Spring 4-1-2018

Impact of Suspended Particles on Bacterial Concentrations in Great Bay Estuary Oysters

Audrey Berenson
University of New Hampshire, Durham

INQUIRY journal

www.unh.edu/inquiryjournal SPRING 2018

Research Article

Impact of Suspended Particles on Bacterial Concentrations in Great Bay Estuary Oysters

—Audrey Berenson

Food poisoning caused by the bacteria *Vibrio parahaemolyticus* has recently become a problem in the northeastern United States, particularly in the summer (Gulf of Maine Council on the Marine Environment, 2011; Urquhart, Jones, Yu, Schuster, & Marcinkiewicz, 2016). As climate change causes water temperatures to rise, these pathogenic (infectious) and non-pathogenic (harmless) *V. parahaemolyticus* are becoming more common residents in New England's marine ecosystems, which have previously been chillier and did not provide optimal conditions for the bacteria to thrive (Baker-Austin, Trinanes, Gonzalez-Esçalona, & Martinez-Urtaza, 2017). *V. parahaemolyticus* commonly lives in raw oysters due to the oyster's active filtration of the water around them. This is especially concerning due to how much New England residents love eating raw seafood. My research, funded by a Summer Undergraduate Research Fellowship (SURF), was an exploration of the mechanism of *V. parahaemolyticus* contamination of oysters in Great Bay Estuary, where illnesses have yet to occur.

The lab that I work in is particularly interested in how the bacteria are getting into the oysters. The oysters filtering the bacteria out of the water seems like a probable culprit, but we are further exploring the role of particles floating around in the water, such as plankton and sediment (Lovell, 2017). We want to know whether concentrations of these suspended particles influence bacterial concentrations in oysters.

The author collects Great Bay oysters before storing them in a cooler for later analysis in the lab.

When oysters filter the water to collect organic material to eat, they also collect any suspended particles in the water column, including plankton, algae, plant material, and sediment. Therefore, they are likely collecting and accumulating any bacteria that cling to the particles. Human activity, such as boating, swimming, fishing, and harvesting shellfish, can cause sediment and plankton on the bottom of the bay to be resuspended into the water, where it floats around until it settles to the bottom again or gets filtered by shellfish. Previous research in our lab has shown that *V. parahaemolyticus* and other *Vibrio* species like to hang out in the sediment at the

bottom of the bay, but my SURF project focused on the particles that are floating in the water and are more likely to be filtered by shellfish (Jones, Striplin, Mahoney, Cooper, & Whistler, 2010). I hypothesized that increased suspended particles can increase *V. parahaemolyticus* concentrations in oysters.

My Journey to SURF

I am fascinated by microbiology, particularly pathogenic microorganisms. I now have big dreams to research infectious disease someday, but I didn't know that during my first two years of college. I started out as a genetics major, but switched to the biomedical science: medical microbiology program after my first year. I knew from the start that I wanted to work in a research lab, so I set out to find a job or internship at the end of my first year. I applied to work on a multidisciplinary research project exploring water quality, even though I had no prior lab experience.

I wasn't offered the position for the project on water quality, but I really connected with the interviewer, Dr. Stephen Jones, who offered me a position in his research lab working with *Vibrio* bacteria. Dr. Jones is a professor of natural resources at the University of New Hampshire (UNH) and the principal investigator (PI) in the lab where I've worked on a variety of projects over the past two years. The primary project I am involved with is *Vibrio* surveillance in Great Bay. *Surveillance* is a term that is used in microbiology and infectious disease research to describe the active monitoring of a disease/disease-causing microbe in an area. We go out in a boat every two weeks in the warm months and every four weeks in the cooler months to collect oysters, plankton, sediment, and water samples for analysis. We use this data to determine where the *Vibrio* bacteria concentrate in the ecosystem. I've also worked on counting and categorizing plankton from the bay, measuring *Enterococci* and *Escherichia coli* bacterial concentrations in water samples from the area, and using microbial source tracking to determine where fecal contamination is coming from in samples from several New England states.

