

Sunlight inactivation of *Campylobacter jejuni* and *Salmonella enterica*, compared with *Escherichia coli*, in seawater and river water

Lester Sinton, Carollyn Hall and Robin Braithwaite

ABSTRACT

The inactivation of *Campylobacter jejuni* and *Salmonella enterica*, compared with *Escherichia coli*, was determined in 100l chambers of seawater and river water located at an outdoor site. The chambers (paired with dark controls) were seeded with waste stabilization pond effluent and laboratory-cultured pathogens, and exposed to sunlight in summer and winter experiments. All sunlight inactivation (k_S) rates, as a function of cumulative global solar radiation (insolation), were far higher than the corresponding dark (k_D) rates, with a ranking (and average k_S rates for seawater and river water, respectively) of: *C. jejuni* (3.23; 2.34) > *S. enterica* (0.51; 0.37) > *E. coli* (0.34; 0.26). All the T_{90} (time to 90% inactivation) values were higher in winter than in summer, but there was far greater similarity between the summer and winter S_{90} (insolation needed for 90% inactivation) values. The rapid inactivation of *C. jejuni* was attributed to a high susceptibility to photooxidative damage. The results suggest that, in sunlight-exposed waters, *E. coli* will be a more conservative indicator for *C. jejuni* than for *S. enterica*, and *C. jejuni* transmission as a pathogenic agent is less likely than for *S. enterica*.

Key words | *Campylobacter jejuni*, *Escherichia coli*, river water, *Salmonella enterica*, seawater, sunlight inactivation

Lester Sinton (corresponding author)
 Carollyn Hall
 Robin Braithwaite
 Institute of Environmental Science and Research,
 P.O. Box, 29-181, Christchurch,
 New Zealand
 Tel.: +64-3-351-6019
 Fax: +64-3-351-0010
 E-mail: lester.sinton@esr.cri.nz

INTRODUCTION

In shallow fresh and saline surface waters, the principal factor affecting the survival of enteric bacteria is the level of exposure to sunlight, although there is a contribution from factors such as starvation, grazing by heterotrophic nano-flagellates, temperature and salinity. Reviews of the effects of these factors include those of Harm (1980), Gameson & Gould (1985), Jagger (1985), Rozen & Belkin (2001), and Sinton (2005).

Most of the studies reviewed above involved enteric indicators. They suggest that, where the source is untreated sewage, fecal coliforms (including *E. coli*) are inactivated in sunlight more rapidly than enterococci, although this difference is less marked in fresh waters (Fujioka *et al.* 1981; Gameson 1986; Davies-Colley *et al.* 1994; Sinton *et al.*

1994, 1999, 2002). The opposite occurs when they are from a waste stabilization pond (WSP), because enterococci suffer irreparable sunlight damage in ponds, whereas fecal coliforms are rendered more sunlight resistant (Sinton *et al.* 2002).

There is comparatively little information available on sunlight inactivation of bacterial pathogens, such as *Salmonella* spp., and *Campylobacter* spp. Three field studies have demonstrated lower inactivation of *Salmonella* spp. compared with *E. coli* in surface waters (Rhodes & Kator 1988; Borrego *et al.* 1990; Mezrioui *et al.* 1995), although they did not include measurement of sunlight. Pommepuy *et al.* (1992) and Johnson *et al.* (1997) noted the bactericidal effect of sunlight on *Salmonella* spp. in marine waters, but did not

quantify the sunlight dose. Other experiments involving partially- or unquantified exposure to natural sunlight have produced conflicting results, with *S. enterica* being reported as both more (Davies & Evison 1991; Chandran & Hatha 2005) and less (McCambridge & McMeekin 1981) susceptible than *E. coli*.

Similarly, the effects of natural sunlight on *Campylobacter* spp. do not appear to have been quantified. In laboratory-based Petri dish microcosms, *C. jejuni* has been shown to be highly susceptible to UV-C (Butler *et al.* 1987) and UV-B (Obiri-Danso *et al.* 2001) radiation. Indirect evidence of sunlight effects was also presented by Obiri-Danso *et al.* (1999), who showed that *Campylobacter* was present in saline waters in the morning (presumably as a result of overnight entry) but absent in the afternoon after half a day of sunlight exposure. In a review of several estuarine studies, Jones (2001) also noted that both seasonal distribution and diurnal variation in *C. jejuni* counts showed significant correlations with patterns of sunlight exposure.

In this paper, we present the results of a two-year field study, designed to determine inactivation rates of *C. jejuni* and *S. enterica* in relation to sunlight “dose” (insolation), in seawater and river water, compared with *E. coli*. The pathogens were selected for study because both are important causes of gastroenteritis in New Zealand, with reported incidences being around 400 and 40 per 100,000 for *C. jejuni* and *S. enterica*, respectively (Anon 2003). Although contaminated food is probably an important transmission route, both pathogens have been detected in natural waters in New Zealand (McBride *et al.* 2002; Eyles *et al.* 2003), and water transmission is a suspected contributor to disease incidence (Stehr-Green *et al.* 1991; Simmons & Smith 1997).

