Point Loma Nazarene University

Addressing San Diego's Bacterial Public Health Crisis in Two Ways: Assessment of Antibiotic Resistance in Fecal Indicator Bacteria and Development and Field-testing of Inquiry-based unit for Classroom Education

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By

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The thesis of Brody John Pettek is approved, and it is acceptable in quality and form for publication:

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2025

I dedicate this thesis to my wife, Taylor Pettek,

to all the staff and professors, and to all those

who helped me achieve this monumental task.

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Abstract of the Thesis

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It has been well documented that the San Diego area has seen an increase in bacteria in its waterways. Due to its close negative correlation with human health, it is important to attempt to address this public health crisis. This study aims to better understand this problem by assessing the amount of antibiotic resistance present in fecal indicator bacteria *Enterococcus*. Water samples were taken from Mission Bay California, a populated manmade bay, twice a week for 6 weeks. From this ocean sample two samples were created, one control solution, and one solution was treated with a solution containing 1mL of 1.6% Ampicillin set to the minimum inhibitory concentration (MIC) to ensure that remaining Enterococcus were considered resistant. After testing and analysis was complete it was determined that 3% of *Enterococcus* samples collected survived the MIC of ampicillin, and were considered to be resistant. This study also attempts to address this public health crisis through public education. Sixth grade students participated in three days of inquiry-based lessons that challenged them to determine what factors affect how much bacteria enters the water, and why that is a problem. During this unit students followed the scientific process where they read articles to gain background knowledge, investigated a question through a lab protocol, analyzed multiple sources of data, and synthesized a conclusion. A mixed methods convergent study that featured both quantitative data from assessments and qualitative data from student written responses showed that they increased their understanding of these topics after participation in this unit.

Introduction

San Diego county is currently home to over three million people and is the second largest county in California (County of San Diego Health and Human Service Agency, 2020). In addition to its native population, San Diego is one of the most popular tourist destinations in the United States with as many as 30 million visiting each year (Kelley, 2019). One of the largest and most popular attractions is Mission Bay Park which spans 4,235 acres split among beaches, parks, protected marine bay, and the Pacific Ocean. Roughly 15 million people visit and utilize this space each year participating in activities such as swimming, surfing, sailing, sunbathing, and celebrating (City of San Diego Parks and Recreation, 2024).

Despite San Diego's overwhelming popularity, there is growing concern about the quality and safety of the water in San Diego County, including increased presence of *Enterococcus* bacteria. This genus of bacteria is the most commonly used Fecal Indicator Bacteria (FIB) due to its high concentration in contaminated marine waters, and close correlation with human health (Byappanahalli et al., 2012). Enterococcal infections cause symptoms that affect the skin, and gastrointestinal and respiratory systems (Fleisher et al., 2010). *Enterococcus* bacteria are found in highest concentrations near storm drains, river mouths, or lagoons especially after rain events due to inadequate sewage or stormwater treatment that stem from spills, infrastructure malfunction due to age, or overload from heavy rainfall (Burgmann et al.; 2018; Korajkic et al., 2019). In addition, surf zones or areas with large amounts of water column disturbance have been shown to have increased detectable concentrations of *Enterococcus* due to redistribution into the water column (Le Fevre & Lewis, 2003). Surf zones like these are common along the shorelines of San Diego. Due to the high accumulation and negative impact on human health, the concentration of *Enterococcus* is closely monitored to maintain public health by both the city of San Diego, as well as non-profit organizations like the Surfrider Foundation. Data collected by these organizations have documented increased concentrations of *Enterococcus* after rain events, as well as near areas with poor sewage or stormwater treatment like the Tijuana River near Imperial Beach. In addition to Imperial Beach and the Tijuana River, these organizations show that Mission Bay stands out with consistently high levels of Enterococcal bacteria, with rates consistently above healthy norms (Leduc, 2024; *County of San Diego*, 2024). Despite posted warnings and news stories, surfers and beach goers are often seen entering these contaminated waters, thereby exposing themselves to these pathogens.

Additionally, these bacterial infections are becoming more difficult to treat due to an increase in antibiotic resistance factors. Worldwide, reports have suggested that more bacterial populations are becoming resistant to a growing number of antibiotics. These reports show that due to large amounts of antibiotics in circulation, there is stronger selection pressure which creates more opportunities for resistance factors to persist (Llor & Bjerrum, 2014; Patini et al., 2020). Thus, it is important to determine if this selection pressure has caused more marine bacteria to conserve these genes as well. Antibiotic-resistant *Enterococcus* have been found to be widespread across different marine environments in other parts of the world, with the highest concentrations of resistant *Enterococcus* found in the water column, followed by animal digestive tracts, and then in beach sand (Korajkic et al., 2020). Testing done at the Tijuana River estuary near San Diego, an area noted for its lack of sewage treatment, identified numerous antibiotic resistance genes present in samples they collected. This suggests that urban wetlands, like Mission Bay, may become reservoirs for antibiotic-resistance genes (Cummings et al., 2010). Antimicrobial resistance has been noted as one of the largest threats to human health worldwide, as researchers race to develop antibiotics to attack bacteria in new ways (Llor & Bjerrum, 2014). Treatment of antibiotic-resistant strains of *Enterococcus* is challenging, requiring more severe antibiotics and more detailed and complex treatment plans (Gilbert et al., 2023).

The purpose of this study is to address these public health concerns in two ways. First, to determine the percentage of *Enterococcus* bacteria collected from Mission Bay that are clinically resistant to two common antibiotics. Second, by educating middle school students in the classroom about the relationship between bacteria and human health, our local watershed, and the factors that influence the number of bacteria in our waterways.

Literature Review

Enterococcus is a relatively small genus of bacteria, originally thought to be a part of the *Streptococcus* genus. It contains two main groups of clinically significant species, *E. faecalis* and *E. faecium*. These two species are quite similar in the infections they cause and are rarely treated differently from each other. All *Enterococcus* species are gram-positive and are either spherical or ovoid cells arranged in pairs or chains. They are catalase negative and most are homofermentative specializing in the production of lactic acid (Hardie & Whiley, 1997). A systematic review of occurrence studies shows that *Enterococcus* bacteria are successful in the digestive systems of animals but are also uniquely suited to survive in freshwater and marine ecosystems, as well as on aquatic and terrestrial vegetation, and in beach sand and soil. *Enterococcus* are uniquely able to survive at higher salt concentrations compared to other FIB providing them with an advantage in extraenteric environments (Byappanahalli et al., 2012).

Despite these advantages, once released from the gastrointestinal tract, *Enterococcus* bacteria, like many others, can be stressed by sunlight. The time for sunlight to reduce the population by 90% varies and depends on geographic and seasonal factors with more rapid reduction time in warmer water with low turbidity (2 hours), and lower population reduction time reported in cooler water (35 hours) which is similar to winter water temperatures in San Diego (Byappanahalli et al., 2012). Other natural stressors to *Enterococcus* include high levels of salinity with faster reduction occurring in marine water compared to freshwater (Anderson et al., 2005), nutrient starvation during the transition between animal gastrointestinal systems and an oligotrophic environment (Byappanahalli et al., 2012), and, finally, via predation by bacterivorous protozoa (Boehm et al., 2005). Disinfection of wastewater is the most viable method of reducing *Enterococcus* bacteria. In the United States it is most commonly done through treatment with chlorine followed by UV light. This treatment has been found effective in reducing both *Enterococcus* and other pathogenic bacteria (Berg et al., 1978).

The unique ability of *Enterococcus* bacteria to thrive in both the nutrient-rich digestive systems of animals, extraenteric habitats like soil/sand, and marine ecosystems makes this genus so successful compared to other bacteria. Studies consistently confirm that Enterococcal concentration is higher in marine ecosystems with inadequate sewage treatment, specifically near storm drains, river mouths, and lagoons compared to marine environments free of these pollutants (Burgmann et at., 2018; Korajkic et al., 2019). This aligns well with the findings above suggesting that *Enterococcus* species have a competitive advantage in these areas. There are generally two ways *Enterococcus* may enter these ecosystems. First, when discharged from the digestive systems of animals, (such as humans or dogs) and relocated to wastewater. If left untreated, the bacteria may survive and re-enter the environment. Alternatively, if waste or pollution containing *Enterococcus* is left in the terrestrial environment due to a variety of factors including homelessness, and is then washed untreated into storm drains, this *Enterococcus* is able to persist and survive the transition into marine environments such as Mission Bay. *Enterococcus* is also able to survive if deposited in beach sand and soil where they can survive for a longer period of time creating a sink, or area of increased bacterial concentrations, where beachgoers may come into contact with it (Byappanahalli et at., 2012). These sinks have also been shown to redistribute bacteria in the water column when disturbed by waves; *Enterococcus* concentrations were shown to be higher in areas where wave action was occurring compared to outside of the surf zone (Le Ferve & Lewis, 2003). All these findings match with the observed increase in *Enterococcus* concentrations in Mission Bay, where multiple sources of untreated stormwater and waste enter the bay to serve as a source of bacteria, large amounts of soil to serve as a sink, and wave action due to recreational boating to redistribute stored bacteria.

