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Clearing Up Murky Waters: Clarifying the Relationship Between Indicator Organisms and Disease in Recreational Water Settings

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Clearing Up Murky Waters: Clarifying the Relationship Between Indicator Organisms and Disease in Recreational Water Settings

By

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University of California, Berkeley

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Professor Alan Hubbard

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Abstract

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Doctor of Philosophy in Epidemiology

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Many infectious bacterial and viral agents exist in the world and are located in areas where humans may come into contact with them. Food, water, and environmental locations may be contaminated with infectious material, and detecting the presence of these harmful biologic agents is of import to public health agencies. One method that has been used to determine if infectious agents may be present in food or water is measurement of "indicator" bacteria or viruses. Indicator organisms are easily measured bacteria or viruses whose presence in water or food is thought to parallel the potential presence of infectious agents in the same food or water samples. Because there are so many potential bacteria (or viruses) that may infect a sample, it is impractical to test for all of them; rather, measurement of a single indicator organism may be more feasible.

Indicator bacteria have been used to determine if marine waters at beaches across the United States are safe for swimming. Guidelines issued by the U.S. Environmental Protection Agency (U.S. EPA) have focused on determining when recreational waters may pose a risk of excess gastrointestinal illness among swimmers when compared to non-swimmers. However, marine environments are very complex, and tidal patterns, solar inactivation, water temperature, and many other factors all can influence the presence or absence of indicator and infectious microorganisms in the water. Research has indicated that indicator organisms may be useful in predicting gastrointestinal illness in marine environments, but other health outcomes have been less studied. In order to verify that indicator organisms do track well with infectious organisms, a systematic review and meta analysis was conducted to determine if indicator organisms can predict a different health outcome, skin infection. Once the link between indicator organisms and health outcomes was established, the next goal was to explore different methods to strengthen the relationship between indicator organisms and health. Currently, the U.S. EPA advises that a single bacterial indicator, *Enterococcus*, be measured in marine waters. A binary cutoff of above or below 104 colony forming units per 100 mL is used to advise whether a beach is unsafe or safe for swimming. In order to improve prediction of illness at beaches using indicator organisms, several methods were considered. Flexible statistical modeling techniques, such as

SuperLearning, were used, as well as consideration of multiple biological and physical indicators at the same time. The final aim was to examine the potential sources of the infectious agents, as well as the indicator bacteria, at Avalon beach in Southern California.

The results of this investigation suggest that indicator bacteria can be quite useful in predicting human illness, but perform better under certain conditions. The systematic review and meta-analysis showed that there was a strong relationship between certain indicator organisms and skin infections in marine water settings. Higher concentrations of total coliform, fecal coliform, *E. coli*, *Enterococcus*, and fecal *Streptococci* were associated with increased risk of skin related illness in marine waters. These findings support the biological plausibility of using indicator organisms to predict illness, even in a complicated, dynamic environment such as a marine beach. The second investigation found that application of the U.S. EPA guidelines at Avalon Beach did not accurately predict when waters were unsafe for swimming. However, use of flexible statistical methods (SuperLearner) greatly improved prediction of gastrointestinal illness over traditional modeling methods, such as logistic regression. Further improvements were seen when, instead of using a single indicator organism, combinations of biological and physical indicators were used. By combining physical and biological indicators, it was possible to identify circumstances when elevated concentrations of *Enterococcus* predicted excess gastrointestinal illness in swimmers. When solar radiation levels were low, indicator bacteria concentrations were more strongly associated with adverse health outcomes, whereas higher solar radiation levels were protective. This finding is biologically plausible because it is thought that solar radiation can directly damage indicator bacteria as well as pathogens and render them non-viable. Thus, under high solar radiation conditions, indicator organisms as well as infectious organisms would be inactivated. The final analysis examined groundwater flow as a potential risk to swimmers at Avalon beach. Because of a leaking sewage infrastructure at Avalon, it is thought that groundwater flux might be transporting raw sewage contents into the ocean water. Sewage is known to carry potentially high levels of pathogenic organisms, and thus groundwater flow levels might pose a direct threat to swimmers. When groundwater flow was higher, the incidence of gastrointestinal illness was elevated among swimmers who swallowed water, relative to swimmers who swallowed water on days when groundwater flow was lower. Additionally, the relationship between groundwater flow and solar radiation was similar to that seen with *Enterococcus* and solar radiation. When solar radiation levels were high, groundwater flow was less predictive of excess gastrointestinal illness, as would be expected. When traditional analysis methods were used to relate traditional and rapid indicators to illness, relationships were much stronger when groundwater flow was high versus when groundwater flow levels were lower. In conclusion, the results of these analyses suggest that indicator organisms can be used to predict health outcomes in recreational water settings, but that their performance may be greatly improved by using flexible modeling techniques as well as other indicators, such as solar radiation.

This thesis is dedicated to my parents, Katherine and Robert, as well as my sister, Audrey. Proofreading everyone's papers over the years has really paid off.

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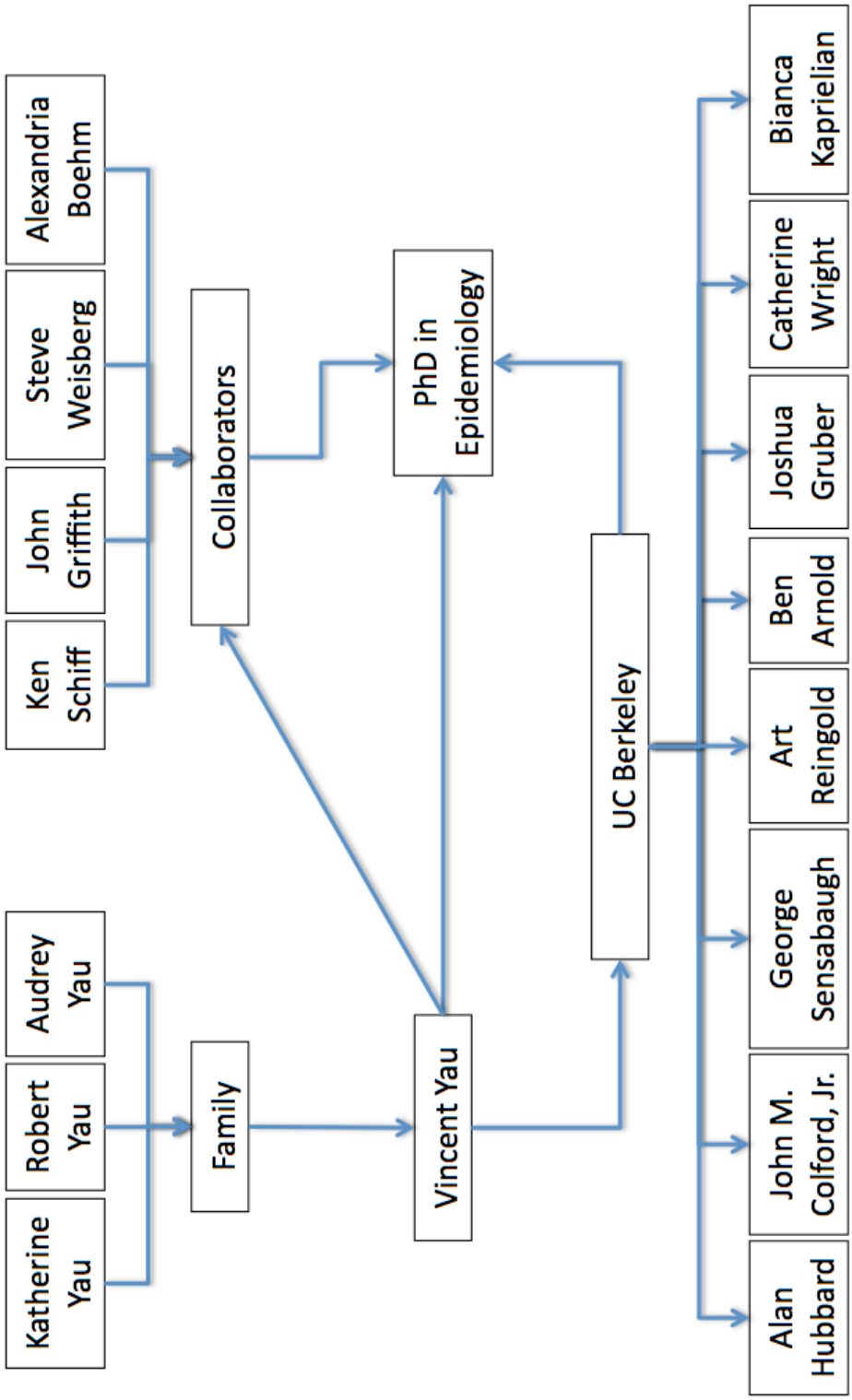
There are many individuals who were “instrumental” to the creation of this thesis. Below is a Directed Acyclic Graph to represent a few of them, though there were many others who belong here.



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Examining the use of bacterial indicators and physical indicators to predict safety of recreational water exposure. Data analyzed using STATA and R statistical programs.

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Assisted in a research study conducted on patients undergoing cardiovascular surgery. Analyzed serum samples for antibiotic levels to determine whether or not antibiotic concentrations were optimal throughout extended surgeries.

Publications

- Yau V, Wade T, deWilde C, Colford, J. Skin-related symptoms following exposure to recreational water: A systematic review and meta-analysis. Journal on Water Quality, Exposure and Health 2009 1: 79-103. Participation: first author, abstracted relevant epidemiological and statistical data from primary literature on water quality, health effects, and microbial indicators in order to conduct a meta-analysis, conducted statistical analyses, and generated relevant figures.
- Burkirwa H, Yau V, et al. Assessing the Impact of Indoor Residual Spraying on Malaria Morbidity Using a Sentinel Site Surveillance System in Western Uganda. Am. J. Trop. Med. Hyg. 2009; 81(4): 611-614. Participation: Conducted all statistical analyses, using time-series methods. Collected data from the field and analyzed for preliminary reports.
- Maiteki-Sebuguzi C, Jagannathan P, Yau V, Clark T, Njama-Meya D, Nzarubara B, Talisuna A, Kamya M, Rosenthal P, Dorsey G, Staedke S. Safety and tolerability of combination antimalarial therapies for uncomplicated falciparum malaria in Ugandan children. Malaria Journal 2008; 7:106. Participation: Generated all models and conducted all statistical analyses to determine if antimalarial medications were associated with adverse health events.
- Caffarelli A, Holden J, Baron E, Lemmens H, D'Souza H, Yau V, Olcott 4th C, Reitz B, Miller C, Van der Starre P. Plasma Cefazolin Levels During Cardiovascular Surgery: Effects of Cardiopulmonary Bypass and Profound Hypothermic Circulatory Arrest. J Thorac Cardiovasc Surg 2006; 131(6): 1338-43. Participation: Conducted lab work and biological tests to determine antibiotic concentrations in patient blood samples.
- Allen E, Ding J, Wang W, Pramanik S, Chou J, Yau V, Yang Y. Gigaxonin-Controlled Degradation of MAP1B Light Chain is Critical to Neuronal Survival. Nature 2005; 438:(7065):224-8. Participation: Ran western blots and aided with lab work to determine protein concentrations.

Relevant Courses

- PH252C (Intervention Trial Design) Material covered: Design of clinical and population-level field trials (blinding, randomization, sample size calculation, recruitment, adverse effects monitoring, adherence, surrogate outcomes)
- PH142A (Introduction to Probability and Statistics in Biology and Public Health) Material covered: Descriptive statistics, probability, probability distributions, point and interval estimation, hypothesis testing, chi-square, correlation and regression.
- PH241 (Statistical Analysis of Categorical Data) Material Covered: Measures of association, causal inference, confounding interaction, binary regression, logistic regression.
- PH252B (Modeling the Dynamics of Infectious Disease Processes) Material Covered: standard infectious disease models (Markov chains, etc).
- PH248 (Statistical/Computer Analysis Using R) Material Covered: use of R for descriptive methods, simulation techniques, linear models, and estimation.
- PH252 (Epidemiological Analysis) Material Covered: matched data, spatial analysis, logistic and Poisson regression, survival analysis, Kaplan-Meier estimation, survival distributions, parametric and semi-parametric survival analysis models.
- PH252D (Introduction to Marginal Structural Models) Material Covered: use of inverse probability treatment weighting with respect to marginal structural models, data simulation, directed acyclic graphs, use of algorithms for best model fitting.
- EDUC275H (Hierarchical and Longitudinal Modeling) Material Covered: statistical approaches to hierarchical linear and generalized linear models for longitudinal or clustered data.
- PH242C (Longitudinal Data Analysis) Material Covered: regression models to estimate effects with data following subjects over time, disease incidence modeling for linear and non-linear models, survival analysis, linear models, logistic and Poisson regression, matrix algebra.
- PH252C (Intervention Trials) Material Covered: Design, logistics, and analysis of randomized controlled trials.
- PHC240B (Survival Analysis) Material Covered: Analysis of longitudinal data, use of advanced algorithms to estimate causal effects.
- Stat 153 (Introduction to Time Series) Material Covered: Analysis of correlated/temporal data with various time series models

Work Experience

9/10 – 9/10 **Volunteer.** Communicable Disease Control Unit, San Francisco Department of Public Health

Helped respond to pertussis outbreak in California. Communicated with other health departments, traced close contacts of suspected/confirmed cases, recorded case information into main database.

9/03 – 6/05	**Unit Secretary**. Operating Room, Stanford Hospital Responsible for mediating all communications between nursing units and the Operating Room. Collected information about emergency and trauma cases, arranged for proper surgical tools to be sent to the operating room for specific procedures, and contacted physicians and nurses necessary to perform the surgery. Editor and distributor of the final surgery schedule.
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CHAPTER 1: INTRODUCTION

1.1 Use of Indicator Organisms to Track Pathogens

1.1.1 History and Background

The concept of an indicator organism arose late in the 19th century, in the context of water safety. *Salmonella*, a genus of bacteria with over 2000 species, including *Salmonella typhi* and *Salmonella paratyphi*, was known to be associated with severe illness, ranging from typhoid fever to gastroenteritis (CDC 2010b, CDC 2010c, CDC 2010d). However, testing for *Salmonella* was extremely difficult at that time. Not only was the test very slow, but it was also very difficult to use (Kornacki 2011). Because *Salmonella* was associated with human fecal material, it was thought that measuring other bacteria associated with fecal material might give an "indication" of whether fecal contamination might be present. If fecal contamination was detected, it would then be logical to assume that there was a higher probability of *Salmonella* being in the water. The bacterium chosen was *E. coli*, which was found to be ubiquitous in fecal material. Compared to *Salmonella*, *E. coli* was much easier to measure and thus became the first "indicator" or "index" organism. Because *E. coli* was part of a group of organisms known as "coliforms," the idea of testing for *E. coli* was expanded to testing for the presence of broad groups of coliform bacteria (Cornell 2007).

Since that time, the use of indicator organisms has been adopted by a number of fields to determine the safety of a variety of products and environmental conditions. Because of the wide variety of pathogens that may be involved in food or water safety, testing for all of them individually is impractical. For instance, over 100 human enteric viruses may be transmitted by human feces, and testing for each of these on a regular basis is extremely labor-intensive (Puig 1994). Instead, measuring a single indicator that may suggest fecal contamination or the presence of spoilage organisms may be more useful. Depending on the pathogens or organisms thought to be involved in each situation, different indicator organisms are used. Ideally, the indicator organism shares biological characteristics with the pathogens of interest, with the difference being that the indicator can be measured more rapidly, inexpensively, and easily than the pathogens/organisms of interest. Similarities between the indicator organism and the pathogen should include similar patterns of growth and die-off in the given environment, ensuring a correlation between the concentrations of the two organisms. Additionally, similar susceptibility to human interventions (e.g. chlorination) is desirable. Other favorable traits for an indicator organism are that it is not itself pathogenic, it does not naturally live in the environment being tested, and the source of the indicator organism is the same as the pathogen(s) of interest (e.g. feces). Three major fields that use indicator organisms to assess safety are wastewater discharge, food safety, and recreational water quality monitoring.

1.1.2 Food Safety

In the food safety field, indicator organisms are used for a variety of tasks. Indicator organisms are used to determine how hygienic food production facilities are, before, during, and after food processing. Tests may attempt to detect fecal contamination of food preparation

surfaces. In addition, indicator organisms may be used to determine if the raw materials or ingredients are contaminated. For instance, there have been studies correlating the presence of indicator organisms with *E. coli* levels on beef carcasses across multiple lots of carcasses (Siragusa 1998, Siragusa 2001). A third application of indicator organisms occurs after a food product has traveled through a "biocidal critical control point." Examples of biocidal critical control points are pasteurization of dairy products or use of a smokehouse to treat meat. Traces of coliform bacteria, yeast, or mold in any sample of these products taken after processing would indicate that there was a fault in the processing step (Kornacki 2011).

In some circumstances, the presence of pathogens is not what is of interest when using indicator organisms. For example, indicator organisms can also be used to determine if there are non-pathogenic microflora that might accelerate spoilage of a food product. Many factors can contribute to spoilage of a food product, ranging from the ingredients used or the pH of the product, to the temperature at which the food is stored. In order to determine if microflora are present, testers may take a normally refrigerated product and incubate it at a temperature that promotes growth of microorganisms that typically cause spoilage (Kornacki 2011).

Several common indicator organisms are used to determine if food is safe. Some of the bacteria used are encompassed within the family Enterobacteriaceae. *E. coli*, for example, is often used. Other indicator bacteria are within the genus *Enterococci*, such as *E. faecalis* and *E. faecium.* Bacteriophages, such as coliphage, F-specific phage, and *Bacteroides*, may also be used to determine if viral agents may be present. Because these bacteriophages have similar characteristics to viruses and are present in human feces, they are good candidates to serve as indicators of viral presence.

1.1.3 Wastewater

Exposure to wastewater has always been a major concern for human health. Many disease-causing infectious agents are transmitted via the fecal oral route, and many viruses, parasites, and bacteria are present in sewage. Examples of the infectious agents that may be found in fecal material include Hepatitis A virus (HAV), Norwalk-like viruses, adenoviruses, *Giardia lamblia*, *Cryptosporidium parvum*, and *E. coli* (Scott 2003). Wastewater may be reclaimed or "recycled" for domestic use, but it may also be piped to a marine outfall.

Wastewater reclamation has been in use in California since the 1890's, and there are currently over two hundred reclamation plants in California. Wastewater is commonly used to irrigate golf courses and food crops, and it is sometimes discharged into waters where recreation takes place. Most reclaimed wastewater is used in agriculture, and regulations exist to ensure that reclaimed water used in this setting meets certain standards. For example, if wastewater is intended to be used for agriculture, the median number of coliforms should be ≤ 2.2 per 100 mL for samples taken over a seven-day period (Pescod 1992). More stringent requirements exist for reclaimed wastewater used in areas where human contact is more likely, such as playgrounds and schoolyards.

Marine outfalls are pipelines or tunnels that discharge water from a variety of sources out to sea. Municipal wastewater being discharged may receive no treatment or only primary treatment, based on the assumption that the enormous volume of the ocean will adequately dilute any pathogens. Many of these outfalls are located far from shore in order to ensure that pathogens are unlikely to travel back to shore, where infection of humans could take place (Beder 1989). In addition to being reclaimed or piped out to sea, wastewater can also leech into groundwater and be carried into water sources where humans may make contact with the transported wastewater.

Because wastewater is often reused, standards based on indicator organisms have been developed to determine when wastewater is relatively safe. Many different indicator organisms have been considered. Total coliforms, which are lactose-fermenting bacteria that grow at 37º C while not forming spores, are one traditional indicator. A subset of total coliforms is fecal coliforms, which are the bacteria capable of growing at an elevated temperature, 44.5º C. A third traditionally used indicator is *Enterococcus*, as measured using the EPA 1600 method (Rose 2004).

Although filtration and disinfection of wastewater does take place before reuse of the water, there is evidence that reclaimed water may still contain enteric viruses or protozoans such as *Giardia lamblia* and *Cryptosporidium parvum*. Even more concerning is that these pathogens have sometimes been detected when indicator bacteria were not detectable (Rose 2004). One study estimated that if removal efficiencies at disinfectant and treatment plants were not met, then wastewater discharged into recreational impoundments might meet regulatory guidelines only 25% of the time (Tanaka 1998). This finding is particularly concerning given the large number of persons who are exposed to recreational waters each year.

1.1.4 Recreational Water Settings

Safety risks related to exposure to recreational waters contaminated with sewage have been recognized in the United States since the 1920's. The American Public Health Association conducted the first reviews of disease associated with exposure to recreational waters at the time (Simons 1922). However, further studies were not undertaken until almost thirty years later, when two major epidemiologic studies were undertaken by the United States Public Health Service (Stevenson 1953). In a 1948 study, it was seen that swimmers in Lake Michigan experienced higher overall illness rates than non-swimmers. In 1949, a second study was conducted in Dayton, Kentucky along the Ohio River, and again swimmers were found to have higher risk of illness than non-swimmers. However, while swimming was predictive of illness, the coliform bacteria being used as indicators to regulate water quality at the time were not strongly predictive of illness.

A great deal of controversy over the usefulness of measuring indicator organisms in recreational water settings was generated in 1959, when Moore conducted a five-year study across 43 beaches in the United Kingdom. The study showed that there were few if any risks to public health associated with swimming in sewage contaminated waters, even if a beach was obviously contaminated (Moore 1959). After a decade of debate, the U.S. Environmental

Protection Agency (U.S. EPA) decided that there were few conclusive epidemiological studies establishing the usefulness of indicator organisms in recreational water settings. Since then, many epidemiologic studies showing associations between indicator organisms and certain illnesses have been conducted (Pruss 1998). The illnesses most strongly associated with levels of indicator organisms have been gastrointestinal illness (GI) and acute febrile respiratory illness (AFRI). The indicator organisms most strongly associated with these symptoms were *Enterococci,* fecal *Streptococci*, thermotolerant coliforms, and *E. coli* (Pond 2005). Following a review of all the literature available at the time, the World Health Organization (WHO) concluded that the risk of enteric illnesses and AFRI was associated with indicator organism concentrations (WHO 2003, WHO 2005).

Since 2003, several studies have investigated the impact that pollution at beaches may have on those who use marine and fresh bodies of water for recreation. It has been estimated that visitors spend two billion person days each year at coastal recreational waters alone. Globally, the WHO has estimated that there are in excess of 120 million cases of GI illness and 50 million excess cases of severe respiratory illness attributable to exposure to contaminated recreational waters (Shuval 2003). These illnesses are estimated to cost three million disability adjusted life years (DALYs), and the economic impact is estimated to be approximately 12 billion dollars annually. In the U.S. alone, approximately 129 million individuals visited the beach or some other waterside location from 2000-2001. In one study of two beaches located in southern California, it was estimated that the economic impact associated with visiting polluted beaches was over 3.3 million dollars (Dwight 2005). Over a third of this cost was attributed to GI illnesses, nearly 1 million dollars was due to acute respiratory illnesses, and the remaining third was due to ear and eye infections. A similar study examining data from 28 beaches in southern California estimated that there were between 627,800 and 1,479,200 excess cases of GI illness due to swimming in contaminated beach water in a single year (Given 2006). The health care costs associated with these excess GI illnesses were estimated to be between 21 and 51 million dollars annually.

While the risks of GI and AFRI illnesses have been positively associated with the presence of indicator organisms, few studies have examined the relationship between the presence of indicator organisms and other health outcomes. Ear, eye, and skin infections have been linked to the presence of indicator organisms, though the evidence has been limited (Pruss 1998).

1.2 Potential Pathogens in Recreational Water Settings

1.2.1 Viruses

As mentioned above, there are over 100 human enteric viruses that may be transmitted through feces (Puig 1994, Mara and Horan 2003), and they are of particular concern because viral infections typically require very low infectious doses (Murray 2008, Haas 1999). The viruses potentially transmitted by feces include adenoviruses, HAV, hepatitis E virus (HEV), coxsackieviruses, and echoviruses.

There are 51 different antigenic types of human adenoviruses recognized, and they are known to be commonly found wherever human fecal waste is present. They are characterized by above average resistance to disinfecting agents and varied pH levels. In addition, the adenoviruses are known to survive outside the human body for extended periods of time, and can remain infectious despite normal UV treatment of wastewater (Thompson 2003). The symptoms associated with adenovirus infections are usually respiratory, but depending on the subtype, gastroenteritis, conjunctivitis, and rash may be seen as well (Pickering 2009). The incubation period for adenovirus infections is typically less than 10 days, though it may be longer. Adenoviruses are known to be much more stable than HAV in seawater (Pond 2005). In addition, it is thought that adenoviruses may survive longer in marine waters than more fragile viruses, such as enteroviruses, and thus persist and can cause infection if ingested (Enriquez and Gerba 1995).

Non-polio enteroviruses are RNA viruses that include the coxsackieviruses, echoviruses, and other enteroviruses. They are very commonly acquired, second only to the common cold, and often produce similar symptoms (CDC 2011a). The symptoms range from mild flu-like symptoms to skin rash. Incubation periods can range from two days to two weeks, and they are typically associated with fresh water outbreaks of disease (Pond 2005).

HAV and HEV are both RNA viruses for which humans are the only reservoir. HAV produces acute symptoms, but not chronic infection or illness like hepatitis B or C viruses. Hepatitis caused by HAV typically resolves without treatment. HEV infections often resolve, but sometimes do become chronic (CDC 2009c, CDC 2009d). Like the viruses discussed above, HAV and HEV are often transmitted through the fecal-oral route. HAV is known to survive outside the human host for extended periods of time (i.e. up to months). The symptoms caused by HAV and HEV include fever, jaundice, vomiting, nausea, abdominal pain, and other non-specific symptoms. Several studies have demonstrated transmission of HAV via water, especially in the setting of rivers contaminated by wastewater. Large-scale waterborne outbreaks of HEV have not been reported, although it is thought to be possible (Pond 2005).

1.2.2 Protozoans

Two of the most common protozoans causing GI infections in humans are *Cryptosporidium parvum* and *Giardia duodenalis* (formerly known as *Giardia lamblia)*. *C. parvum* is often found in the GI tracts of humans and animals. It produces oocysts that are widely found in lakes, streams, and in runoff, and can survive outside of an animal host for very long periods of time (CDC 2010e). *Cryptosporidium* is one of the most common causes of waterborne illnesses in recreational settings, and has also been shown to cause outbreaks of drinking water related disease. Symptoms include nausea, abdominal cramps, fever, and dehydration. Outbreaks of *Cryptosporidiosis* are most often associated with swimming pools, although several infections due to exposure to a stream draining onto a beach have been reported (Pool 2005).

Giardia, like *Cryptosporidium*, has human as well as several animal hosts. It also can survive in the environment for weeks or months, and can be transmitted via recreational and drinking water. Transmission is via the fecal-oral route, and symptoms include diarrhea,

abdominal cramps, and nausea (CDC 2011b). *Giardia* is also an extremely common cause of outbreaks of waterborne disease. Most waterborne outbreaks of *Giardia* have been related to exposure to recreational fresh water settings (Pond 2005).

1.2.3 Bacteria

Many bacterial pathogens have been shown to cause waterborne outbreaks of disease, including *E. coli*, *Helicobacter pylori*, *Campylobacter*, *Salmonella*, *Shigella*, and *Vibrio*.

There are many strains of *E. coli*, although five pathogenic groups are of most interest with regard to human GI illness: enteroaggregative *E. coli* (EAggEC), enterohaemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), and enterotoxigenic *E. coli* (ETEC). Symptoms of *E. coli* gastrointestinal infection can include bloody diarrhea, depending on the strain involved. *E. coli* can survive in surface water (Wang and Doyle 1998). Most outbreaks of *E. coli* related to recreational water have involved fresh water settings (Pond 2005).

Helicobacter pylori is a bacterium capable of producing chronic infection human beings, and has been shown to increase the risk of gastric ulcers and some cancers. The prevalence of *H. pylori* infection is high worldwide, ranging from 30-40% in the U.S. to up to 70% in many developing countries (CDC 2009b). Humans are the only known reservoir, although it has been shown that *H. pylori* can survive in fresh water and in biofilms (Mazari-Hiriart 2001).

Campylobacter spp. consists of 15 species whose reservoir is the GI tract of warm-blooded animals. *Campylobacter* grows best at the body temperature of a bird. Birds suffer no significant illness, but in humans, *Campylobacter* is one of the most common causes of diarrheal illness. Because they are present in large numbers at many beaches, *Campylobacter* concentrations could potentially be quite high as well. However, *Campylobacter* is very fragile, and cannot survive very long periods of time in dry environments (CDC 2010a). It is often found in sewage, and has been isolated from surface waters (Van Breemen 1998). *Campylobacter* has also been cultured from sediment at beaches at a number of locations (Obiri-Danso and Jones 2000, Jones 1990).

While human *Salmonella* infections are often caused by contaminated food, *Salmonella* has also been found in marine waters (Polo 1998). Symptoms of *Salmonella* infection include diarrhea, fever, and abdominal cramps. It is estimated that, in the U.S., 1.4 million *Salmonella* infections occur each year (CDC 2010b, CDC 2010c).

Shigellosis (infection with one of the species of *Shigella*) may cause diarrhea, fever, and abdominal cramps. Symptoms often begin one to two days after infection and often resolve on their own. Transmission of *Shigella* is via the fecal oral route, and food-borne outbreaks of shigellosis have been documented (CDC 2009f). Shigellosis may also be acquired through exposure to contaminated recreational waters. Most of the cases attributed to contaminated recreational water are in fresh water circumstances (Pond 2005).

Vibrio parahaemolyticus and *Vibrio vulnificus* are part of the same family of bacteria that causes cholera. Both of these *Vibrios* live in seawater and require the salty environment to survive. Infection can cause vomiting, diarrhea, and abdominal pain. Both of these *Vibrios* can also cause skin infections if they come into contact with an open wound (CDC 2009a, CDC 2009e).

