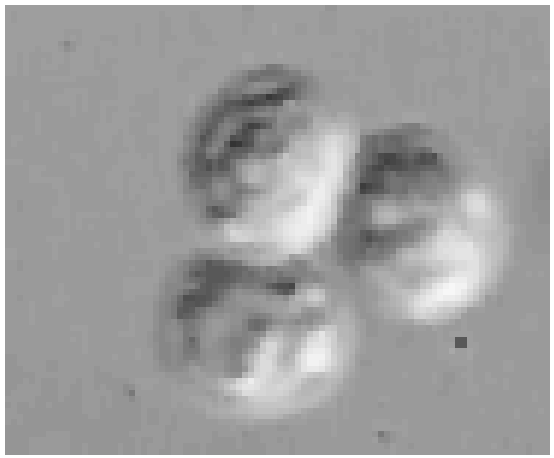
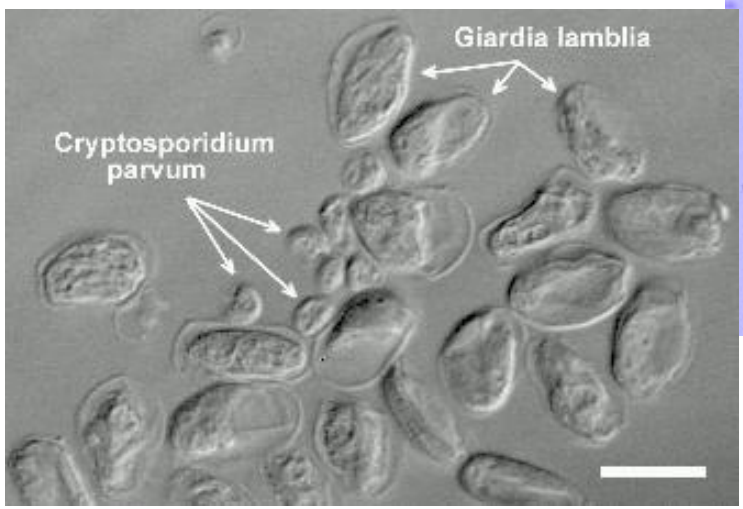
Occurrence and Survival of Protozoan Parasites





Rebecca L. Ives
Joan B. Rose, Ph.D

Photo Credit: H.D.A. Lindquist, U.S. EPA

Overview

- Study Objectives
- Background Information
  - Organisms of interest
  - Method
- Occurrence Study:
  - Lower Grand River Watershed
  - CSO / Retention Basin Sampling
  - River Raisin Watershed

Experimental Goals

- Occurrence of *Cryptosporidium* spp. and *Giardia* spp. in urban and rural Michigan waters receiving CSO discharges
- Survival of *Cryptosporidium* in natural Michigan waters at temperatures found in environment

Background: Enteric Protozoa

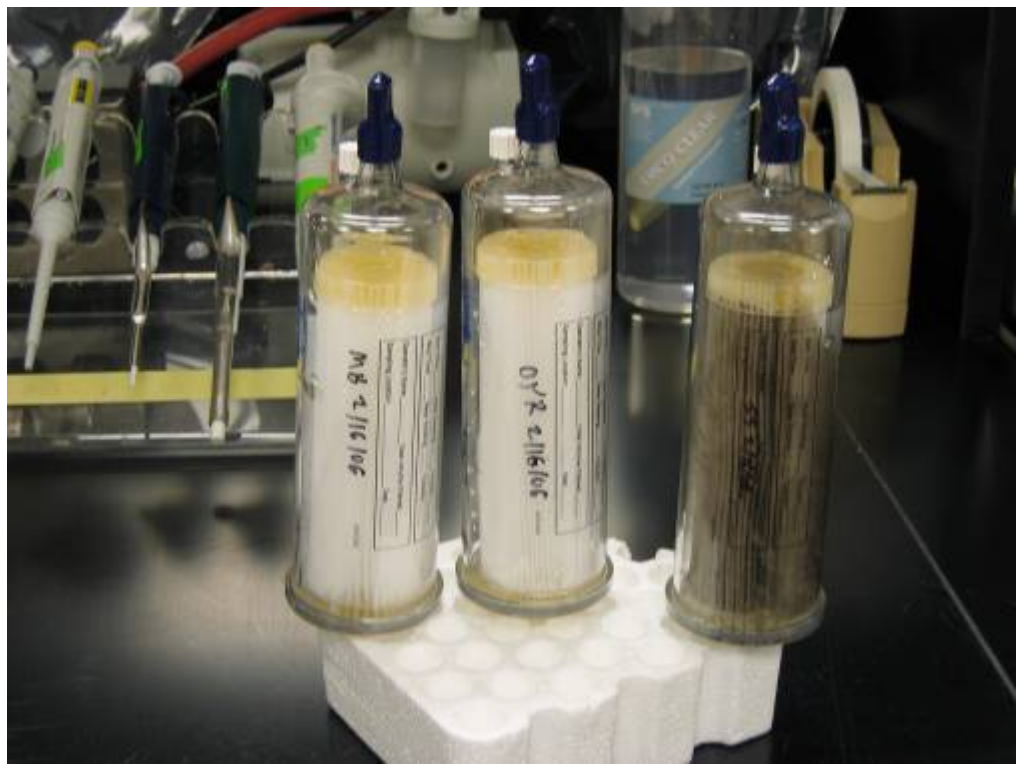
- *Cryptosporidium* and *Giardia*
- Single-celled, obligate intracellular parasites
- **Spread by the Fecal-oral route**
  - Protective exterior structure – chlorine resistance
  - *Cryptosporidium* = oocyst
  - *Giardia* = cyst
  - Collectively = (oo)cyst