A common feature of many survival studies is that they have involved inoculation of microcosms with pure cultures, in the absence of the fecal material in which they would normally enter natural waters. Accordingly, our study involved the use of WSP effluent seeded with *C. jejuni* and/or *S. enterica* prior to injection into large (100l) chambers. This effluent type was selected because WSPs are used in over 70% of New Zealand sewage treatment plants (Unpublished CoSINZ database, NZ Ministry of Health).

METHODS

Field work

The experiments were conducted at an outdoor research site at Lincoln, (latitude 43°S), near Christchurch, New Zealand. Seven experiments (Table 1) were conducted over two years. A modification of the approach described in Sinton *et al.* (1994, 1999, 2002) was used. The water was held in circular, plastic, open-topped, 120l chambers, lined with foil on the outside, and filled to a depth of 390 mm (100l per chamber). Except for experiment 5 (Table 1), each sunlight-exposed chamber was paired with a dark control. Each chamber contained two small bilge pumps, one continually stirring the water, and the other fitted with a plastic hose, and operated only for sample collection.

To regulate temperature, the chambers were placed in a 400l water jacket, which was continually stirred with a bilge pump and cooled with ice to maintain a chamber water temperature of 14°C. This temperature was selected because it is the mean annual temperature of both the fresh water and seawater collection sites – respectively, the LII River (a spring-fed stream with a mean flow of 0.9 m³ sec⁻¹) and the Lyttelton Harbour shoreline. This approach also removed temperature as an experimental variable.

For each experiment, 95l of water was pumped into each chamber and, on the hour after the water surface was first exposed to direct sunlight, 5l of WSP effluent (from the Christchurch sewage treatment plant) was added to give a 5% mixture. The effluent was first seeded with a laboratory-cultured mixture of six *C. jejuni* or six *S. enterica* strains (see below). The chamber contents were stirred for 3 min and the first sample was collected. For the *C. jejuni* analyses, subsequent samples were collected every 15 or 30 min for 3 h (in the summer and winter experiments, respectively), and on the hour for 8 h for *E. coli* and *S. enterica*. Samples were held in the dark at 6–8°C, and were transferred to the laboratory within 30 min.

Global (i.e. diffuse plus direct) solar radiation (GSR) was measured on site with a LI-COR LI-200SA pyranometer connected to a LI-COR LI-1000 datalogger. Chamber water temperatures were monitored with a digital thermometer. Dissolved oxygen levels were monitored with a TPS dissolved oxygen meter fitted with a YSI 5739 probe.

Table 1 | Summary of experimental program. All experiments were conducted over 8 daylight hours

Expt no.	Season, weather	Bacteria	Water type	Comments
1	Summer, clear sky all day	<i>C. jejuni</i> , <i>E. coli</i>	River water	15 min <i>C. jejuni</i> sampling interval for 2 h, followed by hourly sampling
2	Summer, some morning cloud	<i>C. jejuni</i> , <i>E. coli</i>	Seawater	15 min <i>C. jejuni</i> sampling interval for 2 h, followed by hourly sampling
3	Winter, high cloud all day	<i>S. enterica</i> , <i>E. coli</i>	River water	Hourly sampling
4	Winter, thin high cloud all day	<i>S. enterica</i> , <i>E. coli</i>	Seawater	Hourly sampling
5	Winter, partly cloudy all day	<i>C. jejuni</i> , <i>E. coli</i>	River water, seawater	30 min <i>C. jejuni</i> sampling interval for 2 h, followed by hourly sampling; No dark control*
6	Summer, morning cloud	<i>S. enterica</i> , <i>E. coli</i>	River water	Hourly sampling
7	Summer, morning cloud	<i>S. enterica</i> , <i>E. coli</i>	Seawater	Hourly sampling

*The dark control results from experiments 1 and 2 were used to adjust the sunlight inactivation data in experiment 5.

Preparation of pathogen seeds

E. coli was present in the WSP effluent in sufficiently high counts for the experiments (a median of 2.6×10^4 CFU 100 ml^{-1}), but *C. jejuni* and *S. enterica* counts (a median of $< 4 \text{ } 100 \text{ ml}^{-1}$ for each species) were too low for defining survival curves. Accordingly, the effluent was seeded with a laboratory-cultured mixture of either *C. jejuni* or *S. enterica*. Each seed was a mixture of six isolates from river water samples collected in a microbiological survey of New Zealand rivers (McBride *et al.* 2002). The isolates were confirmed as *C. jejuni* and *S. enterica* (subsp. *enterica* serovar Brandenburg) by polymerase chain reaction (unpublished primers, ESR, Christchurch) and by serotyping (Remel Agglutinating serum), respectively. The mixed isolate approach was selected on the assumption that it would provide more representative inactivation rates of *C. jejuni* and *S. enterica* than if single strains were used. Each isolate was sub-cultured only four times prior to use.

