Nitrate enrichment does not affect enteropathogenic Escherichia coli in aquatic microcosms but may affect other strains present in aquatic habitats

Meredith T. Davis¹,², Adam D. Canning³, Anne C. Midwinter² and Russell G. Death¹

¹ School of Natural Sciences, Massey University, Palmerston North, Manawatu, New Zealand
² Molecular Epidemiology and Veterinary Public Health Laboratory—Hopkirk Research Institute, School of Veterinary Science, Massey University, Palmerston North, Manawatu, New Zealand
³ Centre for Tropical Water and Aquatic Ecosystem Research (TropWATER), James Cook University of North Queensland, Townsville, Queensland, Australia

ABSTRACT

Eutrophication of the planet’s aquatic systems is increasing at an unprecedented rate. In freshwater systems, nitrate—one of the nutrients responsible for eutrophication—is linked to biodiversity losses and ecosystem degradation. One of the main sources of freshwater nitrate pollution in New Zealand is agriculture. New Zealand’s pastoral farming system relies heavily on the application of chemical fertilisers. These fertilisers in combination with animal urine, also high in nitrogen, result in high rates of nitrogen leaching into adjacent aquatic systems. In addition to nitrogen, livestock waste commonly carries human and animal enteropathogenic bacteria, many of which can survive in freshwater environments. Two strains of enteropathogenic bacteria found in New Zealand cattle, are K99 and Shiga-toxin producing Escherichia coli (STEC). To better understand the effects of ambient nitrate concentrations in the water column on environmental enteropathogenic bacteria survival, a microcosm experiment with three nitrate-nitrogen concentrations (0, 1, and 3 mg NO₃-N/L), two enteropathogenic bacterial strains (STEC O26—human, and K99—animal), and two water types (sterile and containing natural microbiota) was run. Both STEC O26 and K99 reached 500 CFU/10 ml in both water types at all three nitrate concentrations within 24 hours and remained at those levels for the full 91 days of the experiment. Although enteropathogenic strains showed no response to water column nitrate concentrations, the survival of background Escherichia coli, imported as part of the in-stream microbiota did, surviving longer in 1 and 3 mg NO₃-N/L concentrations (P < 0.001). While further work is needed to fully understand how nitrate enrichment and in-stream microbiota may affect the viability of human and animal pathogens in freshwater systems, it is clear that these two New Zealand strains of STEC O26 and K99 can persist in river water for extended periods alongside some natural microbiota.

Subjects Biochemistry, Ecology, Microbiology, Freshwater Biology, Aquatic and Marine Chemistry
Keywords E. coli, Enteropathogenic, Eutrophication, Microcosm, Nitrate, Water quality

How to cite this article Davis MT, Canning AD, Midwinter AC, Death RG. 2022. Nitrate enrichment does not affect enteropathogenic Escherichia coli in aquatic microcosms but may affect other strains present in aquatic habitats. PeerJ 10:e13914
http://doi.org/10.7717/peerj.13914
INTRODUCTION
The provision of clean water and sanitation for all by 2030 is one of the United Nations Sustainable Development Goals (United Nations, 2015; United Nations, 2020). Although increased access to consistently sanitised water has reduced the number of drinking water related outbreaks in many developed countries, there are currently 1.8 billion people worldwide lacking safely managed drinking water supplies (United Nations, 2020) including portions of New Zealand (Ministry of Health, 2018). In many regions, sources of drinking water are contaminated with faeces and nutrients as a result of current and historical wastewater, agricultural, and industrial practices (Baron et al., 2002; Mallin et al., 2015; Jalliffier-Verne et al., 2016; United Nations, 2016). Source water quality is particularly important in areas where water sanitation measures are absent or prone to failure, where microorganisms are resistant to the sanitation treatments available, and/or where recreational, food irrigating, and/or food harvesting waters are affected (Hrudey, Hrudey & Pollard, 2006; Jones et al., 2013; Viñas, Malm & Pettersson, 2019). To achieve the United Nation’s 2030 goal, preventing and remediating source water pollution in combination with increased water sanitation infrastructure is necessary (United Nations General Assembly, 2015; United Nations, 2020).

Globally, agriculture uses more than 70% of all freshwater abstractions, with livestock farming using disproportionately more water than other agricultural products (e.g., grain and vegetables) by weight (Pimentel et al., 2004). Once used, that water commonly re-enters the environment polluted with both nutrients and faeces (Pimentel et al., 2007; Khan & Mohammad, 2014). In New Zealand, land use has changed considerably over the last few decades, moving away from beef and sheep farming to a more intensive pastoral dairy model (Weeks et al., 2016; Julian et al., 2017). Between 1990 and 2015, New Zealand agriculture documented a 600% increase in fertiliser use, a 90% increase in irrigated agricultural land, and a 70% increase in the number of dairy cattle, with at least 65% of animal-derived nitrate leachate into freshwater ecosystems originating from dairy farms (Stats, 2017). As a result, New Zealand’s freshwaters have amassed considerable levels of faecal and nutrient pollution (Gluckman, 2017; Stats, 2020).

Waterborne disease is a not just a third world problem. The largest drinking water acquired campylobacteriosis outbreak ever documented happened in 2016 in Havelock North, New Zealand (Gilpin et al., 2020). More than 5,500 people using an unchlorinated town water supply developed symptoms of gastroenteritis after the water was likely contaminated with sheep faeces (Gilpin et al., 2020).

In New Zealand, between 2013 and 2018, just 32% of 364 monitored drinking water sites met the Escherichia coli (E. coli) standards at all times (Ministry of Health, 2018). Median nitrate-nitrogen (NO$_3$-N) levels at 44% of monitored sites were greater than 3 mg NO$_3$-N /L, a standard limit used by Regional Councils for reporting, and 19% of sites failed to meet safe drinking water standards for nitrate with levels greater than 11 mg NO$_3$-N /L, at least once (Stats, 2020). Additionally, it is estimated that 50% of New Zealand’s rivers (by length) are above 0.44 mg NO$_3$-N/L (ANZG, 2018). A recent report by Environment Canterbury (New Zealand) Regional Council (2020) concluded that the
observed deterioration of regional ground water quality in the region was a direct result of agricultural land use. Of the 202 wells tested for NO$_3$-N across the region 47% were actively deteriorating and 28% were unchanged (Tregurtha, 2020). Of those, 32% had nitrate levels of greater than 5.65 mg NO$_3$-N/L, half of the maximum acceptable value (MAV) (Ministry of Health, 2018), and 6% were above the maximum acceptable value of 11.3 mg NO$_3$-N/L. In addition to high NO$_3$-N levels, half (102 wells) exceeded the maximum allowable levels of E. coli between 5% and 50% of the time (Tregurtha, 2020).

