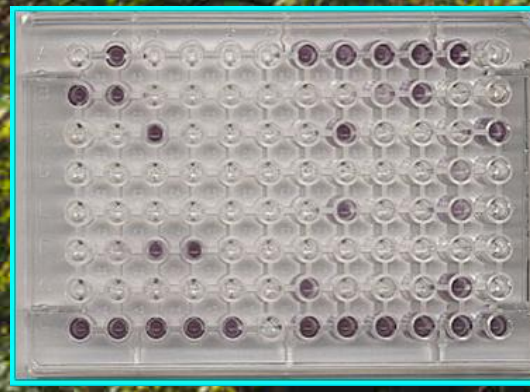
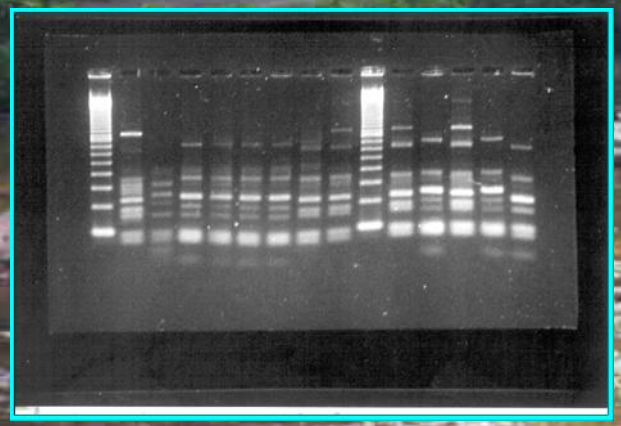
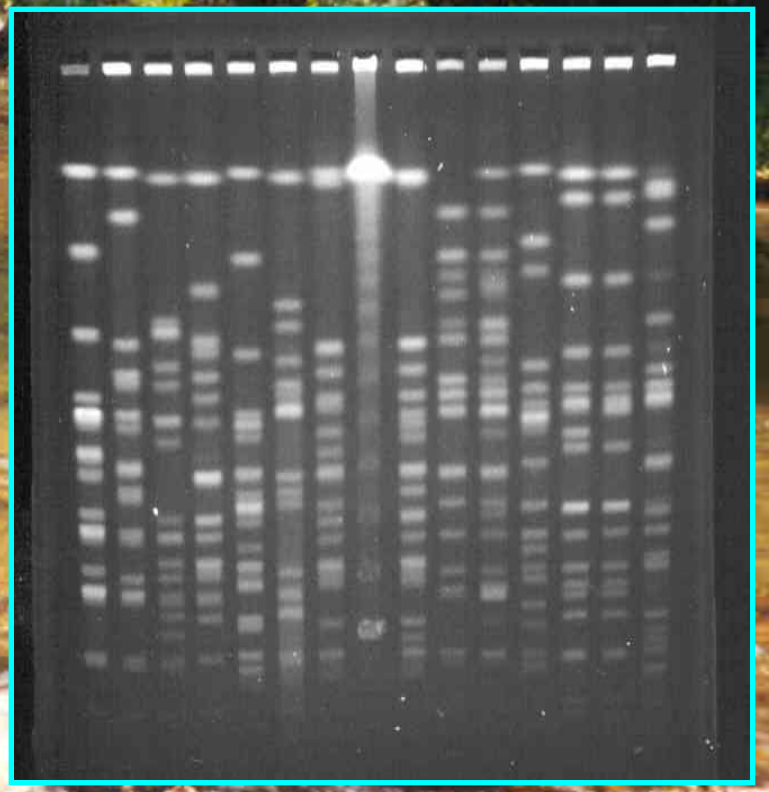
Development and Application of Microbial Source Tracking Technology