Common Characteristics Affecting Epidemiology

- **Shed in the feces of infected animals and humans**
- Low infective dose: 1-10 (oo)cysts
- (Oo)cysts immediately infectious
- Environmental dispersal
- (Oo)cysts are stable, survive long periods in environment
- **Zoonotic**

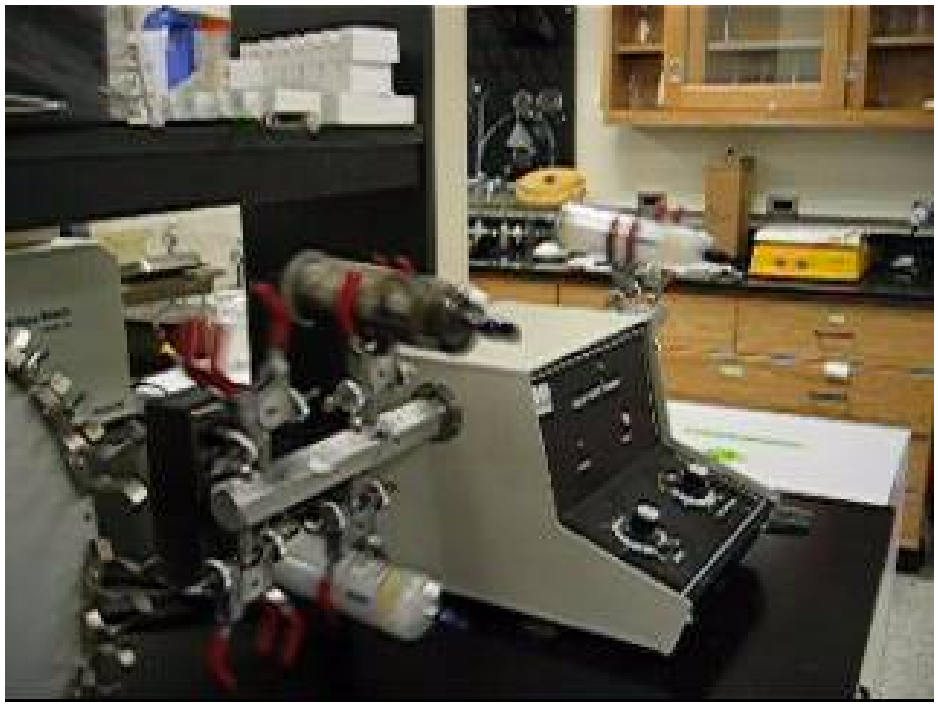
Occurrence: EPA Method 1623

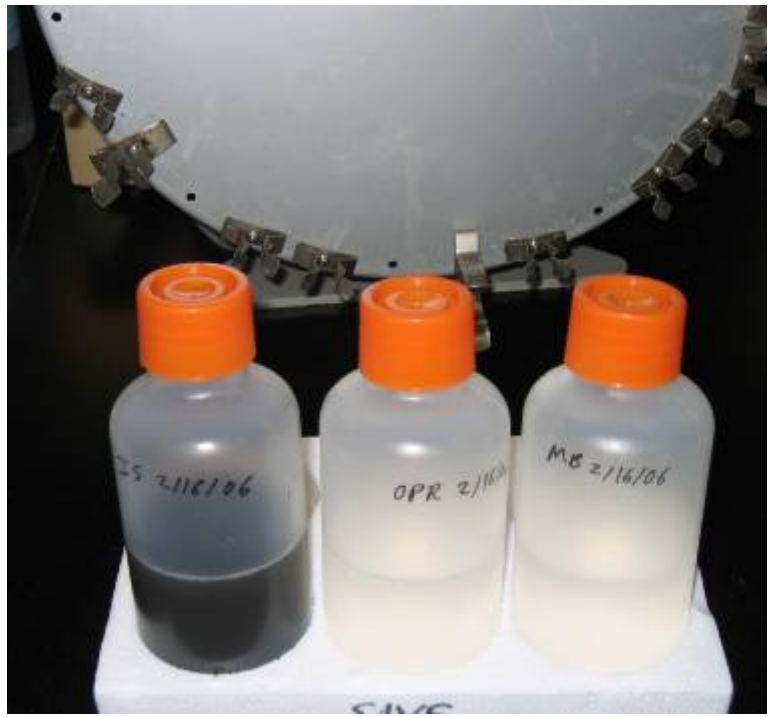
1. Filtration



Occurrence: EPA Method 1623

2. Elution





Occurrence: EPA Method 1623

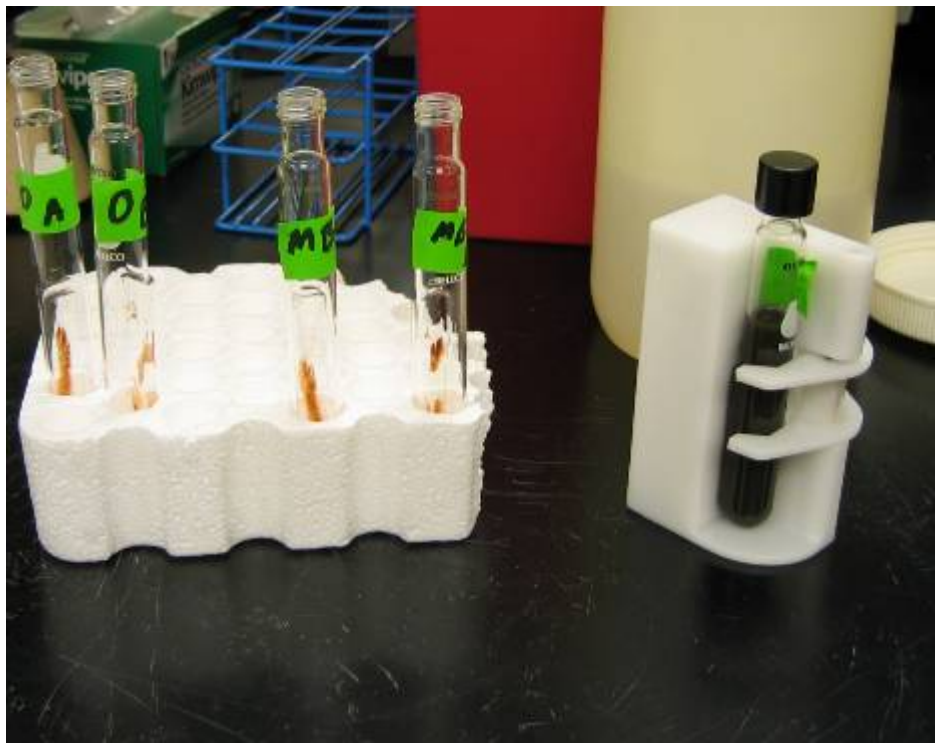
3. Centrifugation

Occurrence: EPA Method 1623

1. ImmunoFluroscent Assay (IFA): **Separation**

Occurrence: EPA Method 1623

1. IFA: **Separation**

