An Overview of the Bacteriological Examination of Freshwater Mussels

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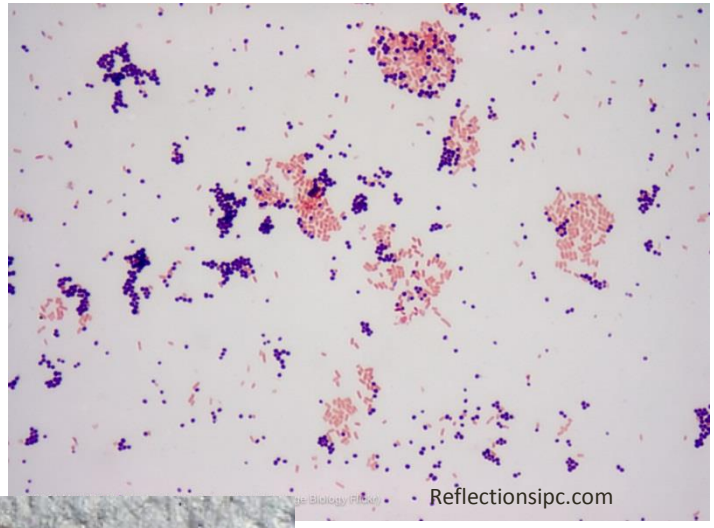
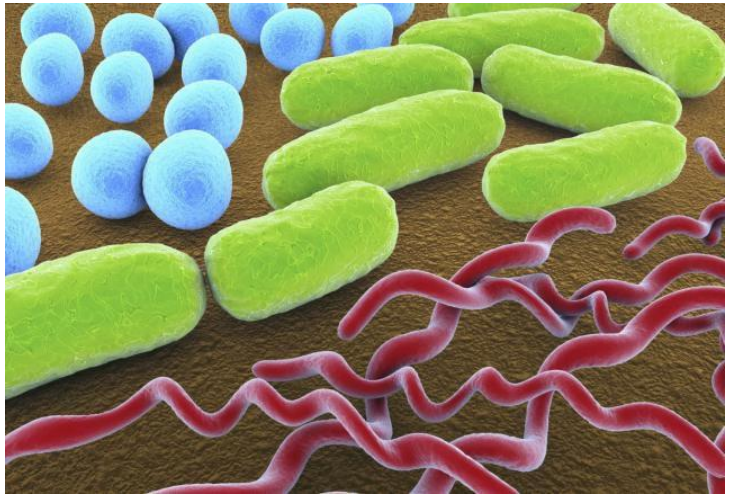
Bacteria Overview

- Unicellular, Microscopic
  - Cell wall
  - DNA
- Inhabit Every Environment
  - 1 Million Bacterial Cells in a mL of water
- Nearly impossible to truly sterilize
- Some species can go from division to reproducing in 10 minutes
- *E. coli* can travel 25 times its body length in 1 second



Bacteria Overview

- Beneficial, Commensal or Parasitic
  - changing conditions
- Complex interactions between bacterial species can cause disease
  - May not be as simple as one species



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Bacteriology at the LFHC

- Routine Health Inspections
- Identify pathogens in wild populations
  - Informed decisions regarding brood stock collection
- American Fisheries Society Bluebook
- Specific Pathogens
  - *Yersinia ruckeri*
  - *Aeromonas salmoncida*
  - *Edwardsiella ictaluri*
  - *Renibacterium salmoninarum*

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Bacteriology at the LFHC

- *Yersinia ruckeri*
  - Causes disease in salmonids
  - High mortality rates
  - Identified from freshwater mussels

Bacteriology at the LFHC

- *Aeromonas salmonicida*
  - Warm, cool and coldwater fish
  - High mortalities
  - Identified from freshwater mussels