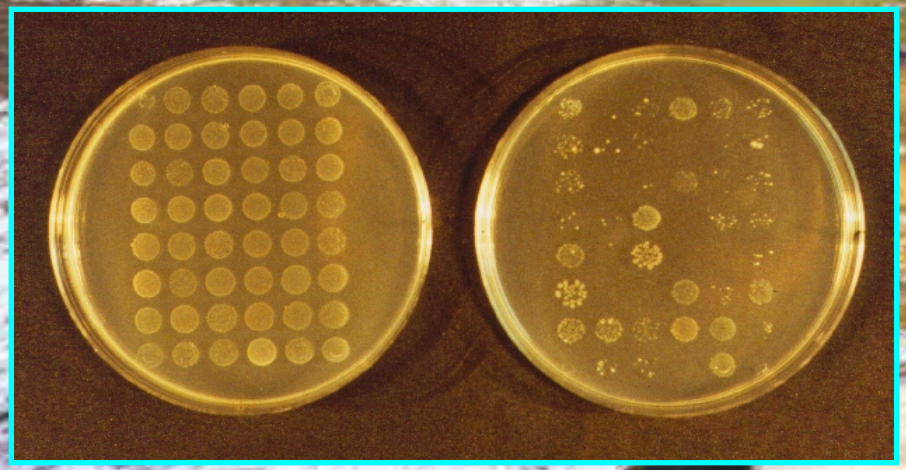
Charles Hagedorn

Virginia Tech







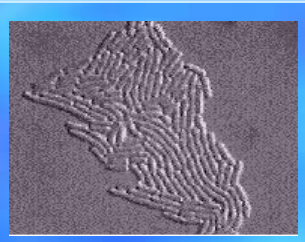
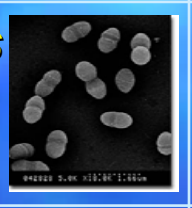


Acknowledgements

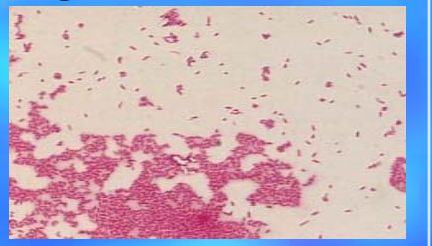
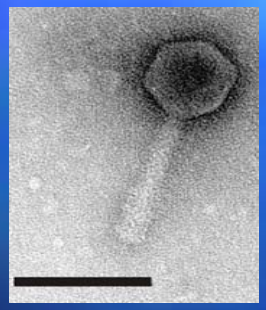

Source Tracking Methods:
Where are we now?
Where do we go from here?


Source Tracking Options
Bacterial
Viral
Protozoal
Chemical

Bacterial Targets

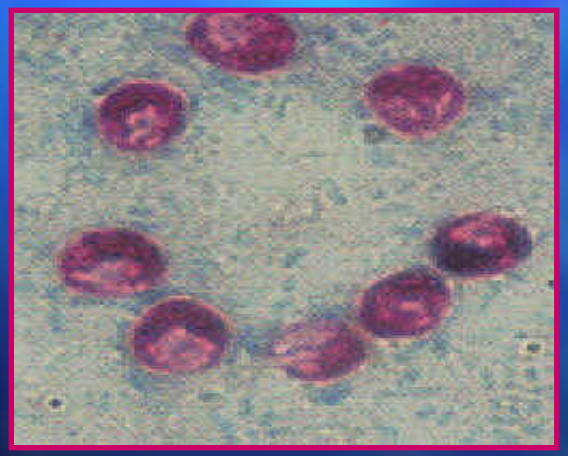
Bacteria	Advantages	Disadvantages
Total/Fecal Coliforms	■Used extensively as fecal indicators	■Ecology, prevalence, resistance to stress differ from pathogens
E.coli 	■Not usually pathogenic to humans ■Present at concentrations higher than pathogens	■May not be a good indicator in tropical/subtropical environments
Enterococcus 	■Especially useful in marine environments and recreational waters	■Found in environmental reservoirs ■Regrowth possible
Bacteroides/Bifido. 	■Less common in animals ■Human isolates ferment sorbitol ■Evidence of recent contamination	■Survivability in environment is variable ■Culture methods not well defined
Clostridium perfrigens 	■Good for prediction of viruses or remote fecal pollution	■Persistent in environment

