

Water quality, metagenomics and the microbial community in DOC campground water

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DEPE Workshop
Allan Wilson Centre
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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
- Promotes recreation, e.g. through provision of campgrounds
- General increase in waterborne gastroenteritis within NZ in past 20yrs: 18,000 – 34,000 cases per year



Increasing incidence of serious infectious diseases and inequalities in New Zealand: a national epidemiological study

Michael G Baker, Lucy Telfar Barnard, Amanda Kvalsvig, Ayesha Verrall, Jane Zhang, Michael Keall, Nick Wilson, Teresa Wall, Philippa Howden-Chapman

Lancet 2012; 379: 1112-19
Published Online
February 20, 2012

Summary

Background Although the burden of infectious diseases seems to be decreasing in developed countries, few national studies have measured the total incidence of these diseases. We aimed to develop and apply a robust systematic method for monitoring the epidemiology of serious infectious diseases.



^mEpiLab



Contents

- **Objectives**
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Objectives

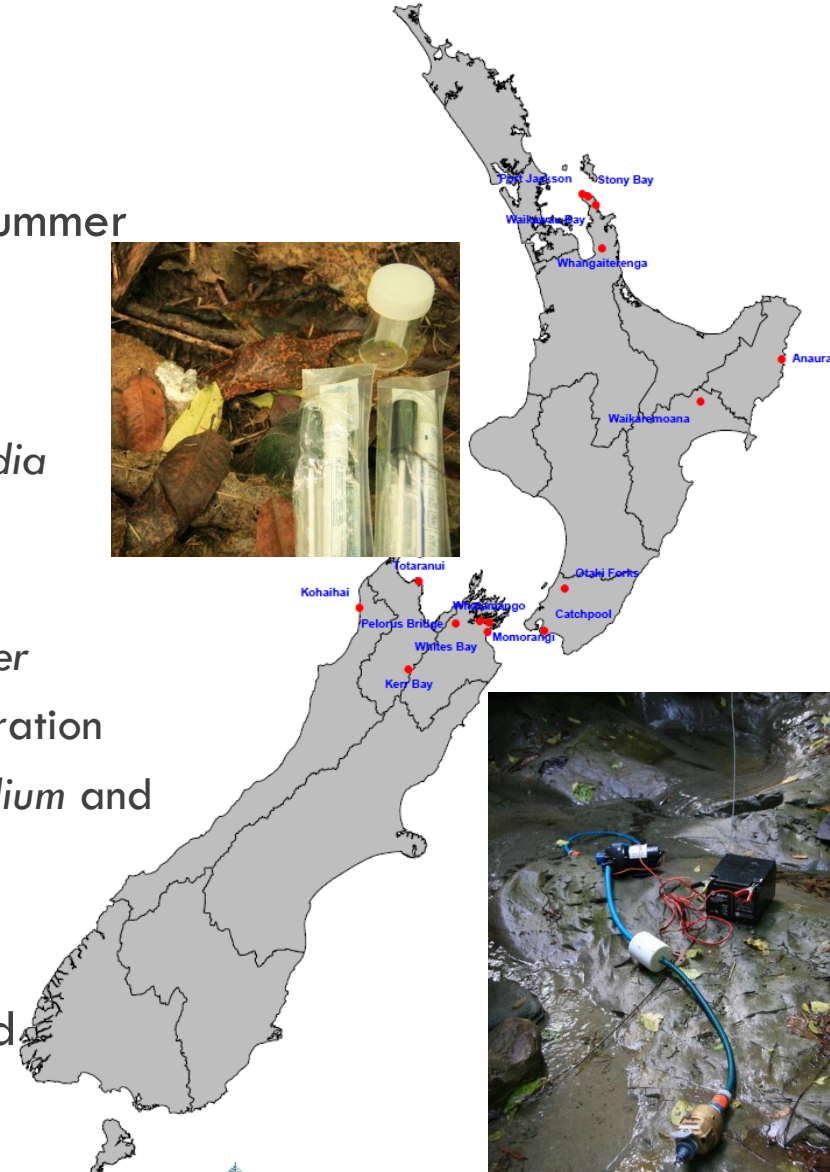


- Describe the metagenome of campground water
- Perform waterborne pathogen source tracking



