Microbial Source Tracking at Berry Springs Pools

Water Resources Division
Technical Report
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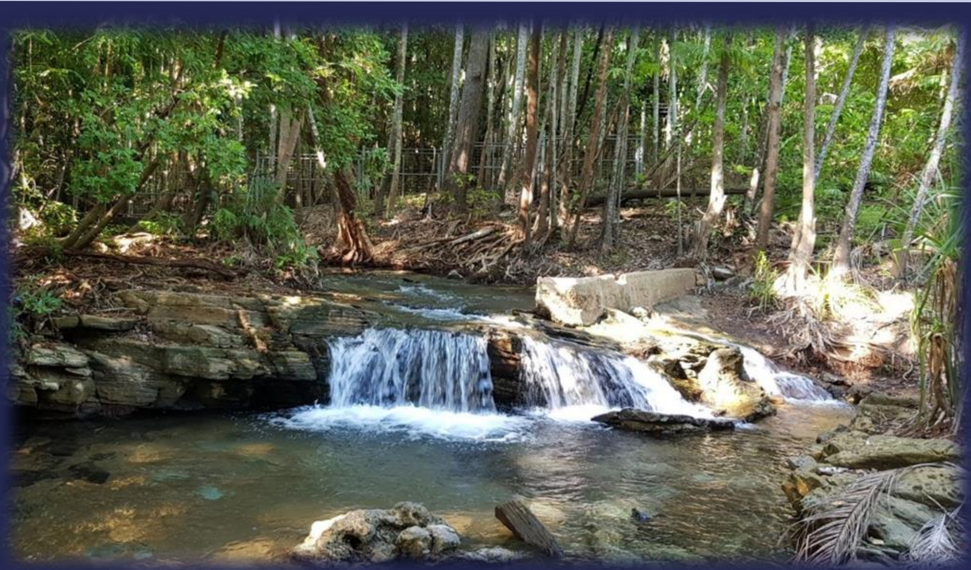
Berry Springs Pools – what is the problem?



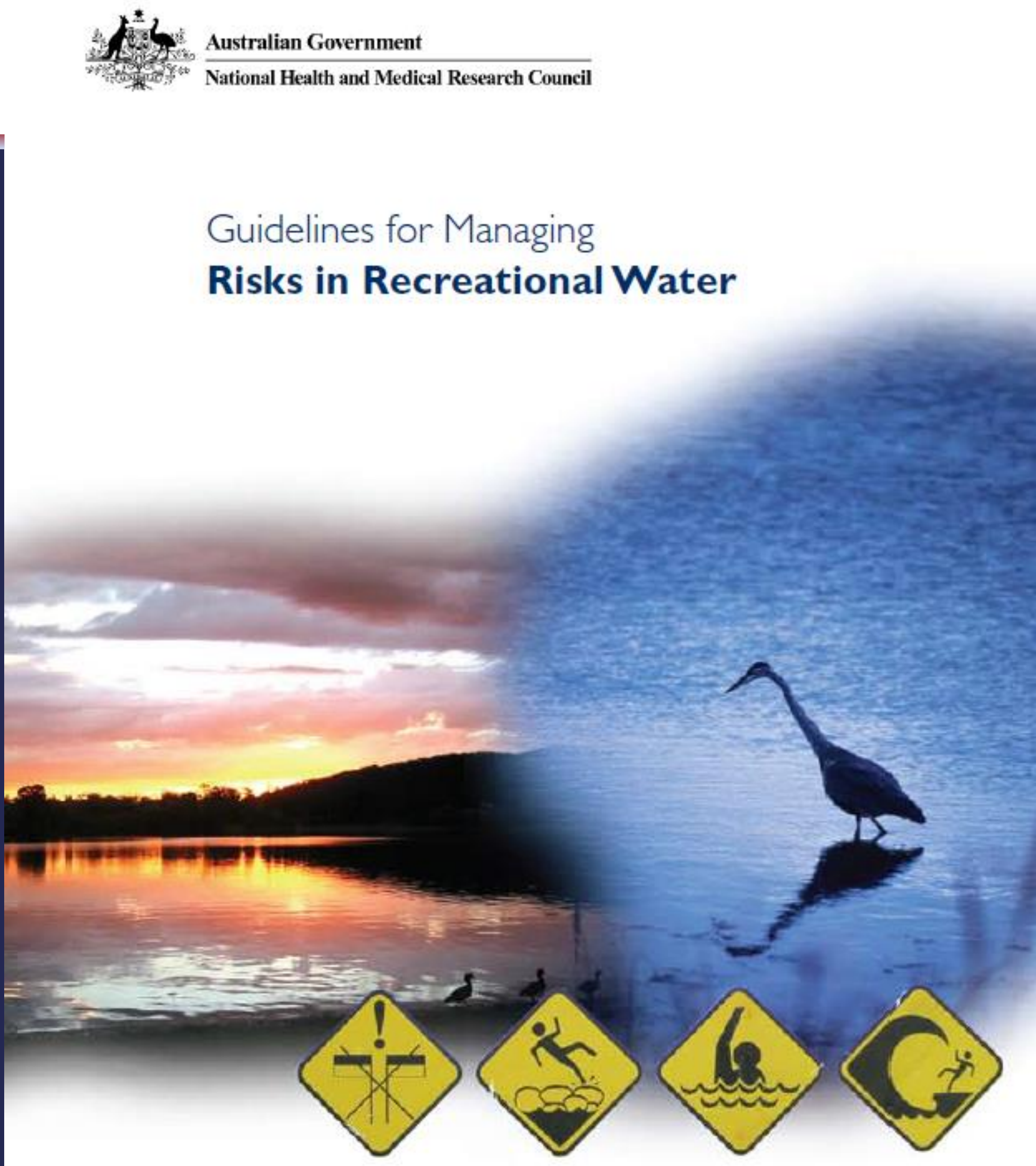
- Microbiological water quality fluctuates with low water quality forcing temporary pool closures
- The reasons for fluctuations are not clear

- Microbiological water quality is assessed using bacterial faecal indicators i.e. measuring enterococci levels

Routine microbial water quality monitoring for recreational waters

NHMRC Guidelines for Managing Risks in Recreational Water (2008)

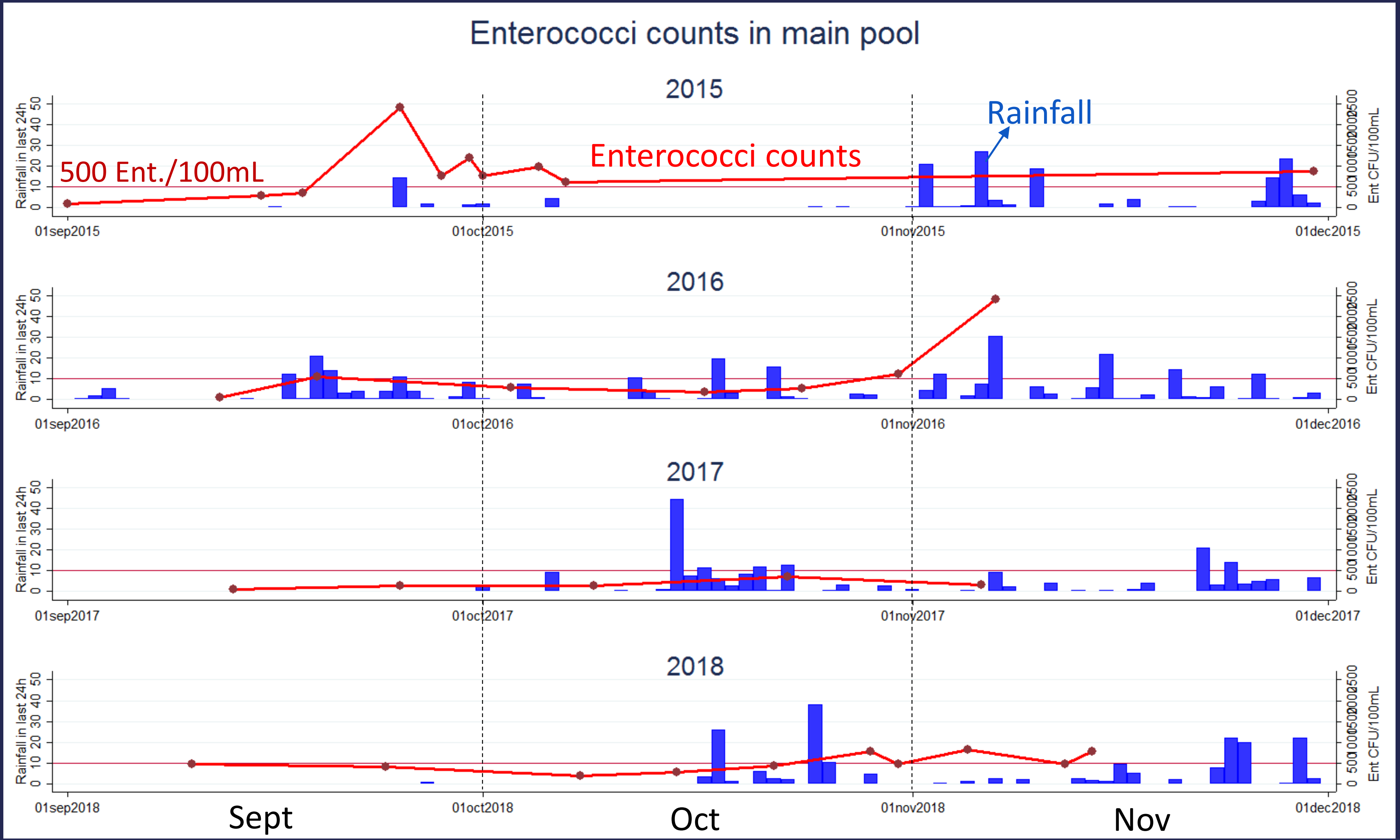
- *“Faecal indicators are organisms that act as surrogates for potential pathogens (disease-causing organisms) associated with faecal contamination.”*
- *“Enterococci are currently considered the most suitable indicator for both marine and fresh recreational water.”*



Australian Government
National Health and Medical Research Council

Guidelines for Managing
Risks in Recreational Water

Enterococci levels fluctuate during build-up with peaks above 500 CFU/100mL forcing temporary pool closures



Previous work on enterococci levels in Berry Springs pools

- **NTG report 1967**
  - Elevated enterococci levels at main pool particularly in mornings
  - Leak in clay pipes btw toilets + septic?
- **NTG report 1992**
  - Highest enterococci levels in build up
  - Significant correlation btw enterococci + enteric pathogen salmonella in pools
  - Source native fauna? ➔ same salmonella serovars as from wildlife + different to humans
- **NTG report 2010**
  - Management recommendations and discussion of potential sources:
    - Rainfall associated i.e. land runoff containing wildlife faecal matter incl. from bats?
    - Point sources incl. septic tank/pipes, swimmers?
    - Groundwater contaminated by septic tanks?
    - Wildlife Park runoff from washing down of enclosures?

Berry Springs Source Tracking Project - Outline

Overall Aim was to compare microbial contributions to pools from different sources to help identify sources of enterococci

1. **Statistical multivariable analysis of existing enterococci data from 2010 to 2017**

2. **Collect new data in 2018**
   - Water samples from 10 sites in Berry Springs Nature Park and Wildlife Park
   - Five sampling rounds from May to Nov 2018

Berry Springs Source Tracking Project – Outline cont.

