Implementation of a Scat Monitoring Program to Determine *Giardia* and *Cryptosporidium* Concentrations in Wildlife Fecal Deposits in the Protected Bull Run Watershed, OR

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May 3, 2012

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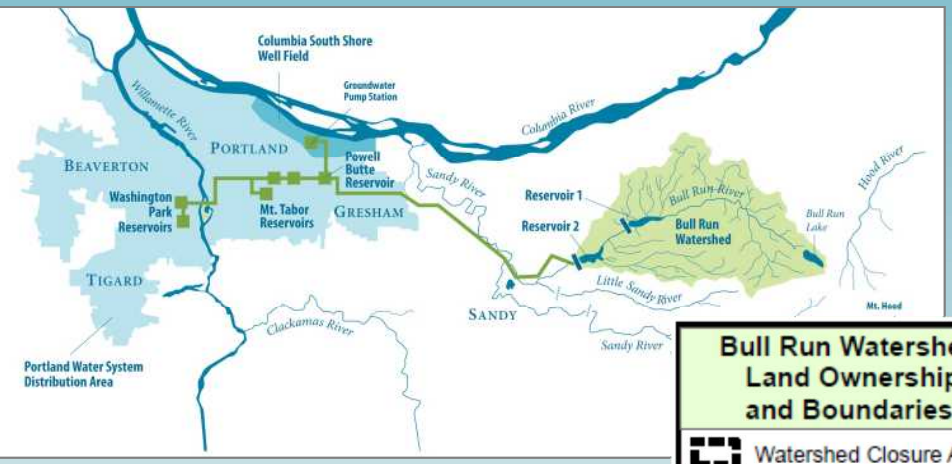
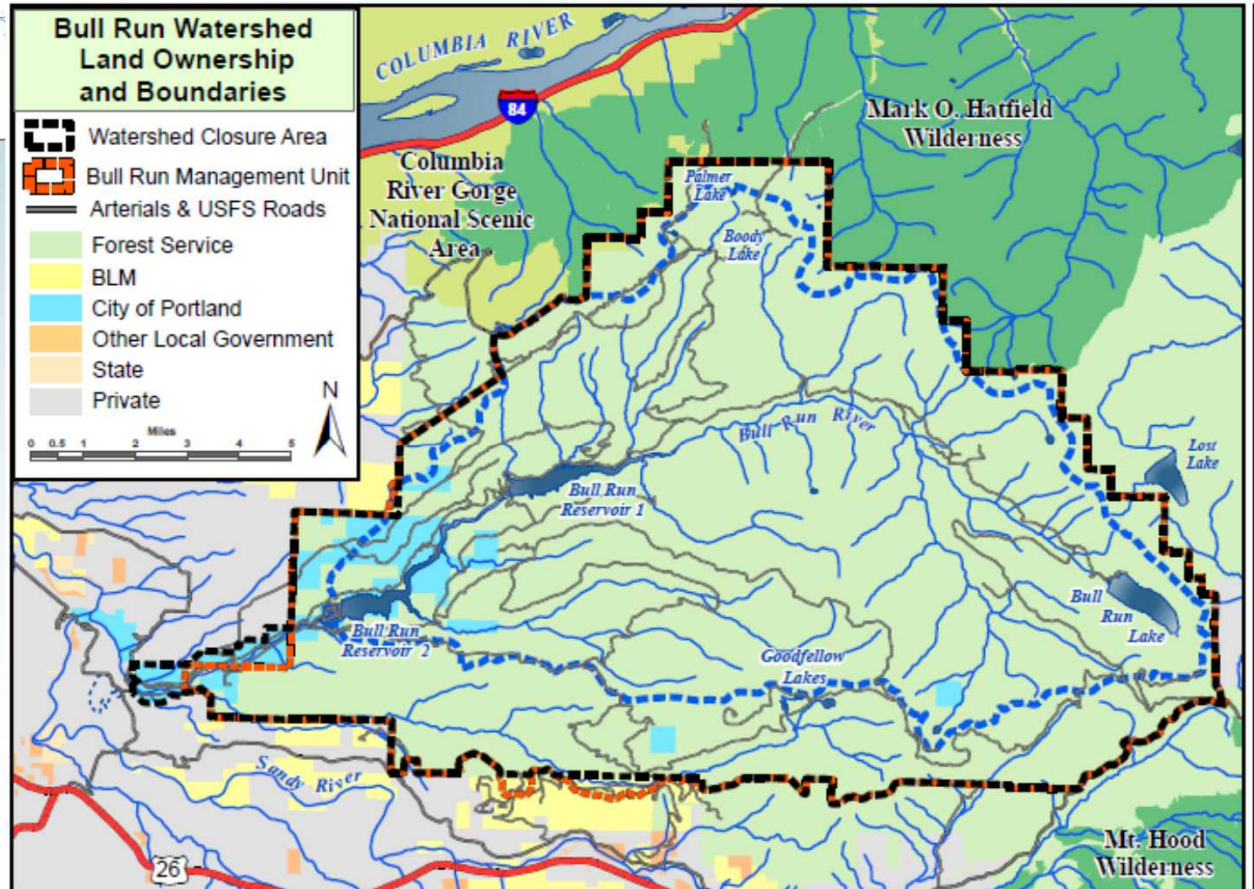
Bull Run Watershed



