

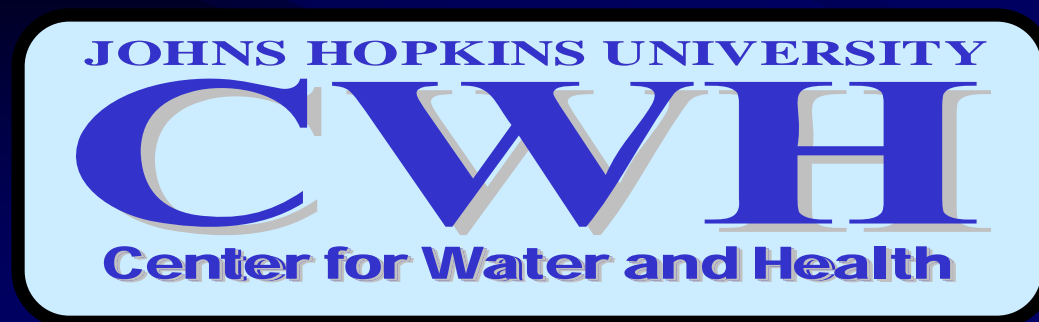
Efficacy of Engineered Wetlands in Removal of Human Protozoan Enteropathogens; Fact of Fiction?

Thaddeus K. Graczyk

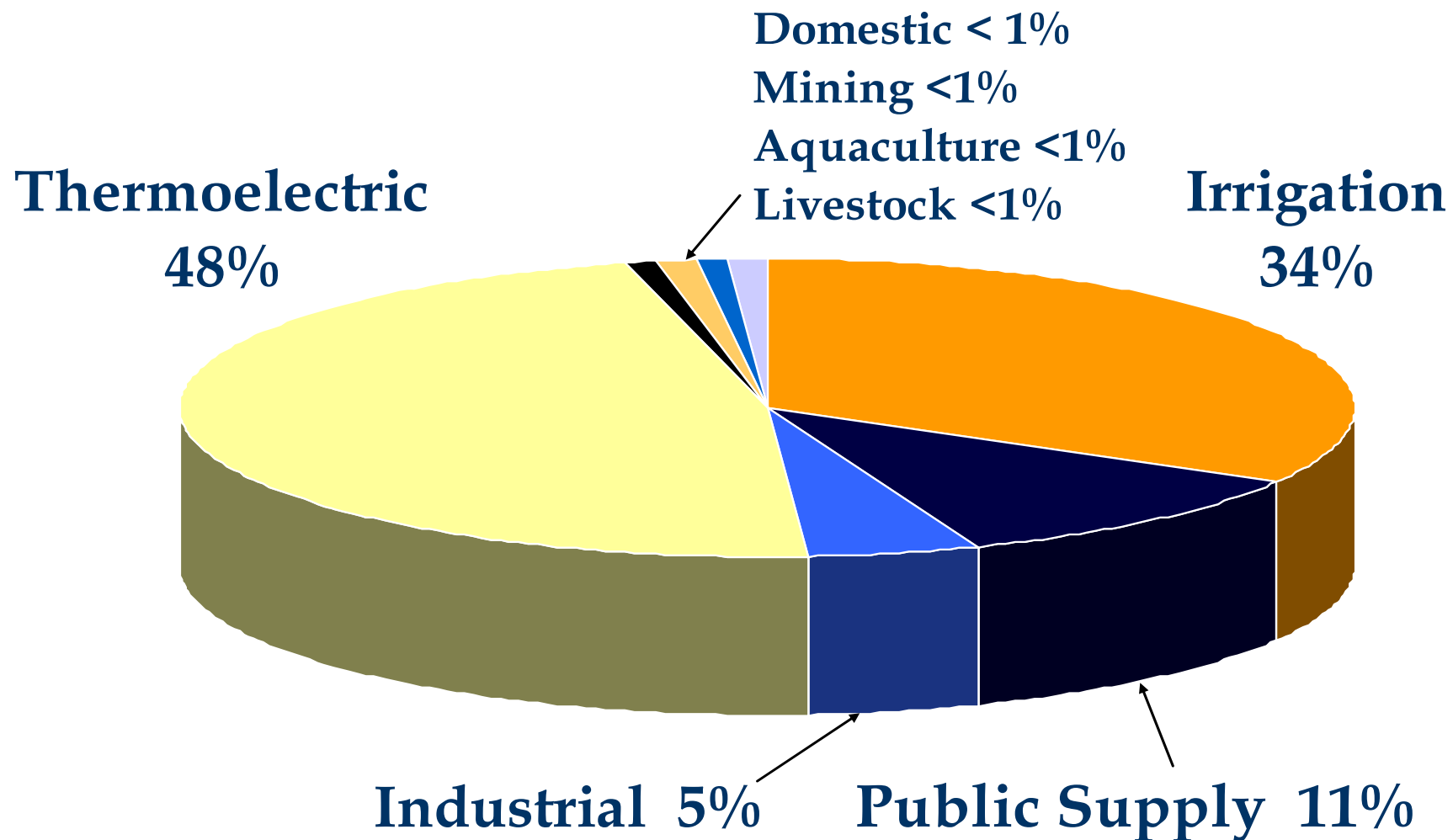
Department of Environmental Health Sciences

Department of Molecular Microbiology Immunology

Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA



Total Water Withdrawals in the United States

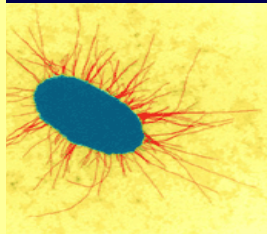


Source: USGS (2004)

Categories of Organisms of Public Health Significance in Wastewater

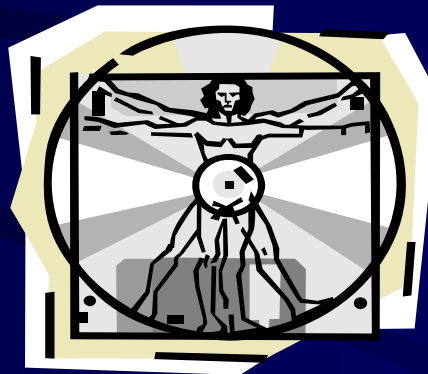
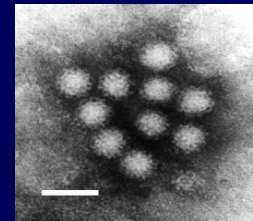
Bacteria