- Water collection for:
  - **Water Chemistry** (*ECMU*)
    - Water stable isotopes
    - Elemental fingerprinting analysis (semi-quant method)

    ➔ compare aquifer vs rainfall/surface water contributions
  - **Enterococci culture** (*Berrimah Labs*)
  - **Molecular microbiology**
    - Extract microbial DNA from water (*ECMU*) to
      - sequence bacterial DNA (16s rRNA gene tag) (*ACE*)

      ➔ compare microbial contributions from different sources to pools (Bayesian source tracking)
      - perform faecal indicator PCRs (*B.theta*, Hf183) (*ECMU*)

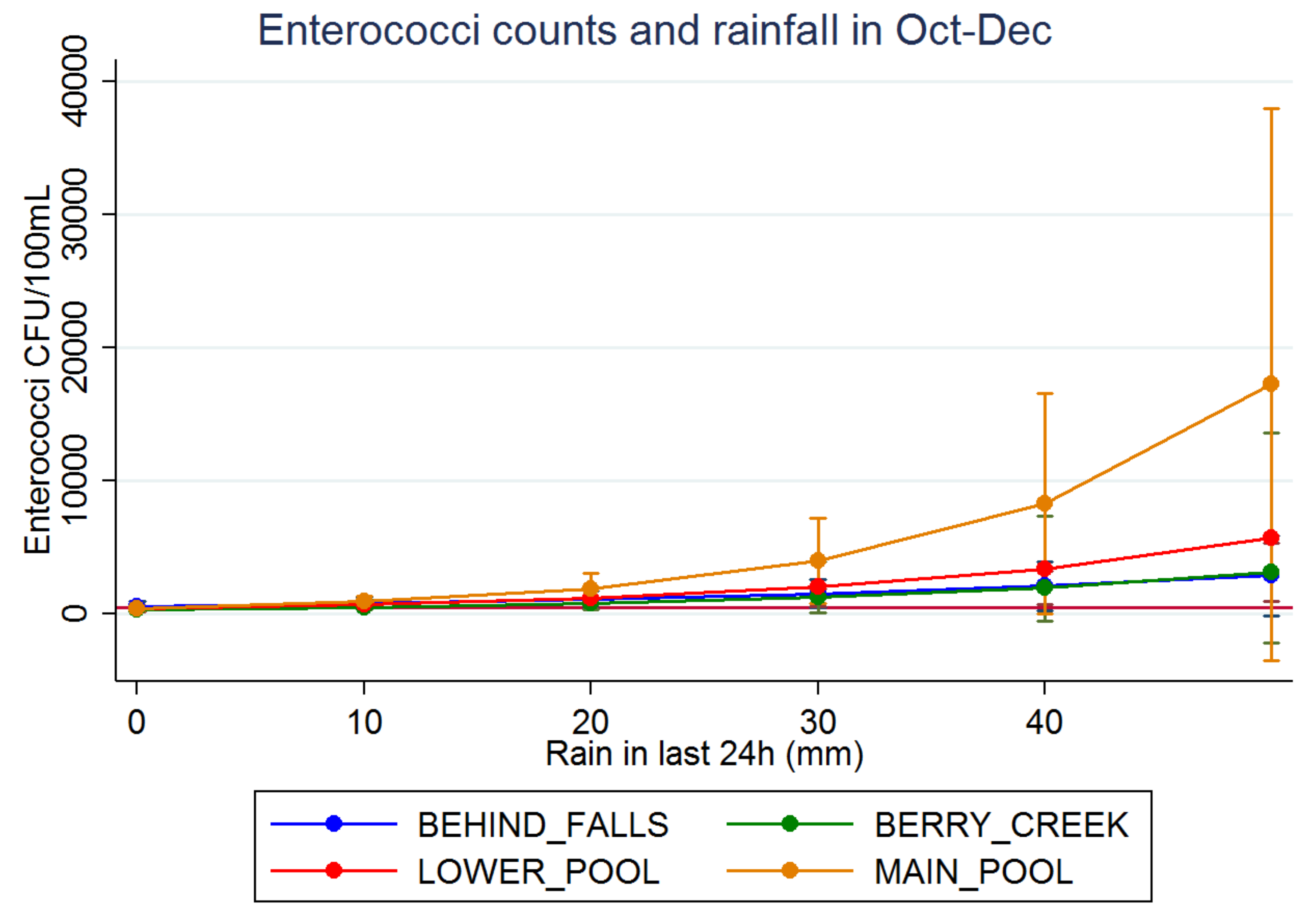
      ➔ detect DNA of faecal indicators which are more human specific than enterococci
  - **Data Analysis** (*ECMU*)
    - Bayesian source tracking + model to associate enterococci counts with biotic and abiotic factors

1. Results of enterococci data analysis, 2010 to 2017

The model indicates that:

- The build-up has the highest enterococci counts
- The more rain, the higher the enterococci counts
- Main Pool strongest increase in counts with more rainfall
- Berry Creek least increase

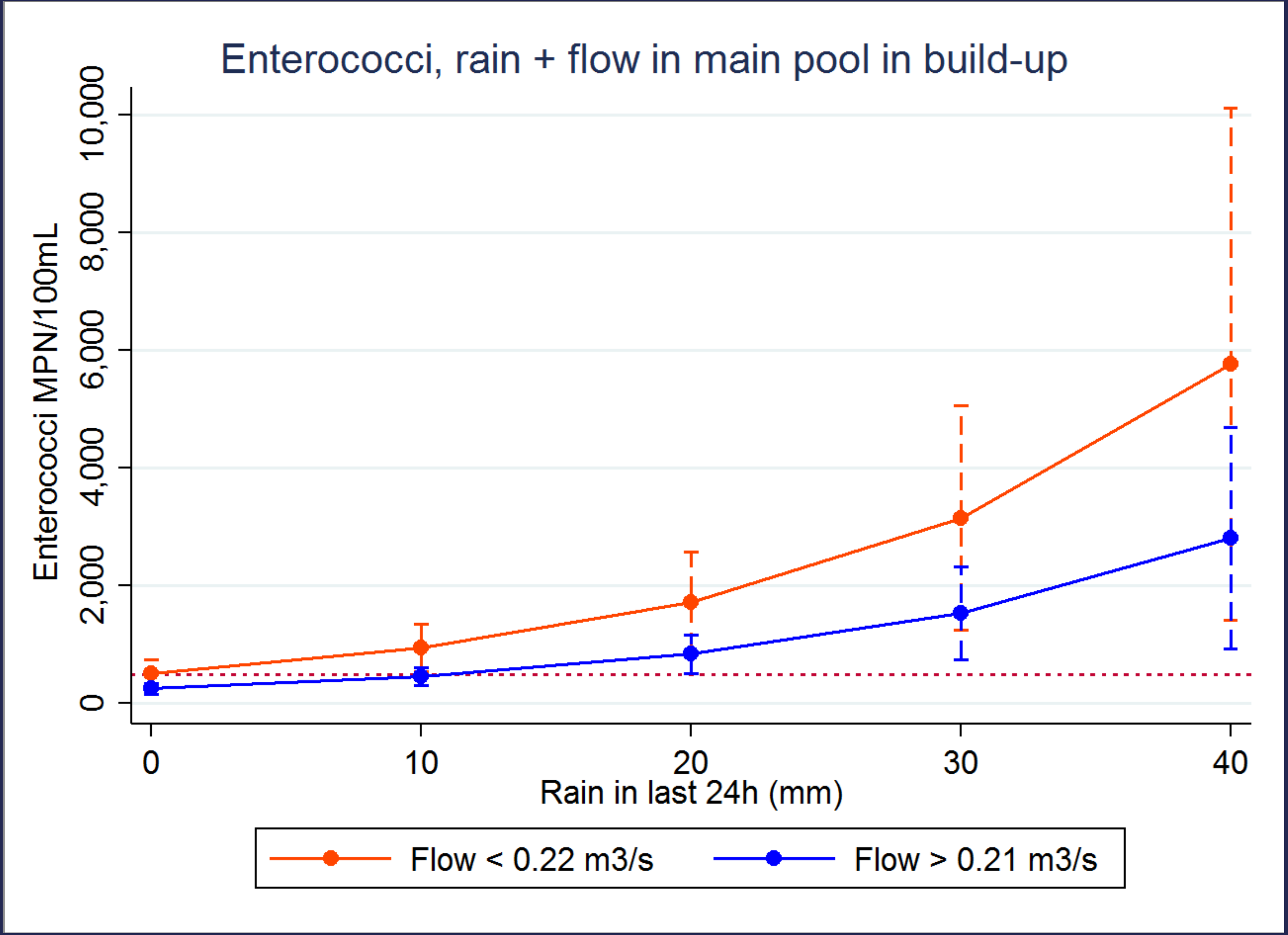
Negative binomial multivariable model in Stata-14 with outcome enterococci counts and predictors quarter (P<0.05), flow cat (P<0.05), site (P<0.05) & rainfall in last 24h (centred at mean)(P<0.001) incl. interactions rain x sites (P<0.05); robust standard errors accounting for years



1. Results of enterococci data analysis, 2010 to 2017 *cont.*

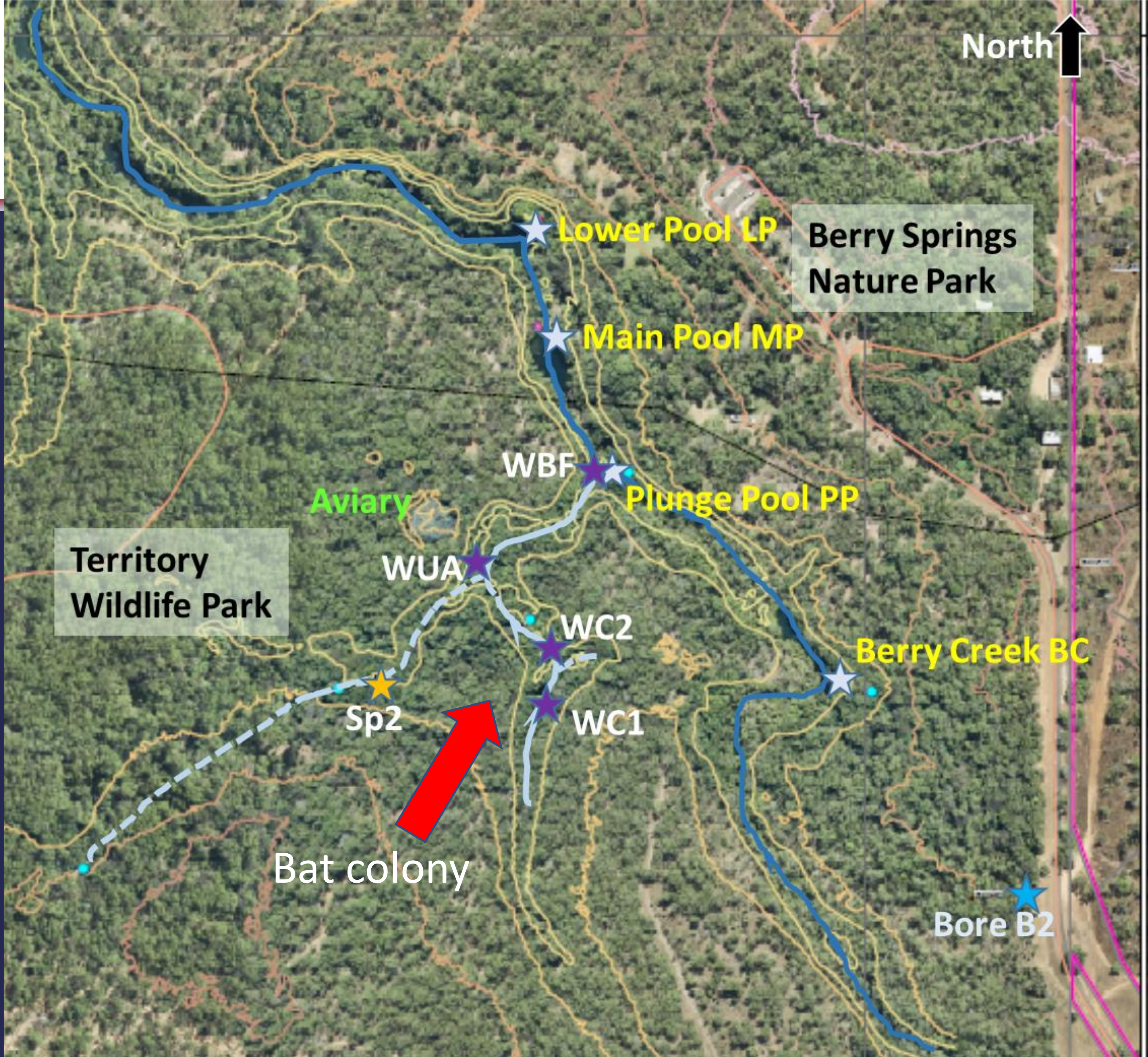
The stronger the flow or the more previous rain events, the less enterococci after rain

