

Bather Density and Levels of *Cryptosporidium*, *Giardia*, and Pathogenic Microsporidian Spores in Recreational Beaching Waters



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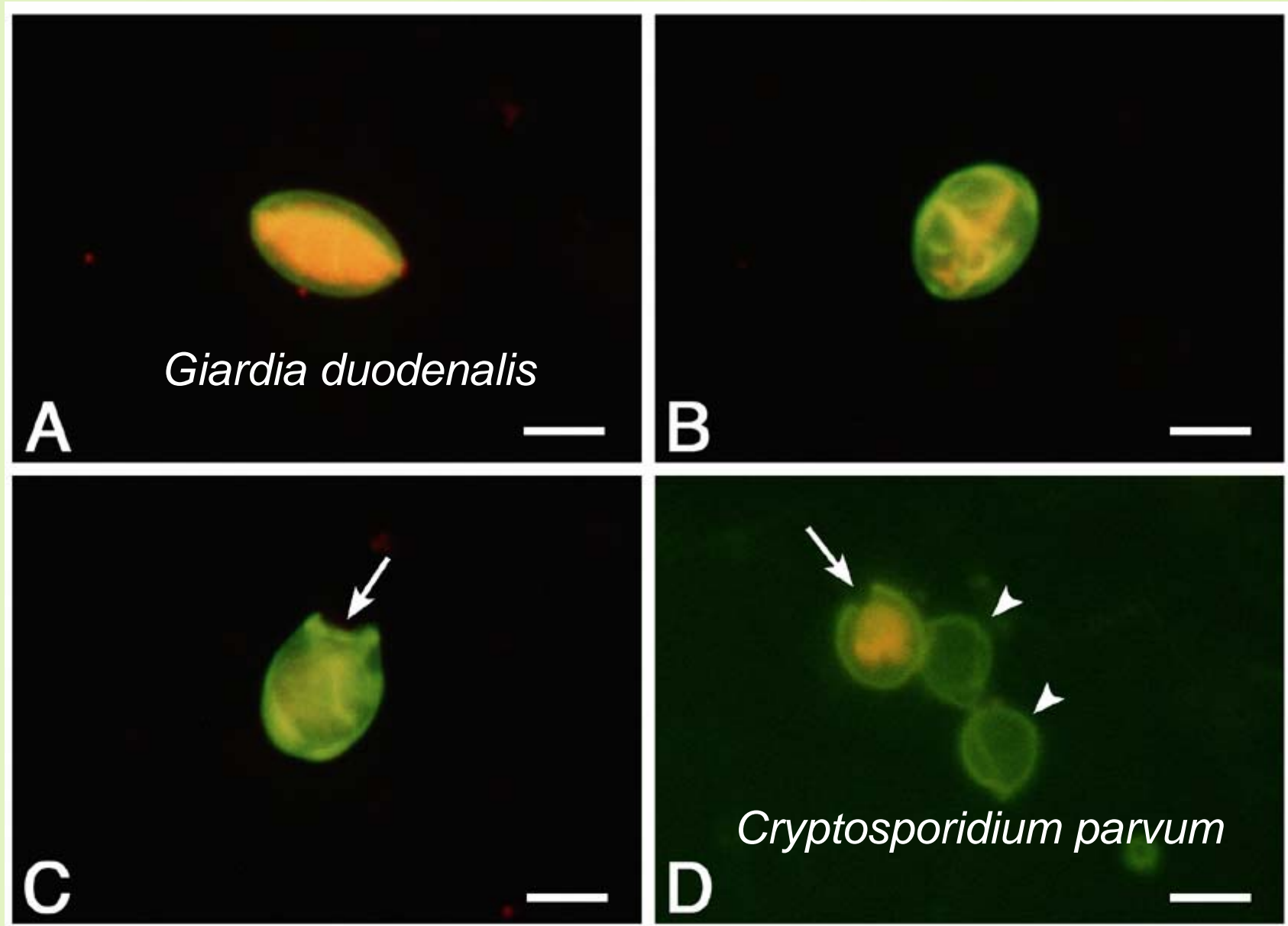
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Study Concept (surface recreational waters)

- ✿ Fecal indicator organisms (*E. coli*, *enterococci*, total and fecal coliforms)
- ✿ Sedimentation in water column
- ✿ Sediment re-suspension (bathers, boat traffic, dredging, runoff, tides, etc.)
- ✿ Microbial load (fecal accidents, anal residue, diapered children)
- ✿ Bather numbers vs fecal coliform levels
- ✿ *Cryptosporidium*, *Giardia*, and *microsporidian spores* vs. fecal coliforms; lack of correlation
- ✿ A need for better water quality parameters
- ✿ Technology for quantitative assessment of viable waterborne *Cryptosporidium*, *Giardia*, and *microsporidia*

The Graczyk's Lab: **Multiplexed Fluorescent *In Situ* Hybridization (FISH) for *Cryptosporidium* and *Giardia***