1.3 Current Environmental Protection Agency (EPA) Regulations

Given the many potential pathogens that may be associated with fecal contamination of recreational water, use of indicator organisms to monitor water safety seems to be warranted. Due to financial and logistic constraints on many local health departments, constant monitoring of each beach in the U.S. for a host of infectious agents is impractical. After collecting information from a variety of studies, in 1986 the U.S. EPA issued new recommendations for indicator organism levels in recreational water settings (Cabelli 1983, Dufour 1984). Prior recommendations had been based on fecal coliforms, and had been set at 200 fecal coliform organisms per 100 mL (U.S. EPA 1976).

In fresh water, enterococci or *E. coli* were identified as the best indicators for predicting GI illness. The U.S. EPA decided to use a binary cutoff describing acceptable and unacceptable water quality. This cutoff was based on the decision that when swimmers were compared to non-swimmers, if 19 excess cases of gastroenteritis would occur for every 1,000 swimmers relative to non-swimmers, the water quality was unacceptable. The indicator organisms chosen for fresh water settings were enterococci and *E. coli.* The cutoff that represented 19 excess cases per 1,000 swimmers for enterococci was a geometric mean of at least five samples over a 30-day period that exceeded 33 colony-forming units (cfu) per 100 mL. For *E. coli*, if the geometric mean of at least five samples over 30 days exceeded 126 cfu/100 mL, then the beach should post a no-swim advisory. For marine waters, the indicator organism of choice was enterococci. If the geometric mean of at least five samples taken over 30 days exceeded 35 cfu/100 mL, then a swimming advisory should be posted at that beach. In addition to the geometric mean approach, if a single sample taken at the beach had levels of enterococci that exceeded 104 cfu/100 mL, then an advisory should be posted as well (U.S. EPA 1986, Wade 2003).

Since the issuing of the 1986 regulations, several criticisms have been leveled. In a major study conducted by the EPA, results from three heterogeneous marine sites were used to calculate the appropriate regulatory guidelines for marine waters. Data from all sites were pooled to create one dataset. Original analyses were conducted using linear regression, fitting concentrations of enterococci with the difference in GI incidence between swimmers and non-swimmers. Cutoffs representing 19 excess cases of GI per 1,000 swimmers were then derived from the linear models. The results were found to be highly sensitive to what data were retained for analysis and what data were excluded. A reanalysis of the data found significant heterogeneity between study locations, indicating that one nationwide guideline may not perform the same at all beaches (Fleisher 1991). Recently, the EPA set a goal of publishing new recreational water quality criteria by October 2012, in response to a lawsuit between the Natural Resources Defense Council, the U.S. EPA, the National Association of Clean Water Agencies, the County of Los Angeles, and the Los Angeles County Flood Control District. The lawsuit,

settled in August 2008, focused on forcing the EPA to meet mandatory duties required by the Clean Water Act (U.S. EPA 2011).

1.4 Rapid Methods for Measuring Indicator Organisms

1.4.1 Limitations of Culture-Based Indicator Organism Measurement

One major limitation to the use of indicator organisms to regulate beach water quality is that the time needed to measure indicator organisms is quite long. For culture-based methods, the amount of time needed to grow the indicator organisms and count them may take ≥ 24 hours (Wade 2006). An additional factor is the time necessary to collect and transport the sample to a laboratory. The resultant delay between sample collection and notification of beachgoers makes it very difficult to issue beach swimming advisories that accurately reflect the amount of contamination in the water. It is known that beach water quality can change from day to day (Boehm 2002). The major concern is that on high risk days, swimmers may not be receiving the notification that the water is unsafe for swimming. In addition, acting on delayed information may mean that beaches are closed on days when water quality is good. These false positive alerts could have negative impacts on tourism and local businesses.

Investigating the effects of delayed announcements is possible when using data from prospective cohort studies. These large epidemiologic studies recruit study participants and measure water quality multiple times on the same day. Enumeration of the indicator organism concentrations using slower methods is possible because water samples are collected roughly at the same time the swimmer enters the water. Because many study days are consecutive, it is also possible to assign a swimmer on day one the indicator concentration level measured on day two, and determine if that swimmer's health status is better predicted by their actual exposure (day one indicator organism levels) or their day two indicator organism levels. When data from prospective cohort studies conducted at Doheny Beach in southern California were taken and made to reflect the time lag necessary to measure traditional culture-based methods, the relationship between indicator organisms and health was substantially weakened (Colford, unpublished data). However, positive associations were seen between same day indicator organism levels and health, highlighting the need for a more rapid method to measure indicator organisms.

1.4.2 Rapid Methods

In order to remedy this situation, methods that allow for the rapid measurement of microorganisms have been developed. For instance, polymerase chain reaction (PCR) methods have been developed that allow the quantification of some indicator bacteria in less than two hours (Santo Domingo 2003). Rapid methods were used in one EPA study conducted in 2003, which measured both *Enterococcus* and *Bacteroides* concentrations using a quantitative polymerase chain reaction (QPCR) method. Significant associations were found between *Enterococcus* levels and GI illness at a Lake Michigan beach, and the relationship grew stronger as the swimmers spent more time in the water (Wade 2006).

Rapid methods offer clear advantages over culture-based methods with respect to time. However, if new regulations based on rapid methods are formulated, technical difficulties may arise as local departments attempt to procure instruments, train staff, and develop standardized protocols.

1.5 Alternative Statistical and Graphical Methods

1.5.1 SuperLearning

As the U.S. EPA moves towards developing new regulatory guidelines in 2012, several other potential improvements in methodology should be explored. In addition to the use of rapid methods for measuring indicator organisms, improvement of statistical methods may be necessary to develop guidelines that accurately reflect the acceptable/unacceptable cutoff of 19 excess cases of GI illness per 1,000 swimmers. As alluded to above, Fleisher et al. reported in 1991 that the choice of statistical models may greatly influence where an acceptable/unacceptable cutoff point for an indicator is drawn. Most statistical models used in water quality studies focus on linear relationships between indicator organism concentrations and disease. However, a linear relationship may not best capture the risk of swimmers exposed to varying concentrations of indicator organisms. The optimal curve could instead look logarithmic, or take some other form.

Thus, examining other modeling techniques could yield statistical models that better represent the relationship between indicator organism concentrations and health of swimmers. A model that could consider both linear and non-linear modeling approaches and choose the most appropriate method, given the data, would be a useful tool. An even more useful tool would take the linear and non-linear approaches and combine them into an overall model. The aforementioned approach was taken and developed into a tool called the "SuperLearner" by biostatisticians at the University of California, Berkeley (van der Laan 2007, Polley 2010). The SuperLearner Algorithm is an estimator that combines a given list of candidate statistical models to form a composite estimator.

1.6 Goals for Dissertation

1.6.1 Relationship of Indicator Organisms With Skin Symptoms

The literature relating indicator organisms to health outcomes has been inconsistent. Some studies at some beaches have demonstrated significant relationships between indicator organisms and illness (Wade 2006, 2008) while other studies have seen no such relationships (Colford 2007). In general, there is a consensus that indicator organisms can predict GI and AFRI under many circumstances. However, because the findings have not always been consistent, additional evidence linking indicator organisms to illness would help bolster the biological plausibility of using indicator organisms in recreational water settings. Additionally, it is quite plausible that contaminated waters may increase the risk of illnesses other than GI and AFRI, but reports of indicator organism-illness relationships for ear, eye, and skin infections tend to be infrequent or poorly documented. In order to clarify this relationship, this dissertation

begins with a systematic review of the literature relating indicator organisms to skin conditions such as rash and skin infection. After amassing data from all studies reporting relationships between skin conditions and indicator organisms, meta-analytic techniques were used to synthesize the results across the studies. A new technique to meta-analyses is also used, the Ratio of Odds Ratios (ROR). Because some studies report odds ratios from beaches with low indicator organism concentrations, and some studies report odds ratios from beaches with high indicator organism concentrations, it would make sense to compare and contrast OR's from these different beaches. It would be expected that with higher indicator organism levels, more fecal contamination would be present, and higher OR's associated with swimming would be seen. Thus, ROR's offer a method to compare synthesized results between beaches.

1.6.2 Improving Performance of Indicator Organisms

Once the relationship between indicator organisms and human health is more clearly established, the second goal of this dissertation is to consider several novel ways to improve prediction of illness at beaches. The first method involves using the SuperLearner method to compare traditional modeling approaches to newer, more flexible approaches, as well as to the performance of the SuperLearner itself. Another potential improvement that will be investigated is the use of multiple indicators in combination. Single indicator approaches may predict illness to a certain degree, but it is plausible that by measuring two indicators at once, prediction of illness might be improved. In addition to considering multiple biologic indicators, easier to measure physical indicators, such as solar radiation and salinity, will be considered. Because physical conditions often influence the survival of indicator and pathogenic organisms, combining physical information with biologic information also could potentially improve prediction of illness over use of a single indicator like *Enterococcus*. Finally, graphical methods often used in genetic analysis will be used to visualize and compare a variety of cutoffs for pairs of indicators considered together.

1.6.3 Groundwater Transport of Sewage as Health Risk

The final goal of this dissertation is to examine the role that groundwater plays in risk of illness at Avalon Beach, in southern California. A leaking sewage infrastructure, combined with groundwater flux, is thought to channel large amounts of sewage into waters frequented by beachgoers at Avalon. Avalon has historically been one of the most contaminated beaches in California. Analyses conducted in this section examine the hypothesis that increased groundwater flow is associated with increased risk of gastrointestinal illness. Another hypothesis involves examining indicator organism performance when groundwater flow is higher versus when it is lower. If groundwater is conveying raw sewage onto the beach, it is likely that pathogen levels will also increase when groundwater flow levels are high. It is expected that under these circumstances, indicator organisms should strongly predict illness, while when groundwater flow levels are lower, indicator organism-health associations should be attenuated.

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CHAPTER 2: SYSTEMATIC REVIEW AND META-ANALYSIS, SKIN INFECTION

2.1 Introduction

The following section attempts to bolster the already biologically plausible relationship between indicator organisms and health outcomes. While GI infections and AFRI have been relatively well linked to indicator organism concentrations, research on other health outcomes such as ear, eye, and skin infections has been limited. In addition, many of the studies that have examined skin related symptoms were relatively small, and thus were underpowered. This systematic review of the skin disease and indicator organism literature attempts to summarize and synthesize the results across many papers. The primary goal of this investigation is to quantify the association between microbial indicator organisms used to monitor recreational water quality and skin-related health outcomes in non-outbreak conditions in both marine and freshwater settings. The findings of this investigation were published in the journal Water Quality, Exposure and Health under the title "Skin-related symptoms following exposure to recreational water: A systematic review and meta-analysis" (Yau 2009). Co-authors on this paper included Tim Wade, from the U.S. EPA, Carol K. deWilde, and John M. Colford, Jr. This chapter has been subjected to review by the National Health and Environmental Effects Research Laboratory and approved for publication. Approval does not signify that the contents reflect the views of the Environmental Protection Agency.

2.2 Abstract

Background: Exposure to contaminated recreational waters (defined by levels of fecal and other types of indicator bacteria) is associated with adverse health outcomes. The principal health outcome studied previously has been gastrointestinal illness. Although many studies included reports of frequent skin complaints (e.g. rash or itch) following recreational water exposure, no systematic reviews have examined the association between indicator organism levels and skin-related symptoms.

Methods: Twenty relevant peer-reviewed studies were identified. The relative risks (swimmers vs. non-swimmers) of skin-related symptoms among those exposed to recreational water with bacterial indicator concentrations above threshold levels were determined using meta-analysis. Similarly, the relative risks (swimmers vs. non-swimmers) of skin-related complaints after exposure to water with bacterial indicator concentrations below threshold levels were determined. The ratio of these odds ratios (ROR) was then computed for each indicator.

Results: The risk of skin-related symptoms was significantly elevated in marine water with high levels of total coliforms ROR 1.86, (95% CI 1.21, 2.87); fecal coliforms ROR 1.45 (95% CI 1.02, 2.07); *E. coli* ROR 1.98, (95% CI 1.43, 2.75); enterococci ROR 2.04 (95% CI 1.34, 3.09) and fecal streptococci ROR 1.70 (95% CI 1.07, 2.71). However, no significant associations with water quality indicators were demonstrated for the freshwater indicators examined (total coliform, fecal coliform, *E. coli*).

Conclusions: Swimmers exposed to marine water at high levels of several indicator bacteria experience a significant increase in skin-related symptoms compared to non-swimmers. This relationship was not demonstrated in freshwater settings.

2.3 Methods

2.3.1 Literature Search

The literature search was done using five electronic databases: PUBMED (http://www.ncbi.nlm.nih.gov/pubmed/), BIOSIS (http://apps.isiknowledge.com/), Web of Science (http://apps.isiknowledge.com/), EMBASE (http://openaccess.dialog.com/med/), and ProQuest Dissertation and Theses (http://proquest.umi.com/pqdweb) for all dates until August 2008. The search terms used included key words "water and health," "water and fecal," "water and feces," "water and indicator," "recreational water and health," as well as permutations of the above keywords. We also contacted experts in the field and reviewed the citations of relevant studies for other relevant studies.

After gathering all available studies, we reviewed the titles and abstracts and retained relevant ones for full-text review. Studies were retained if the abstract and title pertained to health effects with respect to swimming in fresh or salt waters, and if the abstract or title suggested that microbiological quality of the water was measured. Studies published in all languages were considered, as long as the title and abstract were available in English.

2.3.2 Selection Criteria

Studies were included in this review if they met the following criteria:

Water exposure: Studies related to marine (ocean) or fresh (lakes, rivers) waters were included, but studies involving swimming pools and other treated bodies of water were excluded. All forms of water contact were included (swimming, sporting events, bathing, etc.)

Water quality measures: Studies had to include at least one numeric measure of the microbiologic quality of the water. Studies without quantitative measures of microbiologic water quality were excluded.

Health outcomes: In addition to a measure of microbial water quality, a measure of human health associated with microbial water quality had to be reported. After full-text review, if no measure of skin related symptoms (irritation, rash, infection, itchiness, etc.), etc.) was reported, the study was excluded.

Study design: Because the purpose of this review was to determine the relationship between microbial indicators and skin-related outcomes under non-outbreak conditions, only studies that dealt with endemic situations were considered for this review. Studies were also required to report data on a control group (otherwise we could not calculate a measure of relative risk).

Study characteristics: If a publication was based on data that had been previously published, the most recent analysis was abstracted and the earlier publication was excluded. Also, only peer-reviewed publications were retained.

2.3.3 Data Abstraction

The data were abstracted independently by two authors (C.K.d. and V.Y.). For each study, the following data were abstracted: microbial water quality measure (type and numerical value), water type (marine or fresh), population studied, geographic location, study size, study design, how skin symptoms were defined, covariates included for, comparison group, information on swimming exposure (type and duration), relative risks, and confidence limits. If a measure of relative risk was not reported, then data were abstracted and used to calculate the odds ratio and its 95% confidence interval. If a publication reported data from several study sites, or the same site over several years, data for each site were abstracted and treated as a separate study (Table 2). If a study did not report a measure of relative risk comparing swimmers to a non-swimmer comparison group, but had a different comparison group (*e.g.,* swimmers in waters with minimal contamination, Haile 1999), those relative risks were extracted instead. Three indicator organisms (total fungi, *Candida*, and enteroviruses) were excluded from analysis because too few studies (< 2) examined their relationship to-skin related outcomes.

A total of eight microbial indicator organisms were included as part of the marine water meta-analysis and six as part of the fresh water meta-analysis (Table 2). The indicator organisms included in the marine water analysis were total coliforms, fecal coliforms, fecal streptococci, *E. coli*, enterococci, *Klebsiella*, *P. aeruginosa*, and staphylococci. For the fresh water analysis, total coliforms, fecal coliforms, fecal streptococci, *E. coli*, enterococci, and staphylococci were included. Indicator organisms were selected for analysis if two or more studies examined them in relation to skin-related outcomes. Even though some indicator organisms are currently recommended for use (*i.e.,* U.S. EPA recommends enterococci and *E. coli* for fresh waters), this recommendation is based on their association with gastrointestinal illness (Cabelli 1983), not other symptoms. Thus, both currently recommended indicator organisms as well as indicator organisms not in current use were included.

2.3.4 Cut-off Points For Threshold Values

Values to define high bacterial levels were obtained for total coliforms, fecal coliforms, fecal streptococci, and enterococci, and these cut-off points were analyzed to determine if there was an association between these levels and skin-related health outcomes. The California State Water Resources Control Board (1990a) has recommended that a cut-off point of 10,000 cfu / 100 mL be used for total coliforms in marine waters, and a cut-off point of 400 cfu / 100 mL for fecal coliforms (California State Water Resources Control Board 1990b). Haile (1999) recommended a cut-off point of 35 cfu / 100 mL for *E. coli*, while Ogan (1994) recommended a cut-off point of 35 cfu / 100 mL for fecal streptococci, and the EPA recommendation for a cut-off point using enterococci was 35 cfu / 100 mL (U.S. EPA 1986). California cut-off points were not selected preferentially; rather, any cut-off points found were considered. Alternative cut-

points were not found, potentially because non-recommended indicator organisms are not as well researched.

Cut-off points for freshwater indicator organisms were also obtained from the literature. The California Department of Public Health (2000) has recommended a fecal coliforms cut-off point of 200 cfu / 100 mL, while a cut-off point for total coliforms of 1,000 cfu / 100 mL was recommended by the San Diego Water Board (2007). For fecal streptococci, a cut-off point of 100 cfu / 100 mL was recommended by Wiedenmann et al. (2006); however, no studies were identified with fecal streptococci levels higher than this cut-off, so no comparison could be made.

Cut-off points proposed by the EPA and WHO were chosen preferentially, but when there were no established guidelines, cut-off points recommended in the literature were used instead. In marine settings, the EPA provided a recommended cut-off point for enterococci, but for the remaining fresh and marine indicators, there were no WHO or EPA recommended values. No cut-off points were found for concentrations of *Klebsiella*, *P. aeruginosa*, or staphylococci in marine waters, and no recommended cut-off point was found for staphylococci concentrations in fresh waters. Thus, no ROR was calculated, and only odds ratios comparing swimmers to control groups were computed for these indicators through meta-analysis.

2.3.5 Data Analysis

Separate analyses were used to examine each combination of microbial indicator organism and water type (marine and fresh). If a study reported microbial indicator values over a range, the median value of that range was used in our analyses. Exposure categories were formed by defining thresholds for high exposure based on cut-off points listed in US EPA and WHO criteria or guidelines recommended for safe recreational water (U.S. EPA 1986, WHO 2001), and if those were unavailable, cut-off points recommended in the literature were used (San Diego 2007, Haile 1999, California Department of Public Health 2000, Wiedenmann 2006, Ogan 1994, Wade 2003).

If a study reported multiple relative risks from a single study site, the highest exposure measure and its relative risk were used for analysis, consistent with the prior work of Wade et al. (2003). This prevented a single study from receiving more weight solely because of the number of results presented. However, if a study reported findings from multiple, independent study sites, the microbial indicator level and relative risk from each study site were recorded and used for analysis. Also, if a certain beach was studied one year and then studied again in a subsequent year, those study results were recorded separately. One potential concern related to treating each site in a report as a separate observation lies in the fact that multiple findings from those sites might, theoretically, not be independent of each other. However, pooling such sites might not be appropriate because of differences in the swimming populations that utilized the beaches and in the indicator organism levels that were present at the time. Rather than combining potentially different populations and sites, we analyzed them as separate observations (Table 2).

2.3.6 Meta-Analysis of Study Site Results

We calculated a summary relative risk of skin-related outcomes for each microbial indicator organism level (*i.e.* one odds ratio for swimmers vs. non-swimmers above the indicator cut-off point and one odds ratio for swimmers vs. non-swimmers below the cut-off point using fixed-effects models if no heterogeneity was present; otherwise, random-effects models were utilized. Heterogeneity of study results was assessed for each analysis using the Q statistic (DerSimonian and Laird 1986).

A binary variable was created to categorize the data into the sites with mean indicator levels below the cut-off point and those above. We then compared the odds ratio of skin-related outcomes for exposure above the cut-off point to the odds ratio of skin-related outcomes below the cut-off point. The mean difference between the log relative risks was taken, and the null hypothesis being tested was that the difference was equal to zero. The difference was then exponentiated to create the ratio of odds ratios (ROR). A ROR above 1.0 suggests an increased risk of skin-related symptoms among those exposed to indicator levels above the cut-off as compared to those exposed to indicator levels below the cut-off point (Altman 2003). For example, if the OR for swimmers vs. non-swimmers was 5.0 above the indicator cut-off point and 2.0 below the cut-off point, then the ROR would be reported as 2.5 (=5.0 / 2.0).

2.3.7 Heterogeneity

Sources of heterogeneity that might explain the variability between the results of different studies were investigated by using a random-effects meta-regression model (Thompson and Sharp 1999). The outcome being modeled was the natural log of the relative risk for skin-related outcomes, and predictor variables were indicator variables for whether or not a study adjusted for a particular covariate (available covariates were: gender, respondent, socio-economic status, age, health or allergy history, visitor or native status, ethnicity, food consumption, knowledge of beach health hazards, place of residence, marital status, use of randomization, exposure activity at the beach, insect repellant use, sunblock use, physical weather and wave data, beach density, presence of animals or boats, and swimming history), study size, study type, and geographic location of the study. The final model was chosen by excluding covariates with p-values < 0.2.

Analyses were performed using Stata 10.0 for the Macintosh (Stata Corporation 2008).

2.4 Results

A total of 3,468 titles and abstracts were reviewed for relevance, and 47 of these were retained for full text review. Of these, 20 studies (Table 1) were retained for final analysis. Twenty-seven studies were excluded because three studies included no information or inadequate information on microbiological water quality (Amson 1991, New Jersey 1988, Baylet 1984), 16 were excluded because skin outcome data were not reported or were not adequately reported (Balarajan 1991, Bandaranayake 1995, Bonilla 2007, Cheung 1991, Dufour 1984, Fattal 1987, Fleisher 1996, Harrington 1993, Kocasoy, McBride 1998, Philipp 1985, Seyfried 1985b, Wiedenmann 2006, Foulon 1983, Kueh 1995, Marino 1995), one study was excluded because the indicator organism used was not reported in any other study (Pilotto 1997, cyanobacteria),

three studies were excluded because they did not generate any relevant primary data (Burke 2002, Pruss 1998, Robinton 1966), two studies were excluded because the same results were published elsewhere (Haile 1996, Zmirou 1990), and one study was excluded because data on a control group were not reported (Stevenson 1953).

2.4.1 Characteristics of Included Studies

The 20 studies retained for inclusion in the meta-analysis had study populations ranging from 104 to 23,241 individuals. Nine of the 20 studies were conducted in freshwater settings, while the remaining 11 studies were conducted in marine water settings (Table 1). One publication, Cabelli 1983, reported data from two separate studies (one in Louisiana, USA, and one in Egypt).

2.4.2 Study Designs

There were five different types of studies represented in the sample of 20 included studies: three randomized controlled trials, two cross-sectional studies, one retrospective cohort study, one case-control study, and 13 prospective cohort studies.

Ten of the prospective cohort studies were traditional cohort studies (Cabelli 1979, Cabelli 1983, Cheung 1990, Haile 1999, Prieto 2001, Seyfried 1985, Von Schirnding 1992, Colford 2007, Wade 2008, Alexander 1992). These studies recruited individuals on the beach and collected information on their water exposure that day. Follow-up of these individuals for skin-related illness was conducted 3 to 35 days after exposure. At least one water sample was collected on the day of exposure. In all but one study, swimmers were compared to non-swimmers with respect to skin related illnesses. Haile (1999) instead compared swimmers in waters with higher levels of contamination to swimmers in waters with minimal levels of contamination.

The remaining three prospective cohort studies (Dewailly 1996, Fewtrell 1992, Medema 1995) were conducted in the context of an athletic event. Exposed individuals were athletic event participants (triathletes, canoeists, and surfers) while non-swimmers were individuals present at the same event who had no water exposure (employees, etc.). Water samples were collected during the event, and follow-up for skin-related symptoms occurred 5-9 days after the events.

The study by Lee et al. (2002) was one of the cross-sectional studies, and water sampling, current skin-related illness status, and history of river exposure were collected on the same day. The comparison groups were those with exposure to highly contaminated water vs. lower water contamination. In the study by Dwight et al. (2004), surfers who surfed at least once a week were interviewed at two different beaches (one highly contaminated and one less contaminated) and were asked about symptoms in the past three months and exposure history for that time. Mean monthly indicator organism levels were provided by local health agencies.

Among the randomized trials, Jones et al. (1991) randomly assigned individuals to swimming or non-swimming behavior. Skin-related symptoms were assessed three days and

three weeks after exposure, and water quality was assessed the day of exposure. Van Asperen (1997) also randomized individuals to swimming or not swimming and assessed skin symptoms one week after exposure. Water quality was measured five minutes before exposure. The study by Wiedenmann et al. (2006) was similar to both of the above studies: individuals were randomized to exposure or non-exposure and outcomes were measured one week after the study.

One retrospective cohort study by Ferley et al. (1989) collected data on health status and water exposure the week prior to health symptoms. Water quality was assessed by collecting samples in advance of the health surveys. Samples were collected two days per week, and the concentrations measured on those days were extrapolated to the adjacent days.

The case-control study conducted by Charoenca et al. (1995) measured water quality at various beaches and then enrolled patients with staphylococcal skin infections and determined their seawater contact ten days before.

Eleven studies recruited both adults and children (Cabelli 1979, Cabelli 1983, Seyfried 1985, Ferley 1989, Cheung 1990, Von Schirnding 1992, Haile 1999, Prieto 2001, Colford 2007, Wiedenmann 2006, Wade 2008), while five studies recruited only adults (Jones 1991, Dewailly 1986, Fewtrell 1992, and Medema 1995, Dwight 2004). Four studies focused only on children (Alexander 1992, Lee 2002, Van Asperen 1997, Charoenca). Definitions of outcomes and exposures for all the included studies are in Table 3.

Most studies used non-swimmers as the comparison group, but the comparison populations differed. Thirteen studies chose to use beach-goers who had no water exposure (Cabelli 1979, Cabelli 1983, Cheung 1990, Prieto 2001, Seyfried 1985, Von Schirnding 1992, Colford 2007, Alexander 1992, Ferley 1989, Jones 1991, Wade 2008, Van Asperen 1997, Wiedenmann 2006), while three studies used employees at a sporting event or athletes with no water exposure at the same sporting venue (Medema 1995, Fewtrell 1992, Dewailly 1986). Haile (1999), Lee (2002), and Dwight (2004) used exposure to less contaminated waters as a comparison group for individuals exposed to highly contaminated waters.

2.4.3 Exposure Assessment and Definitions

If studies did not report direct observation of water exposure, it was assumed that self-report was used instead. For fifteen of the twenty studies, exposure was determined by self-report (Cabelli 1979, Cabelli 1983, Seyfried 1985, Dewailly 1986, Ferley 1989, Cheung 1990, Alexander 1992, Von Schirnding 1992, Haile 1999, Prieto 2001, Lee 2002, Colford 2007, Dwight 2004, Charoenca 1995, Wade 2008). The definition of exposure differed from study to study. The most common definition was head immersion or facial contact (Cabelli 1979, Cabelli 1983, Cheung 1990, Haile 1999, Jones 1991, Colford 2007). The next most common definition was any contact with the water (Alexander 1992, Seyfried 1985, Von Schirnding 1992, Ferley 1989). Three studies defined exposure as participation in a water-related sporting event (Medema 1995, Fewtrell 1992, Dewailly 1986). (Table 3)

2.4.4 Meta-Analysis of Study Site Results: Marine Water

For all bacterial indicator organisms tested, the odds ratios (of illness in swimmers vs. non-swimmers) at sites with low indicator levels were significantly smaller than the odds ratios at sites with elevated indicator levels. The ROR comparing the odds ratios among swimmers in waters with high concentrations of enterococci vs. the odds ratio among swimmers in water with low concentrations was 2.04 (95% CI 1.34-3.09). For total coliforms, the ROR was 1.86 (95% CI 1.21-2.87). Studies with fecal coliform levels above 400 cfu / 100 mL had odds ratios that were 1.45 times larger than studies with lower indicator organism levels (95% CI 1.02-2.07). For *E. coli*, the ROR was 1.96 (95% CI 1.38-2.79). Studies with elevated fecal streptococci had an elevated odds ratio that was significantly different than a ROR of 1 (ROR = 1.70, 95% CI 1.07-2.71). (Figure 1)

2.4.5 Meta-Analysis of Study Site Results: Fresh Water

Analyses of the cut-off points for fecal coliforms and total coliforms revealed no significant associations. For total coliforms, the ROR was 1.17 (95% CI 0.75-1.84). For fecal coliforms, the ROR was 1.69 (95% CI 0.88-3.27). The same conclusion was reached with *E. coli* (ROR = 0.62, 95% CI 0.03-13.55), although the number of sites included was small (n = 3).

2.4.6 Meta-Analysis of Indicators Without RORs: Marine and Fresh Water

Three marine indicator organisms (*Klebsiella*, *P. aeruginosa*, and staphylococci) and one freshwater indicator organism (staphylococci) had no recommended cut-off point, and so no ROR was calculated. However, regression was used to determine if there was a linear relationship between concentration level of the indicator organism and the study OR. For *Klebsiella*, the OR associated with a one hundred cfu increase in concentration per 100 mL was 1.16 (95% CI 0.98-1.37). For *P. aeruginosa*, the OR was 1.28 (95% CI 0.30-5.44). For marine staphylococci, the OR associated with a one hundred cfu increase in concentration per 100 mL was 1.02 (95% CI 0.97-1.06). For freshwater staphylococci, the OR associated with a one hundred cfu increase in concentration per 100 mL was 1.74 (95% CI 0.17-17.72).

