Project SQUIRT

Students QUalitatively Investigating Rad Tunicates

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Goals

To

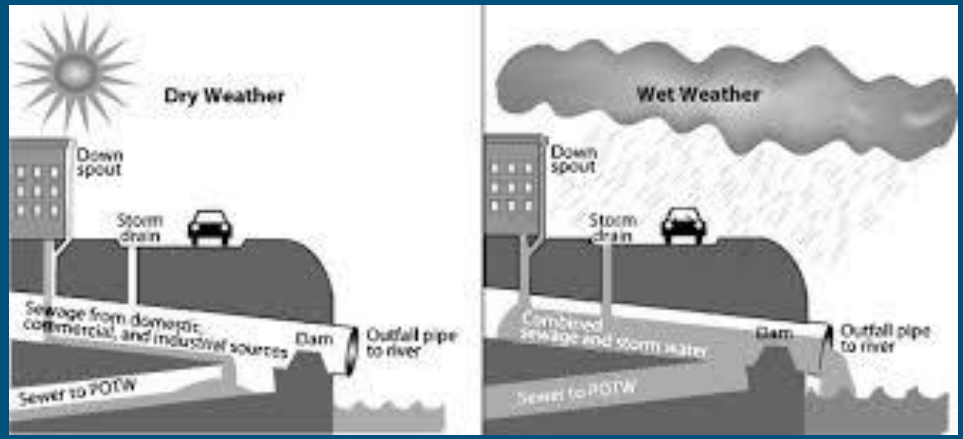
-expose students to scientific thinking/process
-provide mentorship (BioBus scientists –> students)
-teach core concepts in biology
-environmental science
    -biodiversity
    -evolution
    -selection
    -resilience
-connect science to the real-world/real-life/social justice

Why NYC's Waterways?

CSO- Combined sewer overflow

-CSOs are a system of pipes underneath all of NYC that collect rainfall, sewage waste, and industrial water waste

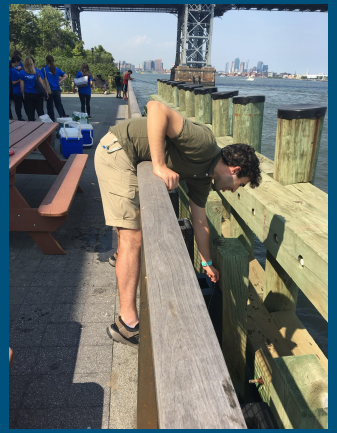
-Waste collected in pipes enter NYC's waterways once there is 0.33 in. of rainfall, polluting our rivers



Why sea squirts?

- Accessible: plentiful population all over NYC coasts
  - We used one species of solitary ascidian, *Molgula manhattensis*
- Seawater bacteria are notoriously difficult to culture, squirt gut microbiome may teach us about Hudson River water bacteria content
- Squirts selectively harbor certain bacteria in their gut microbiome
  - Evidence has suggested that ascidians may harbor bacteria that release toxic metabolites in high stress/high disturbance environments in order to fight off competition
- Experimenting with squirts offers students a plethora of skills
  - Invertebrate biology: sampling, dissection, etc.
  - Microbiology: sterile technique, culturing, analyzing results, etc.
- Room to grow: connect to locality, CSO events, etc