The Graczyk's Lab: Multiplexed Fluorescent *In Situ* Hybridization (FISH)

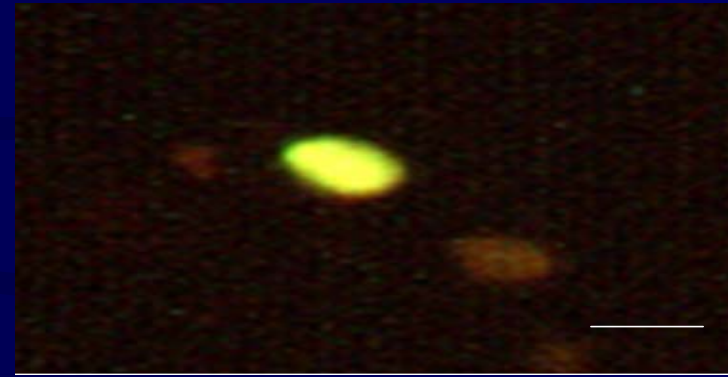
E. hellem Hester et al. (2000) *J Eukaryot Microbiol* **47**:299-308.

Graczyk et al. (2007) *J Clin Microbiol* **45**:1255-60

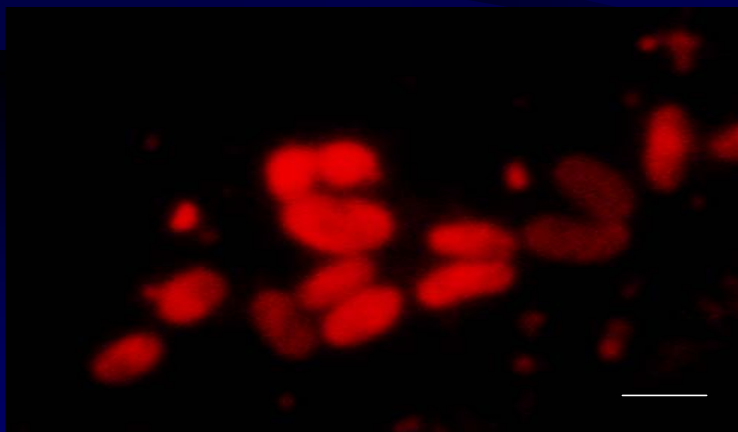
E. hellem



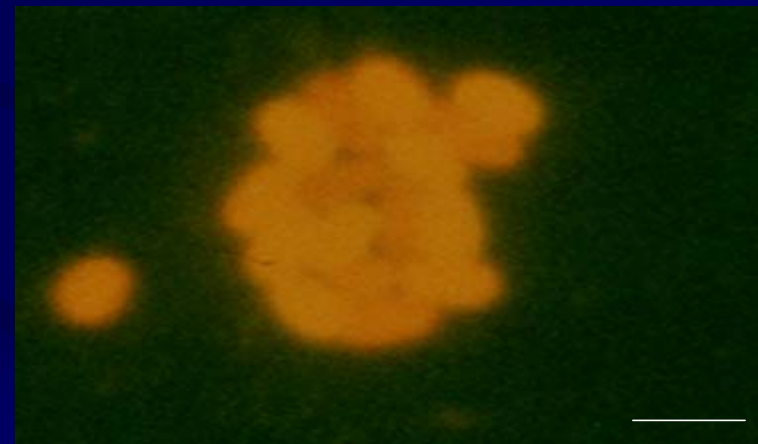
E. bieneusi



E. intestinalis



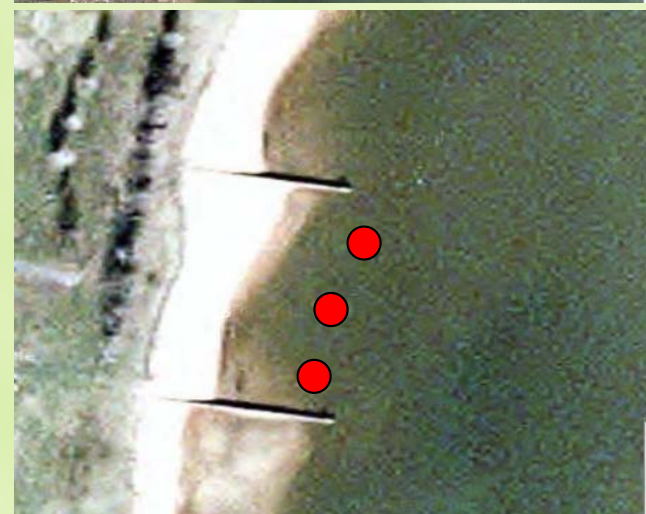
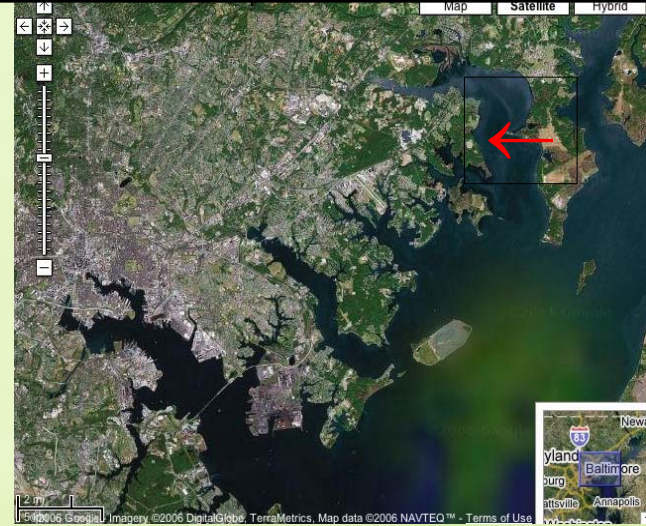
E. cuniculi



