American Alligator Bacterial Source Tracking in Coastal South Carolina

Michelle A. Johnston, Ph.D.
John A. Knauss Marine Policy Fellow
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Purpose

- To determine if American alligators (*Alligator mississippiensis*) are a source of fecal coliform and potential pathogens in coastal South Carolina surface waters

Outline

- Introduction
- Hypothesis
- Objectives
- Design and Methods
- Results
  1. Bacterial Identification
  2. Genotypic Fingerprinting
  3. Bacterial Source Tracking
- Conclusions
- Acknowledgements

Water Quality Degradation

- Half of population in coastal communities
- Bacterial pollution
- Various contributing sources
- Potential pathogen evaluation with fecal coliform indicators
- Helps detect unsafe water conditions

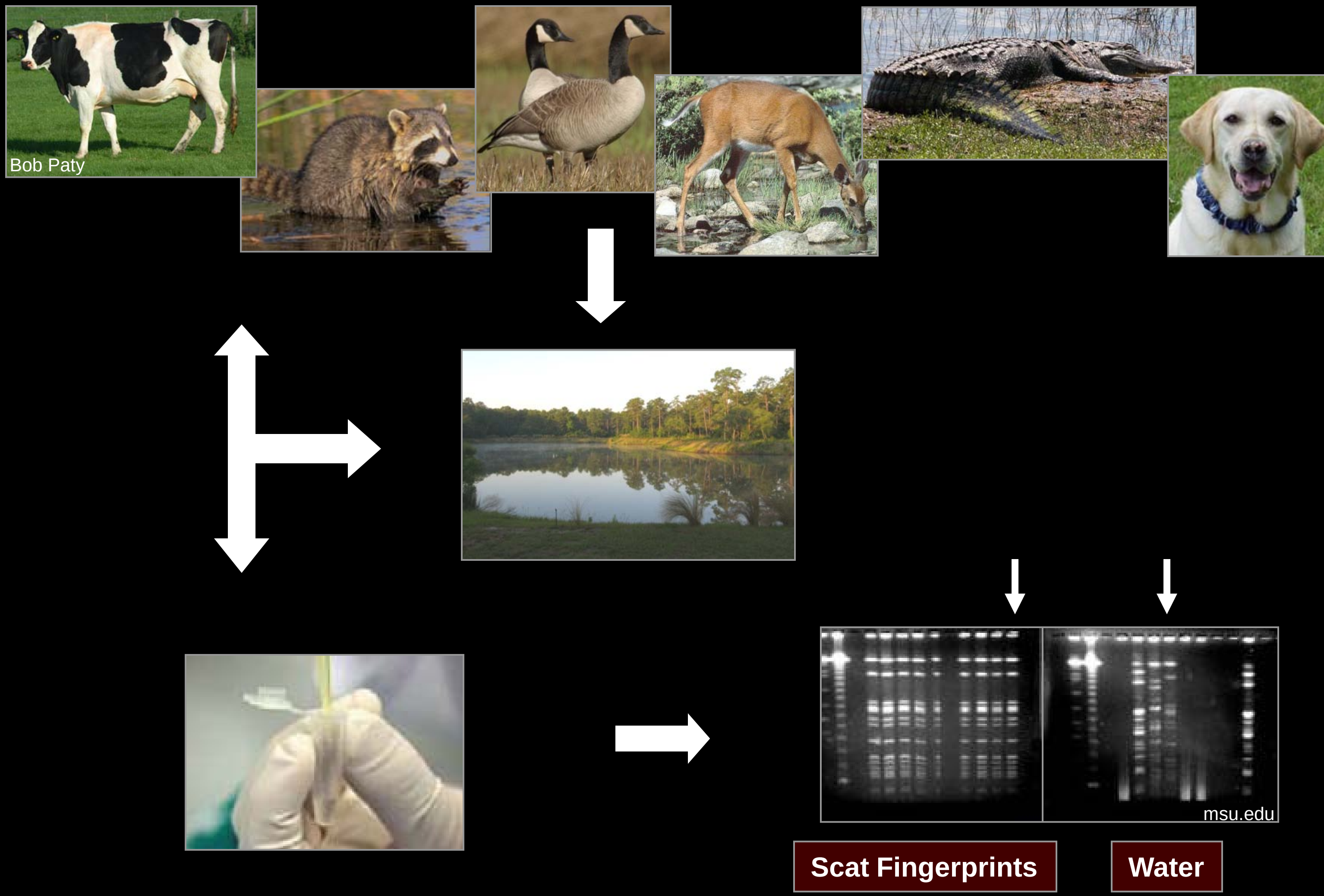
Indicator Limitations

- Regulatory microbiology
- Oversimplification
- EPA evaluation of methods
- No source identification
- Identification for management
  - Endotherms main source

American Alligator

Alligator mississippiensis

Bacterial Source Tracking (BST)



Hypothesis and Objectives

- H_1: The American alligator is a source of bacterial contamination in South Carolina surface waters

  i. Determine if alligators contribute to fecal coliform bacterial water quality degradation
  ii. Determine density of alligator fecal coliforms
  iii. Evaluate bacterial identification tools and BST fingerprinting tools
  iv. Determine fingerprint reproducibility and stability
  v. Evaluate BST database stability

Design and Methods: Study Area

Alligator Sampling Techniques

Distress Call

Alligator Sampling Techniques

Snare and Roll

Alligator Sampling Techniques

Cloacal Swab

Alligator Sampling Techniques

Water Sample, Tag, and Release

- Palmetto Bluff
  - 2006 (n=31)

Research Component 1

Bacterial Identification

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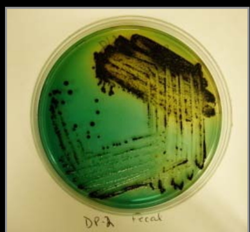
Sample Processing

