The Effects of Salinity, pH, and Dissolved Oxygen on the Sensitivity of PCR Identification of the *T4* Bacteriophage in Estuarine Water

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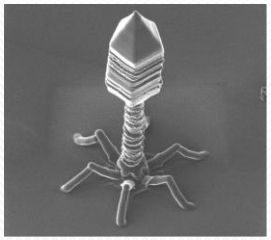
Bacteriophages

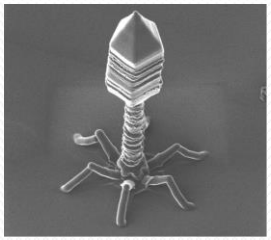
- Bacteriophages are host specific viruses that infect bacteria.
- *Escherichia coli* is a common bacterium that is found in fecal contaminated water.
- The T4 bacteriophage has been shown to infect, replicate within and subsequently lyse *E. coli,* spreading new virus particles into the environment.



Bacteriophages as Indicators

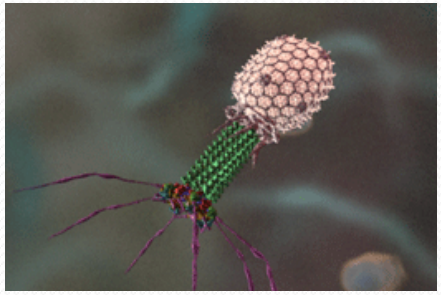
While an excellent bacterial pollution indicator in *drinking and waste water settings*, little is known about:

- the survival and persistence of bacteriophages in natural freshwater and saline settings.
- bacteriophage survival and persistence in high flux waterways



Bacteriophages as Therapy

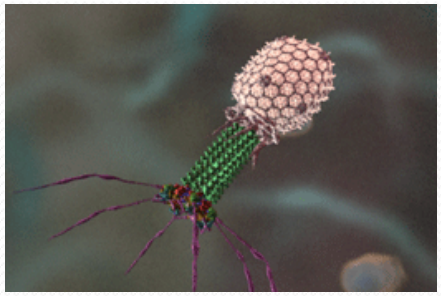
- Bacteriophages have potential in *limiting aquatic bacteria through their lytic* properties.
- Phage therapy has potential for use to *control disease in aquaculture systems.*



Bacteriophages as Therapy

Advantages

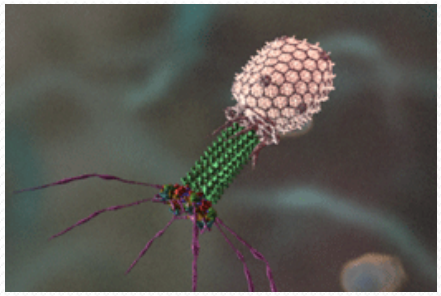
- *self-replication*;
- *increased* concentrations as infection *persists*;
- *highly selective to host*, which *prevents harm* to beneficial, naturally occurring microflora.
- *evolve faster* than bacteria (not static)



Bacteriophages as Therapy

Disadvantages

- Phages are *sensitive to temperature, chemical treatments and salinity*.
- *Coliphage occurrence may be significantly different between freshwater, estuarine and coastal locations*



Long Term Research Plan

- *Isolate* and quantify local bacteriophages using the "PCR"
- *Identify* local bacteriophages by genetic fingerprinting
- Build a *catalogue* of local bacteriophages
- *Test feasibility of phage therapy* in local bacterial blooms.

PCR Identification

We have developed (PCR) protocol for identifying the presence of T4 bacteriophage in water samples.

Targets two genes in the T4 genome:

- Open Reading Frame 23, (OFR 23) which encodes for a major capsid protein
- Open Reading Frame 43, (OFR 43) which encodes for the T4 DNA polymerase.

Sensitive to detect 5 virus particles (230 viruses per milliliter of sample)

Sampling Sites

Waccamaw River (High Flux)

