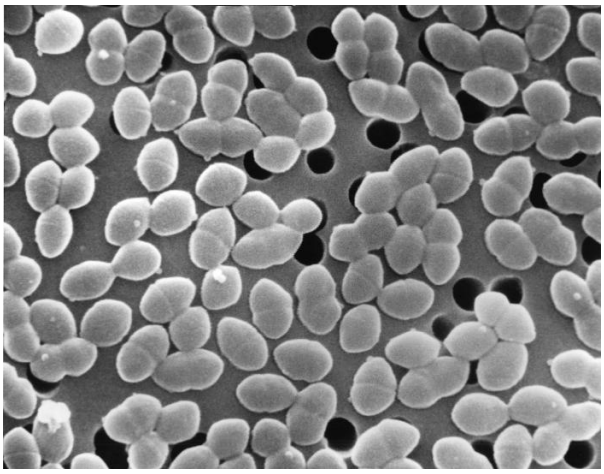
Can *Crassostrea virginica* Filter *Enterococcus faecalis*?



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Abstract

Enterococcus faecalis is a bacterium which is capable of causing a wide variety of different infections and diseases. Oysters, a filter feeder, are capable of filtering bacterium and other pollutants. Eight experiment tanks were set up, four with oysters and four without to serve as the control to see the filtration capacity of the *crassostrea virginica*. The results showed that the oyster tanks had a significant decrease in *enterococcus faecalis* colonies. We concluded that *crassotrea virginica* does have an impact on *enterococcus faecalis* concentration; we presume is due to the enterococci size cells matching the filtration range of the *crassotrea virginica*.

Using the statistical hypothesis test, the derived t-test equals 2.16, exceeds the critical value of t=1.943 with degrees of freedom at 6. Therefore, the null hypothesis is rejected and it is concluded that the mean MPN for the experimental group was significantly lower than the mean MPN of degrees of freedom the control group. In terms of the research problem, it appears that oysters significantly lower the amounts of MPN in experimental tanks.

Introduction

Bodies of water in New York City as well as numerous water bodies all over the world, are unsafe to swim in due to *entercoccus faecalis* which originates from human waste (Gilmore, 1994). *Enterococcus faecalis* can enter the environment from sewage pollution points, such as CSO's (combined sewage overflow) or drain runoff systems (Shiaris, 1987). Being that *enterococcus faecalis* is capable of causing gastroenteritis; this could make recreational bodies of water unsafe to swim in. It is also worth noting that the approximate amount of *enterococcus faecalis* levels present in many lakes and water bodies are unknown due to the fact that neither the government nor the private sector is capable of monitoring every beach and lake (Fliesher, 1993).

This experiment attempts to find a solution to the problem of waterways contaminated with *enterococcus feacalis* and observing whether *crassotrea virginica* could affect *enterococcus*

faecalis levels and filtering bacteria. Our hypothesis is that oysters can signifigantly lower *enterococcus faecalis* levels.

Being that *enterococcus faecalis* is a bacterium that originated from human waste, other pathogens that originate from human waste may be present because of very similar characteristics *enterococus faecalis* shares with other pathogens (Doyle, 2006). Therefore, gastroenteritis, ear infections, dysentery, typhoid fever and even hepatitis A can be present with high levels of *enterococus faecalis* (Frenso, 2009).

Background

There has been a study done on all gastroenteritis-associated hospital discharges during 1996–2007. The study marked how many people were admitted to a hospital for the treatment of gastroenteritis. There were nearly 110,000 hospitalizations per year in epidemic seasons for gastroenteritis (Lopman, 2007). The approximate number of the amount of people who visit a beach regularly in the US are 89 million individuals (Dwight, 2007). The Centers for Disease Control and Prevention (CDC) have reported 21 recreational water outbreaks back in 2000 (Lee, 2002).