- Long history of source water protection

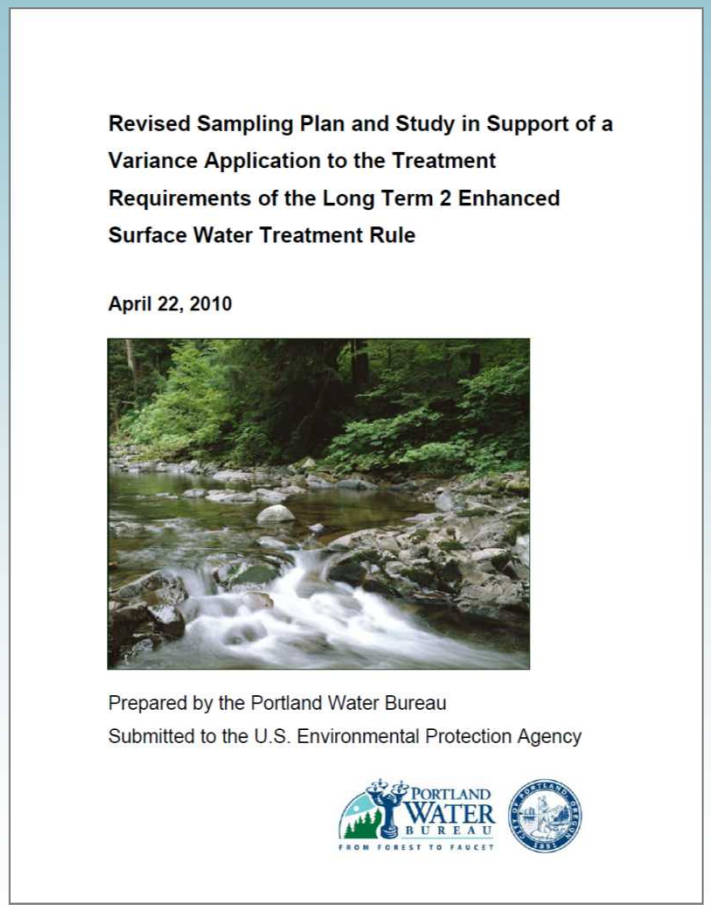
- BRWMU/Closure area
- No public entry
- No recreational use
- No development
- No timber harvest since 1993



Background

- Long Term 2 Enhanced Surface Water Treatment Rule (LT2 Rule)- additional *Cryptosporidium* treatment for higher risk drinking water systems
- Unfiltered water systems must add minimum of 2-log removal regardless of monitoring results
- Portland Water Bureau (PWB) elected to pursue a variance to the LT2 Rule treatment requirements for the Bull Run watershed
- LT2 Variance Sampling Plan and Study developed in consultation with EPA

LT2 Variance Sampling Plan and Study



- One year intensive *Cryptosporidium* monitoring at the raw water intake
- Scheduled and event based monitoring at upstream watershed locations- potential *Cryptosporidium* hot spots
- Adaptation of the Pathogen Catchment Budget Model