Campylobacter
Salmonella
Shigella
E. coli
Vibrio



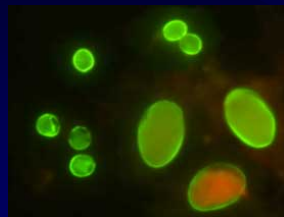
Viruses

Noroviruses
Hepatitis A virus
Rotavirus
Coronaviruses



Protozoa

Cryptosporidium
Giardia
Microsporidia,
Cyclospora,
Toxoplasma

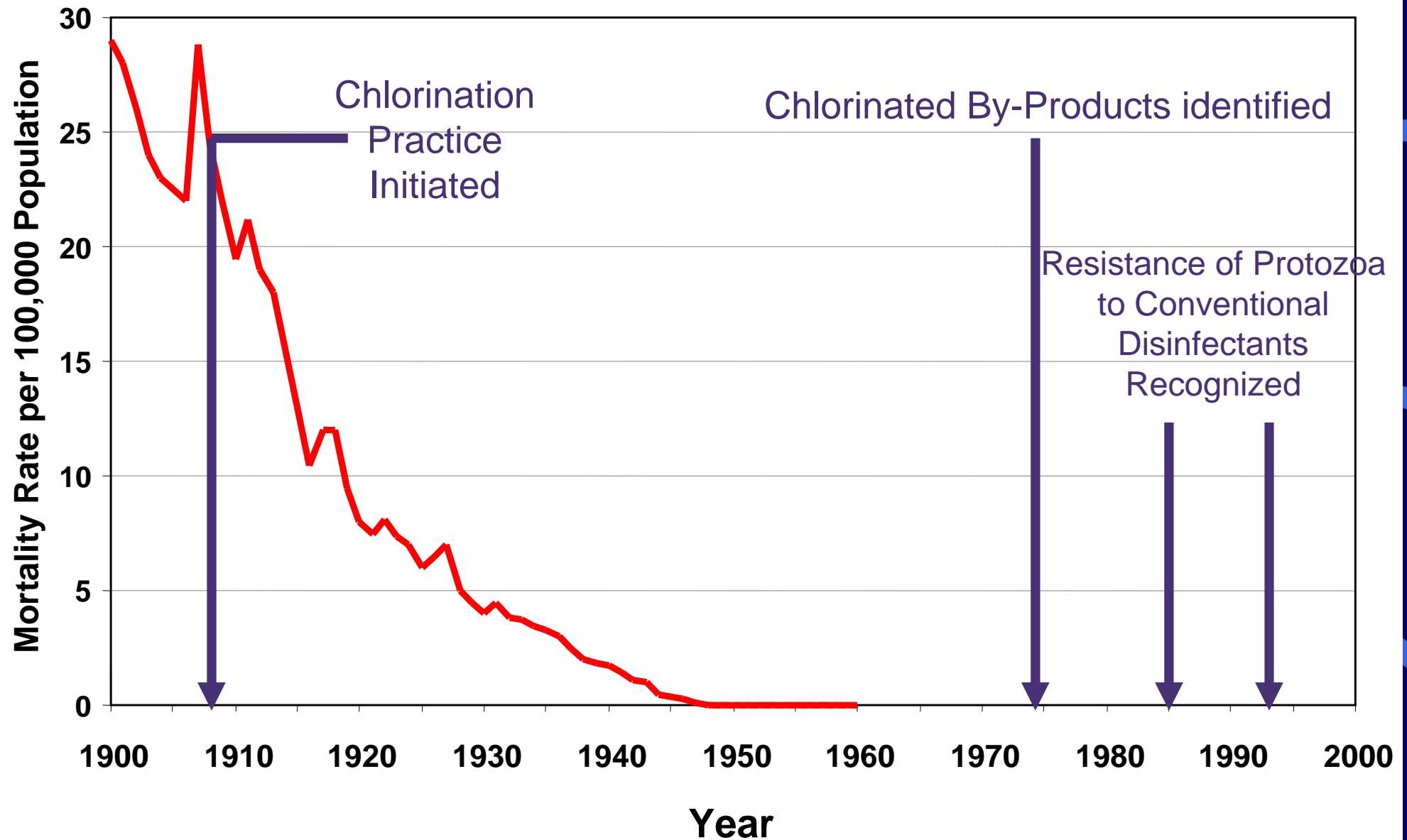


Helminths

Ascaris
Trichuris



Mortality Rate for Typhoid Fever in the United States



BACK RIVER WASTEWATER TREATMENT PLANT

Back River









Comparison of Microbial Inactivation or Removal Efficacy by Selected Disinfectants and Filtration Processes

| Disinfectants | Bacteria | Viruses | Protozoa | Overall Rating |
|-------------------------|----------------|----------------|----------------|----------------|
| Free chlorine | Excellent | Excellent | Fair/Poor | Good |
| Chloramines | Fair | Poor | Very Poor | Poor |
| Chlorine dioxide | Good/Excellent | Good/Excellent | Fair | Good |
| Ozone | Excellent | Excellent | Good/Excellent | Good/Excellent |
| Ultraviolet irradiation | Good/Excellent | Good | Good/Excellent | Good/Excellent |

Filtration Processes

| | | | | |
|---------------------------|-----------|-----------|-----------|-----------|
| Granular Media Filtration | Good | Fair | Good | Good |
| Low-Pressure Membranes | Excellent | Excellent | Excellent | Excellent |