One freshwater indicator organism, *Enterococcus*, had a recommended cut-off point, but all available studies had indicator organism concentrations above the cut-point. For a 100 cfu / 100 mL increase in *Enterococcus* concentrations in freshwater settings, the OR was 0.88 (95% CI 0.57-1.36). A similar situation occurred with freshwater streptococci, but instead all studies had indicator concentrations lower than the recommended 100 cfu / 100 mL cut-point. A linear regression was performed, and the OR associated with a 100 cfu / 100 mL increase in concentration was 3.83 (95% CI 0.60-24.39). Linear regression was chosen in lieu of other models because the data were relatively sparse, and thus it was not obvious if the data were clearly linear or non-linear. While more complicated splines and exponential models could have been fit, the interpretation of the coefficients of these models would have been more complicated.

2.4.7 Heterogeneity

Heterogeneity was detected in several of the analyses ($p < 0.2$), and to explore sources of heterogeneity, meta-regression was used. Factors that were considered were adjustment by the authors for any confounders, or adjustment for a variety of confounders (gender, respondent to survey, socioeconomic status (SES), age, history of health and allergies, visitor or native status, ethnicity, food consumption, knowledge of beach hazards, place of residence, marital status, exposure activities at the beach, insect repellant use, sunblock use, physical and weather data, density of individuals at the beach, presence of boats or animals, and swimming history). These covariates were coded as indicator variables, with a "1" value indicating that the study adjusted for that covariate, and a "0" value for studies that did not adjust for that covariate.

For freshwater settings, the sources of heterogeneity for the fecal coliform meta-analysis were retrospective cohort study design (OR = 2.42, 1.08-5.41) and gender (OR = 2.42, 0.76-6.61). These odds ratios can be interpreted as the single retrospective cohort study (Ferley 1989) reported odds ratios that were 2.42 times greater than odds ratios reported from other study design types, and that studies that adjusted for gender reported odds ratios that were 2.42 times greater than odds ratios reported from studies that did not adjust for gender. For fecal streptococci, the main source of heterogeneity was study size.

For marine settings, the primary sources of heterogeneity in the meta-analysis considering *E. coli* were adjustment for visitor/native status of the study participants (OR = 1.80, 95% CI 1.13-2.88) and ethnicity (0.54, 95% CI 0.28-1.07). For enterococci, the main contributors to heterogeneity were adjustment for visitor/native status (OR = 2.10, 95% CI 1.09-4.06), exposure category below or above 35 cfu / 100 mL (OR = 1.38, 95% CI 0.84-2.27), gender (OR = 0.17, 95% CI 0.07-0.46), socioeconomic status (OR = 5.66, 95% CI 1.53-20.8), and age (OR = 0.36, 95% CI 0.20, 0.67).

2.4.8 Publication Bias

A statistical test for publication bias (Begg 1994) suggested that for some marine indicator organisms, publication bias may have been present (marine fecal streptococci = 0.001, marine fecal coliforms p = 0.017, marine enterococci p = 0.001, marine *E. coli* p = 0.001, marine *Klebsiella* p = 0.003, marine *P. aeruginosa* p = 0.009, marine staphylococci p = 0.08). The Begg test plots study effect size against a measure of the study's standard error or sample size, and determines if the study effect sizes are symmetrically distributed around the overall summary effect. If these plots are not symmetrically distributed, it is likely that publication bias may be present. This suggests that the summary relative risks reported in this study may be overestimates.

A further analysis was done using the trim and fill method proposed by Duval and Tweedie, which non-parametrically attempts to account for the effects of publication bias and create an unbiased summary effect estimate (Duval 2000). For fecal streptococci in marine settings, publication bias was suspected in studies with indicator levels greater than 35 cfu / 100 mL. The trim and fill analysis gave a random effects OR of 1.97 (95% CI 1.37, 2.84) for studies with indicator levels greater than 35 cfu / 100 mL (previous unadjusted summary OR was 2.25, 95% CI 1.51-3.36). The ROR for marine fecal streptococci, adjusted for publication bias,

becomes 1.49 (95% CI 0.97-2.30). For marine fecal coliforms, after adjusting for potential publication bias, the new ROR was 1.33 (95% CI 0.95, 1.88). For marine enterococci, the adjusted ROR was 1.31 (95% CI 0.86, 1.98). For marine *E. coli*, the ROR adjusted for publication bias was 1.76 (95% CI 1.22, 2.54). For marine *Klebsiella*, no ROR was calculated, but an adjusted summary OR was calculated to be 1.38 (95% CI 1.1, 1.74). For marine *P. aeruginosa,* there was no ROR to adjust, but the adjusted OR was 1.36 (95% CI 1.12, 1.65). For marine staphylococci, the adjusted OR was 1.80 (95% CI 1.26, 2.56).

2.5 Discussion

2.5.1 General Conclusions

There are several microbiological indicator organisms that are associated with skin-related health conditions in marine waters. This review has provided some evidence that skin-related health conditions are associated with exposure to contaminated recreational waters. All marine indicator organisms showed statistically significant associations, with enterococci demonstrating the strongest association between bacterial levels and skin symptoms (ROR = 2.04, 95% CI 1.34-3.09). Cut-off points for freshwater indicators did not demonstrate statistically significant associations with skin-related outcomes, but were suggestive of an association. However, we found few published studies that have examined indicator organisms and skin-related outcomes in freshwater situations. The small number of freshwater studies is probably an important factor in the lack of significant findings for freshwater indicators. For the freshwater analyses, the number of study sites per indicator ranged from a low of three to a high of nine sites. However, for marine studies, the minimum number of sites for any one indicator was nine sites and the maximum was twenty.

For these indicators, predefined cut points were used. Other cut points may have maximized the ROR, but such data exploration would have to be accounted for with penalized p-values for multiple comparisons. Additionally, looking for cut-points that would maximize the risk would be better done with primary study data, rather than in a meta-analysis setting, which suffers from more potential biases than individual studies.

Skin ailments (e.g. rashes, skin infections and irritation) among swimmers could arise from a wide variety of causes, ranging from physical irritation to infection. However, because our review observed a higher rate of skin ailments at marine sites with higher levels of fecal contamination, a cause independent of physical irritation is suspected. Skin ailments among swimmers may be caused by a wide variety of pathogenic microorganisms, some of which would be naturally occurring and not necessarily associated with fecal indicator bacteria (e.g. cyanobacteria, cercarial dermatitis, seabather's eruption caused by zooplankton) (Burke 2002). However, other pathogens that could cause skin irritations, such as such as *Pseudomonas*, *Staphylococcus*, and adenovirus, could co-occur with fecal indicators associated with runoff, sewage discharge or through shedding by other swimmers (CDC 2008).

The results from the meta-regressions indicate that there is evidence that controlling for native or visitor status of study participants may be an important factor to consider in future

studies. The marine studies that used *E. coli* and adjusted for visitor/native status reported an odds ratio that was 1.80 times greater compared to studies that did not adjust (95% CI 1.13-2.88), and studies that adjusted for ethnicity had odds ratios that were 0.54 times smaller than studies that did not (95% CI 0.28-1.07). Thus, both native/visitor status as well as ethnicity appear to be important covariates to consider for adjustment. Studies of enterococci also supported the finding that visitor/native status was an important variable to include (OR = 2.10, 95% CI 1.09-4.06), but other explanations of heterogeneity included exposure category below or above 35 cfu / 100 mL (OR = 1.38, 95% CI 0.84-2.27), gender (OR = 0.17, 95% CI 0.07-0.46), socioeconomic status (OR = 5.66, 95% CI 1.53-20.8), and age (OR = 0.36, 95% CI 0.20, 0.67).

Other possible sources of heterogeneity are indicated in the freshwater meta-analyses. Among studies that examined fecal coliforms as an indicator organism, the single retrospective cohort study (Ferley 1989) reported an odds ratio that was 2.42 times greater than studies that did not use the retrospective cohort design (95% CI 1.08-5.41). For studies examining fecal streptococci, it appeared that larger studies tended to report larger odds ratios. For a 1,000 person increase in study size, the odds ratio increased by a factor of 1.04 times (95% CI 0.99-1.09, p-value 0.08).

2.5.2 Biases

Publication bias was seen in several of the sub-analyses. Analysis of marine indicators indicated that with the exception of total coliforms, publication bias was present for all of the indicator organisms. For fecal streptococci, fecal coliforms, and marine enterococci, the summary RORs changed from significant findings to non-significant findings. However, the ROR for marine *E. coli* remained statistically significant. While this might cast doubt on the usefulness of fecal streptococci, fecal coliforms, and enterococci as indicator organisms, the direction of the ROR still indicates an association between indicator concentration and risk of skin conditions in swimmers. However, these findings reinforce the idea that publication bias tends to overstate the association between indicator organism concentrations and the risk of skin disease.

Another source of bias was reported by Fleisher et al. (2006). It was found that swimmers who perceived that there was a health risk associated with swimming in marine waters reported significantly higher rates of skin ailments compared to bathers who did not recognize any health risk associated with bathing in marine waters. Only one study in this systematic review adjusted for this variable (Haile 1999). Thus, there is the potential for participants in other studies to have over-reported their incidence of skin ailments, thus theoretically causing results in those studies to be biased upwards.

Another potential source of bias is the comparison of swimmers to non-swimmers. These populations may have inherent differences that might confound the association between indicators and skin related outcomes. For example, swimmers might be healthier individuals while non-swimmers might be more prone to illness, or perhaps individual with higher SES might be better educated about the risks associated with swimming, while those with lower SES might be more willing to swim and become exposed. Also, swimmers might be more likely to

report symptoms than non-swimmers because they suspected that swimming may have caused the symptoms they experienced.

Another potential source of bias is that some studies relied on individuals to self-report their exposure and outcome status after the study. Swimmers might have poor recall of their exposure status, and they may have been more likely to report symptoms if they knew they had been exposed for long periods of time. This form of recall or "information" bias may have been present in many studies, because few were able to assign defined swimming activities and times to study participants or to employ physicians to assess outcome status in a blinded fashion.

In order to minimize biases associated with comparing disparate study populations, another analysis was conducted calculating ROR measures for studies with internal controls (an OR for high indicator concentration swimmers vs. non-swimmers and an OR for low indicator concentration swimmer vs. non-swimmers). Nine out of ten studies with internal controls demonstrated an elevated odds ratio for skin-associated outcomes in more polluted waters compared to less polluted waters, although only one was statistically significant (Figure 2).

An alternative way to deal with the biases present in various studies is to assign different weights to different studies, with more rigorous and high quality studies receiving more weight and smaller and potentially more biased studies receiving lower weights. While weighting schemes were considered, the method of assigning weights is very subjective. Without a standard, systematic method of assigning weights, the results might be skewed to indicate that a certain indicator was worse or better than the raw data suggest. Rather than weight the data, the authors chose to allow the readers to look at the data and draw their own conclusions.

2.5.3 Suggested further research

It is evident that the current freshwater indicator organism literature with respect to skin-related health outcomes is inadequate. Future studies should consider skin-associated outcomes using both traditional and novel indicators of recreational water quality. Future studies should use a measure of bather density and determine if it has any influence on health-related outcomes. Bather density could be an important variable because higher bather densities might elevate the concentration of bacteria in the water by resuspending sediments or shedding of indicator organisms and/or pathogens.

2.5.4 Limitations

One of the major criticisms of meta-analyses of observational studies is that it is probable that there are biases and confounding factors that have not been adjusted for in the individual studies, and that the populations in each study are not comparable to populations in other studies (Shapiro 1994). This would make any summary measures suspect. In order to deal with the heterogeneity of the data, random effects analyses were used whenever appropriate. It is also possible that studies without significant findings may not have been published. Although every effort was made to find all relevant studies, dissertations, and reports, some studies may not have

been found, and some studies that were relevant may have not published enough data to extract because no significant findings were found.

Another limitation to consider is the vast difference between many of the study populations and sites. The studies included ranged across Asia (Lee 2002, Cheung 1990), Europe (Jones 1991, Alexander 1992, Fewtrell 1992, Ferley 1989, Medema 1995, Prieto 2001, Africa (Von Schirnding 1992) and North America (Cabelli 1979, Cabelli 1983, Seyfried 1985, Dewailly 1986, Haile 1999, Dwight 2004, Colford 2007). Some studies specifically looked for tourists (Cabelli 1983) while other studies dealt with native populations (Dwight 2004).

2.6 Conclusions

The results of this review indicate that skin complaints may be significantly more likely to occur among swimmers exposed to marine waters with levels of total coliforms, fecal coliforms, *E. coli*, enterococci, and streptococci above the recommended cut-off points for these indicator organisms. No statistically significant relationships between indicator organisms and skin conditions were found for freshwater cut-off points.

2.7 Figures and Tables

Table 1: Final list of studies retained, by date of publication, location of study, marine or fresh water exposure, total study sample size, and study design.

Reference	Location	Water Type	Sample Size	Study Type
Cabelli (1979)	USA	Marine	8073	Prospective Cohort
Cabelli (1983)	USA / Egypt	Marine	3778 / 23241	Prospective Cohort
Seyfried (1985)	Canada	Fresh	4537	Prospective Cohort
Dewailly (1986)	Canada	Fresh	120	Prospective Cohort
Ferley (1989)	France	Fresh	5737	Retrospective Cohort
Cheung (1990)	Hong Kong	Marine	18741	Prospective Cohort
Jones (1991)	UK	Marine	276	Randomized Cohort
Alexander (1992)	UK	Marine	703	Prospective Cohort
Fewtrell (1992)	UK	Fresh	516	Prospective Cohort
Von Schirnding (1992)	South Africa	Marine	733	Prospective Cohort
Charoenca (1995)	USA	Marine	106	Case-Control
Medema (1995)	Netherlands	Fresh	395	Prospective Cohort
Van Asperen (1997)	Netherlands	Fresh	104	Randomized Cohort
Haile (1999)	USA	Marine	10459	Prospective Cohort
Prieto (2001)	Spain	Marine	1858	Prospective Cohort
Lee (2002)	Indonesia	Fresh	435	Cross-Sectional
Dwight (2004)	USA	Marine	1873	Cross-Sectional
Wiedenmann (2006)	Germany	Fresh	1759	Randomized Cohort
Colford (2007)	USA	Marine	8797	Prospective Cohort
Wade (2008)	USA	Fresh	21015	Prospective Cohort

Table 2: Six freshwater indicators and eight marine indicators retained for final analysis. "Studies" indicates the number of publications dealing with that indicator and skin disease, and sites refers to the number of beaches/independent locations that were studied in those publications.

Freshwater Indicators	Studies	Sites	Authors/Sites*
Fecal Coliform	6	9	Ferley 1989- 3 Sites Lee 2002- 1 Site Dewailly 1986- 1 Site Fewtrell 1992- 2 Sites Medema 1995- 1 Site Seyfried 1985- 1 Site
Total Coliform	2	4	Ferley 1989- 3 Sites Lee 2002- 1 Site
Fecal Streptococci	4	7	Ferley 1989- 3 Sites Fewtrell 1992- 2 Sites Medema 1995- 1 Site Seyfried 1985- 1 Site
E. coli	3	3	Medema 1995- 1 Site Van Asperen 1997- 1 Site Wiedenmann 2006- 1 Site
Enterococcus	3	3	Wade 2008- 1 Site Van Asperen 1997- 1 Site Wiedenmann 2006- 1 Site
Staphylococci	3	4	Fewtrell 1992- 2 Sites Seyfried 1985- 1 Site Van Asperen 1997- 1 Site

Marine Indicators	Studies	Sites	Authors/Sites*
Enterococci	6	25	Cheung 1990- 9 Sites Cabelli 1979- 2 Sites Cabelli 1983- 10 Sites Haile 1999- 1 Site Colford 2007- 1 Site von Schirnding 1992- 2 Sites
E. coli	4	22	Cheung 1990- 9 Sites Cabelli 1979- 2 Sites Cabelli 1983- 10 Sites Haile 1999- 1 Site
Total Coliforms	7	9	Prieto 2001- 1 Site Jones 1991- 1 Site Cabelli 1979- 2 Sites Alexander 1992- 1 Site Haile 1999- 1 Site Colford 2007- 1 Site Dwight 2004- 2 Sites
Fecal Streptococci	5	14	Cheung 1990- 9 Sites Prieto 2001- 1 Site Jones 1991- 1 Site Cabelli 1979- 2 Sites Alexander 1992- 1 Site
Fecal Coliform	8	18	Cheung 1990- 9 Sites Prieto 2001- 1 Site Jones 1991- 1 Site Cabelli 1979- 2 Sites Alexander 1992- 1 Site Haile 1999- 1 Site Colford 2007- 1 Site von Schirnding 1992- 2 Sites
Klebsiella	2	11	Cheung 1990- 9 Sites Cabelli 1979- 2 Sites
P. aeruginosa	4	13	Cheung 1990- 9 Sites Prieto 2001- 1 Site Jones 1991- 1 Site Cabelli 1979- 2 Sites
Staphylococci	3	11	Cheung 1990- 9 Sites Prieto 2001- 1 Site Charoenca 1995- 1 Site

*Only distinct beaches or sites that were analyzed separately and for which a separate relative risk was reported were counted as a site.

Table 3: Exposure, outcome definitions, and outcome assessment methods for each study included in the meta-analysis. For some studies, limited information was available about outcome assessment methods.

Freshwater Studies	Exposure Definition	Outcome Definition	Outcome Assessment
Seyfried (1985)	Any contact	Skin rash, welts, boils	Self report symptoms, telephone interview 7-10 days after, questionnaire mailed if no contact
Dewailly (1986)	Windsurfers	Infectious and allergenic skin conditions	Self report symptoms, questionnaire 2 days after
Ferley (1989)	Bathing	Skin infections	Self report symptoms, interviewed 1 week after
Fewtrell (1992)	Canoeists	Undefined skin symptoms	Self report symptoms, telephone interview 5-7 day after, questionnaire 1 month after
Medema (1995)	Triathalon- swim	Skin or mucosal symptoms	Self report symptoms
Van Asperen (1997)	Head immersion	Itchy skin, skin rash, present at least 2 parts of the day in the 2 days post trial	Self report symptoms, questionnaire 5 days after
Lee (2002)	Daily, some days, never exposure to water	Doctor inspection for skin conditions	Doctors assessed symptoms, day of study
Wiedenmann (2006)	3 head immersions, 10 minutes in water	Skin infections or cutireactions	Doctors assessed symptoms 1 week after, questionnaire 3 weeks after
Wade (2008)	Waist or higher immersion	Rash or itchy skin	Self report symptoms, telephone interview 10-12 days after

Marine Studies	Exposure Definition	Outcome Definition	Outcome Assessment
Cabelli (1979)	Head immersion, >10min in water	Itchy skin, rash, welts	Self report symptoms, telephone interview 8-10 days after
Cabelli (1983)	Head immersion	Irritations and disturbances of the skin	US: Self report symptoms, telephone interview 7-10 days after, Egypt: Self report symptoms, 1 week follow-up
Cheung (1990)	Head immersion or water touching face	Rashes, skin symptoms exclusive of sunburn	Self report symptoms, telephone interview 7 days after
Jones (1991)	Head immersion	Skin rash	Self-Report symptoms, 3 days after
Alexander (1992)	Any contact	Skin rash, itchy skin	Self report symptoms, telephone and questionnaire 10 days after
Von Schirnding (1992)	Water contact beyond waist	Skin rash, itchy skin, welts	Self report symptoms, telephone interview 4 days after
Charoenca (1995)	Seawater contact 10 days before study	Staphylococcal skin infections, cultured	Patients reporting to clinic with skin infections recruited
Haile (1999)	Head immersion	Skin rash	Self report symptoms, telephone interview 9-14 days after
Prieto (2001)	Self report swimming activity, no definition reported	Skin irritation, itching	Self report symptoms, telephone interview 7 days after
Dwight (2004)	Surfers in polluted beaches	Self reported skin infection	Self report symptoms, interview
Colford (2007)	Head or face under water	Skin rashes, infected cuts/scrapes	Self report symptoms, telephone interview 14 days after

Table 4: Additional study information, by freshwater indicator. Water sampling method, laboratory analysis method, suspected contamination source, and comparison groups.

Freshwater Indicator	Study	Collection Method	Lab Method	Probable Source	Study Groups
Fecal Coliform	Ferley (1989)	2x a week at 5 beaches, 30 cm depth	Spread plate or membrane filter procedure with Tergitol and TTC agar, incubated	Untreated urban domestic sewage	Swimmers vs. Non-Swimmers
	Lee (2002)	Water sampled at survey time	None Stated	Pulp Mill, Treated Waste, Point Source	Village with high pollution vs. Village with low pollution
	Dewailly (1986)	One day, 8 sites sampled	None Stated	Sewage	Windsurfers vs. Non-water exposed
	Fewtrell (1992)	Day of activity	None Stated	Several Upstream Sewage Treatment Plants	Canoeists vs. Non-canoeists
	Medema (1995)	Samples of 3 sites, 30 cm below surface	Dutch Standard methods	None Stated	Swimmers vs. Non-swimmers
	Seyfried (1985)	Sample Beaches 2-3x a day, water and sediment at depth of at least 50 cm	Water: MPN (Most Probable Number) using Standard Methods	None Stated	Swimmers vs. Non-Swimmers
Total Coliform	Ferley (1989)	2x a week at 5 beaches, 30 cm depth	Spread plate or membrane filter procedure with Tergitol and TTC agar, incubated	Untreated urban domestic sewage	Swimmers vs. Non-Swimmers

	Lee (2002)	Water sampled at survey time	None Stated	Pulp Mill, Treated Waste, Point Source	Village with high pollution vs. Village with low pollution
Fecal Streptococci	Ferley (1989)	2x a week at 5 beaches, 30 cm depth	Poured plates using D. coccosel agar	Untreated urban domestic sewage	Swimmers vs. Non-Swimmers
	Fewtrell (1992)	Day of activity	None Stated	Several Upstream Sewage Treatment Plants	Canoeists vs. Non-canoeists
	Medema (1995)	Samples of 3 sites, 30 cm below surface	Dutch Standard methods	None Stated	Swimmers vs. Non-swimmers
	Seyfried (1985)	Sample Beaches 2-3x a day, water and sediment at depth of at least 50 cm	Water: MPN (Most Probable Number) using Standard Methods, and membrane filter m-*Enterococcus* agar (Difco)	None Stated	Swimmers vs. Non-Swimmers
E. coli	Medema (1995)	Samples of 3 sites, 30 cm below surface	Dutch Standard methods	None Stated	Swimmers vs. Non-swimmers
	Van Asperen (1997)	Day of exposure, multiple sites, 250 ml samples	Dutch Standard Methods	Treated sewage	Primary School children randomized
	Wiedenmann (2006)	Sampled every 20 min	MUG Hydrolysis, microtiter plate method	Treated and untreated municipal sewage,	Randomized bathers vs. non-bathers

				agricultural runoff, waterfowl contamination	
Enterococcus	Wade (2008)	Samples shin and waist deep	EPA Membrane Filtration Method 1600, and QPCR	Treated Sewage (Point Source)	Swimmers vs. Non-Swimmers
	Van Asperen (1997)	Day of exposure, multiple sites, 250 ml samples	Dutch Standard Methods	Treated sewage	Primary School children randomized
	Wiedenmann (2006)	Sampled every 20 min	MUD Hydrolysis and formazan formation	Treated and untreated municipal sewage, agricultural runoff, waterfowl contamination	Randomized bathers vs. non-bathers
Staphylococci	Fewtrell (1992)	Day of activity	None Stated	Several Upstream Sewage Treatment Plants	Canoeists vs. Non-canoeists
	Seyfried (1985)	Sample Beaches 2-3x a day, water and sediment at depth of at least 50 cm	Water: Gelman Filters, incubated on Vogel-Johnson agar, Sand: Enrich in m-Staphylococcus broth, spread on Vogel-Johnson agar	None Stated	Swimmers vs. Non-Swimmers
	Van Asperen (1997)	Day of exposure, multiple sites, 250 ml samples	Dutch Standard Methods	Treated sewage	Primary School children randomized

Table 5: Additional study information, by marine indicator. Water sampling method, laboratory analysis method, suspected contamination source, and comparison groups.

Marine Indicator	Study	Collection Method	Lab Method	Probable Source	Study Groups
Enterococci	Cheung (1990)	3 samples per beach, 1m deep	Membrane filtration, incubated on media	Human sewage discharge, stormdrains, livestock waste	Swimmers vs. non-swimmers
	Cabelli (1979)	Sample several times per day, chest depth 4 in below surface	Membrane filter	Sewage	Swimmers vs. Non-swimmers
	Cabelli (1983)	Multiple samples, Chest Depth, just below surface	Membrane Filter, mE medium	US: None Stated, Egypt: Raw sewage	Swimmers vs. Non-swimmers
	Haile (1999)	Daily ankle depth samples	Membrane filtration	Storm Drain Runoff	Swimmers in more polluted water vs. swimmers in less polluted water
	Colford (2007)	Daily, hourly sampling	Membrane Filtration, chromogenic substrate method, and qPCR	Non-point source, human contamination minimal	Swimmers vs. non-swimmers
	Von Schirnding (1992)	Day of trial, samples before and during trial	Standard membrane filtration methods	Septic tank overflows, stormwater run-off, fecal contamination in river water	Swimmers vs. Non-swimmers
E. coli	Cheung (1990)	3 samples per beach, 1m deep	Membrane filtration	Human sewage discharge	Swimmers vs. non-swimmers

			media	livestock waste	
	Cabelli (1979)	Sample several times per day, chest depth 4 in below surface	mC	Sewage	Swimmers vs. Non-swimmers
	Cabelli (1983)	Multiple samples, Chest Depth, just below surface	Membrane Filtration, mTEC medium	US: None Stated, Egypt: Raw sewage	Swimmers vs. Non-swimmers
	Haile (1999)	Daily ankle depth samples	Membrane filtration, Hach Method 10029	Storm Drain Runoff	Swimmers in more polluted water vs. swimmers in less polluted water
Total Coliforms	Prieto (2001)	30 cm below surface	Standard Methods	Sewage Systems	Swimmers vs. Non-Swimmers
	Jones (1991)	Sampled every 20 min at surf, 30 cm, chest depth, and 50 m off-shore.	None Stated	None Stated	Bather vs. Non-Bather Randomized
	Cabelli (1979)	Sample several times per day, chest depth 4 in below surface	Most Probable Number, mC procedure	Sewage	Swimmers vs. Non-swimmers
	Alexander (1992)	2 samples at waist depth	Standard Methods	Sewage	Swimmers vs. Non-Swimmers

	Haile (1999)	Daily ankle depth samples	Membrane Filtration	Storm Drain Runoff	Swimmers in more polluted water vs. swimmers in less polluted water
	Colford (2007)	Daily, hourly sampling	Membrane Filtration and chromogenic substrate method	Non-point source, human contamination minimal	Swimmers vs. Non-Swimmers
	Dwight (2004)	None Stated	None Stated	Untreated Urban Runoff (non-point source)	Polluted vs. Non-polluted beach
Fecal Streptococci	Cheung (1990)	3 samples per beach, 1m deep	Membrane filtration, incubated on media	Human sewage discharge, stormdrains, livestock waste	Swimmers vs. non-swimmers
	Prieto (2001)	30 cm below surface	Standard Methods	Sewage Systems	Swimmers vs. Non-Swimmers
	Jones (1991)	Sampled every 20 min at surf, 30 cm, chest depth, and 50 m off-shore.	None Stated	None Stated	Bather vs. Non-Bather Randomized
	Cabelli (1979)	Sample several times per day, chest depth 4 in below surface	mSD	Sewage	Swimmers vs. Non-swimmers
	Alexander (1992)	2 samples at waist depth	Standard Methods	Sewage	Swimmers vs. Non-Swimmers
Fecal Coliform	Cheung (1990)	3 samples per beach, 1m deep	Membrane filtration	Human sewage discharge	Swimmers vs. non-swimmers

			media	livestock waste	
	Prieto (2001)	30 cm below surface	Standard Methods	Sewage Systems	Swimmers vs. Non-Swimmers
	Jones (1991)	Sampled every 20 min at surf, 30 cm, chest depth, and 50 m off-shore.	None Stated	None Stated	Bather vs. Non-Bather Randomized
	Cabelli (1979)	Sample several times per day, chest depth 4 in below surface	Most Probable Number	Sewage	Swimmers vs. Non-swimmers
	Alexander (1992)	2 samples at waist depth	Standard Methods	Sewage	Swimmers vs. Non-Swimmers
	Haile (1999)	Daily ankle depth samples	Membrane Filtration	Storm Drain Runoff	Swimmers in more polluted water vs. swimmers in less polluted water
	Colford (2007)	Daily, hourly sampling	Membrane Filtration and chromogenic substrate method	Non-point source, human contamination minimal	Swimmers vs. Non-Swimmers
	Von Schirnding (1992)	Day of trial, samples before and during trial	Standard membrane filtration methods	Septic tank overflows, stormwater run-off, fecal contamination in river water	Swimmers vs. Non-swimmers

Klebsiella	Cheung (1990)	3 samples per beach, 1m deep	Membrane filtration, incubated on media	Human sewage discharge, stormdrains, livestock waste	Swimmers vs. non-swimmers
	Cabelli (1979)	Sample several times per day, chest depth 4 in below surface	mC procedure	Sewage	Swimmers vs. Non-swimmers
Staphylococci	Cheung (1990)	3 samples per beach, 1m deep	Membrane filtration, incubated on media	Human sewage discharge, stormdrains, livestock waste	Swimmers vs. non-swimmers
	Prieto (2001)	30 cm below surface	Standard Methods	Sewage Systems	Swimmers vs. Non-Swimmers
	Charoenca (1995)	None Stated	Gelman membrane filtration, Vogel-Johnson Medium used with incubation	None Stated	swimmers at polluted vs. less polluted

Figure 1: Summary of Meta-Analysis Results, Odds Ratios, and Ratio of Odds Ratios (ROR). Number of individual sites with reported odds ratios is given next to each indicator, as well as the number of total studies included in each subanalysis in the Studies column.