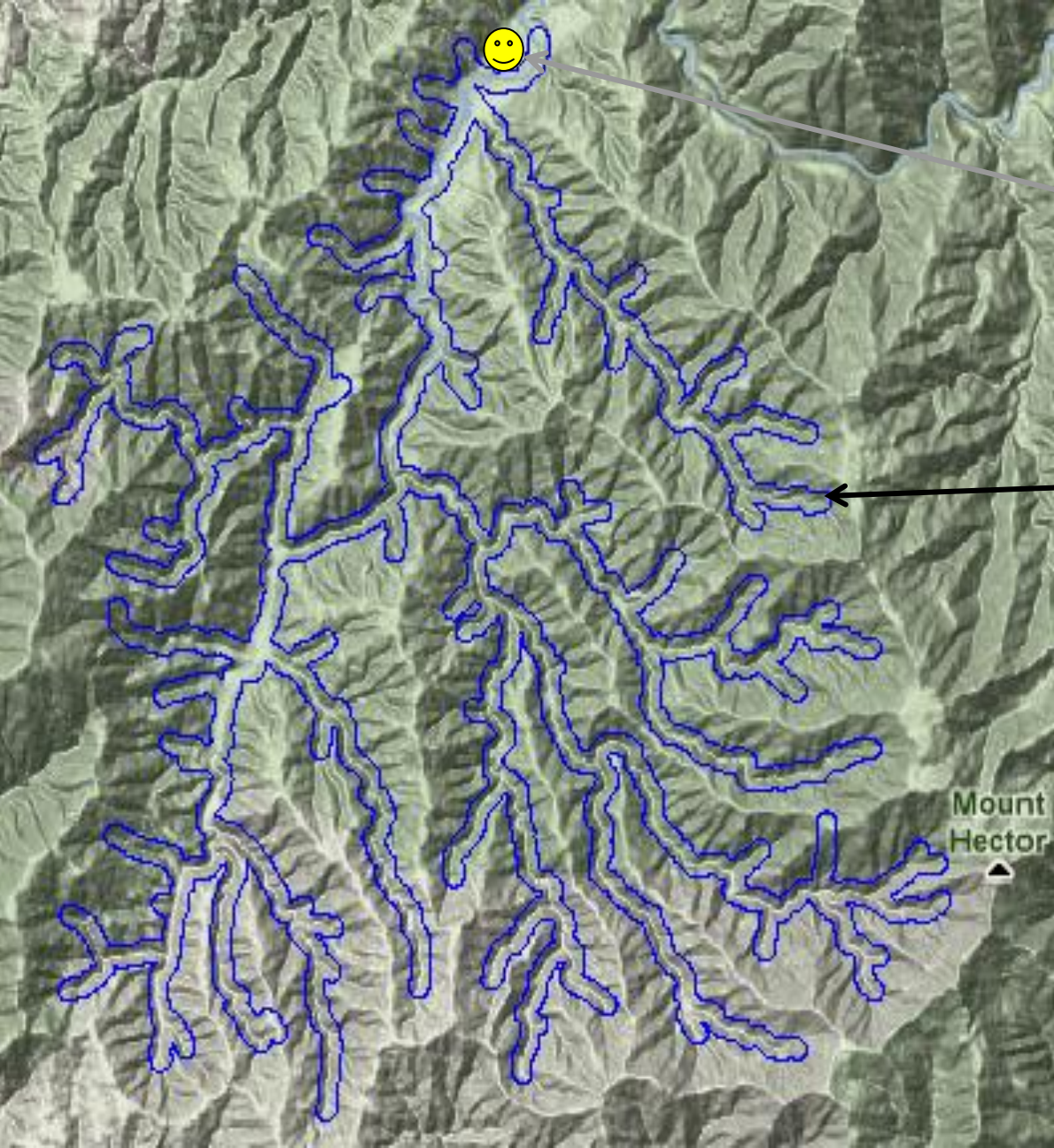
Materials

- 15 campgrounds managed by DOC
- Campgrounds to be sampled 4 times in 2 summer seasons
- Samples collected:
 - Faecal scoop for *Cryptosporidium* and *Giardia* screening
 - Faecal swab *Campylobacter* isolation
 - 100mL water for screening of *Campylobacter*
 - 100mL water for screening of *E. coli* enumeration
 - 100L water filtered on site for *Cryptosporidium* and *Giardia* screening
 - 2L water for metagenome analysis
- Physical characteristics of each campground catchment i.e. land cover, land use ...