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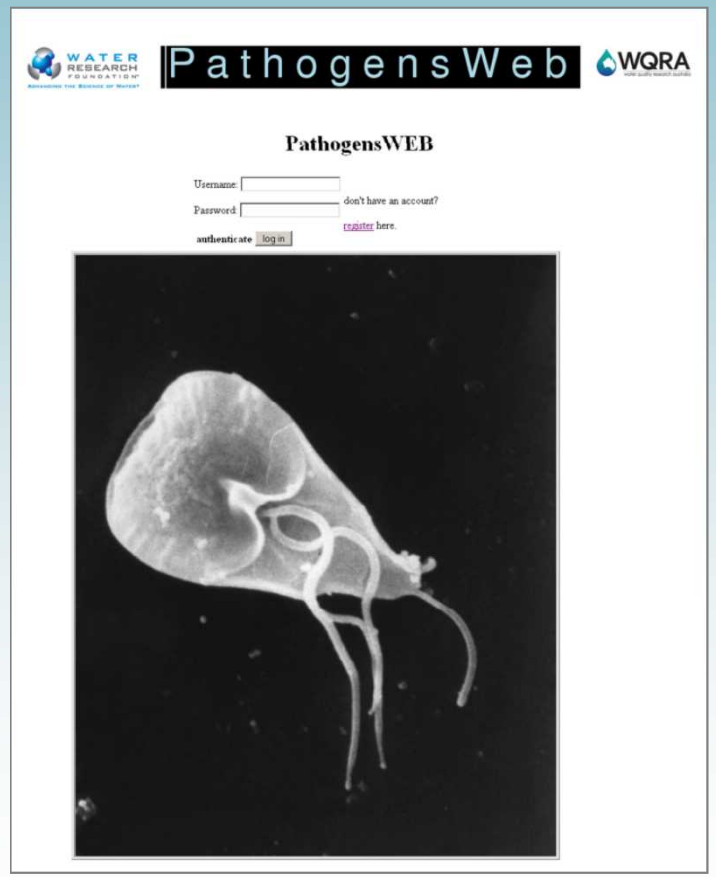
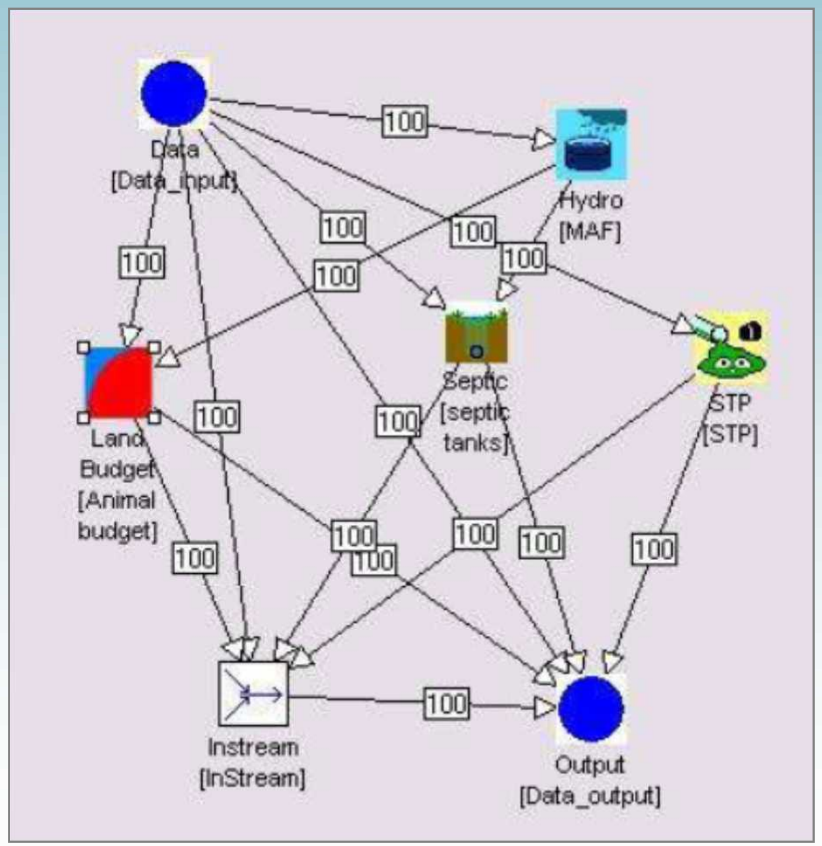
Pathogen Catchment Model



- Processed-based mathematical model to quantify pathogen and fecal indicator loads within watersheds
- Uses GIS land use data, IHACRES hydrologic model, microbial inactivation rates, animal density & pathogen shedding intensities to predict event-based *Cryptosporidium*, *Giardia* & *E. coli* loads
- Journal of Water & Health 5(2):187-208. Ferguson, Croke, Beatson, Ashbolt & Deere, 2007