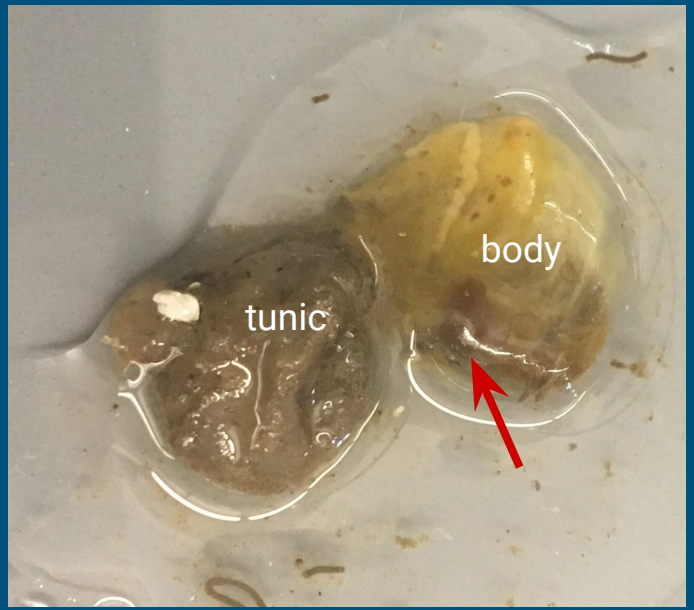
Experiment: Sampling

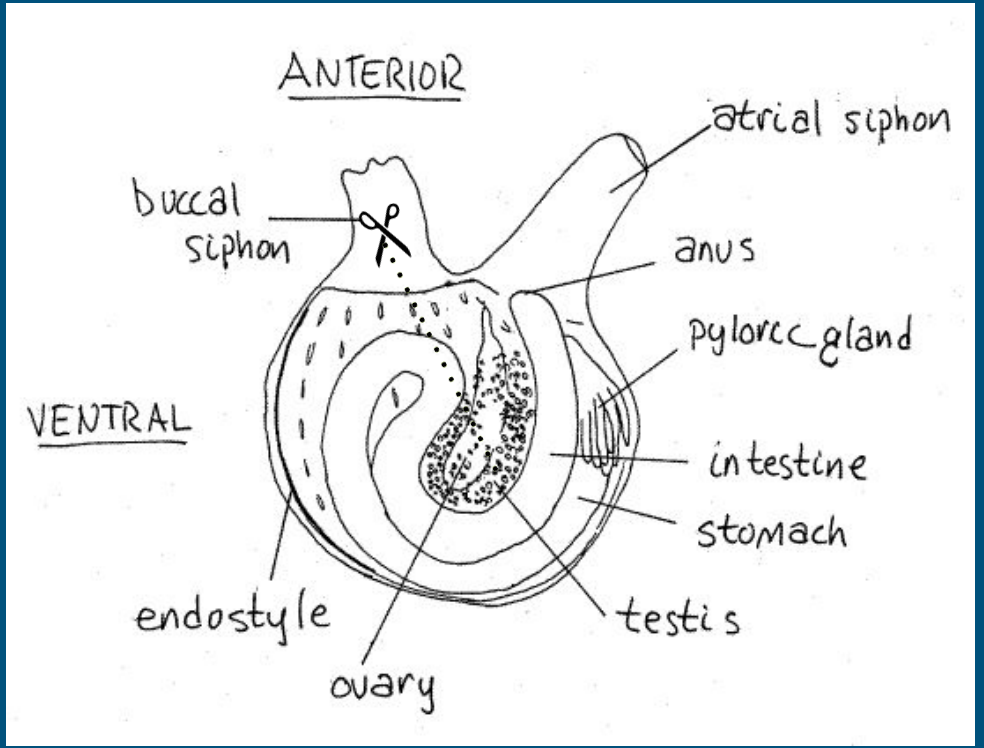


-Collected animals (*Molgula manhattensis*) and water samples at LES Ecology Center, Pier 25, and Battery Park
-Kept at room temperature in of seawater during transportation

Dissection

Dissect body away from the “tunic,” then open up intestine (red arrow) and swab

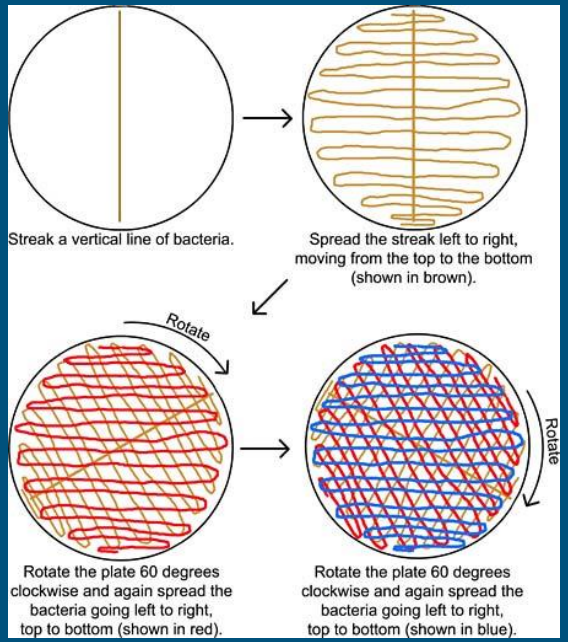




Culturing

-Plated onto LB, LB-Amp, OXOID using block streaking method

Media	Qualities
Nutrient agar	available, general purpose medium supporting growth of a wide range of non-fastidious organisms
LB	available, nutritionally rich, commonly used to grown & maintain E.coli
LB-Ampicillin	available, used to select for ampicillin-resistant bacteria
OXOID	available, used to identify total coliform from fecal coliform