Bacteriology at the LFHC

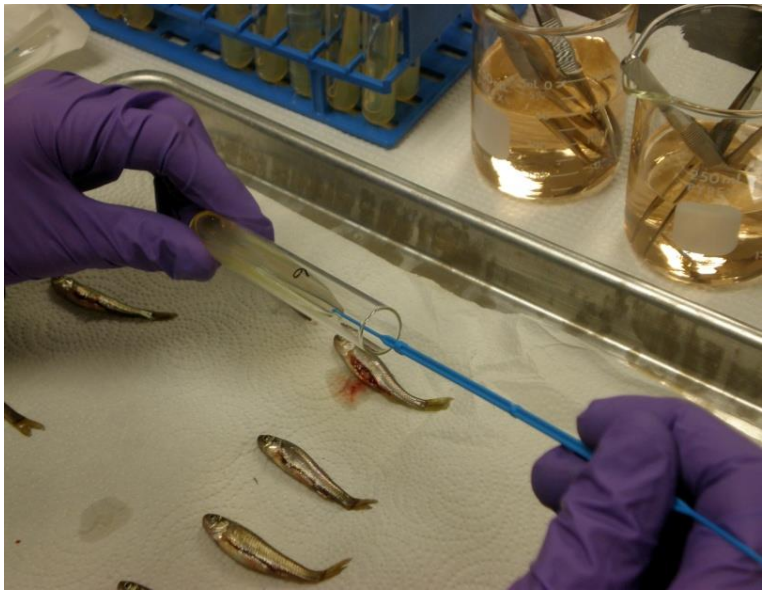
- *Edwardsiella ictaluri*
  - Primarily Channel Catfish
  - “Hole in the Head” Disease
  - High Mortality Rates

Bacteriology at the LFHC

- *Renibacterium salmoninarum*
  - Salmonids
  - Bacterial Kidney Disease
  - Large Mortality Events if Stressed

Bacteriology at the LFHC

- Culture bacteria
  - Kidney
- Tryptic Soy Agar (TSA)
- Incubate 23°C
- Isolate
  - Pure Cultures
- Identify
  - Morphology (Gram Stain)
  - Motility
  - Biochemical Tests
  - Carbohydrate Fermentation
- Identification
  - Biolog
  - PCR
  - DFAT



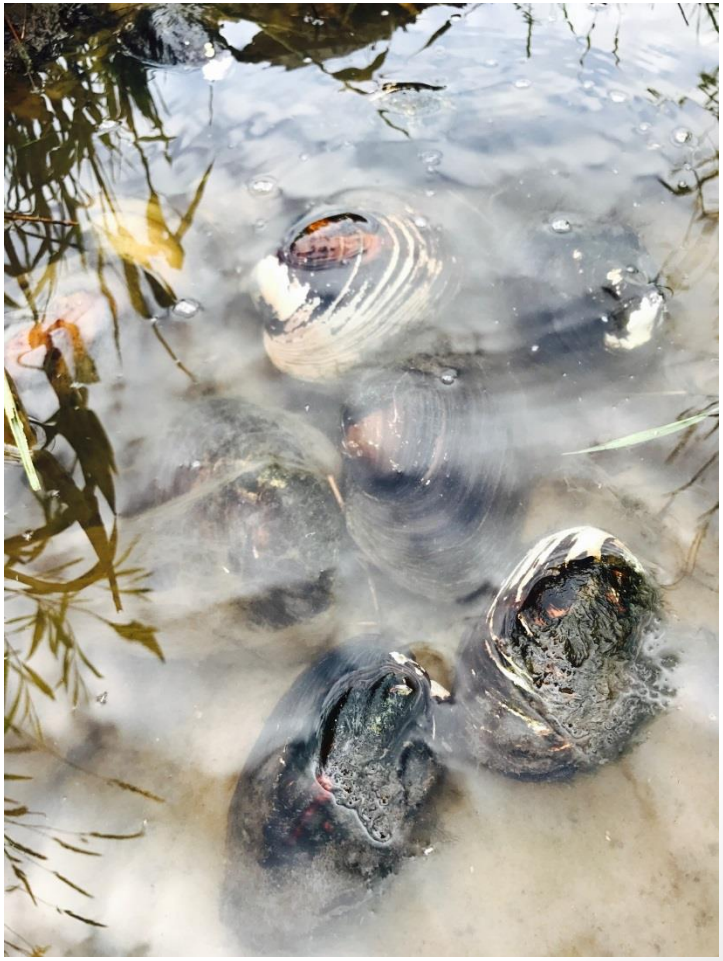
Beka McCann

Bacteriology

- Fish
  - Specific bacteria
  - Standardized Methods
- Marine Mussels
  - Economic Impact
- Freshwater Mussels
  - Largely unknown
  - Lack of consensus

Hurdles to Studying Mussel Bacteriology

- Difficult to study
  - Aquatic Environment
    - Contamination
    - Bioconcentrators
    - Food Source
  - Relationship to Mussels
    - Symbiotic
      - Mutualism
      - Commensal
      - Pathogen
    - Incidental
      - Acquired during siphoning