The six *C. jejuni* strains were cultured separately in Preston's broth (ISO 1995) to a mid log phase, and mixed to provide equal counts of each strain. Then, 1 ml of the appropriate dilution of the mixture was added to the 5 l of WSP effluent seed to give an initial count in the chamber water of between 10^5 and 10^6 CFU 100 ml^{-1} . The procedure for the six *S. enterica* strains was similar, except that the culture medium was selenite cysteine broth (Merck), and the target concentration in the chamber was between 10^6 and 10^7 CFU 100 ml^{-1} .

Laboratory assays

Fecal coliforms were enumerated by membrane filtration (Millipore HA, $0.45 \mu\text{m}$ pore size) and incubation on mFC agar (BBL) at 44.5°C for 24 h (APHA 1998). Typical blue fecal coliform colonies were counted, the membranes were transferred to nutrient agar containing 4-methylumbelliferyl-beta-D-glucuronide (Difco) and incubated at 35°C for 4 h. Fecal coliform colonies exhibiting fluorescence under a UV-A lamp were counted as *E. coli* (USEPA 1991).

Enumeration of *C. jejuni* was by multiple tube dilution in Preston's broth (ISO 1995), using a 5×5 tube dilution series (10 ml to 0.001 ml volumes). The 10 ml samples were first processed by membrane filtration (Millipore HA,

0.45 μm pore size). The membranes were added to the tubes and stirred vigorously. Incubation was at 42°C for 48 h. Confirmation (all tubes were tested) was by streaking on Exeter agar (Oxoid) and incubation for a further 48 h at 42°C. Plates exhibiting characteristic, pinkish, convex, round, slightly mucoid colonies, were counted as positive for *C. jejuni*.

In preliminary experiments, enumeration of *S. enterica* by multiple tube dilution in selenite cysteine broth (Merck) was repeatedly found to produce counts that were too variable to reliably establish survival curves. Accordingly, the membrane filtration procedure for fecal coliforms was followed, but with incubation on XLD agar (Merck) containing 40 mg l⁻¹ novobiocin (for suppression of *Proteus* spp.) at 35°C for 24 h. Using this method, the seeded *S. enterica* colonies were clearly visible as black colonies against background non-salmonellae. Randomly-selected colonies were confirmed as salmonellae by streaking on triple sugar iron agar (Merck) and lysine decarboxylase agar (Oxoid), and confirmed as urease negative, according to the procedures in APHA (2001).

Calculation of inactivation parameters

The approach described in Sinton *et al.* (1994) was adopted. Inactivation rates in the dark chambers were determined by fitting a linear regression line to the (log_e-transformed) counts to derive a dark inactivation rate coefficient (k_D), in log_e units h⁻¹. In chambers exposed to sunlight, the percentage survival (p) at exposure time t was defined as $p = 100N/N_0$, where N is the CFU count, and N_0 is the initial count. Each p value was corrected for dark inactivation as described in Sinton *et al.* (1999). Because the summer and winter experimental temperatures were the same, the summer dark control chamber results were also used to correct the winter *C. jejuni* experiments (experiment 5, Table 1). Where the survival curves displayed a recognizable “shoulder” (Harm 1980), this was quantified using a dimensionless parameter, n_S , and the sunlight inactivation coefficient (k_S) was derived from the slope of the log-linear section of the inactivation curve (i.e. omitting the shoulder points), plotted against insolation, as described in Sinton *et al.* (1994).

The S_{90} and T_{90} values (respectively, the insolation and time taken to achieve a 90% reduction in CFUs) were also

calculated. The dark T_{90} was derived directly from the mean k_D value, as $2.303/k_D$. The S_{90} and sunlight T_{90} values were derived directly from the regression line fitted to all the log_e-transformed counts in the sunlight chambers (i.e. including the shoulder points), plotted against insolation and time, respectively.

RESULTS AND DISCUSSION

Dark inactivation

The median initial *E. coli* counts in both the dark and sunlight-exposed chambers were around 1.3×10^3 CFU 100 ml⁻¹. The equivalent initial counts for the seeded *C. jejuni* and *S. enterica* were 1.1×10^5 and 3.0×10^6 CFU 100 ml⁻¹, respectively. Inactivation rates for all three bacteria were markedly slower in the dark than in the sunlight-exposed chambers (Table 2). Dark inactivation of the pathogens was faster than for *E. coli* in both seawater and river water, but no other consistent dark inactivation patterns emerged. This probably reflects the difficulty in quantifying slow dark inactivation rates over short periods (8 h, in these experiments).

Inactivation of enteric microbes in dark, natural water microcosms has been largely attributed to the predatory, lytic, and grazing effects of the microbiota in natural waters (Toze 2004; Sinton 2005). In our study, these effects were possibly increased by the presence of residual effluent microbiota. These effects are likely to increase at both higher oxygen levels (Toze 2004), and at higher temperatures, possibly in combination with increased bacterial metabolism in low nutrient environments (Gameson & Gould 1985). However, in our study, temperature was stabilised at 14°C, and dissolved oxygen levels remained at 8–9 ppm throughout the experiments, which largely eliminated these factors as experimental variables.