Allochthonous human or animal pathogenic organisms in aquatic habitats are a primary cause of disease in humans and animals (Schwarzenbach et al., 2010; Jenkins, Ahmed & Barnes, 2021), commonly associated with faecal contamination and typically assessed by monitoring water column E. coli levels as a proxy (Odonkor & Ampofo, 2013). One cow/pig/sheep excretes approximately twice the E. coli per day a human does, some of which may be human and/or animal entero-pathogenic strains (Jamieson et al., 2003; Avery, Moore & Hutchison, 2004). Shiga-toxin producing E. coli (STEC) are one type of zoonotic bacteria commonly hosted asymptotically by ruminants and shed through their faeces (Cooley et al., 2013). Escherichia coli strains, especially strains expressing Shiga-toxins capable of causing diarrhoea, enterohaemorrhagic disease or haemolytic uraemic syndrome in humans are clinically and economically significant emerging zoonoses both in New Zealand and globally (Centers for Disease Control and Prevention, 2018).

Instead of identifying human or animal pathogenic organisms directly in aquatic systems, non-specific E. coli in the water column are monitored, as their presence is assumed to be representative of the extent of recent faecal contamination (Odonkor & Ampofo, 2013). However, E. coli, including enteropathogenic strains such as STECs, are able to persist for months or years outside a host (Fairbrother & Nadeau, 2006; Fremaux, Prigent-Combaret & Vernozy-Rozand, 2008; Ahmed, Gyawali & Toze, 2015). Two strains of STEC, responsible for the majority of human disease globally, O157 and O26 are frequently associated with environmental, produce, and/or water contamination (Centers for Disease Control and Prevention, 2018; Joseph et al., 2020).

Other E. coli strains hosted by ruminants (e.g., enteropathogenic K99) are a leading cause of diarrhoeal disease and mortality in neonatal livestock (Brunauer, Roch & Conrady, 2021).

In addition to E. coli, other microorganisms (e.g., viruses, protozoa, and other bacteria) responsible for both human and animal disease present in livestock faeces can enter adjacent aquatic systems where environmental stores may be created (Soller et al., 2016; Salman & Steneroden, 2015).

Understanding the mechanistic drivers of enteropathogenic bacteria in aquatic systems is complex. Livestock dominated catchments frequently display heavily modified geography; altered hydrological cycling, significant soil compaction, and/or increased erosion (Germer et al., 2010). Additionally, reduced riparian cover, agrochemical application, and nutrient leaching is common (Germer et al., 2010; Molina et al., 2017). These modifications may affect the movement, virulence, and/or persistence of human and animal pathogens (Strawn et al., 2013). This is concerning, as many livestock hosted microorganisms are potentially...
zoonotic and shed through excreta, urine and faeces (Cleaveland, Laurenson & Taylor, 2001).

Of these changes, eutrophication is one of the most pervasive, impacting aquatic systems globally (Frumin & Gildeeva, 2014). Nutrient enrichment can affect aquatic microorganisms directly by relieving nutrient-limited growth constraints or indirectly through reduced predator suppression (Haller et al., 2009; Zimmer-Faust et al., 2017). Experimentally manipulating aquatic environmental conditions and assessing the growth and persistence of organisms of interest is a useful way to gain a better understanding of the individual drivers of waterborne bacteria.

Studies of in-stream microbial communities have suggested that predation by, and competition with, in-stream microbiota are the primary factors limiting E. coli survival in the water column (Wanjugi & Harwood, 2013a; Korajkic et al., 2019b). Despite extensive research into E. coli, including work showing that non-toxigenic E. coli can take up nitrate (Taabodi et al., 2019), the influence of concentrations of nitrate (the most abundant form of dissolved inorganic nitrogen in waterways Sigman et al., 2001) on the growth and persistence of E. coli in aquatic systems, remains largely unexamined.

This study aimed to use a microcosm experiment to investigate the effects of ambient nitrate concentrations in the water column on survival rates of two enteropathogenic strains of E. coli, one affecting calves (K99) and one affecting humans (STEC O26), in both a sterile environment and in the presence of in-stream microbiota. We hypothesised there would be a positive relationship between water column nitrate concentrations and pathogenic E. coli reproduction and persistence. We also anticipated that the presence of in-stream microbiota would mediate this relationship, as microbial predation and/or competition may limit the survival of the pathogenic E. coli (Wanjugi & Harwood, 2013b). Although E. coli stores are most commonly found in benthic substrates (Havelaar & Melse, 2003; Muirhead et al., 2004), this experiment was focused on the effects of water column nitrate concentrations as this is the most common sample type used in monitoring drinking and recreational waters (World Health Organization, 2011; Ministry for the Environment, 2020).

**MATERIALS & METHODS**

**Escherichia coli strains**

We used E. coli K99 (ESR 3020 (ESR—NZRM culture collection)) and STEC O26 (NZRM 3537 (Browne et al., 2018; ESR—NZRM culture collection)), as model organisms and monitored the number of colony forming units (CFU) present in the water column, to measure persistence, over 91 days. A pilot experiment demonstrated that there was no measurable difference between the survival rates of two different STEC strains (e.g., STEC O157 and STEC O26 (Supplementary Information)), therefore, a single STEC strain, O26, was chosen for use in the microcosm.

In addition to the artificially inoculated strains (i.e., K99 and STEC O26), background E. coli, imported as part of the microbiome in the stream water used, was present in all the wells using unsterilised water. The background E. coli were present in the control wells at
each of the three NO\textsubscript{3}-N concentrations and were monitored in the same manner as the inoculated wells to determine whether they responded to NO\textsubscript{3}-N concentration.

**Microcosm experiment**

The nitrate concentrations examined (1 and 3 mg NO\textsubscript{3}-N/L) were selected to align with the proposed New Zealand maximum nitrate levels for riverine ecosystem health (*i.e.*, 1 mg NO\textsubscript{3}-N/L) and three times that level (*Essential Freshwater Science and Technical Advisory Group, 2019; Canning, 2020*).

Throughout the experiment the water temperature was maintained at \(\sim10\, ^\circ\text{C}\), mimicking a typical New Zealand river’s average winter water temperature (*Scott & Poynter, 1991*). The temperature-controlled room housing the microcosms had standard UV grow lights set on a timer to mimic a typical winter day length in Palmerston North, New Zealand (-40.393560, 175.633072): 9.5 hr of light and 14.5 hr of dark. New Zealand streams experience the greatest rainfall in winter and consequently the greatest loading of faecal pollution (*Phiri et al., 2020*).