Sara Erickson

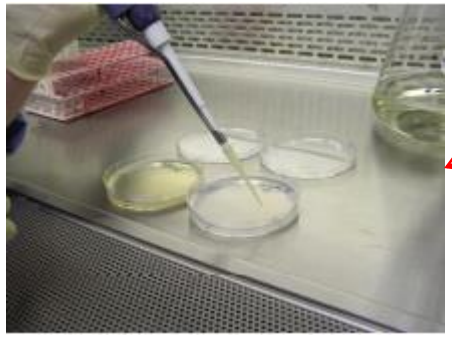
Hurdles to Studying Mussel Bacteriology

- Sample Collection
  - Organs
    - Visceral Mass
      - Removed
      - Externally Disinfected
      - Homogenized
  - Fluids
    - Hemolymph
- Growth Media
  - Culture living bacteria
  - May require more specialized media
  - Slow growing bacteria may be outcompeted
- Metagenomics
  - Highly sensitive DNA based assay
  - Can be expensive

Starliper, Powell and Garner 2009


Starliper, Powell and Garner 2009


Starliper, Powell and Garner 2009


Starliper, Powell and Garner 2009

Hurdles to Studying Mussel Bacteriology

- Obtain more background information
  - Geographical Areas
  - Unionid Species
  - Sample Type
  - Mussels in Culture
    - May be critical in our understanding of Unionid diseases
- Mussel Mortality Event
  - Examined quickly
  - Very few answers historically

Bacteriology Surveys of Unionid Mussels

- Began as a survey of Unionids from the upper Mississippi River
  - Tony Goldberg (University of Wisconsin)
  - Diane Waller (USGS)
  - Sara Erickson (USFWS)
- Mortality event in Clinch River allowed for comparison of the culturable bacterial communities
  - Jordan Richard (USFWS)

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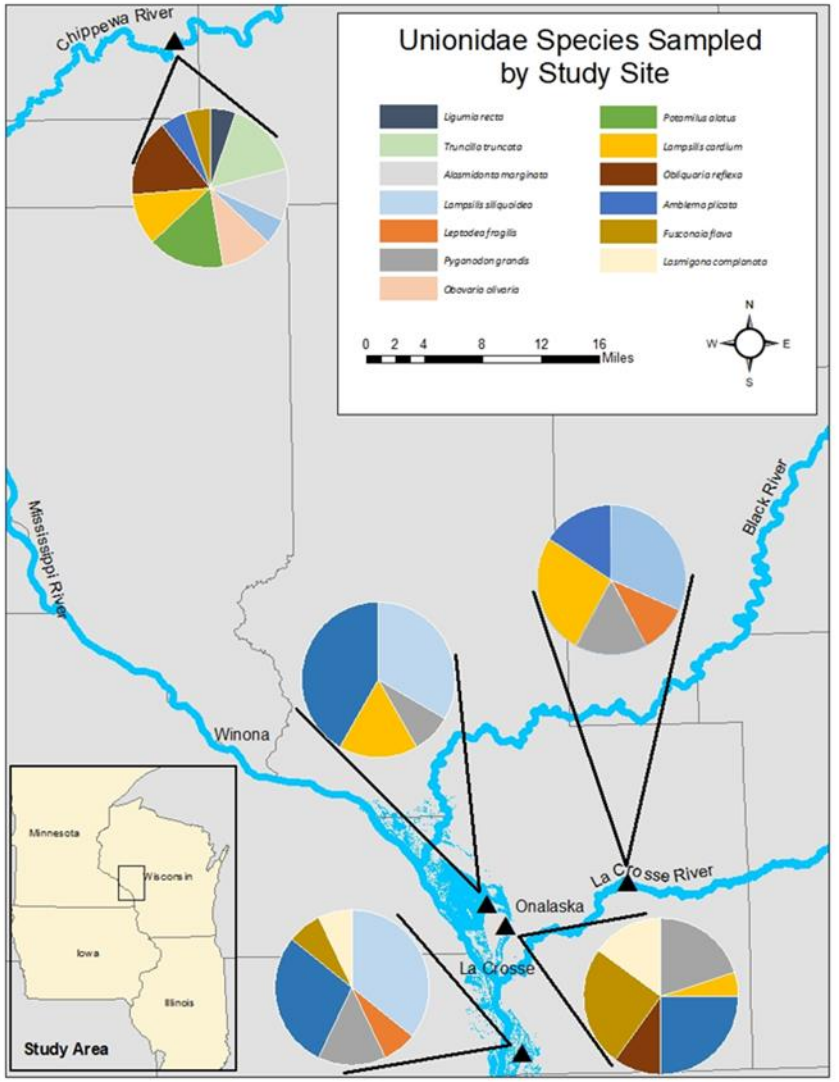
A Survey of Unionid Mussels from the upper Mississippi River