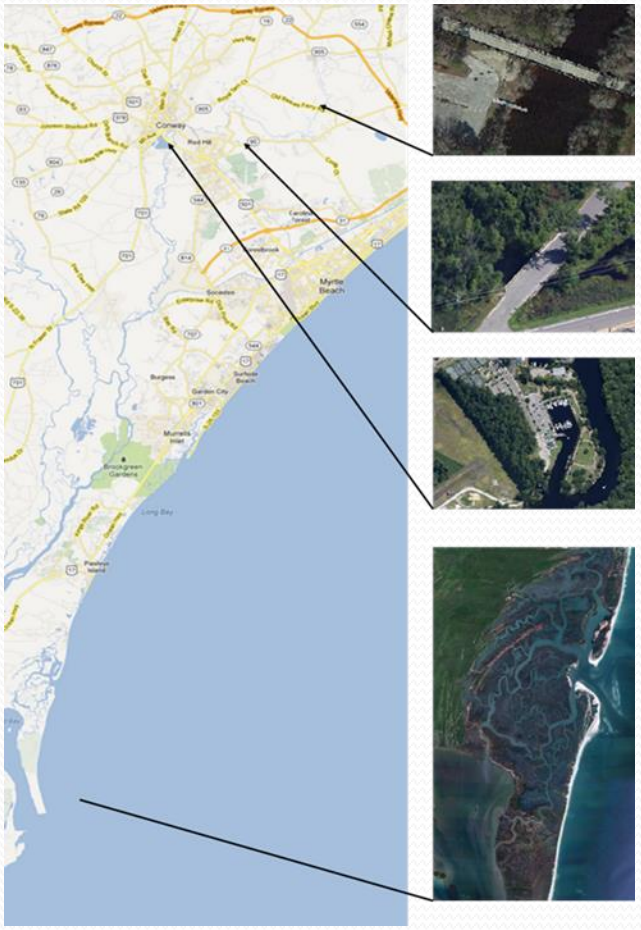
- Reaves Ferry
- Conway Waterfront
- Hagley Landing
- Wachesaw Landing

Waccamaw River backwater (Low Flux)

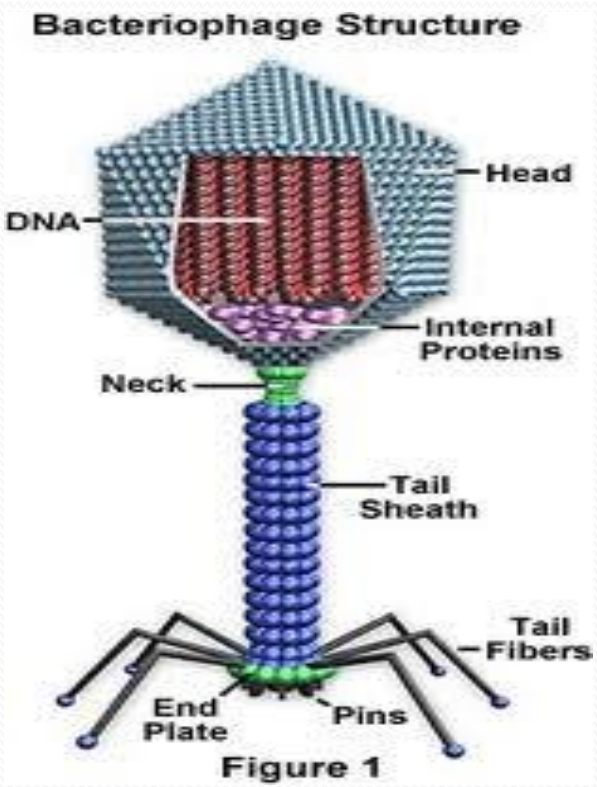
- Sterritt Swamp
- Conway Waterfront Swamp

North Inlet (Tidal)