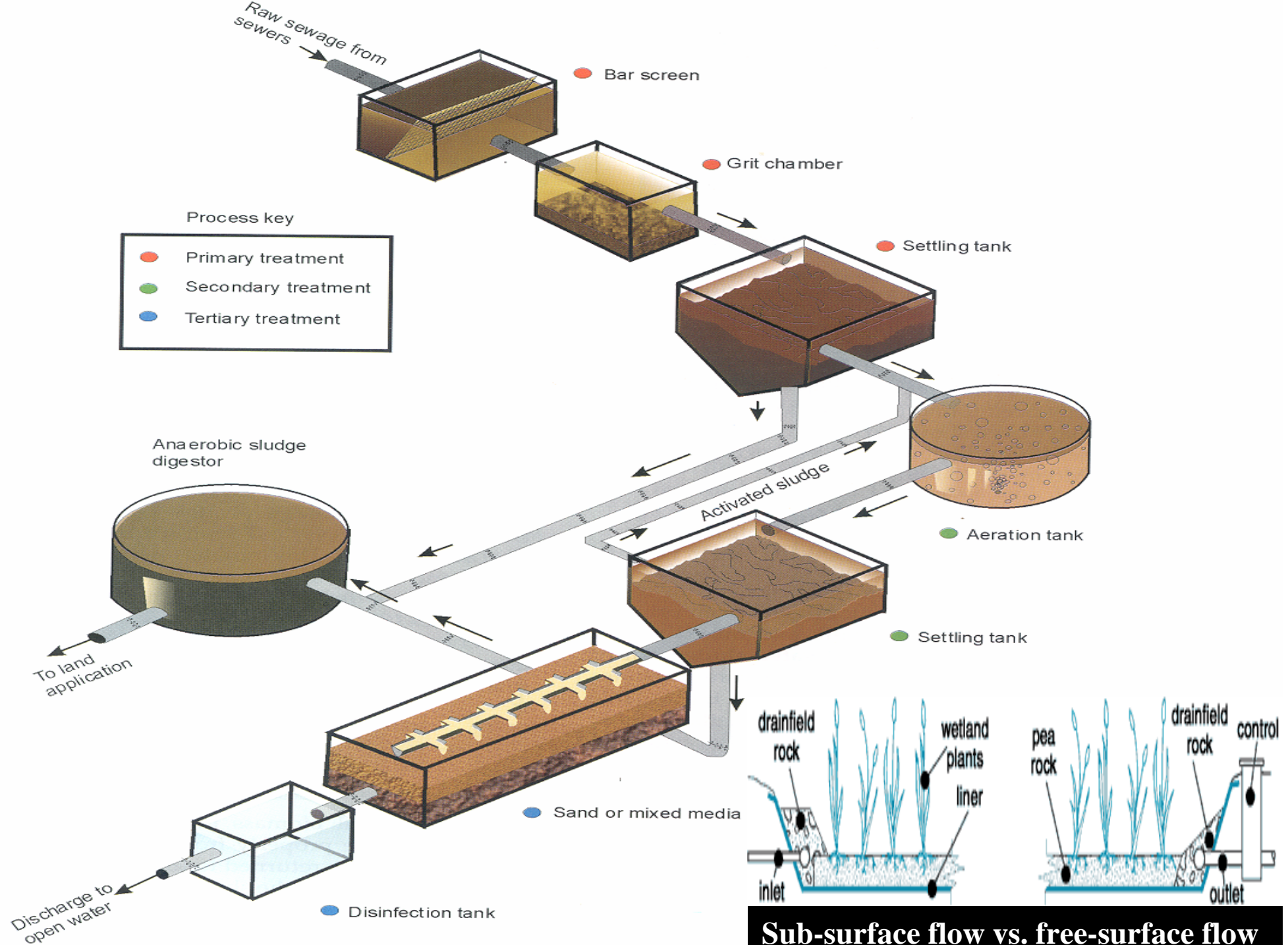


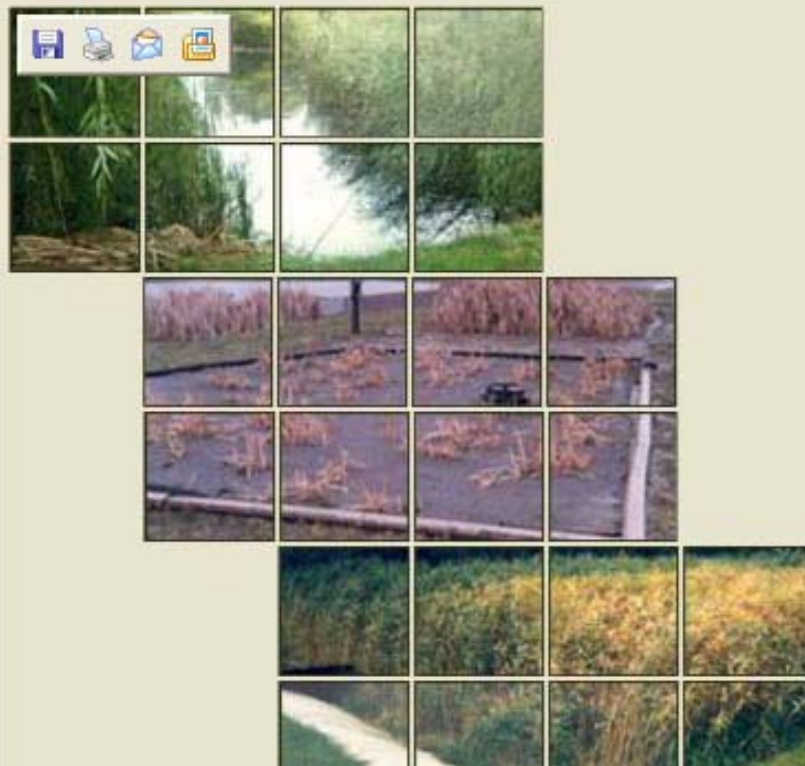
FIGURE 21.3 Schematic of the treatment processes typical of modern wastewater treatment (from Pepper *et al.*, 1996.)

Sub-surface flow vs. free-surface flow
“removal” vs. “source-tracking”

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Back to Nature

Constructed Wetlands for Low-Cost Wastewater Treatment and Nature Conservation



Constructed wetlands are marshes built to treat contaminated water. They have four key components:

- Soil and drainage materials (such as pipes and gravel)
- Water
- Plants (both above and below the water)
- Micro-organisms

Constructed wetlands purify the water that flows through them. Compared to conventional treatment methods, they tend to be simple, inexpensive, and environmentally friendly. Constructed wetlands may be used to treat water from many different sources:

- Sewage (from small communities, individual homes, and businesses)
- Stormwater
- Agricultural wastewater (including livestock waste, runoff, and drainage water)
- Landfill leachate
- Partially treated industrial wastewater
- Drainage water from mines
- Runoff from highways

Warning: The information in this website is entirely drawn from a 1993 publication, and has not been updated since the original publication date. Users are cautioned that information reported at that time may have become outdated.

United States
Environmental Protection
Agency

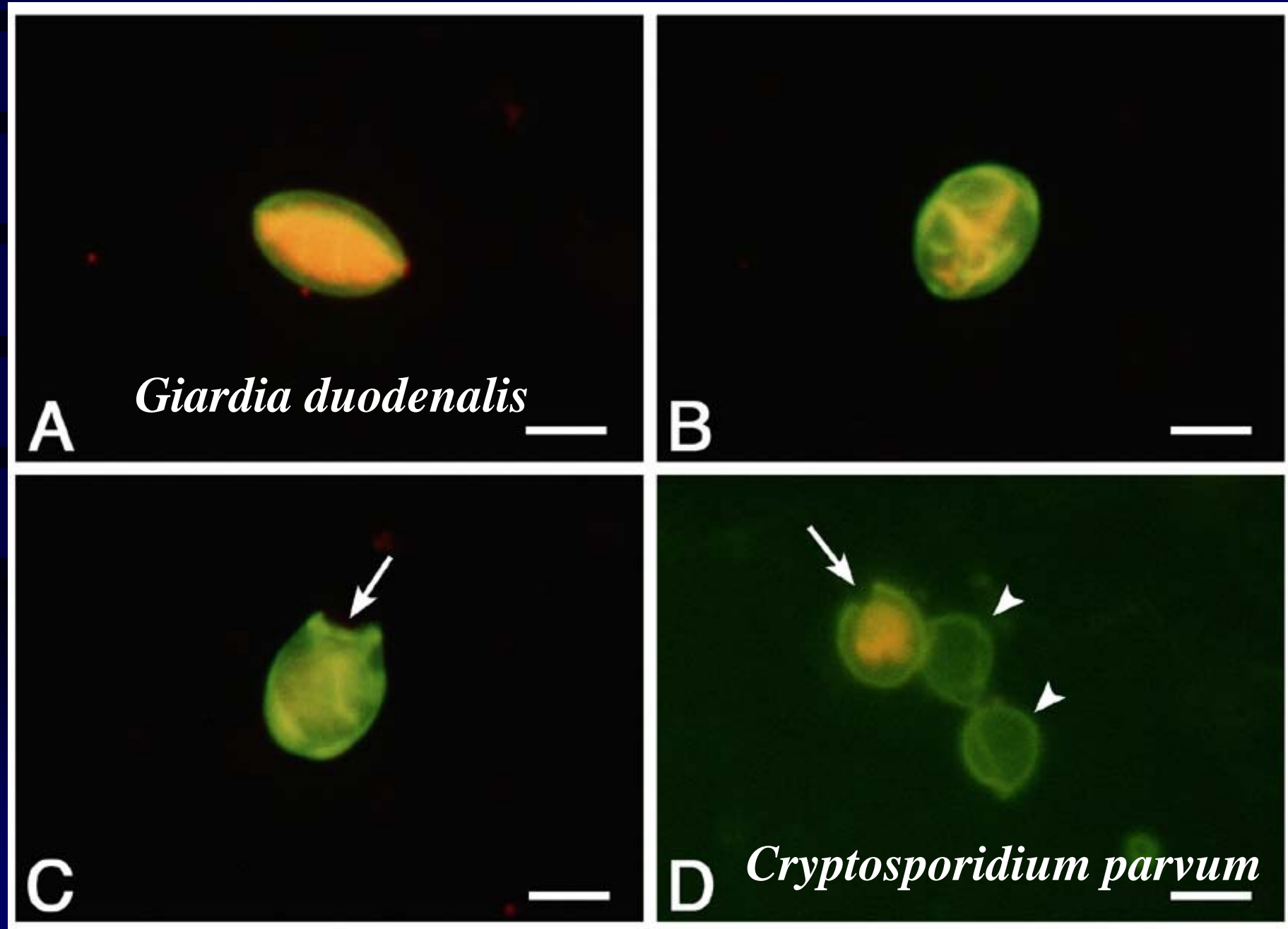
EPA832-R-93-005
September 1993

Constructed Wetlands for Wastewater Treatment and Wildlife Habitat

17 Case Studies



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



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

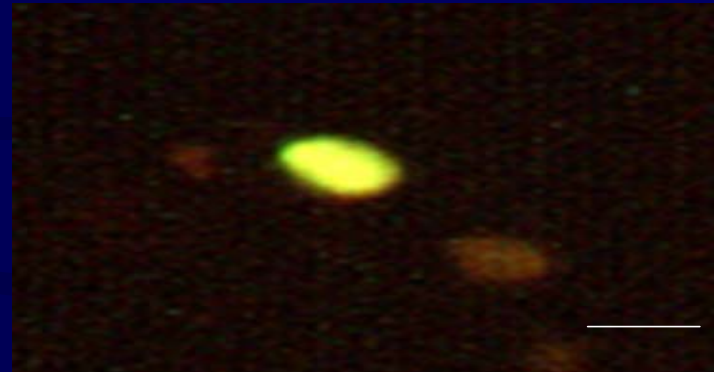
E. hellem Hester et al. (2000) *J Eukaryot Microbiol* 47:299-308.