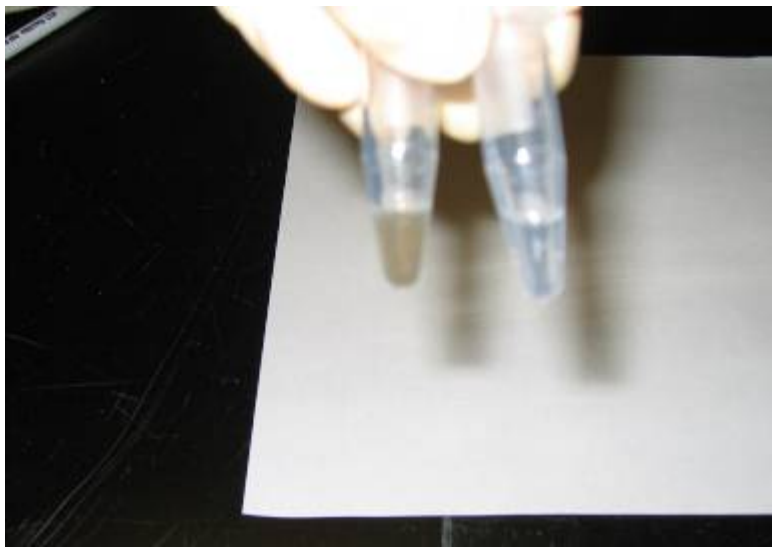


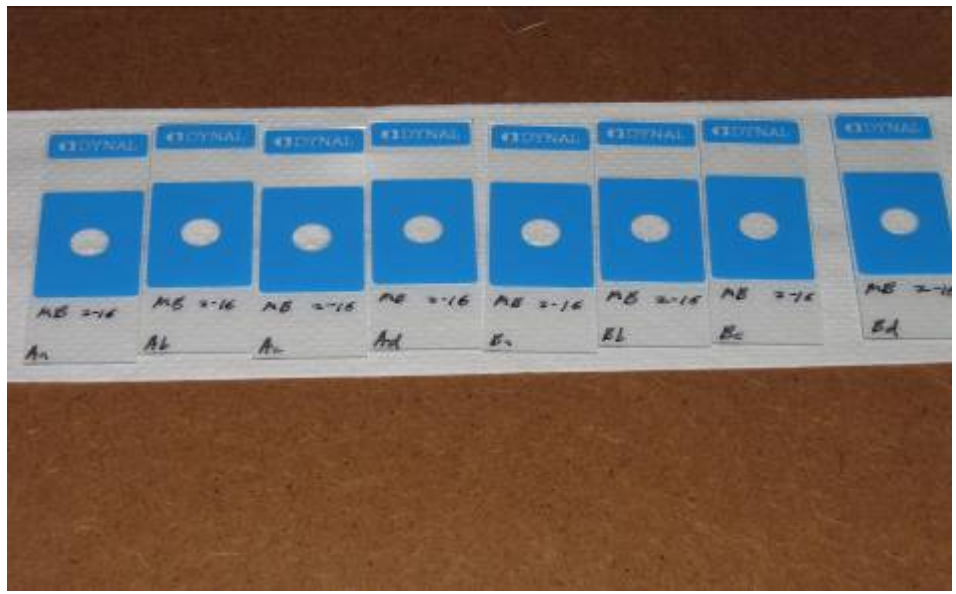
Occurrence: EPA Method 1623

1. IFA: **Separation –** wash step

Occurrence: EPA Method 1623

2. IFA: Dissociation of bead-(oo)cyst complex
3. IFA: **Application to slides and staining**

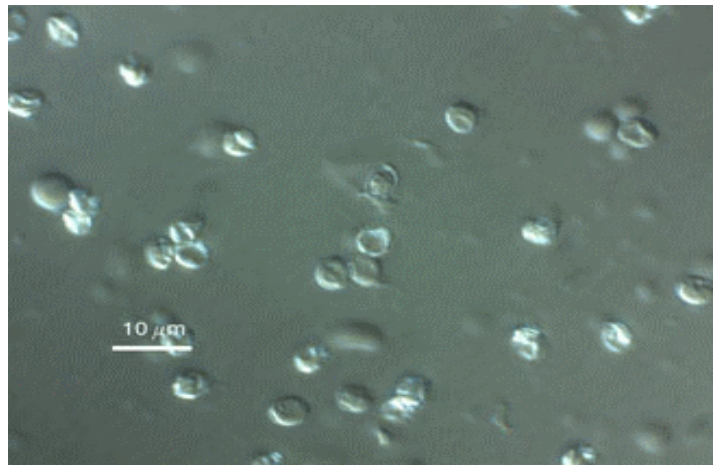




Occurrence: EPA Method 1623

4. Enumeration
via fluorescence microscopy & (DIC) microscopy

Stains:
FITC –green
DAPI - blue



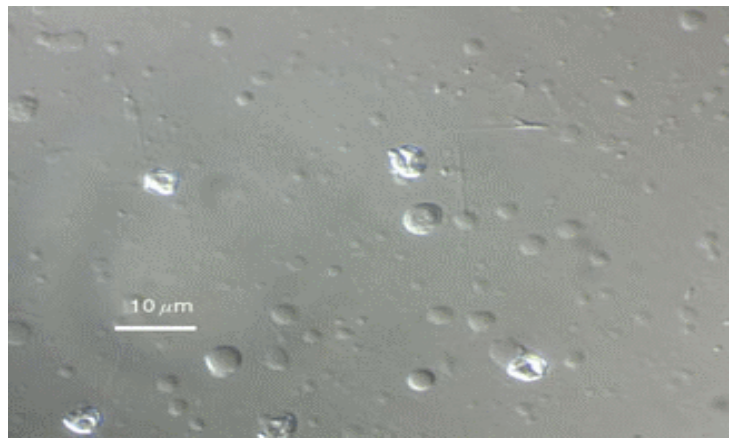


Photo Credit: H.D.A. Lindquist, U.S. EPA

Lower Grand River Watershed Sampling Sites

- Study Period: April 2005 to August 2006
- Survey of 17 sites – Recreational areas