Footnotes:
Meta-Analysis summary results. 1. Total coliform cut-off: San Diego Water Board (2007). 2. Fecal coliform cut-off: California Department of Public Health (2000). 3. *E. coli* cut-off: U.S. EPA (1986) 4. total coliform cut-off: California State Water Resources Control Board (1990a). 5. fecal coliform cut-off: California State Water Resources Control Board (1990b). 6. *E. coli* cut-off: Haile (1999). 7. *Enterococcus* cut-off: U.S. EPA (1986). 8. streptococci cut-off: Ogan (1994). 9. ROR is the ratio of odds ratios from high vs. low indicator settings. [1] Lee 2002, [2] Ferley 1989, [3] Fewtrell 1992, [4] Medema 1995, [5] Seyfried 1985, [6] Dewailly 1986, [7] Jones 1991, [8] Cabelli 1979, [9] Alexander 1992, [10] Colford 2007, [11] Wade 2008, [12] Prieto 2001, [13] Haile 1999, [14] Cheung 1990, [15] von Schirnding 1992, [16] Cabelli 1983, [17] Dwight 2004, [18] Van Asperen 1997, [19] Wiedenmann 2006, [20] Charoenca 1995.

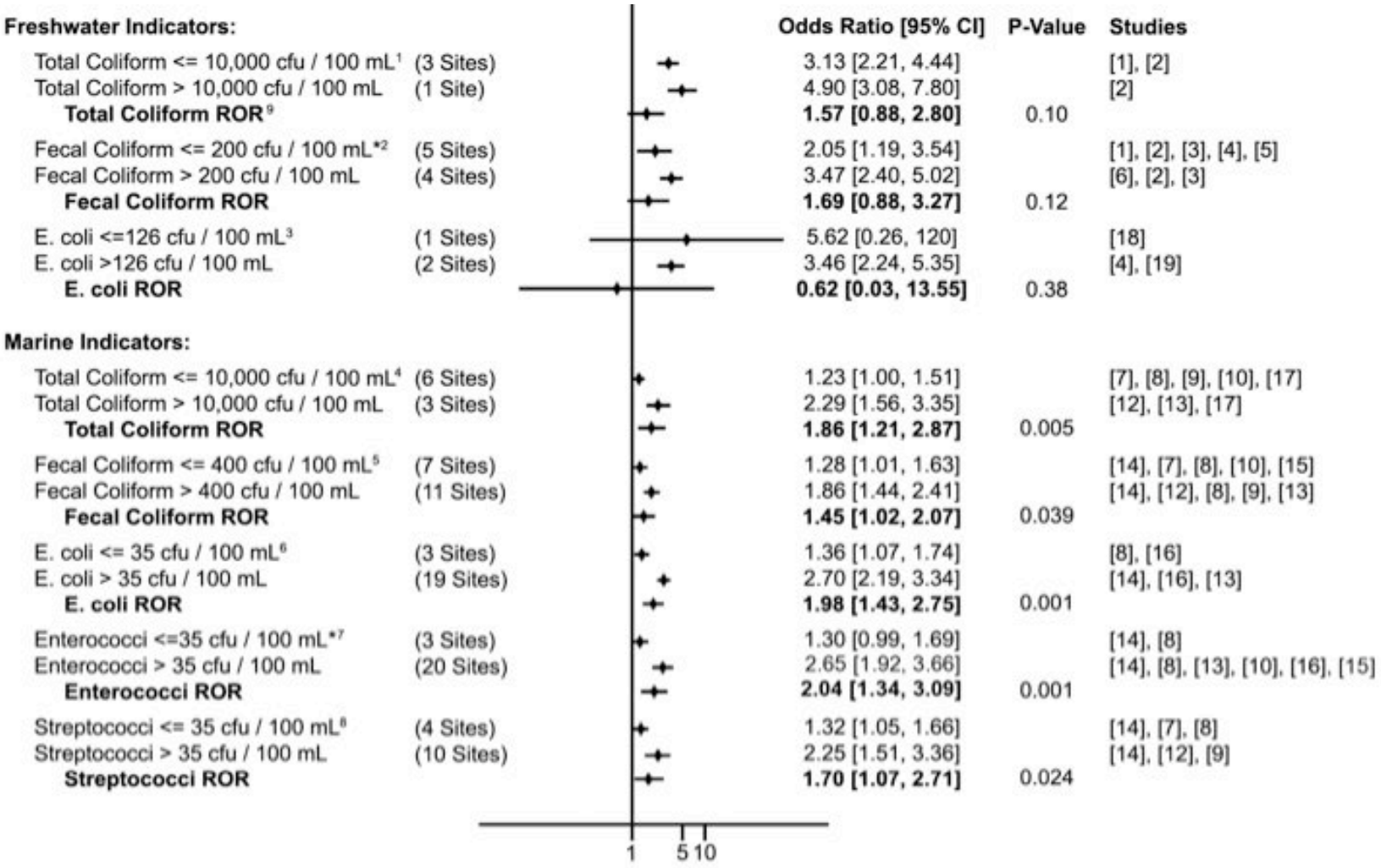

Freshwater Indicators:
Odds Ratio [95% CI]
P-Value
Studies
Total Coliform <= 10,000 cfu / 100 mL[1] (3 Sites) 3.13 [2.21, 4.44] [1], [2]
Total Coliform > 10,000 cfu / 100 mL (1 Site) 4.90 [3.08, 7.80] [2]
Total Coliform ROR[9] 1.57 [0.88, 2.80] 0.10
Fecal Coliform <= 200 cfu / 100 mL*[2] (5 Sites) 2.05 [1.19, 3.54] [1], [2], [3], [4], [5]
Fecal Coliform > 200 cfu / 100 mL (4 Sites) 3.47 [2.40, 5.02] [6], [2], [3]
Fecal Coliform ROR 1.69 [0.88, 3.27] 0.12
E. coli <=126 cfu / 100 mL[3] (1 Sites) 5.62 [0.26, 120] [18]
E. coli >126 cfu / 100 mL (2 Sites) 3.46 [2.24, 5.35] [4], [19]
E. coli ROR 0.62 [0.03, 13.55] 0.38
Marine Indicators:
Total Coliform <= 10,000 cfu / 100 mL[4] (6 Sites) 1.23 [1.00, 1.51] [7], [8], [9], [10], [17]
Total Coliform > 10,000 cfu / 100 mL (3 Sites) 2.29 [1.56, 3.35] [12], [13], [17]
Total Coliform ROR 1.86 [1.21, 2.87] 0.005
Fecal Coliform <= 400 cfu / 100 mL[5] (7 Sites) 1.28 [1.01, 1.63] [14], [7], [8], [10], [15]
Fecal Coliform > 400 cfu / 100 mL (11 Sites) 1.86 [1.44, 2.41] [14], [12], [8], [9], [13]
Fecal Coliform ROR 1.45 [1.02, 2.07] 0.039
E. coli <= 35 cfu / 100 mL[6] (3 Sites) 1.36 [1.07, 1.74] [8], [16]
E. coli > 35 cfu / 100 mL (19 Sites) 2.70 [2.19, 3.34] [14], [16], [13]
E. coli ROR 1.98 [1.43, 2.75] 0.001
Enterococci <=35 cfu / 100 mL*[7] (3 Sites) 1.30 [0.99, 1.69] [14], [8]
Enterococci > 35 cfu / 100 mL (20 Sites) 2.65 [1.92, 3.66] [14], [8], [13], [10], [16], [15]
Enterococci ROR 2.04 [1.34, 3.09] 0.001
Streptococci <= 35 cfu / 100 mL[8] (4 Sites) 1.32 [1.05, 1.66] [14], [7], [8]
Streptococci > 35 cfu / 100 mL (10 Sites) 2.25 [1.51, 3.36] [14], [12], [9]
Streptococci ROR 1.70 [1.07, 2.71] 0.024
1
5
10

Figure 2: Studies with internal control groups (OR comparing swimmers to non-swimmers in water with lower than recommended indicator levels versus OR comparing swimmers to non-swimmers in water with higher than recommended indicator levels). Each study has a ROR reported, and if multiple studies are present for a given indicator, the ROR's from each individual study are meta-analyzed into a summary ROR.

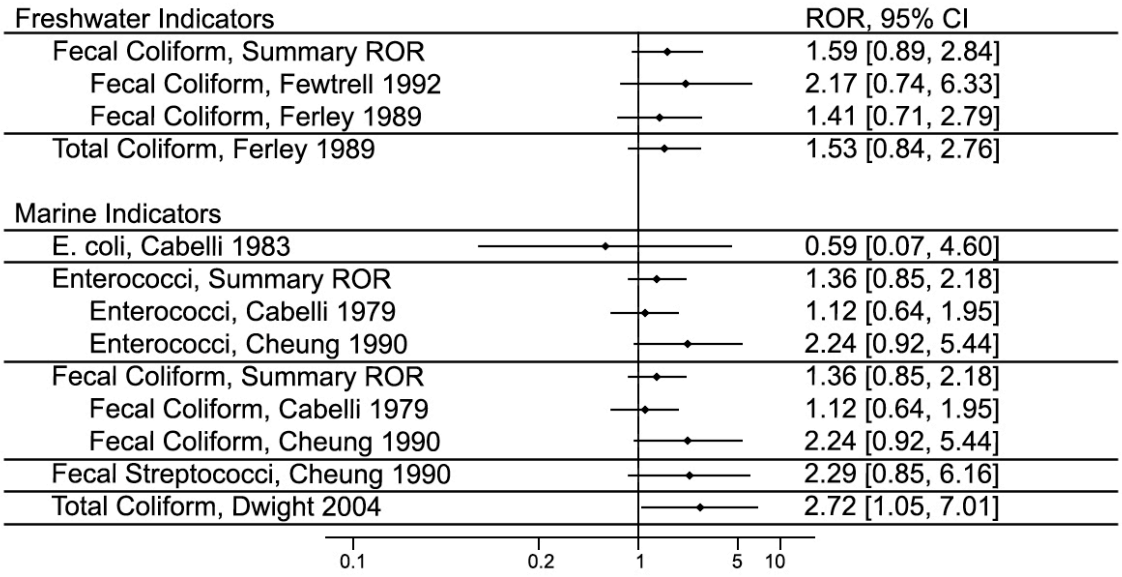


Figure 3: Microbial indicator concentrations by indicator. Mean, median, and minimum/maximum values for each microbial indicator are reported.

		Indicator Concentration (cfu / 100 mL)		
Freshwater Indicators:		**Mean**	**Median**	**Range**
Total Coliform <= 10,000 cfu / 100 mL	(3 Sites)	1079	786	10-2440
Total Coliform > 10,000 cfu / 100 mL	(1 Site)	2446	24461	24461-24461
Fecal Coliform <= 200 cfu / 100 mL	(5 Sites)	69	76	6-133
Fecal Coliform > 200 cfu / 100 mL	(4 Sites)	889	762	285-1749
E. coli <=126 cfu / 100 mL	(1 Sites)	68	68	68-68
E. coli >126 cfu / 100 mL	(2 Sites)	153	153	136-170
Enterococcus >33 cfu / 100 mL	(3 Sites)	297	83	37-770
Staphylococci (n/a cut-point)	(4 Sites)	45	13	3-151
Streptococci (n/a cut-point)	(7 Sites)	31	15	13-82
Marine Indicators:				
Total Coliform <= 10,000 cfu / 100 mL	(6 Sites)	744	574	37-2022
Total Coliform > 10,000 cfu / 100 mL	(3 Sites)	10667	10000	10000-12000
Fecal Coliform <= 400 cfu / 100 mL	(7 Sites)	95	77	8-254
Fecal Coliform > 400 cfu / 100 mL	(11 Sites)	797	565	400-3166
E. coli <= 35 cfu / 100 mL	(3 Sites)	11	15	2-15
E. coli > 35 cfu / 100 mL	(19 Sites)	1311	269	35-10400
Enterococci <=35 cfu / 100 mL	(4 Sites)	22	28	7-31
Enterococci > 35 cfu / 100 mL	(21 Sites)	1250	168	35-9160
Streptococci <= 35 cfu / 100 mL	(4 Sites)	21	24	4-32
Streptococci > 35 cfu / 100 mL	(10 Sites)	159	101	40-500
Klebsiella (n/a cut-point)	(11 Sites)	205	105	4-943
P. aeruginosa (n/a cut-point)	(13 Sites)	11	5	0.07-46
Staphylococci (n/a cut-point)	(11 Sites)	952	921	100-2963

Figure 4: Summary of Meta-Analysis Results, Odds Ratios. Forest plot for OR's from various studies relating *E. coli* in freshwater settings to skin ailments. Results divided by level of indicator concentration.

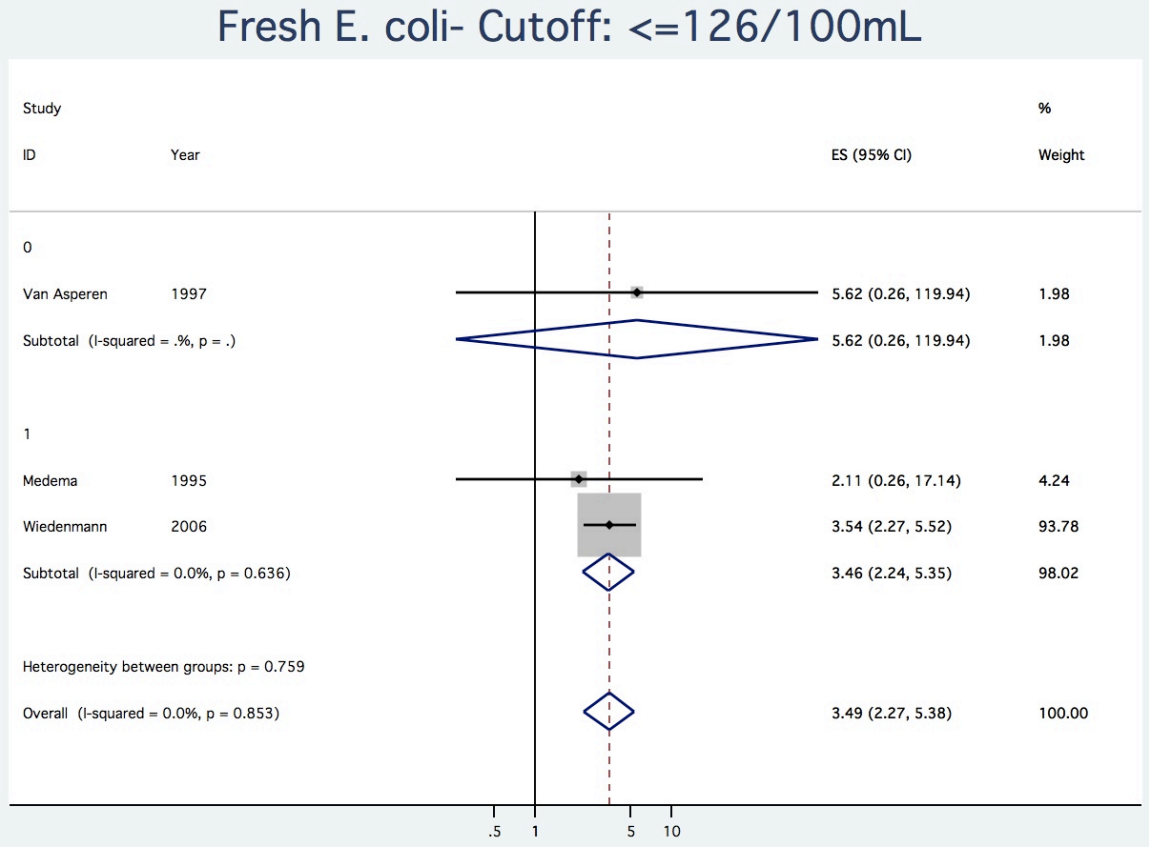


Figure 5: Summary of Meta-Analysis Results, Odds Ratios. Forest plot for OR's from various studies relating *Enterococcus* in freshwater settings to skin ailments. Results divided by level of indicator concentration.

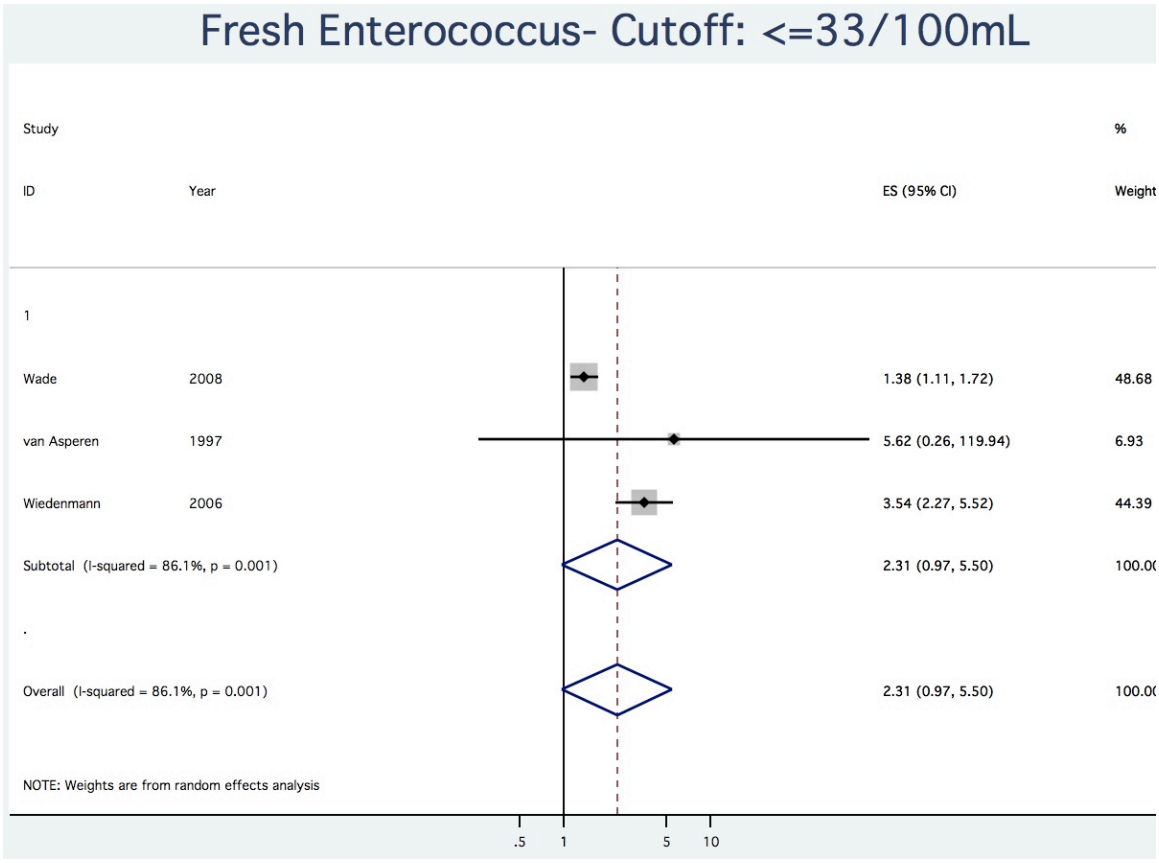


Figure 6: Summary of Meta-Analysis Results, Odds Ratios. Forest plot for OR's from various studies relating fecal coliforms in freshwater settings to skin ailments. Results divided by level of indicator concentration.

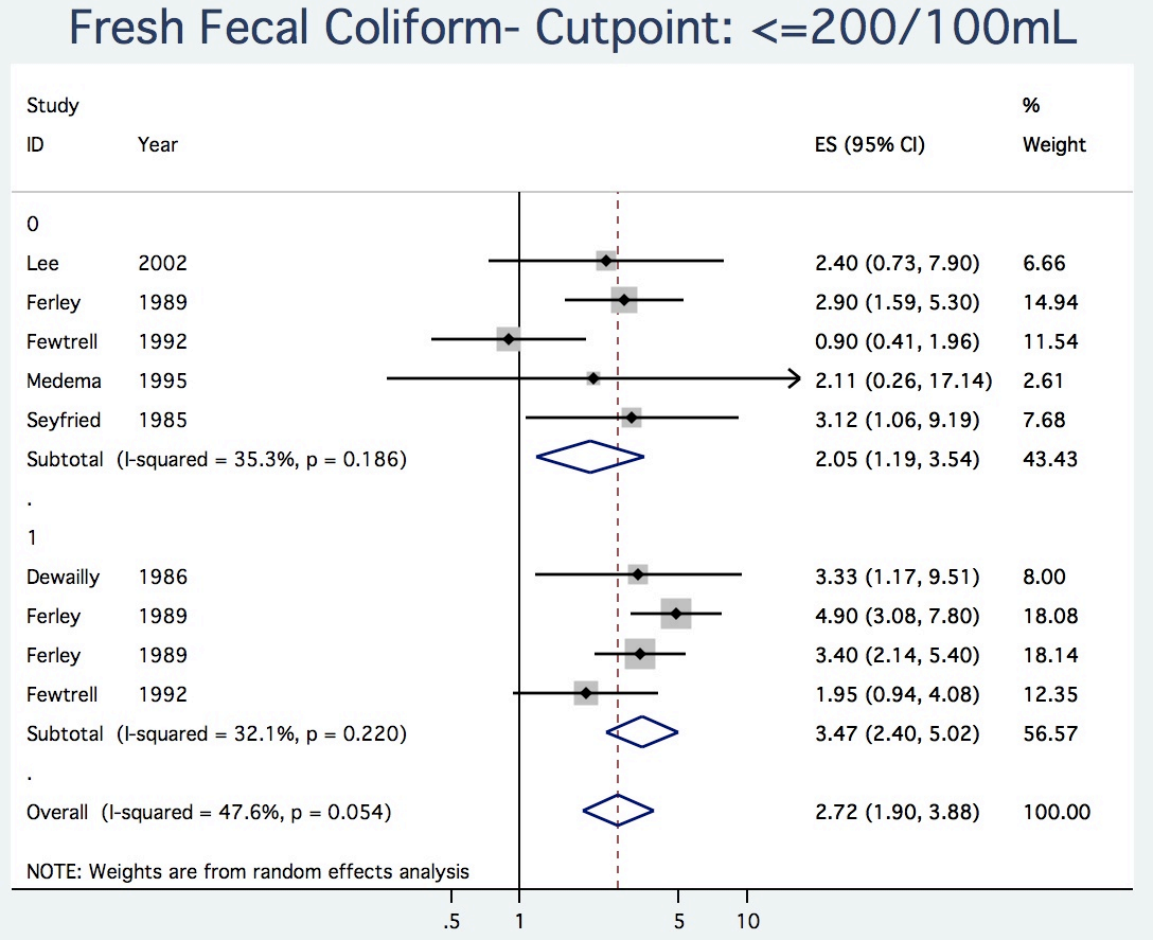


Figure 7: Summary of Meta-Analysis Results, Odds Ratios. Forest plot for OR's from various studies relating *Streptococci* in freshwater settings to skin ailments.

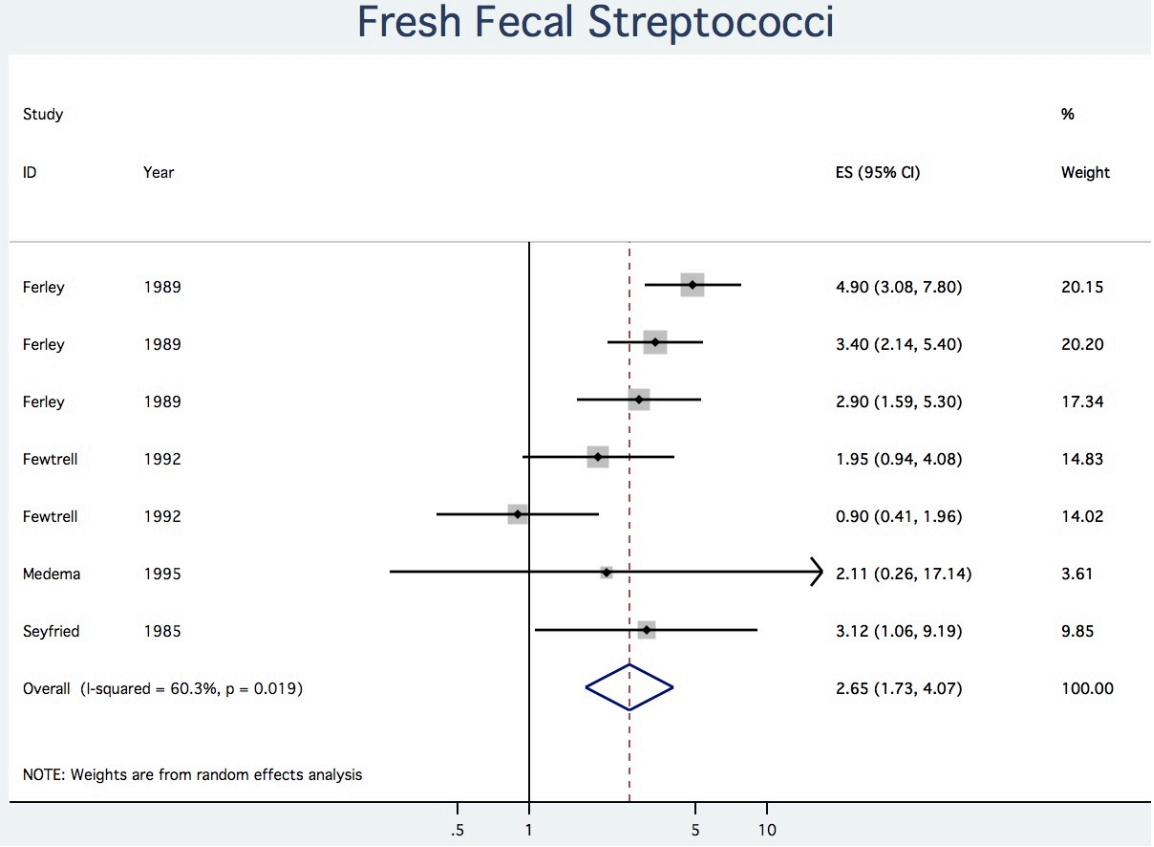


Figure 8: Summary of Meta-Analysis Results, Odds Ratios. Forest plot for OR's from various studies relating *Staphylococci* in freshwater settings to skin ailments.

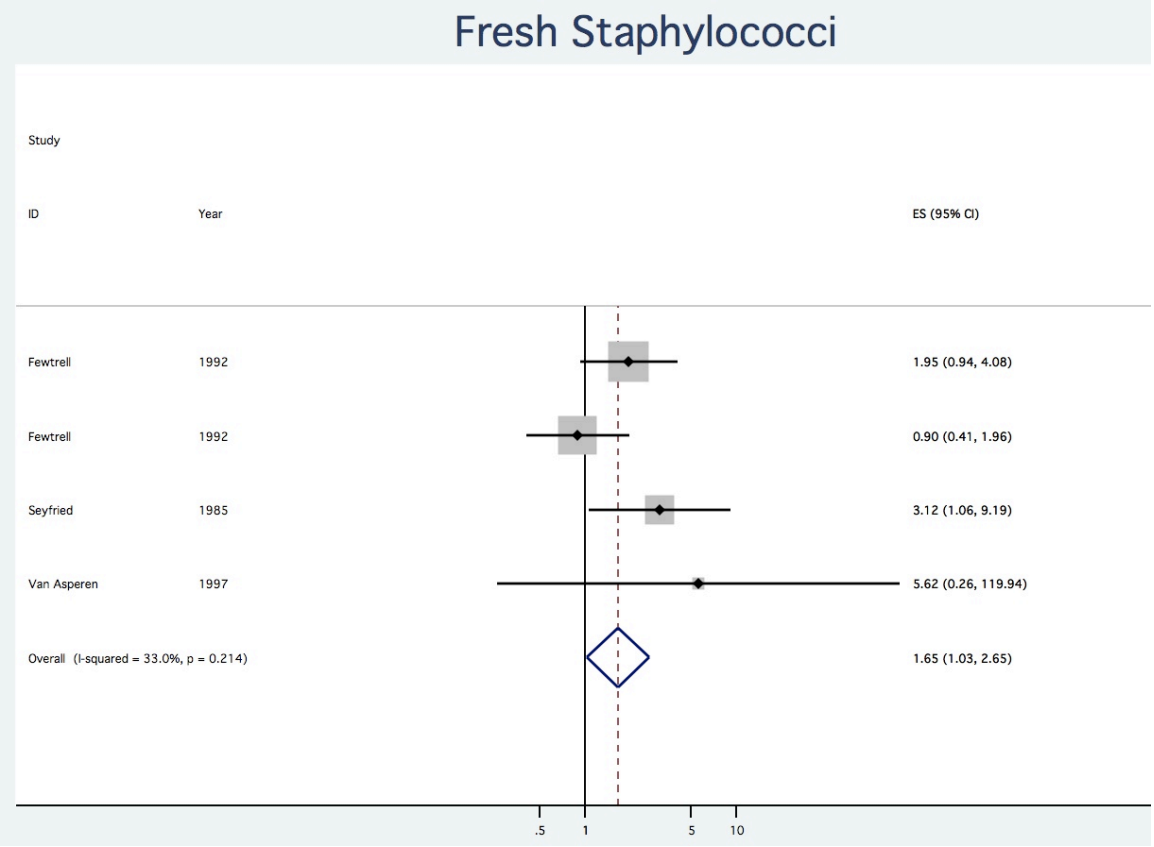


Figure 9: Summary of Meta-Analysis Results, Odds Ratios. Forest plot for OR's from various studies relating total coliform in freshwater settings to skin ailments. Results divided by level of indicator concentration.