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

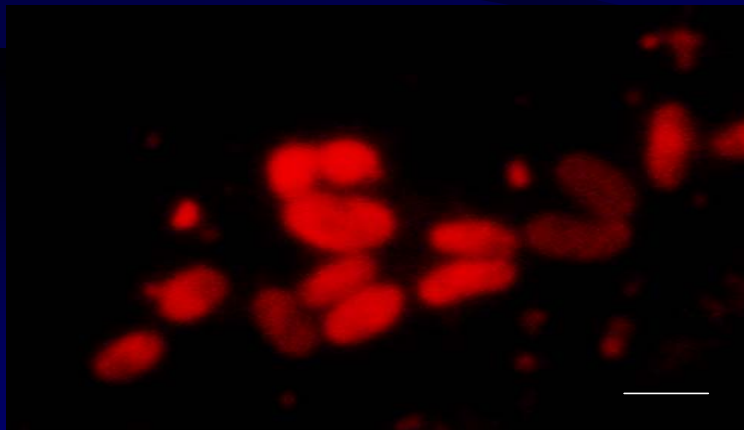
E. hellem



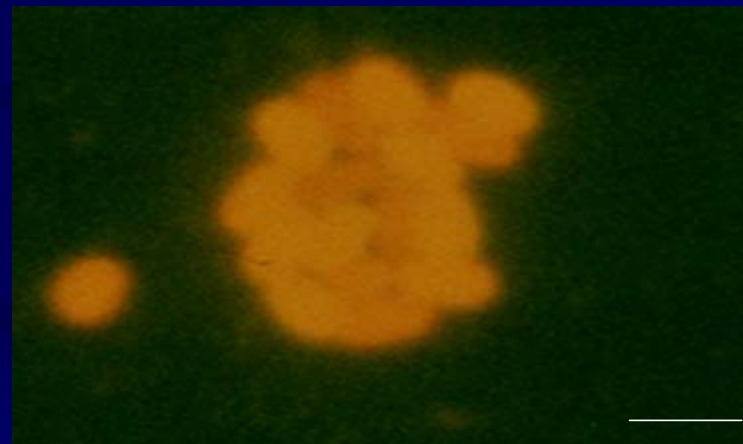
E. bienensei



E. intestinalis



E. cuniculi



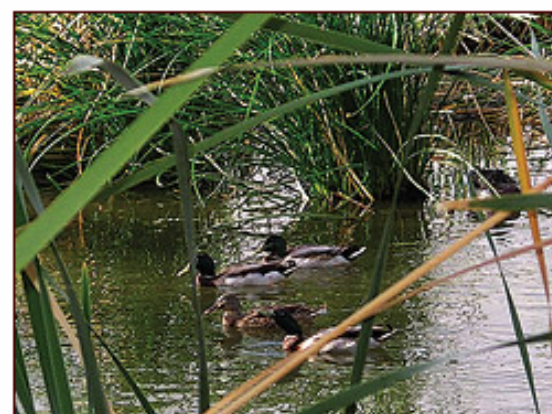
ES&T News

Engineered wetlands may pose risks to wildlife

Havens for wildlife in the parched southwestern U.S., wetlands constructed to receive sewage treatment plant discharges also boast a sterling record for slashing nutrient levels, suspended solids, and biological oxygen demand in effluents. But after two decades of research on treatment wetlands, scientists have scarcely investigated whether pesticides and metals in sewage effluent pose a risk to wildlife. Research published in this issue of *ES&T* (pp [603-611](#)) indicates for the first time a potential exposure pathway for a complex mix of the nearly 50 inorganic and organic contaminants found in a treatment wetland.

Larry Barber and his colleagues at the U.S. Geological Survey (USGS) analyzed water and fish tissue samples from the Tres Rios Demonstration Constructed Wetlands located near Phoenix, Ariz. The 10-acre site receives secondary sewage effluent from industrial, residential, and medical sources. Contaminants flowing through the site are eliminated through photodegradation, volatilization, biodegradation, sorption to sediments, and uptake by plants, says Barber.

Indeed, the researchers found that the wetlands reduced contaminant concentrations by 40-99% between the



John Thullen, USGS

Treatment wetlands outperform sewage treatment plants when it comes to removing contaminants, but scientists say that exposure to



January 15, 2006
Vol. 40, Iss. 2

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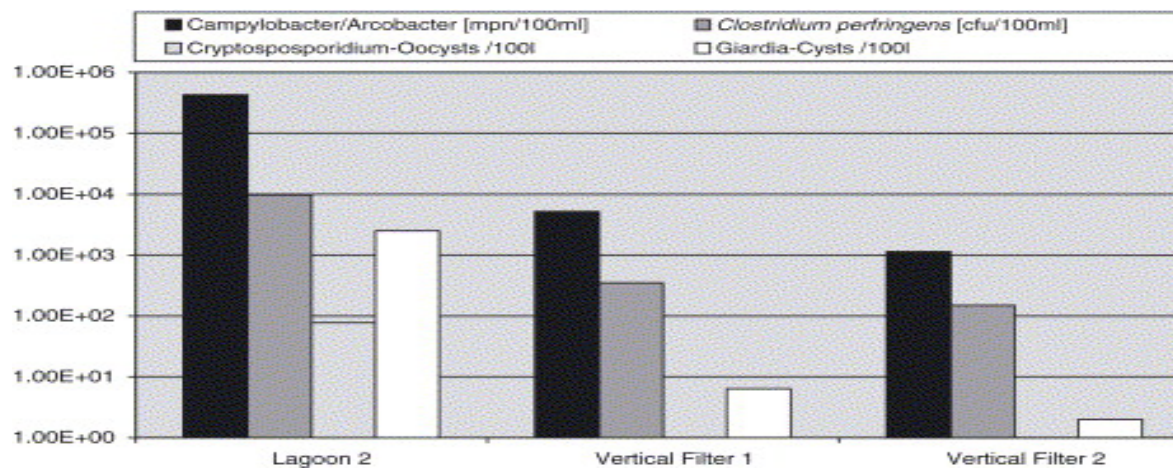
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Ulrich et al. 2005. Water Res 39; 4849-58

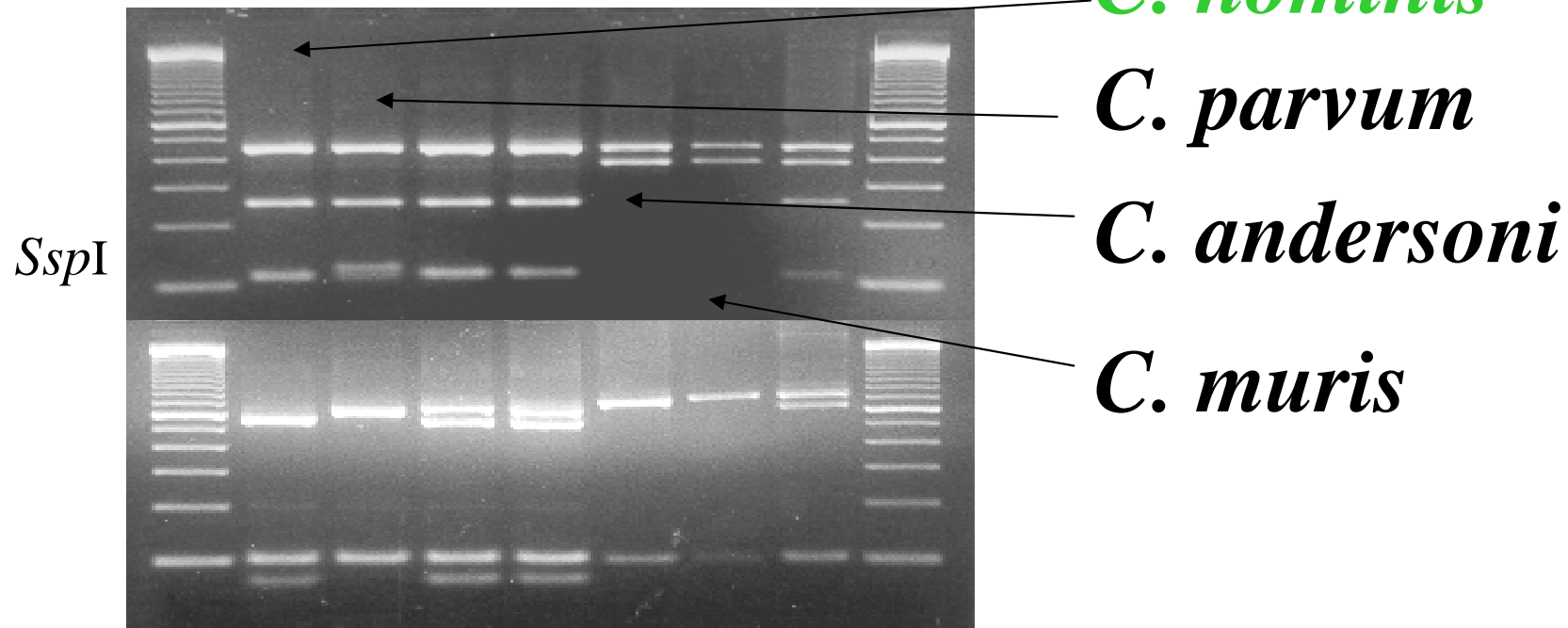
| Microorganisms | Water | | | | Sediment | | | |
|-----------------------|--|----------------|---------|--|--|----------------|---------|--|
| | Log reduction/(day log ₁₀ day ⁻¹) | R ² | p-value | | Log reduction/(day log ₁₀ day ⁻¹) | R ² | p-value | |
| Fecal coliform | -0.256 | 0.899 | 0.0001 | | -0.151 | 0.986 | 0.0001 | |
| <i>S. typhimurium</i> | -0.345 | 0.769 | 0.0019 | | -0.312 | 0.916 | 0.0001 | |
| Coliphage | -0.397 | 0.983 | 0.0001 | | -0.107 | 0.867 | 0.0008 | |
| PRD-1 | -0.198 | 0.865 | 0.0008 | | -0.054 | 0.930 | 0.0001 | |
| <i>Giardia</i> | -0.029 | 0.490 | 0.187 | | -0.370 | 0.861 | 0.022 | |