Enterococcus bacteria are clearly present in our waterways; they also work well as FIB. Their presence in the waterways can indicate that there may be other more pathogenic bacteria present as well. While *Enterococcus* are usually commensal bacteria, some species are opportunistic human pathogens (Morrison et al., 1997). As mentioned above, *Enterococcus* alone are also of clinical significance in regard to public health. *E. faecalis* and *E. faecium* both cause common hospital-borne infections such as urinary tract infections, central nervous system infections, abdominal infections, and pelvic infections (Byappanahalli et al., 2012). Outside of the hospital, a public health survey that sampled 1,303 people found that those who regularly entered the ocean were 1.76 times more likely to report gastrointestinal illness, 4.46 times more likely to report respiratory illness, and 5.91 times more likely to report a skin infection compared to those who did not regularly enter the ocean (Fleisher et al., 2010). While most of these infections do not require clinical treatment, patients may seek medical attention if they do not resolve on their own. Traditionally, *Enterococcus* infections were treated with penicillin or another cell wall-active agent in addition to an aminoglycoside and are relatively easy to treat (Miller et al., 2014). Recent recommendations for non-resistant strains include treating with penicillin G or Ampicillin. Systemic infections such as cystitis are treated with nitrofurantoin, amoxicillin, and fosfomycin (Gilbert et al., 2023).

However, some strains of *Enterococcus* can carry resistance genes that provide tolerance to a wide range of antibiotics and are of much more clinical significance. One study examined the quantity of antibiotic resistance genes of *Enterococcus* samples collected from recreational marine ecosystems in an area similar to Mission Bay. They found that 54% of the *Enterococcus* collected featured genes that provide resistance to one of the tested antibiotics (tetracycline and vancomycin), and concluded that recreation marine environments could be reservoirs of antibiotic resistance and virulence genes (Santiago-Rodrigues et al., 2013). A systematic review by Korajikc (2020) of antibiotic resistance in marine *Enterococcus* found that the proportion of collected species that featured resistance varied between reports, however ampicillin (24.2% of collected specimens) was the most common resistance factor, while vancomycin (2.4% of collected specimens) was the least. Additionally, they determined that *Enterococcus* collected from the water column had the highest percentage of antibiotic resistance (18.8%), followed by animal feces and tissue (14.8%), and then sediment (9.4%) (Korajikc et al., 2020). Locally, quinolone resistance genes were isolated from samples collected from the surface sediments of the Tijuana river estuary (Cummings et al., 2010).

Analysis of modern multi-drug resistant (MDR) *Enterococcus* has been traced to a clade that coincides with the introduction of antibiotics as a treatment method. This clade has been shown to feature increased mobile genetic elements and alterations in metabolism which creates an exceptionally malleable genome when faced with multiple selective pressures (Lebreton et al., 2013). These highly mobile genetic elements allow genes to be passed easily from individual to individual, thus allowing Enterococcal bacteria to quickly acquire resistance and pass it along. For example, *Enterococcus* have 5 common penicillin binding proteins (PBP's) that have been shown to provide tolerance to β-lactams like penicillin and ampicillin, as well as vancomycin. There are also many plasmid-mediated genes that provide antibiotic resistance through differing mechanisms including modification of drug targets, inactivation of antibiotic agents, overexpression of efflux pumps, and the presence of a complex cell envelope (Miller et al., 2014). Treatment of these resistant strains require stronger, more aggressive antibiotics that can be more damaging to the patient. For systemic infections resistant to β-lactam antibiotics, a combination of ampicillin-sulbactam or vancomycin is a common treatment method. For strains resistant to vancomycin a treatment plan must be made by an infectious disease consultant because they require more complex treatment methods (Gilbert et al., 2023).

The problems caused by large amounts of infectious bacteria in San Diego waterways can also be addressed by effectively educating the next generation. Research-based classroom teaching has been shown to increase public awareness of environmental issues and therefore can be an important tool to address this problem (Sola, 2014). Current research suggests that science education should allow students to take ownership of their own learning rather than a teacher providing direct information. This is a practice known as inquiry-based teaching (Abdi, 2014). Many pedagogical methods fall under the umbrella of inquiry-based learning; however teachers should provide students with the opportunity to solve problems as a method to develop new learning. In science, students should be involved in the process, or nature, of science in which they must develop questions based on a given problem, then attempt to solve that problem by collecting and analyzing data, and forming conclusions (Constantinou et al., 2018). To do this, students can either collect their own data or be given data sets to analyze, such as those made available by Michigan State University Data Nuggets website (Data Nuggets, 2025).

Another pedagogical method that falls within inquiry-based learning is experiential learning. This concept requires educators to provide students with relevant real-world experiences to increase student interest and engagement in the activity. In the science classroom, these experiences can be experimental labs, real world data sets, or trips outside of the classroom. Experiences like these can not only build content knowledge, but also soft skills like critical thinking, and data fluency (Alkan, 2016). If designed correctly, these experiences provide students with an understanding of how these skills and concepts fit into science as a whole and are also significantly more engaging for the students (Jack & Lin, 2017; Kong, 2021). Higher engagement from experiential learning can also increase student motivation, as well as their attitude toward science as a whole (Weinberg et al., 2011). Thus, effective education places a heavy emphasis on student engagement. Another way to increase student engagement is to connect learning to their personal interests. It has been shown that when educators connect unit or lesson storylines to student interests or hobbies, students are more engaged and have more success (Penuel et al., 2022). Additionally, connecting classroom learning to the local community has also been shown to increase students' engagement, and thus student learning (Rittenburg et al., 2015). This can take the shape of small partnerships with local businesses to display art, or larger scale projects that involve students in solving local issues.

Research questions

Enterococcus bacteria have been well studied, and are the only FIB recommended by the Environmental Protection Agency for brackish water or marine water due to the close correlation with human health and their tolerance for brackish marine conditions. There is substantial water quality testing in place for most major cities, as well as by nonprofit groups that show high levels of *Enterococcus* present in San Diego waterways. These results match well with what is known about the unique characteristics of the *Enterococcus* genus showing that these bacteria collect in marine areas with poor sewage treatment. There is also a great deal of research on antibiotic resistance factors found in the gene pool, including resistance genes that confer tolerance to most classes of antibiotics. Some studies have begun to look at the presence of these antibiotic resistance genes collected from marine *Enterococcus*, but results have been inconsistent and have varied from place to place. The last published study to examine antibiotic resistance in the San Diego, California area revealed, through amplification, a strong presence of these genes in collected samples but no testing has been done in the 15 years since (Cummings et al., 2010). Due to their clinical significance, and the number of people exposed to these pathogens each year, it is important to address the public health concern of elevated bacteria levels in the San Diego area by both conducting water testing focused on antibiotic resistance in areas where people enter the water and by educating students to increase public awareness of this issue. The purpose of this study was to answer these research questions:

A. First, what percentage of *Enterococcus*, collected from Mission Bay, is clinically resistant to ampicillin and vancomycin? I hypothesized that there would be a large proportion of *Enterococcus* collected that would be resistant to at least one antibiotic due to their high occurrence in the area, and documented history of antibiotic resistance.

B. Second, is it possible to increase student awareness of local water quality issues through a short series of engaging lessons? I hypothesized that after completing a short series of inquiry-based lessons students will gain a better understanding of the role of factors that influence the amount of *Enterococcus* bacteria in our waterways.

Part I: Assessment of Antibiotic Resistance in FIB

Part I Methods

Research setting

Bacterial specimens were collected from Mission Bay in San Diego, California. The collection site receives freshwater from Rose Creek, a small urban creek, where it meets the larger body of Mission Bay over a sandy ocean floor (Appendix A). Mission Bay is a large man-made bay with relatively limited water movement and circulation. It is exposed directly to the Pacific Ocean, San Diego River, numerous smaller sources of urban runoff, as well as contaminants from recreational activities and urban development. The sample site is approximately two miles from the ocean and was chosen specifically because of its reported high *Enterococcus* values (Leduc, 2024).

Data collection

Water samples (100 mL) were collected twice weekly from the same location, at the same time of day (6:45 AM) for 4 weeks. Water samples were collected in sterile disposable IDEXX 120 mL plastic vials (Catalog No. WV120SBAF-200) to eliminate contamination. Samples were collected by walking approximately 3 meters into the bay, in water approximately

60 cm deep. Careful attention was made to ensure minimal disturbance of sediments, and to ensure collection was limited to the water column with minimal sediment.