  - 3 chosen for systematic surveillance, all downstream of Grand Rapids
- Deer Creek Park – 22 samples
  - Furthest site upstream
- Riverside Park - 19 samples
- North Beach Park – 19 samples
  - Lake site, North of Grand River mouth


Muskegon Airport
North Beach Park
Mouth of Grand River
Riverside County Park
Deer Creek Park
Retention Basin Discharge
Grand Rapids Wastewater Treatment Plant
Plaster Creek
Gerald Ford Int'l Airport
Image © 2007 TerraMetrics
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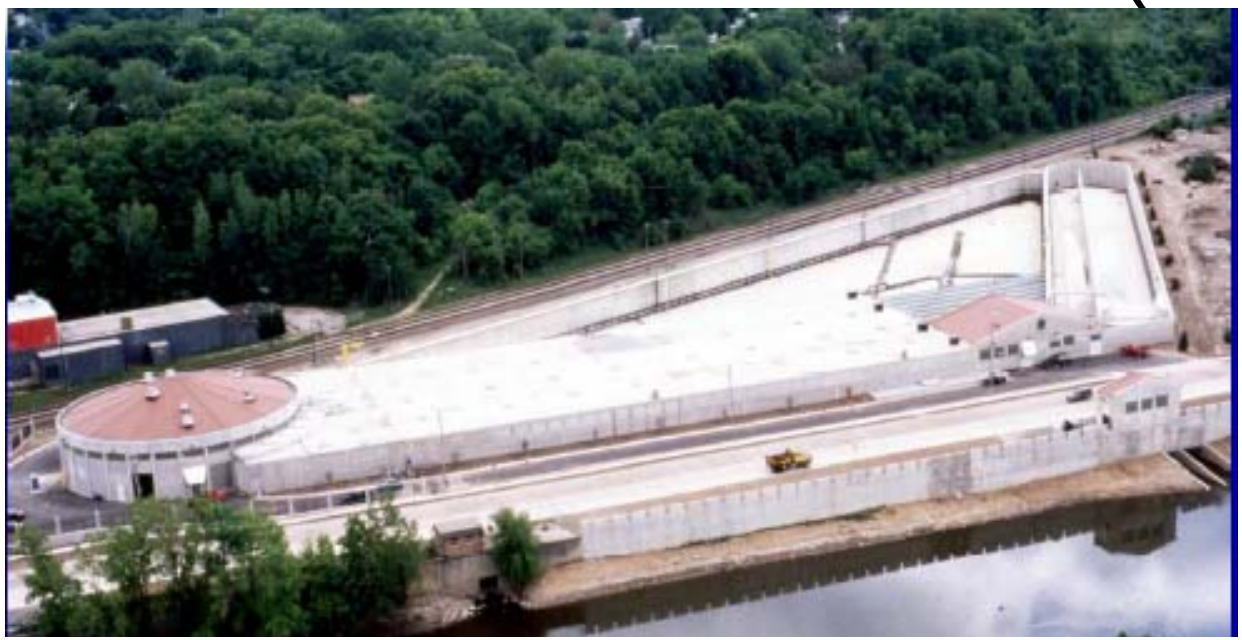
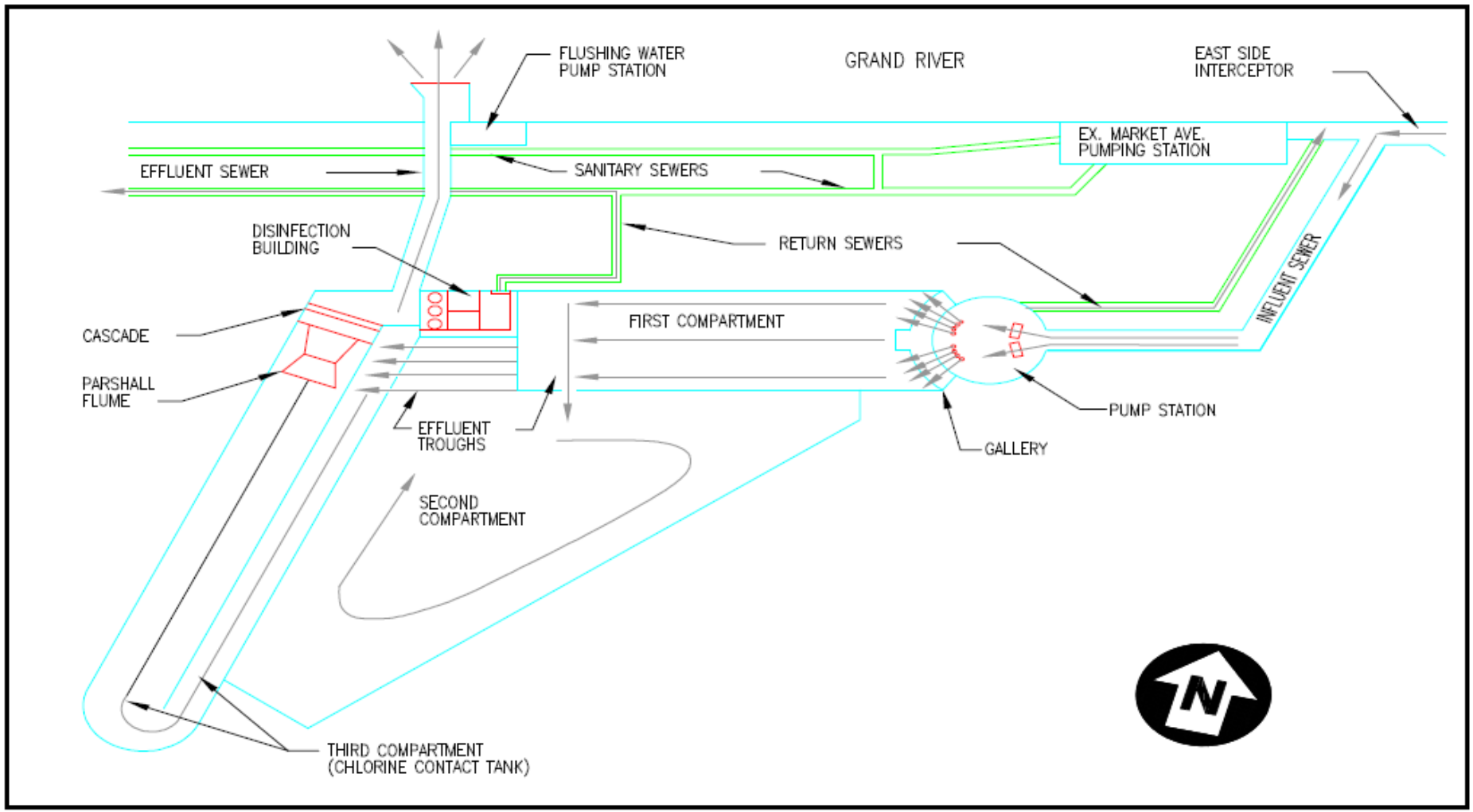
8.68 mi
Google
Pointer 42°59'06.61" N 85°52'06.96" W elev 634 ft
Streaming 100%
Eye alt 29.25 mi

