Isolating *Aeromonas* from Precipitation and the Atmosphere

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Purpose of Study

- Collect rainwater, hail and wind samples
- Isolate and identify *Aeromonas* and other closely related genera within those samples
- Investigate how these bacteria move throughout the environment and the atmosphere

What is *Aeromonas?*

- An ubiquitous aquatic bacterium
- Known to portray many roles in the environment
- Disease-causing pathogen of fish, animals, and humans

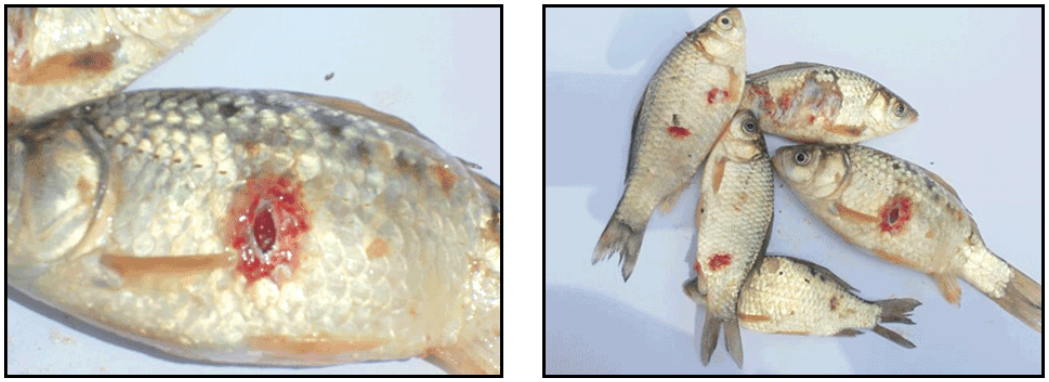
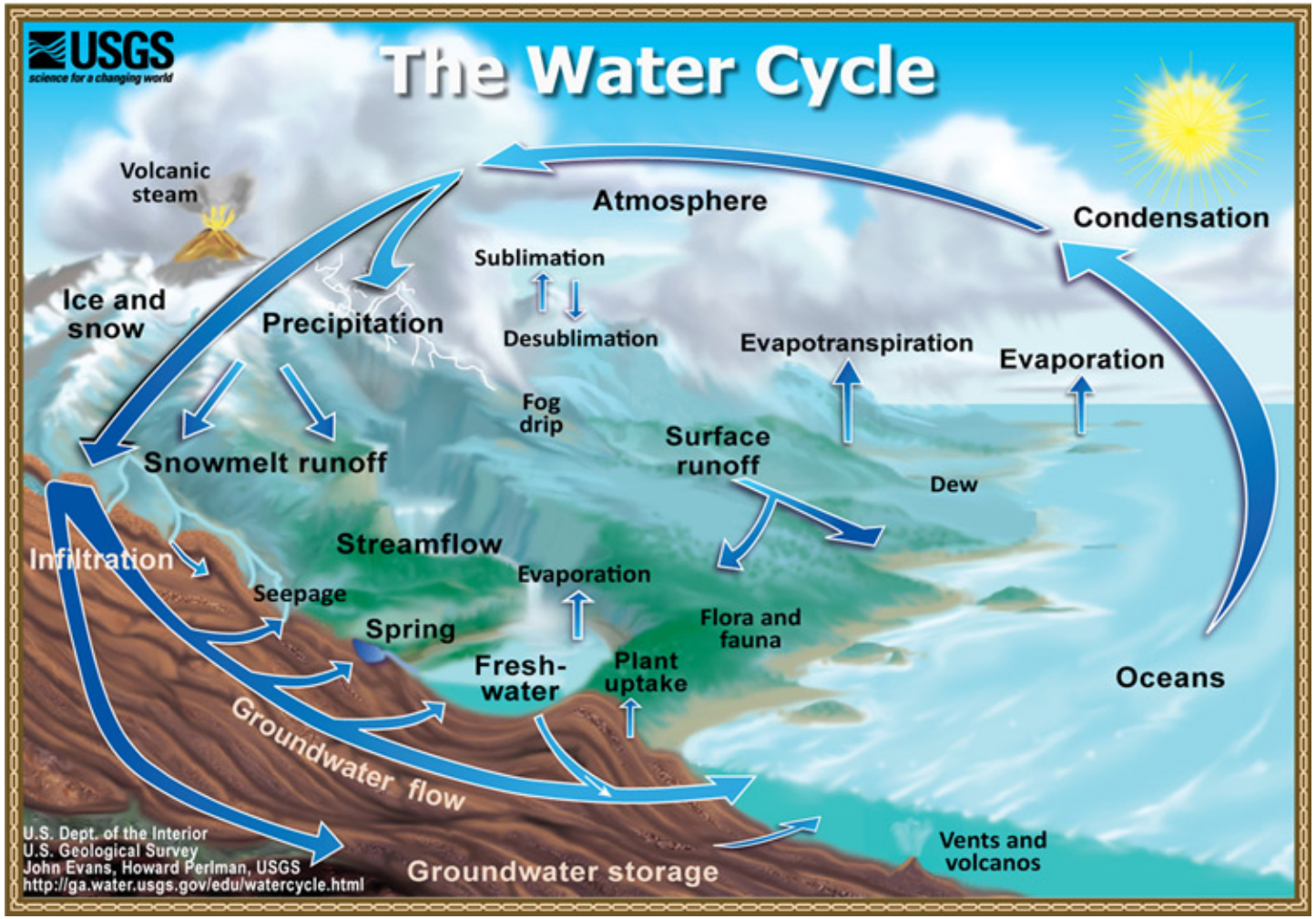