Sunlight inactivation

A comparison of the T_{90} values in Table 2 shows that all the inactivation rates in sunlight were far higher than in the dark, and the survival curves in Figure 1 show that inactivation was directly related to the amount of insolation

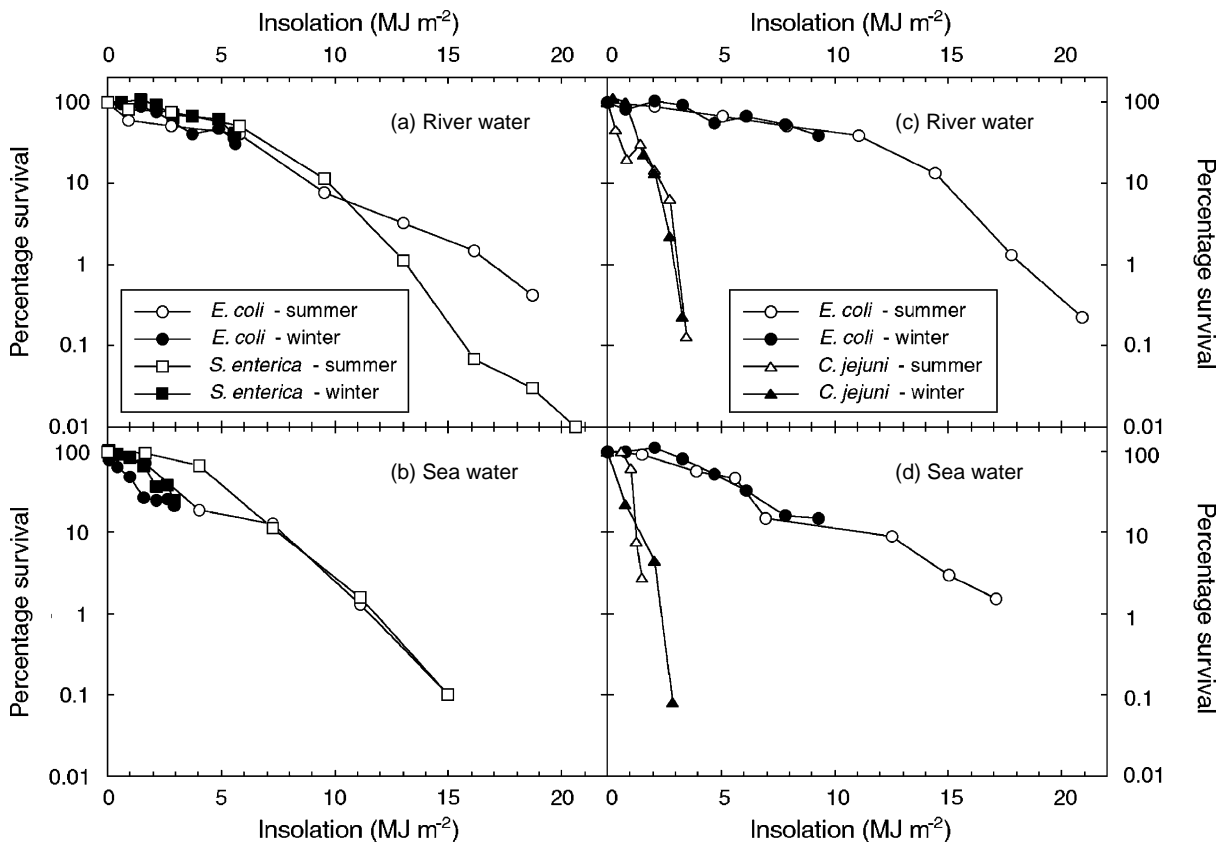
Table 2 | Key inactivation parameters for *E. coli*, *S. enterica*, and *C. jejuni* in sunlight-exposed river water and seawater

Indicator or pathogen	Water type	Dark inactivation parameters		Sunlight inactivation parameters				n_s	k_s ($m^2 MJ^{-1}$)		
		k_D (h^{-1})	T_{90} (h)	T_{90} (h)		S_{90} ($MJ m^{-2}$)			Winter	Summer	
<i>E. coli</i> *	River water	0.004	548	17.3	3.85	18.6	10.2	1.22	5.96	0.17	0.35
	Seawater	0.007	322	9.19	3.44	7.69	8.31	1.28	2.02	0.30	0.38
<i>S. enterica</i>	River water	0.034	67.4	26.8	4.81	20.4	7.15	1.43	10.3	0.17	0.56
	Seawater	0.011	210	12.6	2.39	5.60	6.36	1.19	5.60	0.46	0.55
<i>C. jejuni</i>	River water	0.028	82.6	1.58	0.80	1.65	1.68	20.4	NS [†]	2.74	1.93
	Seawater	0.066	35.0	1.26	0.78	1.28	1.38	2.87	13.3	2.46	3.99

 k_D – Dark inactivation coefficient. k_s – Sunlight inactivation coefficient. T_{90} – Time taken to achieve a 90% reduction in CFUs. S_{90} – Insolation needed to achieve a 90% reduction in CFUs. n_s – Shoulder constant.

*Mean of two experiments.

†No shoulder.