Market Avenue Retention Basin (MARB)

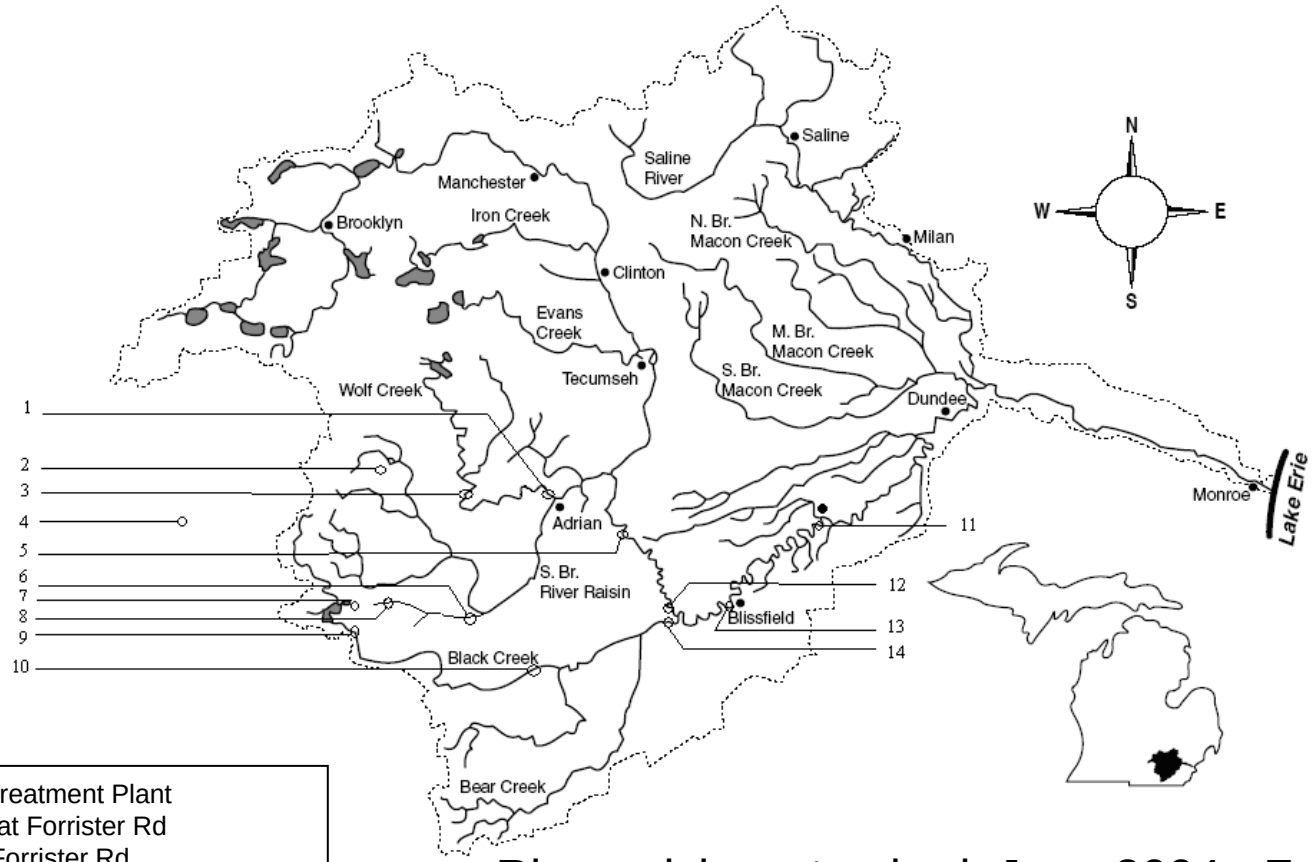


Municipality	Retention Basin	Year Constructed	Basin Capacity (MG)	Covered/ Uncovered	Type of Facility	Construction Cost	O & M Cost	Design Criteria
Grand Rapids, MI	Market Avenue RB	June 1992	30.5		offline	$30 million	$40,000	10 yr-1 hr storm
		First Compartment	10.68	covered				
		Second Compartment	16.68	uncovered				
		Third Compartment	3.14	uncovered				

Market Avenue Retention Basin (MARB)



River Raisin Watershed:



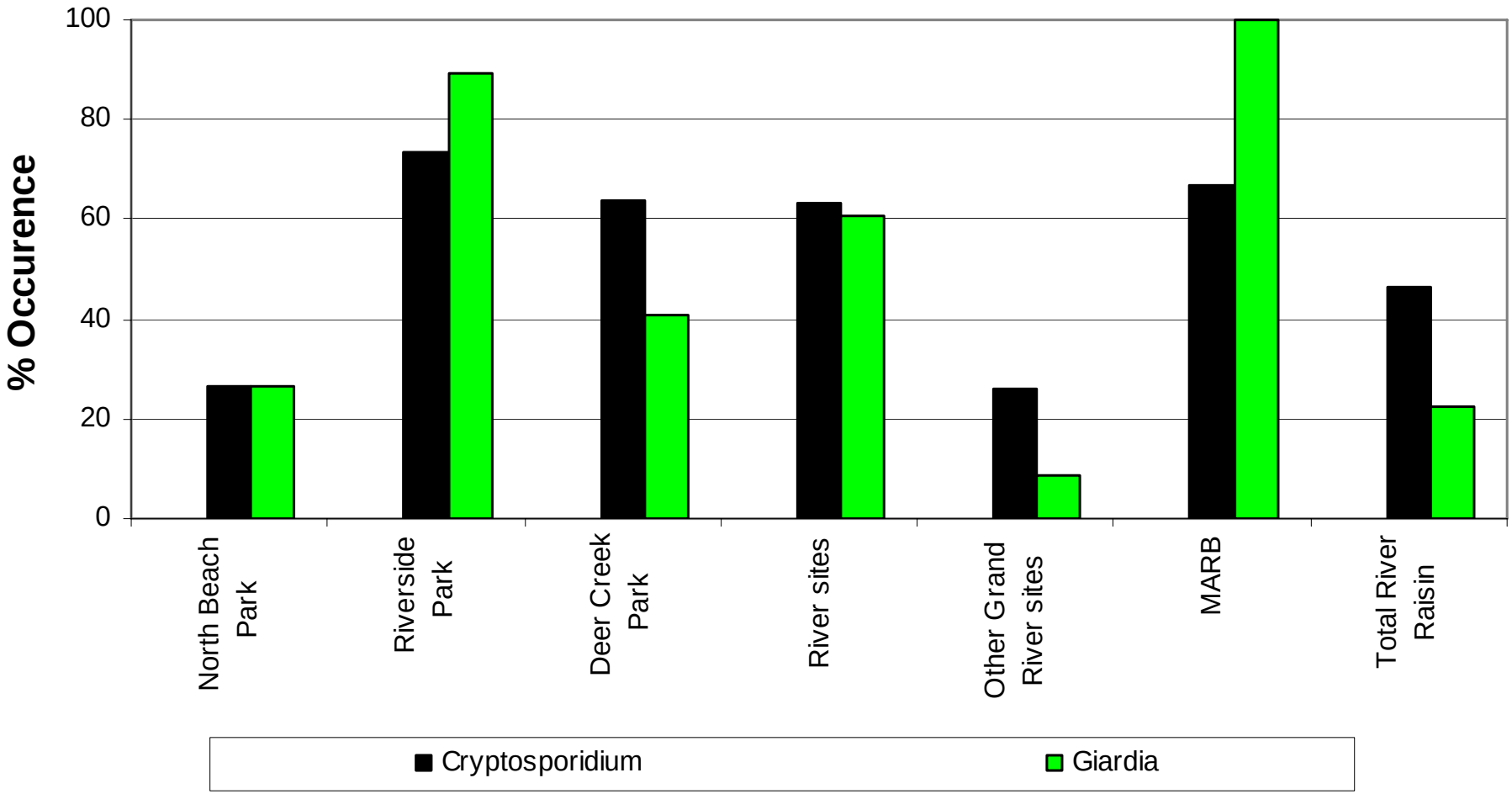
1. Adrian Water Treatment Plant
2. Milk Drain Tile at Forrister Rd
3. Wolf Creek at Forrister Rd
4. St. Joseph Creek at Beecher Rd
5. Main Branch River Raisin at Deerfield Rd
6. Stony Creek at Gorman Rd
7. Rice Lake Drain at Haley Rd
8. Stony Creek at Seneca Rd
9. Black Creek at Medina Rd
10. Black Creek at Morse Rd
11. Deerfield Water Treatment Plant
12. Main Branch River Raisin at Crockett Rd
13. Blissfield Water Treatment Plant
14. Black Creek at Crockett Rd

River raisin watershed: June 2004 - February 2005.