→ System gets flushed with more rain and flow



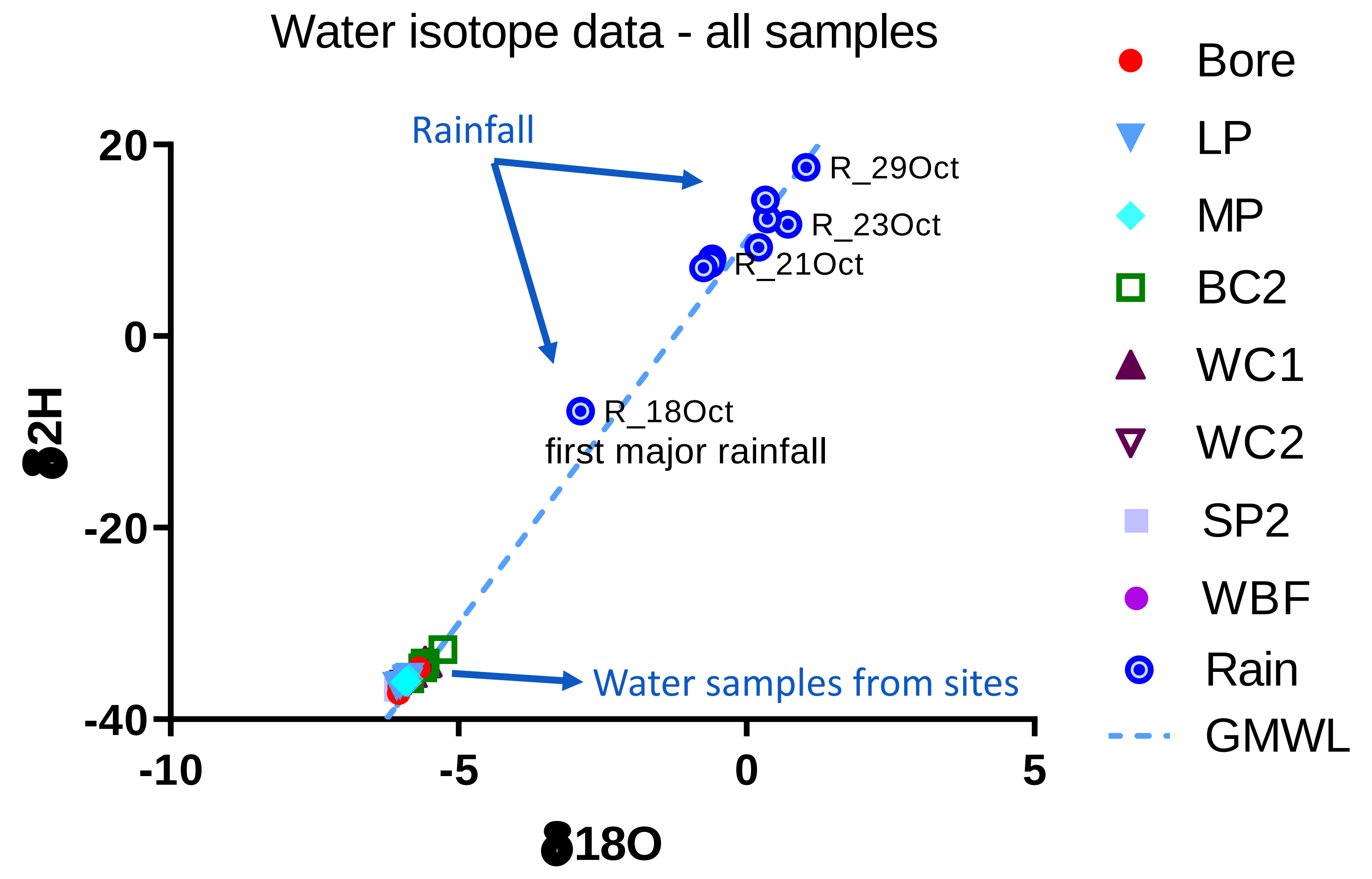
2. New Data Collection

- **1st round: 1 May 2018**
  - before opening of pools
  - only time Sp2, Berry Ck under bridge sampled ➔ dry after that
- **2nd round: 5 Sept 2018**
  - no rain yet
- **3rd round: 15 Oct 2018**
  - no rain yet
  - bats have arrived
- **4th round: 18 Oct 2018**
  - 12 hours after 1st rain (23mm)
- **5th round: 19 Nov 2018**
  - day after closure of pools
  - only time PP sampled



Results – Water Chemistry
Stable Water Isotopes ($\delta^{18}O$ and $\delta^{2}H$)

- Only small + scattered rainfall events Sept-Nov 2018


Water isotope data - all samples

Rainfall

R_18Oct
first major rainfall

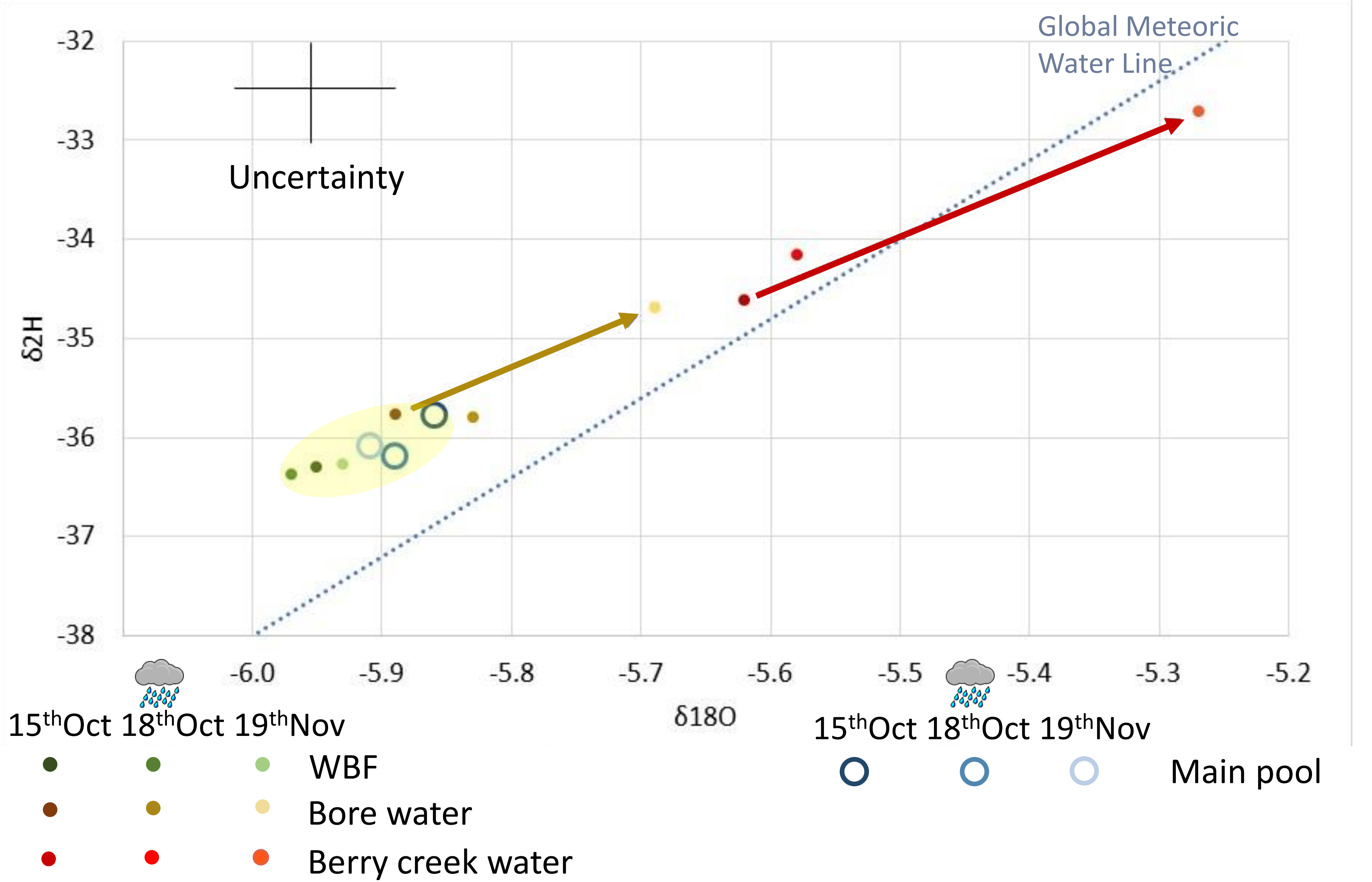
Water samples from sites

Results – Water Chemistry
Stable Water Isotopes ($\delta^{18}O$ and $\delta^{2}H$)

- Water isotopes from WBF + pools hardly changed with rainfall ➔ within assay uncertainty

- Berry creek changed with rainfall

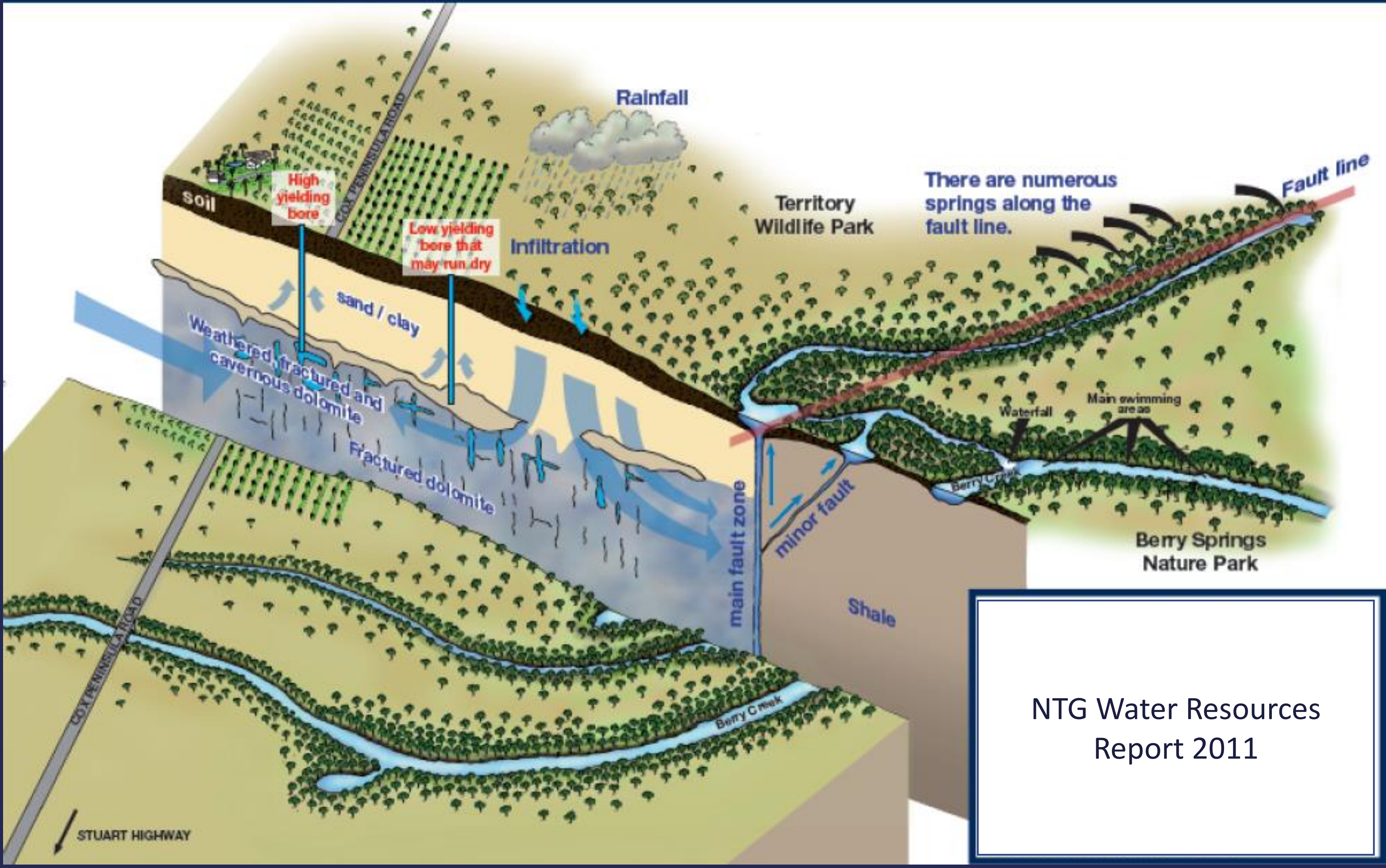
- Bore (RN009485- 18m depth) changed with rainfall ➔ leak along casing or because bore accesses aquifer in dolostone?