- Debidue Creek,
- Crabhaul Pocket Ponds
- Bart's Bridge



DNA Extraction

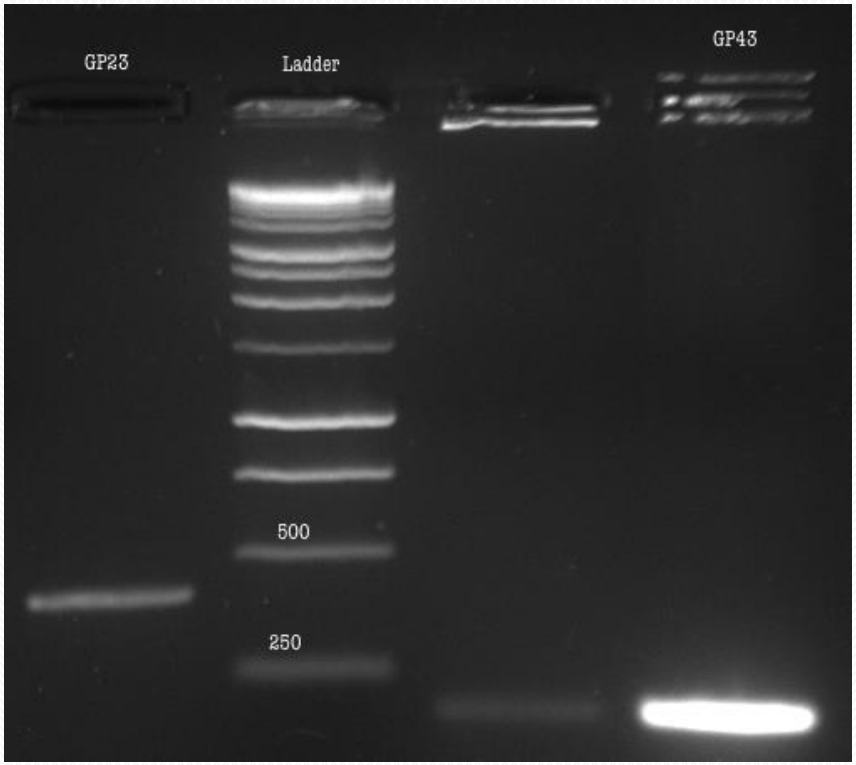


- 70 uL of sample and 7 uL of proteinase K were added to a micro centrifuge tube and were incubated at room temperature for 45 minutes on an orbital shaker to expose T4 DNA.
- Samples were exposed to a constant temperature of 96.1^{o}C for 10 minutes in a hot block to inactivate proteinase K and any remaining phage samples.

PCR Amplification and Testing

- In a large PCR tube, 25uL of GoTaq Hot start master mix, 2uL of each L primer (23L and 43L) , 2uL of each R primer (23R and 43R) and 21uL of sample DNA were combined.
- The genetic material was amplified using the T4 program on a Biorad personal thermocycler.
- After amplification, the samples were run out on a 1% agarose gel stained with ethidium bromide at 100 volts for 1 hour. The gel was imaged using a 100 base pair ladder as reference.

T4 Positive result



GP23, a major capsid protein of 403 base pairs;

GP43, the core DNA polymerase of T4 replisome consisting of 198 base pairs

Results

Overall Identification

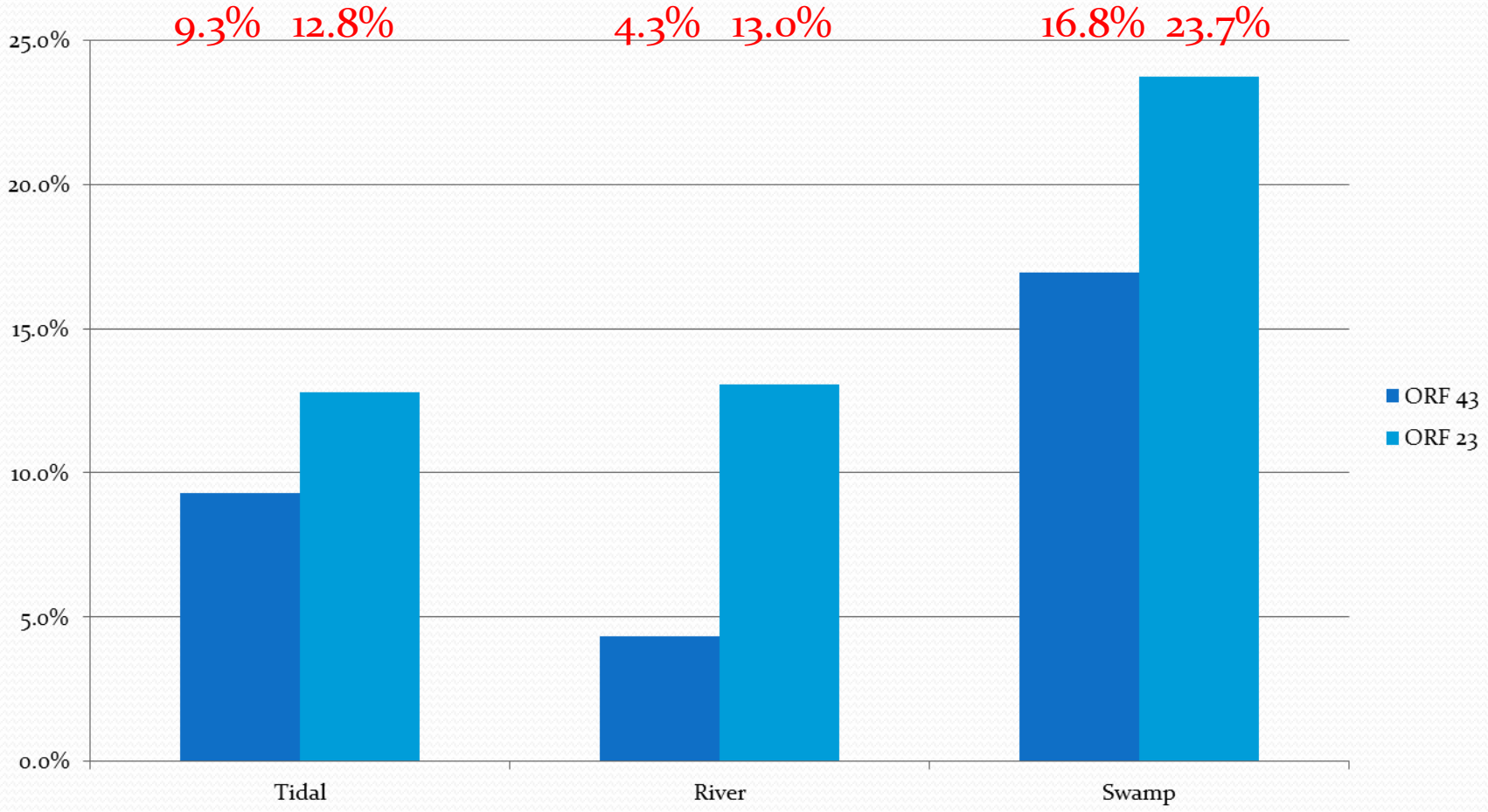
Site	# of Samples	# of ORF 23 Detected	# of ORF 43 Detected	% ORF 23 Detected	% ORF 43 Detected	Flux Rate
Hobcaw A	48	4	6	8.33%	12.50%	Tidal
Hobcaw B	32	4	5	12.50%	15.63%	Tidal
Hobcaw C	6	0	0	0.00%	0.00%	Tidal
Lower River	6	0	0	0.00%	0.00%	High
Reeve's Landing	24	1	3	4.17%	12.50%	High
Conway Waterfront	16	1	3	6.25%	18.75%	High
Conway River Swamp	33	7	10	21.21%	30.30%	Low
Sterrit Swamp	26	3	4	11.54%	15.38%	Low