- Survey 14 Unionid Species (101 Individuals) in the upper Mississippi River basin
  - Black Sandshell
  - Deertoe
  - Elktoe
  - Fatmucket
  - Fragile Papershell
  - Giant Floater
  - Hickory Nut
  - Pink Heelsplitter
  - Plain Pocketbook
  - Three-horn Wartyback
  - Three Ridge
  - Wabash Pigtoe
  - White Heelsplitter
  - Butterfly

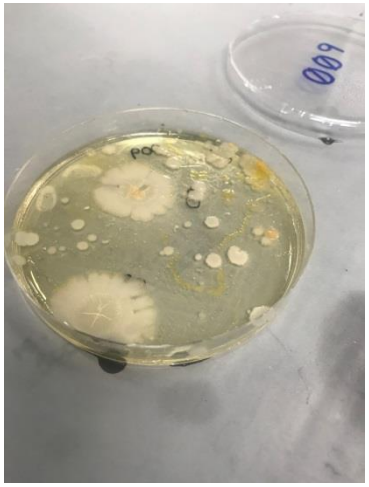


A Survey of Unionid Mussels from the upper Mississippi River

- Hemolymph
  - Nonlethal sample
  - Relative disconnect with the aquatic environment
  - Circulatory system

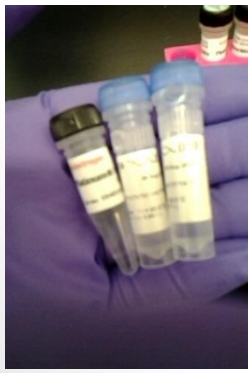
A Survey of Unionid Mussels from the upper Mississippi River

- Open the Mussels with reverse pliers
- Sterilize adductor muscle with 70% Isopropyl Alcohol
- Draw Hemolymph
  - Anterior Adductor muscle
- Place two drops onto Tryptic Soy Agar (TSA)
- Incubate at 23°C for one week



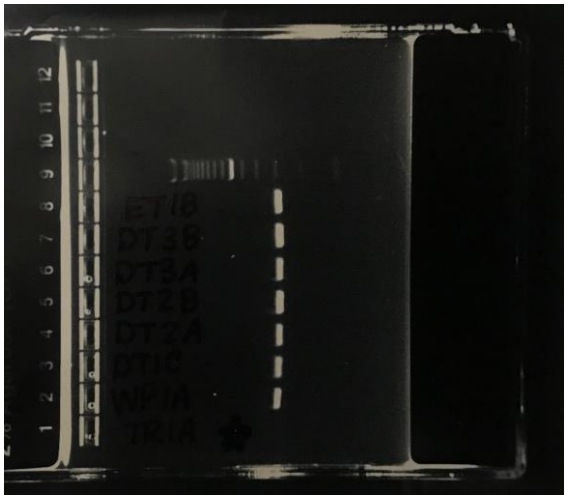
A Survey of Unionid Mussels from the upper Mississippi River

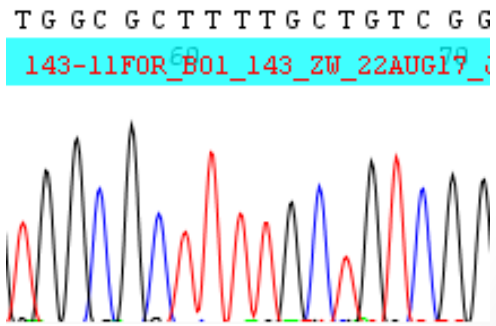
- Sample colonies
- Extract DNA
  - Prepman Ultra Reagent
- PCR primers targeting 16S rRNA gene
  - Regularly used in bacterial taxonomy



A Survey of Unionid Mussels from the upper Mississippi River

- Sanger Sequencing
  - Whitney Genetics Lab
- BLASTn search





A Survey of Unionid Mussels from the upper Mississippi River

- 180 bacterial isolates identified from 74 individuals
  - Cultured bacteria from 73% of individuals sampled
- No obvious pathogens detected
- Trends.....
  - *Bacillus* spp. isolated from 23% of individuals (23 of 101)
  - *Aeromonas* spp. isolated from 16% of individuals (16 of 101)
- *Yersina ruckeri* and *Aeromonas salmonicida*
  - Certifiable fish pathogens
  - Several isolations and locations

A Survey of Unionid Mussels from the upper Mississippi River

- *Bacillus* spp.
  - Hearty group of bacteria
    - Wide ranges of temperature, pH, UV, salinity, etc.
    - Variety of environments
    - Can be pathogens to plants and animals
  - Produce antibiotic and bacteriostatic compounds
  - Breakdown metals and chemicals
  - Many *Bacillus* spp. (including our isolates) convert urea into calcium carbonate
    - Used for “living concrete” that can heal itself
    - Probiotic to help chickens produce thicker egg shells