Berry Springs Aquifer

Pools fed by

- Berry Creek in dolomite
- Creek from Wildlife Park forest ➔ springs due to water forced to surface along fault line btw dolomite (south) + shale (north)

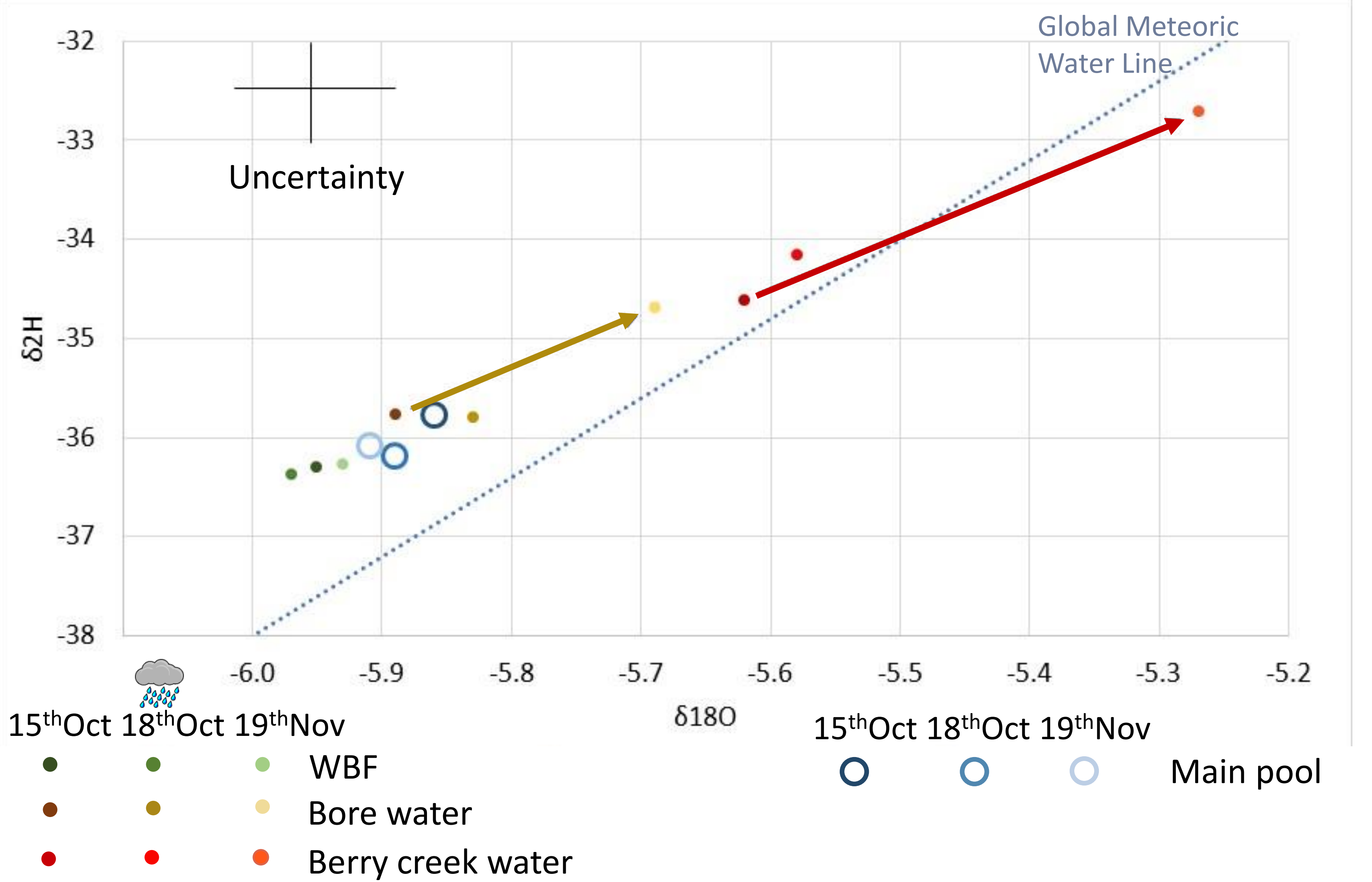


NTG Water Resources Report 2011

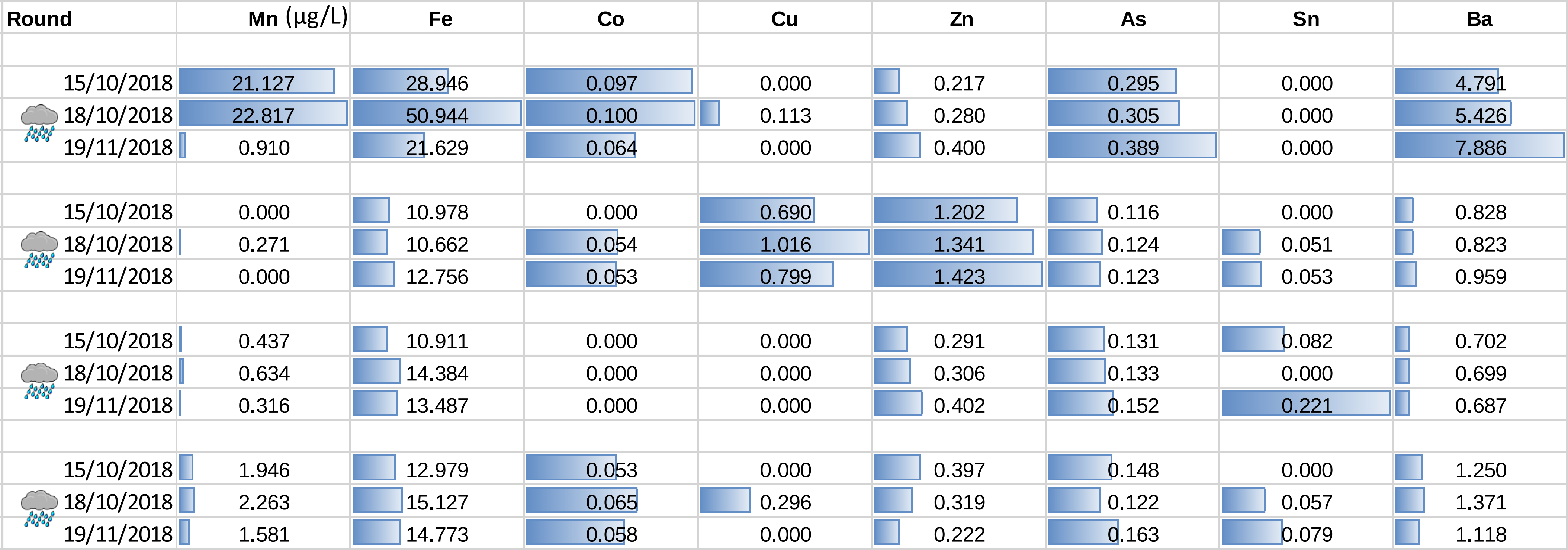
Results – Water Chemistry
Stable Water Isotopes ($\delta^{18}O$ and $\delta^{2}H$)

- Isotopes from WBF + pools hardly changed with rainfall
- Bore + Berry ck changed with rainfall

➔ Mass balance suggests that pools mainly fed by water from Wildlife Park creek/springs



Results – Water Chemistry
Elemental fingerprinting: selected elements



	Round	Mn (µg/L)	Fe	Co	Cu	Zn	As	Sn	Ba
Berry Creek	15/10/2018	21.127	28.946	0.097	0.000	0.217	0.295	0.000	4.791
	🌧 18/10/2018	22.817	50.944	0.100	0.113	0.280	0.305	0.000	5.426
	19/11/2018	0.910	21.629	0.064	0.000	0.400	0.389	0.000	7.886
Bore water	15/10/2018	0.000	10.978	0.000	0.690	1.202	0.116	0.000	0.828
	🌧 18/10/2018	0.271	10.662	0.054	1.016	1.341	0.124	0.051	0.823
	19/11/2018	0.000	12.756	0.053	0.799	1.423	0.123	0.053	0.959
WBF	15/10/2018	0.437	10.911	0.000	0.000	0.291	0.131	0.082	0.702
	🌧 18/10/2018	0.634	14.384	0.000	0.000	0.306	0.133	0.000	0.699
	19/11/2018	0.316	13.487	0.000	0.000	0.402	0.152	0.221	0.687
Main Pool	15/10/2018	1.946	12.979	0.053	0.000	0.397	0.148	0.000	1.250
	🌧 18/10/2018	2.263	15.127	0.065	0.296	0.319	0.122	0.057	1.371
	19/11/2018	1.581	14.773	0.058	0.000	0.222	0.163	0.079	1.118

Berry Creek: More Mn, Fe, Ba (high in clays)

➔ Mn, Fe redox sensitive i.e. more soluble under anoxic conditions when water stagnant

Bore water: More Cu, Zn ➔ casing?

➔ Mass balance based on Ba also supports that springs contributed large amount to pools

Water Chemistry - Conclusions

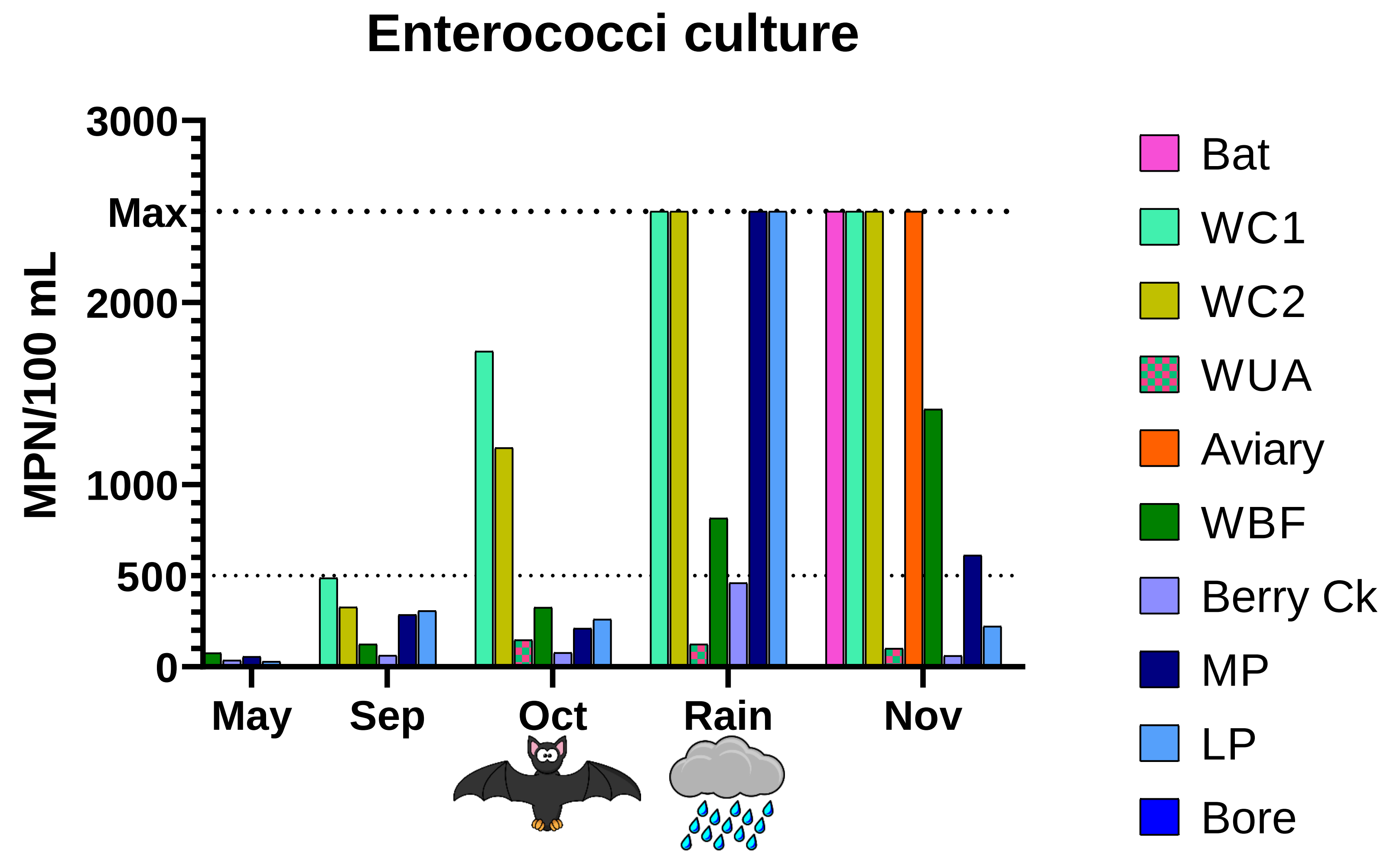
- Rainfall events were small and spread over 11 days

→ rain likely evaporated or retained in soil and did not contribute significantly to pools

- Precise mass balance based on stable isotopes not possible as only small differences