I loved working in the lab from the beginning. Fun coworkers, opportunities to work on a variety of projects, and working with bacteria have made it a fabulous experience, but I couldn't always make a personal connection to the research we were doing. Even after my SURF proposal was approved and I began my project, I wasn't sure how interested I was in environmental microbiology; I was more interested in medical microbiology! I wasn't entirely sure how all of this connected back to me and my big dreams to impact the world. I knew the experience was worth it and that I was a part of something great, and I was getting lots of valuable experience, gaining lab skills, and making connections with my coworkers and others I met along the way. I knew that the research that we were doing was contributing to addressing issues for both the oyster farming industry and public health, but I longed for a deeper, more personal connection to what I was doing. I found that connection when I attended the EcoHealthNet program at Tufts University in June 2017.

EcoHealthNet ended up changing my life, along with my views on my research project. The program was limited to undergraduate and graduate students who were involved in research and had interests in infectious disease, antimicrobial resistance, and zoonotic diseases (diseases that can spread between animals and people, such as West Nile virus or *Salmonella*). I got to spend a week at Tufts

University learning about ecohealth, a new field that researches the connections between environmental health and the health of all living things, including microbes, wildlife, and people. The program focused on how people impact the world, which then impacts the way diseases emerge and impact people (as well as wildlife). After a week of learning and hearing about all the incredible research the other students were doing, meeting top researchers, and being inspired, I fell in love with the idea of ecohealth, and I realized that my project directly connected to this idea. I was suddenly so much more interested not only in the benefits of doing a research project but also in the results and analysis and what it all meant.

How to SURF

In order to gather the data needed for my project, I spent ten weeks collecting samples from the Oyster River and Nannie Island, two different sites in Great Bay. Every other week a Ph.D. student, some lab technicians, and I would go out in a boat dressed in clothes we could get covered in oyster goo and mud.

The author uses an oyster rake in the waters of Great Bay.

We collected at least twelve medium-to-large oysters at each site, along with a Whirlpak (sterile plastic baggy) of sediment from the bottom of the bay, a Nalgene bottle of water from the surface, a second Nalgene bottle of water from just below the surface for my project, and a small bottle of bay water with concentrated plankton in it. We collected the oysters with an oyster rake, which really just looks like a giant pair of salad tongs.

The sediment was collected off the oysters when they were pulled up with the rake. The plankton was concentrated with a big, clear, plastic box with a filter on the bottom, called the Schindler-Patalas (which we lovingly call “Patty”). We also came armed with a couple of coolers to put the samples in, and a speaker so we could listen to classic rock while we got eaten alive by greenhead flies.

We processed the bottles of water, small bottles of concentrated plankton, baggies of sediment, and baskets of oysters in Jackson Estuarine Laboratory at Adams Point on Great Bay. I processed the suspended sediment samples, which were specific to my project. The oyster, plankton, sediment, and additional water samples were processed by both my lab mates and me.

First, I recorded the weights of the clean filters. Then, I filtered 280 mL of each water sample through a different filter. After that, I dried the filters overnight in a drying oven so that the water would evaporate, and I could weigh the filters again to determine the mass of only the dried sediment. Once I did this, I placed the filters into a muffler furnace, which burned off the organic material, including

plant matter, plankton, and algae, at a very high temperature (550°C). What remained on the filters was only the inorganic sediment, such as rocks, sand, and clay, which was also weighed. This gave an estimate as to how much sediment (in mg/L) was suspended in the water column at the time of collection, as well as the proportion of inorganic to organic sediment, determined by comparing the mass of the dried sediment to the mass of the sediment left after burning in the muffler furnace.

The oysters, plankton, water, and sediment were all processed a little differently initially, but they all ended up being diluted out in a nutrient broth so that the bacteria could grow and we could determine the bacterial concentrations. All samples were set up in a three-tube most-probable-number (MPN) scheme, which is a type of serial dilution (each set of three tubes is ten times more dilute than the last) that allows for estimation of bacterial concentrations.

