	NO. BACTERIA PER ML					
	E. coli			*S. typhimurium*		
	REP 1	REP 2	REP 3	REP 1	REP 2	REP 3
0	1.39×10^8	1.45×10^8	1.85×10^8	1.09×10^8	8.8×10^7	1.24×10^8
55.4	1.40×10^8	1.53×10^8	1.50×10^8	1.25×10^8	1.21×10^8	1.17×10^8
114.5	1.26×10^8	1.36×10^8	1.39×10^8	1.29×10^8	1.26×10^8	1.35×10^8
174.8	7.6×10^7	7.7×10^7	7.35×10^7	1.30×10^8	1.31×10^8	1.35×10^8
244.3	8.1×10^6	5.75×10^6	3.2×10^6	1.19×10^8	1.05×10^8	9.85×10^7
310.4	1.34×10^6	1.45×10^6	1.75×10^6	4.56×10^7	5.02×10^7	4.77×10^7
573.0	1.60×10^5	1.68×10^5	1.55×10^5	3.02×10^6	2.74×10^6	3.07×10^6
726.8	7.48×10^4	1.20×10^5	8.0×10^4	7.0×10^5	6.0×10^5	5.5×10^5

VIII. Publications

VIII. Publications

1. J. McCambridge and T.A. McMeekin.
   The effect of temperature on the activity of predators of *Salmonella typhimurium* and *Escherichia coli* in estuarine water.
   Aust. J. Mar. Freshwater Res. 6: 851-855.

2. J. McCambridge and T.A. McMeekin.
   Relative effects of bacterial and protozoan predators on survival of *E. coli* in estuarine water samples.
   Appl. Environ. Microbiol. 40: 907-911.

3. J. McCambridge and T.A. McMeekin.
   Effect of solar radiation and predacious microorganisms on survival of faecal and other bacteria.
   Appl. Environ. Microbiol. (in press).