The experiment used 90 microcosm wells (30L \(\times\) 10D \(\times\) 20W cm, containing 3L of water) made in-house. Treatments were replicated five times and randomly assigned to wells.

Two types of water were used in the experiment, (1) sterile stream water, unfiltered and containing cellular debris and other native chemicals, and (2) intact stream water, with the water column microbiome intact. A pilot experiment demonstrated that there was no measurable difference between the use of highly filtered Milli-Q water and unfiltered sterile stream water on K99 or STEC O26 growth or persistence (*Supplementary Information*).

Stream water was collected from the Turitea stream, Palmerston North, New Zealand (40.393728°S, 175.632937°W). The Turitea is a third order, stony bottom stream, with a five year *E. coli* median attribution band rating of E (*i.e.*, the lowest/worst ranking). It typifies the worst 25% of New Zealand streams (*LAWA, 2020*). Because it drains low intensity agriculture it was less likely these strains would be novel to the in-stream microbiota.

Stream water was left intact until NO\textsubscript{3}-N levels measured less than 0.1 mg/L (within \(\pm5\%\), \(+0.1\) mg/L) on a TriOS NICO nitrate meter (KISTERS AG, Germany) and then was sterilised or used intact. Stream water was autoclaved in a Getinge autoclave (Getinge AB, Gothenburg, Sweden).

Potassium nitrate (KNO\textsubscript{3}) powder (Thermo Fisher Scientific, Waltham, MA) was mixed into each well until the target NO\textsubscript{3}-N concentration was established (within \(\pm5\%\) \(+0.1\) mg/L), measured with a TriOS NICO nitrate meter. The excess nitrate enriched stream water (intact and sterile) was saved in the cold room and used to replace the water removed for culturing. Once the nitrate levels were established, 30 of the wells were inoculated with \(\sim300\) CFU each of a single enteropathogenic *E. coli* strain (*i.e.*, K99 or STEC O26), control wells were not inoculated. The *E. coli* levels and NO\textsubscript{3}-N concentrations of the water column in each microcosm were examined on 35 occasions, every 24 hrs for the first seven days then every 72 hrs until day 91. Colony counts above 500 CFU per 10 ml of water were too many to count, for this reason a 500 CFU/10 ml maximum was instituted. Nitrate concentrations were maintained by adding KNO\textsubscript{3} as needed.
Sample collection and bacterial culturing

All of the *E. coli* settled out of the water column in less than 24 hrs (as previously seen in the pilot experiment (Supplementary Information)), so all wells were briskly agitated with a sterile glass stirring spoon to resuspend the *E. coli* in the water column immediately prior to sample collection. Water column sample aliquots were diluted 1:10, 1:100, 1:1,000, 1:10,000, 1:100,000, and 1:1,000,000 with sterile MilliQ H$_2$O to a final volume of 100 ml. Water samples of 10 µl were processed for each K99 and STEC O26 well. For negative control wells in sterile stream water 100 ml was sampled to ensure there was no contamination/growth. In the control wells containing intact stream water, background *E. coli* levels were monitored in the same method as the experimental wells with between 5–100 ml of water sampled, in increasing volumes until there were no *E. coli* grown. An additional 100 ml sample was processed from each control well 24 h after 0 CFU/100 ml was reached. All water samples less than 100 ml in size were diluted with sterile Milli-Q to a final volume of 100 ml. That 100 ml of water was then vacuum filtered through a single sterile 0.45 µm cellulose ester membrane filter (Merck KGaA, Darmstadt, Germany) and cultured.

Bacterial culturing followed United States Environmental Protection Agency method 1603 (*EPA US, 2015*). Each filter was placed onto a Difco Modified mTEC Agar (VWR, Radnor, PA, USA) plate, incubated at 37.5 °C for two hours, and then incubated at 45 °C for 18-20 h. Following incubation, colonies resembling *E. coli* (red/magenta colonies) were counted. Colony counts were calculated in CFU/10 ml and the amount removed from each vial or mesocosm for culture was replaced with an equal amount of the same water type containing the appropriate NO$_3$-N concentration.

Identification of *E. coli* present in the intact stream water microbiome

*Enterobacter cloacae*, commonly found in mammalian faeces is a bacterium that may produce the β-glucuronidase enzyme (*Pearez, Berrocal & Berrocal, 1986*). This enzyme is responsible for the red/magenta colony colour used to identify *E. coli* on Modified mTEC agar. It is also the gene typically targeted to identify *E. coli* using molecular methods. Therefore, they are easily mistaken for *E. coli* when using either of these methods. To ensure the identity of the colonies that were counted as *E. coli* were in fact *E. coli*, 96 colonies were randomly chosen from the background *E. coli* cultures across the 10–25 days for further characterisation. The selected colonies were purified on plate count agar (Merck KGaA, Darmstadt, Germany) and identified by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (Bruker, Billerica, CA, USA) using the “on slide formic acid extraction” method (*Lévesque et al., 2015*).

Sample processing for molecular testing

To confirm the colonies grown from the inoculated intact river water microcosms were STEC O26 or K99, not imported background *E. coli*, 192 colonies (e.g., 96 potential STEC O26 and 96 potential K99) phenotypically identified as *E. coli* by their red/magenta colour on Modified mTEC Agar were randomly selected from across the cultures, up to and including day 91. Additionally, to ensure no contamination of the control wells had
Table 1  Details of the oligonucleotide primers used in this study.

| Gene | Primer sequences                      | Product size | Reference                  |
|------|--------------------------------------|--------------|-----------------------------|
| uidA | Forward: 5′ AGTGTGATATCTACCGCTT-3′   | 84 bp        | Anklam et al. (2012)        |
|      | Reverse: 5′ AGAACGGTTTGTGGTTAATCAG-3′ |              |                             |
| wzy  | Forward: 5′AGCGTATGTTGATATATTTAATGGC-3′ | 141 bp       | Anklam et al. (2012)        |
|      | Reverse: 5′AATGTGGTCCCAAAGAATGAA-3′  |              |                             |
| K99  | Forward: 5′TATTATCCATTGGTGATTTG-3′   | 314 bp       | Roosendaal, Gaastra & De Graaf (1984) |
|      | Reverse: 5′GGTATCCCTTTAGCACGTATTTTCC-3′ |             |                             |

occurred during sampling, 90 colonies (e.g., 45 from STEC O26 intact control wells and 45 from K99 intact control wells) were randomly selected from across the intact control well cultures, up to and including day 25. Colonies were purified on Modified mTEC Agar. Genomic DNA was extracted from each purified colony using a boil preparation protocol; two or three colonies were suspended in 1 ml of Milli-Q H_2O and heated at 100 °C for 10 min then centrifuged at 13,000 rpm for 5 min. The supernatant was aliquoted and used as DNA template.