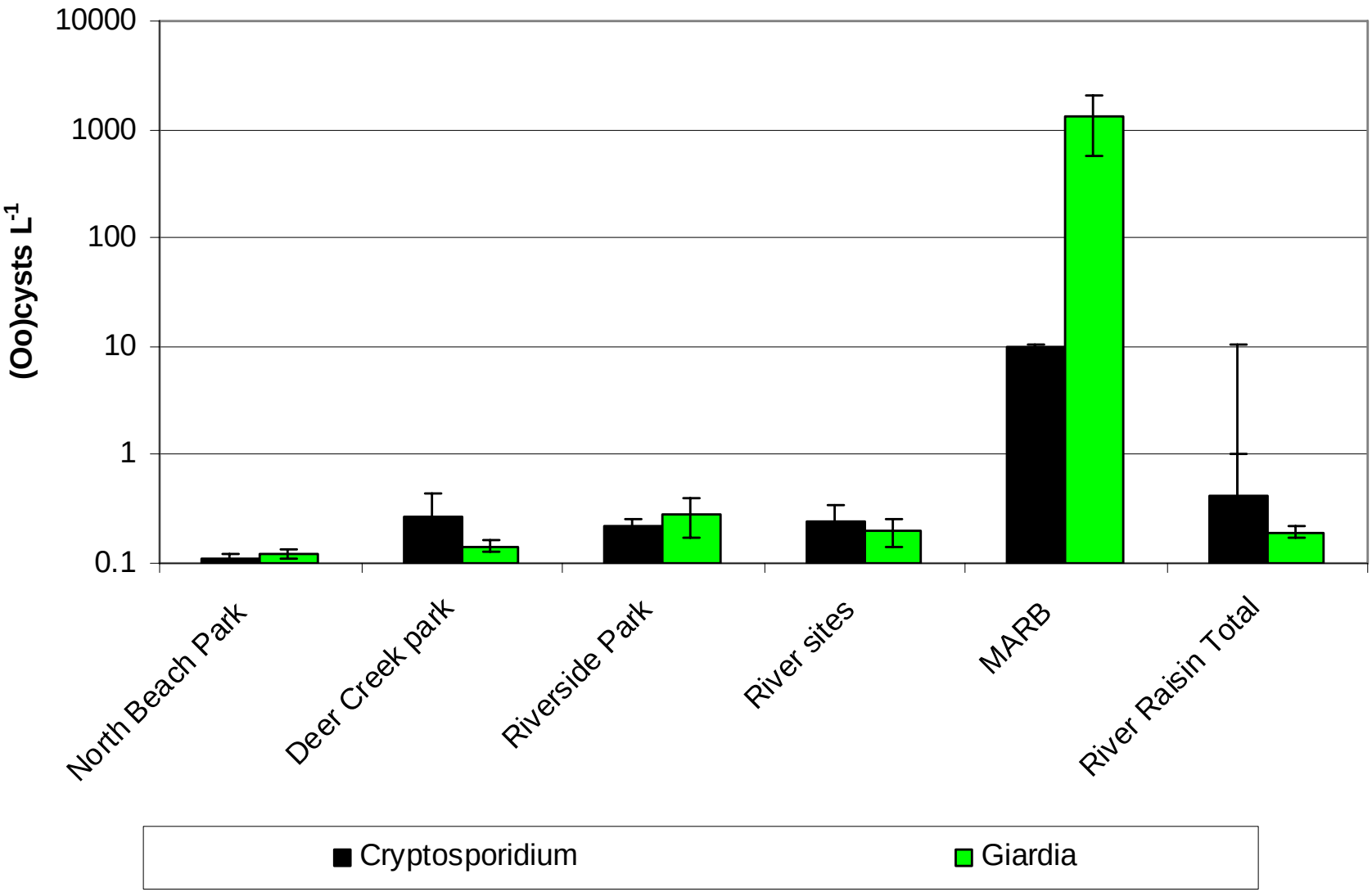
- 3 surface water treatment plant intakes
- 4 tributary creeks
- 1 field drainage structure