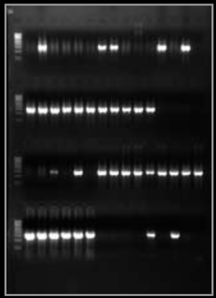
Culture



DNA Extraction

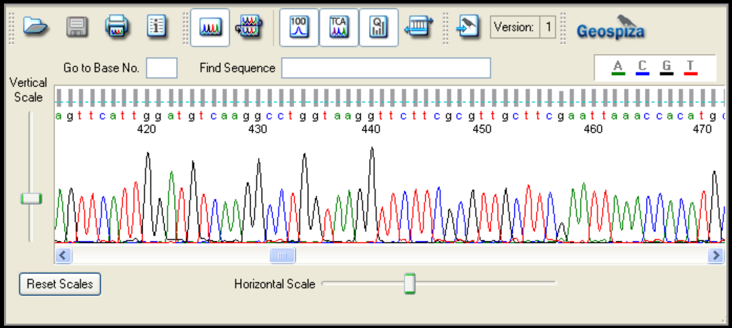
PCR

PCR Products



Purification

16S rRNA gene Sequencing

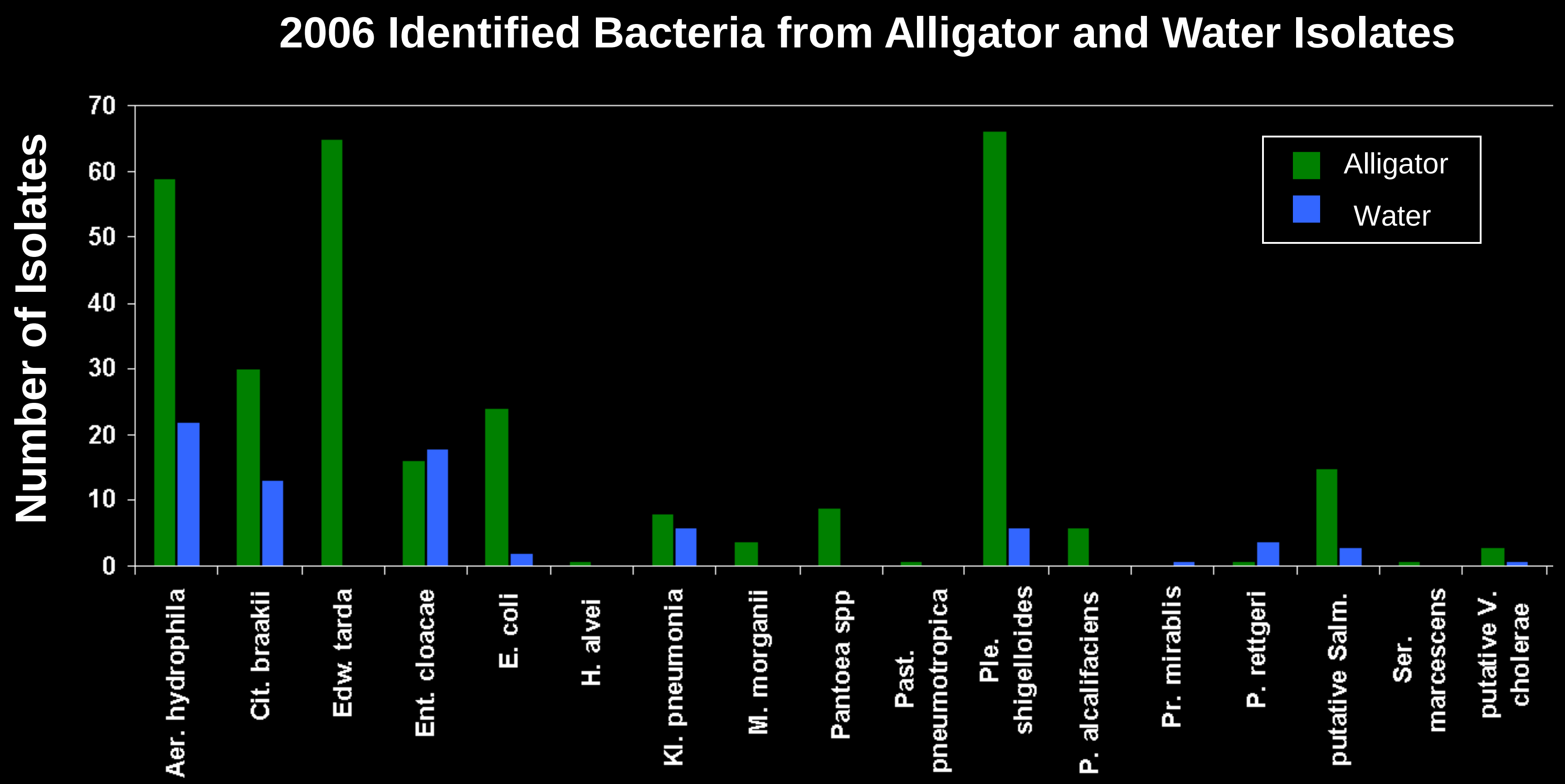


Sequencing Identification

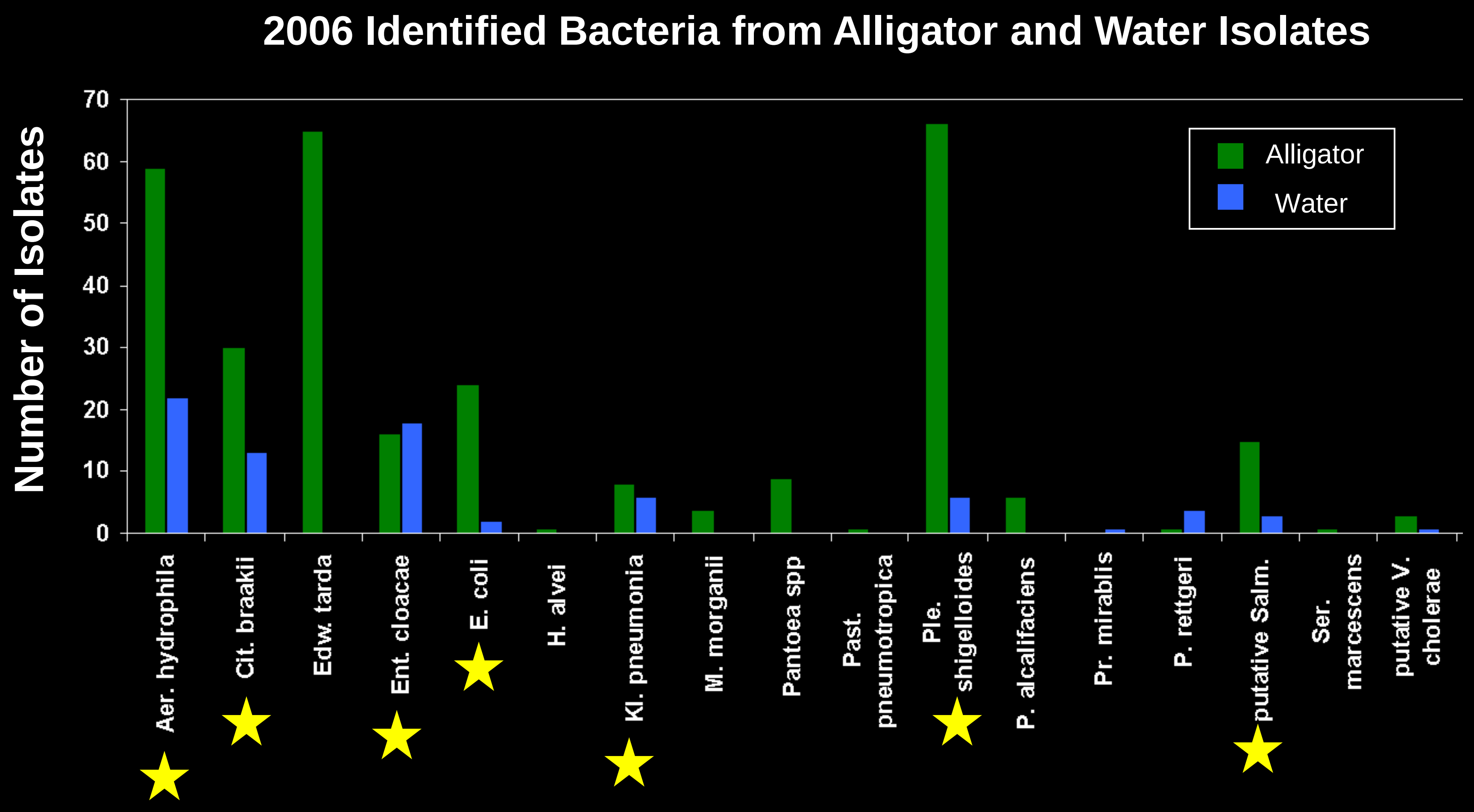
NCBI

Nat. Center for Biotech. Info., Basic Local Alignment Search Tool

Bacteria Identification Results



Bacteria Identification Results



Bacterial Density Results

- Fecal Coliform Most Probable Number (MPN):

Species	Fecal Coliform MPN (Density/g feces)	Source
Alligator	**8.0×10^{9}**	Johnston et al., 2010