PCB Model Consist of Five Modules



- Animal Budget
- Sewage Treatment Plant
- Septic Systems
- Hydrologic
- In-stream Processes

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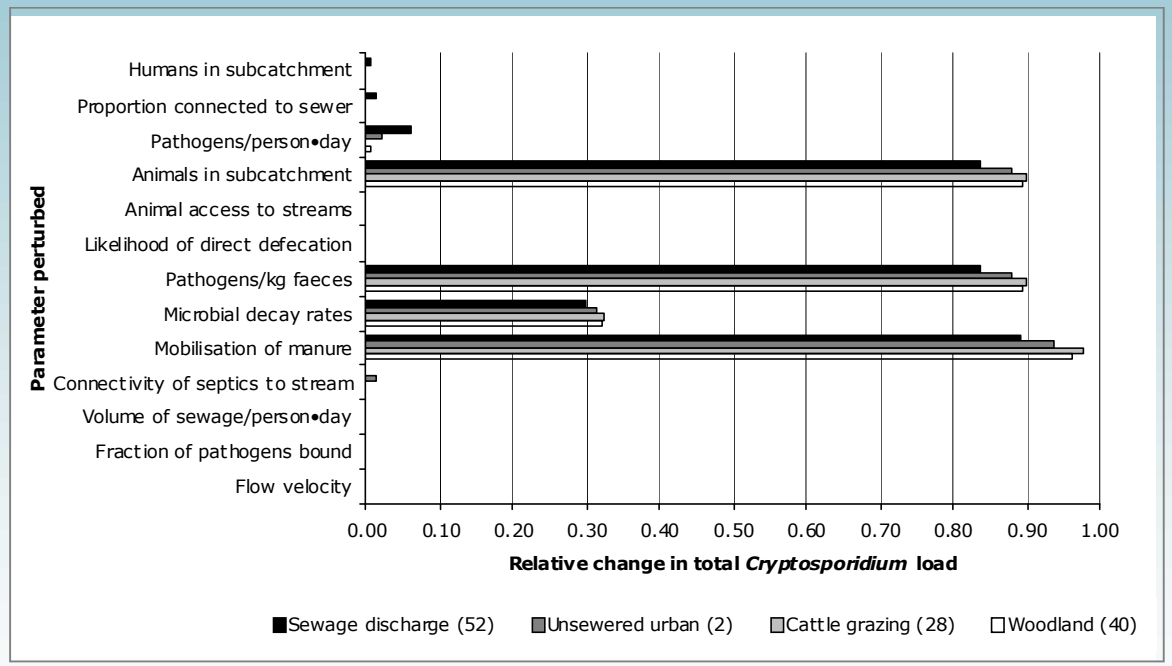
Animal Budget- Problems of Data Availability



Adapted from Christobel Ferguson & Barry Croke presentation for PWB, Nov. 2009

- PCB model is sensitive to Animal Budget
- Lack of wildlife information
- Lack of wildlife pathogen data
- Wildlife is only potentially significant source of pathogens in the Bull Run watershed

Wildlife Investigations

- Local wildlife biologist (Phil Rickus, David Evans & Associates) identified wildlife that are most abundant, produce largest fecal loads, and/or use aquatic & riparian habitats
- Target wildlife population estimates
- Daily manure production and likelihood of deposition in waterways
- Habitat use
- Birthing seasons

Target Wildlife

Species	Bull Run Estimate	
	Dry Season (June–September)	Wet Season (October–May)
Black-tailed deer (*Odocoileus hemionus columbianus*)	150	80
Roosevelt elk (*Cervus elaphus roosevelti*)	45	20
Bobcat (*Lynx rufus*)	40	30
Cougar (*Puma concolor*)	10	7
Coyote (*Canis latrans*)	70	50
Black bear (*Ursus americanus*)	35	15
River otter (*Lontra canadensis*)	6	5
American beaver (*Castor canadensis*)	22	22
Snowshoe hare (*Lepus americanus*)	7,920	7,920
Canada goose (*Branta canadensis*)	18	5
Small rodents[1]	204,000	204,000

[1] Estimates based on four common species that live in a variety of habitat types in the watershed: bushy-tailed wood rat (*Neotoma cinerea*), Douglas's squirrel (*Tamiasciurus douglasii*), western deer mouse (*Peromyscus maniculatus*), and creeping vole (*Microtus oregoni*).