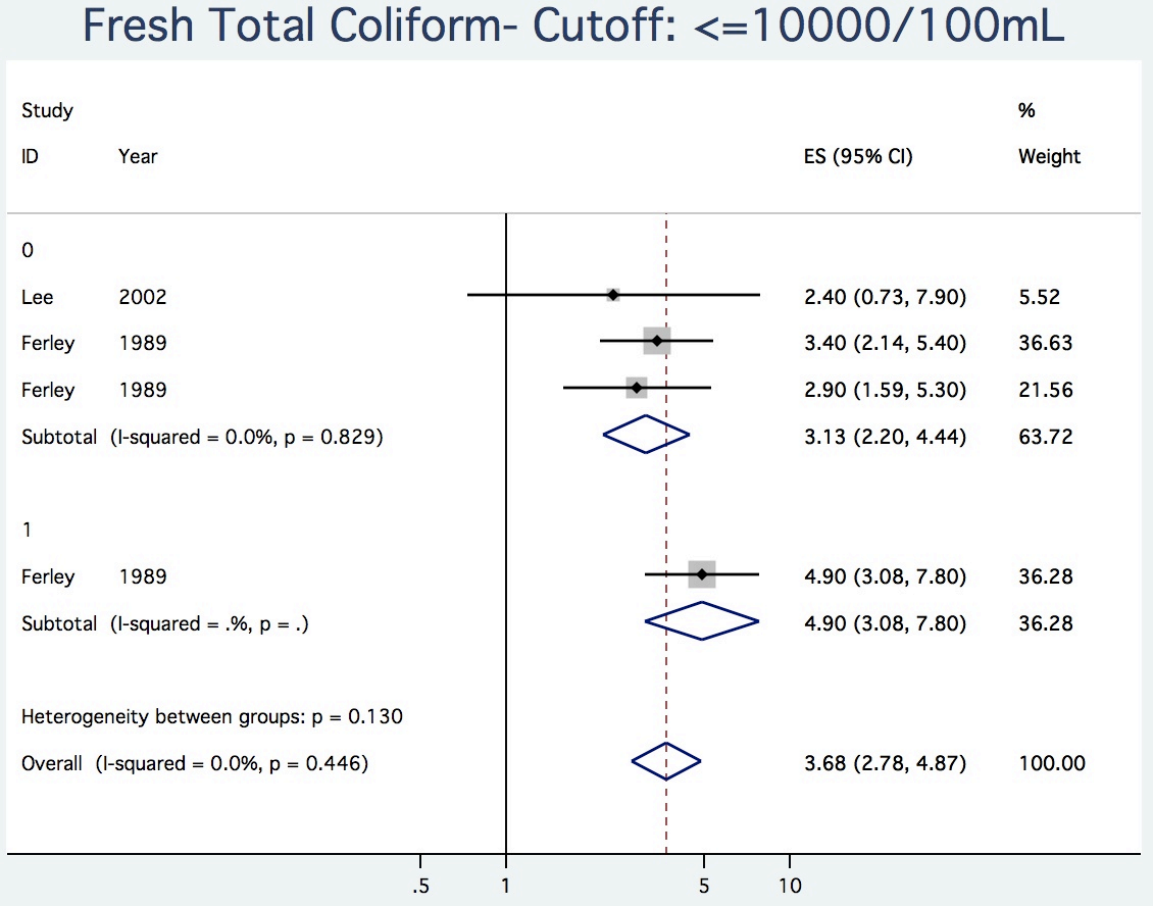


Figure 10: Summary of Meta-Analysis Results, Odds Ratios. Forest plot for OR's from various studies relating *E. coli* in marine settings to skin ailments. Results divided by level of indicator concentration.

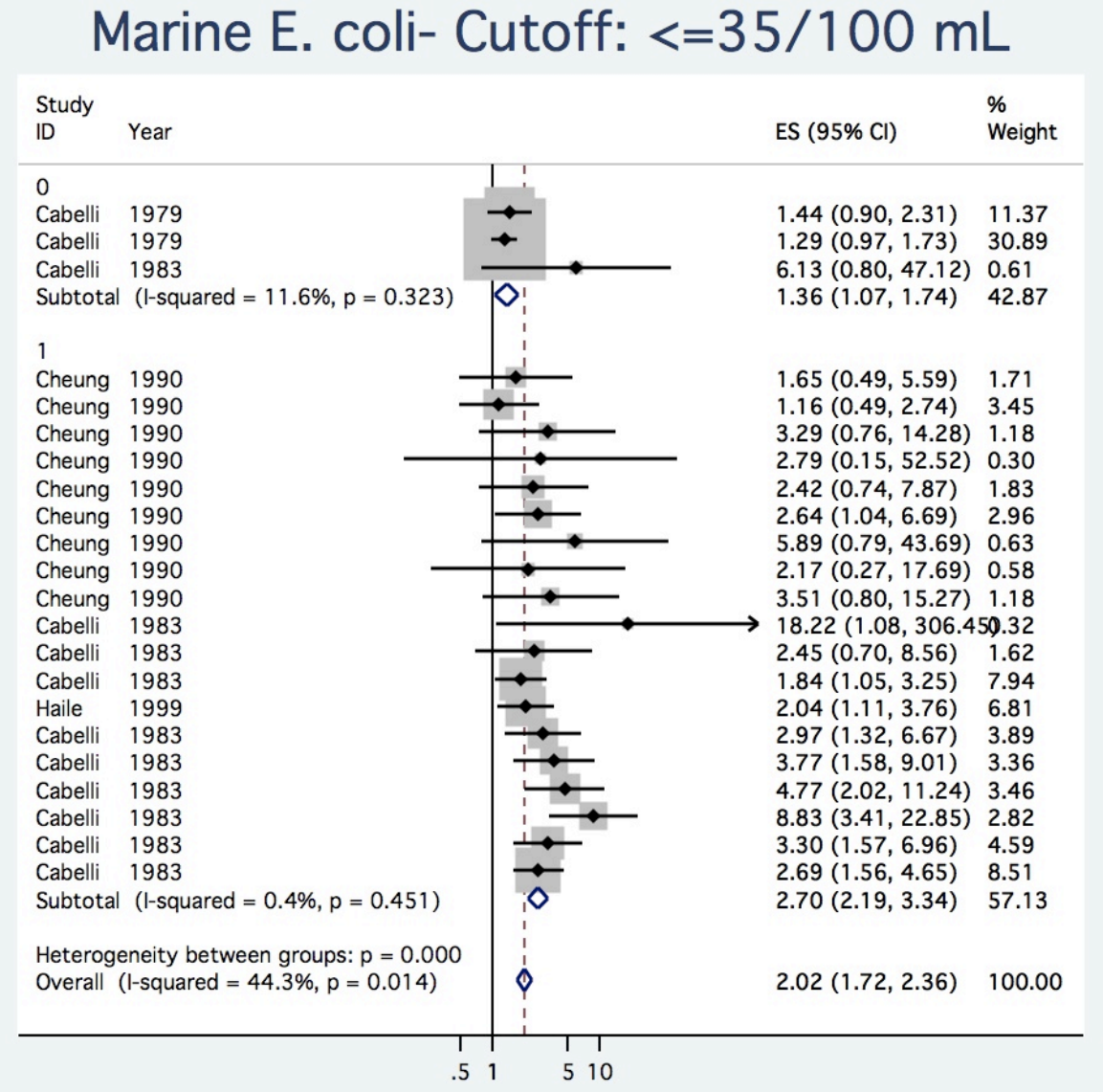


Figure 11: Summary of Meta-Analysis Results, Odds Ratios. Forest plot for OR's from various studies relating *Enterococcus* in marine settings to skin ailments. Results divided by level of indicator concentration.

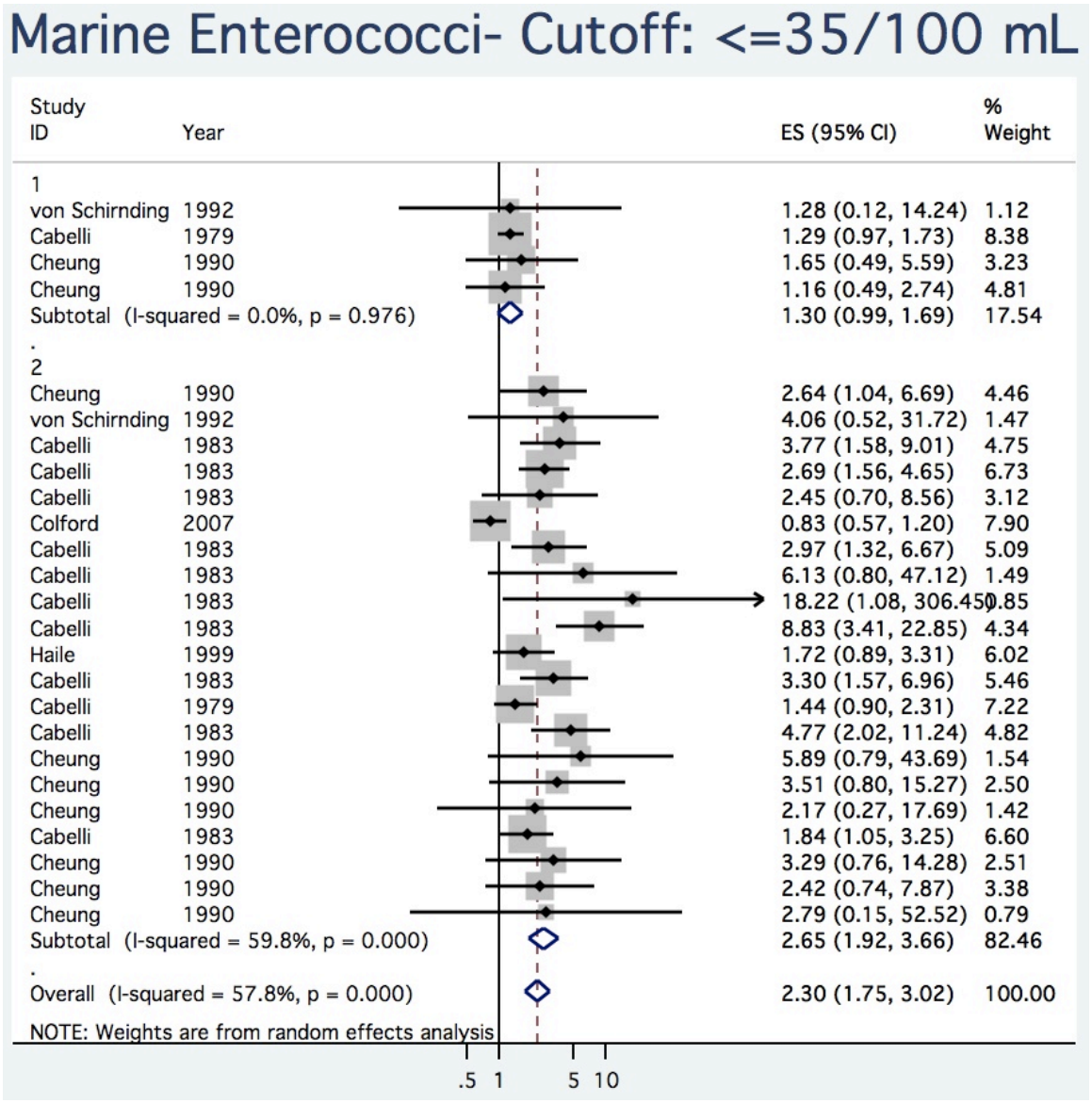


Figure 12: Summary of Meta-Analysis Results, Odds Ratios. Forest plot for OR's from various studies relating fecal coliforms in marine settings to skin ailments. Results divided by level of indicator concentration.

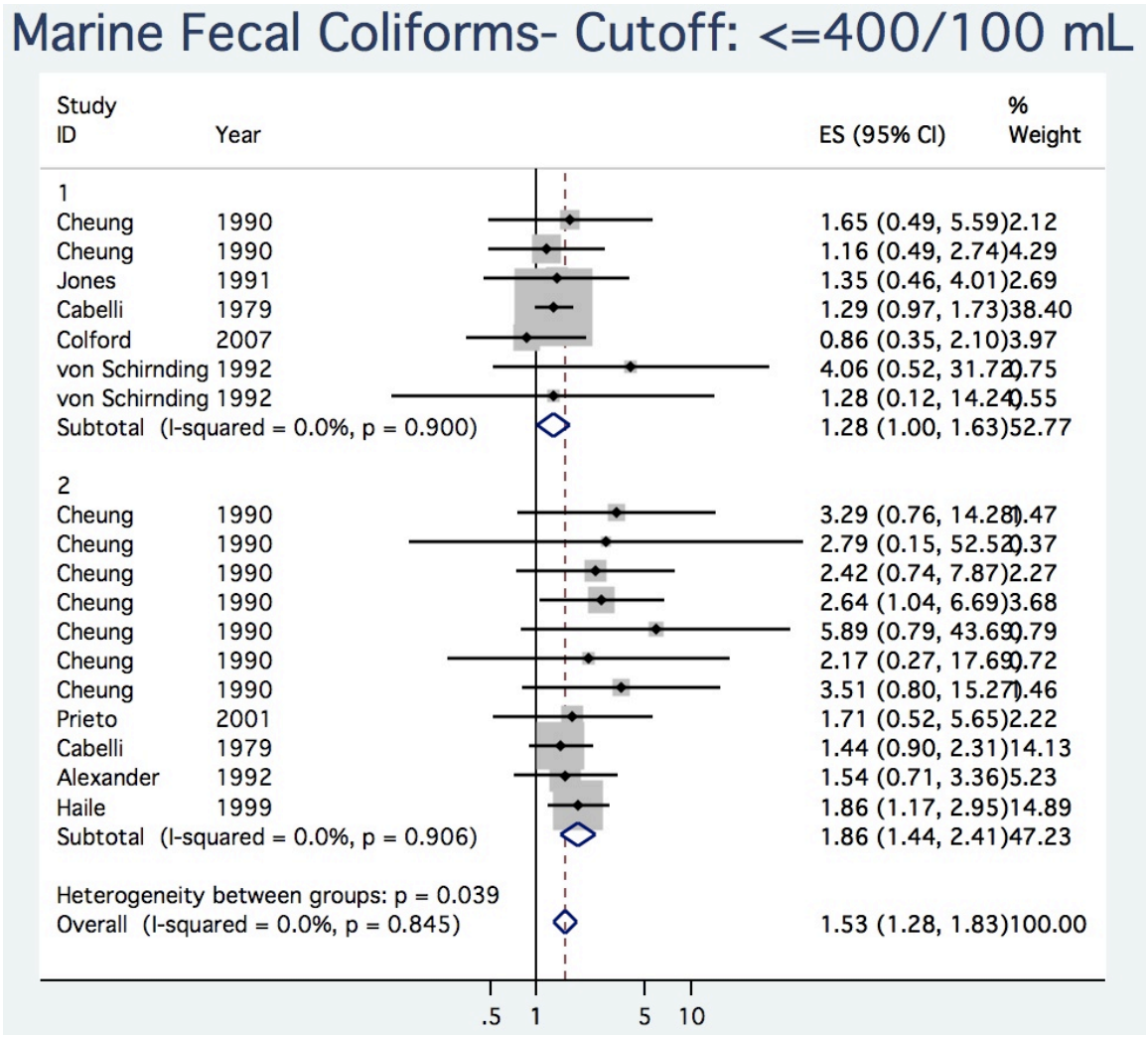


Figure 13: Summary of Meta-Analysis Results, Odds Ratios. Forest plot for OR's from various studies relating fecal *Streptococci* in marine settings to skin ailments. Results divided by level of indicator concentration.

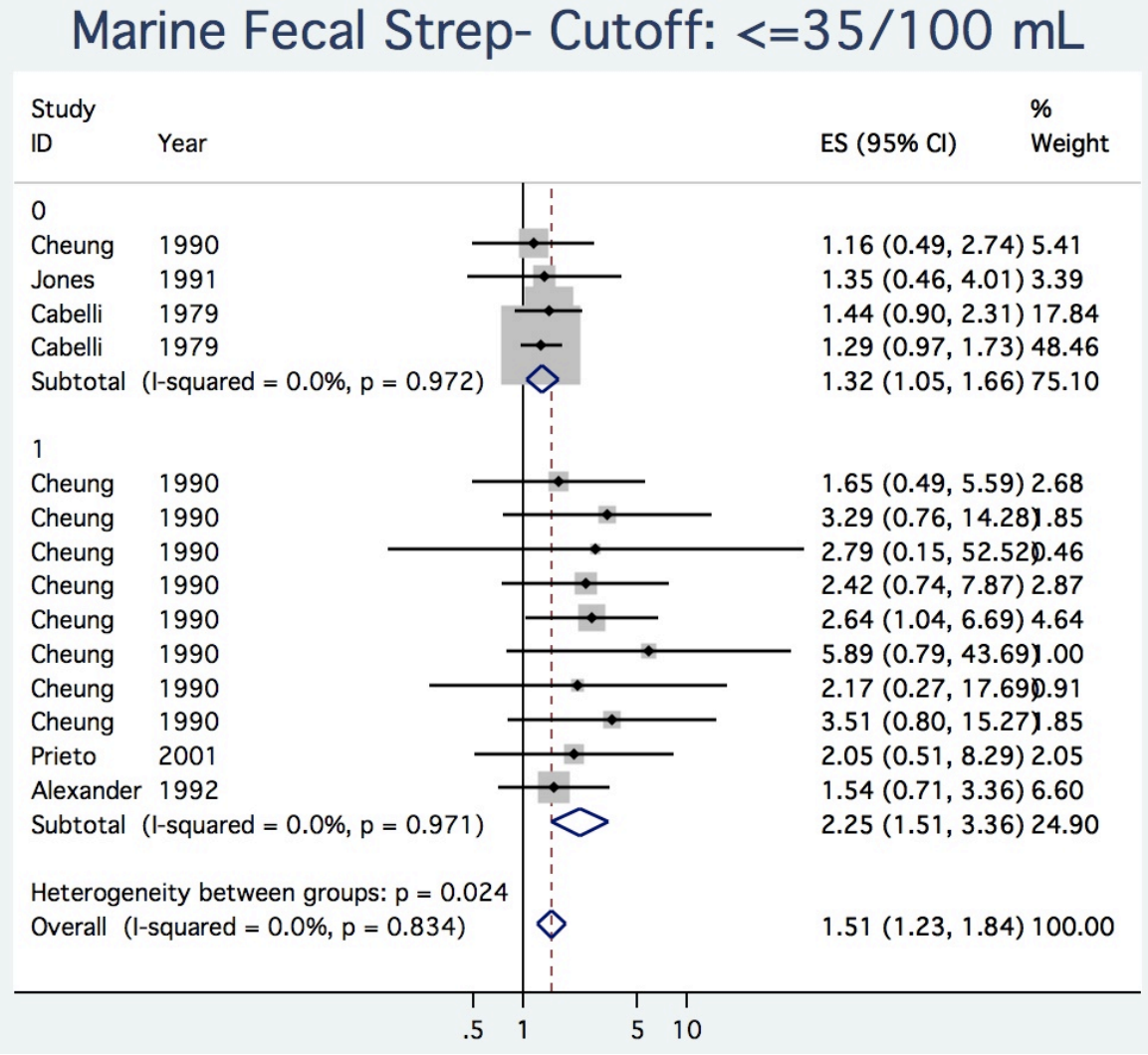


Figure 14: Summary of Meta-Analysis Results, Odds Ratios. Forest plot for OR's from various studies relating *Klebsiella* in marine settings to skin ailments.

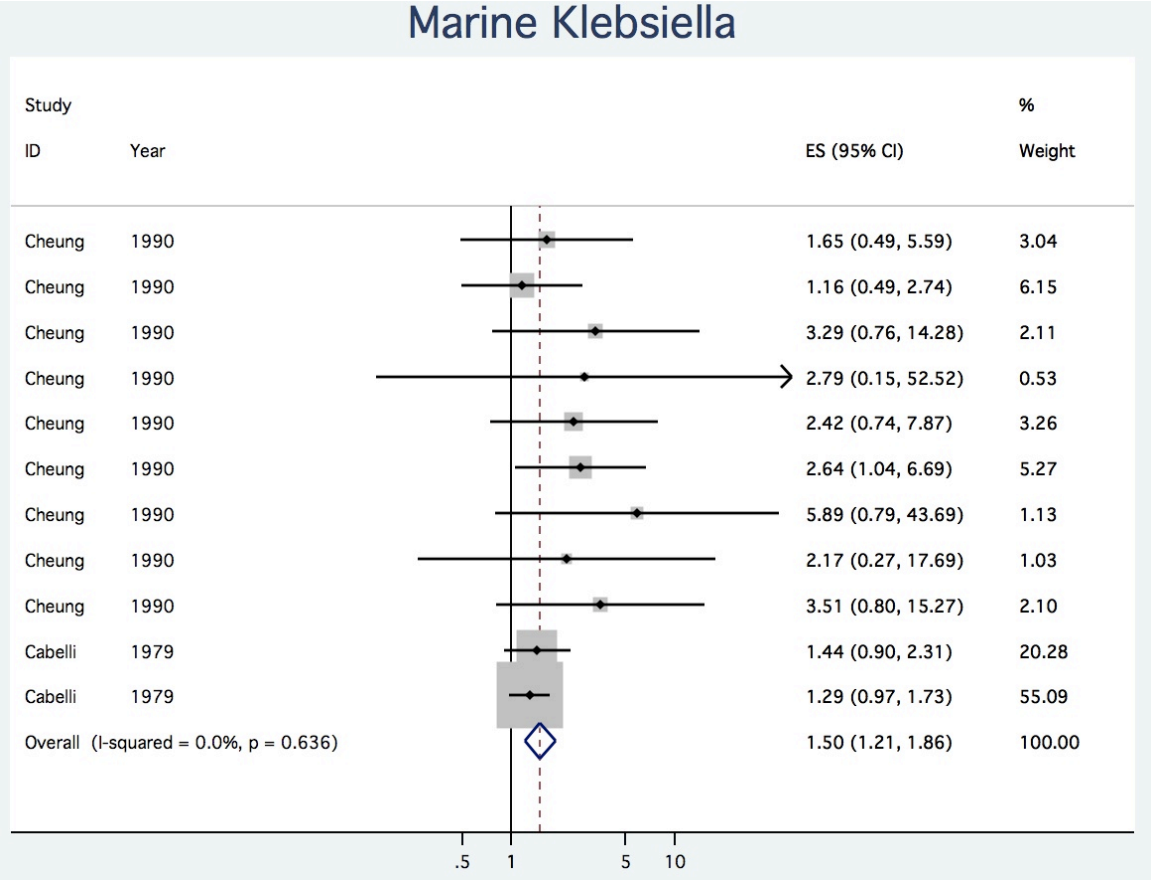


Figure 15: Summary of Meta-Analysis Results, Odds Ratios. Forest plot for OR's from various studies relating *P. aeruginosa* in marine settings to skin ailments.

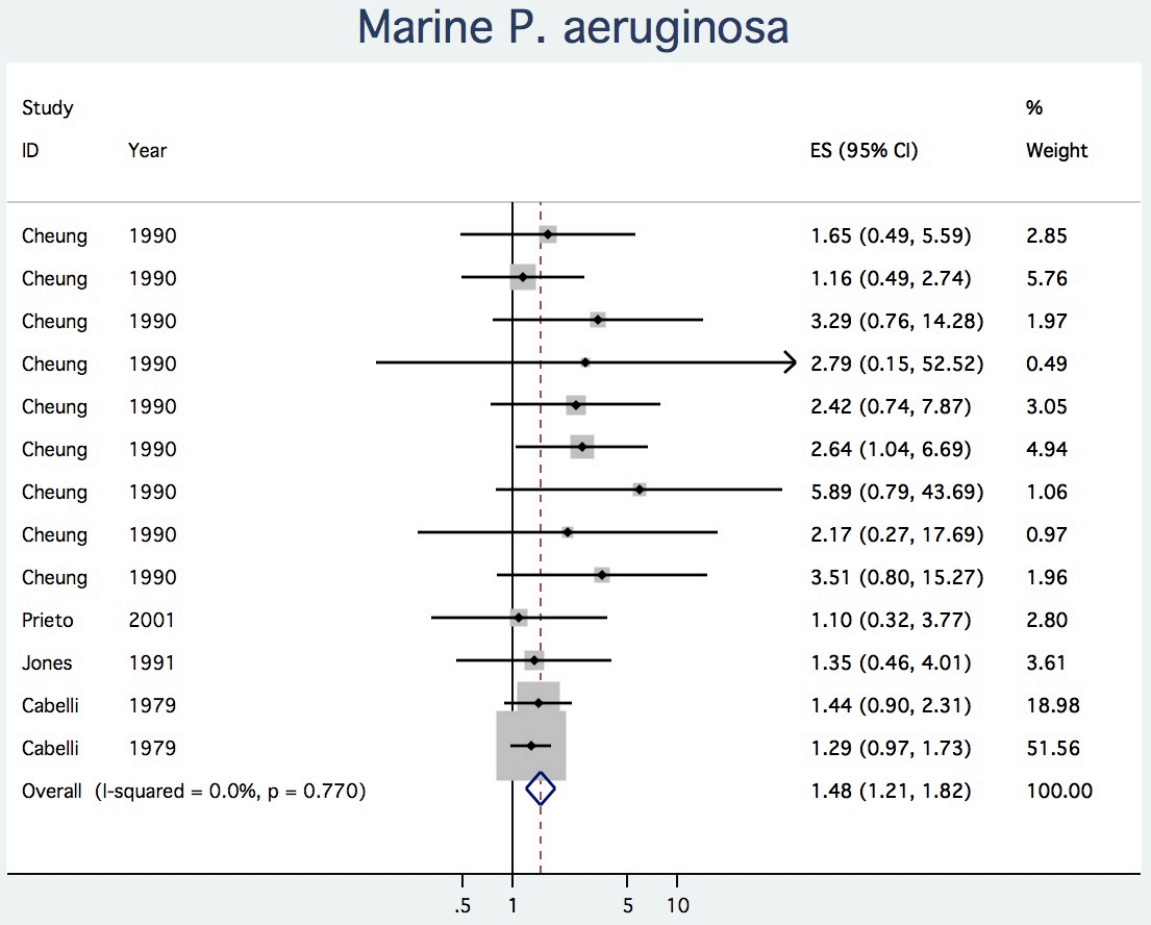


Figure 16: Summary of Meta-Analysis Results, Odds Ratios. Forest plot for OR's from various studies relating *Staphylococci* in marine settings to skin ailments.

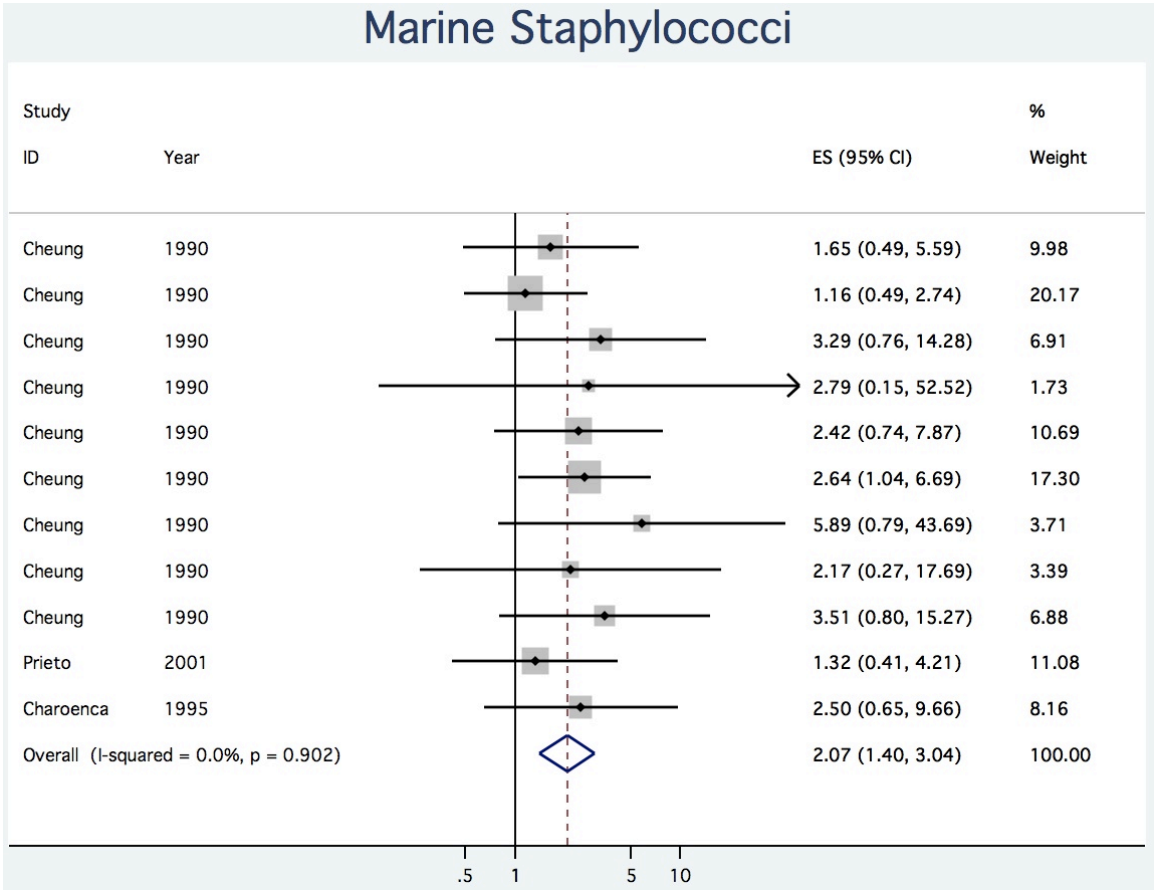
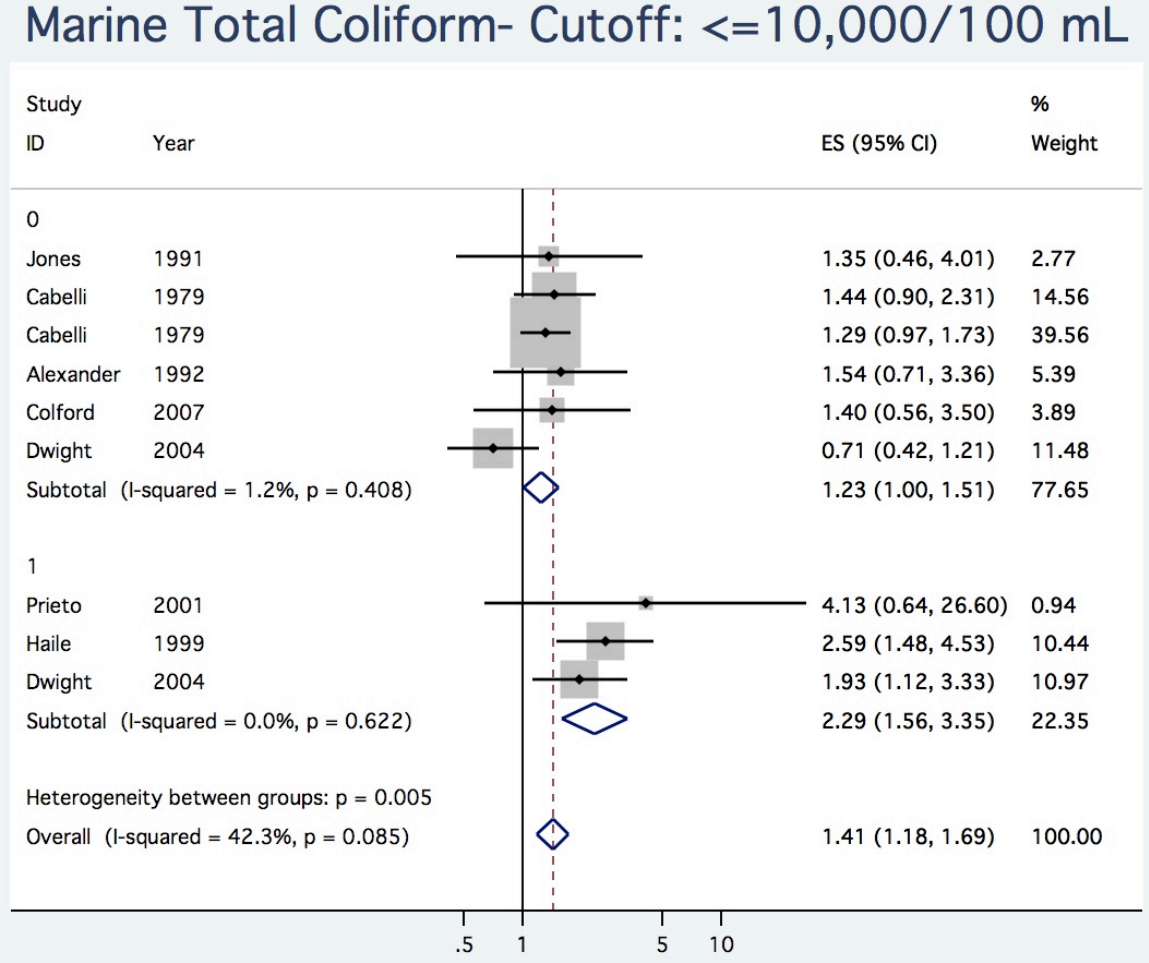


Figure 17: Summary of Meta-Analysis Results, Odds Ratios. Forest plot for OR's from various studies relating total coliforms in marine settings to skin ailments. Results divided by level of indicator concentration.