Duck	3.3×10^{7}	Schueler and Holland, 2000
Human	1.3×10^{7}	Schueler and Holland, 2000
Dog	2.3×10^{7}	Schueler and Holland, 2000
Turtle	1.6×10^{6}	Harwood et al., 1999
Cow	2.3×10^{5}	Schueler and Holland, 2000

Research Component 2

Genotypic Fingerprinting

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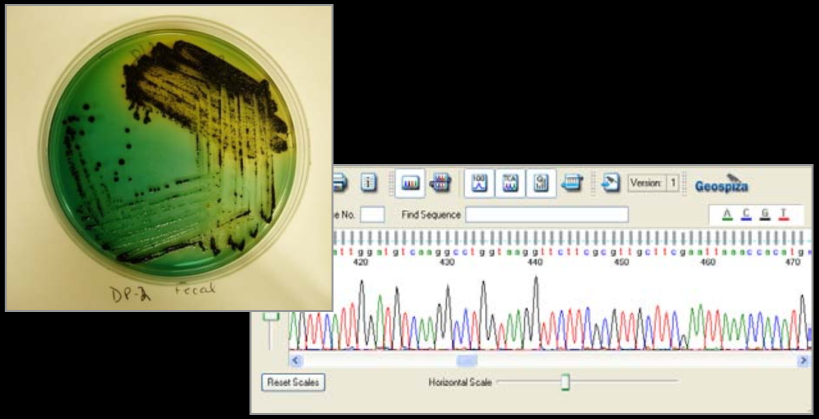

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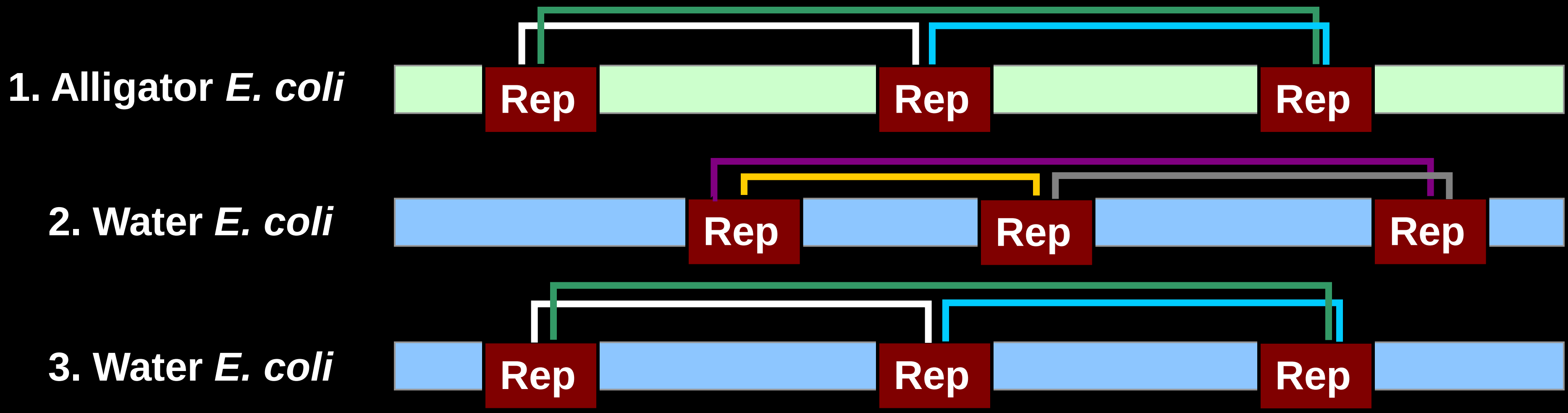
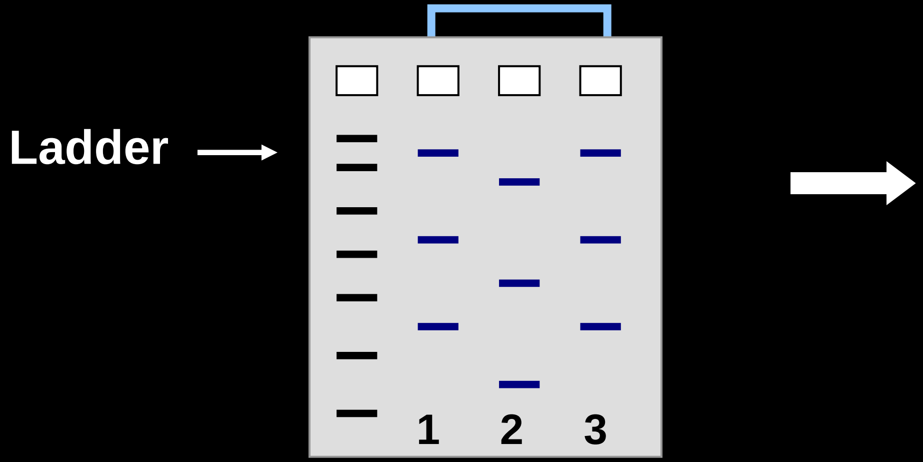
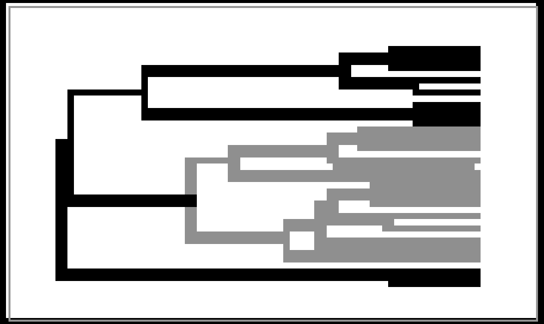
Genotypic Fingerprinting