**Figure 1** | Sunlight inactivation, compared to *E. coli*, of *S. enterica* in (a) river water and (b) seawater, and *C. jejuni* in (c) river water (d) seawater. Each experiment was conducted over 8 hours of daylight.

received (i.e. sunlight “dose”). Although there was no difference between the *E. coli* and *S. enterica* sunlight inactivation (k_S) rates in the winter river water chamber, the overall k_S ranking, and average (mean of summer and winter) k_S rates for seawater and river water, respectively, were: *C. jejuni* (3.23; 2.34) > *S. enterica* (0.51; 0.37) > *E. coli* (0.34; 0.26). *S. enterica* was inactivated up to 1.6 times faster than *E. coli*; *C. jejuni* was inactivated up to 16 times faster.

Because of low counts of WSP effluent pathogens, the experiments involved comparisons of laboratory-cultured *S. enterica* and *C. jejuni* with *E. coli* assayed directly from the effluent. The potential loss of resistance to environmental stresses by laboratory strains as a result of repeated sub-culturing has been discussed by Fux *et al.* (2005), and the two pathogens may thus have been inactivated faster than those occurring naturally in effluent. To lessen this effect, isolates from natural waters were used, and sub-culturing was minimised prior to mixing with the effluent.

The shape of survival curves will also be influenced by the culture and enumeration methods. For example, counts of sunlight damaged cells may increase if resuscitation steps, anaerobic incubation, or oxygen scavengers are used (Khaengraeng & Reed 2005), which may result in some flattening of sunlight inactivation curves.

As recorded in Sinton *et al.* (1994, 1999, 2002), there was far greater similarity between the summer and winter S_{90} (insolation-dependent) parameters than the equivalent T_{90} (time-dependent) parameters. This suggests that S_{90} s are reasonably robust parameters for comparing inactivation between seasons. The summer T_{90} s were lower than the winter values for all three bacteria.

The *E. coli* S_{90} s were lower (faster inactivation) than in Sinton *et al.* (2002), probably because of the greater sunlight penetration associated with shallower chambers (390 mm compared with 560 mm) and more dilute WSP mixtures than in the earlier study (5% compared with 10%). Similarly, the increase in sunlight inactivation with increasing salinity (Table 2; Figure 1) was less marked than in Sinton *et al.* (2002), probably because of variations in effluent and water clarities between experiments on separate days. Nevertheless, in 4 of the 6 comparisons in Table 2, the k_S values show faster sunlight inactivation in seawater compared with river water, confirming the synergistic effects on bacterial inactivation of

sunlight and salinity (Davies & Evison 1991; Šolić & Krstulović 1992; Sinton *et al.* 2002).

Differences in clarities of the seawater/WSP effluent mixtures between different days, together with differences in the length of previous exposure to sunlight of the WSP effluent *E. coli* population, may also explain the differences between the *E. coli* curves in Figure 1 (b) and (d). However, the equivalent curves for river water were very similar.

The survival curves for all three bacteria exhibited a “shoulder” (n_S ; Table 2), representing the number of “targets” needing to be hit before a CFU is inactivated (Harm 1980). However, n_S values should be treated with caution, because they are the most variable of the parameters. Shoulder size selection is partially subjective and, in the calculation method given in Sinton *et al.* (1994), n_S is highly dependent on the slope of the subsequent log-linear inactivation phase. Thus, a steep inactivation phase explains the large n_S value in the *C. jejuni* in the winter river water, which in fact represents only 1 h of winter morning sunlight (1 MJ m⁻² insolation).

The slightly faster *S. enterica* inactivation (k_S) rates compared with *E. coli* (ranging from unity in the river water in winter to around 1.5 times faster in the other chambers) is in agreement with the findings of Davies & Evison (1991) and Chandran and Hatha (2005). As noted above, sunlight exposure in a WSP renders surviving *E. coli* considerably more sunlight resistant than *E. coli* from sewage, probably as a result of photorepair mechanisms (Sinton *et al.* 2002). This implies that the *S. enterica* strains used here were probably also more sunlight resistant than sewage *E. coli*. Thus, the comparative inactivation ranking of naturally-occurring *S. enterica* and *E. coli* in WSP effluent may depend on whether *S. enterica* also develops sunlight resistance through photorepair. Rhodes & Kator (1988) interpreted evidence of *S. enterica* recovery in estuarine waters as indicating repair of sub-lethal damage, which in turn suggests photorepair of photobiological (DNA) damage. However, although the species possesses the necessary enzyme (photolyase), laboratory studies have so far failed to demonstrate a photorepair mechanism (Lindenbauer & Darby 1994; Baron 1997). This topic warrants further investigation.

The rapid inactivation of *C. jejuni* in the sunlight-exposed fresh and saline waters was the most marked finding in our study. Inactivation was so rapid that it seems

reasonable to assume that, as for enterococci (Sinton *et al.* 2002), sunlight exposure in a WSP will damage *C. jejuni*, and increase post-discharge inactivation in natural waters. This assumption is supported by *C. jejuni* reductions recorded in the Christchurch sewage treatment plant – counts of around 10^4 100 ml⁻¹ in the effluent entering the pond are reduced to <3 100 ml⁻¹ at the outlet (unpublished data, ESR, Christchurch).