Cnn.com


Sbs.com

A Survey of Unionid Mussels from the upper Mississippi River

- Calcium carbonate production
  - *Brevundimonas diminuta*
  - *Pseudomonas stutzeri; P. koreensis; P. putida*
  - *Viridibacillus arenosi*
  - *Lysinibacillus boronitolerans; L. sphaericus*
  - *Caulobacteraceae bacterium*
- *Exiguobacterium* spp. (mostly *E. indicum*)
  - Chondroitin
- *Aeromonas* spp.
  - Ubiquitous
  - Likely secondary pathogens

A Survey of Unionid Mussels from the upper Mississippi River

- Isolates involved in bioremediation
  - 40 mussels (64 isolates)
    - Heavy metals (Chromium, Arsenic, Lead, Cadmium, Mercury, etc.) Polycyclic Aromatic Hydrocarbons (PAH's), PCB's, Atrazine, Aflatoxins, etc.
      - *Bacillus cereus; B. thuringiensis; B. pumilis; B. aquimaris*
      - *Kocuria rosea*
      - *Arthrobacter sulfonivorans; Arthrobacter oxydans*
      - *Microbacterium testaceum; Microbacterium oleivorans*
      - *Xanthomonadaceae bacterium*
      - *Stenotrophomonas chelatiphaga*
      - *Sporosarcina ginsengisoli*
      - *Acidovorax sp.*
      - *Viridibacillus arenosi*
      - *Agrobacterium tumefaciens; Agrobacterium fabrum*
      - *Acinetobacter dispersus ; Acinetobacter haemolyticus; Acinetobacter lwoffii*
      - *Cellulomonas hominis*
      - *Microbacterium petrolearium*
      - *Cellulosimicrobium funkei; Cellulosimicrobium cellulans*
      - *Microbacterium paraoxydans*
      - *Alpha proteobacterium*
      - *Pseudoxanthomonas mexicana*
      - *Sphingopyxis chilensis*
      - *Curtobacterium herbarum*
      - *Stenotrophomonas maltophilia*
      - *Rhodococcus erythropolis*
      - *Variovorax paradoxus*
      - *Pseudoarthrobacter sp*

A Survey of Unionid Mussels from the upper Mississippi River

- Other interesting isolates
  - *Moraxella osloensis*
    - Produces molluscicide
    - Symbiotic with Nematode
      - Toxic to grey garden slug
  - *Pseudomonas syringae*
    - Allows water to freeze at higher temps
    - Used by ski resorts
    - Role in atmosphere
  - *Chromobacterium violaceum*
    - *Produces Violacein*
  - *Pseudomonas entomophila*
    - *Toxic to insects*
    - *Used in agriculture as natural control method*
  - *Stenotrophomonas rhizophila*
    - *Produces osmoprotective substances to help plants*

Mortality event in the Clinch River

- Clinch River
  - Eastern TN, VA
  - Several mortality events
  - Pheasantshell
- Sampled using same methods as UMR

http://orig.jacksonsun.com

Clinch River Results

- Shared between UMR and Clinch River
  - *Bacillus* spp.
    - Including species with high capacity for calcium carbonate production
  - *Aeromonas* spp.
  - *Pseudomonas* spp.
  - *Moraxella osloensis*

Ncwildlife.org

- Some Differences
  - *Yokenella regensbergei* (47%; 9 of 19 mussels)
    - Significance Unclear
    - Isolated from oil contaminated soils
      - Potential to degrade hydrocarbons
    - Reported as the most prevalent isolate in an Ebonyshell mortality event in Tennessee River, Alabama. (Starliper et al 2009)
    - Future work will determine importance of this isolate

Comparison of Clinch River to UMR

- Mississippi River
  - Bacteria associated with degrading toxic substances were isolated from 40% of the mussels
- Clinch River
  - Only a couple isolates

National Park Service

Mussel Bacteriology Conclusions

- Collection baseline data is critical
  - Combination of metagenomic and culture methods
  - Understand the Bacterial Communities
- Work towards identifying pathogens and standard methods
  - Protect the animals brought into the hatchery
- *Yokenella regensbergei* deserves further study
- Similar to marine mussels- captive populations might play an important role in identifying causative agents of disease

Thanks!

- La Crosse Fish and Wildlife Conservation Office (USFWS)
- Upper Midwest Environmental Sciences Center (USFWS)
- Genoa National Fish Hatchery (USGS)
- Whitney Genetics Laboratory (USFWS)

Any Questions?

Mara Koenig