Viral Targets

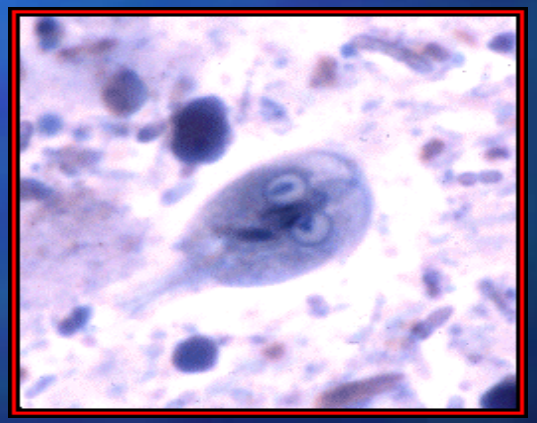
Virus	Advantages	Disadvantages
Bacteroides fragillis bacteriophage 	■Abundant in human feces ■Phages don't replicate in environment ■Presence correlates with presence of human enteric viruses	■Phage found to be absent in some highly polluted environments
F-specific RNA coliphage 	■Group I and II associated with human feces, group IV associated with animal feces ■Easy to perform ■Rapid detection	■Sensitive detection methods required ■Only small percentage of human feces contain phages ■Unreliable in marine waters
Human Enteric Viruses	■Human specific ■No need to detect indicators	■Low numbers in environment ■Over 120 enteric viruses

Protozoan Targets

- ***Cryptosporidium/Giardia***
  - **Direct monitoring of these human pathogens**
  - **Not readily detectable**
  - **Low infectious doses**



Cryptosporidium



Giardia

Chemical Targets

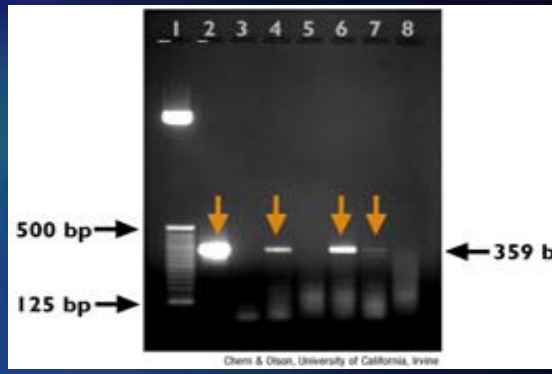
- Caffeine
- Fragrance Agents
- Fluorescent Whitening Agents (Brighteners)
- Fecal Sterols
- Fecal Stanols

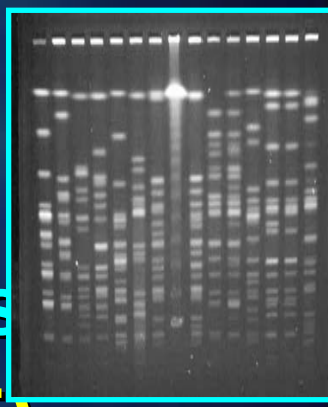
Phenotypic Methods

Method	Library Dependent	Target	Description
Fecal coliform/fecal strep ratio	No	Fecal coliforms/fecal streps	Humans have ratio of >= 4 while animals have ratios below 0.7
ARA	Yes	*E.coli* or *Enterococcus*	Based on antiobiotic resistance patterns unique to different sources of pollution
CUP (BIOLOG)	Yes	*E.coli* or *Enterococcus*	Based on differences in bacterial usage of a wide range of carbon and nitrogen sources
Immunological Methods (sereotyping)	No	*E.coli*	Sereogrouping of organisms based on presence of different somatic O antigenic determinants

Source Tracking Methods: Genotypic

- Ribotyping
- Length Heterogeneity PCR
- Terminal Restriction Fragment Length Polymorphism
- Repetitive PCR
- Denaturing Gradient Gel Electrophores
- Pulse Field Gel Electrophoresis (PFGE)
- Amplified Fragment Length Polymorphism
- Toxin Biomarkers
- Reverse Transcriptase PCR





Genotypic Methods 1.