In the lab, 10 mL of the water sample was placed into three separate 120-mL plastic vials. Into each of these vials, 90 mL of distilled water was added, creating 3 diluted water samples. One prepackaged IDEXX Enterolert reagent (Catalog No. 98-21374-00) was placed into each sample, and mixed until the contents dissolved and the bubbles disappeared. This reagent is premixed, and contains a proprietary defined substrate technology that fluoresces when metabolized by *Enterococcus* bacteria (Budnick et al., 1996). Two different antibiotic solutions were prepared, one containing ampicillin, and one containing vancomycin. These solutions were designed with minimum inhibitory concentration (MIC) to ensure surviving colonies are considered clinically resistant to that antibiotic. The ampicillin antibiotic solution was prepared to contain 16 µL of antibiotic per mL of water (1.6%) . The vancomycin antibiotic solution was prepared to contain 32 µL of antibiotic per mL of water (3.2 %). The ampicillin solution was placed into one of the vials with a diluted water sample and Enterolert reagent, and the vancomycin solution was placed into a new IDEXX vial with a water sample and Enterolert reagent. These three samples - bay sample, bay sample with ampicillin, and bay sample with vancomycin - were placed into separate IDEXX Quanti-Trays (Catalog No. WQT-100) and sealed using the IDEXX Quanti-tray Sealer (Catalog No. 98-0002570-00). Once sealed, Quanti-Trays were placed in an incubator set for 41℃ and left for 24 hours.

After 24 hours, the samples were removed and placed in a dark room to enhance contrast of fluorescence. One at a time, each Quanti-Tray was placed under a UV light. The number of large and small fluorescent wells was recorded for each sample. This assay showing a fluorescent well indicates the presence of living *Enterococcus* bacteria because the organism had metabolized the fluorescent particle within the reagent. The quantity of both the small and large wells was then compared to the IDEXX Quanti-Tray data sheet to produce a most probable number per 100 mL(MPN) of living *Enterococcus* bacteria. To obtain the final MPN of *Enterococcus*, the MPN result provided by the data sheet was then multiplied by 10 to account for the dilution of the water sample. Due to limited supplies a one-week trial period was utilized to determine if any vancomycin resistance was detected.

Data analysis

The independent variable was the presence of antibiotic resistance factors present in the collected *Enterococcus* specimen. The dependent variable was the MPN of bacteria still living after the antibiotic treatment (resistant bacteria). Descriptive statistics (means ± SD) were calculated including a comparison of that MPN to associated risk of illness according to EPA guidelines. Due to the abnormality of the distribution of data set, a Wilcoxon signed-rank paired two-tailed t-test was then inferred to determine the statistical significance of differences between the MPN of clinically antibiotic resistance *Enterococcus* compared to MPN of control *Enterococcus* samples without the treatment of antibiotics. All statistical analyses were done at a significance level of 0.05.

Part I Results

Results from the first week of the study (trial period) indicated a strong presence of Enterococci present in Mission Bay, with two days (3/4/25, 3/6/25) rising above the medium risk as defined by Environmental Protection Agency (EPA) standards, and one day (4/13/25) rising well above the high bacterial risk as defined by EPA standards. During this trial period, some ampicillin resistance was detected, however no clinical vancomycin resistant strains were detected despite high levels of Enterococci present (Table 1, Figure 1). Table 1 and Figure one both show all samples taken during this trial period. To maximize sample size, vancomycin was no longer targeted for the remaining month of data collection.

Table 1

Descriptive statistics for Enterococcus MPN/100mL Control, Ampicillin, and Vancomycin samples

	Ampicillin	Control	Vancomycin
Mean	27.667	287.000	0.000
Std. Deviation	32.192	435.039	0.000
Minimum	0.000	20.000	0.000
Maximum	63.000	789.000	0.000

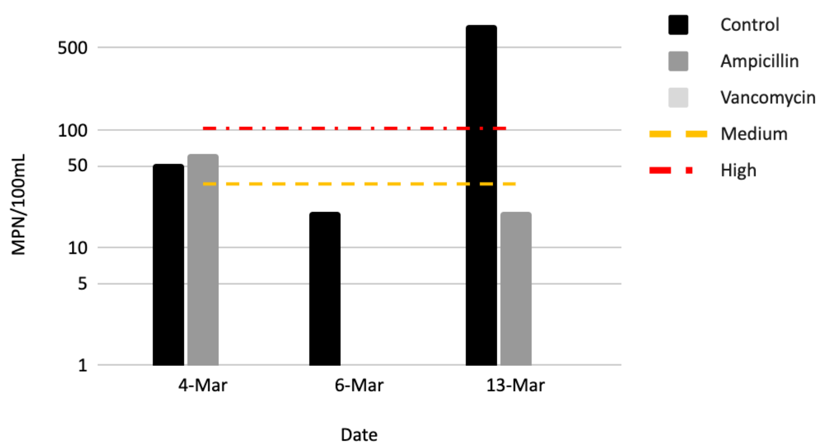

Figure 1

Marine Enterococcus with Ampicillin and Vancomycin Resistance with comparison to Associated Health Risk
Colors indicate bacterial risk according to EPA standards: medium risk yellow (36-104 MPN/100mL), and high risk red (>104 MPN/100mL).

After the trial period, over the next month of testing there were 6 days of detectable Enterococci collected from Mission Bay with a mean of 85.77 MPN/100 mL and standard deviation of 147.8 (Table 2). Significance testing showed there was a statistically significant difference in population size with less detection of ampicillin resistant bacteria as compared to control bacteria, W = 21, z = 2.201, p = 0.018 Two days (3/25 and 4/3) rose above the threshold for medium bacteria risk, and two days (3/13 and 4/1) rose above the threshold for high bacterial risk as shown in Figure 2. Despite high Enterococci detection there was low, but noteworthy, clinical ampicillin resistance detected among that population (~3%).

Table 2

Descriptive statistics for Enterococcus MPN/100mL Control, and Ampicillin samples

	Ampicillin	Control
Mean	3.333	85.778
Std. Deviation	7.071	147.805
Minimum	0.000	0.000
Maximum	20.000	455.000

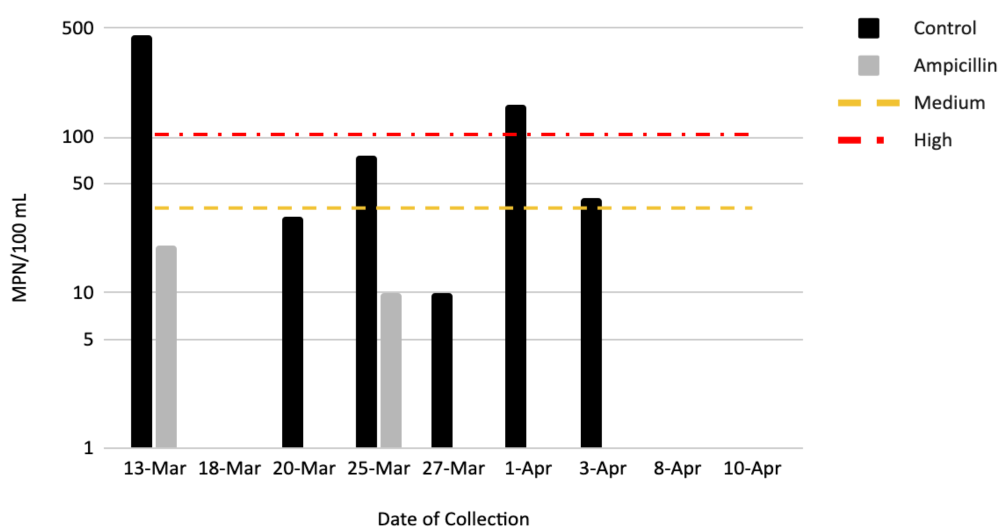

Figure 2

Marine Enterococcus and Ampicillin Resistance with comparison to Associated Health Risk

Enterococci MPN/100 mL for both treatment groups (Control and Ampicillin) in Mission Bay at Rose Creek. Colors indicate bacterial risk according to Environmental Protection Agency standards: medium-risk yellow (36-104 MPN/100mL), and high-risk red (>104 MPN/100mL).

Part I Conclusions

The purpose of Part I of this study was to determine the population of Enterococci, collected from Mission Bay, that exhibit clinical resistance to antibiotics. After one month of testing that featured multiple days of significant Enterococci MPN, roughly 3% of *Enterococcus* collected exhibited clinical resistance to ampicillin, and 0% demonstrated clinical resistance to vancomycin. While the results of the vancomycin test align well with reported results of low rates of vancomycin resistance genes (Korajkic et al., 2020), these results differ slightly from other published results which have reported high prevalence of genes that confer resistance to ampicillin in marine *Enterococcus*. A systematic review of resistance genes in the marine *Enterococcus* denotes that an average of 37.5% of specimens collected featured some resistance gene to ampicillin (Korajkic et al., 2020). Locally, a significant number of quinolone resistance genes were identified in samples collected from surface sediment in the Tijuana River Estuary (Cummings et al., 2010).