Our findings in natural sunlight support the results of laboratory studies which have shown that *C. jejuni* is highly susceptible to short wavelength (UV-B and UV-C) radiation (Butler *et al.* 1987; Obiri-Danso *et al.* 2001). They are also consistent with the field observations of Obiri-Danso *et al.* (1999), who recorded lower afternoon (compared with morning) *Campylobacter* spp. counts in sunlight-exposed waters, and the observations of Jones (2001), who noted that seasonal distribution and diurnal variation in *C. jejuni* counts showed significant correlations with the likely levels of sunlight exposure.

The reasons for the high sunlight sensitivity of *C. jejuni* are unclear. Its sensitivity to UV-B and UV-C wavelengths is likely to be associated with direct DNA (photobiological) damage, and there appear to be no reports of compensating photo- or dark repair mechanisms. However, UV-C wavelengths are removed by the atmosphere, and UV-B radiation comprises only a small proportion of solar energy at the earth's surface. UV-B wavelengths are also more rapidly attenuated in natural waters than longer (UV-A and short-visible) wavelengths, with the result that photooxidative damage becomes more important as a bacterial inactivation mechanism (Sinton 2005). Furthermore, because *C. jejuni* is oxygen-sensitive, the marked difference in inactivation rates between the sunlight-exposed and dark control chambers in this study suggests that this sensitivity is greatly increased in the presence of sunlight, and that its rapid inactivation in natural waters is largely due to high susceptibility to photooxidative damage.

An alternative hypothesis has been advanced to explain sunlight inactivation of enteric bacteria, such as *E. coli*, *S. enterica*, and *C. jejuni*. Gauthier (2000) maintained that solar radiation may induce these normally culturable species to enter a reversible, “viable but non-culturable” (VBNC) state. For example, most or all of the *E. coli* cells confined in fresh and saline water microcosms were reported to enter this state during 72 h of exposure to high

intensity lamps (Barcina *et al.* 1989), and 10 h (Davies & Evison 1991) and 34–57 h (Pommepuy *et al.* 1996) of exposure to solar radiation. The VBNC state is presented as a long-term survival strategy, distinct from other starved, injured, or dormant stages. However, the VBNC hypothesis is highly controversial, with contrary evidence (summarised by Bogosian & Bourneuf 2001) suggesting that so-called VBNC cells are simply injured or dead, and that their apparent resuscitation is due to well-recognised repair processes or the regrowth of a residual culturable fraction.

Using the *C. jejuni* results in Table 2 and Figure 1 as an example, the explanation proposed by Gauthier (2000) would mean that 1–3 h of sunlight exposure caused the entry of most or all of the cells into a VBNC state, whereas 8 h in the dark did not. This would in turn mean that solar radiation significantly enhances the long-term survival prospects of *C. jejuni*. However, this explanation is not supported by the substantial literature – reviewed by Harm (1980), Jagger (1985) and Sinton (2005), amongst others – not only showing that solar radiation is mostly damaging to enteric bacteria, but also elucidating the mechanisms by which this damage occurs, and the cellular processes available to repair some of it. In contrast, no mechanisms appear to have been advanced to explain how sunlight induces entry into a special VBNC state, or how bacteria thereafter protect their DNA and critical cell structures from photobiological and photooxidative damage.

Finally, the rapid inactivation of *C. jejuni* in sunlight-exposed surface waters compared with *E. coli* suggests that the latter species will act as a highly conservative *C. jejuni* indicator. However, this particular indicator: pathogen relationship is complex, with some reviewers (Thomas *et al.* 1999; Jones 2001) noting that changes in *Campylobacter* counts in receiving waters are generally not reflected in those of indicators, and that it is initially difficult to reconcile rapid inactivation with frequent occurrence. Jones (2001) considered that the most likely explanation is the continual input of *Campylobacter* into natural waters as a result of widespread shedding by livestock and wild fowl, and that, in the absence of spores or any physiologically-based protective mechanism, the main survival strategy appears to be the production and excretion of huge numbers of cells in order to ensure that a few will survive long enough to infect another host.

SUMMARY AND CONCLUSIONS

C. jejuni was inactivated in sunlight-exposed river and seawater far more rapidly than *E. coli* or *S. enterica*, probably as a result of high sensitivity to photooxidative damage. Overall, sunlight inactivation of *S. enterica* was slightly more rapid than for *E. coli*, and inactivation of all three bacteria was much faster in sunlight than in the dark. The results suggest that, in sunlight-exposed surface waters, *E. coli* will be a more conservative indicator for *C. jejuni* than for *S. enterica*, and *C. jejuni* transmission as a pathogenic agent is less likely than for *S. enterica*. The results from this study, combined with those in Sinton et al. (1994, 1999, 2002), suggests the following bacterial sunlight inactivation rate ranking (from the greatest to least inactivation): *C. jejuni* > *S. enterica* > sewage *E. coli* > WSP enterococci > sewage enterococci > WSP *E. coli*. However, the individual inactivation rates will vary according to the salinity of the receiving water. The possibility of photorepair of *S. enterica* in WSPs (and thus placement within this ranking) requires further study.