| Organism | Potable water supplied wetland | | Wastewater supplied wetland ^b | | Percent reduction |
|-------------------------------------|--------------------------------|-----------------------|--|-----------------------|-------------------|
| | Influent | Effluent | Influent | Effluent | |
| Total coliform (CFU/100 mL) | < 1.0 | 1.3 × 10 ² | 1.7 × 10 ⁴ | 1.1 × 10 ² | 98.8 |
| Focal coliform (CFU/100mL) | < 1.0 | 22.3 | 7.4 × 10 ³ | 45.0 | 98.2 |
| Coliphage (PFU/mL) | < 1.0 | < 1.0 | 2.8 × 10 ² | 4.7 | 95.2 |
| <i>Giardia</i> ^a | < 1.0 | < 1.0 | 14.1 | 0.7 | 87.8 |
| <i>Cryptosporidium</i> ^a | < 1.0 | < 1.0 | < 12.6 | 2.7 | 64.2 |

Thurston et al. 2001. Water Res 35; 1547-51

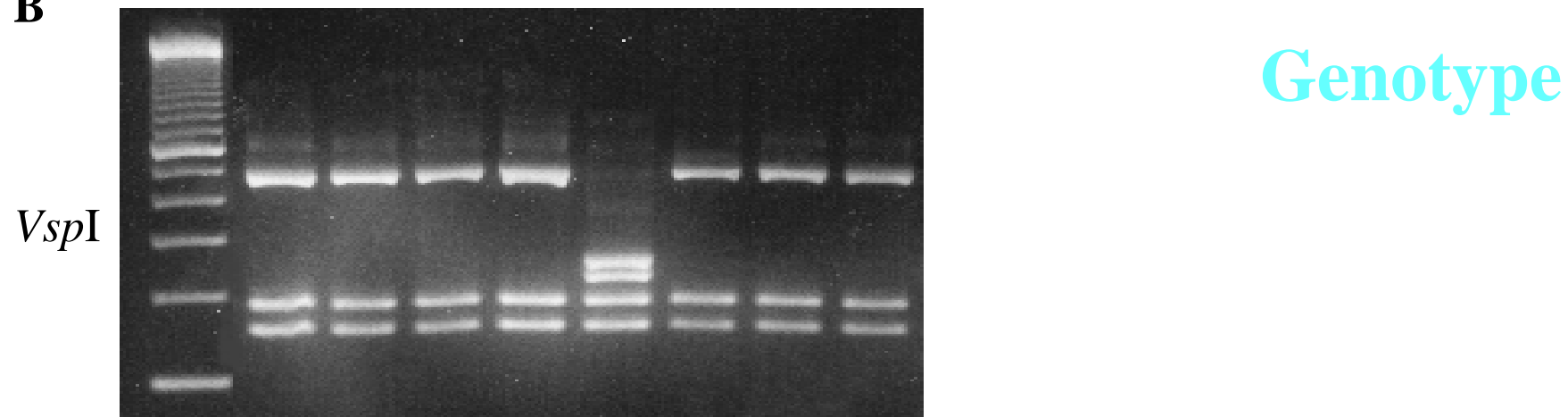
Identification of *Cryptosporidium*; PCR, RFLP

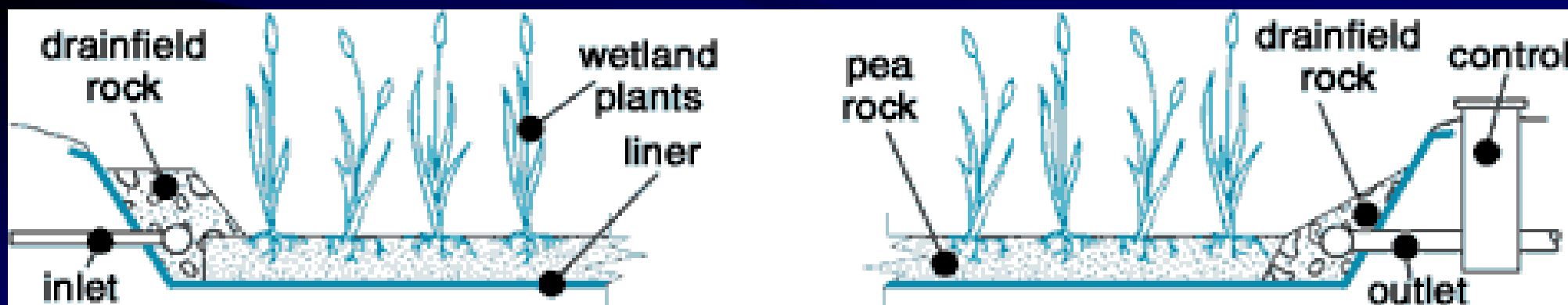
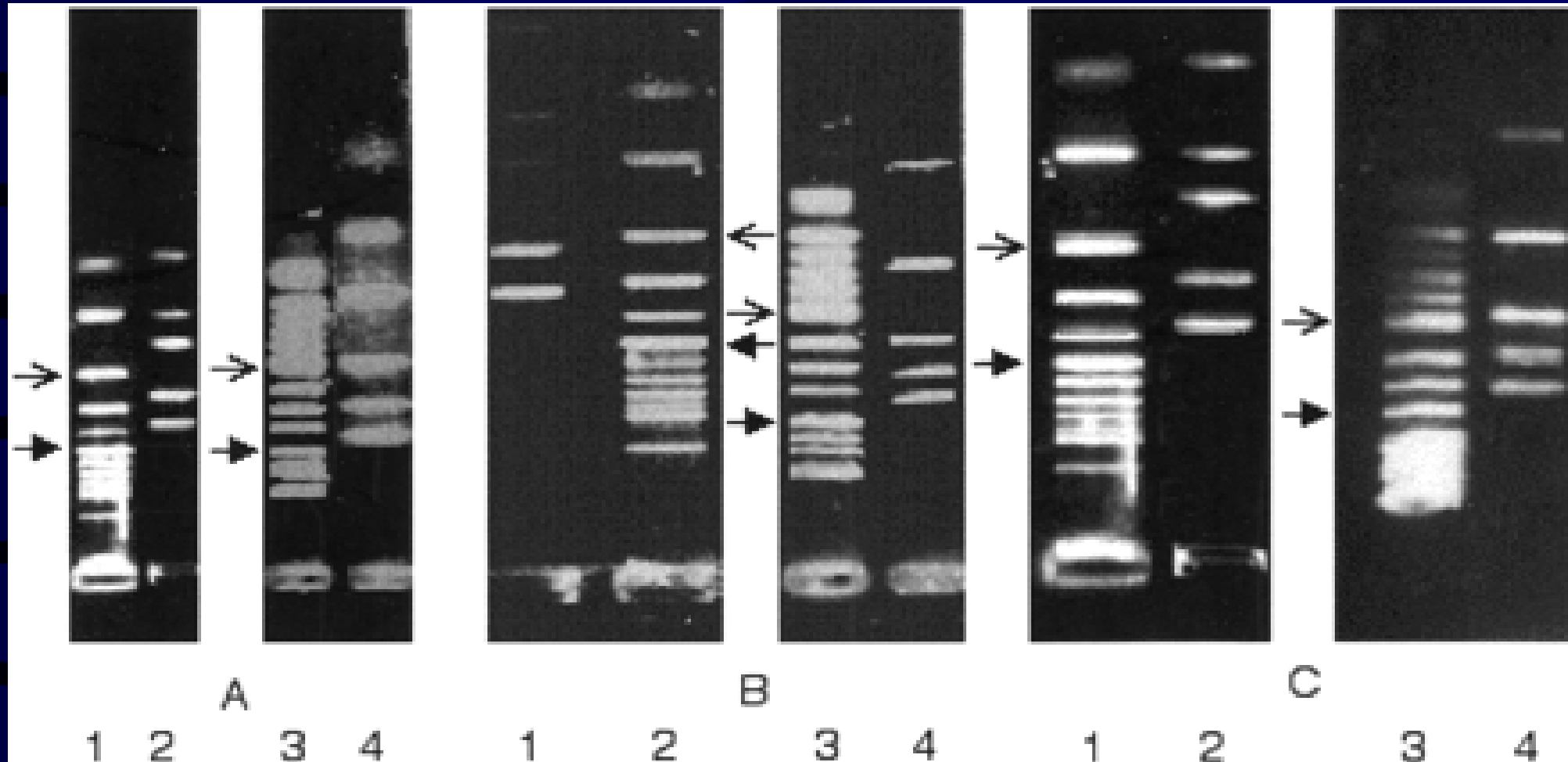
A