It is important to note that the studies above focused on identification and amplification of plasmid genes that may assist in demonstrating resistance to certain antibiotics. One major limitation of this current study was lack of testing for these specific resistance genes. Instead, due to limited time and funding, this study used the MIC concentrations of each antibiotic to ensure that surviving bacteria have survived past the clinical breakpoint to be considered resistant. It is possible that more of these plasmid mediated genes were present in the *Enterococcus* collected, but were not sufficient enough to provide resistance above the clinical breakpoint and thus were not detected. This would help to explain the differences in prevalence of reported resistance genes in previous studies as compared to the lower levels of clinical resistance found in this study.

Further investigation is needed and additional research should focus on collecting additional samples from Mission Bay as well as amplifying the specific genes that confer resistance to antibiotics to get a more detailed understanding of the *Enterococcus* gene pool. Additional testing sites, like Coronado or Imperial Beach, could also be tested due to their proximity to the Tijuana River Estuary. This research could add more evidence to the growing list of environmental concerns facing the San Diego area due to inadequate water treatment, and could encourage public policy makers to invest in solutions like improved sewage or wastewater treatment that directly address the problem.

Part II: Development and Field-testing of Inquiry-based Lessons for Classroom Education

Part II Methods

Research Design

To field-test and to assess the effectiveness of a series of lessons, a mixed methods study that included both qualitative and quantitative data was used. This was chosen to allow a deeper analysis of the research question, by acquiring a more holistic view of student understanding that combines both responding to multiple choice-type questions as well as answering open-ended questions (Damyanov, 2023). This convergent parallel study provided two mutually exclusive data sets so that the analysis of the qualitative responses could be used to explain the quantitative assessment scores.

Study Site and Participants

A three-day series of 58-minute lessons designed for this study was taught in a 6th grade science classroom in a large public suburban middle school in the San Diego area about 15 minutes from the ocean. The school population was evenly mixed between male and female, and features 43.3% of students from minority backgrounds, and 56.7% of students who are white (California Department of Education, 2025). The classroom consisted of thirty students aged 11-12, three of which were English Learners (ELs). Students had previous classroom knowledge about cells, and the water cycle, but no classroom experience with bacteria or watersheds. The author of this study had no prior experience with this particular group of students, but the classroom teacher was present to assist and support. Students completed all work on laptops provided by the school. Lessons were taught during regular class time during the last three days of school, and no additional credit was given for completed work. No identifying data was collected from students who participated in these lessons.

Lesson Design

Both the pre-and post-assessment and the classroom activity were created for this study. The first day, students completed the pre-assessment which consisted of three short questions each assessing a major concept: 1) the connection between bacteria and human health, 2) watersheds, and 3) how bacteria move through the watershed (Appendix B). After the 3-minute assessment, students were introduced to the topic via short video clips showing closed beaches near the Tijuana River (NBC News 7 San Diego, 2024 , 00:00 - 00:46 & Fox 5 News San Diego, 2025, 00:00 - 2:31) They then completed a jigsaw reading activity where some students read an article created by the author of this study either about bacteria or watersheds (as shown in Appendix D) in order to provide some background information. The day concluded with a short group discussion, and then students responded to this question: "How could bacteria affect our watershed?" to summarize their learning for the day.

Before the lesson began in the morning on the second day, water samples were collected by the author of this study in the same fashion as in Part I. At the beginning of class, students were then asked if they could explain why the beach in the video was closed, and were introduced to a water quality testing lab as a way to solve the problem. Students worked in groups to test for the amount of *Enterococcus* present in the ocean water, as well as the nearby lagoon; each group of students processed one sample of either ocean water or lagoon water as shown in Appendix C. In an effort to increase student interest, both nearby sample areas were chosen because they were familiar to students. Finally, they examined additional evidence from other sources as shown in Appendix E. The first data set detailed the *Enterococcus* concentrations with changing rainfall. The second data set focused on the effect of poor nearby sewage treatment and *Enterococcus* concentration at nearby beaches.

On the final day, the results from the previous day's test were recorded by the teacher and then provided to the students. Students then analyzed that data. They then combined this new data as well as the other evidence they had examined the previous day, to answer the lab question about what factors influence the number of bacteria in our waterways (Appendix C). Finally, they took a post-assessment that mirrored that pre-assessment they completed at the start of the first day. A complete summary of all lesson plans can be found in Appendix F.

Quantitative Data Collection and Data Analysis

Quantitative data was collected using the pre-assessment, taken on the first day of lessons before any instruction, and identical post-assessment taken at the end of the three-lesson unit (Appendix B). The quantitative assessment was scored out of seven points distributed among the 3 questions. One point was possible for question one to show a basic understanding of the watersheds. Four points were possible for the next question, with one point for each of the four correct answers to demonstrate understanding of bacteria and their effects on our ecosystem. Finally, two points were possible for the third question for each correct answer demonstrating knowledge of how bacteria can reach our oceans. Descriptive statistics and a 2-tailed t-test were used to analyze the total assessment scores.

Qualitative Data Collection and Analysis

Qualitative data was collected during day two and three of the lesson series (Appendix C). The first question required students to write down patterns they noticed in data depicted in two graphs, one that showed the relationship between rainfall and *Enterococcus* bacteria MPN, and a second that showed the effect of sewage treatment and *Enterococcus* bacteria MPN (Appendix D). The second question asked students to use data from the classroom lab as well as data from the graphs they were given to write a conclusion about what factors affect the number of bacteria in our oceans. Responses to both questions were analyzed and coded using an emergent coding scheme based on the data (Table 3) which includes a student example of each scoring category for reference.

Table 3
Qualitative Data coding scheme

Question 1: Draw or summarize at least one additional piece of data below.	
Beginning	Answer is missing or contains irrelevant information. *Student Sample: "In the beginning, for both of them, they are both very high, and it slowly gets lower with some spikes up."*
Proficient	Accurately describes a pattern present in the data. *Student Sample: "March 13 has a lot of bacteria. March 13 also had a lot of rain."*
Advanced	Accurately describes multiple patterns present in the data, and connects patterns together. *Student Sample: "I noticed that in the days that it rained more there was a lot more bacteria in the water. Also, some of the days didn't get any rain and there wasn't any bacteria in the water. The areas that had good sewage treatment had safe waters most of the time while the areas that did not have their sewage treated, had most of their days with not safe water."*
Question 2: What factors affect how much bacteria enters the water, and why is this a problem?	
Beginning	Incorrect explanation of a possible cause of bacteria quantity in ocean water. *Student Sample: "I think that rain affects the amount of bacteria in our waterways. I think this is because, there are higher bacteria levels in areas in sewage. This is a problem because this makes it a bad environment for the animals and other people around the sewage."*
Proficient	Correctly identifies one correlation between quantity of bacteria and a possible source. Clearly identifies a problem caused by this increase in bacteria quantity. *Student Sample: " I think that rain affects the amount of bacteria in our waterways. I think this because, when there was a lot of rain there was a lot of bacteria. This is a problem because there are high bacteria levels causing people to get sick or hurt."*
Advanced	Correctly identifies more than one correlation between quantity of bacteria and a possible source. Clearly identifies a problem caused by this increase in bacteria quantity. *Student Sample: "I think that the watershed and waste treatment factors affect the amount of bacteria in our waterways. I think this because, the waste has a lot of bacteria in it and the watershed brings it into a lake or lagoon, or all the way to the ocean. This is a problem because, the more bacteria, the more chance that the water is not safe to do anything with."*

Part II Results

Quantitative results

Results from the pre-assessment (M = 3.138, SD = 1.156) and post-assessment (M = 5.034, SD = 1.21) are shown in Table 4. The students had a statistically significant improvement in their understanding of the factors that influence the amount of bacteria in our waterways after participating in this unit, $t(28) = -7.308$, $p < .001$.

Table 4

Descriptive Statistics of Student Pre/Post Assessment Scores

	Pre-Test	Post-Test
Valid	29	29
Median	3.000	5.000
Mean	3.138	5.034
Std. Deviation	1.156	1.210
Minimum	1.000	2.000
Maximum	6.000	7.000

Qualitative Results

Quantitative analysis of students' scores shows a 30% increase in post-test scores as compared to their pre-test scores (Table 4). This indicates that students increased their understanding of the chosen topics, but does not alone provide enough insight into understanding. Qualitative analysis of student written responses shows that 50% of students reached proficiency, and 30% of students reached advanced understanding based on their written response to question 2 (Table 5). While most students answered both of these questions at the same level (beginning, progressing, advanced) there was some discrepancy across answers, likely due to students missing time in class (Table 6). These results together show that a majority of students successfully increased their understanding of the factors that influence the number of bacteria in the waterways, and how that bacteria can affect their health.