Source	Isolate ID	No.	Source	Isolate ID	No.
Alligator	***Aeromonas hydrophila***	**31**	Chicken	*Escherichia coli*	6
	Aeromonas punctata	**1**	Cow	*Escherichia coli*	5
	Aeromonas veronii	**24**	Dog	*Escherichia coli*	5
	Citrobacter freundii	**92**	Horse	*Escherichia coli*	10
	Enterobacter aerogenes	**3**	Pig	*Escherichia coli*	5
	Enterobacter cloacae	**21**	Raccoon	*Escherichia coli*	1
	Escherichia coli	**41**	Sea Turtle	*Citrobacter freundii*	3
	Klebsiella planticola	**5**		*Enterobacter aerogenes*	1
	Klebsiella pneumoniae	**10**		*Escherichia coli*	1
	Plesiomonas shigelloides	**78**		*Salmonella enterica*	1
	Salmonella enterica	**7**			
	Total	**313**		Total	38

Genotypic Fingerprinting







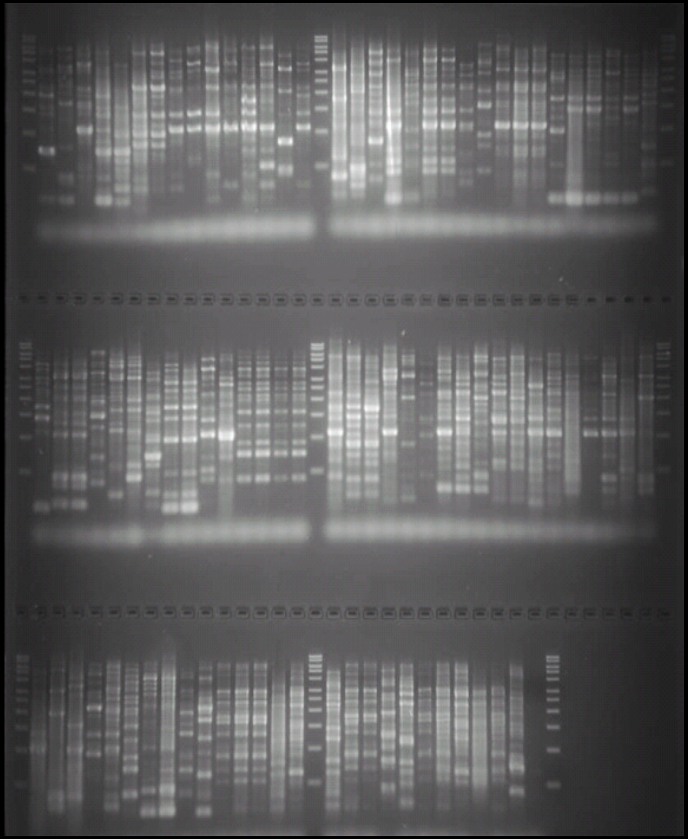
Repetitive Extragenic Palindromic-PCR

- REP-PCR Primers for entire genome:

Primer	Sequence
REP1R-I	5'- III ICG ICG ICA TCI GGC -3'
REP2-I	5'- ICG ICT TAT CIG GCC TAC -3'
ERIC1R	5'- ATG TAA GCT CCT GGG GAT TCA C -3'
ERIC2	5'- AAG TAA GTG ACT GGG GTG AGC G -3'
BOX A1R	5'- CTA CGG CAA GGC GAC GCT GAC G -3'
$(GTG)_5$	5'- GTG GTG GTG GTG GTG -3'
Source:	Versalovic et al., 1994

