Effects of temperature and sand on *E. coli* survival in a northern lake water microcosm

Reyneé W. Sampson, Sarah A. Swiatnicki, Vicki L. Osinga, Jamie L. Supita, Colleen M. McDermott and G. T. Kleinheinz

ABSTRACT

A concern for public health officials is the presence of *Escherichia coli* (*E. coli*), an indicator of fecal contamination, in monitoring recreational waters. While *E. coli* is unlikely to cause disease in humans, its presence may indicate other more pathogenic microorganisms. Many factors can lead to changes in the survival of *E. coli* outside of the animal intestine and may affect the probability of colonizing a new host. Survival of bacteria in recreational water has been linked to water temperature, and most recently to the presence of sand on the beach. This project looked at the survival of an environmental *E. coli* isolate in lake water. Lake water microcosms were placed at 4, 10, 14, or 25°C for up to 36 d and an enzyme-substrate test (Colisure®, IDEXX Corp.) was used to determine the most probable number (MPN) of *E. coli*/100 ml water. *E. coli* numbers at all temperatures declined over the duration of the experiment. The decline was most pronounced at 14°C and was slowest at 4°C. The presence of sand in the microcosm increased the time that *E. coli* survived, regardless of temperature. From a beach management standpoint, these findings indicate that *E.coli* may persist in the environment in cooler water longer than in the warmer water encountered in late summer.

Key words | *E. coli*, recreational water, sand, survival, temperature

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INTRODUCTION

Microbial contamination of recreational waters poses a serious threat to human health, particularly if water is swallowed. The presence of *E. coli* in recreational waters is used to indicate fecal contamination and the possible presence of other, more pathogenic microorganisms such as *Salmonella*, *Shigella*, *Campylobacter*, *Giardia*, *Cryptosporidium* or Norovirus. *E. coli* is most commonly known as a commensal organism of the lower intestine of animals. This primary host environment provides *E. coli* with a warm, constant temperature, in addition to a high concentration of free amino acids and sugars, conducive to bacterial growth. Once excreted from this primary habitat into the external environment, the bacterium may face limited nutrient availability, sunlight, large variations in temperature, pH and moisture, as well as predation (Winfield & Groisman, 2003). All of these factors can lead to a low rate of survival in non-host environments and a low probability of colonizing a new host (Winfield & Groisman, 2003). Given the variations in the physical and chemical characteristics at each location, the survival rate of *E. coli* is uncertain.

Persistence and survival of bacteria in recreational water has been linked to water temperature (Bogosian *et al.* 1996). Several studies reported that cooler water temperatures can increase the ability of *E. coli* to survive in a variety of aquatic conditions (Brettar & Höfle 1992; Smith *et al.* 1994; Bogosian *et al.* 1996) but the perception of the general public, and that of many public health officials', is that cold waters (for example in northern areas such as Lake Superior) would be unsuitable for bacterial survival.

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In addition to the effect of temperature on survival, recent findings suggest that beach sand or sediment may be a reservoir for fecal bacteria in recreational waters (Wheeler-Alm *et al.* 2003). Foreshore beach sand may contain 2–100 times more fecal bacteria than water and is likely to be a major non-point source for beach contamination at several Lake Michigan beaches (Kinzelman *et al.* 2000; Whitman & Nevers 2003).

During some beach monitoring projects *E. coli* levels were found to be unexpectedly high in very cold waters (as cold as 4°C during the swimming season), such as those found in Lake Superior (Sampson *et al.* 2005). Since temperature and the presence of sand apparently play a major role in affecting bacterial concentrations in water, this project further explores the effects of temperature and sand on the survival of *E. coli* in a lake water microcosm. Other researchers have utilized standard laboratory strains of *E. coli* when conducting either microcosm or mesocosm experiments on *E. coli* survival. This study utilizes an environmental *E. coli* isolate obtained from beach water in Lake Superior, WI and examines a wider range of water temperatures than other studies in order to better mimic northern lake environments. Additionally, this study utilized an enzyme-substrate method to enumerate *E. coli* surviving in the microcosm. Other researchers used plate count methods to enumerate *E. coli* and continue to debate the question of culturable versus viable but not culturable organisms. The detection of an enzymatic activity specific to *E. coli* should more accurately enumerate all *E. coli* present and is consistent with methods used during routine beach monitoring. A better awareness of the effect that temperature has on *E. coli* survival in lake water will allow for a more complete understanding of *E. coli* concentrations found during public health beach monitoring efforts.