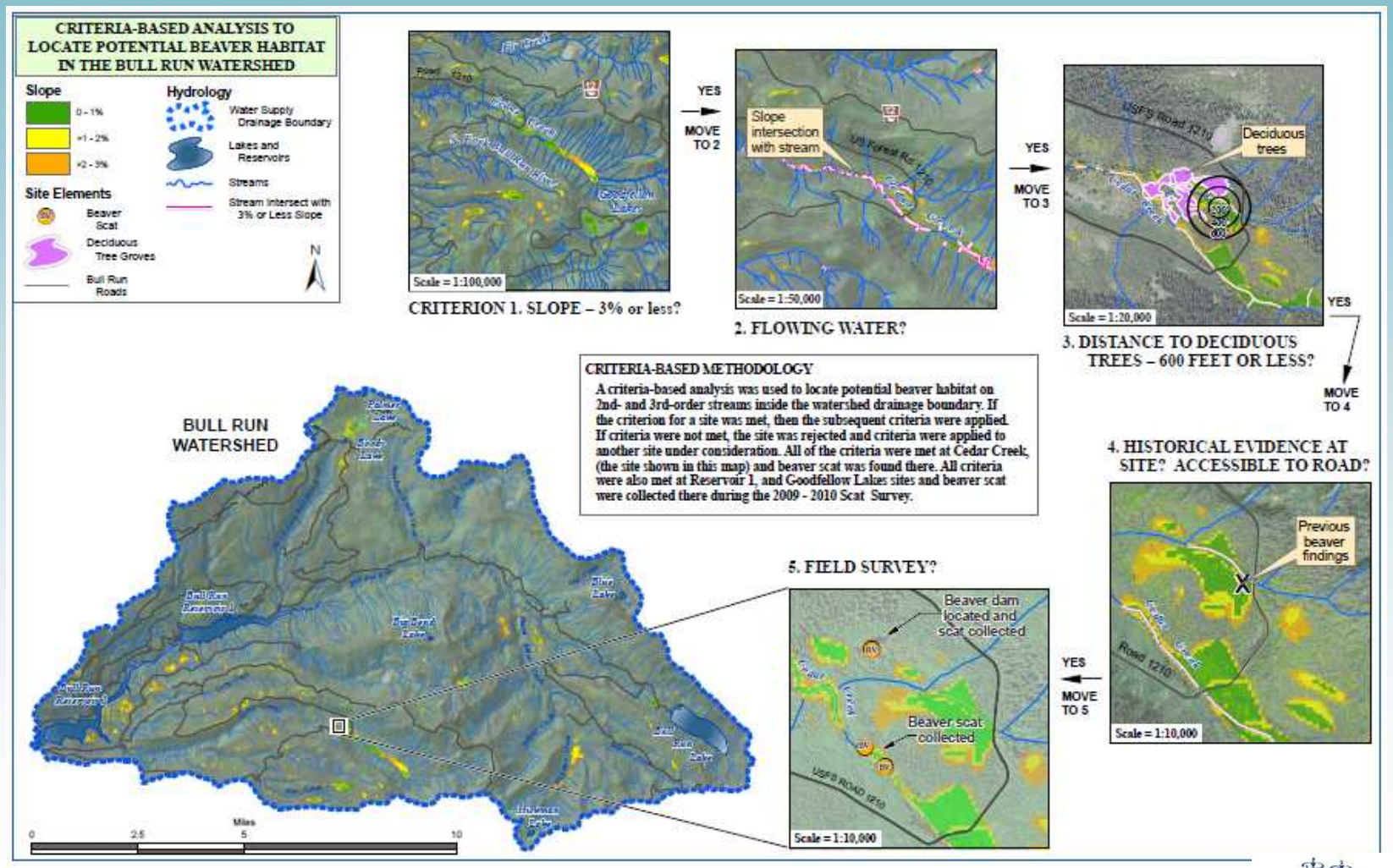
Scat Collection

- Started August 2009
- Scat identification training
- Active and decommissioned roads
- Forest clearings and areas adjacent to roads
- Field examination for freshness
- Samples collected throughout the year
- Higher effort during birthing seasons