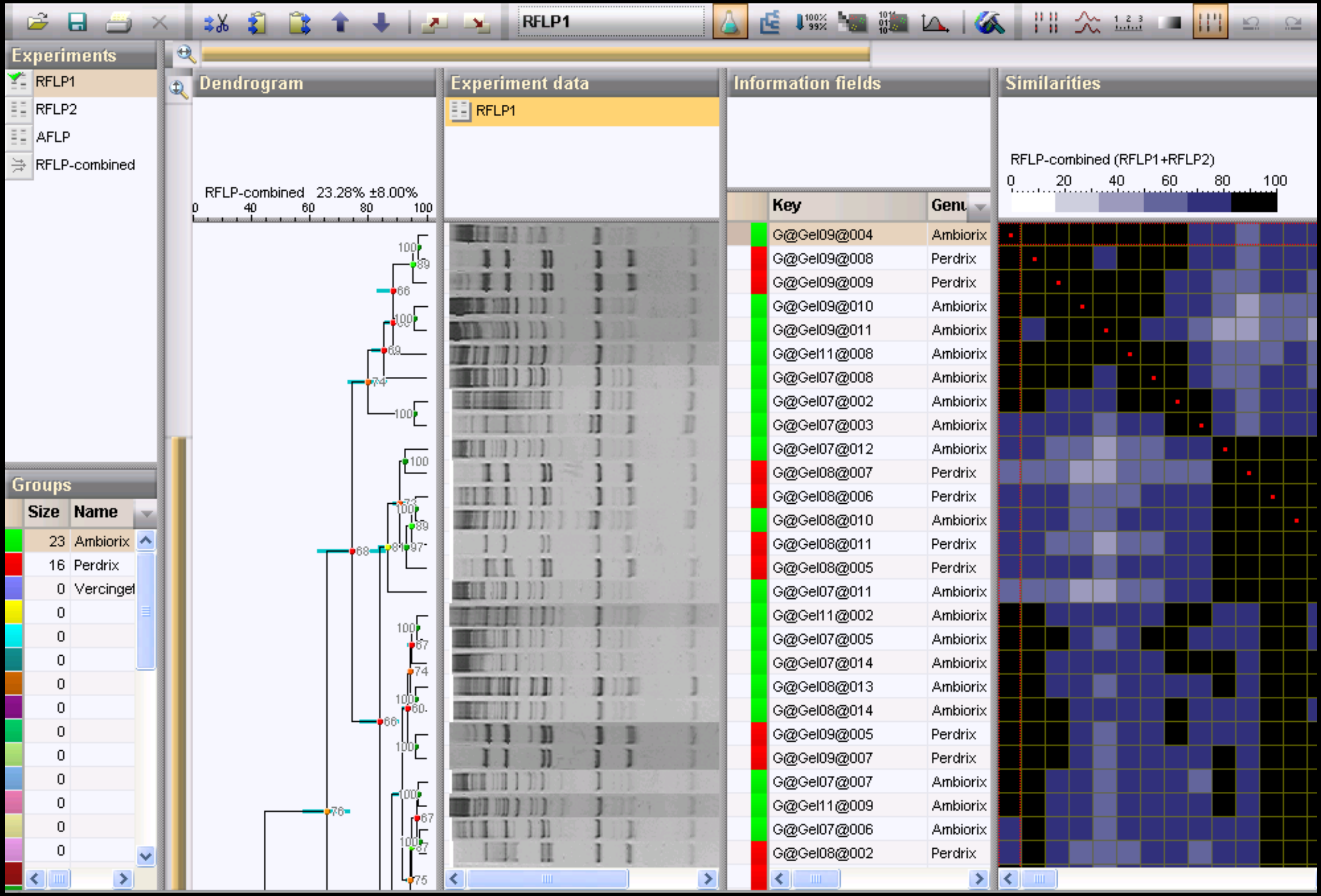
Research Component 2: Methods

- REP-PCR
- Gel Electrophoresis
- EtBr stain and image



Research Component 2: Results

- Gel Compar II Similarity matrix (Pearson Correlation)



Research Component 2: Results

- Percentage of alligator isolates assigned to each group using Jackknife analysis

	Percentage (%) of Alligator Isolates Correctly Assigned to Each Source Group						
Isolate Group	*Aer. spp.*	*Cit. freundii*	*Ent. spp.*	*E. coli*	*Kl. spp.*	*Ple. shigelloides*	*Salm. enterica*
Aer. spp.	**80.4**	3.3	4.6	0.0	0.0	1.3	0.0
Cit. freundii	7.1	**90.2**	4.6	2.4	13.3	1.3	0.0
Ent. spp.	7.1	2.2	**63.6**	2.4	0.0	2.6	0.0
E. coli	3.6	2.2	4.6	**90.2**	20.0	1.3	0.0
Kl. spp.	0.0	0.0	4.6	2.4	**66.7**	0.0	0.0
Ple. shigelloides	1.8	2.2	18.2	2.4	0.0	**93.6**	0.0
Salm. enterica	0.0	0.0	0.0	0.0	0.0	0.0	**100.0**

Values in boldface represent the rate of correct classification (RCC). Average RCC was 84%.

Research Component 2: Results

- Percentage of *E. coli* isolates assigned to each group using Jackknife analysis

Source	Percentage (%) of *E. coli* Isolates Correctly Assigned to Each Source Group							
	Alligator	Chicken	Cow	Dog	Horse	Pig	Raccoon	Sea Turtle
Alligator	**95.2**	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chicken	0.0	**100.0**	0.0	0.0	0.0	0.0	0.0	0.0
Cow	2.4	0.0	**100.0**	0.0	0.0	0.0	0.0	0.0
Dog	0.0	0.0	0.0	**100.0**	0.0	0.0	0.0	0.0
Horse	0.0	0.0	0.0	0.0	**100.0**	0.0	0.0	0.0
Pig	0.0	0.0	0.0	0.0	0.0	**100.0**	0.0	0.0
Raccoon	0.0	0.0	0.0	0.0	0.0	0.0	**100.0**	0.0
Sea Turtle	2.4	0.0	0.0	0.0	0.0	0.0	0.0	**100.0**

Values in boldface represent the rate of correct classification (RCC). Average RCC was 99%.

Research Component 3

Bacterial Source Tracking

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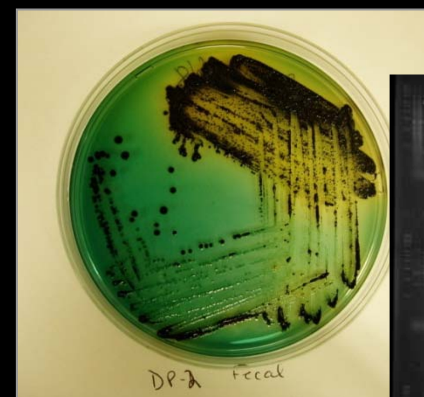