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CHAPTER 3: IMPROVING PERFORMANCE OF INDICATOR ORGANISMS

3.1 Introduction

3.1.1 Single Indicator Vs. Multiple Biological and Physical Indicators

As mentioned above, in order to protect the public from exposure to contaminated recreational water settings, the U.S. Environmental Protection Agency put forward guidelines in 1986. The guidelines stated that in marine recreational waters, the geometric means of at least five samples taken over a 30 day period should not exceed 35 colony forming units (cfu) of *Enterococcus* per 100 mL, and that no single day sample should exceed 104 cfu of *Enterococcus*. In fresh recreational waters, the geometric means should not exceed 33 cfu of *Enterococcus* per 100 mL or 126 cfu of *E. coli* per 100 mL (U.S. EPA 1986, Wade et al. 2003).

However, these recommendations were based on the relationship between levels of a single indicator bacterium and gastrointestinal illness, and did not consider the influence of other potentially important variables. It is not difficult to imagine situations in which an indicator organism works quite well at predicting unsafe swimming conditions, and when it might not work as well. For example, a recent study conducted at Doheny Beach in California found that the relationship between various indicator organisms and a variety of illnesses was greatly strengthened when a sand berm separating the ocean and a stagnant lagoon of water was breached, allowing water in the lagoon to pour into the ocean (John M. Colford, Jr., unpublished data). However, when the sand berm was intact, indicator organism associations with illness were weakened, if not completely nullified.

Situations like these suggest that consideration of multiple variables could greatly improve the ability of regulatory standards to protect the public from unsafe beach waters. These additional variables could range from physical variables (e.g. temperature) to other biological indicators. For example, different guidelines could be used for days when point or non-point sources of sewage were present. Measurement of physical variables carry the advantage of rapidity (biological indicators often take up to a day to culture or process in a lab) and cost savings.

3.1.2 Improved Statistical Methods

Another potential limitation of some of the published literature is that many studies used logistic regression to generate odds ratios (OR's) that can be compared to results from other studies that used the same methodology. However, for the purpose of modeling illness and attempting to predict illness at similar beaches, logistic regression may not always be the optimal statistical approach. Many other prediction algorithms based on different models besides logistic regression exist, including k nearest neighbors, generalized additive models, and algorithms such as the Deletion/Substitution/Addition algorithm. It is probable that a linear function is not the best way to predict gastrointestinal illness in swimming populations.

In addition, odds ratios are not necessarily the best way to assess the predictive power of an indicator organism. It has been demonstrated that even high odds ratios may perform poorly

with respect to classifying individuals as positive or negative for illness (Pepe 2004). In other words, even if an exposure demonstrates a strong OR relating it to disease, many exposed individuals may not actually develop disease. However, other metrics, such as sensitivity and specificity, may be used to compare the predictive performance of single indicator organisms, or even multiple indicator organisms. Another limitation of using the OR as a measure of risk is that the units in which an indicator is measured in can influence the magnitude of an OR. For example, the OR for a 10 cfu /100 mL increase in an indicator can be quite different from the OR for a 1,000 cfu / 100 mL increase in indicator organism concentration. As a result, comparisons between indicator organisms that have been measured in different ways and on different scales may be difficult. Sensitivity and specificity can be used to construct receiver operating characteristic curves (ROC curves), which avoid the problems of non-comparable measurement scales, and also allow readers to determine optimal cutoff points for the indicator organism of interest.

Faced with the above challenges, this paper seeks to improve the predictive relationship between biological indicators and illness in a study conducted at Avalon beach by considering alternative physical variables. The performance of both biological and physical indicators will be assessed using ROC curves. The indicators that are strongest predictors of illness will then be considered jointly, in an attempt to detect effect modification if it exists. A validation analysis is then conducted using traditional regression techniques to corroborate the findings of the previous steps.

3.2 Methods

3.2.1 Study Design

Avalon beach is located on Catalina Island, 26 miles west of Los Angeles. The beach suffers from chronic contamination due to an aging sewage infrastructure that uses salt water to flush the system. This salt water flushing is thought to contribute to heavy corrosion of the sewage pipes (Boehm et al. 2003). At Avalon, a prospective cohort study was conducted using methods comparable to prior studies conducted by other investigators (Wade et al. 2006, Colford et al. 2007, Wade et al. 2008, Wade et al. 2010). Study participants were recruited on the day of their beach visit, and on the same day multiple water samples were collected at varying times and locations on the beaches. Water samples were then analyzed to determine microbial and viral indicator concentrations. At the same time that water samples were collected, physical measurements were made. Information on new illnesses among participants was obtained by phone interview 10 to 14 days after recruitment on the beach.

3.2.2 Recruitment

In order to be eligible, participants had to have 1) no prior participation in the study; 2) a family member older than 18 at the beach; 3) a home address in North America, and 4) no history of face or head contact with ocean or lake water in the past seven days. At Avalon Beach, 7,317 individuals were recruited, and follow-up was completed two weeks later on 6,165 individuals (84%). Other recorded data included the water sampling site closest to the

participant, activities at the beach, prior illnesses or health conditions, level of water contact (body immersion, head immersion, or swallowing water), and demographic data.

Ten to fourteen days after enrollment, participants were contacted by telephone and additional data on demographic, swimming and related activities since initial enrollment, pre-existing health conditions, and any new symptoms experienced since initial enrollment were collected.

3.2.3 Water Sampling and Measurement

Water was sampled at 8 am, 12 pm, and 3 pm from four spatially distinct locations on the beach. Water samples were collected at a depth of 0.5 m on incoming waves. Traditional indicator organisms as well as novel indicators were measured at all three beaches. The traditional culture-based indicator organisms measured were *Enterococcus*, total coliforms, and fecal coliforms. Additionally, qPCR methods for *Enterococcus* were used. Some of the non-traditional indicator organisms measured included adenovirus (PCR), *Bacteroides thetaiotamicron* (QPCR), *Bacteroides* (QPCR), *E. coli* (QPCR), *Enterococcus* (QPCR, Enterolert), enterovirus (QPCR), F-minus coliphage, F-plus coliphage, gull bacteroidales (PCR), human Bacteroidales (PCR), human polyomavirus (PCR), norovirus (PCR), and *Staphylococcus aureus*. (Table 1)

3.2.4 Physical Variables

Extensive physical data were collected at Avalon beach by a research group led by Alexandria Boehm from Stanford University. Variables collected included wind speed, groundwater flow, air temperature and humidity, atmospheric pressure, solar radiation, tidal levels, wind direction, precipitation, salinity, and tidal patterns.

Data on solar radiation and other sky conditions were obtained from the Catalina Island Airport, through a station run by the National Climate Data Center (http://www.ncdc.noaa.gov/oa/ncdc.html, Station 23191). Hourly data were recorded at the station. In order to assign physical variables to swimmers, morning (6 am to 12 noon) averages as well as afternoon averages (12 noon to 6 pm) were created for each physical variable measured.

3.2.5 Health Outcomes Measured

Health outcome data were obtained by examining new symptoms between initial enrollment and date of the phone interview (10 to 14 days later). Symptoms measured included respiratory illness, skin infections, and ear infections among others. The major health outcome of interest for this analysis was highly credible gastrointestinal illness (HCGI). This composite outcome was defined as any of the following symptoms: 1) diarrhea, 2) vomiting, 3) nausea and abdominal cramps, 4) nausea and missed daily activities due to gastrointestinal illness, or 5)

abdominal cramps and missed daily activities due to gastrointestinal illness (as in the study by Wade et al. 2010).

3.2.6 Statistical Methods and Data Analysis

For this analysis, indicator organism concentrations were averaged across the morning sampling periods for each specific collection site. These site-specific AM averages were then assigned as exposures to individuals with water contact at those sites, while those without any water contact were excluded from the analysis. In this way, it is possible to compare those who swam in water with low contamination levels to those who swam in water with high contamination levels. The indicator averages themselves are transformed on the log10 scale.

Data for some indicator organisms were not measured on every study day, leading to missing data. In order to use some of the statistical algorithms (and especially to use multiple indicators at once) only complete data could be considered. Thus, some indicator organisms were removed from consideration. After examining the missingness of the data, a cutoff was made. Only indicators for which missingness was $\leq 50\%$ were retained, allowing for analysis of most indicator organisms, as well as adequate sample size to retain confidence in the statistical methods employed.

Because the primary health outcome of interest was HCGI, infection with a gastrointestinal organism seemed most plausible when a swimmer ingested water (as opposed to skin contact). Thus, analyses focus mainly on swimmers who immersed their heads in the water. While the analysis could have been limited to those who swallowed water, doing so would have greatly reduced the sample size.

Three different statistical approaches were used to address the three primary goals of the study, which were to: 1) assess the performance of various indicators singly and in combination in predicting HCGI, 2) to determine potential effect modifiers and optimal cutoff points for all identified variables, and 3) validate the performance of those cutoff points using traditional analysis methods. The statistical model used for step 1 was the SuperLearner Algorithm, which takes several statistical models and combines them to form a final model that performs as well as, if not better, than any single candidate model. For goal 2, SuperLearner was used in combination with the G-computation estimator to calculate excess risks and cutoffs for various indicator organisms used jointly. For goal 3, a backwards deletion logistic regression model was used (identical to the methodology used in Colford 2007 and Wade 2006, 2008).

The SuperLearner algorithm used here is the same as the one which was explained in the introduction. Briefly, it is an estimator that examines the performance of a given list of candidate statistical models and weights them to form a “super” estimator. In order to fit the best prediction model for HCGI, several candidate estimators were used. The statistical estimators considered were k nearest neighbors (knn), logistic regression (glm), the D/S/A algorithm (DSA), Bayesian regression (Bayes), lasso and elastic-net regularized generalized linear models (glmnet), and generalized additive models (GAM). The DSA algorithm selects an estimator from a set of candidates that minimizes the average cross-validated (CV) risk based on the L2 loss function.

The algorithm searches over a defined candidate estimator space to find the polynomial function that best predicts a univariate outcome (Sinisi 2004). SuperLearner provides both the results of individual candidate estimators, as well as a combined version of all the estimators. The SuperLearner algorithm theoretically performs at least as well as the best single estimator, and often performs better than any single estimator (van der Laan 2007, Polley 2010). Performance in this case is defined as prediction of HCGI using the L2 loss function.

After obtaining the best SuperLearner model by fitting it on the full data, performance of each model using the full data was assessed by plotting an ROC curve and calculating the area underneath the curve (AUC). Recall that on an ROC curve, a model and appropriate cutoff that perfectly predicted illness and non-illness would have perfect sensitivity and specificity, and that point would lie in the upper left corner of the ROC plot. A model that did not predict illness at all (essentially a random guess) would be represented by a curve lying along the 45° degree line beginning at the origin and ending in the upper right corner of the plot.

However, fitting the SuperLearner and the candidate estimators on the full data, and then creating a ROC curve off those predicted values can lead to overfitting of the data. Thus, a second approach was also taken, in which the SuperLearner itself was cross-validated along with all the candidate estimators. Two sets of AUC values were generated, one where the full data was used to fit the SuperLearner and candidate estimators, as well as a set of AUC values when cross-validation was used to fit the SuperLearner and candidate estimators.

3.2.7 Excess Risk and Effect Modification

After assessing the ROC curves and AUC values through the full data and cross-validation, the biological and physical variables with the best predictive ability were considered jointly, in order to determine if combining biological and physical indicator information might prove useful. A variety of cutoff points were created for the biological indicator organisms considered (based on biologically relevant cutoffs, i.e.: 104 cfu/100 mL for *Enterococcus*) and a wide range of cutoff values were considered for physical variables. These binary cutoffs were made to mimic real world beach regulations, which often focus on binary safe/non-safe cutoffs. The performance of those cutoffs was then assessed by calculating excess risks for each binary cutoff. Excess risks were computed using the same SuperLearner Algorithm described above (using full data), with the exception that because continuous data were no longer being used (binary cutoffs for two variables considered jointly), we opted for a simple saturated logistic model. Simple regression on binary variables gives results identical to non-parametric analysis of contingency tables. Linear models were used because they allowed calculation of the excess risk, which is represented by:

P(Illness in the Exposed) – P(Illness in the Unexposed) = Excess Risk

Excess Risk was considered to be a relevant measure because the EPA's current standards are based on a threshold excess risk value of 19 excess cases of highly credible gastrointestinal illness/1,000 swimmers (water quality is thus considered acceptable if an excess risk of less than 19/1,000 swimmers were to get sick when comparing swimmers in potentially

contaminated waters to non-swimmers, but unacceptable if greater than 19/1,000 excess cases would have been seen). The results of this analysis differ slightly from the EPA's definition of excess risk, in that the Excess Risk calculated by this paper compares swimmers in water with high indicator organism levels to other swimmers in water with low indicator organism levels, rather than to non-swimmers. This approach was taken so as to avoid potential confounding, as well as to ensure that the major difference between the swimming populations was the indicator concentration levels. Standard errors for each excess risk were calculated using bootstrap methods (Efron 1981), with the number of bootstrap samples set to 1,000.

3.2.8 Backwards Deletion

In order to validate the potential effect modifiers identified, the traditional analysis method of backwards deletion was employed (Colford 2007 and Wade 2006, 2008). Briefly, the backward deletion method first restricts the study population to those individuals with a certain exposure to water (e.g. those with body contact, those with head immersion, etc.). The next step involves modeling the probability of illness using a logistic regression model, which includes all potential confounding covariates (e.g. study year, age, sex, race, swimming on multiple days, allergies, contact with animals, contact with other sick people, frequency of beach visits, digging in sand, consumption of raw or undercooked eggs or meat). Confounders are removed from the model iteratively until only covariates that change the OR relating indicator concentration to illness by at least 5% (relative change) are left (Rothman 1998). The 95% confidence intervals (CI) are estimated for the OR's by using robust standard errors (Freedman 2010) in order to account for correlation of observations by household. It is assumed that households are independent of each other.

3.3 Results

The health and indicator organism data used in this thesis are still preliminary. Until the data are fully quality assured, the results presented below may be subject to revision.

3.3.1 ROC Curves, SuperLearner Vs. Traditional Methods

To justify the use of the SuperLearner algorithm, ROC curves were first generated for a single indicator organism (*Enterococcus*, measured using the EPA 1600 method, hereafter referred to as *Enterococcus* 1600) using all candidate statistical estimators and area underneath the curve (AUC) values were calculated. Additionally, as a reference point, the performance of *Enterococcus* 1600's 104 cfu/100 mL cutoff was plotted as a single point on the ROC curve. The sensitivity and specificity associated with the 104 cfu/100 mL cutoff for *Enterococcus* 1600 were 0.26 and 0.72, respectively (Figure 1). AUC values using the full data (without cross-validation) ranged from 0.5 (performance similar to a random guess) using logistic regression, the DSA algorithm, and Bayesian regression, up to 0.70 for SuperLearner and K-nearest neighbors (K = 10). When cross-validation was used on the entire approach, the AUC values were attenuated (Figure 2). The highest cross-validated AUC values were found using K-nearest neighbors (K=10, 20) as well as the SuperLearner.

3.3.2 ROC Curves, Single Biological Indicators

Because the SuperLearner outperformed traditional logistic regression and was one of the top performers (using both full data and cross-validated approaches), it was used to calculate AUC values for the other measured biological and physical indicators. As mentioned above, the list of indicators considered was first reduced to indicators with ≤ 50% of the data missing. The candidate list was further winnowed to indicators with a univariate association with HCGI of p-value ≤ 0.2 (through use of simple logistic regression). The indicators, as well as AUC values generated by the SuperLearner using full data, are presented in Table 2. AUC values were also generated using cross-validation, and results are presented in Table 3.

The AUC values presented indicate that the biological indicators that performed best were *Enterococcus*, as measured by the 1600 method, and by QPCR (AUC values of 0.70 and 0.73 with full data, 0.56 and 0.55 under cross-validation). The physical indicators that performed best were solar radiation and UV index (both with AUC values of 0.74 with full data, 0.61 and 0.59 under cross-validation).

3.3.3 ROC Curves, Combination of Traditional Indicators

After determining that the rapid method for measuring *Enterococcus* (QPCR) was the most predictive of HCGI (highest AUC values for biological indicators), a question arose. The next analysis focused on determining whether or not combinations of traditional indicators could perform as well as or outperform the rapid method for measuring *Enterococcus*. The results using only traditional indicators, such as *Enterococcus*, total coliforms, and fecal coliforms, are presented in Tables 4 and 5 (full data and cross-validated approaches, respectively). When traditional methods for measuring *Enterococcus* (EPA 1600) were combined with data from total coliforms, performance was the same relative to the rapid *Enterococcus* method (AUC = 0.73 for full data, AUC = 0.56 under cross-validation). However, no combination outperformed the QPCR method.

3.3.4 ROC Curves, Biological and Physical Indicators

The next goal was to improve prediction by combining biological and physical data. When the biological and physical variables were considered together to determine if prediction could be improved, AUC values were greater than those generated when only single variables were used (Table 6, Table 7). The performance of *Enterococcus* 1600 and QPCR improved more when combined with solar radiation (AUC values of 0.78 for *Enterococcus* 1600, 0.74 for QPCR with full data) than with UV index (AUC values of 0.75 for *Enterococcus* 1600, 0.73 for QPCR with full data). Similar results were seen with the cross-validated approach (AUC values of 0.61 for *Enterococcus* 1600 and solar radiation, 0.59 for QPCR and solar radiation).

3.3.5 Excess Risk, Multiple Cutoffs

Because solar radiation seemed to outperform UV index when combined with biological indicators, only solar radiation was considered in the next step. For each combination

(*Enterococcus* 1600 + Solar Radiation, *Enterococcus* QPCR + Solar Radiation), various cutoffs were considered for both indicators. An excess risk value was calculated, using full data and the SuperLearner, comparing swimmers who had both indicator and solar radiation levels above the cutoff to swimmers who had both indicator and solar radiation levels below the cutoff (Figure 3 and Table 8). This cutoff approach would be similar to regulations issued by the EPA. By looking along a column in Table 8, one can observe how a certain cutoff for *Enterococcus* performs under a variety of solar radiation levels.

The excess risks were highly significant for almost all indicator cutoffs when solar radiation levels were low for *Enterococcus* QPCR, with the exception of the highest solar level (800 w/m^2). The highest excess risk value was 96.5 excess cases of GI illness among those who swam when *Enterococcus* QPCR levels were greater than 50 QPCRCE/100 mL when the 500 and 550 w/m^2 solar radiation level cutoffs were used. For *Enterococcus* 1600, the excess risks were not significant, though a similar pattern emerged. When solar radiation levels were lower, indicator concentrations were more strongly associated with the excess risk of HCGI. The highest excess risk value for *Enterococcus 1600* was 51.8 (35 cfu/100 mL cutoff), when the lowest two cutoffs for solar radiation (500 and 550 w/m^2) were used. The lowest excess risk values were found when solar radiation cutoff levels were at their highest (800 w/m^2).

3.3.6 Traditional Modeling Results

In order to validate these findings, a traditional logistic regression model using a backwards deletion method to adjust for confounders was used. Odds ratios (OR) were calculated for indicator organism associations with HCGI under low solar radiation levels (lower quartile of solar radiation levels, < 637 w/m^2) and under higher solar radiation levels (upper quartile of solar radiation, > 798 w/m^2). Results for a variety of traditional indicator organisms and some rapid methods are presented in Table 9. Various definitions of swimmers were considered as well. Non-swimmers were included as a "control" group, to demonstrate that no indicator organism and illness relationship was seen among those who had no indicator organism exposure.

As expected, no indicator organism and health outcome relationship was seen when non-swimmers were analyzed. Because they had no contact with the water, the indicator organism concentrations in the water would not be expected to predict their health status. However, for swimmers with body, head, and swallow water contact, results were consistently non-significant when solar radiation levels were high. In fact, most adjusted OR values were centered at the null value, 1. In contrast, when solar radiation levels were in the lowest quartile, several significant relationships were seen. Enterococcus QPCR, as seen before, was significantly associated with HCGI among swimmers who had either body or head contact with the water (OR, 95% CI for body contact: 1.63, [1.16, 2.28], head immersion: 1.65 [1.07, 2.54]). For swimmers who swallowed water, confidence intervals were quite wide, indicating small sample sizes in those categories.

3.4 Discussion

The prior analyses suggested five major results. The first is that the 104 cfu/100 mL cutoff for *Enterococcus* 1600 did not have very high sensitivity or specificity at Avalon Beach (0.26 and 0.72, respectively). Second, use of the SuperLearner algorithm for predicting HCGI among swimmers improved the predictive ability of indicators compared to traditional methods like logistic regression, when full data were used. However, when cross-validation was used, those results were attenuated, though findings stayed essentially consistent with those calculated using the full data. Third, *Enterococcus* levels, as measured by QPCR methods, predicted HCGI as well as, if not slightly better than, *Enterococcus* 1600. Fourth, solar radiation appeared to strongly mediate the values for excess risk between *Enterococcus* (1600 and QPCR methods) and HCGI. Last, even traditional analysis methods that generated adjusted odds ratio values demonstrated the mediating effect of solar radiation.

The above findings appear to be biologically plausible. It has been hypothesized that solar radiation inactivates many pathogenic organisms, including bacteria and viruses (Boehm 2009). Thus, one would expect to see a reduced risk of HCGI on days when solar radiation levels are higher. Indeed, the results demonstrate that excess risk values, as well as odds ratio values, decreased as solar radiation levels increased. The results in Table 9 seem to be particularly harmonious, in that virtually every odds ratio relating indicator concentrations to illness on high solar radiation days is centered around 1, indicating that when solar radiation levels are particularly high, indicator organism levels are no longer associated with illness, presumably because of inactivation. However, when solar radiation levels are in the lowest quartile, most OR's seem to demonstrate an elevated risk of illness for those with water exposure (body contact or greater).

There are two additional points concerning the results in Table 9. First, the confidence intervals around the OR's for those who swallowed water are quite wide, indicating a very small sample size. Second, the "non-swimmers" category was included as a negative control. For those without any contact with the water, it is not expected that indicator organism concentrations in the water would predict their odds of illness. Indeed, none of the OR's appear to be elevated. However, some of the OR's appear to be quite low, in particular the OR for *Enterococcus* 1600 on low solar radiation days (OR = 0.69, 95% CI [0.49-0.97]). This statistically significant result does not appear to be biologically plausible, and could be an artifact related to the large number of statistical tests performed.

The problem of testing multiple comparisons can have serious implications in any analytic endeavor. When statistical tests increase in number, as typically occurs when a great deal of information has been gathered and many hypotheses exist, the risk of spuriously finding statistically significant results increases if preventative steps are not taken. Methods that can be employed to prevent this from happening include use of cross-validation or other statistical methods (e.g. the Bonferroni correction, etc.). Cross-validated results calculated in this study corroborated the findings from models fit on the full data, offering additional confidence in the presented results.

One reassuring finding in our study is that the traditional indicator *Enterococcus* 1600 worked quite well at predicting illness when ROC and SuperLearner methods were applied.

However, this indicator organism did not work well under high solar radiation conditions. In fact, the rapid method (*Enterococcus* QPCR) seemed to work even better at predicting HCGI than the culture based method (higher excess risks, as well as odds ratios). This is also reassuring, because the results mirror findings reported in previous studies (Wade 2006, Wade 2010).

While the results are encouraging overall, some limitations of this study need to be taken into account. First, the excess risks calculated were not adjusted for confounding variables. The reason this was not done was because the analysis compared swimmers who immersed their heads in the water to other swimmers who immersed their heads in the water. Because indicator organism concentrations are invisible to the naked eye, it was as if the swimmers were all blinded to their exposure status, similar to conditions found in a blinded controlled trial. Unless demographic variables and other potential confounders could have influenced a swimmer's exposure to indicator organisms, those variables would likely not confound the relationship between indicator organism concentration and illness.

The results in Table 9 seem to support the idea that the excess risk values are not likely to be confounded. The OR's presented in Table 9 are calculated in a similar way to the way excess risk was calculated: swimmers were compared directly to other swimmers. However, the OR's presented were adjusted for potential confounding variables (following traditional methodology used in other studies), and consistent results were seen. OR's were elevated when solar radiation was low, and dropped close to the null when solar radiation levels were high.

However, the comparison of swimmers to other swimmers makes the excess risks more difficult to interpret. A counterintuitive pattern can be seen in the results, especially for *Enterococcus* QPCR levels. As the *Enterococcus* cut point increases, the excess risk values seem to decrease. This finding can potentially be explained by considering the "baseline" or comparison group. For the 35 QPCRCE or CFU cutoffs, swimmers with very low indicator organism concentrations (below 35 QPCRCE/CFU per 100 mL) are being compared to swimmers with higher indicator concentrations (above 35 QPCRCE/CFU per 100 mL). When indicator concentrations are below 35 QPCRCE/CFU per 100 mL, the water can be considered quite clean, but those exposed to water more contaminated than 35 QPCRCE/CFU per 100 mL could be swimming in waters with 36 QPCRCE/CFU per 100 mL or in waters with 200 QPCRCE/CFU per 100 mL. This could be equivalent to comparing swimmers in very clean water to swimmers in moderately to extremely contaminated water. As the cutoffs increase to 50, 104, and 150, the "unexposed" group's indicator exposure levels increase, and thus the risk or odds of illness would be expected to increase. As the "unexposed" group's risk rises, the difference between the exposed and unexposed groups might shrink. This might explain why excess risk does not seem to trend upwards as indicator concentrations cut point levels increase.

Another limitation of these analyses lies in the fact that the statistical estimators that performed the best (KNN and SuperLearner minimized the mean squared error) are not as widely used and understood as logistic regression. G-computation was used to calculate the desired measures of effect, and these more advanced statistical methods may not be accessible to local

regulators. However, the potential improvement in predicting illness with indicator organisms would indicate that looking into alternative estimators is worthwhile.

In summary, the results indicate not only that prediction of HCGI using indicator organisms is aided by considering multiple indicator organisms at once, but that use of different statistical estimators besides logistic regression may lead to substantial improvements in prediction of HCGI. In particular, consideration of solar radiation drastically improved the predictive ability of *Enterococcus* 1600, indicating that performance of currently employed regulatory standards might be improved by considering conditions particular to each beach. Also, the tradition of reporting odds ratios to illustrate the predictive power of an indicator may not be the best metric to use. Instead, ROC curves may be used to assess the predictive power of single and multiple indicator organisms.