**Molecular testing for target genes**

We confirmed STEC O26 and K99 using a polymerase chain reaction (PCR) targeting wzy for O26 and the fimbril subunit for K99 (Table 1) (Roosendaal, Gaastra & De Graaf, 1984; Franck, Bosworth & Moon, 1998; Anklam et al., 2012). The detection limits of the STEC assays have been reported at 10^3 CFU/ml (Anklam et al., 2012) with a specificity to sensitivity ratio at 92%;91% for O26 strains (Browne et al., 2018). The K99 primer has been reported to be highly specific and sensitive (Franck, Bosworth & Moon, 1998) however no exact limits were published. Using a positive control we determined that detection was best when there was at least 2 ng/µl of DNA template per reaction.

Amplification reactions were performed in 20 µl reaction volumes. STEC O26 reactions each contained 0. 5 × iQ PerfeCTa® qPCR ToughMix™, ROX™ (QIAGEN, Düsseldorf, Germany), 1 µM of each primer, and 2.5 µl of DNA template. K99 reactions contained 0. 5 × iQ PerfeCTa® qPCR ToughMix™, ROX™ (QIAGEN, Düsseldorf, Germany), 0.5 µM of each primer, and 3 µl of DNA template.

Thermocycling for both reactions was performed in a SensoQuest labcycler (Biomedizinische Elektronik, Göttingen, Germany) using standard cycling conditions as described in Anklam et al. (2012) and Roosendaal, Gaastra & De Graaf (1984).

Amplification products were visualised using RedSafe™ (iNtRON Biotechnology, Daejeon, Korea) following electrophoresis in 2% Tris-acetate-ethylenediamine tetraacetic acid agarose gels.

**Data analysis**

Statistical analyses were performed in R (R Core Team, 2017). Generalised linear models (GLM; Poisson response) were used to examine the response of K99, STEC O26, and background E. coli (in the control wells containing intact stream water), concentrations to treatment with nitrate, water type (sterile or containing in-stream microbiome) and
duration of treatment. Post hoc Tukey’s honestly significant difference (HSD) tests were performed on an ANOVA using the AICcmodavg package (Mazerolle, 2017) to identify significant factors associated with background E. coli persistence. Plots were made in ggplot2 (Wickham, 2016).

RESULTS
Molecular testing and identification
All 96 potential STEC O26 colonies tested for wzy O26 contained the gene and all 96 K99 were positive for the fimbril subunit locus confirming their identities. The 90 background E. coli colonies were negative for wzy O26 and the K99 fimbril subunit but positive for uidA.

Additionally, all but three of the 96 colonies chosen from the background E. coli cultures were confirmed as E. coli by MALDI-TOF. The three that were not E. coli were identified as Enterobacter cloacae.

Microcosm results
Duration, NO\textsubscript{3}-N concentration, and water type (sterile or containing in-stream microbiota) had no measurable effect on STEC O26 or K99 growth or persistence (Table S2). Both strains attained 500 CFU/10 ml of water within 24 h of inoculation and maintained that level for the full 91 days. However, the survival of the background E. coli/E. cloacae group, imported as part of the in-stream microbiome in the intact stream water, increased non-linearly with NO\textsubscript{3}-N concentration and decreased with time (Figs. 1 & 2).

Post hoc comparisons using the Tukey HSD test identified differences in background E. coli and E. cloacae group survival in NO\textsubscript{3}-N concentrations of 3 mg/L NO\textsubscript{3}-N and 1 mg/L NO\textsubscript{3}-N were similar (Tukey’s HSD: $df = 2$, P adj. = 0.153); but that survival in 0 mg/L NO\textsubscript{3}-N was significantly shorter than in 1 mg/L (Tukey’s HSD: $df = 2$, P adj. = 0.000) and 3 mg/L NO\textsubscript{3}-N (Tukey’s HSD: $df = 2$, P adj. =0.000) (Fig. 3, Table S2). The background E. coli/E. cloacae group were either no longer culturable or dead by day 10 in 0 mg/L NO\textsubscript{3}-N; but survived up to 15 days longer in 1 and 3 mg/L NO\textsubscript{3}-N with significant differences in survival rate a result of duration (Table S3).

DISCUSSION
This study is novel in that it investigated the effects of nitrate enrichment on animal and human enteropathogenic E. coli strains as well as in-stream sourced E. coli and E. cloacae group survival in the water column. It is also novel in its use of New Zealand sourced enteropathogenic E. coli strains in combination with New Zealand water column microbiota to determine whether in-stream microbiota do in fact remediate enteric/pathogenic bacterial pollution as has been hypothesised (Wanjigi & Harwood, 2013a; Ravva, Sarreal & Mandrell, 2014a). The effects of nutrient pollution and eutrophication on human and animal enteropathogenic E. coli strains in freshwater systems are poorly understood. One reason for this is that recreational water samples are rarely put through the additional testing necessary to identify specific enteropathogenic strains of E. coli as it can be time
Averaged background *E. coli/E. cloacae* group die-off rates in the intact stream water at 0, 1, and 3 mg NO₃-N/L concentrations.

Consuming and costly (Cooley et al., 2013; Tarr et al., 2019). However, enteropathogenic bacteria can be identified quickly and accurately using other techniques; molecular and MALDI-TOF (Anklam et al., 2012; Ragupathi et al., 2018).

That there was no measurable difference in enteropathogenic *E. coli* growth in response to increased nitrate concentrations was surprising; extending the length of the experiment and/or reducing the number of bacteria introduced should be investigated further. That K99 and STEC O26 were able to persist and grow in the microcosms regardless of NO₃-N levels is an important finding supported by other studies where STECs, notably O157, have demonstrated extended persistence in aquatic environments (Korajkic et al., 2019b; Wang, Deering & Kim, 2020).

Interestingly, the background *E. coli/E. cloacae* group imported as part of the microbiome in the intact stream water did respond to NO₃-N concentration. The response was not linear with increasing enrichment; treatments with 1 mg NO₃-N/L having the highest retention and longest persistence time followed by 3 mg NO₃-N/L and then 0 mg NO₃-N/L. This may be due to death or the organisms no longer being culturable; viable but not culturable bacterial cells are understood to occur in laboratories (Ding et al., 2017; Liu et al., 2017). That said, further work on characterising the background *E. coli/E. cloacae* group to better understand their response to in-stream eutrophication is needed.