ACKNOWLEDGEMENTS

The authors thank Dr Robert Davies-Colley, National Institute of Water and Atmospheric Research, New Zealand, Dr Andrea Donnison, AgResearch, Hamilton, New Zealand, Drs Andrew Hudson and Brent Gilpin, Institute of Environmental Science and Research, Christchurch, New Zealand, and the journal's referees, for their valuable comments and criticisms. This research was funded from the New Zealand Public Good Science Fund, administered by the Foundation for Research, Science and Technology.

REFERENCES

- American Public Health Association, American Water Works Association & Water Environment Federation 1998 *Standard Methods for the Examination of Water and Wastewater*, 20th edition. APHA, AWWA, WEF, Washington DC.
- American Public Health Association 2001 *Compendium of Methods for the Microbiological Examination of Foods*, 4th edition. APHA, Washington DC.
- Anon 2003 *Notifiable and other diseases in New Zealand: Annual Report 2003*. Report No. FW0426. Institute of Environmental Science and Research Limited, Porirua, New Zealand.
- Barcina, I., Gonzalez, J. M., Iriberry, J. & Egea, L. 1989 Effect of visible light on progressive dormancy of *Escherichia coli* cells during the survival process in natural fresh water. *Appl. Environ. Microbiol.* **55**, 246–251.
- Baron, J. 1997 Repair of wastewater microorganisms after ultraviolet disinfection under seminatural conditions. *Water Environ. Res.* **69**, 992–998.
- Bogosian, G. & Bourneuf, E. V. 2001 A matter of bacterial life and death. *EMBO Rep.* **2**, 770–774.
- Borrego, J. J., Cornax, R., Moriñigo, M. A., Martinez-Manzanare, E. & Romero, P. 1990 Coliphages as an indicator of faecal pollution in water. Their survival and productive infectivity in natural aquatic environments. *Water Res.* **24**, 111–116.
- Butler, R. C., Lund, V. & Carlson, D. A. 1987 Susceptibility of *Campylobacter jejuni* and *Yersinia enterocolitica* to UV radiation. *Appl. Environ. Microbiol.* **53**, 375–378.
- Chandran, A. & Mohamed Hatha, A. A. 2005 Relative survival of *Escherichia coli* and *Salmonella typhimurium* in a tropical estuary. *Wat. Res.* **39**, 1397–1403.
- Davies, C. M. & Evison, L. M. 1991 Sunlight and the survival of enteric bacteria in natural waters. *J. Appl. Bacteriol.* **70**, 265–274.
- Davies-Colley, R. J., Bell, R. G. & Donnison, A. M. 1994 Sunlight inactivation of enterococci and fecal coliforms within sewage effluent diluted in seawater. *Appl. Environ. Microbiol.* **6**, 2049–2058.
- Eyles, R., Niogi, D., Townsend, C., Benwell, G. & Weinstein, P. 2003 Spatial and temporal patterns of *Campylobacter* contamination underlying public health risk in the Taieri River. *N.Z. J. Environ. Qual.* **3**, 1820–1828.
- Fujioka, R. S., Hashimoto, H. H., Siwak, E. B. & Young, R. H. F. 1981 Effect of sunlight on survival of indicator bacteria in seawater. *Appl. Environ. Microbiol.* **41**, 690–696.
- Fux, C. A., Shirliff, M., Stoodley, P. & Costerton, J. W. 2005 Can reference laboratory strains mirror 'real-world' pathogenesis? *Trends Microbiol.* **13**, 56–63.
- Gameson, A. L. H. 1986 *Investigations of Sewage Discharges to Some British Coastal Waters. Chapter 8. Bacterial Mortality, Part 3*. Report No: TR239. Water Research Centre, Medmenham, UK.
- Gameson, A. L. H. & Gould, D. J. 1985 *Bacterial mortality, Part 2. In Investigations of sewage discharges to some British coastal waters. WRc Techn. Rep. TR 222*. WRc Environment, Medmenham, UK.
- Gauthier, M. J. 2000 Environmental parameters associated with the viable but non-culturable state. In *Nonculturable Organisms in the Environment* (ed. R. R. Colwell & D. J. Grimes) ASM Press, USA. pp. 87–112.
- Harm, W. 1980 *Biological Effects of Ultraviolet Radiation*. Cambridge University Press, London, England.
- International Organisation for Standardisation 1995 *Microbiology of food and animal feeding stuffs – Horizontal method for detection of thermotolerant. Campylobacter*. ISO, Geneva, Switzerland.