3.5 Figures and Tables

Table 1: Indicators measured at Avalon Beach

Indicator	Lab	Method
Adenovirus	Sobsey	nPCR
B. thetaiotaomicron	EPA	Q-PCR
B. thetaiotaomicron	Noble	Q-PCR
Bacteroides spp.	EPA	Q-PCR
E coli	Bushon	IMS
E coli	Jay	Colilert (96 Well Tray)
E coli	Jay	EPA 1603 Modified m-TEC
E coli	Noble	Q-PCR
E coli	SCCWRP	Colilert (96 Well Tray)
E coli	SCCWRP	EPA 1603 Modified m-TEC
eaeA virulence gene	Sadowsky	HT-Ecoli Virulence Gene Assay
EAF Plasmid	Sadowsky	HT-Ecoli Virulence Gene Assay
Enterococcus spp.	Bushon	IMS
Enterococcus spp.	EPA	Q-PCR Entero1
Enterococcus spp.	Harwood	Q-PCR-Raptor
Enterococcus spp.	Jay	Enterolert (96 Well Tray)
Enterococcus spp.	Jay	EPA 1600
Enterococcus spp.	Moore	TMA
Enterococcus spp.	Noble	Q-PCR Entero1
Enterococcus spp.	Noble	Q-PCR Entero2
Enterococcus spp.	SCCWRP	Enterolert (96 Well Tray)
Enterococcus spp.	SCCWRP	EPA 1600
Enterococcus spp.	Scott	Q-PCR
Enterovirus	Stewart	Q-PCR
Fecal Coliforms	SCCWRP	MF-Fecal Coliform (APHA 9222D)
Fminus coliphage	Sobsey	EPA 1601
Fminus coliphage	Stewart	EPA 1602
Fplus coliphage	Sobsey	EPA 1601
Fplus coliphage	Sobsey	EPA 1601 5 hr
Fplus coliphage	Sobsey	EPA 1601 5 hr+immunoassay
Fplus coliphage	Stewart	EPA 1602
Gull Bacteroidales	Field	PCR
Hepatitis A	Fuhrman	Q-PCR
Human Bacteroidales	Field	PCR
Human Bacteroidales	Ufnar	PCR
Human Polyomavirus	Harwood	PCR
Human Polyomavirus	Harwood	Q-PCR
L. pneumophila	Gast	PCR
Methanogen nif gene	Ufnar	PCR
Norovirus	Sobsey	nRT-PCR
S. aureus	Goodwin	MF-S.aureus (Chrom Agar) Sand
S. aureus	Goodwin	MF-S.aureus (Chrom Agar) Sand MRSA
S. aureus	Goodwin	MF-S.aureus (Chrom Agar) Water
S. aureus	Goodwin	MF-S.aureus (Chrom Agar) Water MRSA
stx 1 & 2 gene	Sadowsky	HT-Ecoli Virulence Gene Assay
Total Coliforms	SCCWRP	Colilert (96 Well Tray)
Total Coliforms	SCCWRP	MF-Total Coliform (APHA 9222B)

Table 2: AUC values for reduced list of biological and physical indicators (univariate associations of $p \leq 0.2$)

All Biological and Physical Variables*, Area Under Curve (AUC) Values**					
Biological	Lab	AUC	Physical	Lab	AUC
Enterococcus QPCR (EPA)	EPA	0.73	Solar Radiation (w/m^2)	Boehm	0.74
Enterococcus (1600)	SCCWRP	0.70	UV Index	Boehm	0.74
Total Coliforms (MF)	SCCWRP	0.68	Wind Speed m/s	Boehm	0.71
sspam20BST2212	?	0.54	Mean Tide Level (feet)	Boehm	0.70
sspam20HBA3011	?	0.54	Groundwater Flow (10 AM - 5 PM)	Boehm	0.65
sspam20HBA3012	?	0.54	# of Swimmers (Head Immersion)	Boehm	0.64
sspam20BST2211	?	0.54	Mean Salinity	Boehm	0.63
B. thetaiotamicron (QPCR)	Noble	0.52	# of Swimmers (Body Immersion)	Boehm	0.62
sspam20BAD2211	?	0.51	# of Swimmers (Any Contact)	Boehm	0.56
sspam20BAD2212	?	0.51	Salinity (Above/Below Median)	Boehm	0.54
			Groundwater (Above/Below Median)	Boehm	0.53
			Precipitation (Binary)	Boehm	0.51
*Reduced to variables with univariate associations with gastrointestinal illness, $p \leq 0.2$					
*AUC's generated by using SuperLearner Algorithm					

Table 3: AUC values for reduced list of biological and physical indicators, Cross-Validated

All Biological and Physical Variables*, Area Under Curve (AUC) Values**					
Biological	Lab	AUC	Physical	Lab	AUC
Enterococcus QPCR (EPA)	EPA	0.56	Solar Radiation (w/m^2)	Boehm	0.61
Enterococcus (1600)	SCCWRP	0.55	UV Index	Boehm	0.59
Total Coliforms (MF)	SCCWRP	0.56	Wind Speed m/s	Boehm	0.60
sspam20BST2212	?	0.50	Mean Tide Level (feet)	Boehm	0.58
sspam20HBA3011	?	0.48	Groundwater Flow (10 AM - 5 PM)	Boehm	0.55
sspam20HBA3012	?	0.50	# of Swimmers (Head Immersion)	Boehm	0.52
sspam20BST2211	?	0.50	Mean Salinity	Boehm	0.54
B. thetaiotamicron (QPCR)	Noble	0.48	# of Swimmers (Body Immersion)	Boehm	0.47
sspam20BAD2211	?	0.45	# of Swimmers (Any Contact)	Boehm	0.48
sspam20BAD2212	?	0.44	Salinity (Above/Below Median)	Boehm	0.50
			Groundwater (Above/Below Median)	Boehm	0.48
			Precipitation (Binary)	Boehm	0.46
*Reduced to variables with univariate associations with gastrointestinal illness, $p \leq 0.2$					
*AUC's generated by using Cross-Validated SuperLearner Algorithm					

Table 4: AUC values for combinations of traditional biological indicators

Combinations of Traditional Biological Indicators*	
Combination	AUC
Enterococcus (1600) + Fecal Coliform	0.71
Total Coliform + Fecal Coliform	0.72
Enterococcus (1600) + Total Coliform	0.73
Enterococcus (1600) + Total Coliform + Fecal Coliform	0.73
*AUC's generated by using SuperLearner Algorithm	

Table 5: AUC values for combinations of traditional biological indicators, Cross-Validated

Combinations of Traditional Biological Indicators*	
Combination	AUC
Enterococcus (1600) + Fecal Coliform	0.53
Total Coliform + Fecal Coliform	0.53
Enterococcus (1600) + Total Coliform	0.56
Enterococcus (1600) + Total Coliform + Fecal Coliform	0.52
*AUC's generated by using Cross-Validated SuperLearner Algorithm	

Table 6: AUC values for combinations of biological and physical indicators

Pairs of Biological and Physical Indicators*	
Combination	AUC
Enterococcus (1600) + Solar Radiation	0.78
Enterococcus (1600) + UV Index	0.75
Enterococcus QPCR (EPA) + Solar Radiation	0.74
Enterococcus QPCR (EPA) + UV Index	0.73
*AUC's generated by using SuperLearner Algorithm	

Table 7: AUC values for combinations of biological and physical indicators, Cross-Validated

Pairs of Biological and Physical Indicators*	
Combination	AUC
Enterococcus (1600) + Solar Radiation	0.61
Enterococcus (1600) + UV Index	0.57
Enterococcus QPCR (EPA) + Solar Radiation	0.59
Enterococcus QPCR (EPA) + UV Index	0.58
*AUC's generated by using Cross-Validated SuperLearner Algorithm	

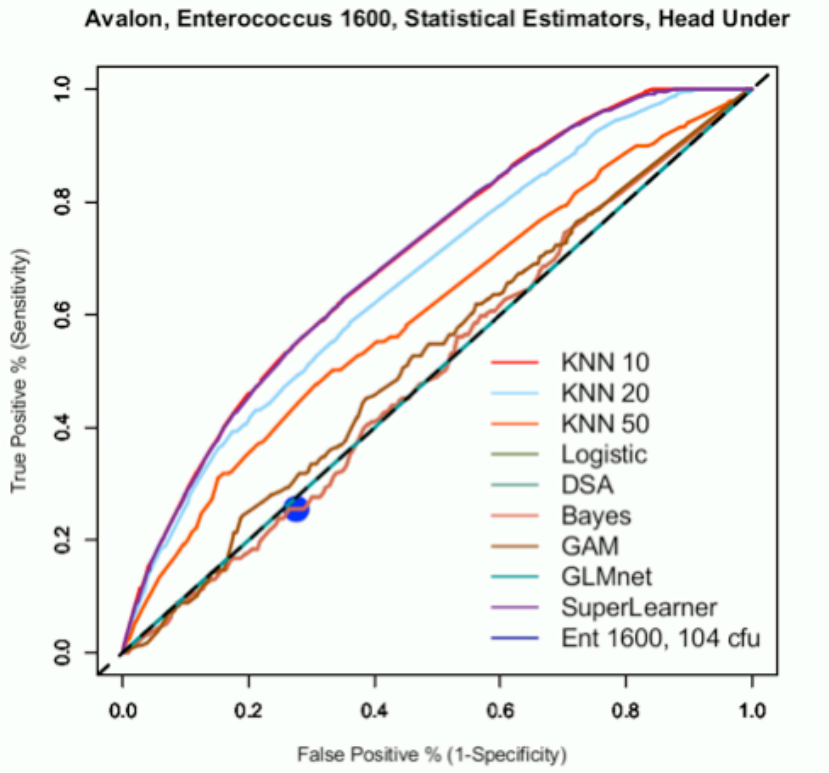
Table 8: Excess Risk values for HCGI and 95% confidence intervals for different cutoffs

Excess Risk: Binary Cutpoints for Enterococcus (1600 and QPCR) vs. Solar Radiation*				
Solar Radiation (w/m2)	Enterococcus 1600 (cfu/100mL)			
	35	50	104	150
800	-2 [-55.1,51.1]	-27.1 [-78.8,24.6]	-16.5 [-90.5,57.5]	-33.6 [-116.7,49.5]
750	1.1 [-36.8,39]	-7.5 [-46.6,31.6]	-3.3 [-52.6,46]	-8.1 [-71.1,54.8]
700	13.4 [-30.3,57]	-5.7 [-50.1,38.8]	-12.7 [-56,30.7]	-3.3 [-54.9,48.2]
650	25.5 [-26.2,77.3]	3.6 [-49.7,57]	-4.1 [-47.7,39.6]	-2.7 [-48.1,42.6]
600	48.9 [-6.3,104.2]	18.2 [-41.3,77.8]	20.6 [-24.4,65.6]	29.3 [-19.7,78.4]
550	51.8 [-3.7,107.3]	21.4 [-38.3,81.2]	27.3 [-19.3,73.9]	44.4 [-7.1,95.9]
500	51.8 [-3.7,107.3]	21.4 [-38.3,81.2]	27.3 [-19.3,73.9]	44.4 [-7.1,95.9]
Solar Radiation (w/m2)	Enterococcus QPCR (QPCRCE/100mL)			
	35	50	104	150
800	21.8 [-26,69.7]	24.5 [-22.8,71.9]	17.4 [-30.4,65.1]	15.8 [-31.8,63.3]
750	**41.2 [0.9,81.5]**	**44.2 [4.8,83.6]**	31.8 [-6.4,70]	30.4 [-7.8,68.6]
700	**71.7 [33.4,109.9]**	**74.7 [38,111.3]**	**50.9 [9.3,92.6]**	**50.6 [9.5,91.7]**
650	**87.9 [53.4,122.5]**	**90.3 [57.9,122.8]**	**64 [22.3,105.8]**	**64.1 [23.3,105]**
600	**92.5 [56.7,128.2]**	**95 [61.7,128.3]**	**73.1 [28.5,117.6]**	**73.8 [30.5,117.1]**
550	**93.5 [54.4,132.7]**	**96.5 [60.7,132.3]**	**73.5 [26.2,120.8]**	**74.8 [29,120.6]**
500	**93.5 [54.4,132.7]**	**96.5 [60.7,132.3]**	**73.5 [26.2,120.8]**	**74.8 [29,120.6]**
*Excess Risks Calculated Using G-Computation Methods, and Parametric Bootstrapping				

Table 9: Logistic regression, Odds Ratios for HCGI adjusted for confounders using backwards deletion.

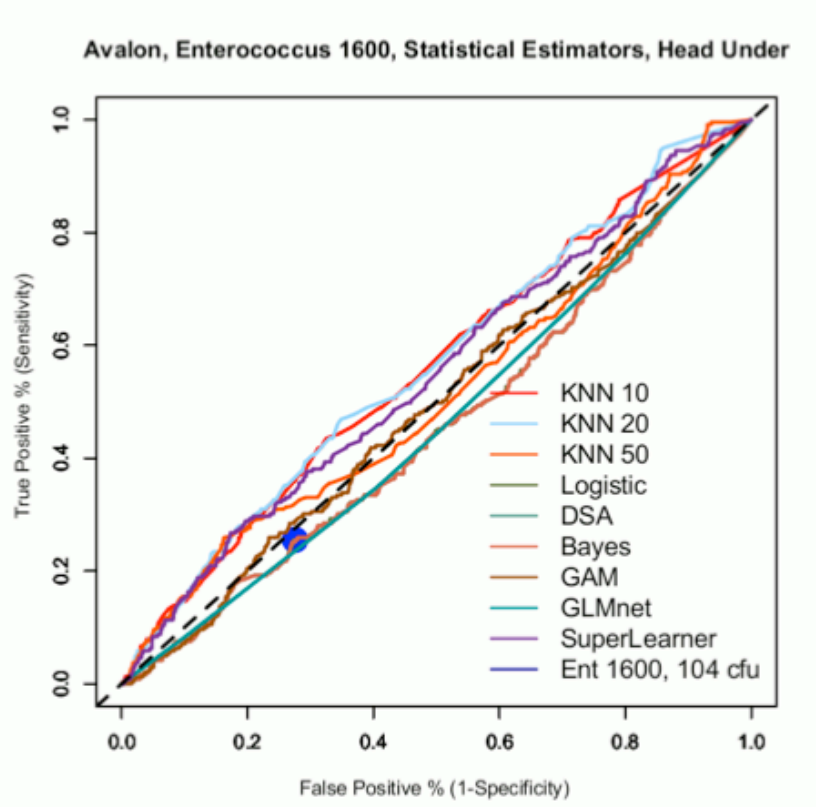
	Low Solar Radiation			
Continuous indicators	Non-Swimmers	Body Contact	Head Under	Swallowed water
EPA 1600	0.69 [0.49,0.97]	1.41 [0.8,2.5]	1.47 [0.83,2.62]	2.64 [0.85,8.27]
EPA 1600 (104)	N/A	1.73 [0.81,3.71]	1.73 [0.72,4.18]	1.46 [0.71,2.99]
Enteroalert	0.67 [0.42,1.08]	1.4 [0.87,2.26]	1.36 [0.81,2.3]	2.06 [0.65,6.53]
Fecal coliform	0.9 [0.63,1.28]	1.96 [0.98,3.89]	**2.99 [1.35,6.61]**	4.54 [0.89,23.02]
Total coliform	0.9 [0.7,1.17]	1.15 [0.65,2.05]	1.48 [0.88,2.47]	2.49 [0.64,9.65]
EPA qPCR (ddct w/inh)	0.78 [0.57,1.06]	**1.63 [1.16,2.28]**	**1.65 [1.07,2.54]**	3.89 [0.68,22.09]
Noble 1 qPCR	0.84 [0.67,1.05]	0.96 [0.72,1.27]	1.12 [0.76,1.65]	1.11 [0.7,1.78]
Noble 2 qPCR	0.73 [0.52,1.04]	0.96 [0.76,1.21]	0.98 [0.75,1.29]	1.18 [0.64,2.16]
	High Solar Radiation			
Continuous indicators	Non-Swimmers	Body Contact	Head Under	Swallowed water
EPA 1600	0.92 [0.54,1.56]	0.98 [0.83,1.15]	0.98 [0.81,1.17]	1.05 [0.73,1.5]
EPA 1600 (104)	N/A	0.98 [0.62,1.54]	0.97 [0.58,1.63]	1.15 [0.43,3.09]
Enteroalert	1.14 [0.69,1.91]	0.98 [0.82,1.17]	1 [0.82,1.24]	0.99 [0.69,1.41]
Fecal coliform	1.1 [0.71,1.69]	1.03 [0.88,1.21]	1.01 [0.86,1.19]	1.04 [0.8,1.34]
Total coliform	1.1 [0.75,1.59]	1.03 [0.83,1.27]	1.03 [0.81,1.29]	1.11 [0.78,1.58]
EPA qPCR (ddct w/inh)	0.96 [0.65,1.43]	1.02 [0.82,1.27]	1.02 [0.83,1.26]	1.26 [0.84,1.9]
Noble 1 qPCR	0.94 [0.72,1.23]	1.04 [0.9,1.19]	1.06 [0.91,1.23]	0.99 [0.74,1.33]
Noble 2 qPCR	0.95 [0.67,1.36]	0.98 [0.84,1.14]	0.98 [0.83,1.15]	0.96 [0.7,1.33]

Figure 1: ROC Curves and AUC Values for *Enterococcus* 1600, Multiple Estimators, Full Data



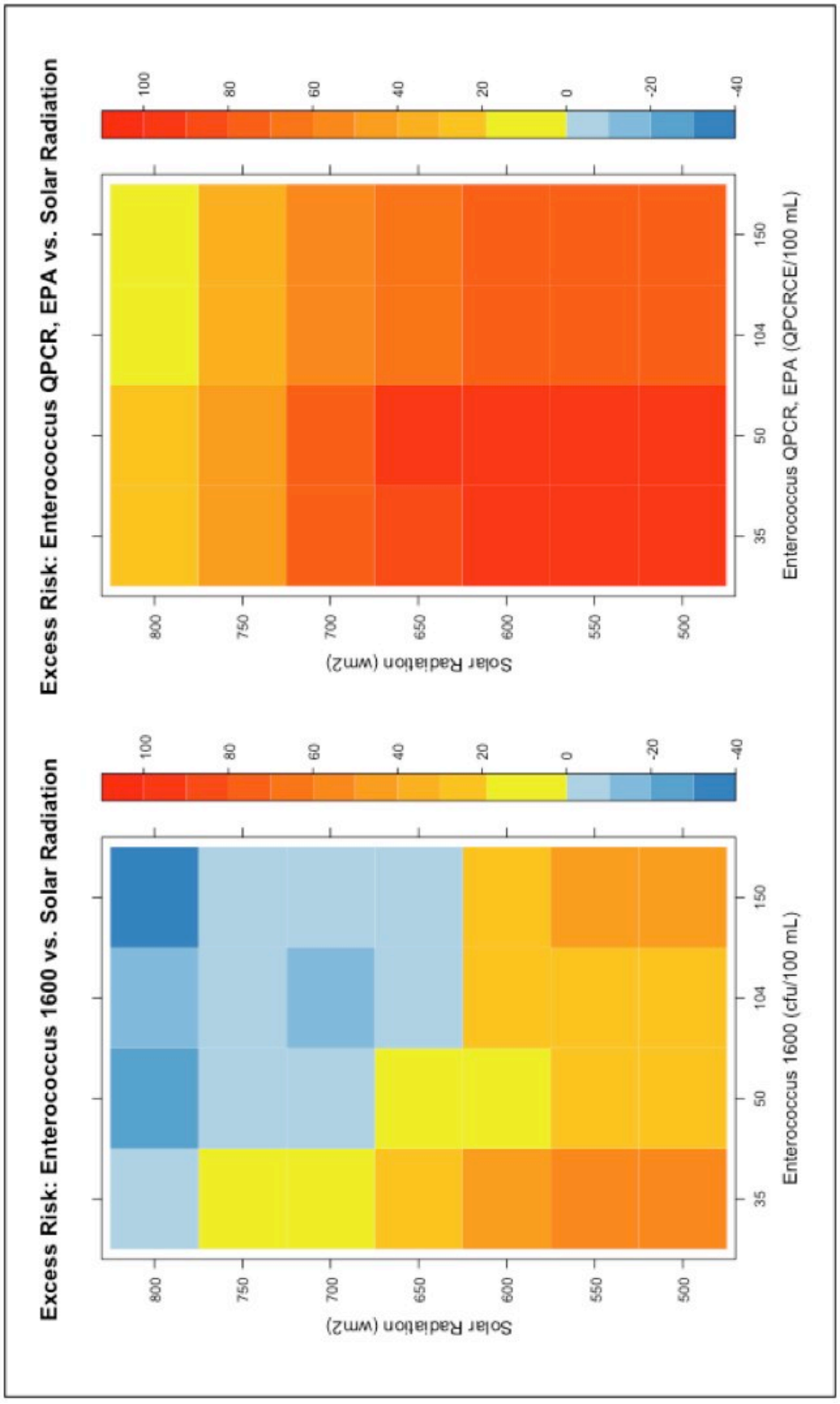
Statistical Estimator	AUC
SuperLearner Algorithm (SuperLearner)	0.70
10 nearest neighbors (KNN 10)	0.70
20 nearest neighbors (KNN 20)	0.67
50 nearest neighbors (KNN 50)	0.60
Generalized Additive Models (GAM)	0.53
Logistic Regression (Logistic)	0.50
D/S/A Algorithm (DSA)	0.50
Bayesian Regression (Bayes)	0.50
Lasso and Elastic-Net Regularized Generalized Linear Models (GLMnet)	0.50

Figure 2: ROC Curves and AUC Values for *Enterococcus* 1600, Multiple Estimators, Cross-Validated



Statistical Estimator	AUC
20 nearest neighbors (KNN 20)	0.57
10 nearest neighbors (KNN 10)	0.56
SuperLearner Algorithm (SuperLearner)	0.55
50 nearest neighbors (KNN 50)	0.51
Generalized Additive Models (GAM)	0.49
Logistic Regression (Logistic)	0.46
D/S/A Algorithm (DSA)	0.46
Bayesian Regression (Bayes)	0.46
Lasso and Elastic-Net Regularized Generalized Linear Models (GLMnet)	0.46

Figure 3: Heat map illustrating excess risk values for a variety of *Enterococcus* and solar radiation cutoffs.



3.6 References

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CHAPTER 4: GROUNDWATER TRANSPORT OF SEWAGE

4.1 Introduction

4.1.1 Sewage Systems at Avalon Beach

Avalon Beach has consistently been rated as one of the most polluted beaches in California. As of 2010, Avalon Beach was included on the "beach bummer" list (beaches with poor summertime dry weather water quality) for nine out of the prior eleven years. Out of the five monitoring stations on the beach, the water quality never improved above a "D" grade during the monitoring periods between April 1st and October 31st in 2009 (Heal the Bay 2010, California Department of Public Health, 2011). A project entitled the "Clean Beaches Initiative Funding for the Avalon Bay Water Quality Improvement Project" was enacted in 2008 to inspect and repair sewers that may have been contributing to the pollution of the beaches. However, even after repairs and inspections were completed, beach water quality remained poor at Avalon Beach. One step that has not been completed, but is thought to be important, is to conduct a sewer infrastructure replacement that includes privately owned sewage systems (Heal the Bay 2010). The sewage systems in Avalon have received a great deal of attention because it is thought that the main source of contamination at Avalon beaches is sewage. The sewer systems are flushed using ocean water, which is thought to degrade the pipes rapidly and lead to leakage.

4.1.2 Groundwater Transport of Sewage to Beach Water

A study conducted by Boehm et al. determined that sewage-contaminated groundwater was a major source of beach pollution at Avalon Beach (Boehm 2009). The source tracking, fate and transport, and modeling study found that, when coupled together, groundwater and solar inactivation could predict concentrations of certain bacteria and viruses in the water. In addition, a separate study that took shoreline samples and subsurface water samples detected human-specific bacteria and enterovirus levels at Avalon Beach, which indicates that human sewage could be a source of fecal bacteria in coastal waters (Boehm 2003). Thus, groundwater at Avalon has been considered an important potential source of infectious material in beach waters.

Other important variables that have been thought to correlate well with infectious organisms and human health are indicator organisms. Traditionally, *Enterococcus* concentrations have been used as a measure of whether or not beach water is safe for swimming. If a single sample of *Enterococcus* is found to contain ≥ 104 colony forming units (cfu) per 100 mL, then an advisory should be posted at that beach, indicating that the water is not safe for swimming (U.S. EPA 1986, Wade 2003). In 2000, a new amendment to the Clean Water Act required that new guidelines be developed using new fecal indicators and more rapid methods of measuring organisms (Public Law 2000).

This study seeks to determine if groundwater flow levels adversely affect the health of swimmers at Avalon Beach. Data collected by Boehm et al. on groundwater flow and solar radiation levels were combined with health and indicator organism data collected during a prospective cohort study that was conducted concurrently. A variety of indicator organisms were measured using traditional and rapid methods. Groundwater flow was explored both as an independent risk factor for adverse health events in swimmers and as a mediating factor of the relationship between indicator organisms and human health.

4.2 Methods

4.2.1 Study Design

The methods and data for the study at Avalon Beach are the same as those described in Chapter 3. In brief, the study was conducted using methods comparable to those used in prior beach studies (Wade et al. 2006, Colford et al. 2007, Wade et al. 2008, Wade et al. 2010). On study days, beachgoers were recruited, and at the same time multiple water samples were collected at different times and locations. Water samples were then processed and bacterial/viral indicator levels were recorded for different sites and times. Study participants were then called 10 to 14 days later to assess new symptoms, as well as demographic information.

4.2.2 Recruitment

Participants were recruited on weekends and holidays over the summer months in 2007 and 2008. At Avalon Beach, 7,317 individuals were recruited over 62 study days, and follow-up was completed two weeks later on 6,165 individuals (84%). In order to be eligible for the study, participants had to have 1) no prior participation in the study; 2) a family member older than 18 at the beach; 3) a home address in North America, and 4) no history of face or head contact with ocean or lake water in the previous seven days. In addition to collecting the above information, other recorded data included the water sampling site closest to the participant, when the participant entered the water (if relevant), activities done at the beach, prior illnesses or health conditions, and level of water contact. Ten to fourteen days after initial enrollment, participants were called and asked for additional data on demographic variables, swimming and related activities since enrollment, pre-existing health conditions, and new symptoms experienced since enrollment.

Only incident cases of illness were considered. Incident illnesses were defined as new cases of any symptoms between the day of enrollment and the date of phone interview (10 to 14 days later). Data on the following incident symptoms were collected: diarrhea, nausea, abdominal pain or cramps, vomiting, rash or itchy skin or skin infection, eye infection, earache or ear infection, urinary tract infection or burning sensation when urinating, fever, cough, sore throat, or highly credible gastrointestinal illness (HCGI). HCGI is a composite symptom defined as any of the following symptoms: 1) diarrhea, 2) vomiting, 3) nausea and abdominal cramps, 4) nausea and missed daily activities due to gastrointestinal illness, or 5) abdominal cramps and missed daily activities due to gastrointestinal illness.

4.2.3 Water Sampling and Analysis

Water samples were drawn from Avalon Beach at 8 am, 12 pm, and 3 pm from four spatially distinct locations on the beach. Water samples were collected at the depth of 0.5 m on incoming waves. Traditional indicators and novel indicators were measured from the water samples.

4.2.4 Groundwater Flow

A well-tested and validated model to determine groundwater discharge was developed by Boehm et al. (Boehm 2009). This model was used to predict groundwater discharge for each hour throughout the summers of 2007 to 2008.

Because groundwater levels are heavily influenced by tidal patterns, instantaneous measure of groundwater may not be informative for characterizing the amount of groundwater flow on a given day. In order to represent the amount of groundwater discharge on a given day, total groundwater flow was summed from 10 AM to 5 PM. This interval was chosen because it was thought to capture the times when indicator data were sampled, as well as when swimmers would be in the water. Volume of groundwater discharge was measured in cubic meters (m^3).

4.2.5 Data Analysis

Three major analyses were conducted in order to determine the role that groundwater played in the health of swimmers at Avalon Beach. First, the incidence of HCGI and diarrhea was examined on days when groundwater flow was high and low. Second, the excess risk of HCGI was calculated comparing individuals who swam on days when groundwater flow and solar radiation levels were above certain cutoffs to individuals who swam on days when groundwater flow and solar radiation levels were below those cutoffs. Finally, adjusted odds ratios were calculated to illustrate the relationship between traditional and rapidly measured indicator organisms and health on days when groundwater flow was high and when it was low.

4.2.6 Incidence of HCGI and Diarrhea, By Day

First, the groundwater total volume information from 10 AM to 5 PM was merged for study days when beachgoers were recruited into the study. Days when groundwater flow was above the median were categorized as “high” and when flow volume was equal to or below the median, the day was categorized as “low” (median value was 162.8 m^3 discharged from 10 to 5 PM). In order to identify incident cases of HCGI and diarrhea, those with baseline illness on the day of study enrollment were excluded. The number of days between recruitment (time = 0) and illness onset was then calculated (maximum = 10-14 days, when the follow-up telephone call was made) for each individual, and incidence per 1,000 swimmers plotted for each day. A line was drawn connecting the incidence points.

Different incidence lines were created for individuals with different swimming behaviors. Swimmers were defined by four categories of increasing intensity of exposure: non-swimmers, swimmers with body immersion (waist or higher), swimmers with head immersion, and swimmers who swallowed water. These definitions were mutually exclusive, meaning that those who swallowed water were not included in the category of swimmers with body immersion.

Significance testing to determine if the incidence of disease on a given day for a certain swim category was different from that for another swim category was done by taking the difference of the two values and bootstrapping the standard error of the difference.

4.2.7 Excess Risk (ER) of HCGI and Effect Modification

The ER measures the risk (or probability) that an exposed individual (swimmer with exposure above a certain cutoff) will become ill, and then subtracts from that value the probability that an unexposed individual (swimmer below the same cutoff) will become ill. It can be expressed not only as the increased probability of illness, but also the number of excess cases per 1,000 swimmers. This enables calculation of the number of excess cases expected on "high-risk" days. The interpretation of the ER is: if swimmers had not swum on high-risk days, how many extra cases of HCGI per 1000 swimmers would have been prevented *among that population* of swimmers. In these analyses, only swimmers who swallowed water were considered.

