

Water quality and the metabiome of DOC campground drinking water

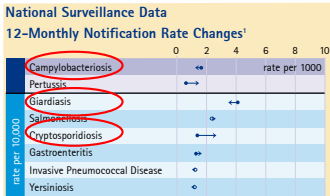
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DEPE Workshop
Allan Wilson Centre

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Preamble



- 780 million people worldwide have no access to safe drinking water (*WHO, 2010*)
- MoH monitors drinking water quality for 91% of New Zealanders
- 18,000 - 34,000 waterborne gastroenteritis cases per year in New Zealand; increasing in past 20yrs (*Baker, 2012*)
- 3 of top 5 notifiable diseases in New Zealand are water-borne (*ESR, 2013*)
- 16S rRNA genes (rDNA) code for 16S rRNA and are highly conserved between different species

Background

- Tourism generates NZ\$2 billion or 18% of export earnings; 9.2% of GDP; 1 in 10 jobs
- Much of tourism is based unique flora and fauna
- DOC protects flora and fauna in 30% (8.5m ha) of New Zealand
- DOC promotes recreation e.g. through provision of campgrounds (+ drinking water)
- Campgrounds not associated with water-borne illness outbreaks ... let us keep it that way!

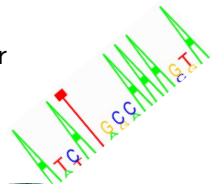


Outline

- Objectives
- Materials
- Lab work
- Findings
- Challenges

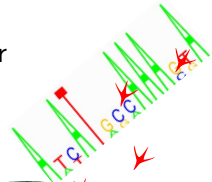
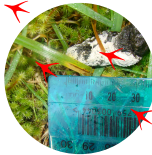
Objectives

- 1 To describe the metagenome of campground water
- 2 To perform waterborne pathogen source tracking



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Materials

- Study sites (map insert): 15 campgrounds managed by DOC
- Four campgrounds classified as 'Serviced' and 11 'Standard'
- Water supply: 12 stream or creek , 1 lake, 1 spring & 1 roof
- Nine had no water treatment, others had UV only (2), filtre only (2), filtre + chemical (1) & filter + UV (1)
- Four rounds of sampling were conducted during 2011/2012 & 2012/2013 summer seasons
- Driving distance of about 23,000km covered during sampling
- Ten campgrounds sampled 4times, three 3times & two 2times

Materials

- Samples collected:
 - Faecal scoop ($n = 669$) for:
 - *Cryptosporidium* and *Giardia* screening
 - Metagenomic analysis (round 4 only)
 - Faecal swab for *Campylobacter* isolation ($n = 669$)
 - 100mL water for *Campylobacter* screening ($n = 103$)
 - 100mL water for *E. coli* enumeration ($n = 103$)
 - 100L water filtered on site for *Cryptosporidium* & *Giardia* screening (in 1st and 2nd rounds only) ($n = 50$)
 - 2L (round 1 & 2) ($n = 50$), 10L (round 3 & 4) ($n = 53$) water for metagenomic analysis

Definitions



What is metagenomics?

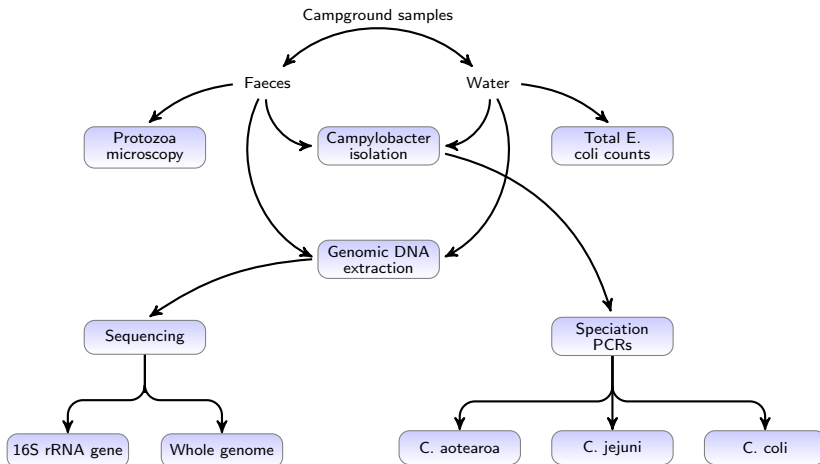
- Study of genetic material recovered directly from environmental samples i.e metagenomes
- Viewed as unbiased method for obtaining genes from all the members of sampled communities
- Commonly metagenomics involves analysis of:
 - Whole genome sequences
 - Gene sequences for components of ribosomal subunits e.g. 16S, 18S and 23S
- Powerful tool for viewing into the microbial macrocosm and factors that affect its composition

Definitions

Why analyse 16S genes?