Species

B



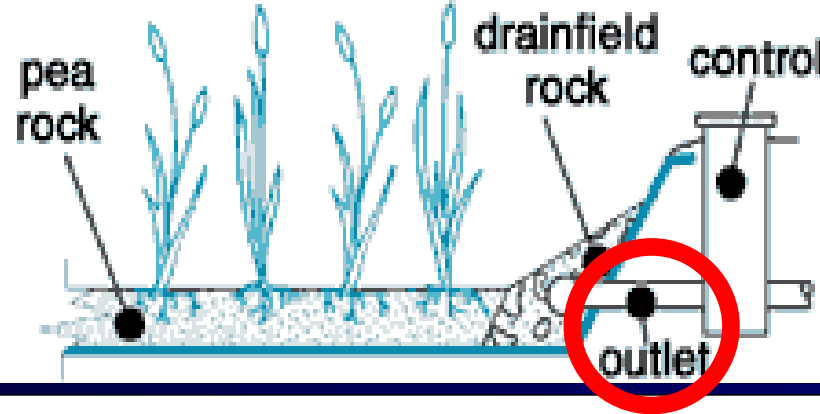
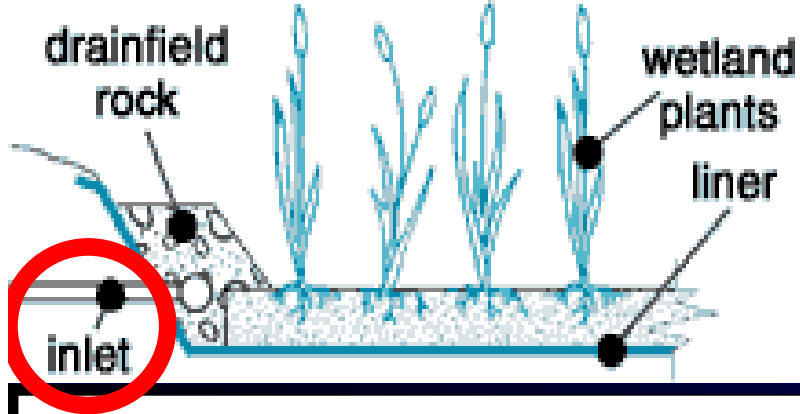


1

2

3

4

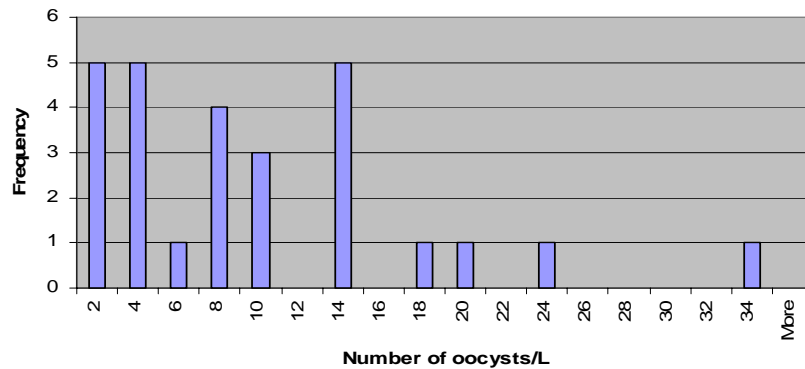


outlet/inlet (%)