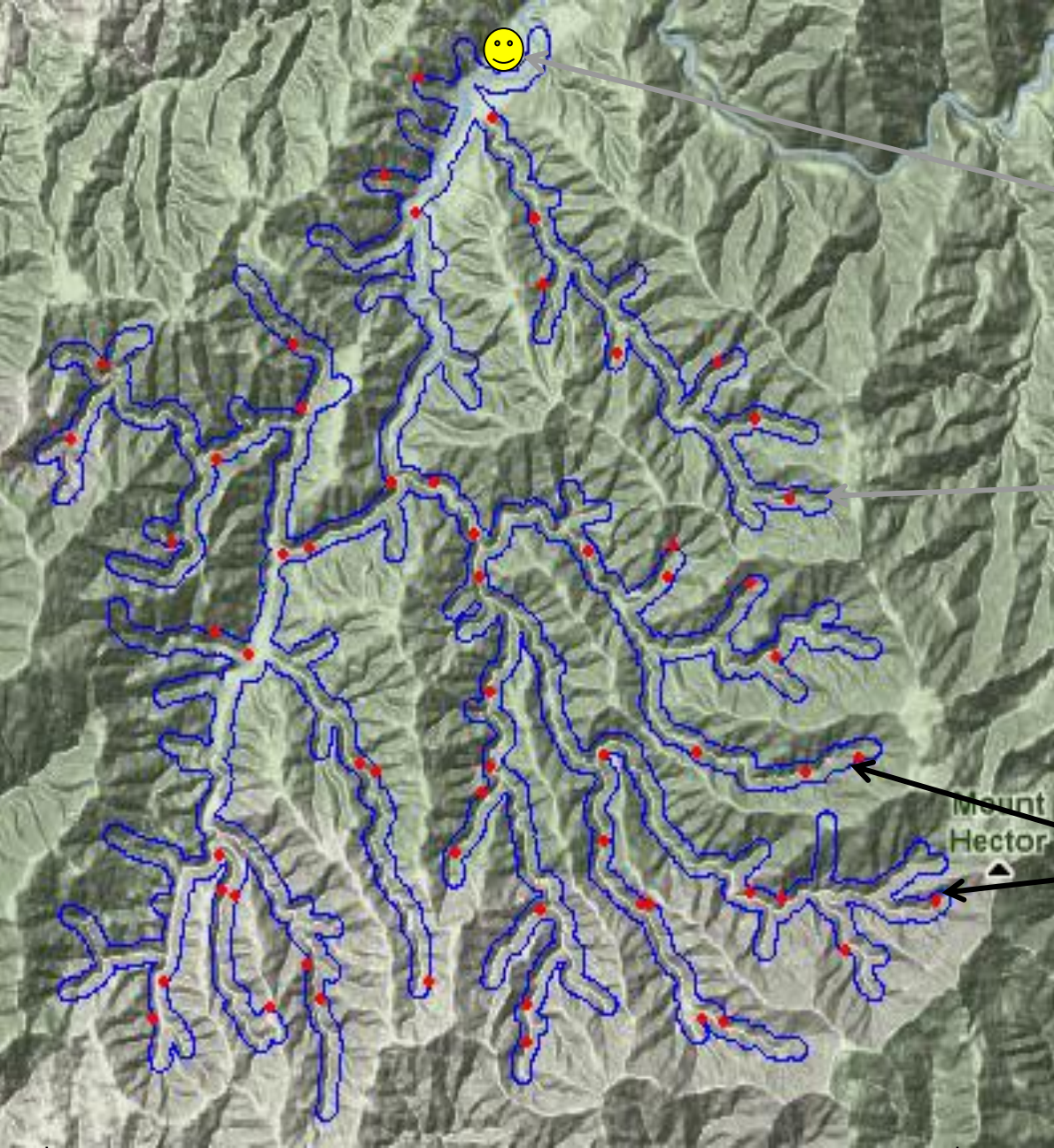
Otaki Forks campground



Otaki Forks campground

100-metre buffer

Mount Hector



Otaki Forks campground

100-metre buffer

10km

Sampling locations

Mount Hector

8km



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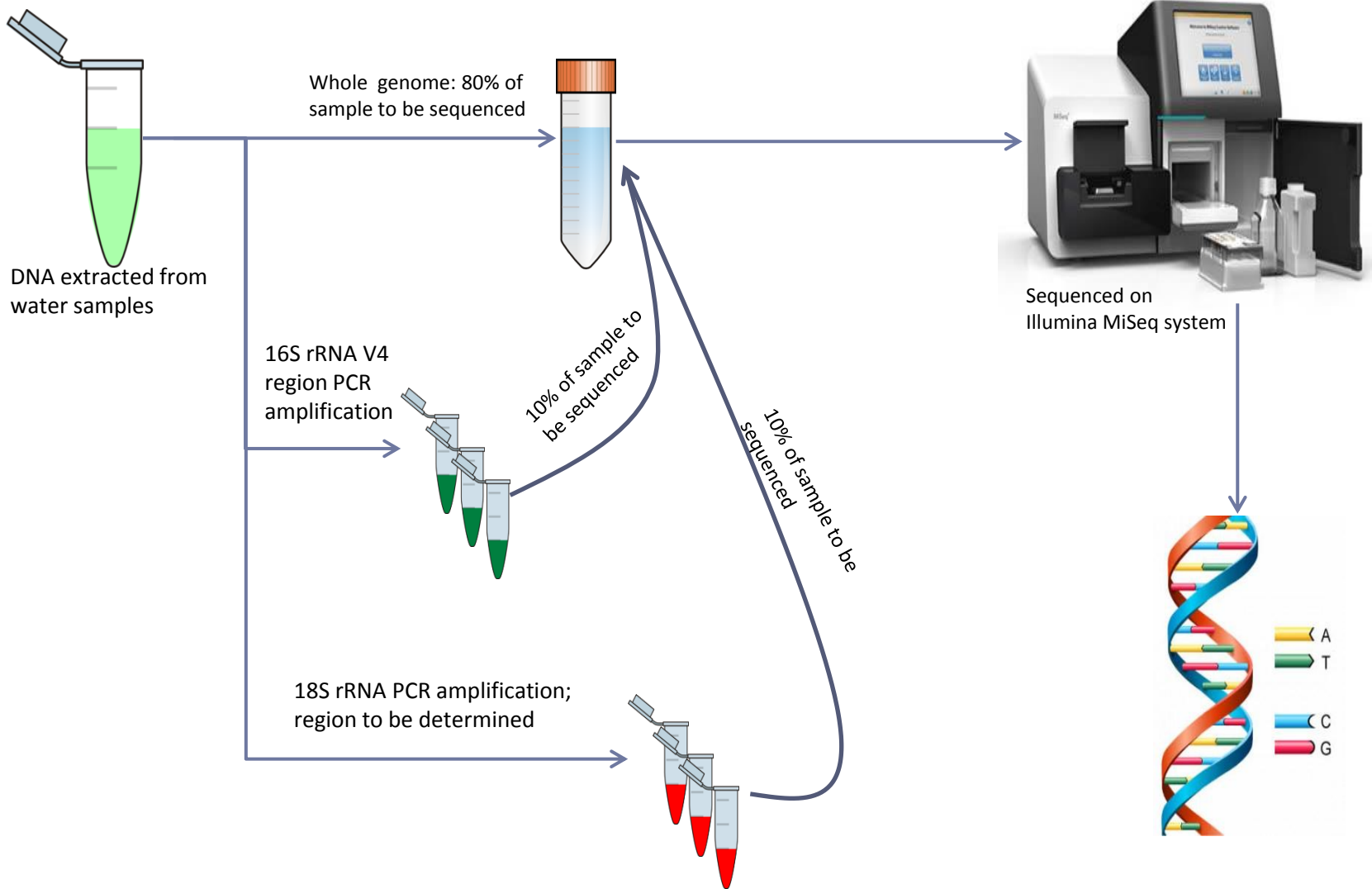


Lab work

- Faeces - *Campylobacter*:
 - Broth enrichment for 48hrs
 - Selective growth on modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) for 48hrs
 - mCCDA positive growth subcultured on horse blood agar for 48hrs
 - DNA extracted from horse blood agar growth
 - *Campylobacter* speciation using PCR
- Faeces & water - *Cryptosporidium* and *Giardia*:
 - Mounting on microscope slide and staining
 - Fluorescence microscopy
- Water:
 - 2litre sample split into 4x500mL subsamples
 - Each filtered separately and filter stored at -80°C
 - DNA extracted from each filter and stored at -20°C
 - Multiplexed 16S rRNA, 18S rRNA and whole genome shotgun sequencing to be performed



Lab work



Lab work

- DNA extraction:
 - Extraction kit from EPICENTRE:
 - Wash off microbes from filter
 - Break down cell wall with lysozyme
 - Degrade RNA with Rnase
 - Clean DNA using isopropanol
 - Issues with obtaining good quality DNA (260/280):
 - Amounts of DNA small in samples?
 - Degraded DNA in samples?
 - Kit not good enough? Will try Zymo kit