METHODS

Lake water from Menominee Park beach on Lake Winnebago, Oshkosh, WI was collected on 11 October 2003 and used as the source of lake water for the microcosms. Background *E. coli* concentrations in lake water used in the microcosms were 10 and 3 MPN/100 ml, respectively. An *E. coli* isolate recovered from water (isolate number LS057) in Lake Superior during the summer of 2003 was used to inoculate the lake water microcosms. This isolate was confirmed to be *E. coli* by biochemical testing (API 20 E, bioMérieux, Inc., Hazelwood, MO) and was serotyped (O:109) by the Department of Veterinary Science Animal Diagnostic Laboratory, Pennsylvania State University. To prepare the microcosms' inocula, a 10 μm loopful of the *E. coli* isolate was placed in 5 mL of nutrient broth and allowed to grow overnight at 37°C. Then 10 μL of this culture was diluted in 9.99 mL of sterile saline solution. One mL of the bacterial suspension was then placed into the microcosm, composed of a 6 L Erlenmeyer flask containing 2 L of lake water along with a stir bar. Additionally, another set of microcosms containing lake water and beach sand were set up with approximately 1 cm of sterilized sand placed in the bottom of the flask with the stir bar. The microcosms were incubated at 4, 10, 14 and 25°C (±1°C) on large stir plates, and were stirred throughout the duration of the experiment. Duplicate microcosms were set up for each temperature.

To assess the survival of *E. coli* in sand, plastic (22 cm × 34 cm) microcosms were filled with sand to a depth of 8 cm at one end of the microcosm. The sand depth gradually tapered and extended to approximately one half the length of the microcosm. The microcosm was then filled with water from Lake Winnebago (see above) to a depth of 3 cm above the sand (at the greatest sand depth, ~4 L of water) and the microcosm was gently rocked to simulate a swash zone on the microcosm "beach". The *E. coli* inoculum (LS057) and sand were prepared as described for the flask experiments. Microcosms duplicates were incubated at 4°C, 10°C or 20°C (±1°C) for 44 d. All microcosms (flasks and plastic) were incubated in the dark. No efforts were made to control pH, oxygen or other physio-chemical factors.

Water was removed from the flask microcosms every 2 d to assess *E. coli* numbers. Dilution series were performed to enumerate bacterial concentrations due to the relatively large number of *E. coli* cells originally added (approximately 1×10^5). For the first several days of the experiment, water samples (10 mL) were diluted 1:100 and 1:1000; thereafter no dilutions or dilutions of 1:10 and 1:100 were performed until bacterial levels decreased.

For *E. coli* enumeration from sand in the plastic microcosms, two grams of wet sand (17.5% moisture)

were removed from the "swash zone" and shaken in 100 mL sterile water for 2 min. The sand/water suspension was allowed to settle for one minute, was shaken for an additional 2 min and allowed to settle again. Immediately after the second settling, the samples were analyzed for *E. coli* numbers.

All water samples were analyzed for *E. coli* using the Colisure® defined substrate (DS) test (IDEXX Corp., Portland, ME: APHA, 1998). This testing procedure is now the most widely used to assess *E. coli* in the recreational waters of the Great Lakes region. Incubation and microbial enumeration of samples were conducted following the manufacturer's recommendations. All results were reported as the most probable number (MPN) of *E. coli* per 100 mL of water. Because dilutions of water samples were made, MPN values were adjusted for the dilution factor. The University of Wisconsin–Oshkosh Lab utilized for all analysis is a Wisconsin State Certified Laboratory with a Quality Assurance plan on file with the Wisconsin State Department of Agriculture, Trade, and Consumer Protection.

Rates of decline of bacterial numbers were calculated by the equation:

$$\text{Rate} = \frac{\text{final count(MPN/mL water)} - \text{initial count(MPN/mL water)}}{\text{hours}}$$

RESULTS AND DISCUSSION

E. coli survival in water with or without the presence of sand

Numbers of *E. coli* at all four temperatures decreased over the duration of this experiment. In flasks held at 10, 14 and 25°C without sand (Figure 1), there was a rapid decrease in *E. coli* numbers during the first week, suggesting that these bacterial cells were undergoing cell death because of a decrease in their fitness. At these three temperatures, rates of decline during the first ten days varied from 5.83×10^5 cells/mL/h at 10°C, to 8.33×10^5 cells/mL/h at 14°C, to 4.17×10^5 cells/mL/h at 25°C (Table 1). In contrast to these relatively high rates of decline, the rate of decline at 4°C during the first week was only 8.33×10^4 cells/mL/h. After the first ten days, the rate of decline of *E. coli* decreased dramatically at 10, 14 and 25°C, and the rate decreased only slightly at 4°C to