Enterococcus faecalis clinical infections include urinary tract infection, bacteremia, bacterial endocarditis, diverticulitis and meningitis (Aher, 2012). Urinary tract infection can cause burning from urination, having to urinate frequently, urge to urinate frequently and abdominal pain (Nicolle, 2008). Bacteremia is characterized by rapid breathing, low blood pressure and fever (Forner, 2006). Bacterial endocarditis can cause fever, malaise, endurance fatigue, heart murmur and weight loss (Amal, 2007). Diverticulitis is capable of causing cramps, and occasionally causing bloody stools (Aldoori, 2002). Meningitis is capable of causing severe headaches, stiffness to the neck and then inflammation (Van de Beek, 2004).

Enterococcus faecalis has the capability of surviving very harsh environments in bodies of water. They are completely capable of an adjusted environment of extreme alkaline of a pH from 10.5 to 11.9 (Flahaut, 1997). They are also able to resist bile salts, detergents, heavy

metals, ethanol, azide and drought. In order to grow, they must be in the temperature range of 10 to 45°C and could survive a temperature of 60°C for 30 min. (Stuart, 2006) Being able to prosper under these hostile conditions, means that *enterococcus faecalis* must have a metabolism that is flexible, flexible enough to burrow down into our digestive systems. *Enterococcus faecalis* are not only capable of only fermentation to produce lactic acid but they can also undergo catabolism (*the breakdown of complex molecules in living organisms*) from a variety of energy sources from carbohydrates, glycerol, lactate, malate, citrate, diamino acids and manyα-keto acids. Given these attributes, *enterococcus faecalis* can survive in digestion tracts, allowing them to infect humans with gastroenteritis and other infections as well. (Wormser, 2006).

The colonies can range 1000-2000 μ in length and appear wet (Napoli, 2012). However, single *enterococcus faecalis* cells range from a 1-0.5 μ size (Murray, 1990). Making them prone to filtration by the *crassotrea virginica*. Filtration by the oyster *crassotrea virginica* was studied and concluded that oysters filters a 1.0 to 12.0 μ particle size range (Haven, 1970). Oysters were found to filter natural occurring particles in the 1.0 to 3.0 μ range (Haven, 1970).

Enterococcus faecalis originates mainly from CSO's, from discharged sewage. Therefore, other bacteriums that come from sewage will most likely follow enterococcus faecalis bacterium's such as Enterobacter, Klebsiella, Citrobacter, Escherichia, Escherichia coli, and these area just a few bacteriums prevalent in fecal matter (Doyle, 2006). Normally, sewage treatment plants would have high levels of enterococci due to a huge amount of fecal matter being discharged. 64-1023 enterococci counts were found at three sewage treatment plants, which can be terrible not only for recreational swimmers, but also the environment. 29 % of the total sampling areas out of 74 locations were labeled either at an unacceptable enterococci level, or a probable danger. (http://www.riverkeeper.org/wp-content/uploads/2010/07/June-2012-WQ-Report.pdf)

Project design chart

Scientific Problem
Can oysters filter *entercoccus faecalis*?
Hypothesis
Oysters can reduce the amount of *enterococcus faecalis* present in contaminated water
Alternative Hypothesis
Oysters can add to the amount of *enterococcus faecalis* present in contaminated water
Null Hypothesis
There is not variation between the control and the experimental groups.
Objective's
Determine if oysters can reduce the amount of *enterococcus faecalis* colonies
Independent Variables
Oysters
Dependent Variables
Levels of enterococcus
Control
Tanks W/O oysters
Constants
Same water
Same tank
Same temperature
Same *enterococcus faecalis* levels
Assumption
Enterococcus can clump together, which can cause them to be filtered by oysters.