Method	Library Dependent	Target	Description
Ribotyping	Yes	*E.coli or Enterococcus*	Genetic fingerprint comes from the genes that code for rRNA which are highly conserved in microbes. DNA is extracted and fragments are separated by gel electrophoresis to form patterns of 4-12 bands
Rep-Box-PCR	Yes	*E.coli*	Conserved sequences in bacterial repetitive elements are used as PCR primers to distinguish among different strains of the same bacterial species
PFGE	Yes	*E.coli or Enterococcus*	DNA fingerprinting using cutting restriction enzymes coupled with electrophoresis analysis
LH-PCR and T-RFLP	No	*Bacteroides Prevotella*	Based on the premise that there are species composition differences in *Bifidobacterium* and *Bacteroides-Prevotella* populations of humans and cows

Genotypic Methods 2.

Method	Library Dependent	Target	Description
DGGE	Yes	*E.coli*	Discriminates between different PCR products of similar size based on changes in electrophoretic mobility which is influenced by melting properties of DNA fragments
AFLP	Yes	*E.coli*	DNA fingerprinting using rare and frequent cutting restriction enzymes coupled with PCR amplification
Toxin Biomarker	No	*E.coli*	Biomarkers are used to detect bacterial contamination by identifying genes that code for toxins in *E.coli* populations
Reverse Transcriptase PCR	No	Enterovirus	Can be used to detect the RNA of any organism whose genome has been sequenced by using primers complimentary to conservative RNA sequences found in the viruses

Category of Criteria	Specific Evaluation Criteria
Tier 1: Measurement Reliability	▪Reproducibility of results ▪Accuracy of correct classification of isolates into correct group ▪Confidence that identified indicator is from presumed source ▪Level of resolution ▪Matrix stability ▪Geographic stability/Temporal stability
Tier 2: Management Relevance	▪Relationship to actual source of contaminations ▪Relationship to public health outcomes ▪Relationship to commonly used water quality indicators ▪Ease of communication to public ▪Ease of communication to management audiences
Tier 3: Cost and Logistics	▪Equipment and lab facilities required ▪Training required ▪Library size required ▪Implementation time ▪Cost of ensuring results are legally defensible ▪Cost per sample/Turnaround time

Method Comparison Studies

- Three MST method comparison studies recently completed:
  - USDA funded at two-year study to compare ARA, PFGE, and RT using *E.coli* and *Enterococcus*
  - USGS funded a program to compare the ability of RT, PFGE, ARA, PCR, and BIOLOG to identify sources of *E. coli* in the waters of Berkeley County WV
  - Southern California Coastal Water Research Project has funded the largest MST methods comparison study comparing ARA, RT, T-RFLP, Rep PCR, CUP, PFGE, F+ coliphage, Viruses, Toxin gene biomarkers

Results of Comparison Studies

- Molecular methods performed better than phenotypic methods.
- RT and PFGE did better than PCR, but PCR is improving.
- Presence-absence methods need quantification.
- Most methods did reasonably well on major or dominant sources, less well on minor sources.
- Libraries need to be larger than presumed.
- No study included all methods, some have not been evaluated at all.

Manager's Dream Table

Method	Short Term	Broad Categories	Specific Sources	Promising
Rybotyping			X	
ARA		X		
PFGE			X	
Nutrient		X	?	X
Box/Rep-PCR			X	
Specific Primer PCR/ VIR	P/A			X
PCR t-RFLP	P/A			?
F+ coliphage		X		
Entero Virus	P/A			X
Adeno Virus	P/A			X

MST is being deployed in the U.S.
(www.epa.gov/owow/nps)

Some 10,000 TMDLs completed to date. Number using MST is increasing…

Rivers and Streams: 19% assessed, 61% good, 39% impaired. Fecals #1 cause of impairment.