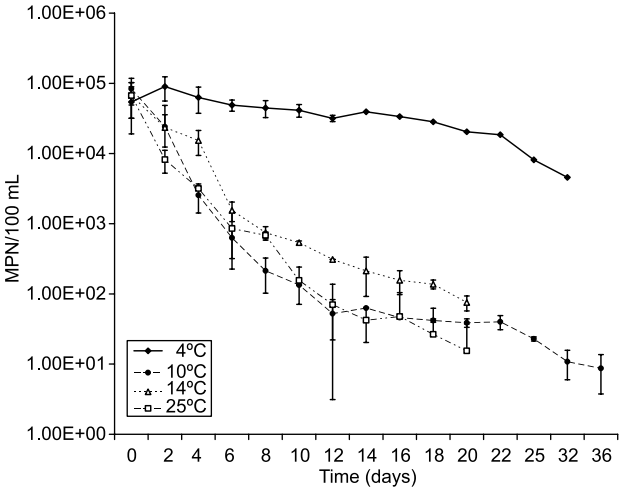

Figure 1 | Survival of *E. coli* introduced into lake water microcosms held at 4, 10, 14 or 25°C (no sand). Points are means of duplicate samples.

5.21×10^4 cells/mL/h. The decline slowed even further during the second and third weeks of the experiment at 10, 14 and 25°C; however, the rate of decline increased at 4°C to 8.93×10^4 cells/mL/h.

In microcosms held at 10, 14 and 25°C with sand (Figure 2), rapid decreases in *E. coli* numbers occurred during the first week, similar to those observed without sand. The presence of sand slowed the rate at which *E. coli* numbers declined at 10, 14 and 25°C (Table 2). Decline slowed to 2.50×10^5 cells/mL/h at 10°C, to 3.75×10^5 cells/mL/h at 14°C and to 4.72×10^5 cells/mL/h at 25°C, indicating that sand may prolong survival. At 4°C decline was most rapid during the first week (2.08×10^5 cells/mL/h) and slowed to 5.56×10^4 cells/mL/h during the third week. Studies with the 4°C microcosm with sand

Table 1 | Rate of *E. coli* decline in water in microcosms without sand present

Temperature	Maximum rate of *E. coli* decline (MPN/mL/h)	Rank of rate decline (1–4)[a]
4°C	8.33×10^4	4
10°C	5.83×10^5	2
14°C	8.33×10^5	1
25°C	4.17×10^5	3

[a]1 = fastest rate of decline and 4 = the slowest rate.

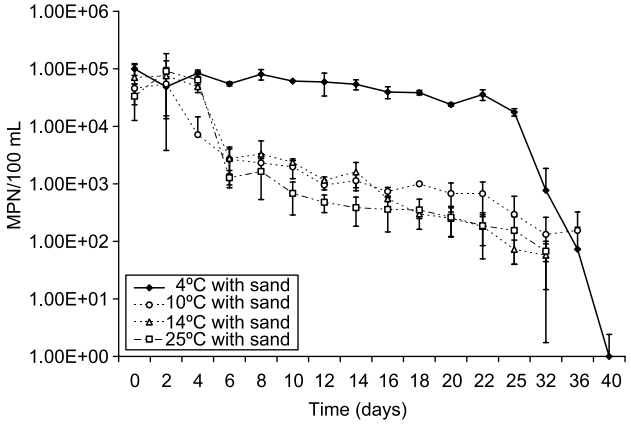

Figure 2 | Survival of *E. coli* introduced into lake water microcosms held at 4, 10, 14 and 25°C with the presence of sand. Points are means of duplicate samples.