Bean/Tiffin Watershed: 1 tributary creek

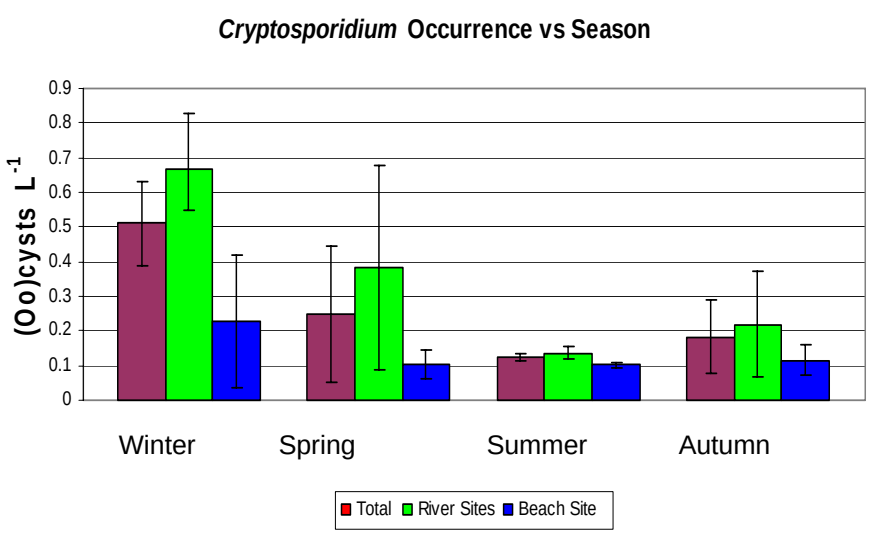
% Parasite Occurrence

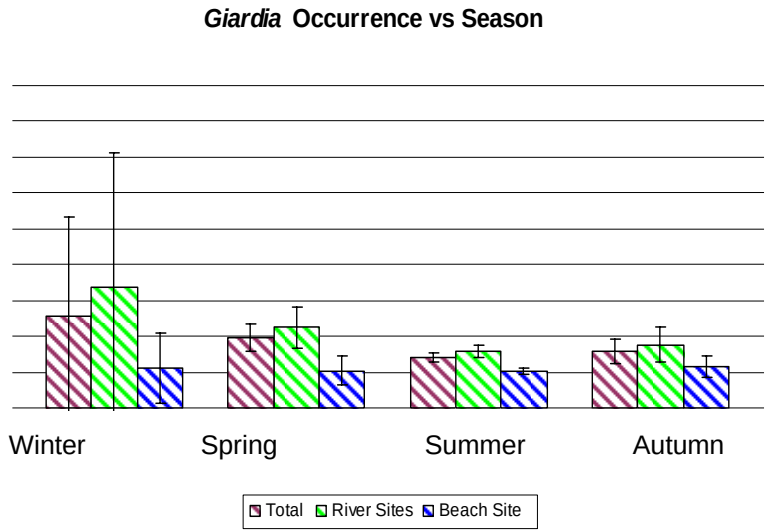


Parasite Occurrence



Grand River Seasonal Parasite Occurrence





Number observations:				
	Winter	Spring	Summer	Autumn
Total	8	18	27	7
River	6	12	18	5
Beach	2	6	9	2

Methods:Survival

- Microcosms of waters from Deer Creek Park
- Microcosms seeded with *Cryptosporidium* (Sterling Parasitology Laboratory, Az)
  - Final concentration ~10^6 oocyst/ml
- 2 microcosms at 25ºC, 2 at 4ºC, aliquots in microcentrifuge tubes at -8ºC
- Positive control: *Cryptosporidium* seeded into sterile distilled water held at 4ºC. Final concentration ~2 x10^7 oocyst/ml

Methods:Survival

- Bleach treatment of aliquot from microcosms
- Dilution series used to infect HCT-8 (human endothelial adenocarcinoma) cell culture in 8-chambered well slides
- Staining of slides
- Most Probable Number (MPN) Analysis

Survival Results

Temperature (ºC)	Sample Seeded with *Cryptosporidium*	Infectivity Assessment (Days)			
		0	14	35	71 (End)
-8	Deer Creek Sample	+	-	-	-
4	Deer Creek Sample	+	+	+	+
25	Deer Creek Sample	+	+	-	-
4	Positive Control	+	+	+	+

Grand River Watershed Observations

- Occurrence
  - *Cryptosporidium* & *Giardia* detected most often in Riverside Park.
  - Cryptosporidium detected in >60% of Grand River and MARB samples - usually at low levels
  - Compared to river:
    - MARB Cryptosporidium concentrations higher by 1 order of magnitude
    - MARB Giardia concentrations higher by 3 orders of magnitude
  - *Cryptosporidium* concentrations: Spring - variable, summer consistently 0.1 – 0.2 L^{-1}
- Survival
  - Infectivity of samples at -8ºC fell below detectable levels after 14 days
  - Infectivity of samples at 25ºC fell below detectable levels after 35 days
  - Samples at 4ºC still infectious by 71 days.

Conclusions

- Low Health Risks at Beach site :
  - 26.3% parasite occurrence at levels between 0.1-0.2 L^{-1}
- The Grand River receives parasites from normal sewage discharges and on occasion receives high levels from CSOs.
- February – August, 2008:
  - The MARB prevented 5.19×10^{9} *Cryptosporidium* oocysts and 6.92×10^{11} *Giardia* cysts from entering the river

Acknowledgements

- Joan B. Rose
- Rose Lab Personnel
- City of Grand Rapids
- NOAA
- MDEQ

Questions?

Occurrence Results

Site	N (sites or events)	% Cryptosporidium (+)	% Giardia (+)	% Cryptosporidium & Giardia (+)	% Either Cryptosporidium or Giardia (+)
Deer Creek Park	22	63.60	40.90	18.20	81.80
Riverside Park	19	73.68	89.47	73.68	94.74
North Beach Park	19	26.32	26.32	10.53	42.11
Other Grand River sites	23	26.00	8.70	8.70	26.00
Total Grand River Watershed sites	83	47.00	40.00	26.50	60.20
River Raisin Watershed (Non - CSO)	28	46.4	32.1	25	53.6
River Raisin Watershed (CSO)	14	50	14.3	7.1	57.1
Total River Raisin Watershed sites	45	46.67	22.22	15.56	53.33
MARB	9	66.7	100	66.7	100

Occurrence Results

Site	*Cryptosporidium* range			*Giardia* range		
	Median	min	Max	Median	Min	Max
North Beach Park	0	0	0.265	0	0	0.48
Deer Creek Park	0.165	0	5.17	0	0	0.45
Riverside Park	0.15	0	0.76	0.28	0	3.3
River Raisin Watershed - Non CSO	0.0785	0	599	0	0	0.763
River Raisin Watershed - CSO receiving sites	0	0	0.893	0	0	0.406
MARB	0	0	26.667	1240	340	4506.667

Grand Rapids CSO Events