An unexpected finding was the speed at which the two enteropathogenic strains formed biofilms. Within 24 h, water column sampling without resuspension of the bacterial biofilms through mechanical agitation resulted in zero *E. coli* colonies grown. This is important from a monitoring perspective as recreational water monitoring only uses samples from the water column for microbial water quality and recreational safety assessments. Streams and rivers are mobile systems where mixing occurs regularly. However, many streams are slow moving or intermittent, and even those with high flows have slower flowing runs and pools. In areas where mixing is reduced, *E. coli* quickly fall out of the water column and adsorb to substrates, periphyton, and/or form biofilms on the water surface (Moreira et al., 2012;
Vogeleer et al., 2014). For this reason, the water column may not be the best substrate to monitor in recreational waterways when attempting to assess the risk of enteropathogenic E. coli strains (Davis et al., 2021). In this experiment water flow and substrates such as rocks or sediment were not used, to ensure the manipulated variables were responsible for any observed differences. Future work should focus on the addition of different substrates, oxygenation levels, and water flow regimes and characterisation of the biofilms and their role in enteropathogenic E. coli survival.

Finally, and most importantly, in-stream microbiota had no measurable effect on the growth or persistence of either K99 or STEC O26. Other studies have suggested that survival of enteric/faecal bacteria, both commensal and pathogenic, may be mediated by aquatic microbes through competition and/or predation (Ravva, Sarreal & Mandrell, 2014b; Korajkic et al., 2019a). A study by Wanjugi & Harwood (2013a) and Wanjugi & Harwood (2013b) found that the presence of in-stream microbiota was the most important factor in remediating E. coli in the water column and sediments, but that STEC O157 displayed extended persistence. While their study used a different STEC serotype, took
place over a much shorter time frame, at higher temperatures (5–10 °C higher than in our experiments), and included sediments, a similar resilience to in-stream microbiota and extended persistence was demonstrated by the enteropathogenic *E. coli* in our study (Wanjugi & Harwood, 2013b). Reductions in colony counts were observed within the first five days Wanjugi & Harwood’s (2013a) study. This did not happen in our study. There was no effect on either enteropathogenic strain that was attributable to in-stream microbiota. Our study used no substrate/sediment, only intact stream water. Considering the speed with which biofilm accumulation occurred, ongoing work is needed to determine whether the addition of benthic substrates could be important to the persistence/remediation of human and animal enteropathogenic *E. coli* strains in aquatic habitats. This next step may help identify an overlooked habitat for in-stream human and animal enteropathogenic *E. coli* strain sequestration or an environment hosting species capable of remediating enteropathogenic *E. coli* from aquatic systems.

While international studies on human pathogens have mixed findings on whether predation and competition in aquatic systems are important limiting factors to persistence or drivers of virulence (Erken, Lutz & McDougald, 2013; Mauro et al., 2013; Schmidt, Shringi & Besser, 2016), neither was found to be a significant factor for human and animal enteropathogenic *E. coli* strains in this experiment. In a realistic scenario, rivers or lakes reaching and maintaining NO₃-N concentrations above 3 mg/L are likely to have other in-stream changes related to eutrophication and ecosystem distress, primarily, periphyton blooms or overgrowth of macrophytes (Camargo & Alonso, 2006). The ramifications of periphyton and macrophyte overgrowth (e.g., fluctuating dissolved oxygen levels and changes in the type/amount of food available) may benefit human and animal pathogenic bacteria that are metabolically diverse, and are capable of surviving in both anoxic and hyperoxic environments. Conversely, bactivorous organisms may not be as adaptable to these conditions, therefore reducing predation (Blount, 2015).
CONCLUSIONS

It is important we understand the potential impacts of rising nitrogen enrichment on our recreational and drinking water sources (Ward et al., 2018; Sprong et al., 2020). Nitrate-nitrogen concentrations at or exceeding those used in this study are being documented in waterways and aquifers, both nationally and globally (Canning, 2020; Tregurtha, 2020). The co-occurrence of elevated NO₃-N concentrations and livestock faeces potentially carrying human and animal pathogens in freshwater systems is a direct result of catchment management and should have us questioning what effect excess nitrogen in our waterways is having on aquatic microbial communities and how that may affect human health (Dodds et al., 2009; Snelder, McDowell & Fraser, 2017). Therefore, aiming to reduce both nutrient and microbial pollution entering freshwater systems is the best way to protect all water for human and non-human life.

ACKNOWLEDGEMENTS

Work was completed in the Ecology Labs, Massey University, Palmerston North, New Zealand. We thank James Connell for his expert health and safety assessment and assistance. Additional thanks to Paul Barrett and Cleland Wallace for sourcing equipment.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding
The authors received no funding for this work.

Competing Interests
The authors declare there are no competing interests.

Author Contributions

• Meredith T. Davis conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
• Adam D. Canning conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.
• Anne C. Midwinter conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.
• Russell G. Death conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.

Data Availability
The following information was supplied regarding data availability:

The raw data is available in the Supplemental Files.

Supplemental Information
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.13914#supplemental-information.
REFERENCES

Ahmed W, Gyawali P, Toze S. 2015. Quantitative PCR measurements of Escherichia coli including Shiga toxin—producing E. coli (STEC) in animal feces and environmental waters. Environmental Science & Technology 49:3084–3090 DOI 10.1021/es505477n.

Anklam KS, Kanankege KST, Gonzales TK, Kaspar CW, Döpfer D. 2012. Rapid and reliable detection of Shiga toxin—producing Escherichia coli by real-time multiplex PCR. Journal of Food Protection 75:643–650 DOI 10.4315/0362-028X.JFP-11-392.

ANZG. 2018. Rational and background information (Ch 8), Australian and New Zealand guidelines for fresh and marine water quality. In: Aquatic ecosystems. 1st edition. Australian and New Zealand governments and Australian state and territory governments, 1–313.

Avery SM, Moore A, Hutchison ML. 2004. Fate of Escherichia coli originating from livestock faeces deposited directly onto pasture. Letters in Applied Microbiology 38:355–359 DOI 10.1111/j.1472-765X.2004.01501.x.

Baron JS, Poff NL, Angermeier PL, Dahm CN, Gleick PH, Hairston NG, Jackson RB, Johnston CA, Richter BD, Steinman AD. 2002. Meeting ecological and societal needs for freshwater. Ecological Applications 12:1247–1260 DOI 10.2307/3099968.