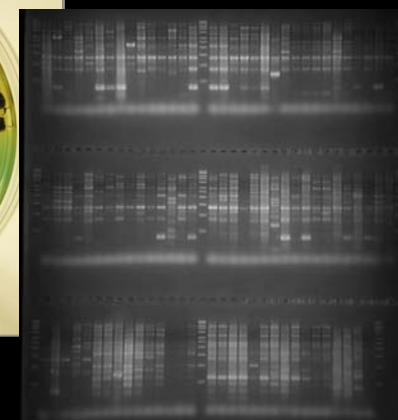

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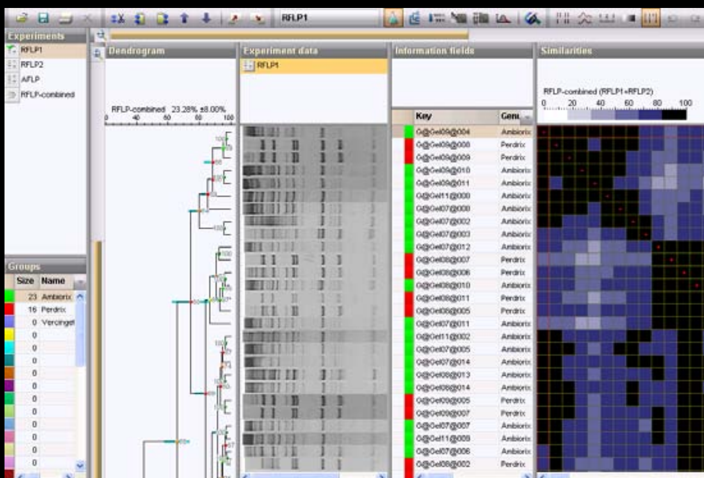
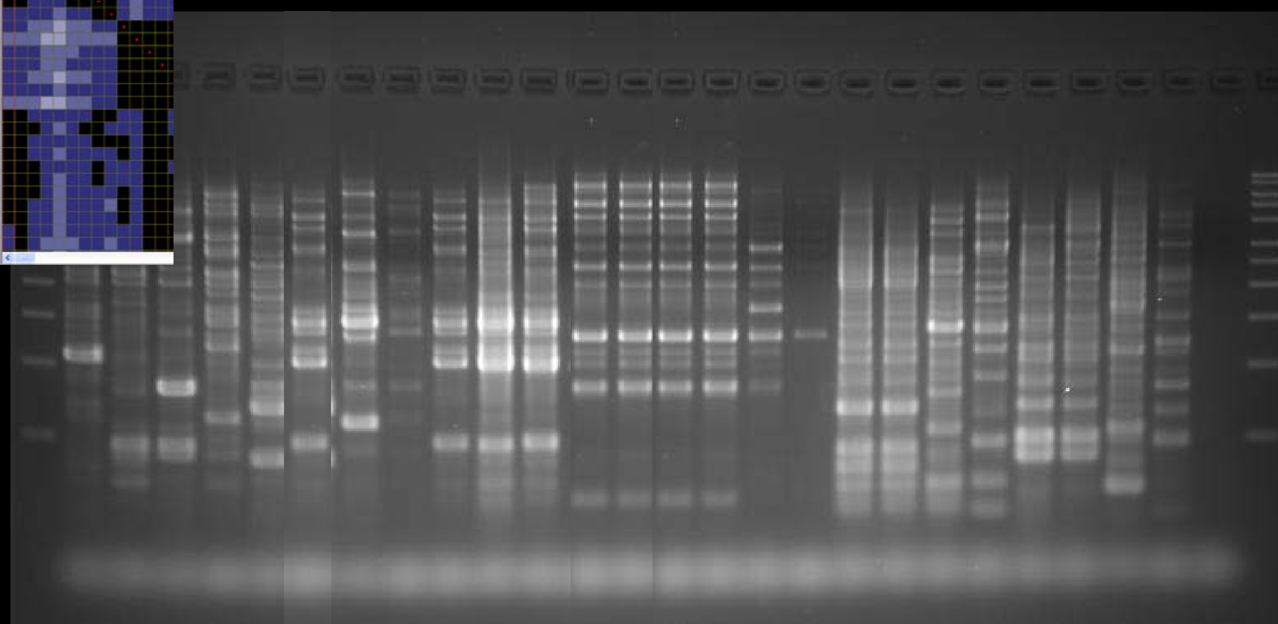
Alligator Sampling

- Palmetto Bluff
  - 2006 (n=31)
  - 2009 (n=4)
- Spring Island
  - 2009 (n=4)

Research Component 3: Results

- Generated 705 alligator and water fingerprints





Research Component 3: Results

- 2006 matches

2006 Bacteria	Water to Alligator Matches	Total Water Isolates	Total Percent (%) Matched
Aer. spp.	2	23	9
Cit. freundii	7	19	37
E. coli	9	24	38
Ent. spp.	3	28	11
Kl. spp.	2	9	25
Ple. shigelloides	3	7	43
Salm. enterica	0	0	0
Pooled	**26**	**110**	**24**

Research Component 3: Results

- 2009 matches

2009 Bacteria	Water to Alligator Matches	Total Water Isolates	Total Percent (%) Matched
Aer. spp.	0	Unknown	0
Cit. freundii	5	Unknown	2
E. coli	4	Unknown	1.5
Edw. tarda	2	Unknown	1
Ent. spp.	0	Unknown	0
Kl. spp.	0	Unknown	0
Ple. shigelloides	6	Unknown	2
Salm. enterica	0	Unknown	0
Pooled	**17**	**272**	**6**

Research Component 3: Results

- MPN from 2009:

Species	Fecal Coliform MPN (Density/g feces)	Source
Alligator	**9.0×10^9**	Current study
Duck	3.3×10^7	Schueler and Holland, 2000
Human	1.3×10^7	Schueler and Holland, 2000
Dog	2.3×10^7	Schueler and Holland, 2000
Turtle	1.6×10^6	Harwood *et al*., 1999
Cow	2.3×10^5	Schueler and Holland, 2000

Conclusions

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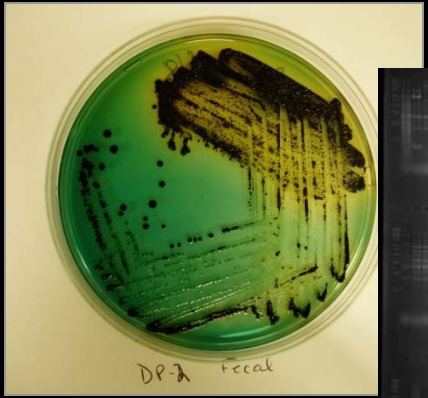