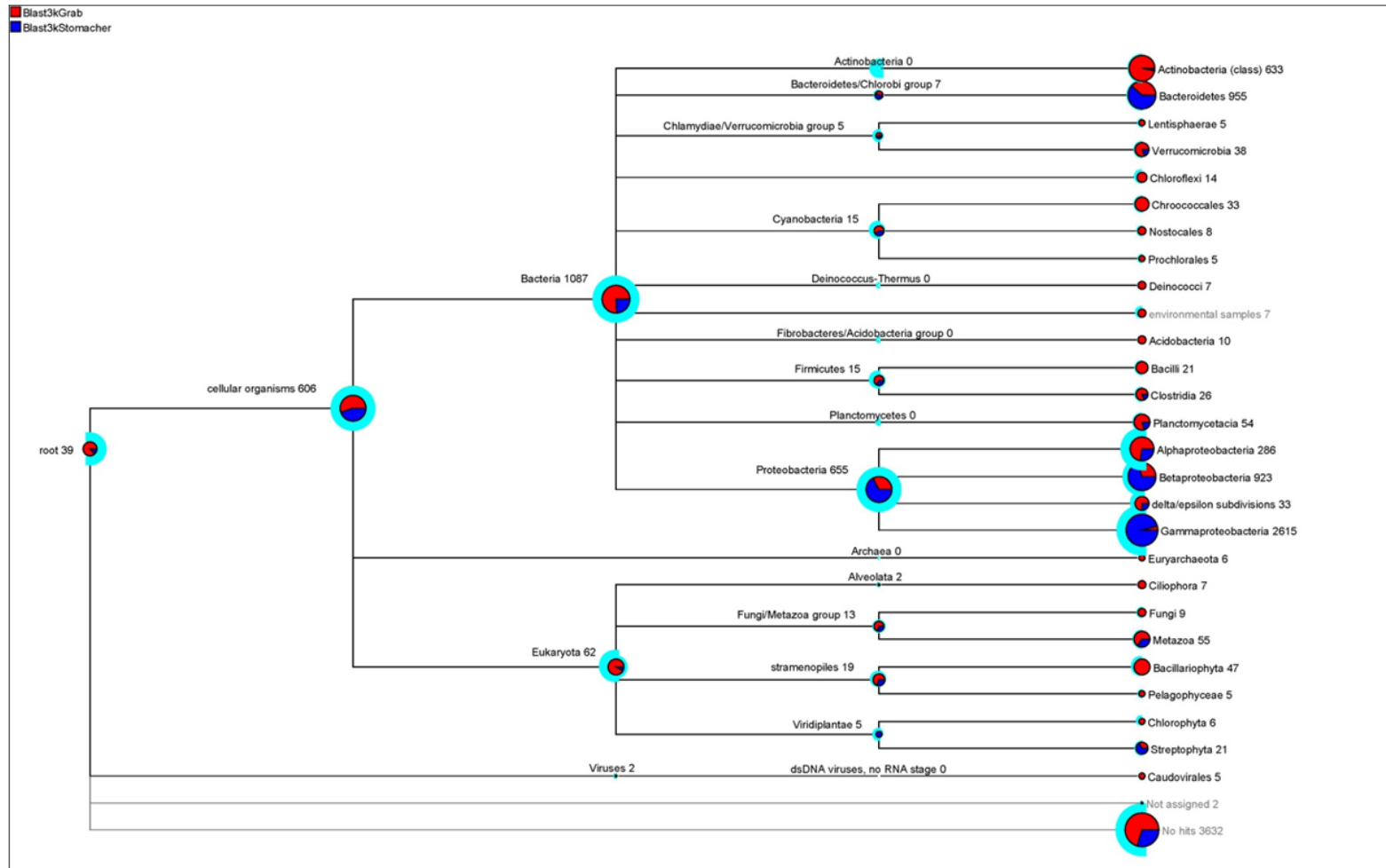
Preliminary findings

Number of *Campylobacter* positive samples on DOC campgrounds during 2011/2012 summer season