Once the dilution tubes were set up, they were incubated overnight so that the bacteria could grow and divide. The tubes with bacteria in them appeared to be cloudy the next day. We streaked the media from the cloudy tubes onto selective agar plates, which select for different species of *Vibrio* bacteria, and differentiate them by the color of the colonies that grow. This helped us to determine whether the bacteria were *Vibrio* or not. The suspected *Vibrio* bacteria were further isolated with more streaking on non-selective agar, which is used to spread the bacteria out more so that isolated colonies can be selected for the next step. After that, they were grown in nutrient broth and tested for the presence of different *Vibrio* genes through a process called polymerase chain reaction.

Once all of this analysis was done, I was able to compare the mass of the suspended particles to the concentration of *V. parahaemolyticus* in the oysters to determine if there was a relationship between the two.

What I Found While SURF-ing

The results of this project show a positive relationship between the mass of suspended particles and the *V. parahaemolyticus* concentration in oysters. This means that as the mass of the suspended particles increased, the bacterial concentration in the oysters also increased. However, this does not necessarily mean that the increased suspended sediment in the water is the sole cause of increased bacteria; it simply means that there is a relationship (Figure 1).

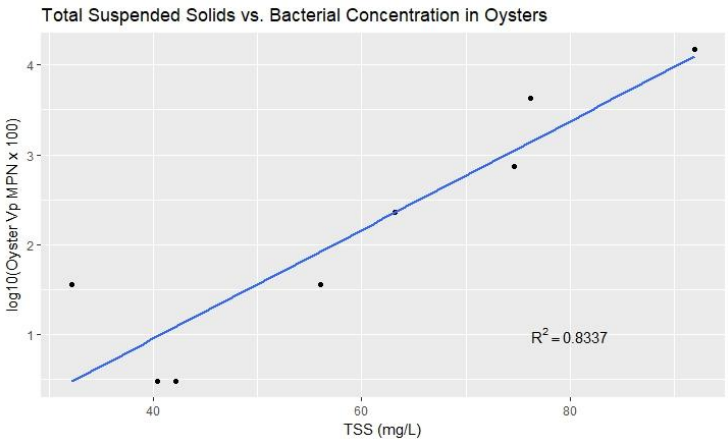

Figure 1. A comparison of the total suspended solids (mg/mL) and the concentration of *V. parahaemolyticus* in oysters. The concentration of *V. parahaemolyticus* was multiplied by 100 before being transformed logarithmically so that all values would be positive and a linear model could be used. Two outliers were excluded so that the R^2 value increased from 0.3731 to 0.8337.

Perhaps even more interesting, this relationship did not apply to all of the suspended solids. Only the mass of the inorganic suspended particles showed this positive correlation with the *V. parahaemolyticus* concentration in the oysters. The mass of the organic suspended particles did not have a clear relationship with the *V. parahaemolyticus* concentration in the oysters. This indicates that perhaps suspended inorganic particles could be a contributor to oyster contamination, while the role of suspended organic particles is more complex.

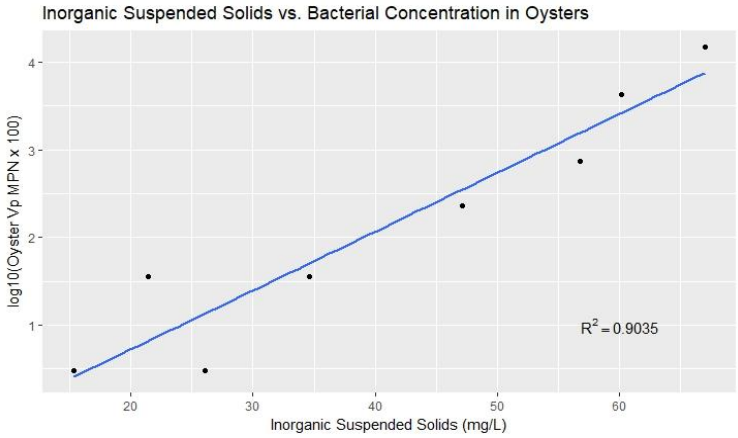

Figure 2. A linear regression demonstrating the strong correlation between the inorganic suspended solids and the concentration of *V. parahaemolyticus* in oysters. The concentration data was transformed in the same fashion as it was in Figure 1. The same two outliers were excluded as well, so that the R^2 value increased from 0.3794 to 0.9035.