Study Site Location



CHESAPEAKE BAY
A Satellite View





❁ Gunpowder State Park- Hammerman Beach Area, Maryland, USA

- ❁ Located on the Gunpowder River,
Baltimore County
- ❁ Offers swimming and water sports



How do they enter the water supply?

- ✿ Agricultural/urban runoff
- ✿ Wastewater treatment plant effluents
- ✿ Leaking septic systems
- ✿ Wildlife
- ✿ Domestic animals
- ✿ Fecal accidents
- ✿ Diapered children



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Methods

Summer 07; 11 consecutive weeks; 33 week samples, 27 weekend samples



- ✿ Bather counts (0-5 scale)
- ✿ Water parameters
 - ✿ Salinity
 - ✿ Conductivity
 - ✿ Dissolved oxygen
 - ✿ Temperature
 - ✿ Tides
 - ✿ Rainfall
- ✿ Water Turbidity
- ✿ Concentration of *C. parvum*, *G. duodenalis*, and microsporidian spores

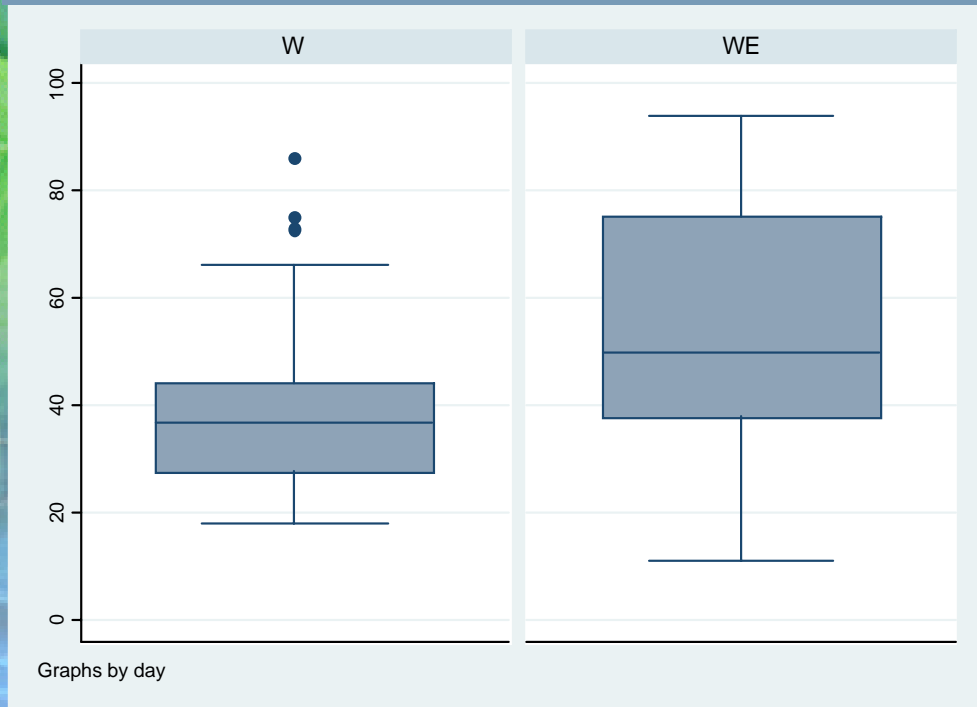


🌿 Bather Score

- ↖️ 🍀 **0 = 0-1 bather**
- 🍀 1 = 2-13 bathers
- 🍀 2 = 14-25 bathers
- ↖️ 🍀 **3 = 26-37 bathers**
- 🍀 4 = 38-49 bathers
- 🍀 5 = 50+ bathers