- Mass balances from both, water stable isotopes and element Ba support the pools being mainly spring-dominated before and after these rainfall events

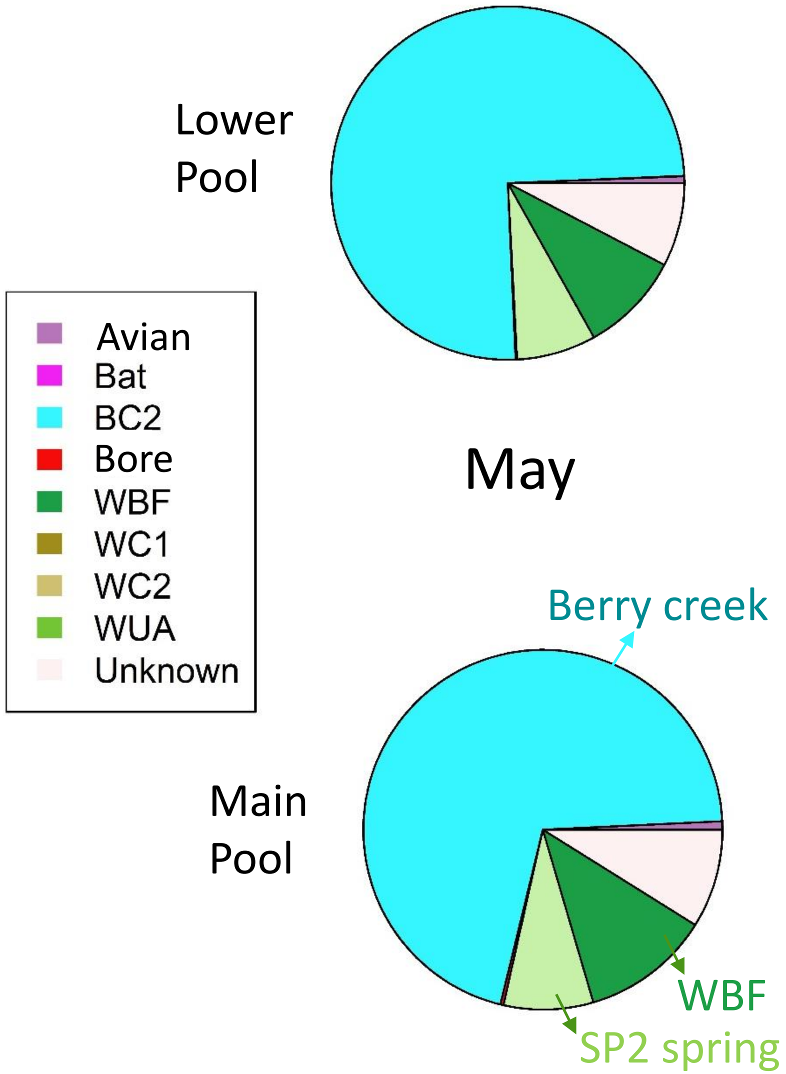
Results – Enterococci culture



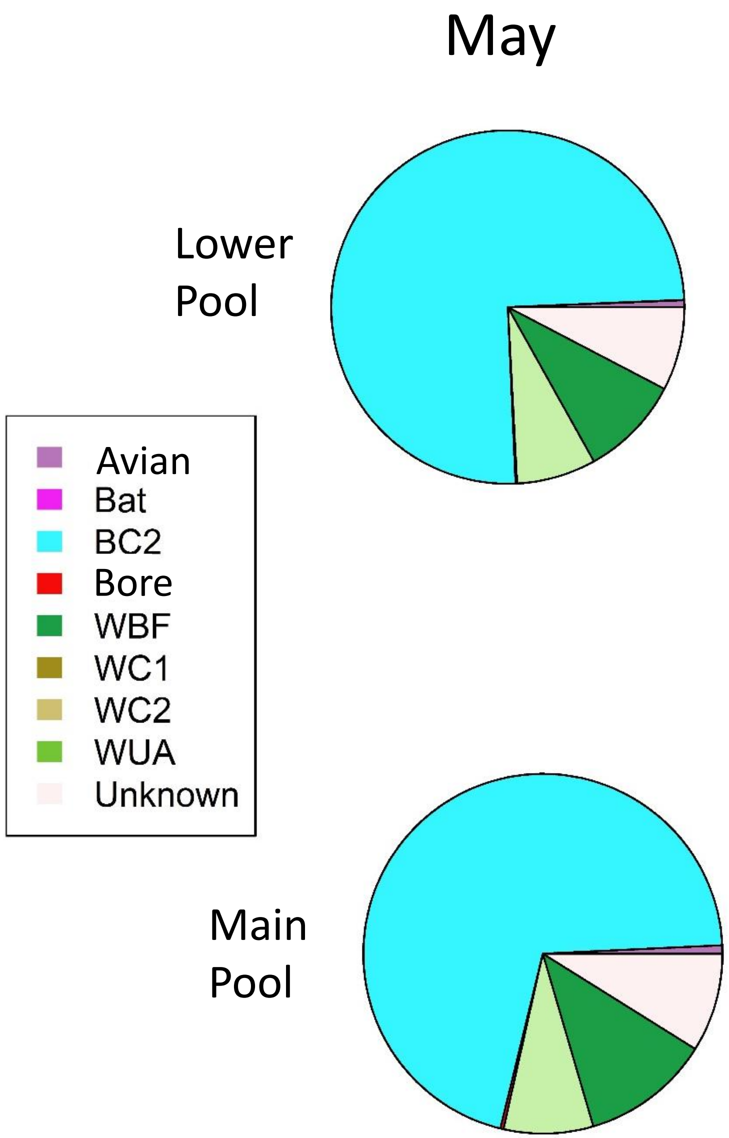
- Increase of enterococci at WC1/2 coincides with bats' arrival in Oct
- After first major rain, max enterococci at WC1/2 + pools
- Counts remain high in Wildlife Park forest in Nov
- Enterococci abundant in aviary pond and bat droppings
- Low counts in Berry Creek
- No enterococci in bore

Results – Bayesian Microbial Source Tracking

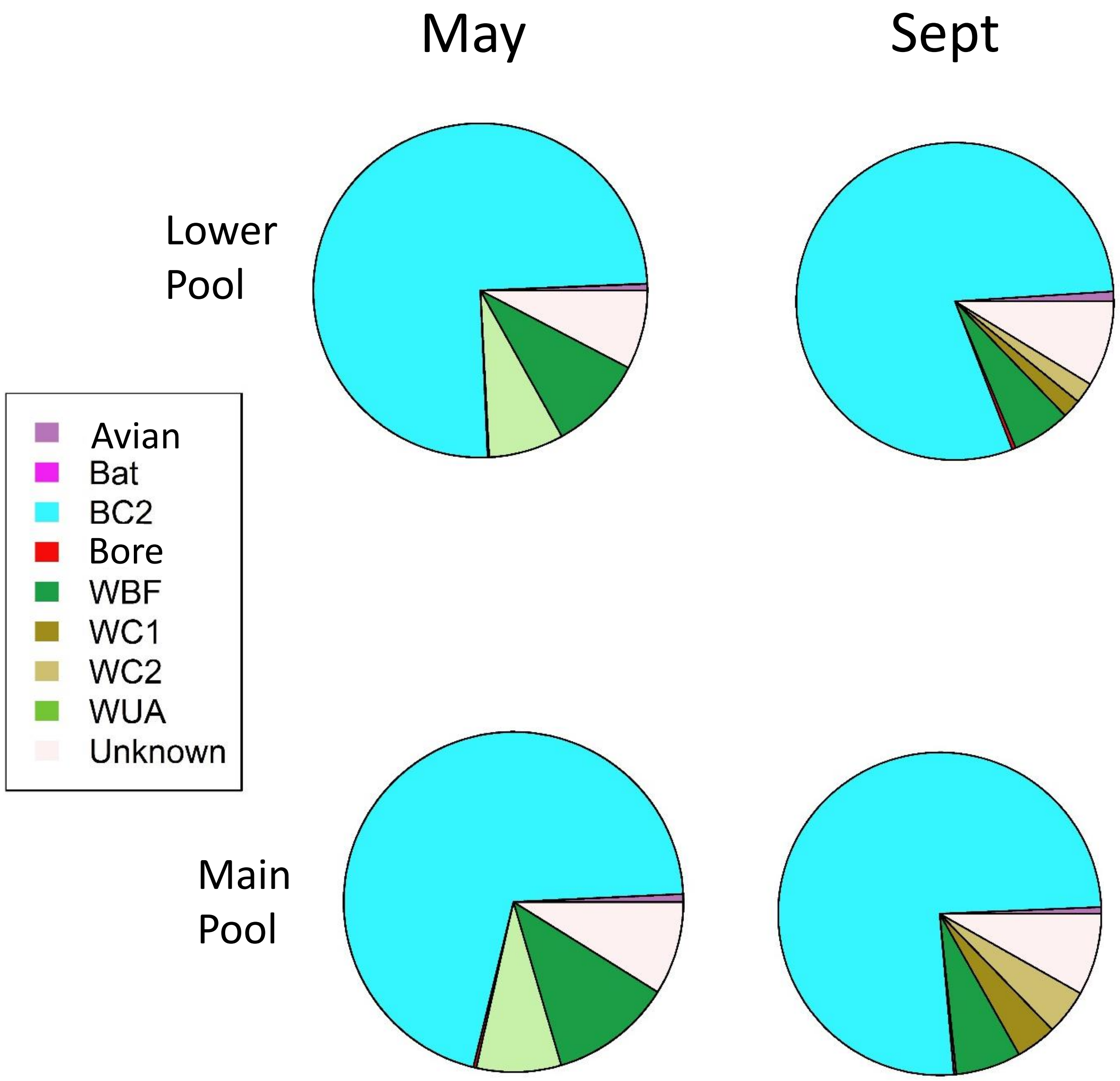
- Results based on DNA from ALL microbes in water
- The pie charts show the microbial contributions from different sources to microbes in pools → different to water isotopes which show the source contributions of actual water masses from springs or surface water to pools
- Pools dominated by microbes which also occur in Berry creek
- Microbes from Wildlife Park creek contributed approx. 15-20% to pool microbes in May
- Very small avian signature in May
- “Unknown” fraction consists of other microbes from sources not sampled



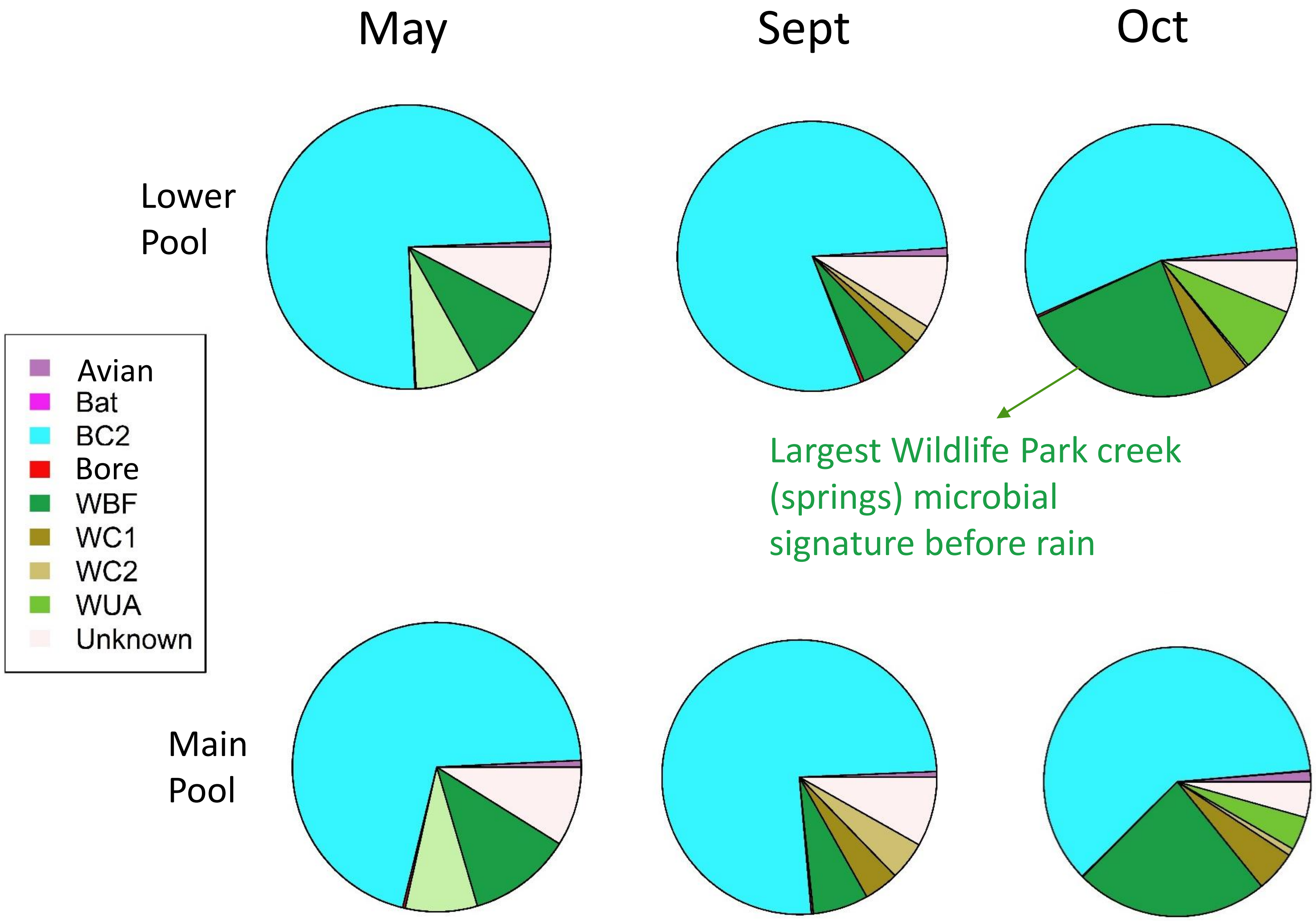
Results – Bayesian Microbial Source Tracking