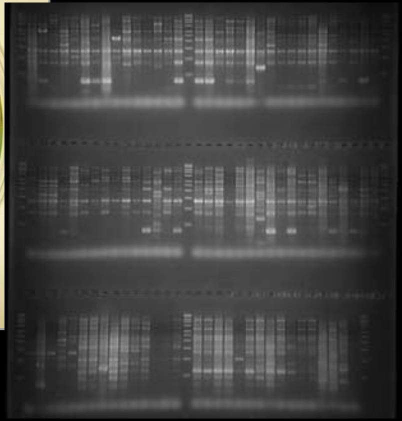

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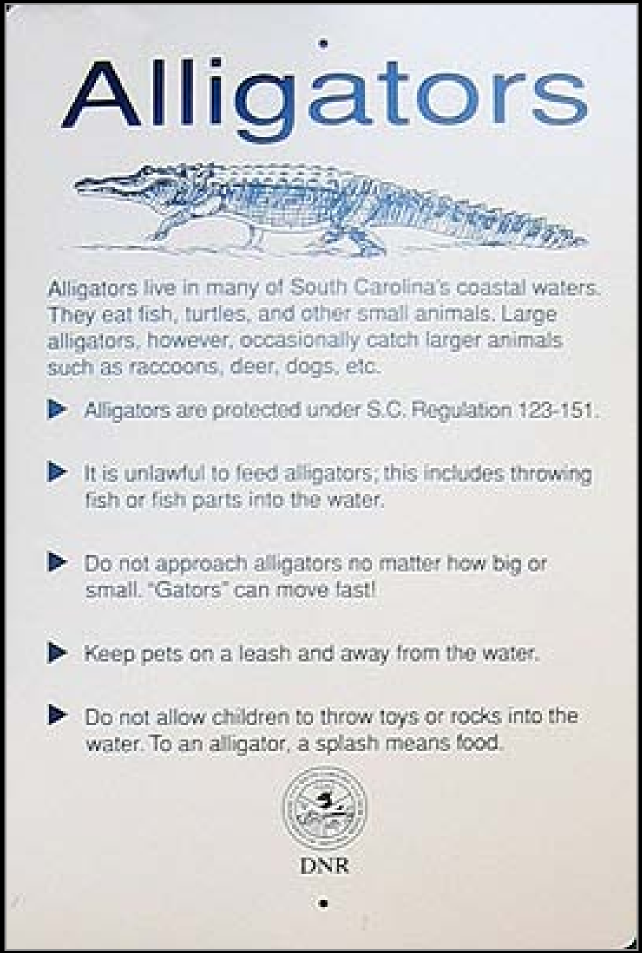
Conclusions

- First study to identify poikilotherms as a source of bacterial pollution in coastal waters
- Alligator fecal coliforms denser than endotherms
- REP-PCR valid BST technique for indicator bacteria and potential pathogens

Conclusions

- Management practices:
  - Hunting
  - Tagging
  - Vegetated buffers



Conclusions

- Potential Collaborations:
  - Caimans: Universidade Federal de Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil

  - GEL Engineering, Charleston, SC

Conclusions

- Cold-blooded sources:
  - Fecal coliforms
  - Potential pathogens
- Concentration of sources
  - Loss of habitat
- Management practices to strive for human and animal balance

Acknowledgements

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- **Committee**:
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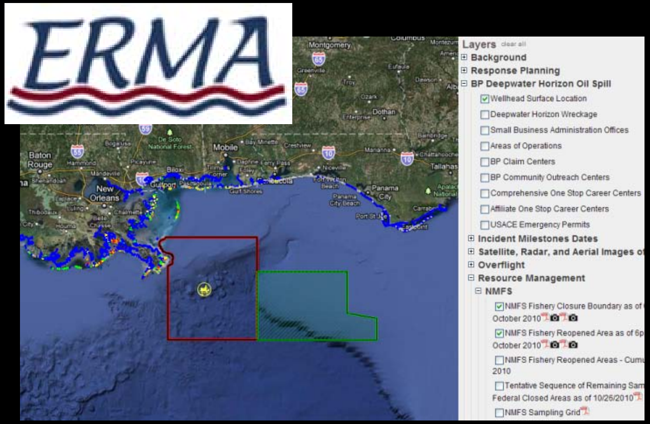
  - Dr. Sean Norman, USC
  - Dr. Dwayne Porter, USC
  - Dr. Heath Kelsey, NOAA-UMCES
- Molecular Microbial Ecology Lab, GIP Lab, NOAA/CCEHBR, Palmetto Bluff Conservancy, SCDNR, Cypress Gardens, and Spring Island - Low Country Institute