	Campground	DOC Region	Campground Type	No of Visits	Faecal Samples	Campy +ve	Water samples	Campy +ve
1	Anaura Bay	East Coast	Recreation Reserve	2	30	14	4	0
2	Catchpool	Wellington	Forest Park	2	13	2	4	0
3	Kerr Bay	Marlborough-Nelson	National Park	1	14	0	2	0
4	Kohaihai	Marlborough-Nelson	National Park	1	10	9	1	0
5	Momorangi	Marlborough-Nelson	Recreation Reserve	2	19	2	4	0
6	Otaki Forks	Wellington	Forest Park	2	26	0	4	0
7	Pelorus Bridge	Marlborough-Nelson	Scenic Reserve	2	20	3	4	1
8	Port Jackson	Port Jackson	Recreation Reserve	1	10	1	2	1
9	Stony Bay	Port Jackson	Recreation Reserve	1	17	10	2	1
10	Totaranui	Marlborough-Nelson	National Park	2	29	10	6	0
11	Waikaremoana	East Coast	National Park	2	35	1	4	0
12	Waikawau	Coromandel	Recreation Reserve	2	18	0	4	0
13	Whangaiterenga	Coromandel	Forest Park	2	13	3	4	0
14	Whاتمango	Marlborough-Nelson	Recreation Reserve	2	19	9	2	1
15	Whites Bay	Marlborough-Nelson	Recreation Reserve	1	11	1	2	0
	Total			27	284	65	49	4