Blount ZD. 2015. The natural history of model organisms: the unexhausted potential of E. coli. Elife 4:e05826 DOI 10.7554/eLife.05826.

Browne AS, Midwinter AC, Withers H, Cookson AL, Biggs PJ, Marshall JC, Benschop J, Hathaway S, Haack NA, Akhter RN, French NP. 2018. Molecular epidemiology of Shiga toxin—producing Escherichia coli (STEC) on New Zealand dairy farms: application of a culture-independent assay and whole-genome sequencing. Applied and Environmental Microbiology 84:e00481-18 DOI 10.1128/AEM.00481-18.

Brunauer M, Roch F-F, Conrady B. 2021. Prevalence of worldwide neonatal calf diarrhoea caused by bovine rotavirus in combination with bovine coronavirus, Escherichia coli K99 and cryptosporidium spp.: a meta-analysis. Animals 11(4):1014 DOI 10.3390/ani11041014.

Camargo JA, Alonso Á. 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. Environment International 32:831–849 DOI 10.1016/j.envint.2006.05.002.

Canning AD. 2020. Nutrients in New Zealand rivers and streams: an exploration and derivation of national nutrient criteria. Wellington, New Zealand: Minister for the Environment DOI 10.6084/m9.figshare.12116460.

Centers for Disease Control and Prevention. 2018. National enteric disease surveillance: Shiga toxin—producing Escherichia coli (STEC) annual report 2016. Available at https://www.cdc.gov/ecoli/surv2016/index.html (accessed on 4 October 2020).

Cleaveland S, Laurenson MK, Taylor LH. 2001. Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. Philosophical Transactions of the Royal Society of London B: Biological Sciences 356:991–999 DOI 10.1098/rstb.2001.0889.
Cooley MB, Jay-Russell M, Atwill ER, Carychao D, Nguyen K, Quiñones B, Patel R, Walker S, Swimley M, Pierre-Jerome E. 2013. Development of a robust method for isolation of Shiga toxin-positive Escherichia coli (STEC) from fecal, plant, soil and water samples from a leafy greens production region in California. PLOS ONE 8:e65716 DOI 10.1371/journal.pone.0065716.

Davis M, Midwinter AC, Cosgrove R, Death RG. 2021. Detecting genes associated with antimicrobial resistance and pathogen virulence in three New Zealand rivers. PeerJ 9:e12440 DOI 10.7717/peerj.12440.

Ding T, Suo Y, Xiang Q, Zhao X, Chen S, Ye X, Liu D. 2017. Significance of viable but nonculturable Escherichia coli: induction, detection, and control. Journal of Microbiology and Biotechnology 27:417–428 DOI 10.4014/jmb.1609.09063.

Dodds WK, Bouska WW, Eitzmann JL, Pilger TJ, Pitts KL, Riley AJ, Schloesser JT, Thornbrugh DJ. 2009. Eutrophication of US freshwaters: analysis of potential economic damages. Environmental Science & Technology 43:12–19 DOI 10.1021/es801217q.

EPA US. 2015. Method 1603: Escherichia coli (E. coli) in water by membrane filtration using modified membrane-thermotolerant Escherichia coli agar (modified mTEC). Available at https://www.epa.gov/sites/production/files/2015-08/documents/method_1603_2009.pdf (accessed on 4 October 2019).

Erken M, Lutz C, McDougald D. 2013. The rise of pathogens: predation as a factor driving the evolution of human pathogens in the environment. Microbial Ecology 65:860–868 DOI 10.1007/s00248-013-0189-0.

Essential Freshwater Science and Technical Advisory Group. 2019. Freshwater Science and Technical Advisory Group (STAG) report to the minister for the environment. Wellington, New Zealand.

Fairbrother JM, Nadeau E. 2006. Escherichia coli: on-farm contamination of animals. Revue Scientifique et Technique 25:555–569 DOI 10.20506/rst.25.2.1682.

Franck SM, Bosworth BT, Moon HW. 1998. Multiplex PCR for enterotoxigenic, attaching and effacing, and Shiga toxin-producing Escherichia coli strains from calves. Journal of Clinical Microbiology 36:1795–1797 DOI 10.1128/JCM.36.6.1795-1797.1998.

Fremaux B, Prigent-Combaret C, Vernozy-Rozand C. 2008. Long-term survival of Shiga toxin-producing Escherichia coli in cattle effluents and environment: an updated review. Veterinary Microbiology 132:1–18 DOI 10.1016/j.vetmic.2008.05.015.

Frumin GT, Gildeeva IM. 2014. Eutrophication of water bodies—a global environmental problem. Russian Journal of General Chemistry 84:2483–2488 DOI 10.1134/S1070363214130015.

Germer S, Neill C, Krusche Av, Elsenbeer H. 2010. Influence of land-use change on near-surface hydrological processes: undisturbed forest to pasture. Journal of Hydrology 380:473–480 DOI 10.1016/j.jhydrol.2009.11.022.

Gilpin BJ, Walker T, Paine S, Sherwood J, Mackereth G, Wood T, Hambling T, Hewison C, Brounts A, Wilson M, Scholes P, Robson B, Lin S, Cornelius A, Rivas L, Hayman DTS, French NP, Zhang J, Wilkinson DA, Midwinter AC, Biggs PJ, Jagroop A, Eyre R, Baker MG, Jones N. 2020. A large scale waterborne
Campylobacteriosis outbreak, Havelock North, New Zealand. *Journal of Infection* **81**:390–395 DOI 10.1016/j.jinf.2020.06.065.

Gluckman P. 2017. *New Zealand’s fresh waters: values, state, trends and human impacts.* Wellington, New Zealand: Office of the Prime Minister’s Chief Science Advisor.

Haller L, Amedegnato E, Poté J, Wildi W. 2009. Influence of freshwater sediment characteristics on persistence of fecal indicator bacteria. *Water, Air, and Soil Pollution* **203**:217–227 DOI 10.1007/s11270-009-0005-0.

Havelaar AH, Melse JM. 2003. *Quantifying public health risk in the WHO guidelines for drinking-water quality: a burden of disease approach.* Bilthoven, Netherlands: Rijksinstituut voor Volksgezondheid en Milieu RIVM.

Hrudey SE, Hrudey EJ, Pollard SJT. 2006. Risk management for assuring safe drinking water. *Environment International* **32**:948–957 DOI 10.1016/j.envint.2006.06.004.

Jamieson RC, Gordon RJ, Tattrie SC, Stratton GW. 2003. Sources and persistence of fecal coliform bacteria in a rural watershed. *Water Quality Research Journal* **38**:33–47 DOI 10.2166/wqrj.2003.004.