Results -Turbidity

Turbidity (FAU) Box Plot by Weekday and Weekend

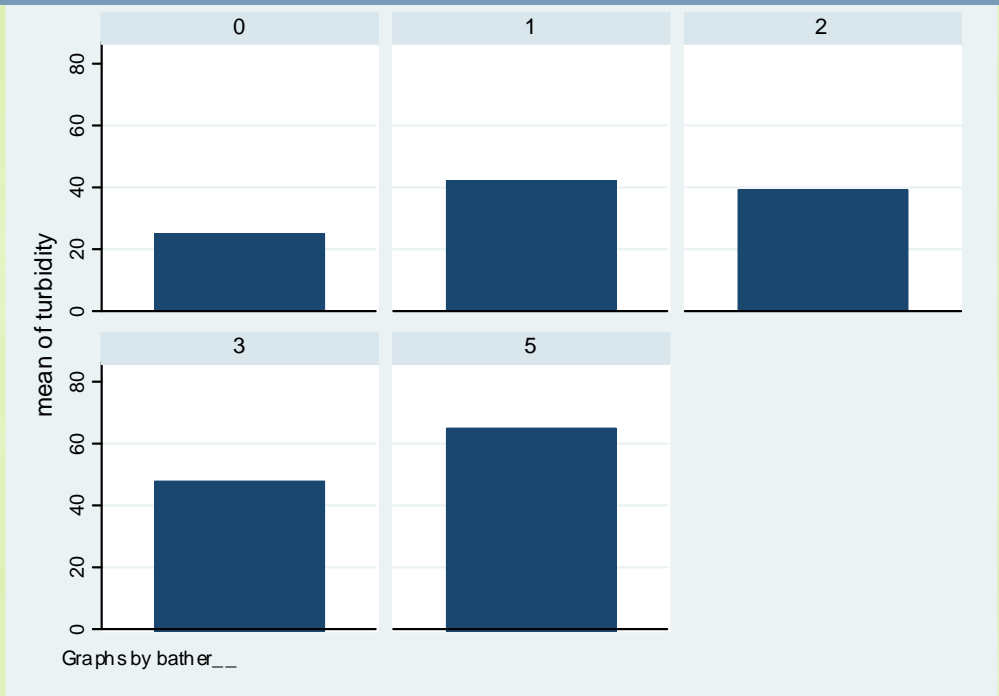


Two sample t-test

Turbidity (W) v Turbidity (WE) $p=0.0321$

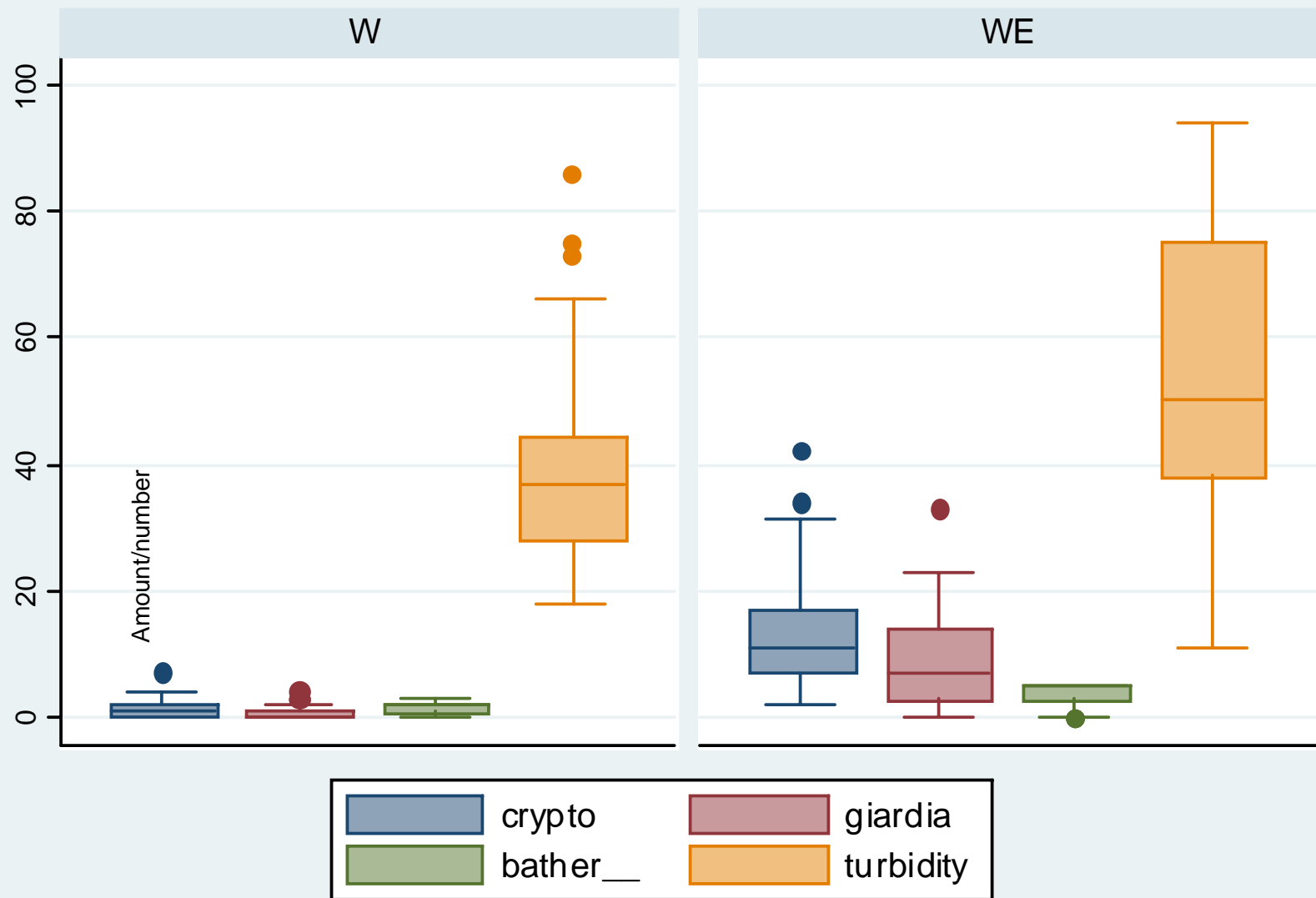
Mean (W)=39.60, Mean (WE)=53.56

Mean Turbidity in Relation to Bathers Score



Pearson Correlation:

Turbidity significantly correlated with bather score; $p=0.0000$



Graphs by day

Two sample t-test:

Crypto (WE) v Crypto (W); $p=0.0000$

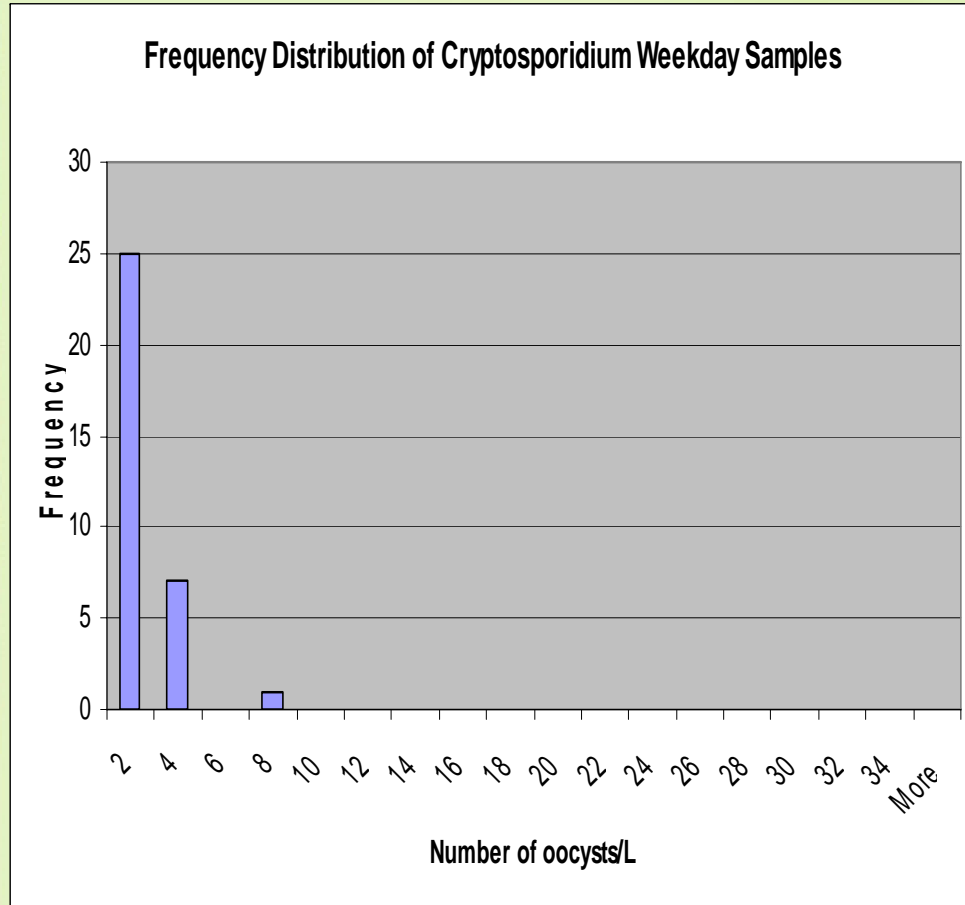
Giardia (WE) v Giardia (W); $p=0.0000$

Turbidity (WE) v Turbidity (W); $p=0.0321$

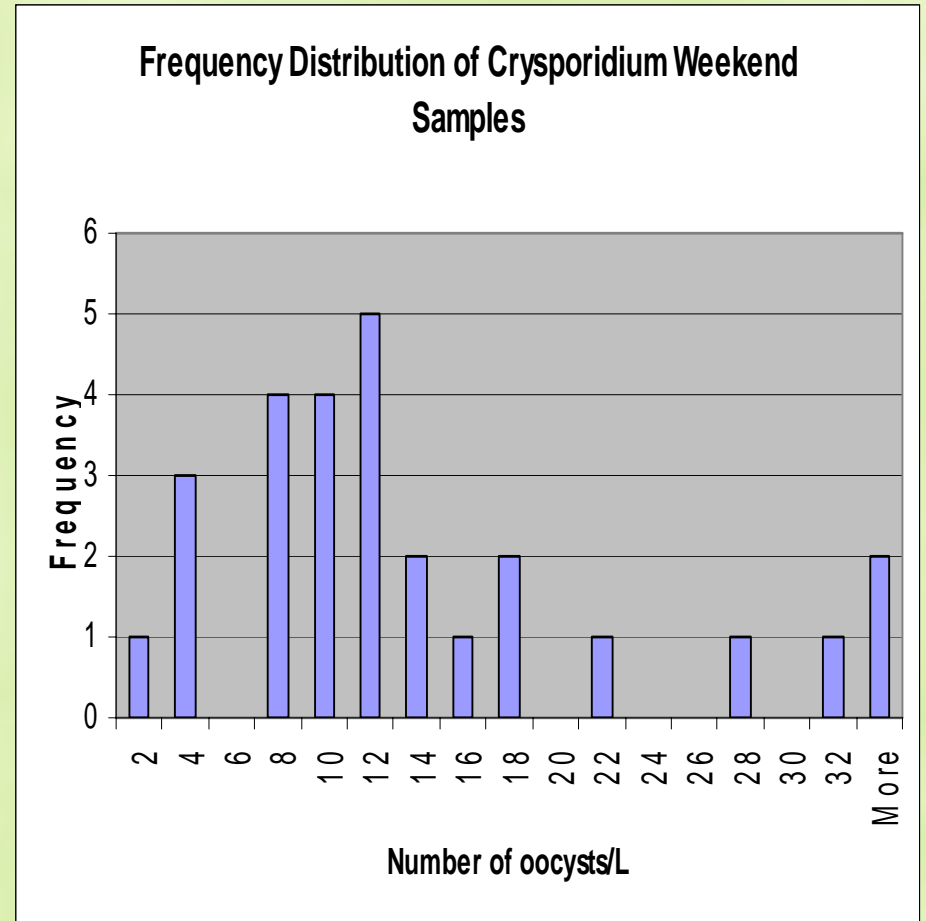
Wilcox signed rank test:

Bather (WE) v Bather (W); $p=0.0001$

Results-*Cryptosporidium*

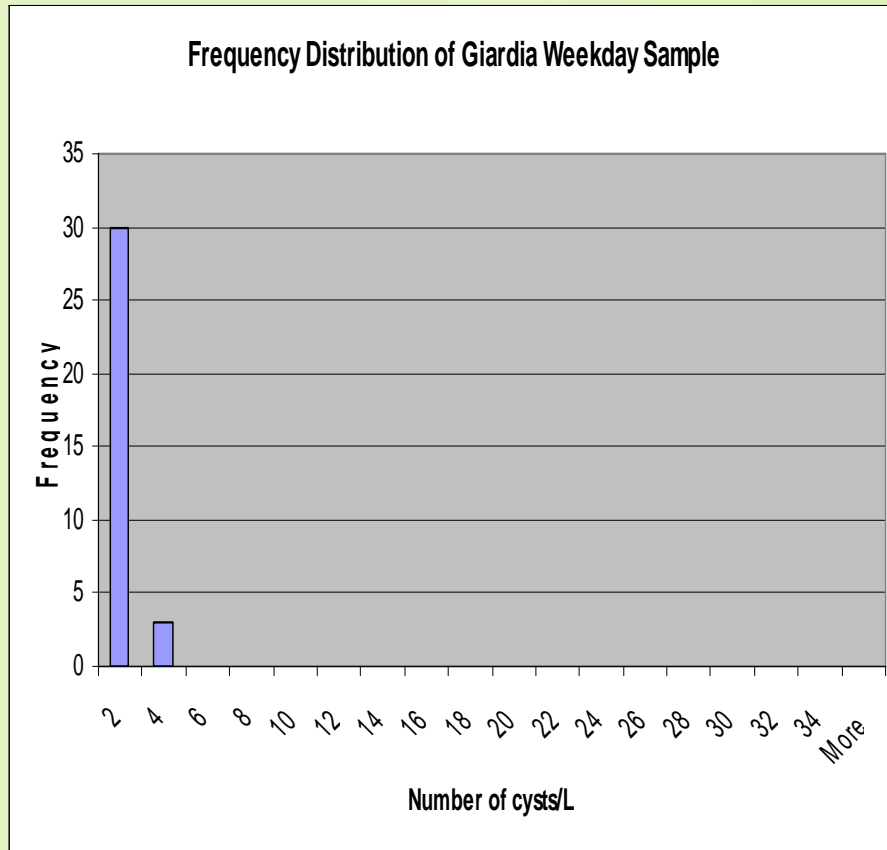


Mean concentration week=
1.51 oocysts/L

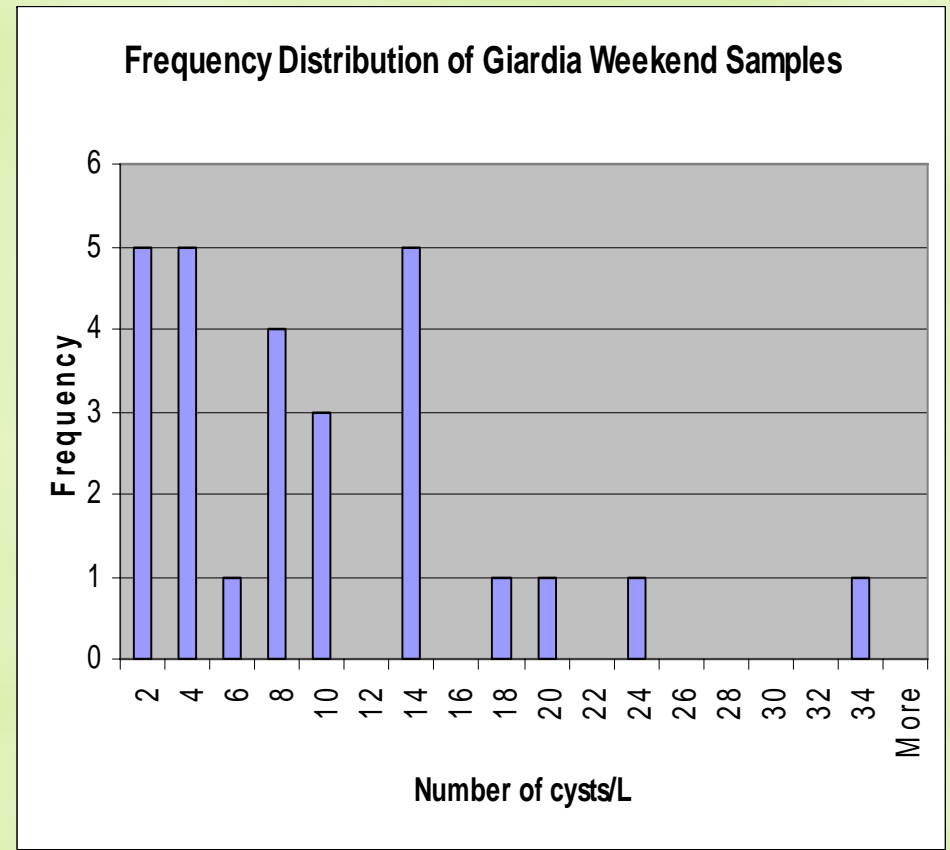


Mean concentration weekend=
13.67 oocysts/L

Results - *Giardia*

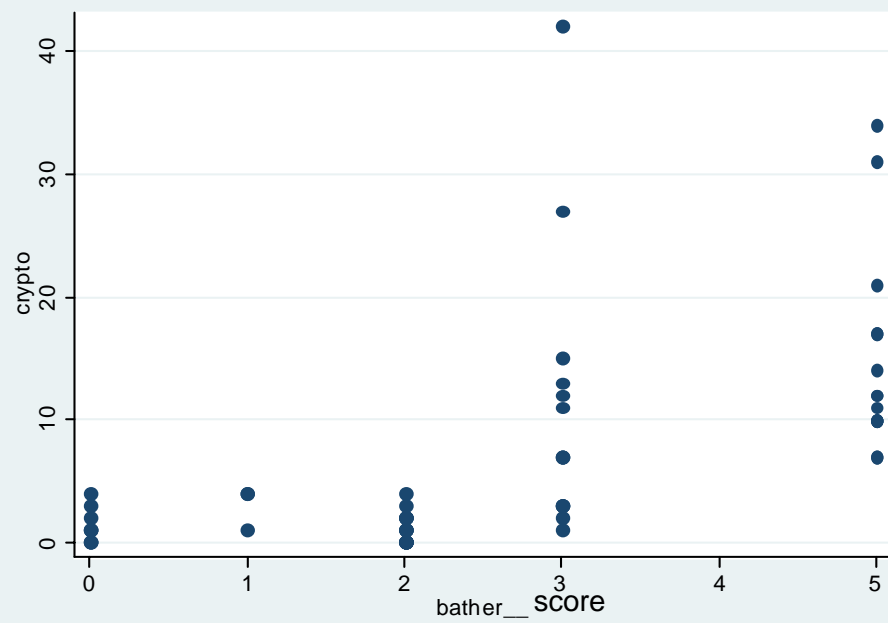


Mean concentration weekday=
0.64 cysts/L

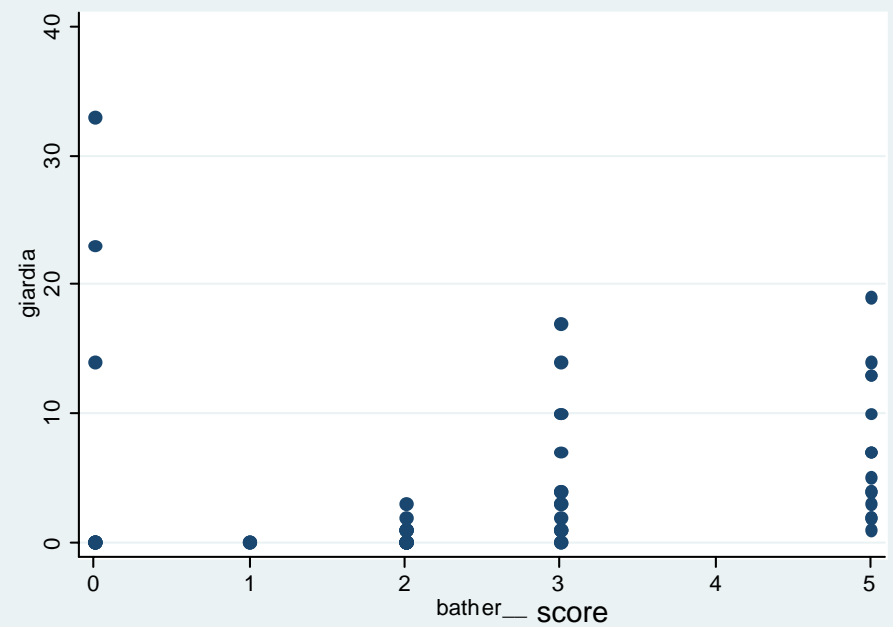


Mean concentration weekend=
9.11 cysts/L

Distribution of *Cryptosporidium* oocysts/L by Bathers Score



Distribution of *Giardia* cysts/L by Bathers Score



Results – Microsporidian spores

	Weekend samples <i>n</i> = 27	Weekday samples <i>n</i> = 33	<i>P</i> -value
Number and (%) of positive samples	16 (59)	10 (30)	0.03
Spore concentration (spores/L)	0 - 16 4.8 + 0.9	0 - 11 1.8 + 0.6	0.04
Bather density score	2 - 5 3.8 + 1.6	0 - 3 1.6 + 1.1	0.001
Water turbidity (NTU)	11 - 88 53.6 + 21.1	18 - 75 39.9 + 15.4	0.04
Rainfall (cm)	0 - 6.0 1.0 + 2.2	0 - 1.8 0.2 + 0.5	NS