Trapping and Baiting of Hares and Rodents

- Initial investigation of potential trapping sites
- Motion-activated camera
- Scientific taking permit from ODFW
- Baiting of wildlife traps
- Daily checking of traps
- Release of native wildlife

Identification of Beaver Habitat



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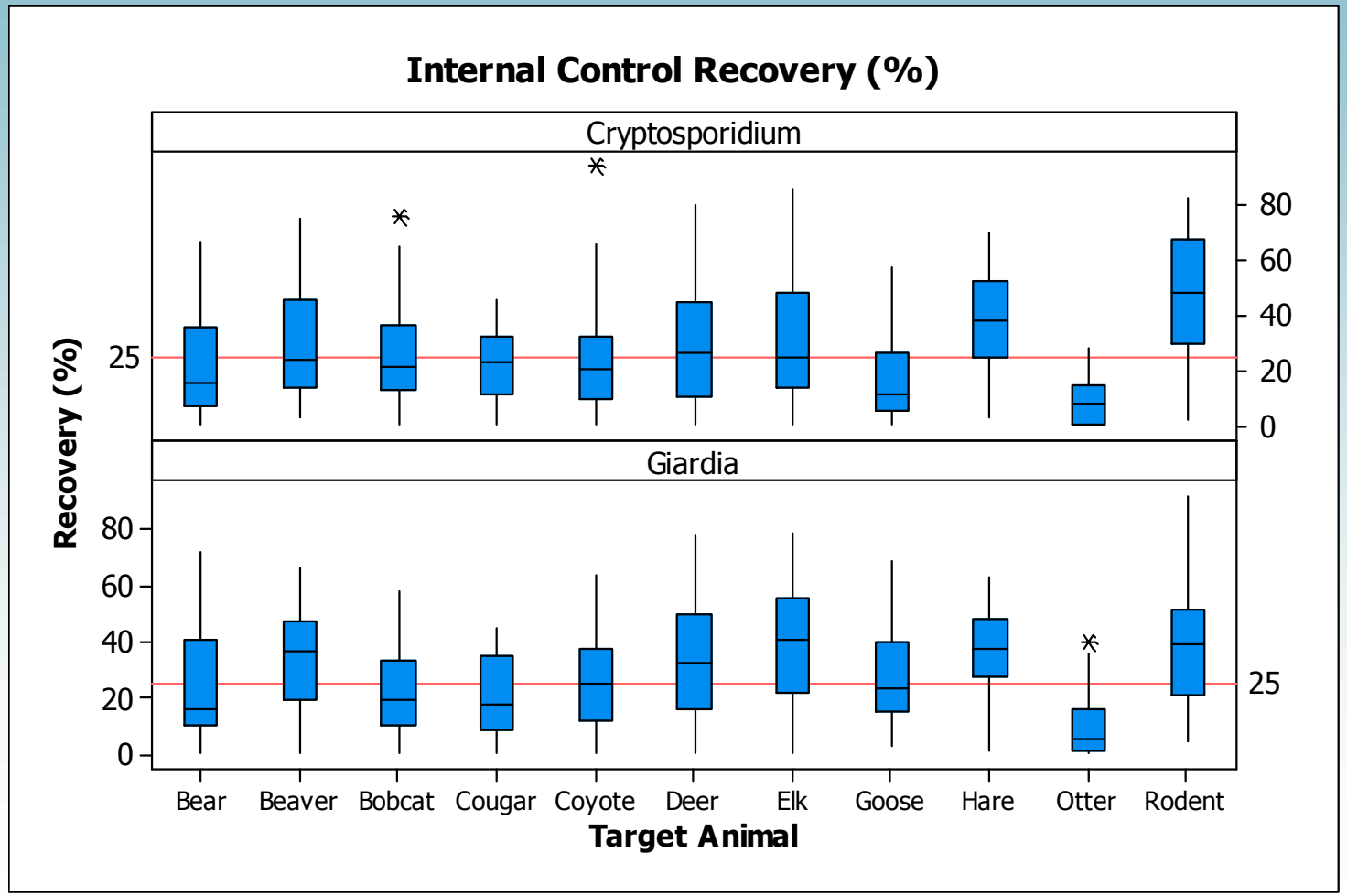
How Many Samples?