Table 5

Summary of Qualitative Student Responses

Question	Proficiency Level	Number of Students (Percentage)
Question 1: Draw or summarize at least one additional piece of data below.	Beginning (missing answers included)	6 (20%)
	Proficient	14 (46%)
	Advanced	10 (33%)
Question 2: What factors affect how much bacteria enters the water, and why is this a problem?	Beginning (missing answers included)	6 (20%)
	Proficient	15 (50%)
	Advanced	9 (30%)

Table 6

Matrix to Summarize Student Responses to Question 1 and Question 2

	Q2 – Students with scores of "Beginning"	Q2 – Scores of "Progressing"	Q2 – Scores of "Proficient"
Q1 – Scores of "Beginning"	2	2	2
Q1 – Score of "Progressing"	3	7	4
Q1 – Scores of "Proficient"	1	6	3

Part II Conclusions

The purpose of this set of lessons was to increase student awareness of the local public health crisis that stems from the high quantity of bacteria in our local watershed. It has been shown that education can help to increase public awareness of environmental topics (Sola, 2014). To answer the question, “Is it possible to increase student awareness of local water quality issues through a short series of engaging lessons?” students participated in a short 3-day series of inquiry-based lessons where they learned about the health effects of bacteria, our local watershed, and factors that increase the number of bacteria in our waterways.

First, students completed a short pre-assessment to provide data on their understanding on the three main topics in this unit. Scores from the pre-assessment indicated they had a minimal understanding of the roles of bacteria in our ecosystems, as well as how it travels through our watershed. During these lessons, student work completion was relatively high, with 93% of students answering at least one question, and 71% of students completing the entire worksheet. This completion rate is high given that the study took place on the last three days of school. Coded analysis of student written responses suggests that 86% of students who participated were proficient in their understanding, and 30% provided responses that suggest they have an advanced understanding of these concepts (Table 5). After completing this short series of lessons students took the same assessment again. Scores from the post-assessment indicated a statistically significant increase. Based on these results, it seems that the three-lesson unit was effective in increasing student understanding of the factors that influence the number of bacteria in our waterway, as well as the role that bacteria play in human health. This small study also provides further evidence of the positive effect of inquiry-based lessons connected to student’s lives on learning.

One major limitation of this study was the time of year the students completed these lessons. Students were asked to complete these lessons over the last three days of school, when other science classes were working on non-academic work. It is reasonable to assume that the excitement from the end of the school year impacted how the students were able to develop new knowledge. Despite the distractions and excitement of the school year ending, engagement in the lessons was high as noted by high turn in rates noted above, as well as observations made by both teachers in the room.

Upon completion and reflection upon this unit, some changes can be made to enhance student learning. First, reworking the assessment to include an opportunity to predict areas on a map that may be more likely to have higher levels of bacteria would provide students a better opportunity to demonstrate their knowledge (Appendix G). Second, more structure should be provided during the additional data investigation on day two to allow students to better understand the trends in the graphs that were provided (Appendix H). Future studies should focus on how this classroom education can change public perception and public awareness.

Thesis Summary

Healthy and clean beaches and oceans are of paramount importance to both San Diego natives and tourists who use these areas for a myriad of recreational purposes. High bacteria levels in these San Diego waterways have been a major threat to public safety for years, and do not appear to be going away any time soon. Urban runoff, increased homelessness, aging sewage treatment infrastructure, and increased storm intensity due to climate change further exacerbate this challenge (Curriero et al., 2001). To continue to understand the extent of this pollution problem, this research examined the quantity of Enterococcus bacteria collected from Mission Bay that carried clinical antibiotic resistance to two different commonly used antibiotics, ampicillin and vancomycin. Three percent of *Enterococcus* bacteria collected was clinically resistant to ampicillin, although zero percent of bacteria collected was resistant to vancomycin.

To address the public perception of this issue, a short series of lessons that focused on understanding the core factors that influence the amount of *Enterococcus* bacteria in our waterways was developed and field tested. After this short series of lessons, middle school students had a better understanding of the factors that influenced the quantity of *Enterococcus* bacteria in San Diego waterways, and the problems the bacteria pose. While this research does not solve the pollution problem in San Diego it can help us to better understand the challenges that we face and provides a method to increase public awareness of this issue.

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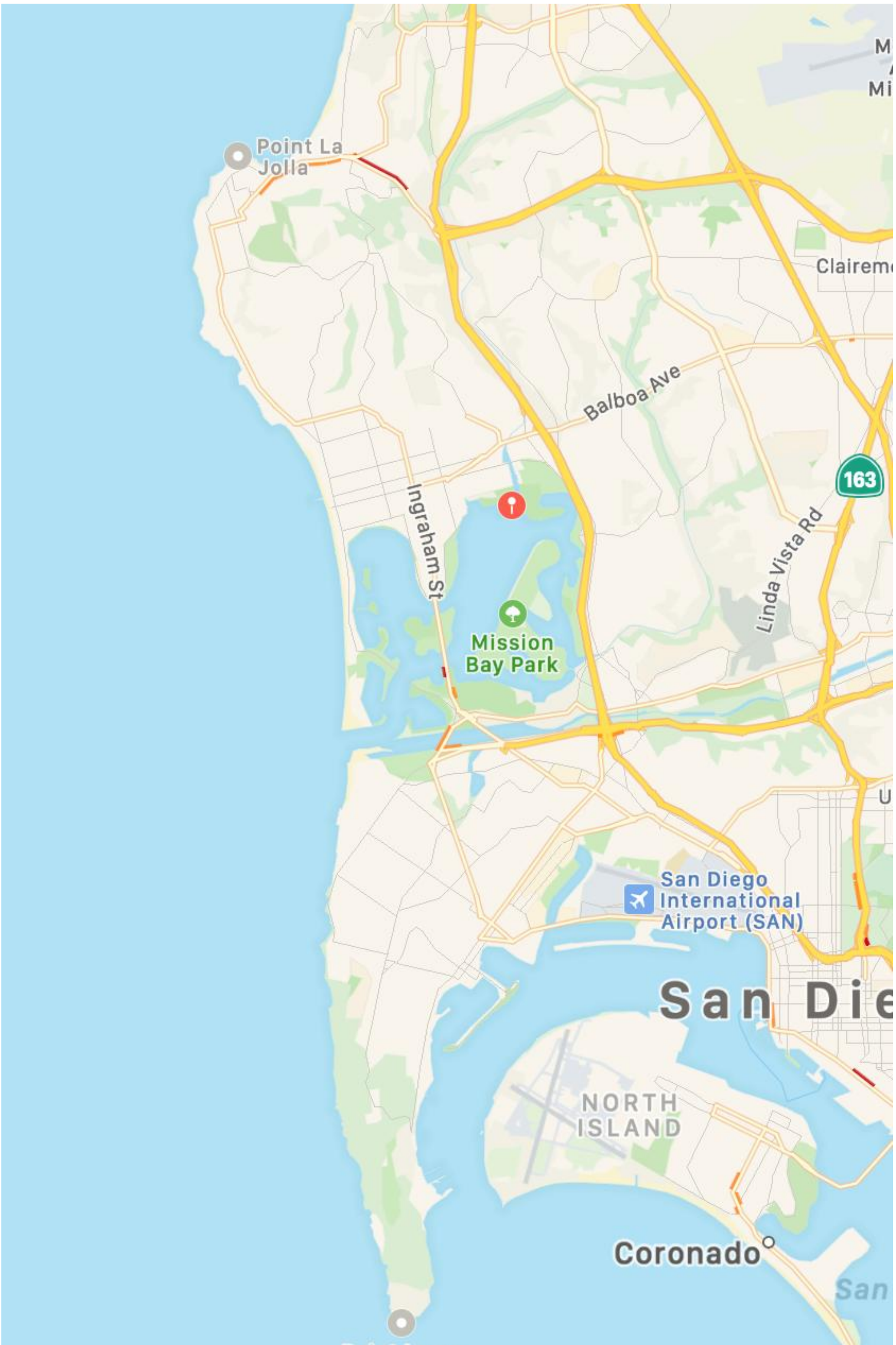
Appendix A: Map indicating sample site in San Diego, California



Appendix B: Pre-and post -Assessment

Watershed Assessment

Please answer these questions to the best of your ability. Good Luck!

1. Email *

____

2. Choose the option that best describes how a watershed works?

Mark only one oval.

- ◯ Water evaporates and then returns to the ecosystem when it rains
- ◯ It works like a bowl that funnels all water to the lowest point (lake or ocean)
- ◯ It works like a machine that pushes water to the ocean or a lake

3. What can bacteria do? Choose all that apply.

Check all that apply.

- ☐ Help plants and animals live
- ☐ Make you sick
- ☐ Breaks down dead things in an ecosystem
- ☐ Survive in different environments

4. What factors affect how much bacteria reaches our ocean? Choose all that apply.

Check all that apply.