Office of National Marine Sanctuaries

Blue Ocean Film Festival, MBNMS



Deepwater Horizon Oil Spill Response

IEEE Conference, Buenos Aires, Argentina

Montana LIVE Dive, TBNMS

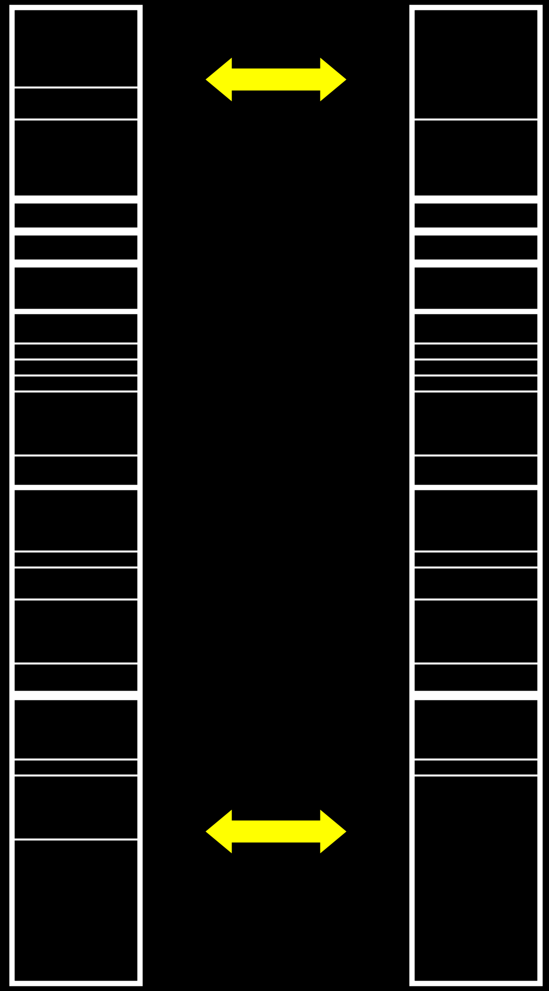
Aquarius 2010 Mission, FKNMS

Later Alligator

Research Component 2: Results

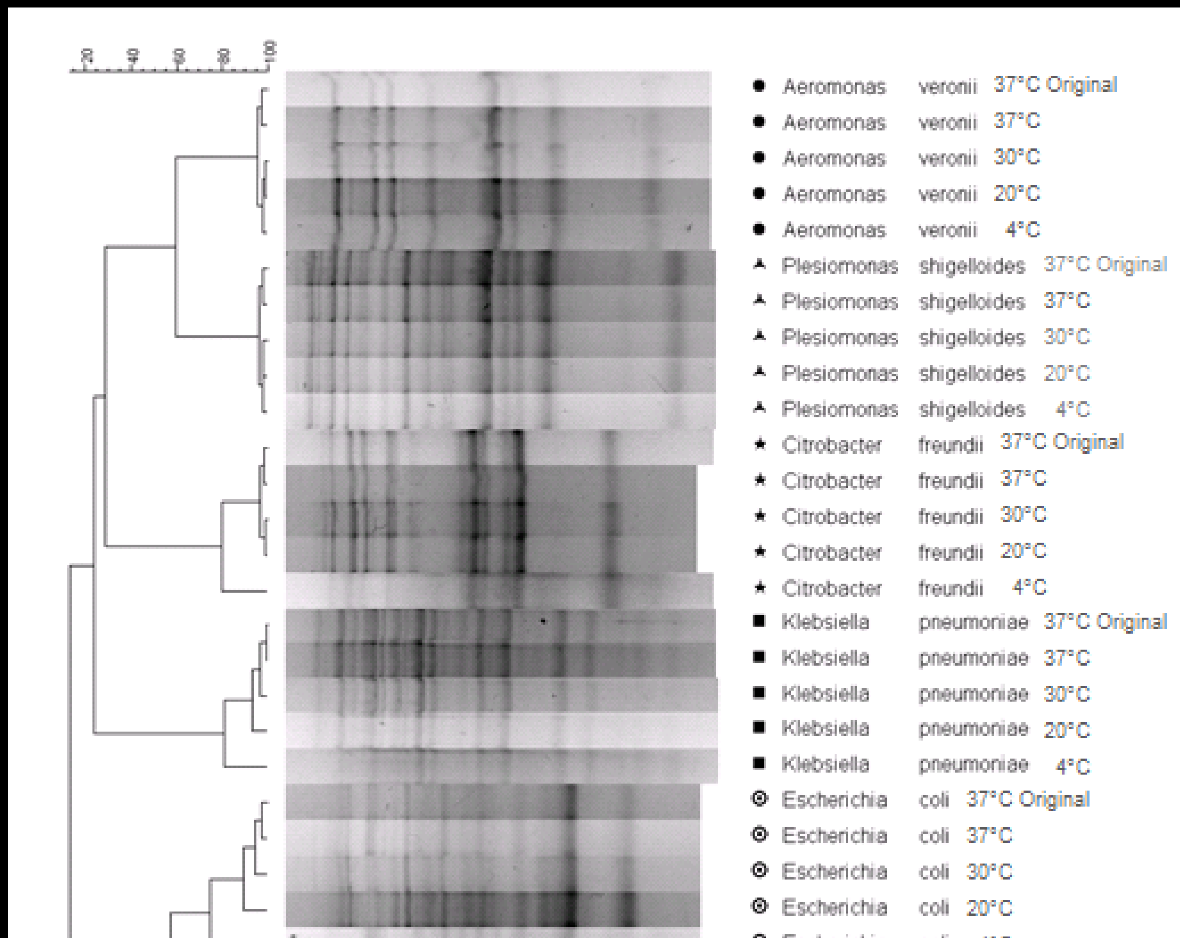
- 90% similarity
  - Repeated control runs
  - 2 band difference

- ≥ 90% similarity cutoff value
  - Matching bands from same source

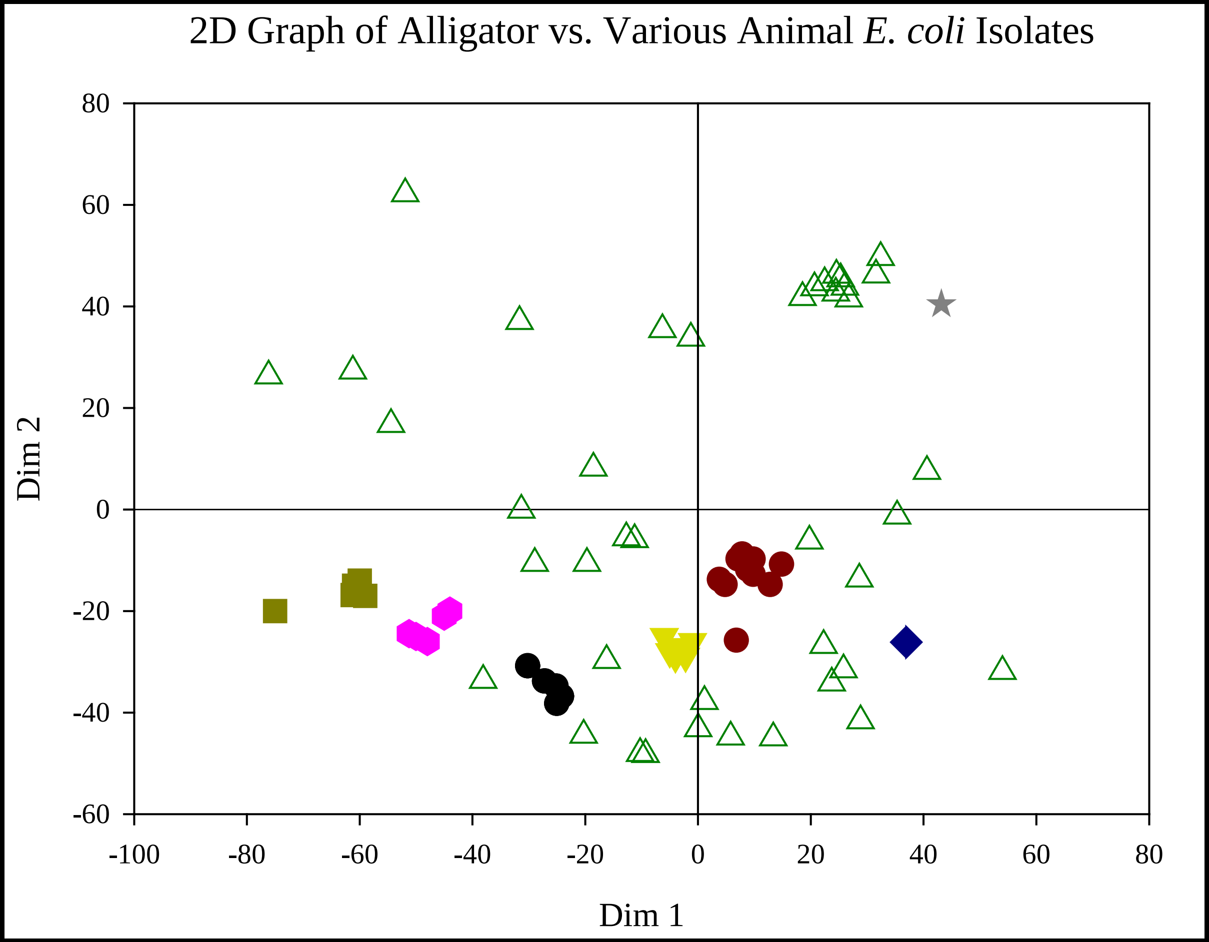


Research Component 2: Results

- Fingerprint stability



Research Component 2: Results



E. coli isolates represent alligator △, chicken ▼, cow ○ , dog ■ , horse ● , pig ⬢ , raccoon ★, and sea turtle ◆ .

Results