Reproduced from http://melissakoski.wordpress.com

Analytical Methods

- *E. coli* enumeration using Quanti-Tray- 4x dilutions
- Enumeration of *Cryptosporidium* oocysts and *Giardia* cysts using immunomagnetic separation and immunofluorescence assay (IMS-IFA)
  - -IMS-IFA is gold-standard method- able to detect few
  - oocysts
  - -Addition of sodium pyrophosphate- a dispersing agent
  - -Addition of diethyl ether to fatty samples
  - -Internal control spike in each scat sample
- Genotyping of positive Cryptosporidium samples

Scat Monitoring QC Results



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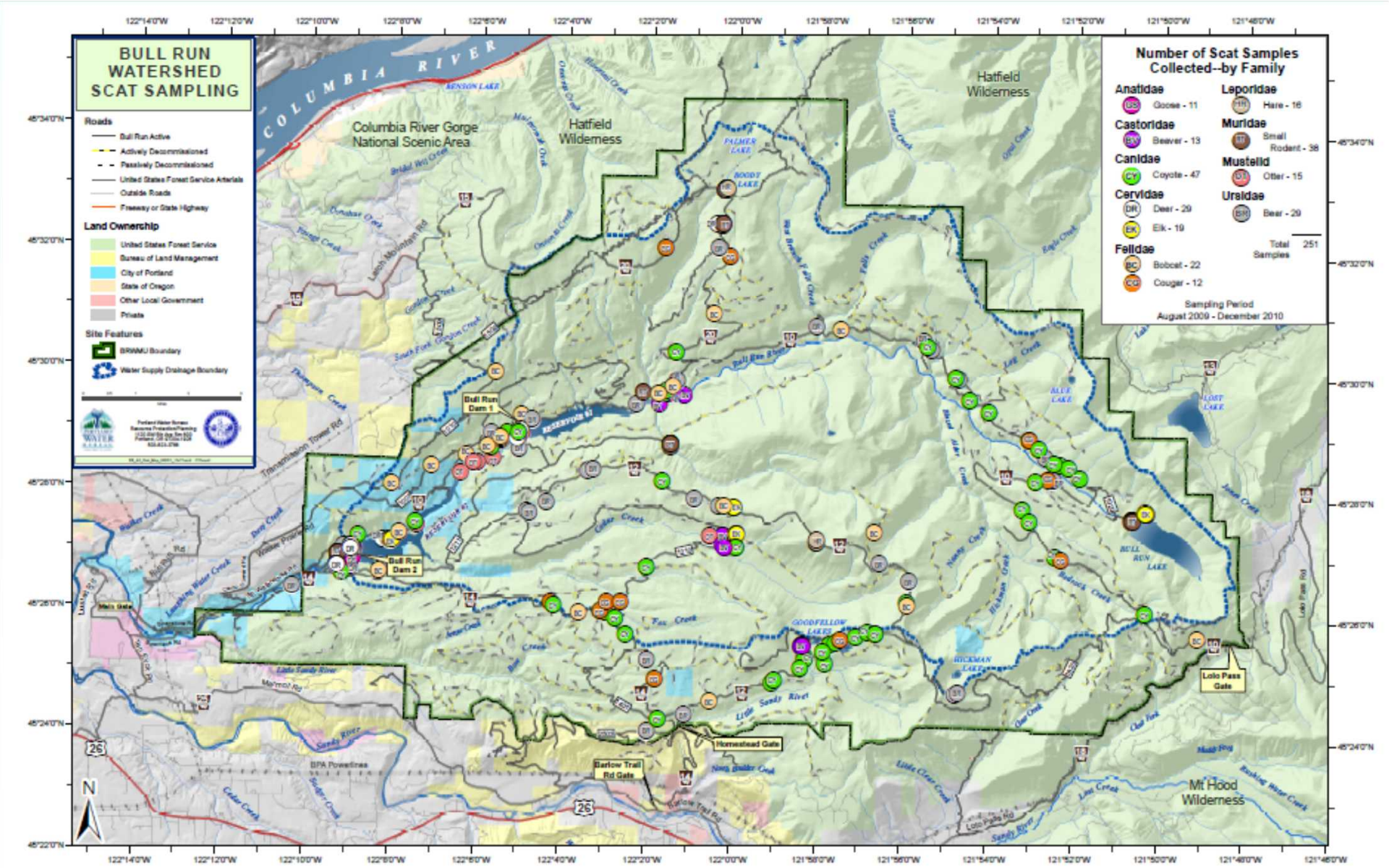
Scat Monitoring Results