Jenkins M, Ahmed S, Barnes AN. 2021. A systematic review of waterborne and water-related disease in animal populations of Florida from 1999–2019. *PLOS ONE* **16**:e0255025 DOI 10.1371/journal.pone.0255025.

Jones S, Greene N, Hueso A, Sharp H, Kennedy-Walker R. 2013. *Learning from failure: lessons for the sanitation sector.* London: UK Sanitation Community of Practice.

Khan MN, Mohammad F. 2014. Eutrophication: challenges and solutions. In: *Eutrophication: causes, consequences and control.* Dordrecht, The Netherlands: Springer, 1–15.

Korajkic A, McMinn BR, Ashbolt NJ, Sivaganesan M, Harwood VJ, Shanks OC. 2019a. Extended persistence of general and cattle-associated fecal indicators in marine and freshwater environment. *Science of the Total Environment* **650**:1292–1302 DOI 10.1016/j.scitotenv.2018.09.108.

Korajkic A, Wanjugi P, Brooks I, Cao Y, Harwood VJ. 2019b. Persistence and decay of fecal microbiota in aquatic habitats. *Microbiology and Molecular Biology Reviews* **83**:e00005-19 DOI 10.1128/MMBR.00005-19.

LAWA. 2020. LAWANZ - Land, air, water Aotearoa. Turitea at No 1 Dairy Water Quality. Available at [https://www.lawa.org.nz/explore-data/manawatu-whanganui-region/river-quality/manawatu/turitea-at-no-1-dairy/](https://www.lawa.org.nz/explore-data/manawatu-whanganui-region/river-quality/manawatu/turitea-at-no-1-dairy/).
Lévesque S, Dufresne PJ, Soualhine H, Domingo M-C, Bekal S, Lefebvre B, Tremblay C. 2015. A side by side comparison of Bruker Biotyper and VITEK MS: utility of MALDI-TOF MS technology for microorganism identification in a public health reference laboratory. *PLOS ONE* **10:**e0144878 DOI 10.1371/journal.pone.0144878.

Liu J, Zhou R, Li L, Peters BM, Li B, Lin C, Chuang T-L, Chen D, Zhao X, Xiong Z. 2017. Viable but non-culturable state and toxin gene expression of enterohemorrhagic *Escherichia coli* O157 under cryopreservation. *Research in Microbiology* **168:**188–193 DOI 10.1016/j.resmic.2016.11.002.

Mallin MA, McIver MR, Robuck AR, Dickens AK. 2015. Industrial swine and poultry production causes chronic nutrient and fecal microbial stream pollution. *Water, Air, & Soil Pollution* **226:**1–13.

Mauro SA, Opalko H, Lindsay K, Colon MP, Koudelka GB. 2013. The microcosm mediates the persistence of shiga toxin-producing *Escherichia coli* in freshwater ecosystems. *Applied and Environmental Microbiology* **79:**4821–4828 DOI 10.1128/AEM.01281-13.

Mazerolle MJ. 2017. Package ‘AICcmodavg.’ R package. Available at https://CRAN.R-project.org/package=AICcmodavg.

Ministry for the Environment. 2020. *National policy statement for freshwater management 2020*. Wellington, New Zealand: Ministry for the Environment.

Ministry of Health. 2018. *Drinking-water standards for New Zealand 2005 (revised 2018)*. Wellington.

Molina MC, Roa-Fuentes CA, Zeni JO, Casatti L. 2017. The effects of land use at different spatial scales on instream features in agricultural streams. *Limnologica* **65:**14–21 DOI 10.1016/j.limno.2017.06.001.

Moreira S, Brown A, Ha R, Iserhoff K, Yim M, Yang J, Liao B, Pszczolko E, Qin W, Leung KT. 2012. Persistence of *Escherichia coli* in freshwater periphyton: biofilm-forming capacity as a selective advantage. *FEMS Microbiology Ecology* **79:**608–618 DOI 10.1111/j.1574-6941.2011.01244.x.

Muirhead RW, Davies-Colley RJ, Donnison AM, Nagels JW. 2004. Faecal bacteria yields in artificial flood events: quantifying in-stream stores. *Water Research* **38:**1215–1224 DOI 10.1016/j.watres.2003.12.010.

Odonkor ST, Ampofo JK. 2013. *Escherichia coli* as an indicator of bacteriological quality of water: an overview. *Microbiology Research* **4(1):**1–11 DOI 10.4081/mr.2013.e2.

Pearez JL, Berrocal CI, Berrocal L. 1986. Evaluation of a commercial β-glucuronidase test for the rapid and economical identification of *Escherichia coli*. *Journal of Applied Bacteriology* **61:**541–545.

Phiri BJ, Pita AB, Hayman DTS, Biggs PJ, Davis MT, Fayaz A, Canning AD, French NP, Death RG. 2020. Does land use affect pathogen presence in New Zealand drinking water supplies? *Water Research* **185:**116229 DOI 10.1016/j.watres.2020.116229.

Pimentel D, Berger B, Filiberto D, Newton M, Wolfe B, Karabinakis E, Clark S, Poon E, Abbett E, Nandagopal S. 2004. Water resources: agricultural and environmental issues. *BioScience* **54:**909–918 DOI 10.1641/0006-3568(2004)054[0909:wraaei]2.0.co;2.
Pimentel D, Cooperstein S, Randell H, Filiberto D, Sorrentino S, Kaye B, Nicklin C, Yagi J, Brian J, O’Hern J. 2007. Ecology of increasing diseases: population growth and environmental degradation. Human Ecology 35:653–668 DOI 10.1007/s10745-007-9128-3.

R Core Team. 2017. The R foundation for statistical computing platform.

Ragupathi NKD, Sethuvel DPM, Inbanathan FY, Veeraraghavan B. 2018. Accurate differentiation of Escherichia coli and Shigella serogroups: challenges and strategies. New Microbes and New Infections 21:58–62 DOI 10.1016/j.nmni.2017.09.003.

Ravva SV, Sarreal CZ, Mandrell RE. 2014a. Strain differences in fitness of Escherichia coli O157: H7 to resist protozoan predation and survival in soil. PLOS ONE 9:e102412 DOI 10.1371/journal.pone.0102412.

Ravva SV, Sarreal CZ, Mandrell RE. 2014b. Strain differences in fitness of Escherichia coli O157: H7 to resist protozoan predation and survival in soil. PLOS ONE 9:e102412 DOI 10.1371/journal.pone.0102412.