Materials

Item	Quantity	Function
Bucket	2	To contain water and decontaminate hazardous waste
15 feet rope	1	To hold the bucket when getting water samples from the Hudson river
Carabineer	1	To hold the bucket and the rope
Portable fridge	1	To contain the water
Ice packs	4	To freeze the water so the water can still hold the bacterium
Centrifuge vile's	>20	To hold specific quantities of water
Gloves (rubber)	1 pair	To protect hands when decontaminating hazardous waste
Data sheet	2	To contain data
Sharpie	1	To label containers
Refregtometer	1	To measure the salinity when collecting water samples from a water body.
Filtered water (comes from the sink)	N/A	To clean materials or aid in decontamination of hazardous waste
Small pipett	2	To clean the refragtometer
Acu wipes	2 boxes	To dry the refragtometer after washing
Finnpipette F1	1	Used to draw an accurate sample of water when evaluating enterococcusa levels.
100ml testing tube	>50	Used to contain an accurate sample for the enterolert trays
Sample water	N/A	Used to run the experiment
Filtered water	N/A	Used to fill the 100 ml bottle, after the insertion of the 10 ml of sample water
Clothing iron	1	Used to seal the enterolert tray to avoid leaking
Cubed board	1	Used to contain the enterolert tray when ironing
Angled stand	1	Used to angle the enterolert tray when ironing
Growing medium	>50	Used to grow/culture bacterium in order to get a reading
incubator	1	Used to warm bacteria to get results
Enterolert tray	>50	Used to contain the sample water
Kitchen white vinegar	2	Used for the sterilization of the fish tanks when first bought or used before in previous experiments
1 foot Flexible air pumps for fish tanks	8	To carry oxygen into the tanks to keep the oysters alive
Air stones	8	Used to support the oxygen tubes by very softly

		pumping oxygen into the tank
Finnpipette tips	>50	Used to put into the finnpipette
White lab coat	1	Used to help protect the skin from the infection of hazardous material
thermometer	8	To measure the temperature
Clorox solution 96 FL Oz	>10	To disinfect the hazardous material
Sharp object	1	To pluck out the enterolert tray that was used during disinfection
Fish tanks 0.75 gal	8	To contain the experiment
oysters	6	To determine whether these creatures can filter the enterococci
Flasks (1000 ml)	2	To contain oyster reserves

Procedure

Steps for getting river water

1. Get bucket and rope.
2. Tie rope onto the bucket.
3. Throw bucket into the water.
4. Get the water carefully.
5. Bring water into the room.

Steps for putting oysters into tanks

1. Get 8 tanks and wash them with a 1 part white vinegar, 10 part water solution.
2. Rinse them with filtered water and dry them.
3. Fill the tanks with suitable aquatic water.
4. Put fish tank oxygen tubes in the tanks to pump oxygen into the tanks.
5. Put 1 oyster in every experimental tank.
6. Put 1 oyster into the 2 reserve flasks in the event of the death of an oyster.

Steps for measuring enterococcus

1. Get the fixed volume pipette and get 10 ml of sample water, using protective gear
2. Put sample water into a 100 ml container
3. Put 90 ml of sterile water into the same container
4. Add growing medium
5. Shake container vigorously up and down until the water looks like urine
6. Put sample water into quanti tray

7. Put the cubed board on-top the angled board facing upward, with the largest well facing upward.
8. Place the quanti tray into the cubed board
9. Use the clothing iron to seal and level the water out
10. Ensure that the quanti tray is sealed
11. Put quanti tray into incubator at 40˚C for 24 hours
12. Remove quanti tray from incubator and put it onto a designated table
13. Count and document how many glowing wells there are with a UV flashlight
14. Use data sheet to determine what the exact amount of enterococci colonies is in the quanti tray
15. Put the quanti tray into decontamination bucket and decontaminate
16. Disinfect all surfaces with a sponge soaked with a 1 part Clorox, 6 parts water.

steps for decontaminating quanti trays

1. Get a decontamination bucket and put 1-6 Clorox solution in the bucket.
2. Put quanti trays in the bucket.
3. Using a sharp utensil, pluck the wells of the quanti tray.
4. Leave the quanti tray in the bucket for 10-15 minutes.
5. Place the decontaminated tray into 2 bio-hazardous waste bags carefully.
6. Throw the waste into the garbage.

Steps for marking down a data graph

1. Go to Microsoft XL.
2. Create data graph that has the x axis representing the control vs experimental tanks both before and after and after the 24 hour period. The y axis should represent the enterococcus level in the most probable numbers (MPN).
3. Write down data according to their axis's.
4. Box all the data.
5. Go to the insert/column/2d column, and select the first graph.