ORF 23
10.5%

ORF 43
16.2%

N=191

Positive Identification



Flow

River – *significant difference* in positive identification ($F_{44,57}$ = 0.601, P > 0.05) between:

- *Low Flux* environment-backwater swamp and primary embanked streamlet (*20.68%*)
- *High flux* tertiary stream sites (*11.11%*)

Estuarine *tidal marsh* (*12.8%*)

- Semi-diurnal tidal flux is high.

This finding is indicative that natural flushing may inhibit the capacity of the bacteriophage to thrive.

Abiotic Factors

Variable	Positive Average	Negative Average	F-Test	Degrees Freedom	P-Value
pH	7.78	7.80	0.069605	(1,76)	0.793
Temperature (°C)	10.36	17.11	8.241235	(1,76)	**0.005**
Dissolved Oxygen (mg/L)	7.96	7.27	2.387397	(1,76)	0.127
Salinity (PSU)	33.91	34.51	1.578205	(1,76)	0.212

Temperature

Temperature showed a *significant inverse relationship* to the proportion of positive results.

- The lower the temperature, the higher occurrence of bacteriophage detection. ($F_{1,76}$=8.241, $p<.005$)
- Somewhat counterintuitive – would expect more bacteria at higher temperatures

Dissolved Oxygen

No significant difference in the rate of bacteriophage detection for water and soil samples ($F_{1,76}$=7.27, p>.127)

- However *significant inverse relationship between water temperature and dissolved oxygen concentration* (R^2 = 0.614, $F_{(1,59)}$ = 93.9, P < 0.001)
- The lower the water temperature, the higher the dissolved oxygen concentration.
- May indicates that positive identification rates are associated with higher oxygen concentrations and not with lower water temperatures.

Precipitation

- 24 Hour Rainfall data collected at Hobcaw Barony shows that

Variable	Positive Average	Negative Average	F-Test	Degrees Freedom	P-Value
24hr rainfall (mm/day)	11.757	2.629	6.02	(1,56)	**0.017**

- *bacteriophage detection increases as precipitation increases* ($F_{1,56} = 6.02$, $P < 0.02$)
- as expected from fecal contamination runoff.

Next Steps

Isolate local bacteriophages using the "PCR" technique

- Expand identification to cover seasonal variation
- **Correlate Bacteriophage with Bacterial presence**
  - Bacterial PCR
  - ***E-coli and coliform counts***
  - *Identify* local bacteriophages by genetic fingerprinting

Build a *catalogue* of local bacteriophages

Test feasibility of phage therapy in local bacterial blooms.

River Samples

	Bacterial Samples	Positive Phage Samples
High Coliform >300 cfu (43)	18	17
Low Coliform <300 cfu (43)	38	7
High *E coli* >100 cfu (23)	2	2
Low *E coli* <100 cfu (23)	54	13

Marsh Samples

	Bacterial Samples	Positive Phage Samples
High Coliform >300 cfu (43)	32	30
Low Coliform <300 cfu (43)	8	2
High *E coli* >100 *cfu (23)*	27	26
Low *E coli* <100 *cfu (23)*	13	4

Questions?