- Jagger, J. 1985 *Solar-UV Actions on Living Cells*. Praeger, New York, USA.
- Johnson, D. C., Enriquez, C. E., Pepper, I. L., Davis, T. L., Gerba, C. P. & Rose, J. B. 1997 Survival of *Giardia*, *Cryptosporidium*, poliovirus and *Salmonella* in natural waters. *Wat. Sci. Tech.* **35**(11–12), 261–268.
- Jones, K. 2001 *Campylobacters in water, sewage and the environment*. *J. Appl. Microbiol.* **90**, 68S–79S.
- Khaengraeng, R. & Reed, R. H. 2005 Oxygen and photoinactivation of *Escherichia coli* in UVA and sunlight. *J. Appl. Microbiol.* **99**, 39–50.
- Lindenhauer, K. G. & Darby, J. L. 1994 Ultraviolet disinfection of wastewater: effect of dose on subsequent photoreactivation. *Water Res.* **28**, 805–817.
- McBride, G., Till, D., Ball, A., Lewis, G., Palmer, S. & Weinstein, P. 2002 *Freshwater microbiology research programme: Pathogen occurrence and human health risk assessment analysis*. Ministry for the Environment, Wellington, New Zealand.
- McCambridge, J. & McMeekin, T. A. 1981 Effect of solar radiation and predacious micro-organisms on survival of fecal and other bacteria. *Appl. Environ. Microbiol.* **41**, 1083–1087.
- Mezrioui, N., Baleux, B. & Troussellier, M. 1995 A microcosm study of the survival of *Escherichia coli* and *Salmonella typhimurium* in brackish water. *Water Res.* **29**, 459–465.
- Obiri-Danso, K., Jones, K. & Paul, N. 1999 The effects of the time of sampling on the compliance of beaches in the north west UK with the EU directive on bathing water quality. *J. Coastal Conserv.* **5**, 51–58.
- Obiri-Danso, K., Paul, N. & Jones, K. 2001 The effects of UVB and temperature on the survival of natural populations and pure cultures of *Campylobacter jejuni*, *Camp. coli*, *Camp. lari* and urease-positive thermophilic campylobacters (UPTC) in surface waters. *J. Appl. Microbiol.* **90**, 256–267.
- Pommepuy, M., Guillaud, J. F., Dupray, E., Derrien, A., Le Guyader, F. & Cormier, M. 1992 Enteric bacterial survival factors. *Wat. Sci. Tech.* **25**(12), 93–103.
- Pommepuy, M., Butin, M., Derrien, A., Gourmelon, M., Colwell, R. R. & Cormier, M. 1996 Retention of enteropathogenicity by viable but nonculturable *Escherichia coli* exposed to seawater and sunlight. *Appl. Environ. Microbiol.* **62**, 4621–4626.
- Rhodes, M. W. & Kator, H. I. 1988 Survival of *Escherichia coli* and *Salmonella* sp. in estuarine environments. *Appl. Environ. Microbiol.* **54**, 2902–2907.
- Rozen, Y. & Belkin, S. 2001 Survival of enteric bacteria in seawater. *FEMS Microbiol. Rev.* **25**, 513–529.
- Simmons, G. C. & Smith, J. 1997 Roof water probable source of *Salmonella* infection. *N.Z. Pub. Health Rep.* **4**, 5.
- Sinton, L. W. 2005 Survival of enteric bacteria in seawater: biotic and abiotic effects. In *Oceans and Health: Pathogens in the Marine Environment* (ed. R. R. Colwell & S. Belkin). Kluwer Academic/Plenum Publishers, USA.
- Sinton, L. W., Davies-Colley, R. J. & Bell, R. G. 1994 Inactivation of enterococci and fecal coliforms from sewage and meatworks effluents in seawater chambers. *Appl. Environ. Microbiol.* **60**, 2040–2048.
- Sinton, L. W., Finlay, R. K. & Lynch, P. A. 1999 Sunlight inactivation of fecal bacteriophages and bacteria in sewage-polluted seawater. *Appl. Environ. Microbiol.* **65**, 3605–3613.
- Sinton, L. W., Hall, C. H., Lynch, P. A. & Davies-Colley, R. J. 2002 Sunlight inactivation of fecal indicator bacteria and bacteriophages from waste stabilization pond effluent in fresh and saline waters. *Appl. Environ. Microbiol.* **68**, 3605–3613.
- Stehr-Green, J. K., Nichols, C., McKewan, S., Payne, A. & Mitchell, P. 1991 Waterborne outbreak of *Campylobacter jejuni* in Christchurch: the importance of a combined epidemiologic and microbiologic investigation. *N.Z. Med. J.* **104**, 356–358.
- Šolić, M. & Krstulović, H. 1992 Separate and combined effects of solar radiation, temperature, salinity and pH on the survival of faecal coliforms in seawater. *Mar. Pollut. Bull.* **24**, 411–416.
- Thomas, C., Gibson, H., Hill, D. J. & Mabey, M. 1999 *Campylobacter* epidemiology: an aquatic perspective. *J. Appl. Microbiol. Symp. Suppl.* **85**, 168S–177S.
- Toze, S. 2004 *Pathogen survival in groundwater during artificial recharge. Wastewater Re-use and Groundwater Quality*. Proceedings of Symposium HS04, held during IUGG 2003, Sapporo. IAHS Publication 285, 70–84.
- United States Environmental Protection Agency 1991 *Test methods for Escherichia coli in drinking waters*. EPA Report No. 600/4-91/016. USEPA, Washington DC.

Available online March 2007