Steps for maintaining Biohazard Safety Level 2

1. Must be personally trained to maintain bio hazardous agents with extreme care.
2. Take extreme caution with sharp objects that are most likely contaminated.
3. Must have a table exclusively meant for putting down bio hazardous agents.
4. Must have a decontamination bucket reserved.

Steps for completing t-test

$$= \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\left(\frac{\sum X_1^2 - \frac{\left(\sum X_1\right)^2}{N_1} + \sum X_2^2 - \frac{\left(\sum X_2\right)^2}{N_2}}{N_1 + N_2 - 2} \right)\left(\frac{1}{N_1} + \frac{1}{N_2} \right)}}$$

$\bar{X}_2$	The mean of the scores of the second group
$\bar{X}_1$	The mean of the scores of the first group
$\sum X_1^2$	The sum of the squares of the first group
$\sum X_2^2$	The sum of the squares of the second group
$\left(\sum X_1\right)^2$	The square of the sum of the scores of the first group
$\left(\sum X_2\right)^2$	The square of the sum of the scores of the second group
N_2	Total number of scores in 2nd group
N_1	Total number of scores in 1st group

Fill in the symbols according to the descriptions provided, and apply the gathered numbers according to the order of operations.

degrees of freedom	significance level					
	20%	10%	5%	2%	1%	0·1%
1	3·078	6·314	12·706	31·821	63·657	636·619
2	1·886	2·920	4·303	6·965	9·925	31·598
3	1·638	2·353	3·182	4·541	5·841	12·941
4	1·533	2·132	2·776	3·747	4·604	8·610
5	1·476	2·015	2·571	3·365	4·032	6·859
6	1·440	1·943	2·447	3·143	3·707	5·959
7	1·415	1·895	2·365	2·998	3·499	5·405
8	1·397	1·860	2·306	2·896	3·355	5·041
9	1·383	1·833	2·262	2·821	3·250	4·781
10	1·372	1·812	2·228	2·764	3·169	4·587
11	1·363	1·796	2·201	2·718	3·106	4·437
12	1·356	1·782	2·179	2·681	3·055	4·318
13	1·350	1·771	2·160	2·650	3·012	4·221
14	1·345	1·761	2·145	2·624	2·977	4·140
15	1·341	1·753	2·131	2·602	2·947	4·073
16	1·337	1·746	2·120	2·583	2·921	4·015
17	1·333	1·740	2·110	2·567	2·898	3·965
18	1·330	1·734	2·101	2·552	2·878	3·922
19	1·328	1·729	2·093	2·539	2·861	3·883
20	1·325	1·725	2·086	2·528	2·845	3·850
21	1·323	1·721	2·080	2·518	2·831	3·819
22	1·321	1·717	2·074	2·508	2·819	3·792
23	1·319	1·714	2·069	2·500	2·807	3·767
24	1·318	1·711	2·064	2·492	2·797	3·745
25	1·316	1·708	2·060	2·485	2·787	3·725
26	1·315	1·706	2·056	2·479	2·779	3·707
27	1·314	1·703	2·052	2·473	2·771	3·690
28	1·313	1·701	2·048	2·467	2·763	3·674
29	1·311	1·699	2·043	2·462	2·756	3·659
30	1·310	1·697	2·042	2·457	2·750	3·646
40	1·303	1·684	2·021	2·423	2·704	3·551
60	1·296	1·671	2·000	2·390	2·660	3·460
120	1·289	1·658	1·980	2·158	2·617	3·373
∞	1·282	1·645	1·960	2·326	2·576	3·291

Steps for getting critical value using statistics

1. plug in numbers according to data graph seen in page 11 (you should get 2.17)
2. You should get 6 degrees of freedom (Total experimental units minus 2 in this case equals 6)
3. Figure out the significance level for the experiment.
4. Calculate the percentage rate of success for the experiment, which should be 90%. Therefore, the null hypothesis is rejected.