Roosendaal B, Gaastra W, De Graaf FK. 1984. The nucleotide sequence of the gene encoding the K99 subunit of enterotoxigenic Escherichia coli. FEMS Microbiology Letters 22:253–258 DOI 10.1111/j.1574-6968.1984.tb00737.x.

Salman MD, Steneroden K. 2015. Important public health zoonoses through cattle. In: Zoonoses-infections affecting humans and animals. Dordrecht, The Netherlands: Springer, 3–22.

Schmidt CE, Shringi S, Besser TE. 2016. Protozoan predation of Escherichia coli O157: H7 is unaffected by the carriage of Shiga toxin-encoding bacteriophages. PLOS ONE 11:e0147270 DOI 10.1371/journal.pone.0147270.

Schwarzenbach RP, Egli T, Hofstetter TB, von Gunten U, Wehrli B. 2010. Global water pollution and human health. Annual Review of Environment and Resources 35:109–136 DOI 10.1146/annurev-environ-100809-125342.

Scott D, Poynter M. 1991. Upper temperature limits for trout in New Zealand and climate change. Hydrobiologia 222:147–151 DOI 10.1007/bf00006102.

Sigman DM, Casciotti KL, Andreani M, Barford C, Galanter M, Böhlke JK. 2001. A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. Analytical Chemistry 73:4145–4153 DOI 10.1021/ac010088e.

Snelder TH, McDowell RW, Fraser CE. 2017. Estimation of catchment nutrient loads in New Zealand using monthly water quality monitoring data. JAWRA Journal of the American Water Resources Association 53:158–178 DOI 10.1111/1752-1688.12492.

Soller JA, Schoen ME, Bartrand T, Ravenscroft JE, Ashbolt NJ. 2010. Estimated human health risks from exposure to recreational waters impacted by human and non-human sources of faecal contamination. Water Research 44:4674–4691 DOI 10.1016/j.watres.2010.06.049.

Sprong RC, van den Brand AD, van der Aa N, van de Ven BM, Bulder AS. 2020. Combined exposure to nitrate and nitrite via food and drinking water in The Netherlands. Bilthoven, Netherlands: Rijksinstituut voor Volksgezondheid en Milieu (RIVM) DOI 10.21945/RIVM-2020-0003.
Stats NZ. 2017. Agricultural production statistics: 2017. Available at https://www.stats.govt.nz/information-releases/agricultural-production-statistics-june-2017-final (accessed on 20 January 2001).

Stats NZ. 2020. Groundwater quality. Available at https://www.stats.govt.nz/indicators/groundwater-quality (accessed on 22 February 2021).

Strawn LK, Fortes ED, Bihn EA, Nightingale KK, Gröhn YT, Worobo RW, Wiedmann M, Bergholz PW. 2013. Landscape and meteorological factors affecting prevalence of three food-borne pathogens in fruit and vegetable farms. Applied and Environmental Microbiology 79:588–600 DOI 10.1128/AEM.02491-12.

Taabodi M, Hashem FM, Oscar TP, Parveen S, May EB. 2019. The possible roles of Escherichia coli in the nitrogen cycle. International Journal of Environmental Research 1:597–602.

Tarr GAM, Lin CY, Lorenzetti D, Chui L, Tarr PL, Hartling L, Vandermeer B, Freedman SB. 2019. Performance of commercial tests for molecular detection of Shiga toxin-producing Escherichia coli (STEC): a systematic review and meta-analysis protocol. BMJ Open 9:e025950 DOI 10.1136/bmjopen-2018-025950.

Tregurtha J. 2020. Annual groundwater quality survey. Kaikora, New Zealand: Environment Canterbury.

United Nations. 2015. United Nations: sustainable development goals. Goal 6: ensure access to water and sanitation for all. Available at https://sdgs.un.org/goals/goal6 (accessed on 10 May 2021).

United Nations. 2016. The sustainable development goals report 2016. New York, NY, USA: United Nations Available at 10.29171/azu_acku_pamphlet_k3240_s878_2016.

United Nations. 2020. The sustainable development goals report. New York.

United Nations General Assembly. 2015. Transforming our world: the 2030 agenda for sustainable development. Division for Sustainable Development Goals: New York, NY, USA.

Viñas V, Malm A, Pettersson TJR. 2019. Overview of microbial risks in water distribution networks and their health consequences: quantification, modelling, trends, and future implications. Canadian Journal of Civil Engineering 46:149–159 DOI 10.1139/cjce-2018-0216.

Vogeleer P, Tremblay YDN, Mafu AA, Jacques M, Harel J. 2014. Life on the outside: role of biofilms in environmental persistence of Shiga-toxin producing Escherichia coli. Frontiers in Microbiology 5:317.

Wang Y-J, Deering AJ, Kim H-J. 2020. The occurrence of shiga toxin-producing E. coli in aquaponic and hydroponic systems. Horticulturae 6:11–36 DOI 10.3390/horticulturae6010001.

Wanjugi P, Harwood VJ. 2013a. The influence of predation and competition on the survival of commensal and pathogenic fecal bacteria in aquatic habitats. Environmental Microbiology 15:517–526 DOI 10.1111/j.1462-2920.2012.02877.x.

Wanjugi P, Harwood VJ. 2013b. The influence of predation and competition on the survival of commensal and pathogenic fecal bacteria in aquatic habitats. Environmental Microbiology 15:517–526 DOI 10.1111/j.1462-2920.2012.02877.x.
Ward MH, Jones RR, Brender JD, De Kok TM, Weyer PJ, Nolan BT, Villanueva CM, Van Breda SG. 2018. Drinking water nitrate and human health: an updated review. *International Journal of Environmental Research and Public Health* **15**:1557 DOI 10.3390/ijerph15071557.

Weeks ES, Death RG, Foote K, Anderson-Lederer R, Joy MK, Boyce P. 2016. Conservation Science Statement 1. The demise of New Zealand’s freshwater flora and fauna: a forgotten treasure. *Pacific Conservation Biology* **22**:110–115 DOI 10.1071/PC15038.

Wickham H. 2016. ggplot2: elegant graphics for data analysis. Available at [https://ggplot2.tidyverse.org](https://ggplot2.tidyverse.org).

World Health Organization. 2011. *Technical guidance on water-related disease surveillance*. Copenhagen: WHO Regional Office for Europe.

Zimmer-Faust AG, Thulsiraj V, Marambio-Jones C, Cao Y, Griffith JF, Holden PA, Jay JA. 2017. Effect of freshwater sediment characteristics on the persistence of fecal indicator bacteria and genetic markers within a Southern California watershed. *Water Research* **119**:1–11 DOI 10.1016/j.watres.2017.04.028.