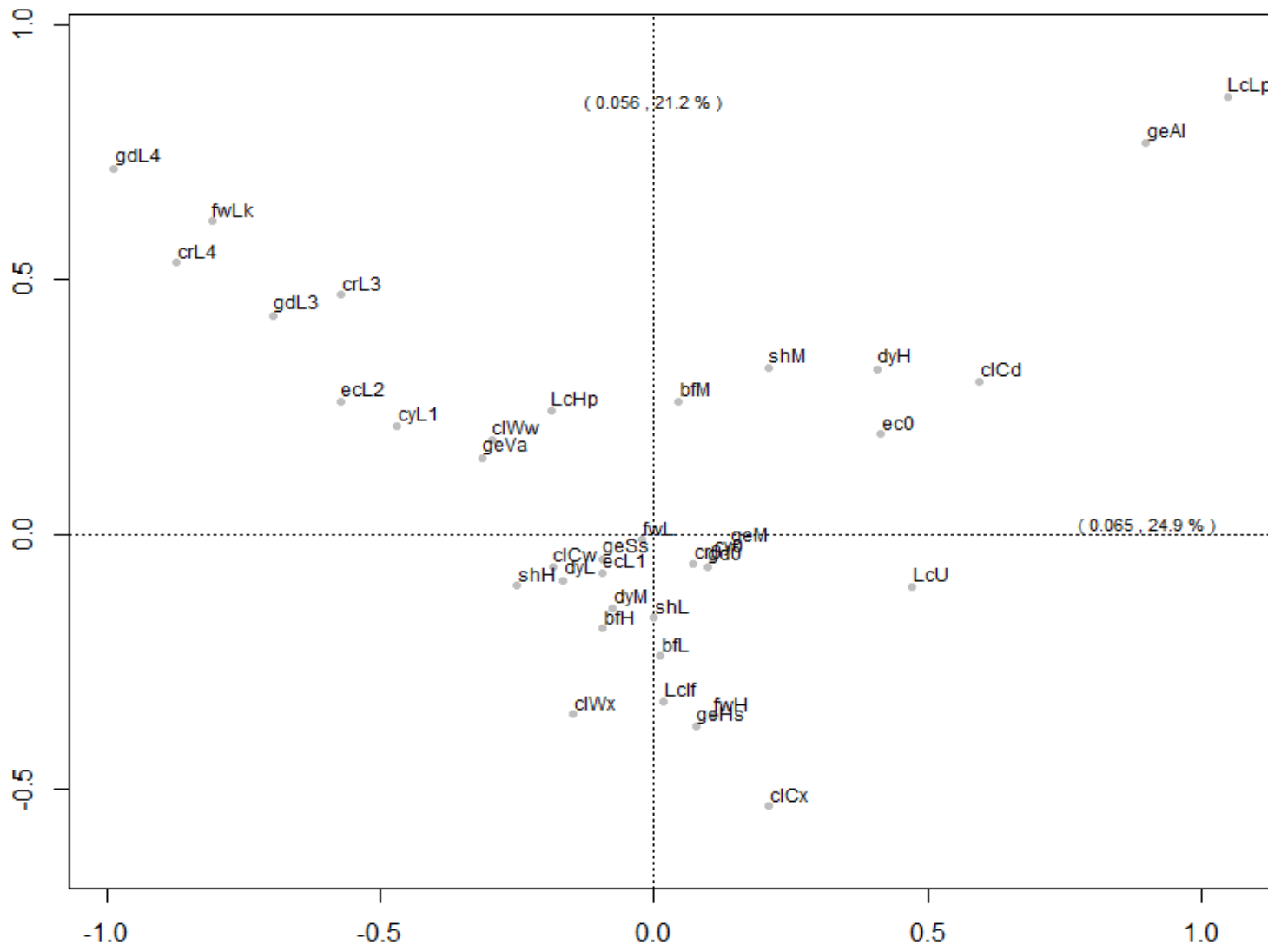
Expected metagenomic analysis

MEGAN output: 'Grab' vs 'Stomacher' samples



Other Work

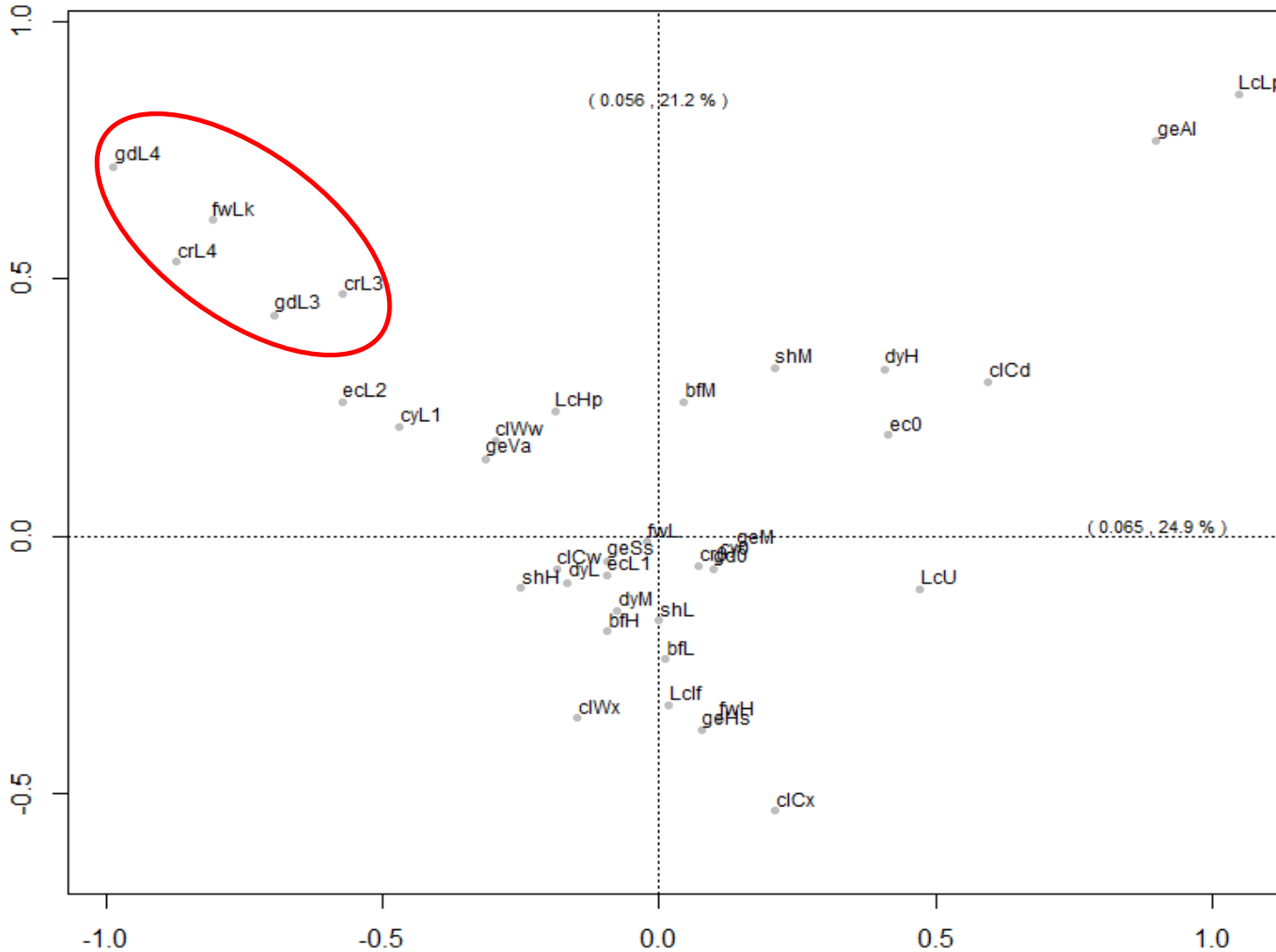
MCA map of catchment variables on 1st and 2nd principal axes