Results

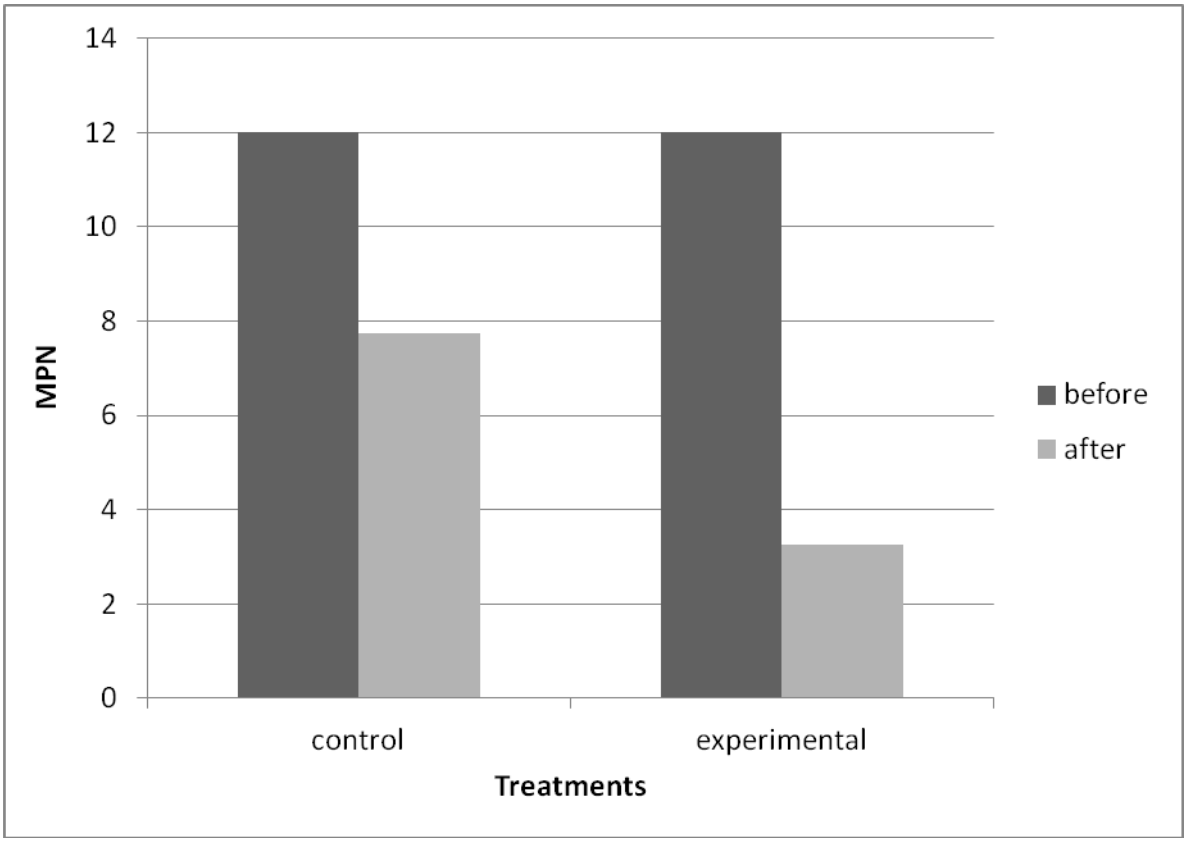

Figure 1

One of the trials of oyster filtration of enterococcus, January 2013. This data was conducted over the summer. The enterococcus seen in the picture has decreased, 2 times as much in the experimental than the control. This is an average of both groups.