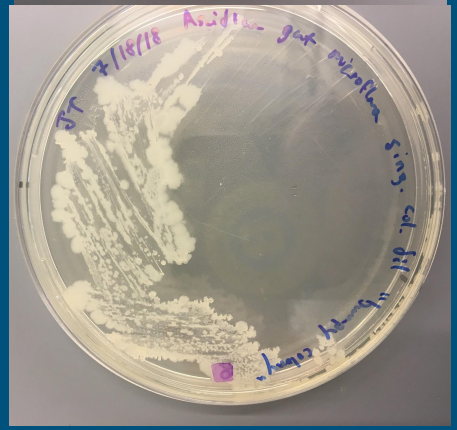


Antibiotic Resistance Assay

-Chose 8 “bacterias of interest” and made single colony streaked plates
-Incubated in rotator for 24 hours at 37°C

-Created liquid cultures from single colony streaked plates in LB-broth (5 mL)
-Applied Kirby-Bauer Antibiotic Disc Assay for preliminary selection

-Penicillin
-Erythromycin
-Neomycin
-Kanamycin
-Streptomycin
-Chloramphenicol
-Novobiocin
-Tetracycline



Kirby-Bauer Antibiotic Disk Assay

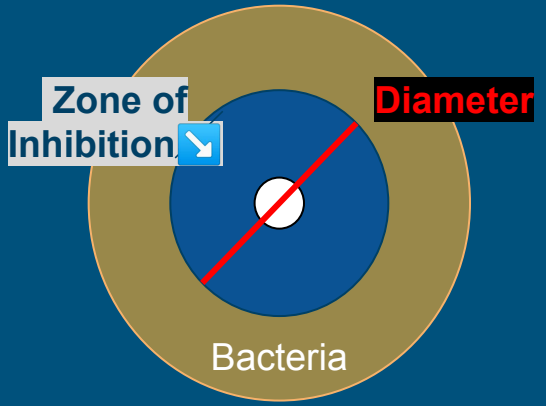
How it works

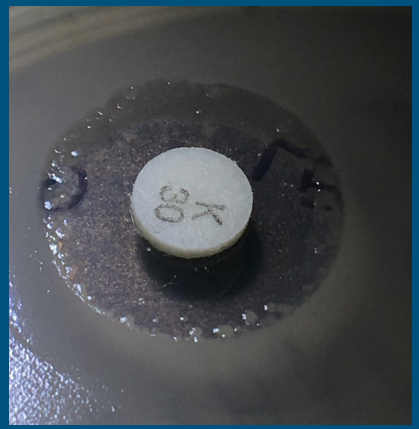
-Kirby Bauer Antibiotic Disc Assay has been historically used to test bacterias' sensitivity to a range of antibiotics
-Wafer discs containing antibiotics are placed on agar plates where bacteria have been plated, and the plate is left to incubate for 24 hours
-If a bacteria is antibiotic susceptible, there will be an area around the wafer where the bacteria have not grown enough to be visible (zone of inhibition)
-Size of zone can be affected by many factors, including the bacteria––because we are using environmental samples, we do not know what bacterias we have isolated and therefore cannot use bacteria-specific ZOIs
-For our ZOI interpretations, we used more general bacteria regulations created by uPenn