The relationship between the mass of the inorganic particles and the *V. parahaemolyticus* concentration in oysters was actually even stronger than the relationship between the mass of all of the suspended particles and the *V. parahaemolyticus* concentration in oysters. Using the program R to produce predictive models for the *V. parahaemolyticus* concentration showed that including the mass of the suspended organic particles actually made the models weaker than when only the inorganic portion of suspended particles was used in the model. This clearly illustrates that the mass of the inorganic particles is a better indicator of *V. parahaemolyticus* concentrations (Figures 2 and 3).

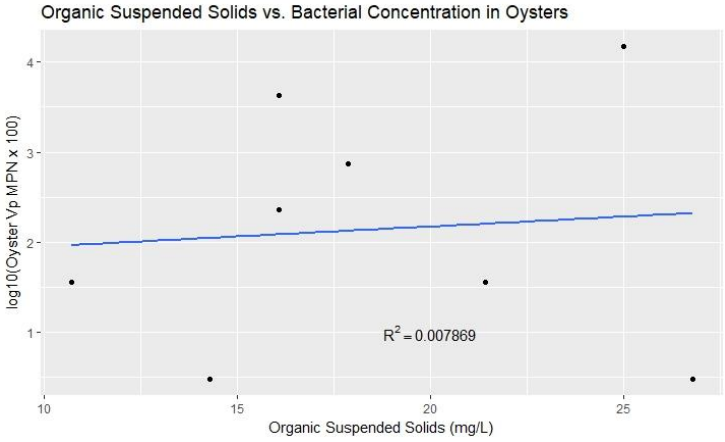

Figure 3. No correlation was found between the organic suspended solids and the concentration of *V. parahaemolyticus* in oysters. The data was transformed in the same fashion as in Figures 1 and 2, and same the two outliers were excluded. The mass of organic suspended solids per volume of water is not a good indicator of *V. parahaemolyticus* concentration in Great Bay oysters.

When oysters filter the water to collect organic material to eat, they also collect any suspended particles in the water column, including plankton, algae, plant material, and sediment. Therefore, they are likely collecting and accumulating any bacteria that cling to the particles. Human activity, such as boating, swimming, fishing, and harvesting shellfish, can cause sediment and plankton on the bottom of the bay to be resuspended into the water, where it floats around until it settles to the bottom again or gets filtered by shellfish. Previous research in our lab has shown that *V. parahaemolyticus* and other *Vibrio* species like to hang out in the sediment at the bottom of the bay, but my SURF project focused on the particles that are

floating in the water and are more likely to be filtered by shellfish (Jones, Striplin, Mahoney, Cooper, & Whistler, 2010). I hypothesized that increased suspended particles can increase *V. parahaemolyticus* concentrations in oysters.

Moving Forward

The initial thought of obtaining funding for my research was scary. I didn't know how to put together a budget form, what to propose in order to get funding, or even how I would go about doing the project itself. However, I did know that I wanted the experience. I was excited when my SURF proposal was accepted, but I was still feeling nervous and a little bit lost. Undertaking a project and making big decisions is difficult when you've never done it before. Not being an expert on environmental microbiology, ecology, and the Great Bay ecosystem didn't help. However, the experience that I gained from this project has been incredibly valuable to me, and I know that it has made me a much better researcher.

Since wrapping up the sample collection for my project in August, I've applied my skills to other projects in the lab and prepared to present my SURF project at the Undergraduate Research Conference at UNH in April 2018. I'm excited to share my research with others.

Working on this project over the past year has reinforced what I learned about myself at the EcoHealthNet conference; I am passionate about public health, infectious disease, and how people impact the planet. I will always remember this past year as my first big project and the year that I finally started figuring out what I want to do. I plan on researching infectious disease as a career, tying it back not only to public health, but also to environmental health. In the meantime, I'll be here in the lab, filtering water samples and collecting bacteria.