Cryptosporidium			
Target Animal	**Positive/Total Samples**	**% Positive**	**Positive Concentration Range (oocysts/g)**
Black-tailed deer	0/70	0.0%	-
Roosevelt elk	0/51	0.0%	-
Bobcat	1/65	1.5%	6942
Cougar	0/26	0.0%	-
Coyote	1/89	1.1%	2
Black bear	0/50	0.0%	-
River otter	0/47	0.0%	-
American beaver	0/48	0.0%	-
Snowshoe hare	0/47	0.0%	-
Canada goose	0/31	0.0%	-
Small rodents	0/72	0.0%	-
Total	**2/596**	**0.3%**	**2-6942**

Giardia			
Target Animal	**Positive/Total Samples**	**% Positive**	**Positive Concentration Range (oocysts/g)**
Black-tailed deer	1/70	1.4%	37
Roosevelt elk	0/51	0.0%	-
Bobcat	2/65	3.1%	111-1856
Cougar	1/26	3.8%	1
Coyote	0/89	0.0%	-
Black bear	2/50	4.0%	1
River otter	0/47	0.0%	-
American beaver	6/48	12.5%	14-1065
Snowshoe hare	0/47	0.0%	0
Canada goose	8/31	25.8%	12-5000
Small rodents	6/72	8.3%	269-2321
Total	**26/596**	**4.4%**	**1-5000**



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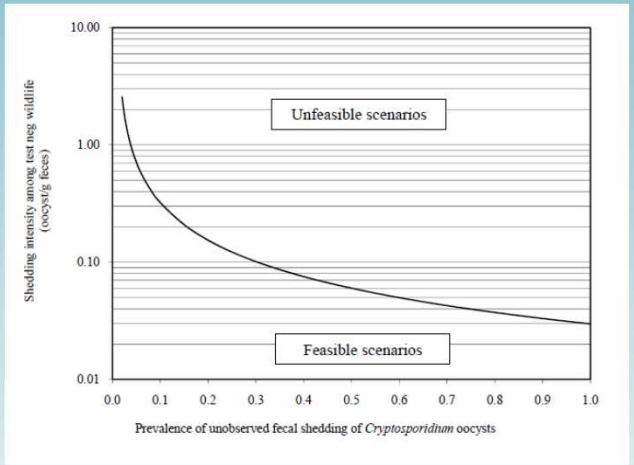
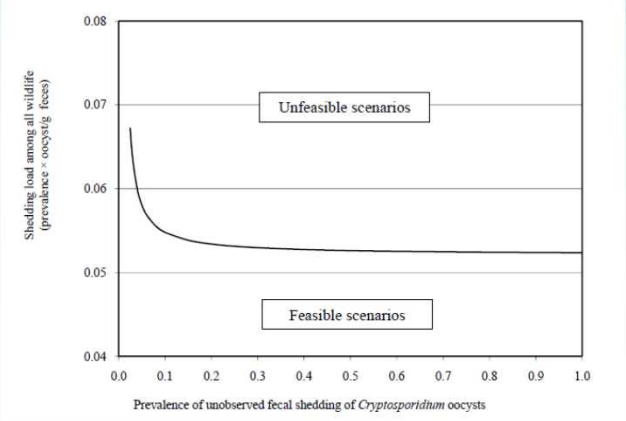
Cryptosporidium Genotyping Results

- Genotyping of coyote sample was unsuccessful due to low number of oocysts in the sample
- DNA sequencing of bobcat sample indicates a novel *Cryptosporidium* isolate
- Most *Cryptosporidium* species are host-adapted- bobcat isolate not likely to be of high public health significance

Estimating Pathogen Loads from a High Proportion of Negative Results

- ½ MDL, corrected for recovery
- Probabilistic worst-case scenario based on scat results (Atwill et al., 2003, Appl. Environ. Microbial. 69(8):4604-4610)
- Arithmetic or geometric mean?
- Estimates differed by ≥ 2 logs





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Conclusions

- Bull Run watershed scat monitoring results are indicative of a very low prevalence of *Cryptosporidium* infection among significant wildlife
- The results provided additional confidence that the level of risk from *Cryptosporidium* in the Bull Run watershed is very low
- More generally, the scat monitoring program highlighted the potential inadequacy of using published values from other regions or extrapolating between species to estimate pathogen loads
- Consistent and robust methods are needed to estimate loading from a high proportion of negative results