- ☐ Rain washes harmful bacteria into the ocean
- ☐ Harmful bacteria is not treated by waste management facility
- ☐ Harmful bacteria grows in the ocean
- ☐ Wind blows harmful bacteria into the ocean

Appendix C: Water Quality Testing Lab Activity

Water Quality Testing Lab

Introduction

Scientists test the amount of bacteria in the water to estimate the risk of a person getting sick if they enter the water. They do this by **testing for the most probable number (MPN) of bacteria** which allows them to estimate that risk. The **higher the MPN the more bacteria in the water and the more likely somebody would get sick**. You are going to test to see if more bacteria is found in lagoon water (freshwater that enters the ocean) or if ocean water has more bacteria.

Question

What factors affect how much bacteria enters the water?

Procedure

1. Teacher will collect two different samples, one from the ocean (Cardiff Reef) and one from a freshwater source (San Elijo Lagoon)
2. Ocean Sample Preparation
    1. Place 10 mL of ocean sample into a sterile container.
    2. Add 90 mL of distilled water into a sterile container.
    3. Add Enteroalert reagent, and mix until completely dissolved.
    4. Pour sample into QUANTI tray, seal, and label.
3. Freshwater Sample Preparation
    1. Place 10 mL of Freshwater sample into sterile container.
    2. Add 90 mL of distilled water into a sterile container.
    3. Add Enteroalert reagent, and mix until completely dissolved.
    4. Pour sample into a separate QUANTI tray, seal, and label.
4. Place the sealed QUANTI trays into an incubator set for 41 degrees C. Leave for 24 hours. *Done by the teacher*
5. After incubation, place the tray under a fluorescent light, and record the number of large, and small wells. *Done by the teacher*
6. Determine MPN using IDEXX data sheet. *Done by the teacher*

Hypothesis

I think that the *type here* sample will contain higher amounts of bacteria because *type here*.

Data

Group Number	Ocean Sample MPN/100mL	Lagoon Sample MPN/100mL
1		
2		
3		
4		
Average		

Additional Data

Click here to find additional data to help with answering our question. Draw or summarize at least one piece of additional data in the space below.

Conclusions

In the space below answer the question, make sure to reference the data from the lab and one additional piece of evidence in your answer.

Question: What factors affect how much bacteria enters the water, and why is this a problem?

I think that *type here* factors affect the amount of bacteria in our waterways.

I think this because, *type here* .

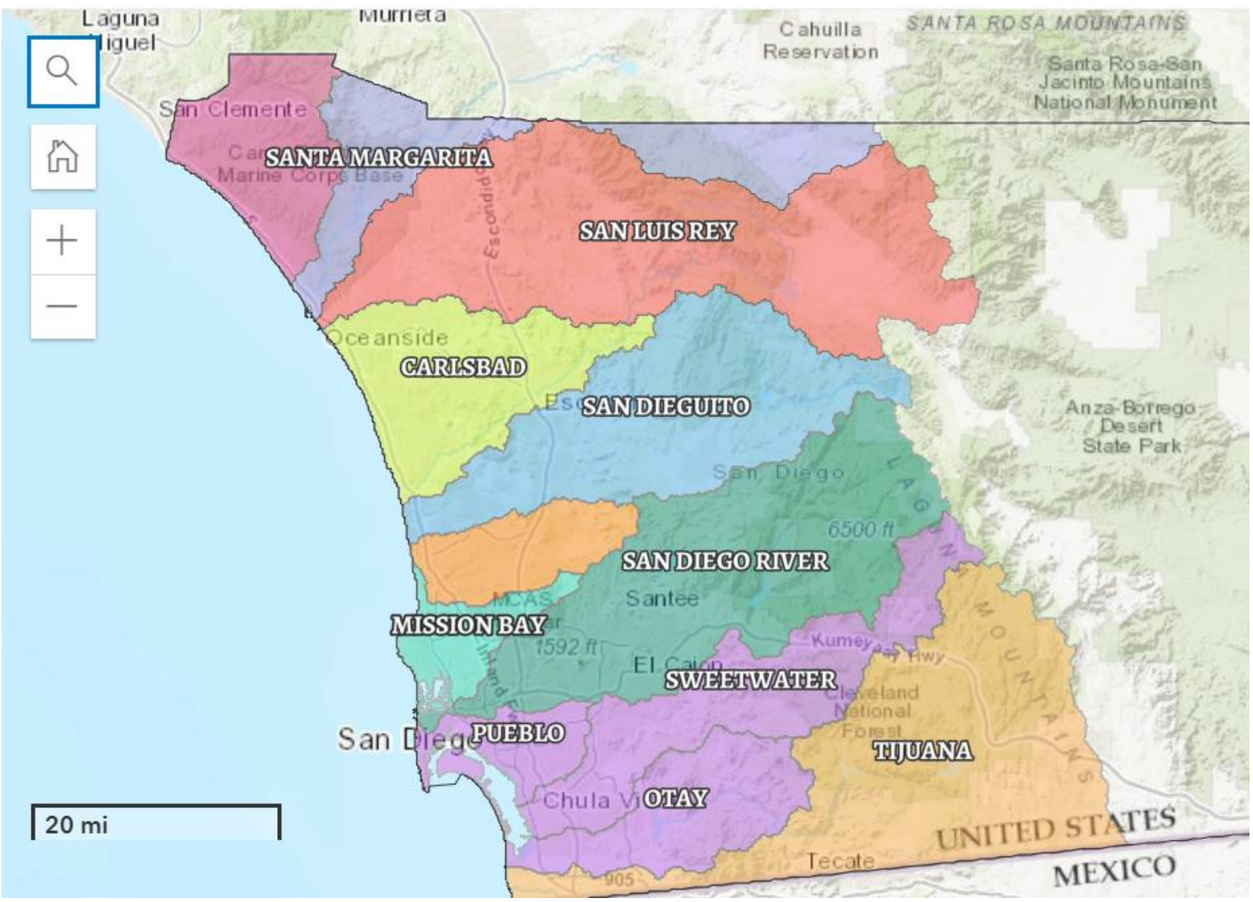
This is a problem because, *type here* .

What Is a Watershed?

Have you ever wondered where all the rainwater goes when it runs off your driveway, the sidewalk, or a nearby hill? That water doesn't just disappear—it becomes part of something called a **watershed**. A watershed is an area of land where all the water that falls, such as rain or melted snow, drains into a common body of water like a **river, lake, or ocean**. You can think of it like a giant bowl or funnel. No matter where a drop of water lands inside the bowl, it eventually flows downhill to the lowest point—just like how water on your roof flows into gutters and then down a drain.

In nature, that "bowl" is made up of **mountains, hills, valleys, forests, neighborhoods, farms, and even cities**. Water flows over the surface (called **runoff**) or travels underground, collecting in streams and rivers before finally reaching a larger body of water. This whole system—the land, the water, and everything that lives on it—is part of the watershed.

Watersheds in San Diego County



San Diego County is home to **11 major watersheds**, each one shaped by the natural land features and the way people have developed the area. These watersheds drain into the **Pacific Ocean**, **San Diego Bay**, and even some **inland lakes and rivers**. Because San Diego has such a variety of land types—from mountains to deserts to coastlines—our watersheds are diverse and unique.

One of the most well-known is the **San Diego River Watershed**, which begins in the mountains east of the city and flows west through Mission Valley, eventually reaching **Mission Bay** and the **Pacific Ocean** near Ocean Beach. This river passes through many neighborhoods, parks, and even shopping areas, so it's especially important to keep it clean.

Another important one is the **San Luis Rey Watershed**, located in the northern part of the county, including towns like **Oceanside, Bonsall, and Valley Center**. It provides habitat for fish like the endangered **steelhead trout** and is an important source of water for nearby farms.

The **Tijuana River Watershed** is different because it crosses the international border between the **United States and Mexico**. This can make it harder to manage pollution and water quality, since it involves cooperation between two countries.

Why Watersheds Matter

Watersheds are **essential to life**. They **collect and carry fresh water**, which we use for things like **drinking, farming, cooking, and cleaning**. In fact, most of the water that comes out of your faucet has traveled through a watershed at some point! In San Diego all of our watersheds eventually lead to the Pacific Ocean. This means that everything that they carry eventually leads to the ocean!

But watersheds do more than move water—they also **support ecosystems**. That means they provide homes for all kinds of living things. In San Diego watersheds, you can find **birds, frogs, lizards, insects, native plants, and fish**. Many of these creatures depend on clean water to survive. If the watershed is healthy, it helps **filter out pollution** naturally, keeping rivers and oceans cleaner and protecting marine life.