I would like to thank my advisor, Dr. Stephen Jones, for being such a valuable resource and mentor throughout this project, as well as before and after! I would also like to thank Meghan Hartwick for being a big supporter of my research as well as an incredible resource when it came time to analyze data. Thank you to my coworkers (past and present) at both Jackson Estuarine Lab and Rudman Hall. I could not have done this without you all. To my family and friends—thank you for listening to me talk about bacteria and oysters for way too long. Last but certainly not least, thank you to the Hamel Center for Undergraduate Research and the contributors to my Summer Undergraduate Research Fellowship (SURF) who made all of this possible: Mr. Dana Hamel, the J. Raymond Hepler Endowed Fund, and Ms. Renee Gilberti. This material is based upon work supported by the NH Agricultural Experiment Station, through joint funding of the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award numbers 233555 and 1004199, and the state of New Hampshire. This work also is supported by funding from the National Science Foundation EPSCoR Program, New Hampshire Sea Grant College Program, National Institutes of Health, and the UNH Graduate School.

References

Baker-Austin, C., Trinanes, J. A., Gonzalez-Escalona, N., & Martinez-Urtaza, J. (2017). Non-Cholera Vibrios: the microbial barometer of climate change. *Trends in Microbiology, 25*, 76-84. doi: 10.1016/j.tim.2016.09.008

Gulf of Maine Council on the Marine Environment. (2011). Microbial Pathogens and Biotoxins. *State of the Gulf of Maine,* (September).

Jones, S., Striplin, M., Mahoney, J., Cooper, V., & Whistler, C. (2010). Incidence and abundance of pathogenic Vibrio species in the Great Bay Estuary, New Hampshire. *Proceedings of the Seventh International Conference on Molluscan Shellfish Safety*, *2*(June), 127–134. Retrieved from https://scholar.google.com/scholar?q=ncidence+and+abundance+of+pathogenic+vibrio+species+in+the+Great+Bay+Estuary&btnG=&hl=en&as_sdt=0%2C30#0

Lovell, C. R. (2017). Ecological fitness and virulence features of *Vibrio parahaemolyticus* in estuarine environments. *Applied Microbiology and Biotechnology, 101*, 1781-1794. doi: 10.1007/s00253-017-8096-9

Urquhart, E.A., Jones, S. H., Yu, J. W., Schuster, B. M., Marcinkiewicz, A. L., Whistler, C. A., et al. (2016) Environmental conditions associated with elevated *Vibrio parahaemolyticus* concentrations in Great Bay Estuary, New Hampshire. PLoS ONE 11(5): e0155018. doi:10.1371/journal.pone.0155018

Author and Mentor Bios

Audrey K. Berenson, from Exeter, New Hampshire, will graduate from the University of New Hampshire (UNH) in 2019 with a degree in biomedical science and a focus in medical microbiology. Audrey received a Summer Undergraduate Research Fellowship (SURF) for 2017 after working in Dr. Steve Jones's lab for over a year. Her research topic on bacteria in local oysters derived from her interest in microbiology. "*Vibrio parahaemolyticus* is odd and fascinating, and I thoroughly enjoyed learning more about it," she says. Audrey also learned that conducting research requires a lot of decision making, both before a project starts and throughout the entire process, as unexpected issues arise and plans need to be adapted and altered. As she moved on to preparing for UNH's Undergraduate Research Conference and writing for *Inquiry,* she hoped others would benefit by learning about microorganisms around us, and how our impact on the world can affect the food we eat. Audrey is planning for a career in infectious disease research. "I would love to take part in research that has an effect on policy to better protect our environment and its inhabitants, including people."

Dr. Stephen Jones is a scientist with the New Hampshire Agricultural Experiment Station, which is part of the University of New Hampshire's College of Life Sciences and Agriculture. Jones has been at UNH for thirty years and is currently a research associate professor in the Department of Natural Resources and the Environment (NRESS) and associate director of the New Hampshire Sea Grant Program. He specializes in environmental microbiology and has been studying the ecology

of *Vibrio* species in estuarine ecosystems since 1989. In his time at UNH, Jones has mentored many undergraduate researchers. He says Audrey's project included all of what is expected in conducting environmental research: problems with measurements, unexpected results, and plenty of life experience. On writing for *Inquiry*, Jones says, "It is very useful for Audrey to learn to write first for scientists and then for broad audiences, and to see and appreciate the differences."