- KEY**
- cr: *Cryptosporidium* (4 levels)
 - gd: *Giardia* (4 levels)
 - ec: *E. coli* (3 levels)
 - cy: *Campylobacter* (2 levels)
 - Lc: Land cover (4 levels)
 - fw: Source of Flow (3 levels)
 - ge: Geology (5 levels)
 - cl: Climate (5 levels)
 - sh: Sheep (3 levels)
 - bf: Beef cattle (3 levels)
 - dy: Dairy cattle (3 levels)

Other Work

MCA map of catchment variables on 1st and 2nd principal axes

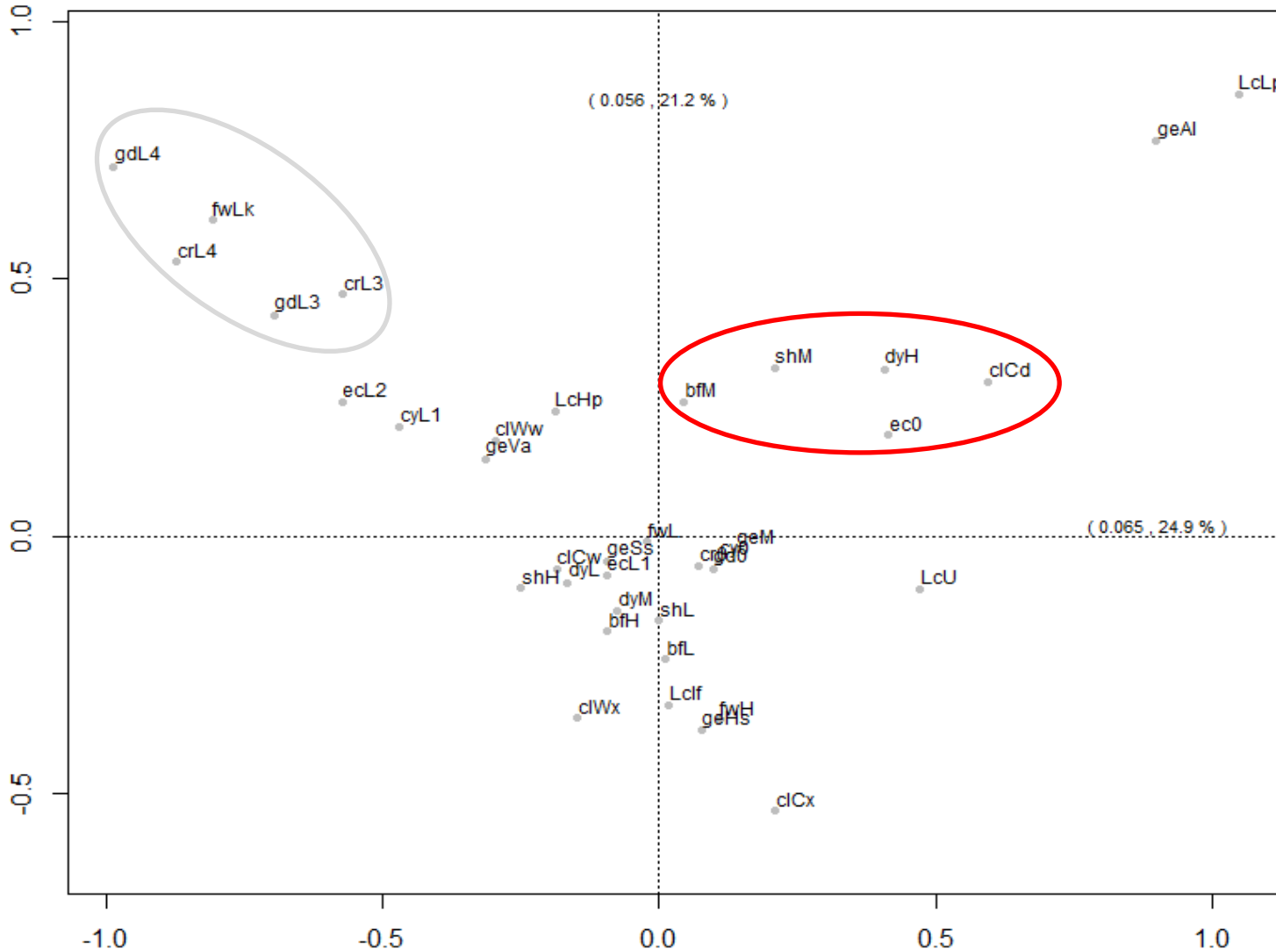


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Thank you

Funding:

Allan Wilson Centre
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