Results – Bayesian Microbial Source Tracking

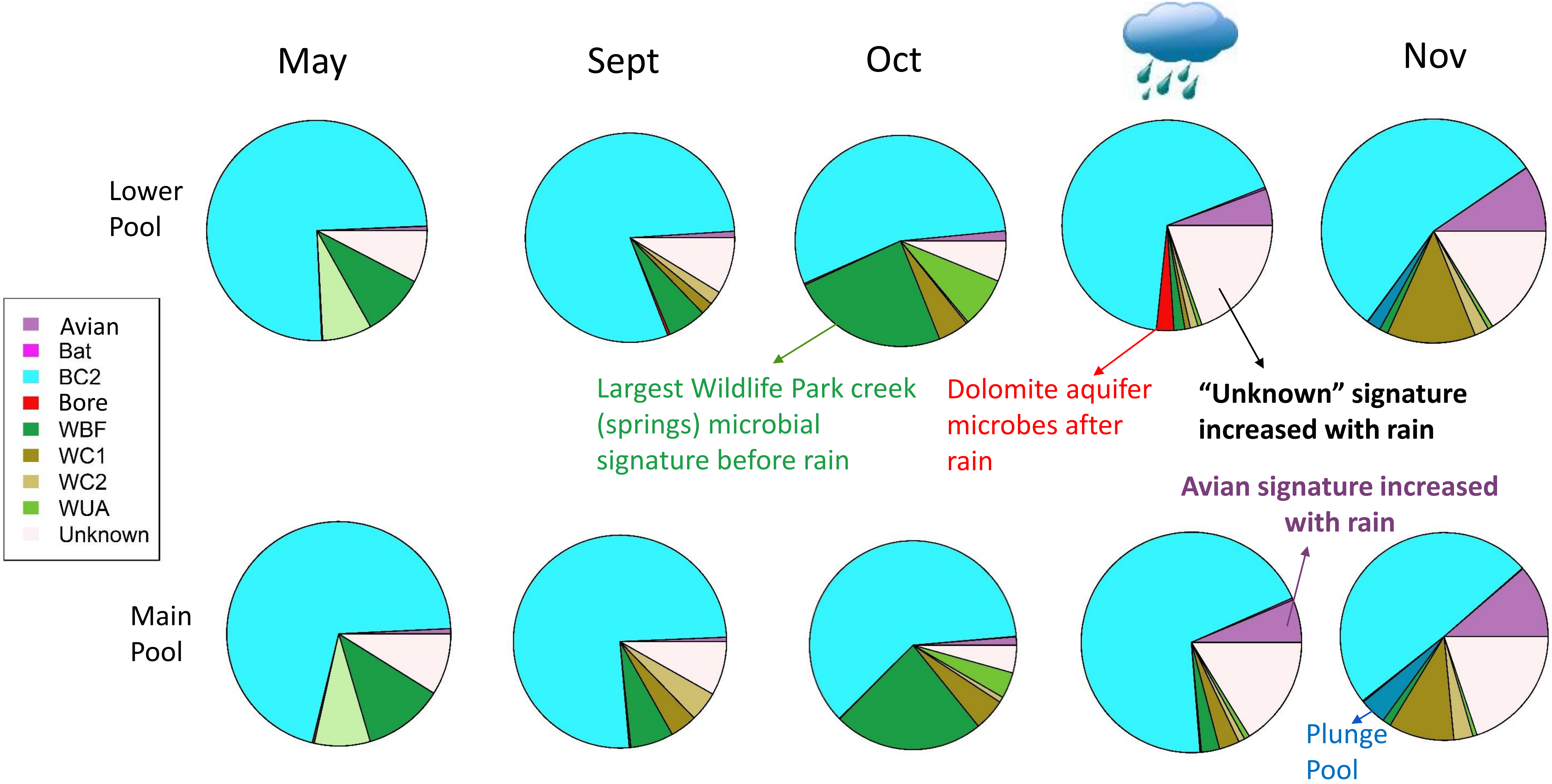


Results – Bayesian Microbial Source Tracking



Largest Wildlife Park creek (springs) microbial signature before rain

Results – Bayesian Microbial Source Tracking



Largest Wildlife Park creek (springs) microbial signature before rain

Dolomite aquifer microbes after rain

“Unknown” signature increased with rain

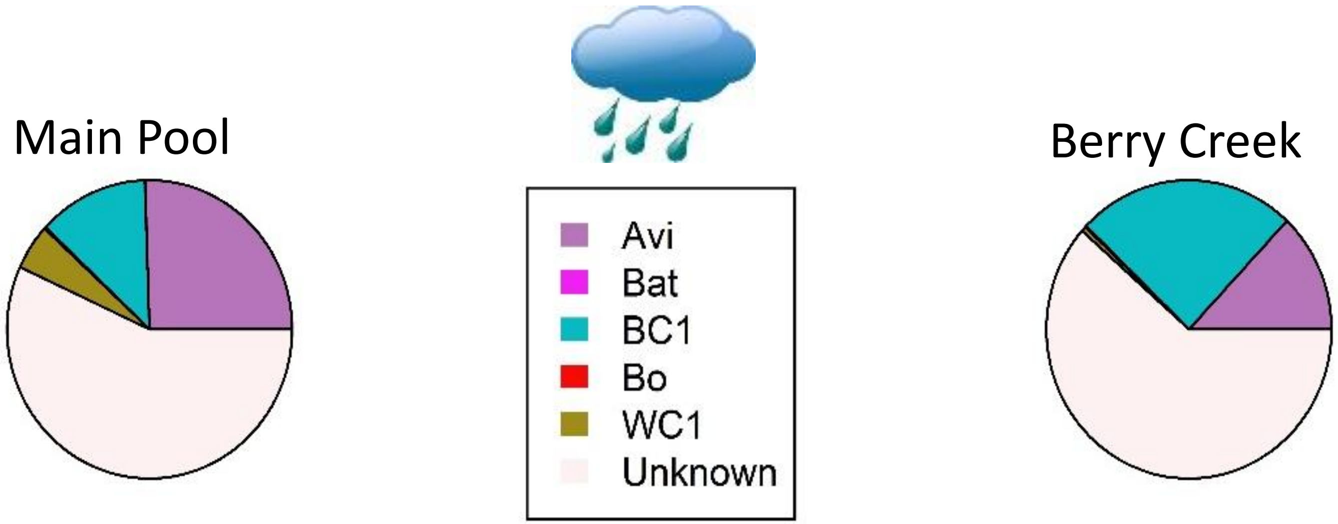
Avian signature increased with rain

Plunge Pool

Results – Bayesian Microbial Source Tracking (MST)

- Avian signature increased with rain ➔ from Aviary pond?
- Avian signature also large in upstream Berry Creek

➔ Avian signature likely not Aviary specific but relates to runoff from soil with droppings of native birds which are also displayed in Aviary



What about the bat droppings?

➔ Microbes from bat droppings contributed < 1% to water microbes (too small for pie charts)

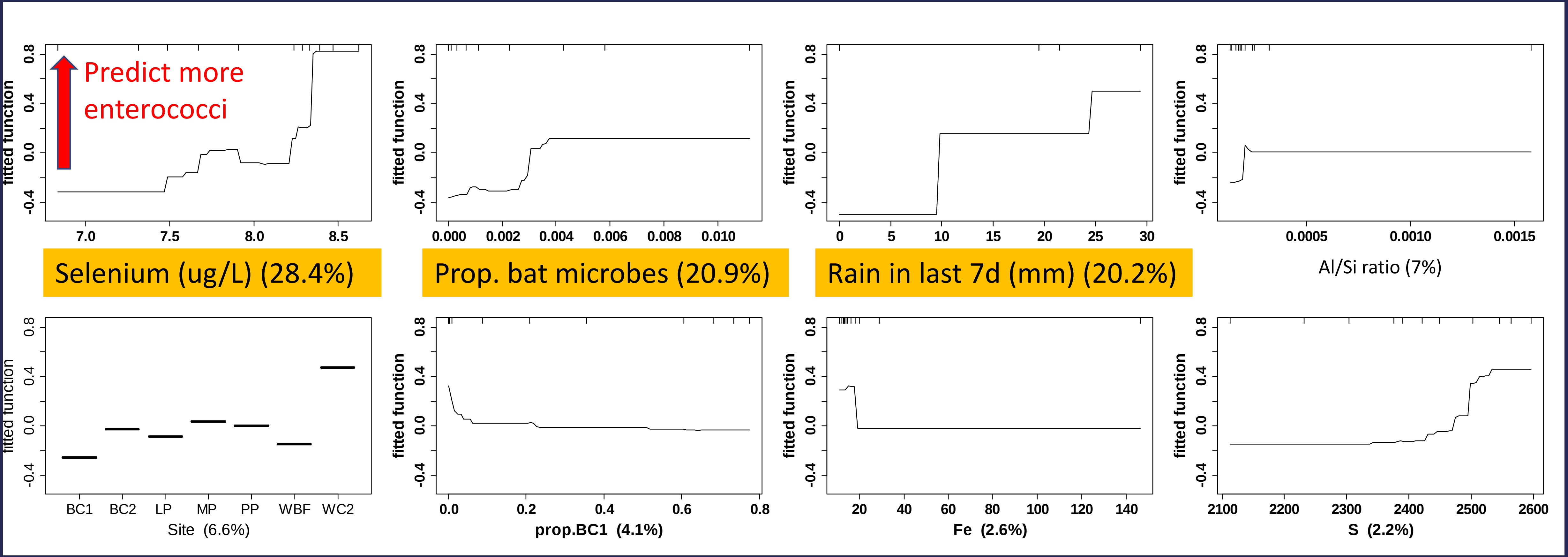
➔ Used MST + elemental fingerprinting data as explanatory variables in multivariable model to predict enterococci counts in water samples

Results – nonlinear multivariable model to predict enterococci counts

- Selenium (Se) contributed most (28%) to model + associated with higher ent counts

➔ Se levels increased every round; occurs in shale ➔ indicator for land runoff in Wildlife Park forest?

- Microbial signature of bat droppings 2nd most important factor (21%) + associated with higher ent counts
- Rainfall in prev 7 days (20%) other important factor with rest of factors considerably less important



Boosted regression tree model (Poisson family) – training data deviance explained 99%; cv test data 95%; cv correlation 97%

Summary of results on drivers of enterococci levels

Enterococci Data Analysis 2010 to 2017

- Enterococci levels strongly associated with rainfall
- Main Pool strongest increase in enterococci upon rainfall, Berry creek least
- The stronger the flow + the more previous rain events, the less the increase after rain

➔ System gets flushed with more rain and water flow

Microbial Source Tracking + multivariable Model

- Land runoff (+ rainfall) are main driver for enterococci counts
- Droppings from bats likely contribute to higher counts
- Enterococci levels not associated with avian microbial contributions in model
- Microbial signatures from sources not captured by sampled sites increased after rain

➔ land runoff in vicinity of pools? microbes resuspended from sediment?