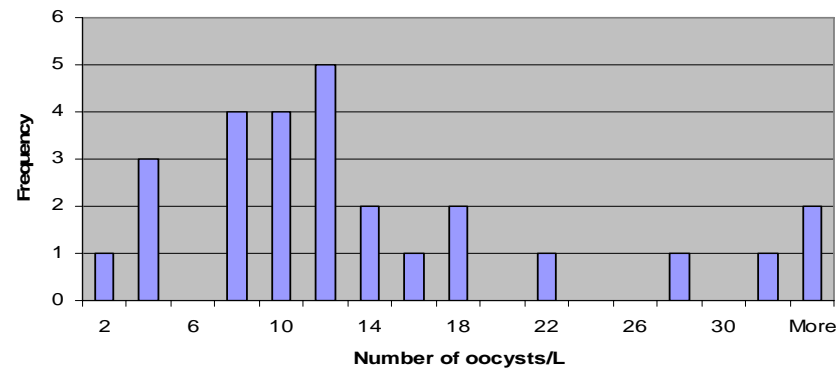
65%

85%

Frequency Distribution of *Cryptosporidium*/inlet

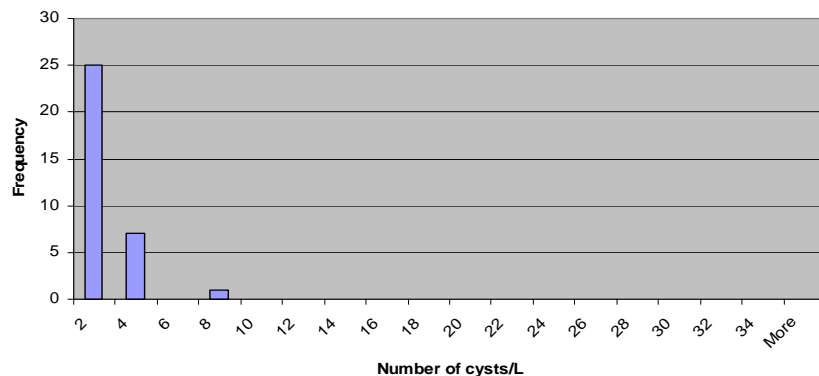


Frequency Distribution of *Cryptosporidium*/outlet

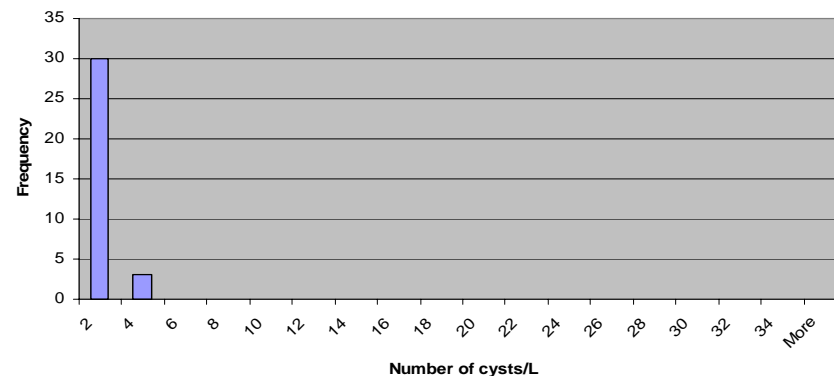


Cryptosporidium

Frequency Distribution of *Giardia*/inlet



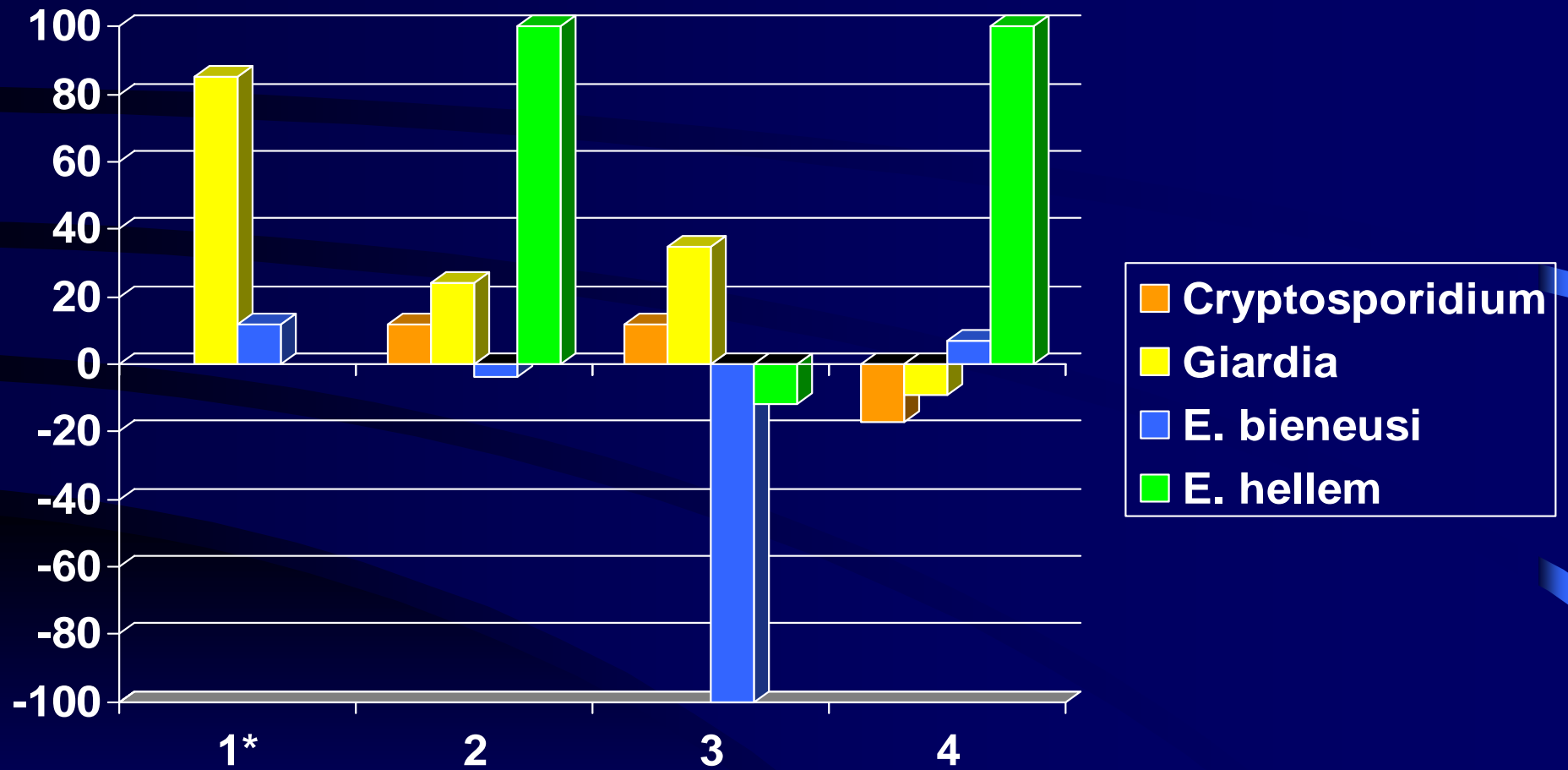
Frequency Distribution of *Giardia*/outlet

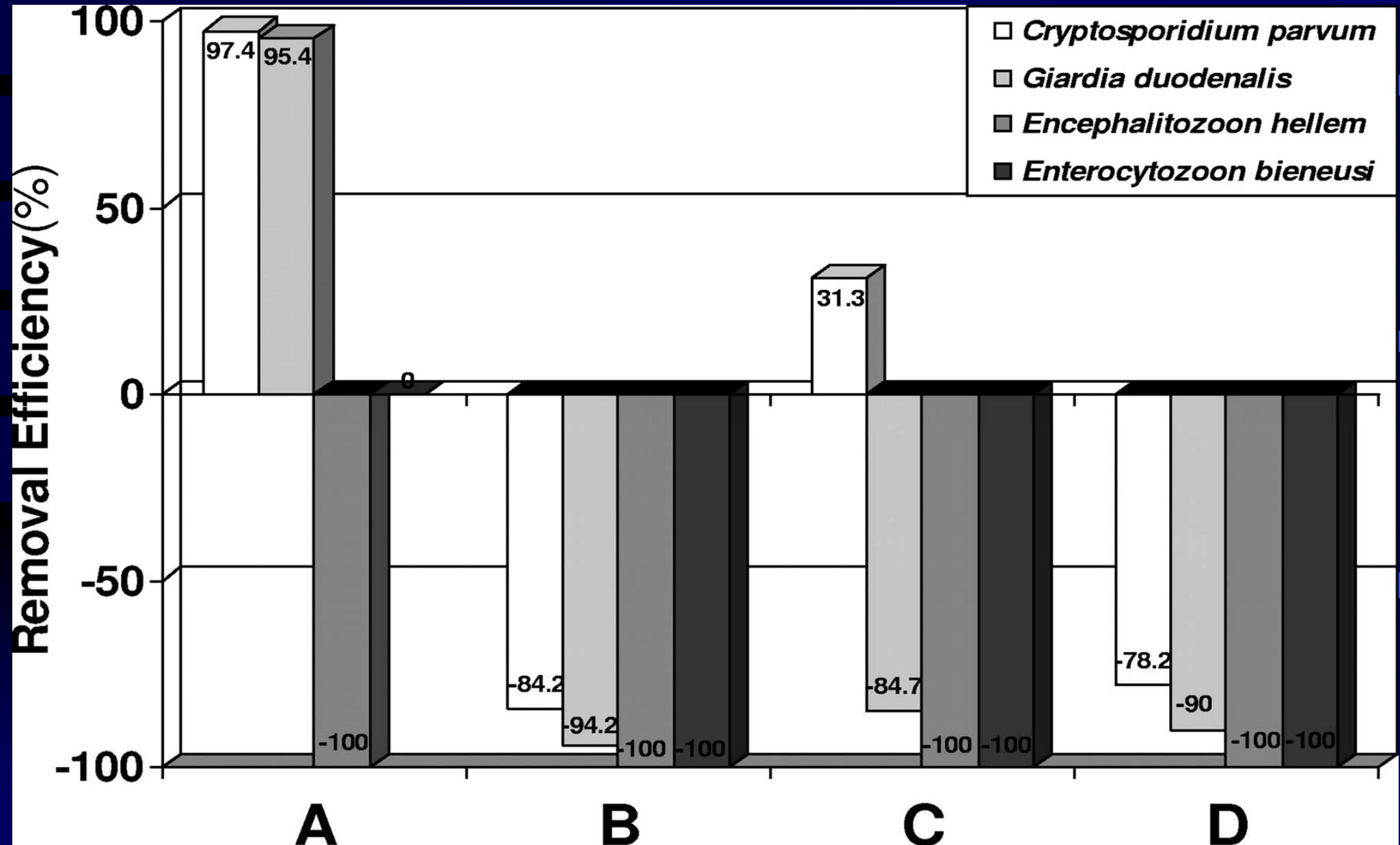


Giardia (Assemblage A)