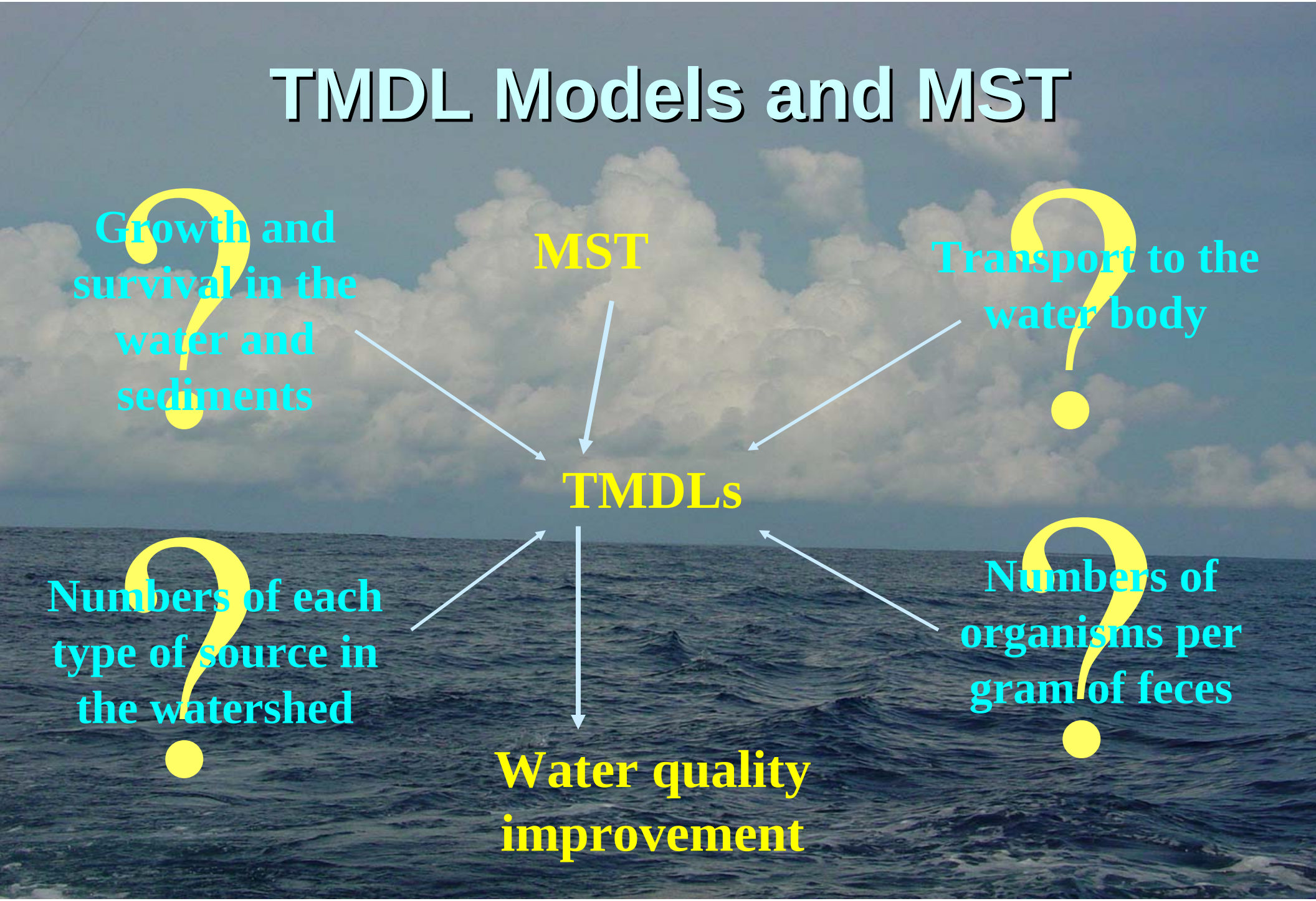
Lakes: 45% assessed, 55% good, 37% impaired, 8% threatened. Fecals #3, after nutrients and metals.

Estuaries: 36% assessed, 49% good, 51% impaired. Fecals #4, after metals, pesticides, and nutrients.

Source Tracking – Where do we go from here?

- Some genius needs to work out a non-library method for major sources.
- Initial method comparison studies were too early; who wants to play again?
- Combine methods to bolster confidence.
- Perform QA/QC on known source libraries.
- Concentrate on locations where remediation efforts are underway.
- Examine the links between sources and receiving waters.

Concentrate on locations where remediation efforts are underway.


TMDL Models and MST
Growth and survival in the water and sediments
MST
Transport to the water body
TMDLs
Numbers of each type of source in the watershed
Numbers of organisms per gram of feces
Water quality improvement


THANK YOU
QUESTIONS?