The Problem with Pollution

Unfortunately, watersheds can also become pathways for **pollution**. When it rains in San Diego, the water runs across rooftops, streets, parking lots, and lawns. If there's **trash, oil, soap, fertilizer, or pet waste** on the ground, the water picks it up and carries it through the storm drain system. Here's the scary part: in San Diego, most storm drains **do not lead to a water treatment plant**. They go straight into nearby creeks, rivers, or the ocean.

This pollution can cause big problems. It can **harm fish and birds**, **close down beaches**, and even make people sick if they swim or surf in contaminated water. In some cases, pollution from just one storm can take days or weeks to clear up.

How You Can Help

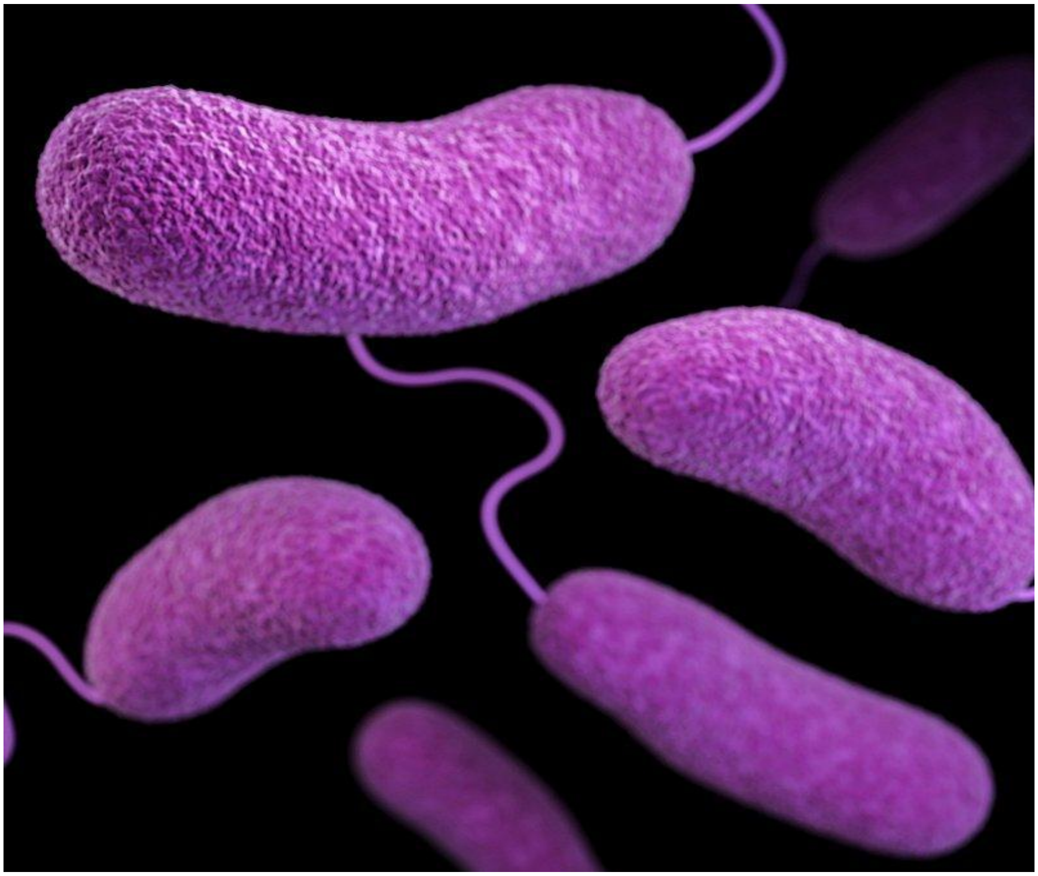
The good news is, everyone—**including you**—can help protect our watersheds. Small actions can make a big difference. For example, **throwing trash in the right place** keeps it from washing into storm drains. **Never pour oil or chemicals down sinks or storm drains**, because they will end up in the water. **Picking up pet waste** keeps harmful bacteria out of rivers and beaches.

Planting **native plants** at school or at home is another great way to help. Native plants like **California poppies, sagebrush, or coast live oak trees** have deep roots that help **absorb rainwater**, **prevent soil erosion**, and **reduce flooding**. They also need less water and fewer chemicals to stay healthy.

Conclusion

A watershed is more than just an area of land—it's a living system that connects **people, water, animals, and the environment**. In San Diego, our watersheds lead to the ocean, the bay, and even across international borders. By protecting our watersheds, we are protecting the health of our cities, our coastlines, and our planet. Every drop of water—and every action you take—matters.

What Are Bacteria?



You’ve probably heard the word “bacteria” before—maybe at the doctor’s office, in a science class, or even on a bottle of hand sanitizer. But what exactly are bacteria, and what do they do? The answer might surprise you!

Bacteria are tiny living organisms. In fact, they’re so small that you need a microscope to see them. Even though you can’t see them, bacteria are all around you—on your skin, in the soil, in food, and even inside your body. But don’t worry! Not all bacteria are bad. In fact, many of them are helpful and even essential to life.

Bacteria are one of the oldest and simplest life forms on Earth. Each one is made up of just a single cell, but they can do some amazing things. Some bacteria can survive in the hottest deserts, the coldest oceans, and even inside volcanoes. Others live inside humans and animals, helping with digestion and keeping our bodies healthy. But here’s something cool: your body is actually full of bacteria—trillions of them! Bacteria also play a huge role in the environment. They help break down dead plants and animals, returning nutrients to the soil. Some bacteria are used to make food like cheese, yogurt, and pickles. Scientists even use bacteria in labs to create medicine and clean up pollution.

The Effects of Bacteria on Human Health

When people hear the word **bacteria**, they often think of getting sick. But did you know that not all bacteria are bad? In fact, bacteria can affect your health in both helpful and harmful ways. Some bacteria make us sick, while others actually help us stay healthy. Let's explore how these tiny living organisms can have such a big impact on our bodies.

Bad Bacteria: The Trouble-Makers

Some bacteria are harmful and can cause illnesses. These are often called **pathogenic bacteria**. They can enter our bodies through contaminated food or water, cuts in our skin, or even from being near someone who is sick. Once inside, they multiply quickly and may release toxins, which are harmful substances that damage our cells.

Some common diseases caused by bacteria include **strep throat**, **tuberculosis**, **urinary tract infections**, and **food poisoning**. To fight these infections, doctors often prescribe **antibiotics**, which are medicines designed to kill or stop the growth of harmful bacteria. However, if antibiotics are used too often or not taken properly, bacteria can become resistant and harder to treat.

Good Bacteria: The Helpers

Not all bacteria are bad! In fact, many bacteria are **essential for our health**. You have **trillions of bacteria living in your body right now**, especially in your intestines. This group of helpful bacteria is called your **gut microbiome**. These bacteria help you digest food, absorb vitamins, and protect your body from harmful germs. Some even produce substances that improve your mood and immune system.

Certain foods, like **yogurt, kefir, and sauerkraut**, contain live helpful bacteria known as **probiotics**. Eating these foods can support the healthy bacteria in your gut and help keep your digestive system balanced.

Keeping the Balance

Your health depends on having the right **balance** of bacteria. If harmful bacteria grow too much, or if helpful bacteria are destroyed (like when you take antibiotics), you might feel sick or have stomach problems. That's why it's important to only take antibiotics when needed and to eat a healthy diet that supports your good bacteria.

In Conclusion

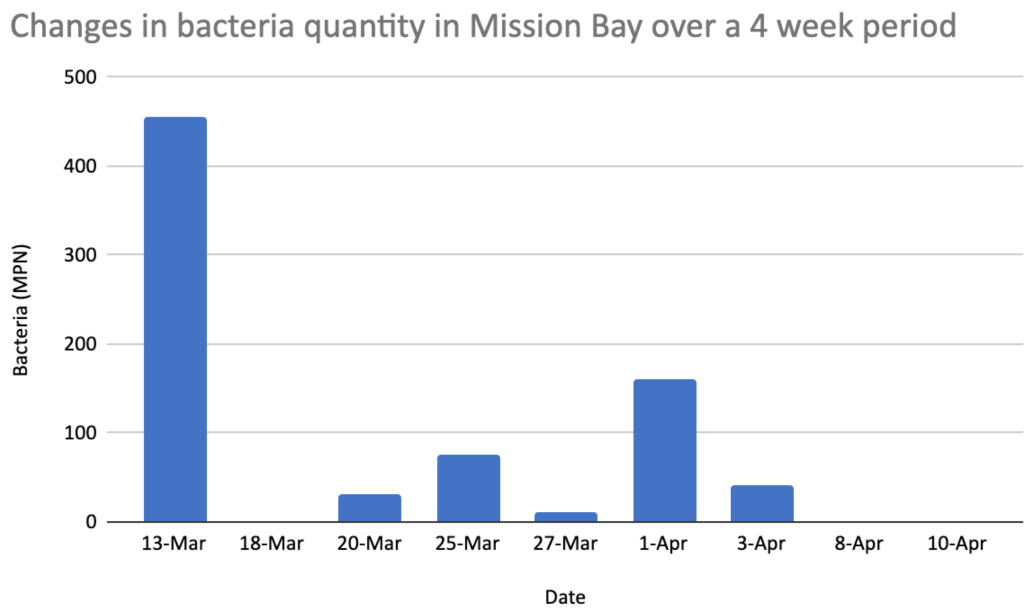
Bacteria are small but powerful. Some can make you sick, while others keep your body working properly. By learning how bacteria affect your health—and how to take care of the good ones—