Interpreting an Antibiotic Disk Assay

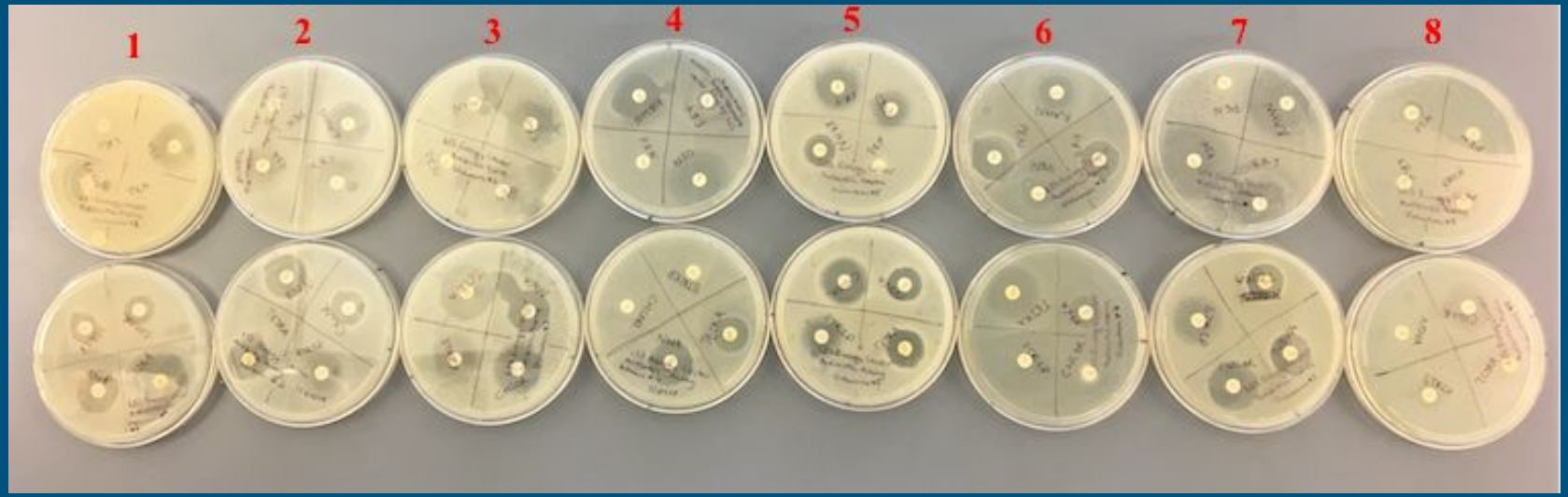
Interpretation of zones of inhibition (in mm) for Kirby-Bauer antibiotic susceptibility test.

		Diameter of zone inhibition (mm)		
Antibiotic	**Disc Conc. (µg)**	**Resistant**	**Intermediate**	**Susceptible**
Penicillin	10	≤11 (uPenn)	12≤21 (uPenn)	≥22 (uPenn)
Erythromycin	15	≤13 (uPenn)	14≤17 (uPenn)	≥18 (uPenn)
Neomycin	30	≤12 (uPenn)	13≤16 (uPenn)	≥17 (uPenn)
Kanamycin	30	≤13 (uPenn)	14≤17 (uPenn)	≥18 (uPenn)
Streptomycin	10	≤11 (uPenn)	12≤14 (uPenn)	≥15 (uPenn)
Chloramphenicol	30	≤12 (uPenn)	13≤17 (uPenn)	≥18 (uPenn)
Novobiocin	30	≤16 (SHC, Hardy)		>16 (SHC, Hardy)
Tetracycline	30	≤14 (uPenn)	15≤18 (uPenn)	≥19 (uPenn)





Antibiotic Disk Assay: Results



Antibiotic Disk Assay: LES Ecol. Ctr. Results

Zone of inhibition measured by diameter (mm)