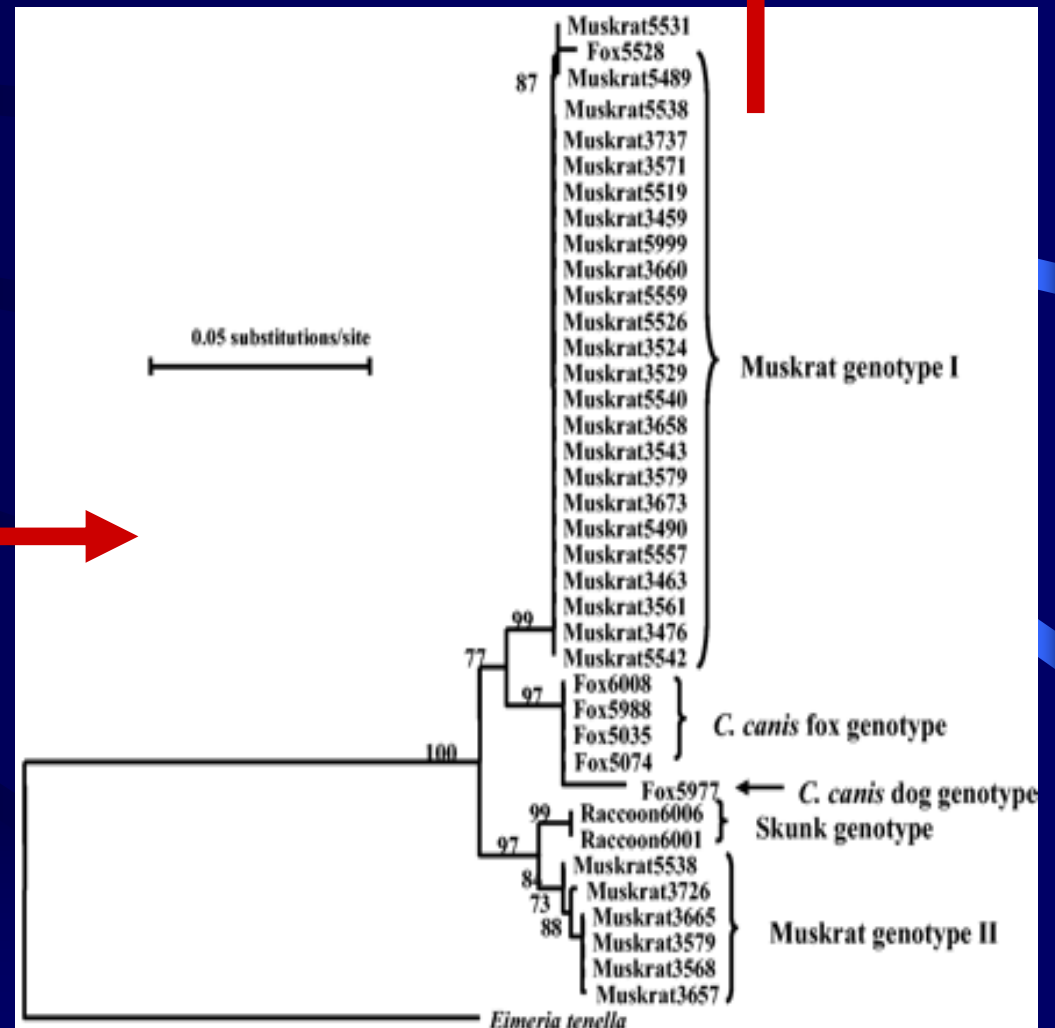
Giardia (Assemblage A, E, and C)

Pathogen removal relative index (inlet - outlet)%





| | | |
|--|---|-----|
| <i>Cryptosporidium felis</i> | AAGCTCGTAG TTGGAATTC GTTAAACCT TATATATAAT ATTTTITTTT AAATATTATT | 60 |
| <i>Cryptosporidium</i> sp. (canine) |AT.-.....-...C...AT.. | 53 |
| <i>Cryptosporidium parvum</i> genotype 1 |AT.A.....-G .TGA..AT.. | 56 |
| <i>C. parvum</i> genotype 2 |AT.A.....-G .TGA..AT.. | 56 |
| <i>C. felis</i> | ATGTAGATT AACATAATC ATATTTTITA AGACTGAATT TTT---AGT TTTGATAATA | 116 |
| <i>Cryptosporidium</i> sp. (canine) | ..A...T... ..-.....-...ATT.A ..-..... | 93 |
| <i>C. parvum</i> genotype 1 | ..A...T... ..-.....-...ATT...TTT... | 102 |
| <i>C. parvum</i> genotype 2 | ..A...T... ..-.....-...AT.A ...T.... | 99 |
| <i>C. felis</i> | TGAATTTTA CTTTGAGAAA ATTAGAGTGC TTAAGCAGG CTTTGCCTT GAATACTCCA | 176 |
| <i>Cryptosporidium</i> sp. (canine) |C.....-.....AG. | 153 |
| <i>C. parvum</i> genotype 1 |A.A..... | 162 |
| <i>C. parvum</i> genotype 2 |A.A..... | 159 |
| <i>C. felis</i> | GCATGGAATA ATAATA--AA AGATTTTAT CTTTTTTTA TTGTTCTAA GATAAAATA | 236 |
| <i>Cryptosporidium</i> sp. (canine) |TT.. ..C... ..G.... | 208 |
| <i>C. parvum</i> genotype 1 |TT.. ..G.... | 217 |
| <i>C. parvum</i> genotype 2 |TT.. ..C... ..G.... | 214 |
| <i>C. felis</i> | ATGATTAAATA GGGACAGTTG GGGGCATTG TATTTAACAG TCAGAGGTGA TATTCTTAGA | 294 |
| <i>Cryptosporidium</i> sp. (canine) |T.....A..... | 268 |
| <i>C. parvum</i> genotype 1 |A..... | 277 |
| <i>C. parvum</i> genotype 2 |A..... | 274 |



Conclusions

- ✓ Wastewater discharges are worldwide risk factors for the introduction of human protozoan enteropathogens into surface waters
- ✓ Demand for high quality drinking and recreational waters rises exponentially due to global demographic growth, reinforcing an urgent need for microbiologically safe reclaimed waters
- ✓ Pathogen source-tracking research in engineered wetlands is deficient, due to the lack of available molecular technology in the past
- ✓ Current technology allows for multiplexed species-specific identification, enumeration, viability assessment, and source-tracking of human protozoan pathogens
- ✓ Public health in developing and developed regions of the world will benefit from changing the conceptual research framework for constructed wetlands from “pathogen removal” to “pathogen source-tracking” efforts. “Removal” assumes that “survived pathogens” originate from pathogens delivered to that wetland from the sewage treatment process, while “source tracking” evaluates the complexity of pathogen ecological interactions
- ✓ Improvements in reclaimed water quality by lowering fecal coliform counts is not a sound solution for human protozoan enteropathogens

Acknowledgements