Figure 1 Fish infected with *Aeromonas hydrophila*. Registered under charity number SC000278 Heriot-Watt University is a Scottish charity UK

Aerobiology

- **Aerobiology:** the science dealing with the occurrence, transportation, and effects of airborne materials or microorganisms (as viruses, pollen, pollutants, or bacteria)
- Plays a fundamental role in transmission of infectious diseases (Fernstrom & Goldblatt, 2013).
- Occurs through "droplet" and "airborne" means
- Environmental factors influence airborne disease transmission

The Hydrologic Cycle

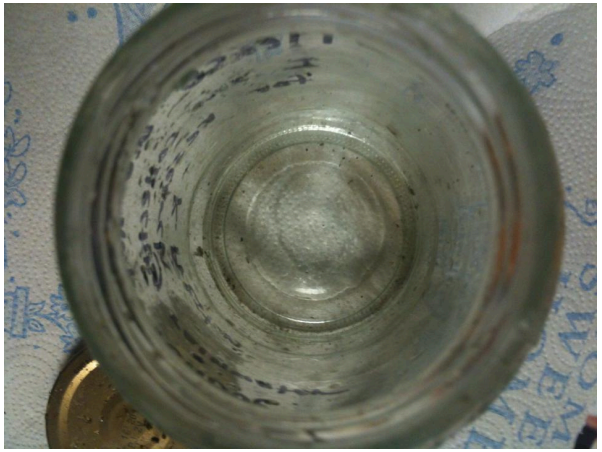


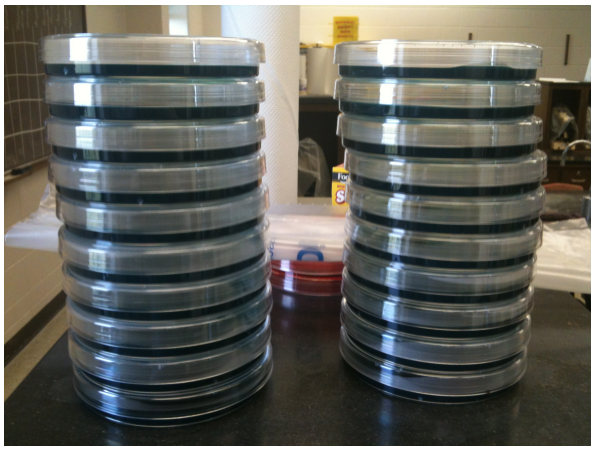
Significance of Study

- Water in the environment is a contamination route for microorganisms (Carvalho et al, 2012).
- Every year 1.8 million people die from diarrheal diseases attributed to unsafe water (WHO, 2004).
- *Aeromonas* associated with diarrheal diseases (Ahmed et al., 2012).
- *Aeromonas* causes gastroenteritis, skin and soft tissue infections and sepsis with lethal course (Khajanchi et al., 2010).
- Antibiotic resistance and virulence traits (Galindo et al., 2006).
- Contamination of rainwater in third world countries