- Ribosomes are found in all living cells and are primary sites of protein synthesis
- Ribosomes consist of two subunits (large and small)
- In bacteria and archaea the small ribosomal subunit contains 16S RNA component (16S rRNA)
- rRNA is essential for protein synthesis
- 16S rRNA genes (rDNA) code for 16S rRNA and are highly conserved between different species
- Hypervariable regions within 16S rRNA gene can provide species-specific signature sequences

Sample processing



Laboratory analysis

- Faeces - *Campylobacter*:
 - Broth enrichment for 48hrs
 - Selective growth on mCCDA for 48hrs
 - mCCDA growth subcultured on horse blood agar for 48hrs
 - DNA extracted from horse blood agar growth
 - *Campylobacter* speciation using PCR
- Faeces and water - *Cryptosporidium* and *Giardia*:
 - Mounting on microscope slide and staining
 - Fluorescence microscopy
- Water:
 - Water samples split into subsamples: 4x500mL (round 1 & 2) or 5x2L (round 3 & 4)
 - Each subsample filtered separately and filter stored at -80 °C
 - DNA extracted from each filter and stored at -20 °C
 - Multiplexed 16S rRNA gene and whole genome shotgun sequencing being performed



Laboratory analysis

Two methods of metagenomic DNA extraction:

- Water DNA extraction (Epicentre Biotechnologies kit):
 - Wash off microbes from filter
 - Break down cell wall with lysozyme
 - Degrade RNA with Rnase
 - Clean DNA using isopropanol
- Faecal DNA extraction (Macherey-Nagel soil kit):
 - Sample lysis by vortexing
 - Precipitate contaminants
 - Bind DNA
 - Clean DNA using wash buffer
 - Elute DNA



Campylobacter isolation

- *Campylobacter* PCR speciation of isolates from 669 faecal samples collected:
 - *C. jejuni* 9.0% (60/669)
 - *C. aotearoa* 9.1% (61/669)
 - *C. coli* 2.2% (15/669)
- *Campylobacter* PCR speciation of isolates from 103 water samples collected:
 - *C. jejuni* 5.8% (6/103)
 - *C. aotearoa* 1.9% (2/103)
 - *C. coli* 1.0% (1/103)

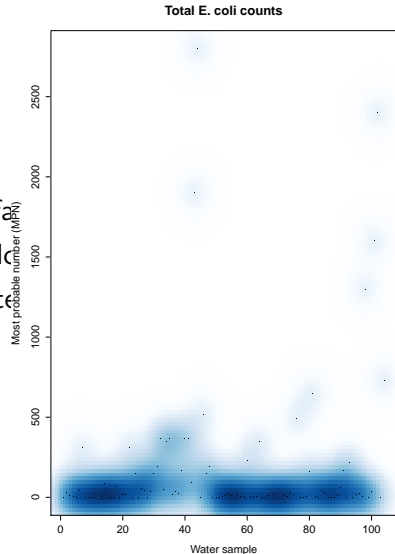
NB: *C. jejuni* & *C. aotearoa* co-detected in 1% (7/669) of faecal and 1.0% (1/103) water samples

Cryptosporidium & Giardia screening

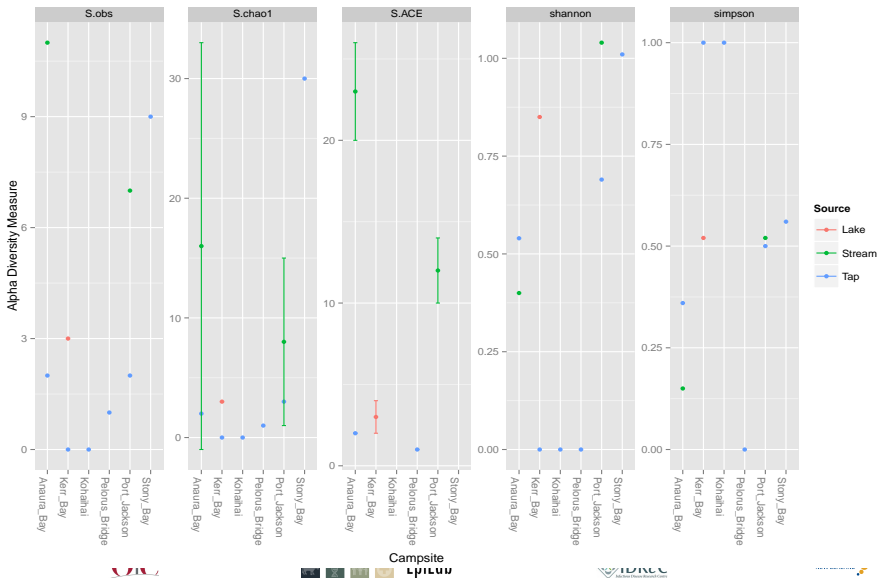
- *Giardia* oocysts were detected in 1.5% (10/669) faecal samples
- Nine samples were from ducks (single campground) and one from passerine
- Six duck samples were sequenced and genotyped
 - Five of the six samples were assemblage BIV; one BIII