	1	2	3	4	5	6	7	8
Pen	6 mm Res	6 mm Res	6 mm Res	6 mm Res	6 mm Res	22 mm Sus	6 mm Res	6 mm Res
Ery	6 mm Res	20 mm Sus	25 mm Sus	11 mm Res	20 mm Sus	19 mm Sus	25 mm Sus	8 mm Res
Neo	17 mm Sus	14 mm Inter	13 mm Inter	17 mm Sus	12 mm Res	15 mm Inter	14 mm Inter	12 mm Res
Kana	22 mm Sus	14 mm Inter	19 mm Sus	24 mm Sus	17 mm Inter	17 mm Inter	22 mm Sus	15 mm Inter
Strep	14 mm Inter	13 mm Inter	20 mm Sus	6 mm Res	13 mm Inter	27 mm Sus	20 mm Sus	12 mm Inter
Chlor	14 mm Inter	17 mm Inter	23 mm Sus	6 mm Res	20 mm Sus	6 mm Res	23 mm Sus	22 mm Sus
Novo	22 mm Sus	22 mm Sus	21 mm Sus	22 mm Sus	17 mm Sus	16 mm Res	19 mm Sus	7 mm Res
Tetra	28 mm Sus	21 mm Sus	27 mm Sus	18 mm Inter	18 mm Inter	23 mm Sus	17 mm Inter	21 mm Sus

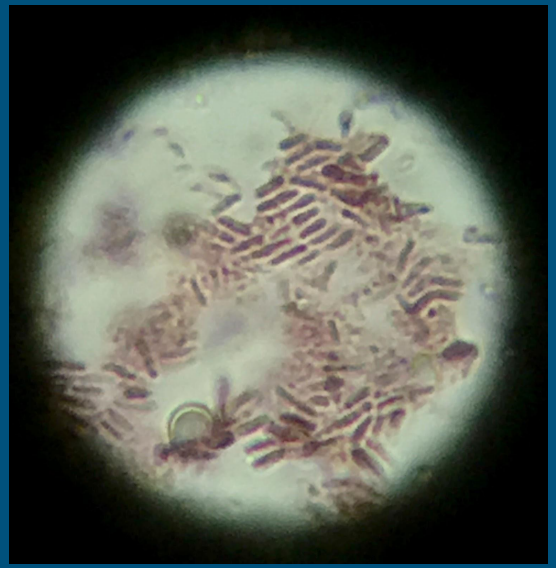
Gram Staining

-A method of staining used to distinguish and classify bacterial species into two large groups

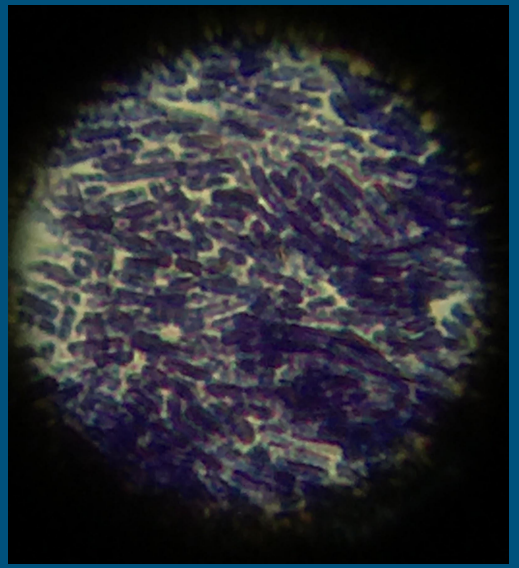
-Gram staining differentiates bacteria by the chemical & physical properties of their cell walls by detecting peptidoglycan, which is present in the cell wall of Gram-positive bacteria

-Gram-negative cells also contain peptidoglycan, but a very small layer of it that is dissolved when the alcohol is added.

Gram **Negative**
(not purple)



Gram **Positive**
(purple)



Summary

- Ascidians (sea squirts!) can be found at subtidal sites throughout Manhattan
  - easy to collect/dissect
- NYC's waterways have distinct bacterial ecologies
  - due to high population, repetitive pollution events, etc.
- Marine gut microbiomes are under-studied and much is unknown about them
  - Sea squirts selectively harbor gut bacteria various reasons
- Seawater and ascidian gut microbiome both contain (varied) antibiotic-resistant bacteria
- Antibiotic disk assays & Gram staining kits yield interesting results and would be possible to repeat and expand with students

Future directions

Geographic, temporal diversity

- More sampling sites in other parts of the city
- Weather, CSO events

Differentiating strains:

- More stains
  - acid-fast stain
  - capsule stain
- Variable culture media
  - MacConkey (bile salt + crystal violet, favors gram neg. bacteria)
- Genetic barcoding? Might work with the right students
  - 16S (of bacteria)
  - 18S+CO1 (of squirts, for conclusive species ID)