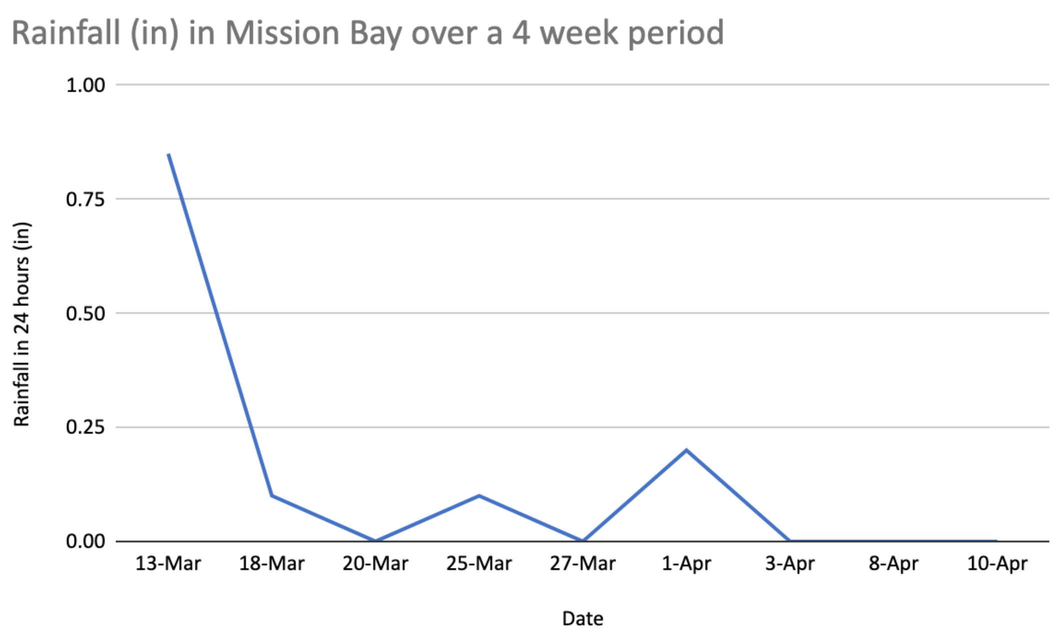
you can stay healthier and help your body stay in balance. So next time you think about bacteria, remember: they're not all bad. Some are your best tiny friends!

Appendix E: Additional Data Student Handout

Additional Data Sets

Data Set 1: Mission Bay *Enterococcus* MPN and Rainfall





Source: Data collected by Mr. Pettek

Data Set 2: Poor Sewage Treatment and Bacterial Level

Poor Sewage Treatment Nearby	
Location	% of Samples that were Safe in the last year
Imperial Beach	27% of samples were safe
Coronado	71% of samples were safe
Mission Bay (Campland)	81% of samples were safe

Good Sewage Treatment Nearby	
Location	% of Samples that were Safe in the last year
Sunset Cliffs National Park	94% of samples were safe
Tourmaline Surf Park	85% of samples were safe
Torrey Pines State Beach	92% of samples were safe

Source: Blue Water Task Force (Surfrider Foundation)

Appendix F: Lesson Plan Overview

Bacteria in Our Waterways Push-In Unit

Goals

Students will use various types of evidence to make a claim about factors that affect the amount of bacteria in their local waterways.

Students will use a lab protocol to determine the factors that influence the amount of bacteria in their local waterways.

Standards

MS-ESS3-1 Earth and Human Activity - Construct a scientific explanation based on evidence for how the uneven distributions of Earth's mineral, energy, and groundwater resource are the result of past and current geoscience processes.

MS-ESS3-3 Earth and Human Activity - Apply scientific principles to design a method for monitoring and minimizing a human impact on the environment.

Outline

Day	Description of Activity	Links
1	Goal: Introduce students to major concepts, (watershed and bacteria), through a jigsaw reading activity. Lesson Plan - Slides (1-7) 1. Begin with short pre-assessment (Watershed Assessment)Watch introduction video 2. Assign student two different groups, each will read one article and take notes on it. 3. Pair up students who read different articles and have them share. 4. Answer final analysis question	Watershed Jigsaw Pre-Assessment Slides
2	Goal: Complete water quality testing lab, and provide	Lab Protocol Surfrider

	students with more evidence to support their claim. Lesson Plan - Slides (8-12) 1. Review intro with students 2. Review procedure and make hypothesis 3. Split students into groups and test samples (ocean vs lagoon) 4. Provide additional evidence	Student Worksheet Additional Data Slides
3	Goal: Analyze data. Make a claim about factors that influence bacteria in our waterways. Lesson Plan - Slides (13-17) 1. Analyze results from previous day 2. Students answer the question using evidence from the lab, and any additional evidence they can. 3. Retake Watershed Assessment	Student Worksheet Slides

Appendix G: Revised Pre/Post Assessment

Watershed Assessment (Revised)

Please answer these questions to the best of your ability. Good Luck!

1. Email *

2. Choose the option that best describes how a watershed works?

Mark only one oval.

- ◯ Water evaporates and then returns to the ecosystem when it rains
- ◯ It works like a bowl that funnels all water to the lowest point (lake or ocean)
- ◯ It works like a machine that pushes water uphill to the ocean or a lake

3. What can bacteria do? Choose all that apply.

Check all that apply.

- ☐ Help plants and animals live
- ☐ Make you sick
- ☐ Break down dead things in an ecosystem
- ☐ Survive in different environments

4. What factors affect how much bacteria reaches our ocean? Choose all that apply.

Check all that apply.

- [ ] Rain washes harmful bacteria into the ocean
- [ ] Harmful bacteria is not treated by waste management facility
- [ ] Harmful bacteria grows by itself in the ocean
- [ ] Wind blows harmful bacteria into the ocean

5. Which beach you think would have the most bacteria in the water? Explain your choice below.

A) A beach near a lagoon
B) A beach not near any storm drains, or sewage treatment plants
C) A beach near a polluted creek after the rain

Appendix H: Revised Student Handout

Water Quality Testing Lab

Introduction

Scientists test the amount of bacteria in the water to estimate the risk of a person getting sick if they enter the water. They do this by **testing for the most probable number (MPN) of bacteria** which allows them to estimate that risk. The **higher the MPN the more bacteria in the water and the more likely somebody would get sick**. You are going to test to see if more bacteria is found in lagoon water (freshwater that enters the ocean) or if ocean water has more bacteria.

Question

What factors affect how much bacteria enters the water?

Procedure

7. Teacher will collect two different samples, one from the ocean (Cardiff Reef) and one from a freshwater source (San Elijo Lagoon)
8. Ocean Sample Preparation
    1. Place 10 mL of ocean sample into a sterile container.
    2. Add 90 mL of distilled water into a sterile container.
    3. Add Enteroalert reagent, and mix until completely dissolved.
    4. Pour sample into QUANTI tray, seal, and label.
9. Freshwater Sample Preparation
    1. Place 10 mL of Freshwater sample into sterile container.
    2. Add 90 mL of distilled water into a sterile container.
    3. Add Enteroalert reagent, and mix until completely dissolved.
    4. Pour sample into a separate QUANTI tray, seal, and label.
10. Place the sealed QUANTI trays into an incubator set for 41 degrees C. Leave for 24 hours. *Done by the teacher*
11. After incubation, place the tray under a fluorescent light, and record the number of large, and small wells. *Done by the teacher*
12. Determine MPN using IDEXX data sheet. *Done by the teacher*

Hypothesis

I think that the *type here* sample will contain higher amounts of bacteria because *type here*.

Data

Group Number	Ocean Sample MPN/100mL	Lagoon Sample MPN/100mL
1		
2		
3		
4		
Average		

Additional Data

Click here to find additional data to help with answering our question. Summarize both pieces of additional data in the space below.

Data Set 1: Mission Bay Enterococcus MPN and Rainfall *I notice that*   Data Set 2: Poor Sewage Treatment and Bacteria Level *I notice that*

Conclusions

In the space below answer the question, make sure to reference the data from the lab and one additional piece of evidence in your answer.

Question: What factors affect how much bacteria enters the water, and why is this a problem?

I think that *type here* factors affect the amount of bacteria in our waterways. I think this because, *type here* . This is a problem because, *type here* .