Total *E. coli* counts in water samples

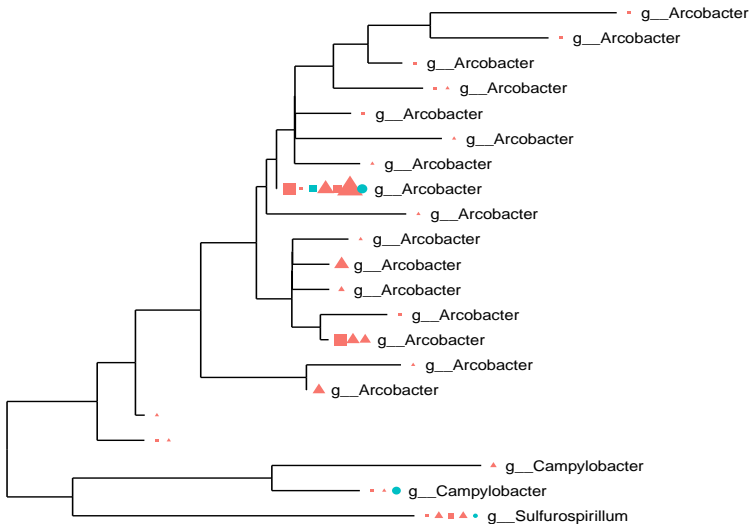
- *E. coli* is used as a proxy for faecal contamination
- Most campground water had low *E. coli* counts
- Eight samples had MPN greater than 1000