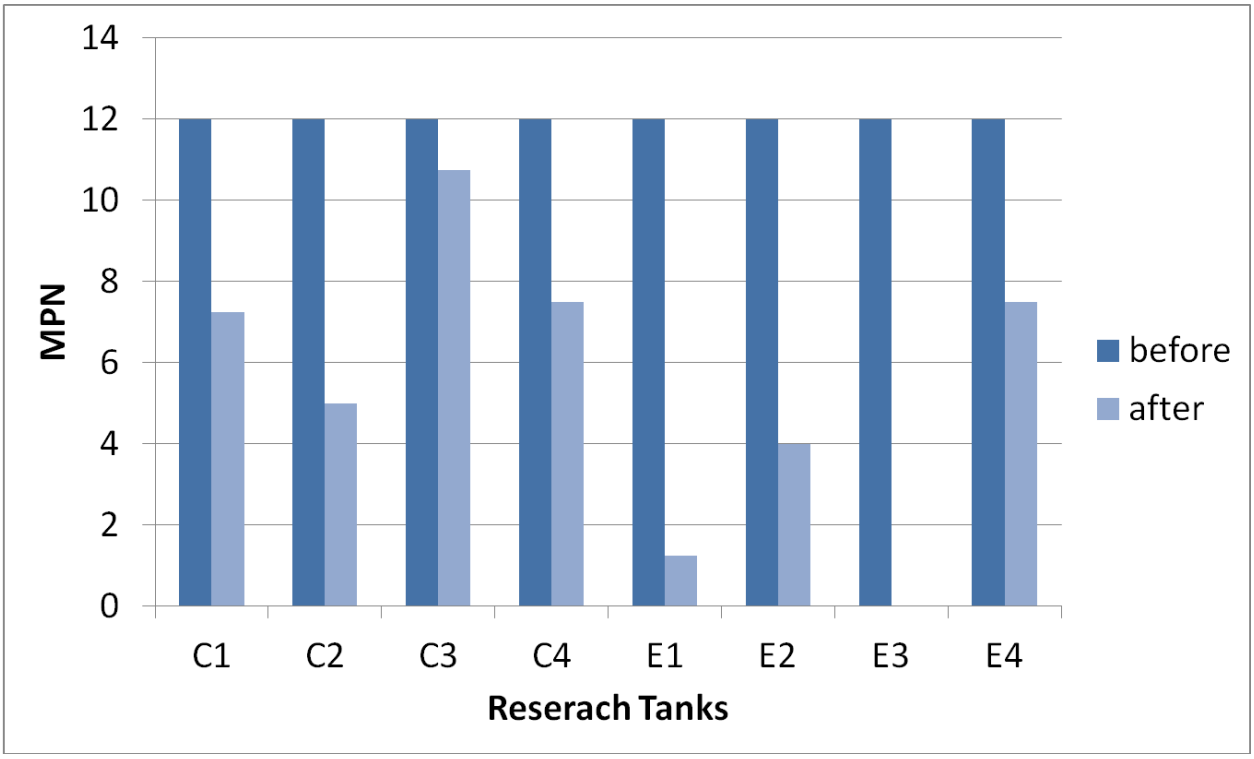

Figure 2

The exact same figure as figure 1, except every enterococcus level is seen in every tank. The C's represent the control group, the E's represent the experimental.

The figure had 8 tanks total, 4 in the control and the other 4 in the experimental. The tanks had Hudson River estuary water inside them and the experimental had 1 oyster in every tank. The enterococcus is measured as soon as the estuary water is inserted into the tanks, and measured again after 24 hours to get two pieces of data that could be compared to one another. A technique that was employed in all the other figures. As seen in the 3 figures, enterococcus dies off regardless of if there's any oysters present. If deprived of any nutrition, they will simply die off.

Using the statistical hypothesis test, the derived t-test equals 2.16, exceeds the critical value of t=1.943 with degrees of freedom at 6. Therefore, the null hypothesis is rejected and it is concluded that the mean MPN for the experimental group was significantly lower than the mean MPN of degrees of freedom the control group. In terms of the research problem, it appears that oysters significantly lower the amounts of MPN in experimental tanks.

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Analysis of results

Using the T-test chart, 90% of the variation can be explained by the oyster treatment, and 10% can be due to random effects. There is a 90% sudden drop of *enterococcus faecalis* could be because oysters can filter a 1.0 to 12 μ particle size. *Enterococcus feacalis* cells are 0.5 to 1.0 μ size, meaning that oysters do have the capability of filtering that particle size. Let alone that that's just a single individual *enterococcus feacalis* cell, *enterococcus feacalis* does also clump together. There is a 10% chance that the sudden drop was due to other reasons unknown.

Conclusion

- Oysters are capable of filtering *enterococcus faecalis*. In the graph seen in the observations, crassostrea virginica are capable of removing over half of all enterococcus faecalis in 24 hours.

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