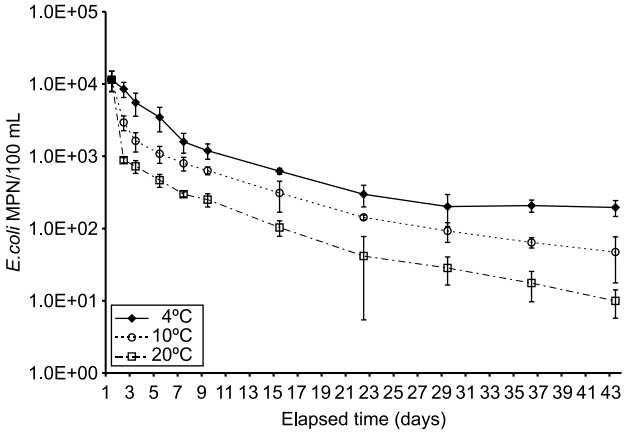

Figure 3 | Survival of *E. coli* from sand recovered from lake water microcosms held at 4, 10 and 20°C with the presence of sand. Points are means of triplicate samples.

were conducted for 40 d to determine the maximum *E. coli* survival without the addition of nutrients. Beginning on day 25 cell numbers began dropping quickly, suggesting that after 25 d cells had lost fitness and could no longer remain viable.

E. coli survival in sand

In the plastic microcosms (designed to more closely mimic a beach swash zone), *E. coli* counts in sand also decreased over the course of the experiment. In sand, there were fewer differences between *E. coli* counts at 4°C and those at other temperatures (Figure 3). While *E. coli* rates of decline in sand were still least in the microcosm held at 4°C, the rate of decline (66.7*E. coli*/100 mL/h) is not substantially different from that at 10°C or at 20°C (Table 3). Greatest rates of decline were seen during the first ten days of the experiment.

Table 2 | Rate of *E. coli* decline in water in microcosms with beach sand present

Temperature	Maximum rate of *E. coli* decline (MPN/mL/h)	Rank of rate decline (1–4)[a]
4°C	2.08×10^5	4
10°C	2.50×10^5	2
14°C	3.75×10^5	1
25°C	4.72×10^5	3

[a]1 = fastest rate of decline and 4 = the slowest rate.

Table 3 | Rates of decline in *E. coli* from sand in microcosm studies

Temperature	Maximum rate of *E. coli* decline (MPN/100 mL/h)	Rank of rate decline (1–3)[a]
4°C	66.7[b]	3
10°C	88.9	2
20°C	163.3	1

[a]1 = fastest rate of decline and 3 = slowest rate of decline.
[b]2 g sand was washed with 100 mL sterile water. Water was analyzed for *E. coli* concentration.

CONCLUSIONS

The results obtained from these *E. coli* survival microcosm studies are similar to those obtained by other researchers using different systems (Brettar & Höfle 1992; Smith *et al.* 1994; Bogosian *et al.* 1996). In all studies, the survival of *E. coli* in water is enhanced at lower temperatures. Bogosian *et al.* (1996) determined that, in nonsterile river water, *E. coli* is able to survive for up to 6 d at 37°C, for 8 d at 20°C, and for 12 d at 4°C. In this study, *E. coli* was able to survive for up to 30 d in nonsterile lake water without sand, and up to 40 d in the presence of sand at 4°C. While water temperature may affect survival rates for *E. coli,* it alone may not be an adequate predictor of *E. coli* concentrations at recreational beaches.

Other laboratory and mesocosm studies have shown that cooler water temperatures and the presence of sand may increase the duration of *E. coli* survival (Rhodes & Kator 1988; Brettar & Höfle 1992; Bogosian *et al.* 1996). This study confirms these findings in a laboratory microcosm designed to more closely mimic a northern freshwater lake environment. Utilization of an environmental isolate of *E. coli* better reflects conditions found in nature. Most other studies have utilized laboratory strains of *E. coli*, and these strains may or may not mimic *E. coli* found in the environment. Environmental isolates have survived for some period of time outside a primary host, and have demonstrated their ability to survive the feast or famine limitations found in the environment at large.

A decrease in water temperature may prolong the ability of *E. coli* to survive in lake water, and may increase the health risk for swimmers. From a beach management standpoint, these findings suggest that beach managers should sample more frequently (and possibly for a longer time) in cooler weather if elevated *E.coli* levels have been reported. In addition, the presence of sand (other particles, or green alga) may enhance survival by providing protection, or a site of higher nutrient concentrations (Brettar & Höfle 1992; Bogosian *et al.* 1996; Whitman *et al.* 2003). This phenomenon may be contributing to the unexpected elevated *E. coli* levels detected in Lake Superior (Sampson *et al.* 2005) and other relatively cold bodies of water by increasing survival time of the organism in the environment. Sand "protection" will likely be the most critical to *E.coli* survival under conditions of fluctuating water and air temperatures, as well as fluctuating nutrient conditions, such as those found in the Lake Superior region. These findings challenge the conventional dogma held by many recreational water managers that *E. coli* will not survive as long at colder temperatures as it will under warmer conditions.

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