Family: Campylobacteraceae



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Source

- Lake
- ▲ Stream
- Tap

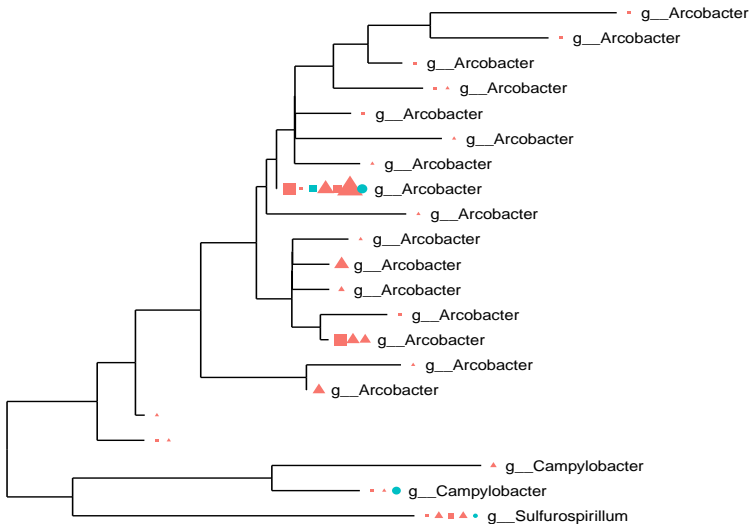
Abundance

- 1
- 25
- 625

Island

- North
- South

Family: Campylobacteraceae



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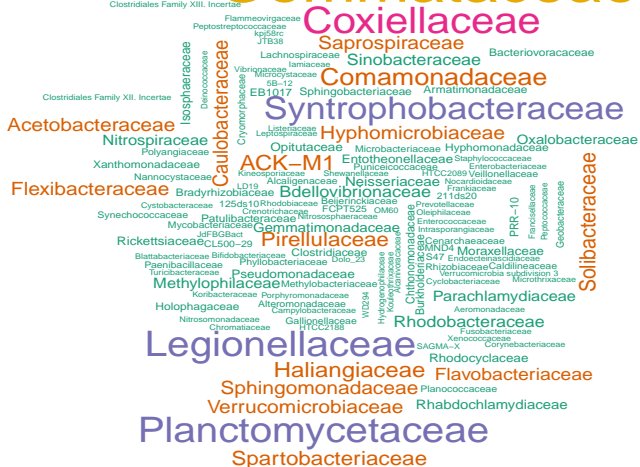
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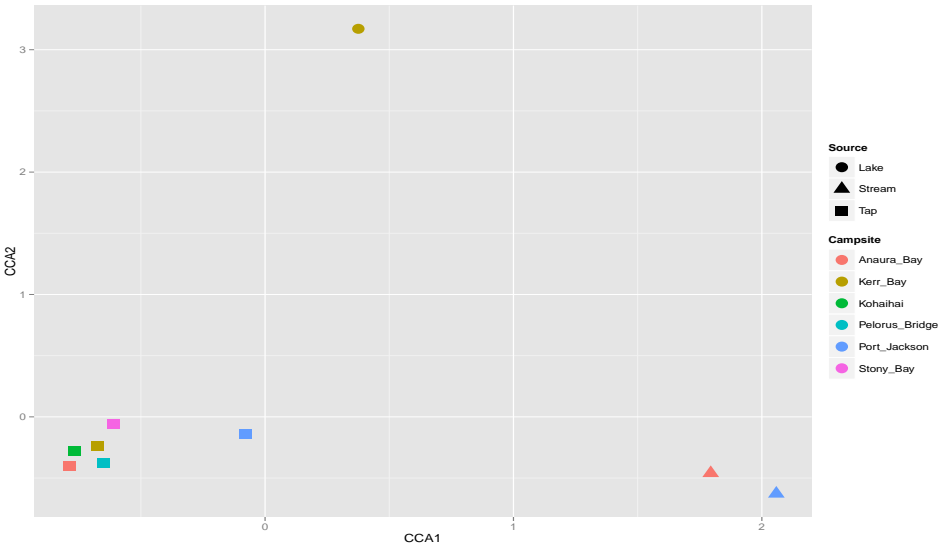
Tap water: top 10% most abundant taxa (Family)

Rhodospirillaceae

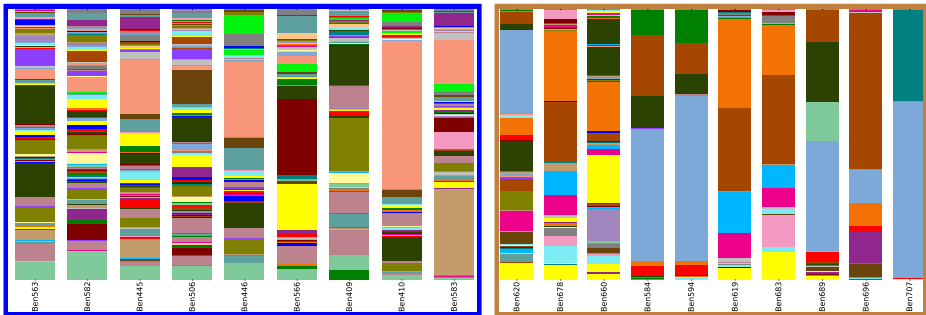
Gemmataceae



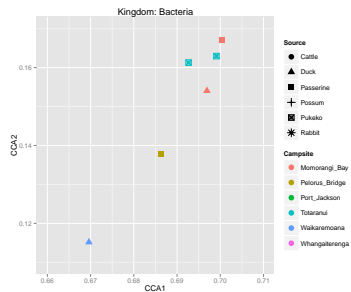
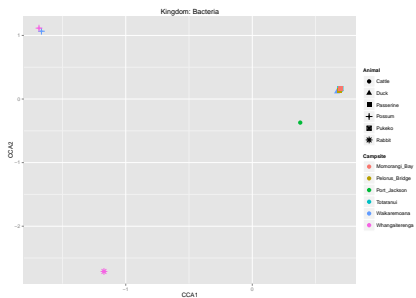
Family: Canonical correlation analysis



Word cloud



Word cloud



Total E.coli counts in water samples



Challenges

- Long driving distances: longest return trip > 1200km (18hrs of driving)
- >10kg of sample on poorly cleared track with slippery steep slope
- In 3rd and 4th rounds water protozoa screening was dropped because of load (>10kg water sample + 15kg filtering equipment)
- DNA extraction proved challenging:
 - Amount of water filtered per filtrate was increased from the recommended 0.1L to 2L (X20!)
 - 560 DNA extractions performed only 78 (14%) were sequenced for 16S rRNA gene; 63 (11%) for whole genome
- Step learning curving in dealing with large amounts of sequenced data (100TB received so far)



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