Rainwater and Wind Collection

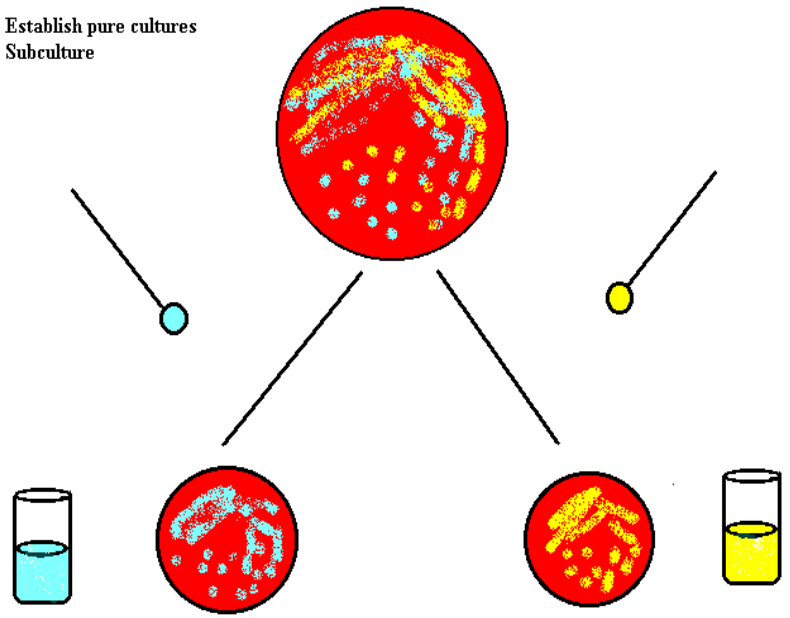
- Rainwater/Hail: sterilized collection jars
- Wind: *Aeromonas* blue medium plates
- *Aeromonas* blue medium with and without ampicillin





Subculturing and Isolating Colonies

- Choose different individual colonies on plate
- Inoculate colonies and subculture by streaking onto TSA (Tryptic Soy Agar) plates
- Continued until pure colonies are isolated

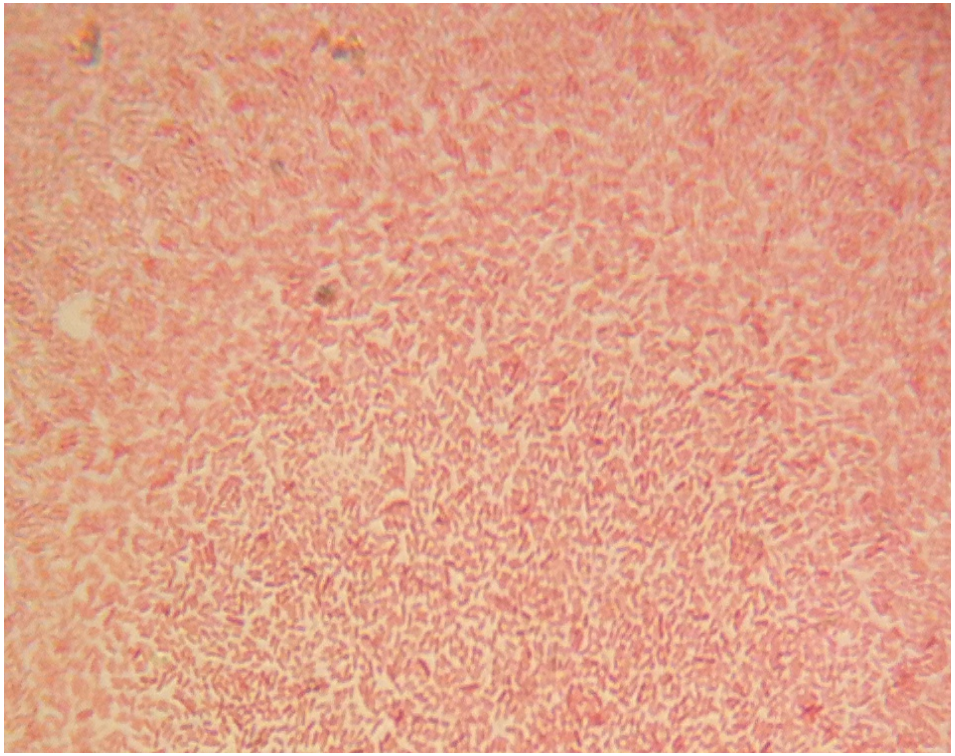


Oxidase Test

- Specifically tests for certain cytochrome C oxidase activity in bacteria
- Positive: dark blue to purple color
- Negative: no color
- Positive = possibility of *Aeromonas*