Sources of enterococci in the tropics

Primary source of enterococci:

- Animals, humans

➔ High survival

1st source:
Guts of host species

Modified from Rochelle-Newall et al., 2015

Sources of enterococci in the tropics

Primary source of enterococci:

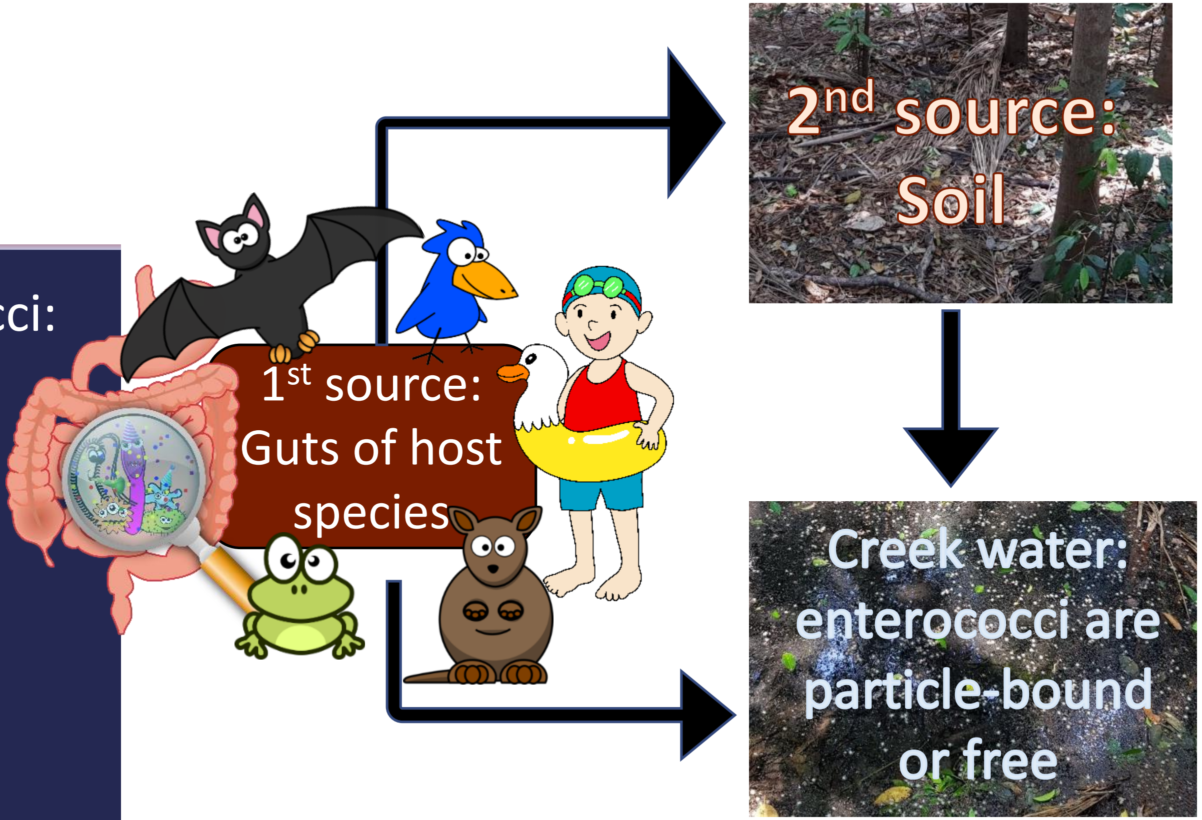
- Animals, humans

➔ High survival

2nd source:

- Soil

➔ Low survival



1st source: Guts of host species

2nd source: Soil

Creek water: enterococci are particle-bound or free

Modified from Rochelle-Newall et al., 2015

Sources of enterococci in the tropics

Primary source of enterococci:

- Animals, humans

→ High survival

2nd source:

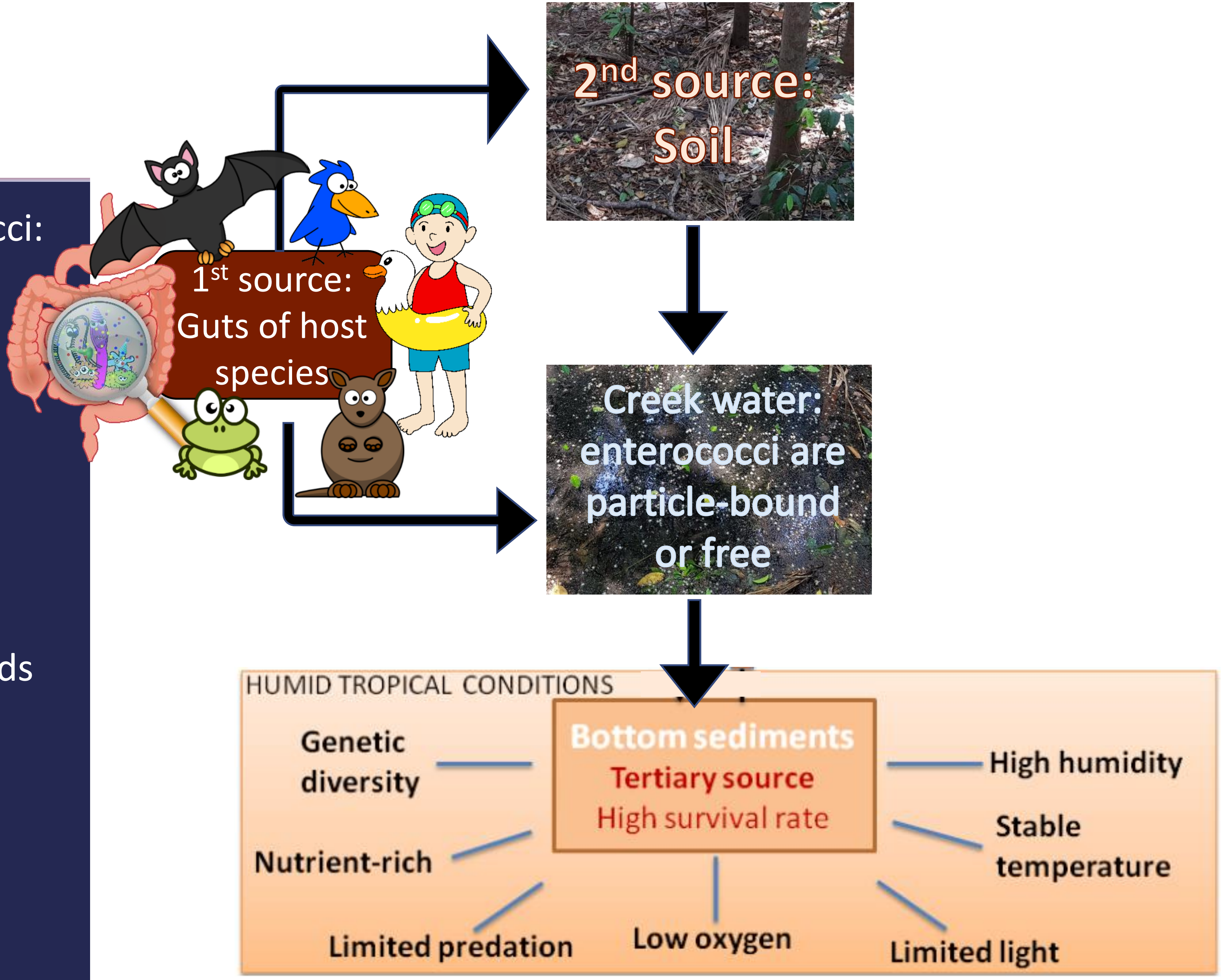
- Soil

→ Low survival

3rd source → in tropics:

- Stream sediment and beds

→ Potentially high survival



1st source: Guts of host species

2nd source: Soil

Creek water: enterococci are particle-bound or free

HUMID TROPICAL CONDITIONS

Bottom sediments

Tertiary source

High survival rate

Genetic diversity

Nutrient-rich

Limited predation

Low oxygen

Limited light

Stable temperature

High humidity

Modified from Rochelle-Newall et al., 2015

Sources of enterococci in the tropics

Primary source of enterococci:
- Animals, humans

→ High survival

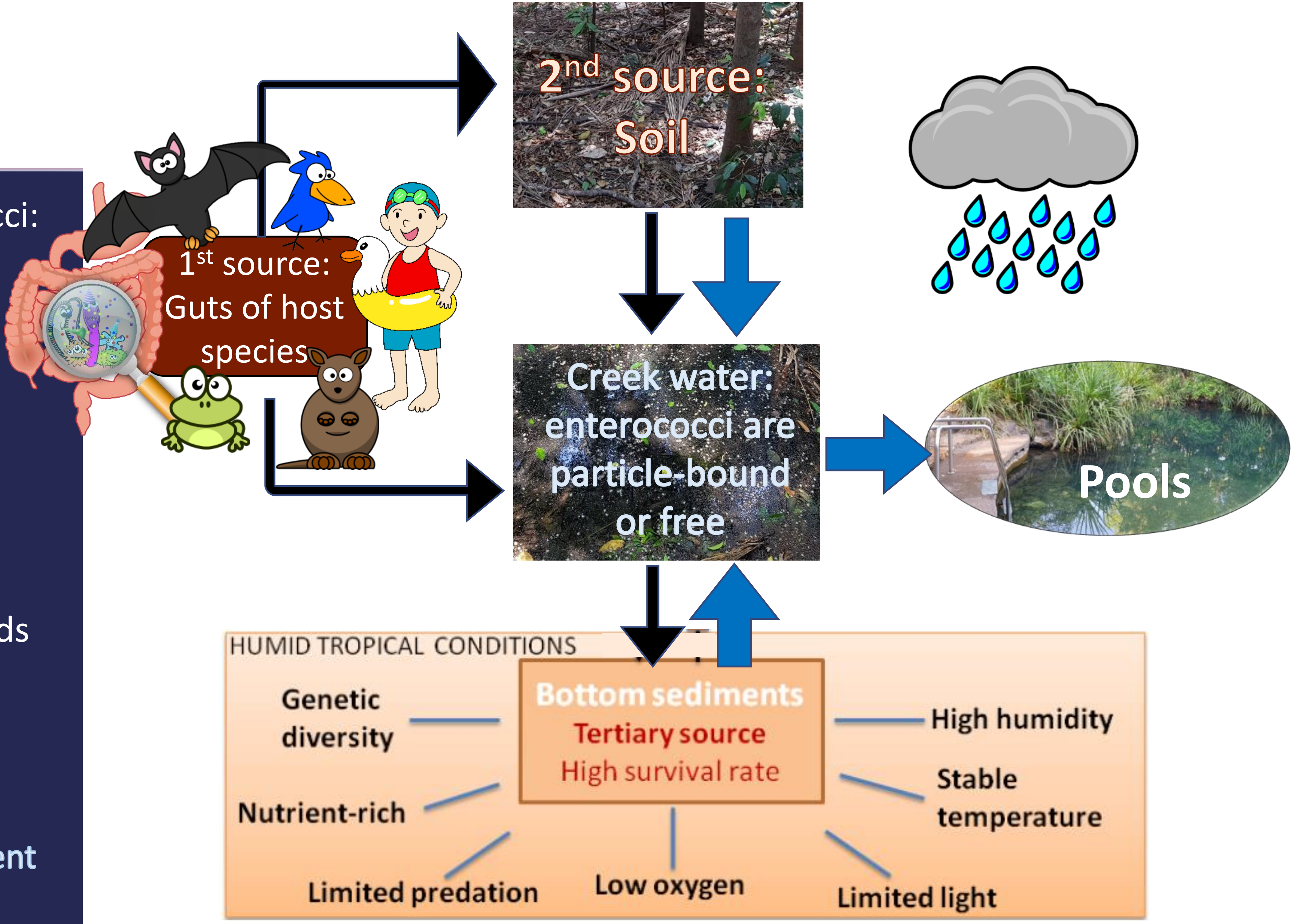
2nd source:
- Soil

→ Low survival

3rd source → in tropics:
- Stream sediment and beds

→ Potentially high survival

→ After intense rainfall
- >> land runoff
- resuspension from sediment
- transfer downstream



1st source: Guts of host species

2nd source: Soil

Creek water: enterococci are particle-bound or free

Pools

HUMID TROPICAL CONDITIONS

Bottom sediments

Tertiary source

High survival rate

Genetic diversity

Nutrient-rich

Limited predation

Low oxygen

Limited light

Stable temperature

High humidity

Modified from Rochelle-Newall et al., 2015

Sources of e
in the tropi

Note in NHMRC guidelines:

"In waters where animals and/or birds are the primary source of faecal material or in situations where environmental proliferation of indicator bacteria may occur, ***the health significance of (these) microorganisms is reduced."***

Does that mean we don't have to worry about enterococci?