Tide (m)	0.17 - 0.67 0.33 + 0.18	0.2 - 0.46 0.29 + 0.12	NS
Water salinity (ppt)	2.1 0.9 + 0.8	0.3 - 2.0 0.7 + 0.6	NS
Water temperature (°C)	26.5 - 32.4 29.6 + 2.0	22.9 - 33.0 30 + 3.0	NS
Dissolved O ₂ (mg/L)	4.9 - 7.9 6.0 + 0.9	4.0 - 7.5 5.7 + 0.9	NS
Water conductivity (FS/m)	51 - 412 181 + 146	70 - 396 165 + 125	NS

Conclusions

- ✿ These enteropathogens are capable of causing waterborne outbreaks
- ✿ Public education should be improved
- ✿ Test waters when bather numbers are greatest
- ✿ Limit number of bathers per area



Conclusions (surface recreational waters)

- ✿ Inadequacy of standard water quality parameters
- ✿ Needs for new water quality parameters or direct testing for specific enteropathogens
- ✿ Two mechanisms for elevated fecal coliform levels (**sediment re-suspension**, bather microbial load)
- ✿ Two mechanisms for elevated fecal levels of *Cryptosporidium*, *Giardia*, and human-virulent microsporidia (**sediment re-suspension**, bather microbial load)
- ✿ **If** this is the case, there should be correlation between fecal coliform counts and waterborne protozoa
- ✿ **If** this is the case, fecal coliforms are as good as direct water testing for waterborne protozoa
- ✿ **If this is the case, no better water quality parameters are needed for surface recreational waters if the water is tested during weekends**

Sunderland, D., T. K. Graczyk, L. Tamang, and P. N. Breysee. 2007. Impact of bathers on levels of *Cryptosporidium parvum* and *Giardia lamblia* cysts in recreational beach water. *Water Research*: **41**, (15); 3483-3489.

Graczyk, T. K., D. Sunderland, L. Tamang, T. M. Shields, F. E. Lucy, and P. N. Breysee. 2007. Quantitative evaluation of the impact of bathers on levels of human-virulent microsporidian spores in recreational water. *Applied Environmental Microbiology* **73**, (13): 4095-4099.



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