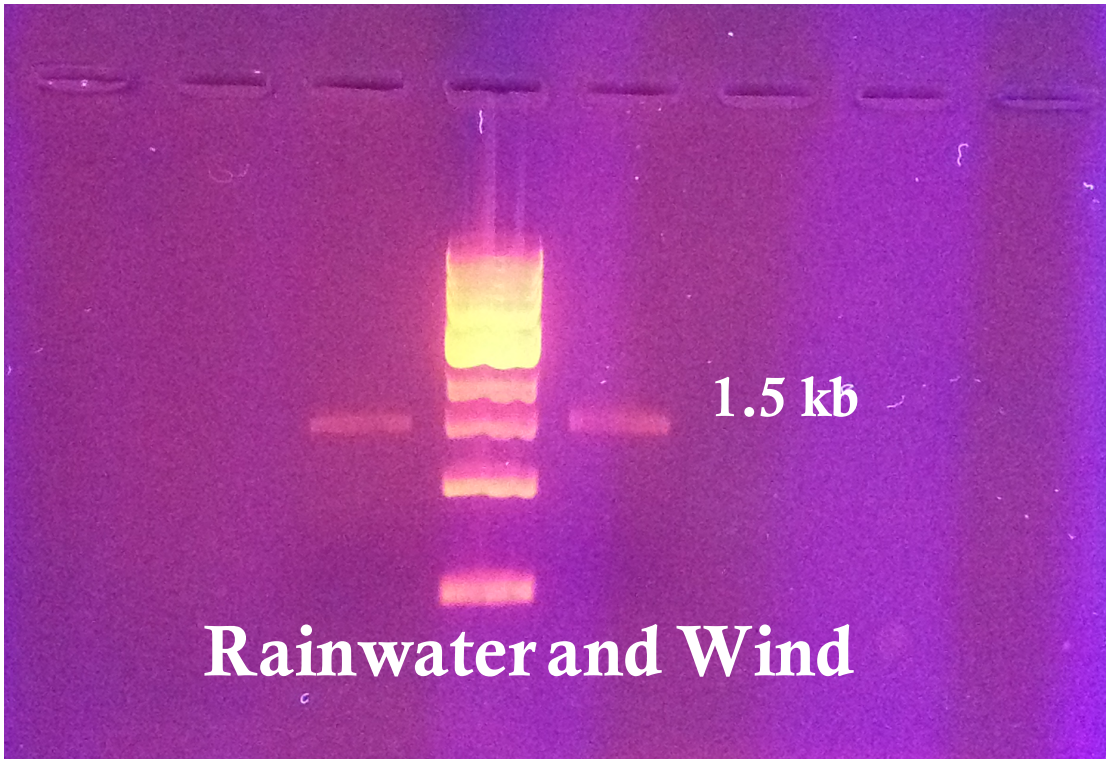
Gram Staining

- *Aeromonas*: Gram negative rods



Colony PCR (Polymerase Chain Reaction)

- Used to copy specific region of *Aeromonas* DNA (16S rRNA)
- Gel electrophoresis



Analysis of Results

- Collected rainwater and wind samples
- Plated and isolated colonies
- Performed oxidase tests and gram staining
- Ran colony PCR on samples
- 15 possibilities of *Aeromonas*

Analysis of Results Cont'd

- Ran PCR purification on possibilities
- Had samples sequenced
- Analyzed sequences through databases
- No *Aeromonas* found
- Possible discovery of new species of bacteria

Table 1. NCBI BLAST Database and RDP Database Comparison for Sequence Samples

NCBI			RDP	
Isolate: source	**Identification**	**% Identity****	**Identification**	**Strength (S_abscore)**
1: wind	*Novosphingobium* clone SeqSEEZ199	99%	*Sphingomonas* 44/40	0.949
2: wind	*Pseudomonas putida*	99%	*Pseudomonas putida*	0.969
3: wind	Uncultured *Novosphingobium* clone SeqSEEZ199	99%	Uncultured Sphingomonadaceae bacterium	0.942
4: rain	*Pseudomonas* MC83	99%	*Pseudomonas* MC83	0.974
5: rain	*Massilia varians*	97%	*Naxibacter* 6981	0.870
6: rain	*Pseudomonas* JSM 2215099	100%	*Pseudomonas fulva*	0.997
7: rain	*Pseudomonas fulva*	100%	*Pseudomonas* 471-1	0.997

**All strength values (E-value) were $p \leq 2 \times 10^{-165}$

*Ampicillin was used in the isolation medium of these strains

Table 1. Continued

NCBI			RDP	
Isolate: source	**Identification**	**% Identity****	**Identification**	**Strength (S_abscore)**
8: rain	*Pseudomonas* 9DLP	99%	*Pseudomonas* PSB1	0.992
9: rain	Uncultured *Pseudomonas*	100%	*Pseudomonas fulva*	0.983
10: rain	*Pseudomonas plecoglossicida*	89%	*Pseudomonas* CPA30	0.520
*11: rain	*Mesorhizobium opportunistum*	94%	*Aurantimonas ureilytica*	0.911
*12: rain	*Mesorhizobium opportunistum*	94%	*Aurantimonas ureilytica*	0.916
*13: rain	*Agrobacterium* H13-3	99%	*Rhizobium* Gls-4	0.972
*14: rain	*Pseudomonas syringae*	96%	*Pseudomonas fluorescens*	0.876
*15: rain	*Pseudomonas syringae*	93%	*Pseudomonas* S1(2011b)	0.552

**All strength values (E-value) were $p \leq 2 \times 10^{-165}$

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Current Status of Project

- Further collection of samples by larger bodies of water
- *gyrB* sequencing
- Eliminate aerobes

Application of Study

- Add to pool of research
- Heightened awareness of microbes in rainwater and atmosphere
- Impact on microbiology and everyday life

References: Literature

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