➔ While land runoff is the likely main source for enterococci, we don't know its health significance and we cannot exclude other sources of enterococci

Primary source
- Animals, hu

➔ High surviva

2nd source:
- Soil

➔ Low survival

3rd source ➔
- Stream s

➔ Potential

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- >> land ru
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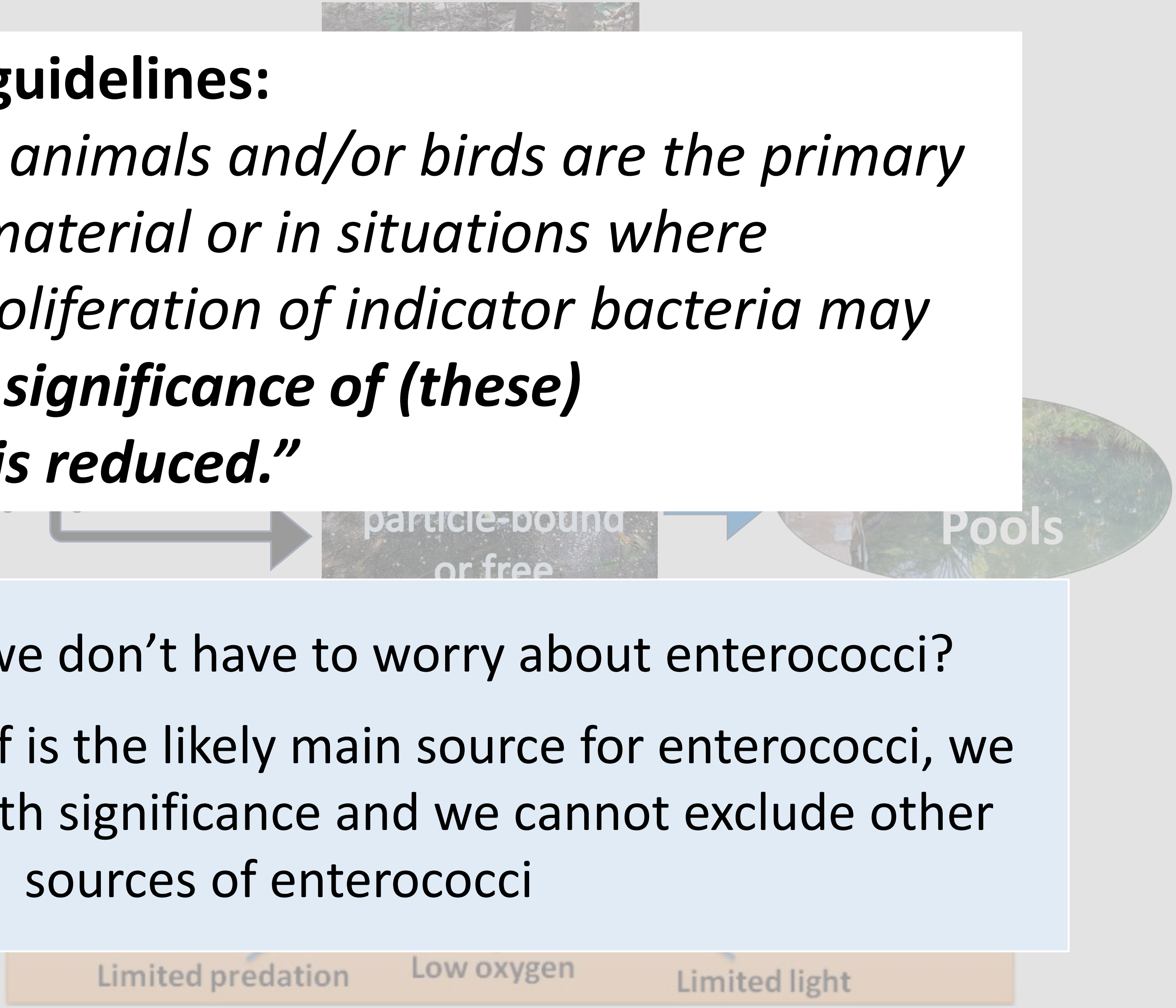
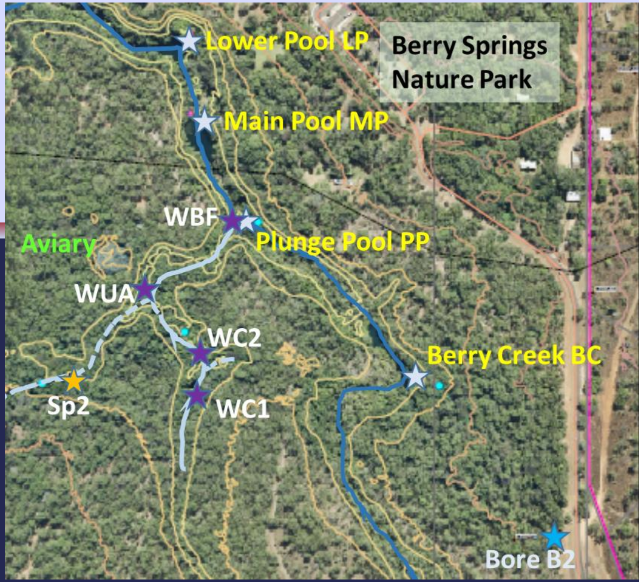


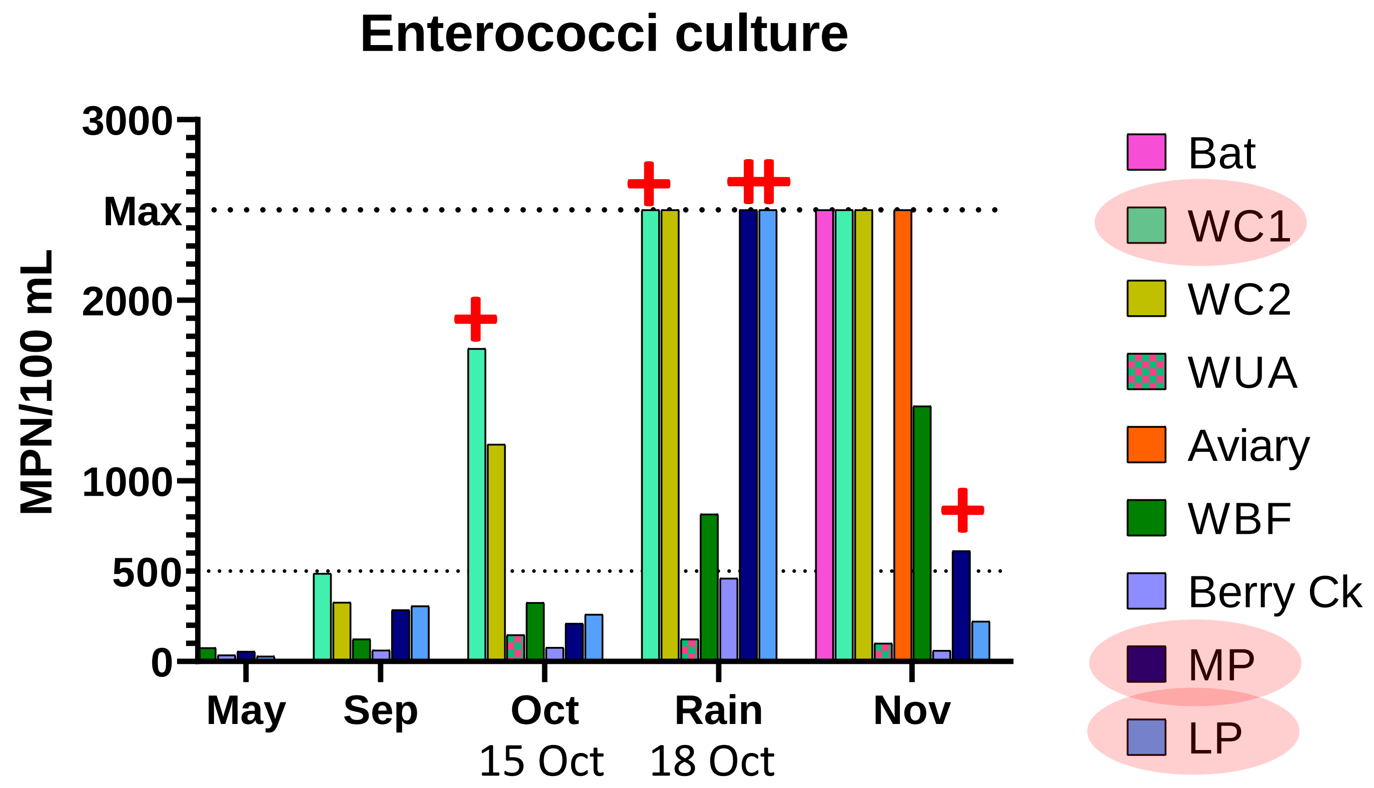
Modified from Rochelle-Newall et al., 2015, Frontiers in Microbiology

Results – human faecal indicator PCRs



- PCR targeting *Bacteroides thetaiotaomicron* (Teng et al., 2004)
- PCR targeting Hf183 *Bacteroides dorei* (Bernhard, 2000)

➔ Human specific but still cross react with some animals (e.g. chickens)



+ Sample(s) positive for *B.theta* and/or Hf183

- No overall association with enterococci
- Negative in bats and aviary pond
- Positive result indicates recent contamination as faster decay with oxygen, light + high temperature
- WC1 was Hf183 positive on 15 + 18 Oct
- Pools were HF183 + *B.theta* pos 18 Oct
- Plunge + main pool *B.theta* pos in Nov

Overall study findings

Results from this study suggest that

- Enterococci levels in pools are likely driven by land runoff incl. bat droppings and poor indicators of human faecal contamination

- Detection of different human faecal markers in pools after major rain event and in Nov suggest traces of human faeces - due to stormwater runoff incl. human excreta from pools' vicinity? Human faeces + toilet paper were observed at plunge pool in Nov

- Potential reasons for Hf183 positive result for WC1 on 15/18 Oct:
  - cross-reactivity of Hf183 assay with animals visiting that site (e.g. scrub fowls?)
  - human excreta close to or upstream of WC1?
  - leaking pipes from a septic tank?
  - less likely: the spring/aquifer itself was contaminated (and contamination was below detection level or absent in other rounds)?

Health significance of findings

- As per NHMRC guidelines unclear as animals likely main source of enterococci
- Enterococci themselves are mainly commensal, some are opportunistic pathogens, especially in hospitals causing e.g. urinary tract infections (Byappanahalli et al., 2012)
- Health significance of bat droppings:
  - Contain *Haemophilus, Salmonella, Clostridia* bacteria, some of which can be pathogenic (Henry et al., 2018) → we detected *Haemophilus* + *Clostridia* in bat droppings and *Clostridia* in creek + pool samples (but no resolution to pathogenic species or strains)
- Hf183/*B.theta* positive samples have been associated with presence of pathogens in some studies (Campylobacter, Enteroviruses…)(Harwood et al., 2014)

❖ *An epidemiological study would be needed to associate enterococci levels in Berry Springs pools with gastrointestinal, skin or respiratory symptoms of visitors*

What more could we do?

- To further explore origin of enterococci:
  - Culture enterococci from pools and sources and sequence their genomes to get a better understanding of where they are coming from
- To further explore reasons for Hf183/*B.theta* positive pool and WC1 samples:
  - Collect a septic tank sample the next time the tank is opened for maintenance purposes to include in microbial source tracking and multivariable model
  - Collect samples near WC1 and animal faeces (e.g. scrub fowls – check for cross-reactivity)
  - Repeat sampling from springs - can a spring only be contaminated for a short period of time?
- Associate faecal markers with presence of pathogens such as enteroviruses, *Campylobacter jejuni* or *Salmonella enterica serovar* similar to NTG report (1992) but using a larger array of pathogens and sensitive high throughput qPCRs (Harwood et al., 2014)

Acknowledgements

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  - Steven Tickell, Des Yinfoo
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  - Simon Ferguson, Donna Jensen, Shael Martin
- ECMU, RIEL CDU
  - Dion Lambrinidis, Zarah Hockey, Anna Padovan

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