Two variables were used to characterize "high-risk" days. The first was solar radiation (measured in watts per meter^2) and the second was cumulative groundwater discharge volume from 10 AM to 5 PM (measured in m^3). These two variables were chosen because Boehm et al. determined that certain bacterial indicator and pathogen concentrations could be predicted using these two variables (Boehm 2009). A variety of cutoffs were considered for both variables. Each variable was divided by a variety of quantiles, starting at 15% of the observations and increasing every 5% up to 85% of the observations, meaning that the potential cutoffs for solar radiation and groundwater were divided at the 15%, 20%, 25%, ..., 85% quantiles.

The ER value for each cutoff was derived by first calculating the probability of HCGI in the high-risk swimming population (above the cutoff for groundwater and solar radiation) and then in the comparison swimming population (below the cutoff for groundwater and solar radiation). Using those probabilities, it was possible to calculate the ER by subtracting one number from the other. The probabilities were calculated using a saturated logistic model. Because groundwater and solar radiation levels had been converted to binary forms (above or below), the probabilities generated from a saturated logistic model are identical to the probabilities developed from a nonparametric model.

In order to derive 95% confidence intervals, a non-parametric bootstrap procedure was used. A distribution of the ER values was created by resampling from the original dataset 1,000 times, recording the ER values, and then creating the lower bound of the 95% confidence interval such that it excluded the lowest 25 ER values (25/1,000 = 2.5%). The upper bound was created in a similar way (excluding the highest 25 ER values).

4.2.8 Indicator Analyses, Adjusted Odds Ratios (OR's)

For indicator organism analyses, water quality indicators were $\log_{10}$ transformed to decrease skewness and make them more normally distributed. The water quality indicators were averaged by beach site location and date, in order to obtain site specific daily concentration values, which were then assigned to swimmers based on their swimming location.

These analyses, like those in the prior section, were restricted to swimmers who swallowed water. The probability of illness with a variety of health outcomes was modeled using a logistic model of the form:

$$\ln[p/(1-p)] = \alpha + \beta_1 I + \gamma \mathbf{X}$$

where I is the continuous value for the indicator organism, and $\mathbf{X}$ is a vector of confounding covariates. OR's were calculated as $OR = \exp(\beta_1)$. This OR corresponds to the increase in odds of illness for an indicator concentration increasing from 0 to 1 on the $\log_{10}$ scale. The model assumes that the relationship between the indicator organism and risk of illness is linear on the log-odds scale.

These models were adjusted for a number of potential confounders (**X**): study year, age, sex, race (white or non-white), swimming on multiple days, allergies, contact with animals, contact with sick people, frequency of beach visits, digging in the sand, and consumption of raw or undercooked eggs or meat. A backwards deletion, or change in estimate algorithm, was used in order to retain only potential confounders that changed the OR by at least 5% (relative change) when the confounder was removed from the model (Rothman 1998). This method is the same as that used in prior studies (Wade 2006, Colford 2007, Wade 2008, Wade 2010).

Indicator analyses were conducted for days when the groundwater discharge volume from 10 AM to 5 PM was above and below the median. First, the relationship between *Enterococcus* (EPA 1600, traditional method) was evaluated with all measured health symptoms. Second, a variety of traditional indicators and rapid methods were related to HCGI risk using adjusted Odds Ratios.

4.3 Results

The health and indicator organism data used in this thesis are still preliminary. Until the data are fully quality assured, the results presented below may be subject to revision.

4.3.1 Water Quality and Groundwater Flow

Over the course of the study, site-specific daily *Enterococcus* concentrations, as measured by the traditional method (culture based, EPA 1600) exceeded the regulatory 104 cfu/100 mL standard on 21 of 61 study days. Out of these 21 days, 10 occurred when groundwater flow levels were below the median, and 11 occurred when groundwater flow levels were above the median. Total groundwater flow volume from 10 AM to 5 PM ranged from 104.9 m^3 to 188.9 m^3.

4.3.2 Incidence of HCGI and Diarrhea

When incidences of HCGI and diarrhea were plotted, peaks in incidence were seen on high groundwater flow days two days after visiting the beach. Incidence of HCGI in swimmers who swallowed water on high groundwater flow days was 30.4 cases per 1,000 swimmers, which

was significantly higher than the incidence in swimmers who swallowed water on low groundwater flow days (8.9 cases per 1,000, p = 0.03). A similar relationship was seen for the outcome diarrhea. On high groundwater flow days, the incidence of diarrhea was 23.1 cases per 1,000 swimmers who swallowed water two days prior, while on low groundwater flow days the incidence was 4 cases per 1,000 swimmers who swallowed water (p = 0.02). These incidence values were calculated based on 896 individuals who swallowed water and were at risk of diarrhea or HCGI two days after their beach visit.

Comparing the incidence of HCGI in those who swallowed water on high groundwater flow days (incidence of 30.4 cases per 1,000 swimmers) to non-swimmers on high groundwater flow days (13.7 cases per 1,000 non-swimmers), the difference in HCGI incidence was suggestive but not significant at the p = 0.05 level (p = 0.07). The difference in incidence of diarrhea between swimmers who swallowed water and non-swimmers was not significant (incidence in swimmers of 23.1/1,000, incidence in non-swimmers of 12.5/1,000, p-value = 0.18). Plots of the incidence of diarrhea and of HCGI are presented in Figures 1 and 2.

4.3.3 Excess Risk of HCGI

Figure 3 presents a graphical representation of the excess risk of HCGI for a number of cutoffs for solar radiation and groundwater discharge. The range of excess risk values ranged from 79.5 excess cases of HCGI / 1,000 swimmers who swallowed water to -90.9 excess cases of HCGI / 1,000 swimmers (negative values indicate a protective effect). Excess risks tended to be elevated when solar radiation levels were lower, as well as when groundwater levels were elevated. The lowest values for excess risk occurred at the maximum considered values for solar radiation (above 85% quintile vs. below 85% quintile). Numerical values, as well as bootstrapped 95% confidence intervals are presented in Table 1.

4.3.4 Indicator Organism Analyses, By Groundwater Flow Volume

When *Enterococcus* 1600 concentrations were related to a variety of symptoms in swimmers who swallowed water, several odds ratios were elevated when groundwater flow was above the median, whereas most odds ratios were near the null value when groundwater flow was below the median. Statistically significant adjusted OR's were found for cough (OR, 95% CI: 2.67 [1.14-6.28), cramps (OR, 95% CI: 1.45 [1.00-2.09]), diarrhea (OR, 95% CI: 1.6 [1.03-2.49]), fever (OR, 95% CI: 1.69 [1.03-2.78]), HCGI (OR, 95% CI: 1.54 [1.07-2.2]), and nausea (OR, 95% CI: 1.97 [1.09-3.55]) when groundwater levels were higher than the median. Conversely, when groundwater levels were below the median, *Enterococcus* concentrations demonstrated no statistically significant relationship with the same symptoms. The only exception to this pattern was for sore throat, for which elevated *Enterococcus* concentrations were associated with increased odds of sore throat when groundwater flow was low (OR, 95% CI: 1.54 [1.04-2.28]), but the odds of illness decreased when groundwater flow was high. Insufficient numbers of swimmers experienced eye infections or urinary tract infections to allow OR's to be calculated.

When multiple indicators were considered, comparing swimmers who swallowed water with higher levels of those indicators to swimmers who swallowed water with lower concentrations of the indicators, a similar pattern to above was seen. When groundwater flow levels were elevated, nearly all OR's for the indicators were greater than 1, whereas when groundwater flow levels were below the median, all adjusted OR's were centered below the null value (OR = 1). When groundwater flow was elevated, statistically significant results were found for *Enterococcus* EPA 1600 (OR, 95% CI: 1.54 [1.07-2.2]), *Enterococcus* EPA 1600 with a binary 104 cfu/100 mL cutoff (OR, 95% CI: 3.8 [1.38-10.49]), and fecal coliforms (OR, 95% CI: 1.32 [1.00-1.72]).

4.4 Discussion

The results presented in the high groundwater flow sections of Figures 1 and 2 are consistent with an infectious cause of diarrhea and of HCGI being in the water. Toxins often act within hours, but several bacterial and viral etiologies of gastroenteritis cause symptoms within one to three days of infection. Norovirus-associated gastroenteritis, for example, has an incubation period between 24 and 48 hours, while enterohemorrhagic *E. coli* has an incubation period between 1 and 10 days, although usually it is around 3-4 days (CDC 2010, CDC 2011).

Water quality did not seem to be markedly different when groundwater flow levels were higher versus when groundwater flow levels were lower. Mean and median concentrations of indicator bacteria were roughly the same whether groundwater flow levels were high or low, and the number of *Enterococcus* exceedences was virtually the same despite differences in groundwater flow. However, the fact that the peaks in disease incidence appeared only when groundwater flow levels are high suggests that even though indicator organism concentrations do not increase, perhaps the concentration of infectious agents that cause diarrhea and HCGI does increase. It is plausible that infectious agents are present in higher concentrations when groundwater flows carrying human sewage leech into the beach water. The higher peaks of diarrhea and HCGI among swimmers who swallowed water is also biologically plausible, as many causes of gastrointestinal illness are acquired via the fecal-oral transmission pathway.

The values for excess risk of HCGI also support the idea that increased groundwater flow is associated with a greater excess risk of HCGI. In Figure 3, two overall patterns seem to emerge. The first was that when solar radiation levels were quite high (817 w/m^2), no cutoff could be found for groundwater flow that corresponded to an elevated excess risk of HCGI. In fact, when a cutoff for groundwater flow at 167 m^3 and a solar radiation cutoff of 817 w/m^2 was considered, those who swam when groundwater flow and solar radiation were above those cutoffs experienced 90.9 *fewer* cases of HCGI per 1,000 swimmers when compared to swimmers who swam when solar levels and groundwater levels were lower. The fact that the trend was present across the entire upper row (817 w/m^2 cutoff for solar radiation) suggests that swimming when solar levels are above 817 w/m^2 is protective compared to swimming when solar levels are below 817 w/m^2. This finding is biologically plausible because solar inactivation is thought to disrupt the DNA and cellular processes of pathogenic organisms (see Chapter 3).

The second major pattern that emerged in the results was that when higher groundwater discharge cutoffs were considered (last columns of Figure 3), regardless of solar level, the excess risk of HCGI appeared to be elevated, except at the highest level of solar radiation. For example, when swimmers who swallowed water when groundwater flows were above 178 m^3 were compared to swimmers who swallowed water when groundwater discharge was lower, risks were elevated almost universally (with the exception of when solar radiation levels were extremely high).

When low solar radiation levels and low groundwater flows were present (bottom left corner of Figure 3), excess risk values were greatly elevated, with some excess risk values being statistically significant. The situation being represented here is essentially the comparison between swimmers who swallowed water virtually uncontaminated by groundwater flow (i.e. very low risk, if it is assumed that groundwater flow increases risk of illness) to swimmers who swallowed water with groundwater concentrations ranging up to 178 m^3 of groundwater discharge from 10 to 5 PM. When the baseline group is a very low risk group who swallowed essentially clean water, it makes sense that individuals who swam in waters that were anywhere from slightly more contaminated to extremely contaminated would have been at substantially higher risk of illness than the low risk population.

The patterns observable in the analyses also support the idea that indicator organism levels are much better predictors of illness when groundwater flow levels are high. None of the indicator organisms considered had an elevated OR (>1) when groundwater flow was low, as opposed to the situation when groundwater flow was high. The results for rapid tests of indicator organisms were not significant, though some were marginally significant when groundwater flow was high (EPA Enterococcus QPCR, with inhibited samples: OR, 95% CI: 1.42 [0.99-2.04]).

In summary, several lines of evidence suggest a relationship between groundwater flow and illness among individuals who were swimming. Because groundwater flow is thought to bring with it raw human sewage, it is hypothesized that when groundwater flow levels are higher, the number of pathogens in the water increases. Among swimmers who swallowed water on days with high groundwater flow vs. low groundwater flow, the incidence of HCGI and diarrhea is significantly higher on the second day after the beach visit ($p = 0.03$ for HCGI, $p = 0.02$ for diarrhea). In addition, when examining the excess risk in individuals who swam on days with higher levels of groundwater flow versus individuals who swam on lower flow days, the excess risk also is elevated. This relationship is attenuated by high solar radiation levels, as would be expected if pathogenic organisms were contaminating the water and causing illness. Finally, traditional indicator approaches appear to be much more strongly associated with illness when groundwater flows are higher, consistent with the hypothesis that indicator organisms are strongly associated with health outcomes when their concentrations follow the concentrations of pathogens in the water.

4.5 Figures and Tables

Table 1: Excess risk of HCGI, by solar radiation and groundwater flow from 10 AM to 5 PM

Excess Risk (cases per 1,000 swimmers)*	Solar Radiation Levels, w/m^3																			
Groundwater Flow (m3 from 10 AM to 5 PM)	461.7		493.8		636.9		698.1		705.5		707.5		715.0		718.8	
123.2	57.2 [-29.2, 143.5]	63.9 [-23.5,151.3]	56.9 [-30.9,144.7]	57.2 [-18,132.4]	4.7 [-70.3,79.6]	-4.4 [-76.9,68.2]	0.3 [-73.2,73.8]	2.3 [-72.4,76.9]								
132.9	63.9 [-13.2,140.9]	**77 [12.8,141.2]**	51.1 [-22.9,125.1]	48.4 [-19.5,116.3]	10.1 [-56.6,76.9]	1.8 [-64.5,68]	9.3 [-52.4,71]	11.1 [-52.2,74.4]								
133.7	66.7 [-12,145.4]	66.2 [-3,135.4]	44.8 [-28.7,118.3]	41.2 [-27.5,109.9]	10.8 [-55.5,77.1]	3.4 [-62.7,69.5]	11 [-51.6,73.7]	13.5 [-51.5,78.4]								
136	71.1 [-8.3,150.5]	**72.6 [6.4,138.7]**	51.9 [-18.6,122.4]	47.9 [-18.6,114.4]	17.4 [-48,82.9]	9.9 [-55.7,75.4]	17.3 [-45.3,79.8]	20.4 [-44.9,85.6]								
139.4	76.8 [-3.7,157.2]	**79.5 [15,143.9]**	59.6 [-9.2,128.5]	55.4 [-10.2,121.1]	25.1 [-40.4,90.7]	17.4 [-48.4,83.2]	24.7 [-38.6,88]	28.8 [-37.8,95.3]								
146.2	70.2 [-9.9,150.3]	**73.3 [10.5,136.1]**	52.4 [-14.6,119.3]	45.5 [-18.3,109.3]	15.3 [-49.4,80]	7.5 [-57.3,72.4]	14.5 [-47.8,76.8]	17.2 [-48.5,82.9]								
149.1	62.5 [-7.5,132.5]	**68 [8,128]**	49.4 [-13.6,112.4]	40.5 [-20.5,101.6]	15.2 [-47.1,77.5]	8.1 [-54.4,70.7]	14.5 [-46.5,75.5]	16.9 [-48,81.8]								
160.6	53.6 [-20.9,128.2]	**65.1 [1.9,128.3]**	47.7 [-18.1,113.5]	39.8 [-24.7,104.2]	17.9 [-46.7,82.5]	11.5 [-53.3,76.3]	18.2 [-43.6,79.9]	21.9 [-44.7,88.4]								
163	60.6 [-12.7,134]	**71.4 [8,134.8]**	54.3 [-11.8,120.4]	46.7 [-18.7,112.1]	24.8 [-40.9,90.6]	18.5 [-47.6,84.5]	25 [-38.3,88.3]	30.4 [-38.6,99.4]								
167.4	54.2 [-19.4,127.9]	**65.1 [1.3,128.8]**	46.2 [-20.1,112.5]	35.2 [-29.9,100.2]	13.3 [-52.2,78.8]	6.9 [-58.8,72.6]	13.5 [-49.7,76.6]	16.7 [-53,86.3]								
169.8	66.7 [-9.9,143.3]	**78.3 [11,145.7]**	60.1 [-10.3,130.5]	50.8 [-20.1,121.8]	28.9 [-42.6,100.5]	22.6 [-49.2,94.4]	29.7 [-40,99.4]	38.9 [-41.3,119.1]								
172.8	41.7 [-39.8,123.2]	59.3 [-12.2,130.8]	35.9 [-41.5,113.3]	27.6 [-51.6,106.8]	15.8 [-61.5,93.1]	11.8 [-65.3,88.9]	20.8 [-54.7,96.4]	34.6 [-55.8,125.1]								
174.6	33.9 [-55.6,123.4]	56.6 [-24.3,137.5]	35.5 [-52.3,123.3]	21.1 [-65.3,107.4]	13.4 [-70.9,97.7]	10.4 [-73.6,94.4]	20.3 [-63.2,103.8]	44.1 [-65.7,153.9]								
175.5	34.3 [-58.8,127.4]	57.4 [-28.4,143.2]	31.8 [-54.8,118.4]	17.9 [-67.2,103.1]	11.6 [-71.8,95]	9 [-74.1,92.1]	18.6 [-64.4,101.5]	42.4 [-67,151.7]								
177.9	40.7 [-60.9,142.3]	61.5 [-30.7,153.7]	36.1 [-57.3,129.4]	17.7 [-76.8,112.2]	10.9 [-82.5,104.4]	8.2 [-85.2,101.7]	18.5 [-76.2,113.1]	56.2 [-89.7,202.2]								
178.4	-6.5 [-103.6,90.5]	17.4 [-68.6,103.3]	-5.9 [-93.3,81.6]	-36.9 [-117.6,43.8]	-42.5 [-121.4,36.5]	-44.8 [-123.1,33.4]	-35.9 [-115,43.2]	-51 [-153.1,51.1]								

Excess Risk (cases per 1,000 swimmers)	Solar Radiation Levels, w/m^3																			
Groundwater Flow (m3 from 10 AM to 5 PM)	736.4		740.8		760.0		761.6		775.5		798.4		816.9							
123.2	11.6 [-52.5,75.7]	-8.7 [-76.9,59.5]	-13 [-82.1,56.1]	-25.2 [-94.7,44.3]	-25.6 [-96.7,45.5]	-21.6 [-98.3,55.1]	-40.8 [-119.1,37.4]									
132.9	15.2 [-42.2,72.5]	-1.7 [-62.4,59.1]	-7.1 [-68.2,54.1]	-18.8 [-78.4,40.8]	-19.2 [-80.7,42.3]	-15.2 [-82.8,52.4]	-34.4 [-103.5,34.6]									
133.7	16.8 [-43.1,76.7]	1.4 [-61.8,64.6]	-5 [-69.2,59.2]	-18.4 [-81.5,44.7]	-17 [-82,48]	-10.8 [-85.8,64.3]	-37.7 [-115.8,40.3]									
136	23.1 [-37.6,83.7]	8.5 [-55.7,72.8]	2.6 [-63.1,68.3]	-11.1 [-76,53.8]	-9 [-76.4,58.5]	-5.4 [-79.2,68.5]	-32.3 [-109.1,44.5]									
139.4	31 [-31.3,93.4]	17.6 [-49,84.2]	12.6 [-56.2,81.4]	-1.5 [-69.9,66.9]	-2.5 [-69.2,64.3]	1 [-72.6,74.6]	-25.8 [-103,51.3]									
146.2	18.3 [-43,79.7]	3 [-62.3,68.2]	0.8 [-67.2,68.8]	-12.9 [-80.3,54.5]	-10.9 [-76.7,54.9]	-7.4 [-80.5,65.6]	-34.3 [-111,42.4]									
149.1	16.6 [-45.1,78.3]	1.6 [-64.7,67.9]	0.8 [-69.1,70.7]	-7.3 [-76.7,62.2]	-5.4 [-73.6,62.8]	-6.1 [-78,65.8]	-32.9 [-108.3,42.4]									
160.6	21.5 [-42.4,85.4]	8.5 [-60.7,77.8]	3.9 [-64.4,72.1]	-4 [-72,64.1]	-2.5 [-69.5,64.4]	-3.7 [-74.5,67.1]	-30.5 [-105,43.9]									
163	29.8 [-36.8,96.4]	18.8 [-54.6,92.1]	14 [-58.3,86.4]	6.2 [-66.1,78.5]	7.5 [-63.8,78.9]	7.9 [-69.4,85.1]	-16.7 [-103.6,70.2]									
167.4	14.6 [-52.5,81.7]	-0.4 [-74.2,73.4]	-5.1 [-78,67.7]	-13 [-86.1,60.1]	-11.6 [-83.3,60]	-18.5 [-96.1,59.1]	**-90.9 [-122.7,-59.2]**									
169.8	36.8 [-41.7,115.3]	29.9 [-62.1,121.8]	25.1 [-66,116.2]	17.3 [-74.3,108.8]	18.6 [-71.7,108.9]	19.7 [-88.9,128.3]	**-89.2 [-120.4,-58]**									
172.8	40.4 [-50.5,131.2]	47.9 [-65.1,160.9]	44.9 [-67.2,156.9]	39 [-73.5,151.5]	41.2 [-70.3,152.8]	63.9 [-90.6,218.4]	-29.3 [-118.6,59.9]									
174.6	53.1 [-59,165.3]	44.4 [-67.6,156.4]	42.1 [-69.2,153.4]	37.2 [-74.5,148.9]	39.3 [-71.6,150.2]	61.5 [-92.8,215.8]	-35.9 [-121.5,49.8]									
175.5	51.4 [-60.4,163.2]	43.5 [-68.2,155.1]	41.3 [-69.6,152.2]	36.8 [-74.6,148.1]	38.7 [-71.9,149.4]	60.8 [-93.2,214.8]	-38.1 [-123.3,47]									
177.9	70.8 [-84,225.6]	62.7 [-92,217.5]	60.5 [-93.9,214.9]	55.9 [-98.8,210.6]	57.8 [-96.4,212]	61.1 [-93.1,215.3]	-36.5 [-123.6,50.6]									
178.4	-43.5 [-153.6,66.6]	-51 [-160.2,58.1]	-53.1 [-161.9,55.8]	-57.4 [-166,51.1]	-55.4 [-163.4,52.6]	-51.9 [-159.4,55.6]	-70.6 [-136.3,-4.9]									

*Swimming defined as swallowing water. Excess Risks presented with [95% Confidence Intervals]

Table 1: Excess Risk of HCGI, comparing swimmers who swallowed water when water conditions were above specified cutoff to swimmers who swallowed water when water conditions were below specified cutoff. 95% confidence intervals calculated by 1,000 fold boostrap.

Table 2: Adjusted Odds Ratios relating *Enterococcus* concentrations and a variety of health outcomes. Traditional EPA 1600 method was used to measure *Enterococcus* concentrations.

Table 2: Enterococcus 1600*, All Symptoms				
Symptom	Groundwater Flow Below Median**		Groundwater Flow Above Median	
	N	Odds Ratio [95% CI]	N	Odds Ratio [95% CI]
Coughs	279	0.56 [0.22,1.46]	115	**2.67 [1.14,6.28]**
Cramps	391	0.87 [0.52,1.48]	396	**1.45 [1,2.09]**
Diarrhea	429	0.96 [0.56,1.64]	397	**1.6 [1.03,2.49]**
Ear Ache	190	0.89 [0.36,2.23]	370	0.87 [0.47,1.58]
Eye Infection	-	-	-	-
Fever	185	1.09 [0.57,2.1]	387	**1.69 [1.03,2.78]**
HCGI	433	0.76 [0.48,1.21]	397	**1.54 [1.07,2.2]**
Nausea	395	0.68 [0.34,1.35]	397	**1.97 [1.09,3.55]**
Skin Rash	340	0.83 [0.43,1.62]	402	1.16 [0.63,2.16]
Sore Throat	283	**1.54 [1.04,2.28]**	363	0.98 [0.63,1.51]
UTI	-	-	-	-
Vomiting	262	0.62 [0.25,1.54]	227	1.05 [0.56,1.99]
*Site Specific Daily Average used to calculate concentration				
**Median value = 162.8 m^3 from 10 AM to 5 PM				

Table 3: Adjusted Odds Ratios relating rapid and traditional indicators to odds of highly credible gastrointestinal illness (HCGI).

Table 3: Multiple Indicators*, HCGI					
Indicators	Lab	Groundwater Flow Below Median**		Groundwater Flow Above Median	
		N	Odds Ratio [95% CI]	N	Odds Ratio [95% CI]
Enterococcus QPCR	EPA	427	0.93 [0.62,1.4]	357	1.27 [0.91,1.78]
Enterococcus QPCR (with Inhibited Samples)	EPA	429	0.89 [0.59,1.36]	359	1.42 [0.99,2.04]
Enterococcus QPCR1	Noble	411	0.71 [0.43,1.18]	307	1.24 [0.87,1.77]
Enterococcus QPCR (with Inhibited Samples)	Noble	233	0.7 [0.44,1.12]	260	1.24 [0.8,1.91]
Enterococcus QPCR2	Noble	429	0.81 [0.59,1.12]	350	1.15 [0.85,1.57]
Enterococcus QPCR2 (with Inhibited Samples)	Noble	433	0.8 [0.57,1.13]	359	1.16 [0.88,1.54]
Enterolert	SCCWRP	433	0.8 [0.52,1.23]	392	1.39 [0.91,2.11]
Enterococcus 1600	SCCWRP	433	0.76 [0.48,1.21]	397	**1.54 [1.07,2.2]**
Enterococcus 1600 (104 cfu/100 mL cutoff)	SCCWRP	433	0.73 [0.21,2.55]	364	**3.8 [1.38,10.49]**
Enterococcus 1600 (35 cfu/100 mL cutoff)	SCCWRP	434	0.5 [0.21,1.15]	361	1.86 [0.84,4.11]
Fecal Coliforms	SCCWRP	433	0.83 [0.58,1.18]	364	**1.32 [1,1.72]**
Total Coliforms	SCCWRP	429	0.88 [0.58,1.32]	360	0.81 [0.53,1.24]
*Site Specific Daily Average used to calculate concentration					
**Median value = 162.8 m^3 from 10 AM to 5 PM					

Figure 1: Incidence of HCGI for a variety of mutually exclusive swim definitions, divided by days when groundwater flow is above and below the median groundwater flow from 10 AM to 5 PM.

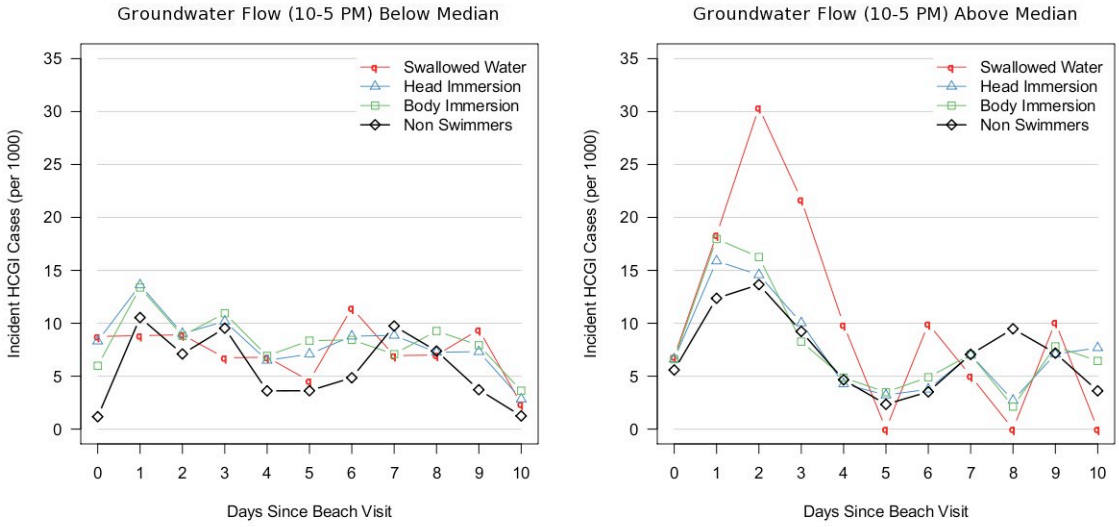


Figure 2: Incidence of diarrhea for a variety of mutually exclusive swim definitions, divided by days when groundwater flow is above and below the median groundwater flow from 10 AM to 5 PM.

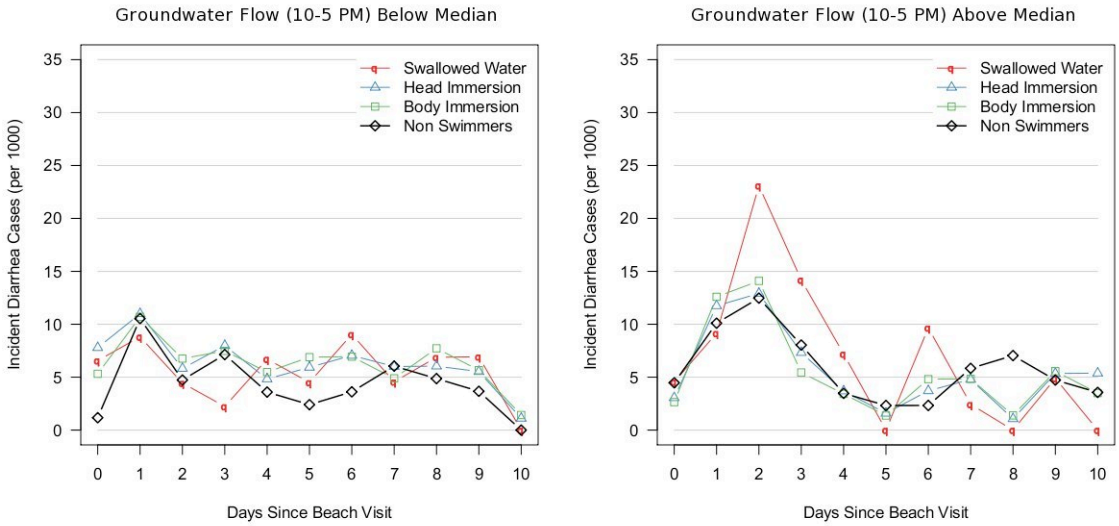
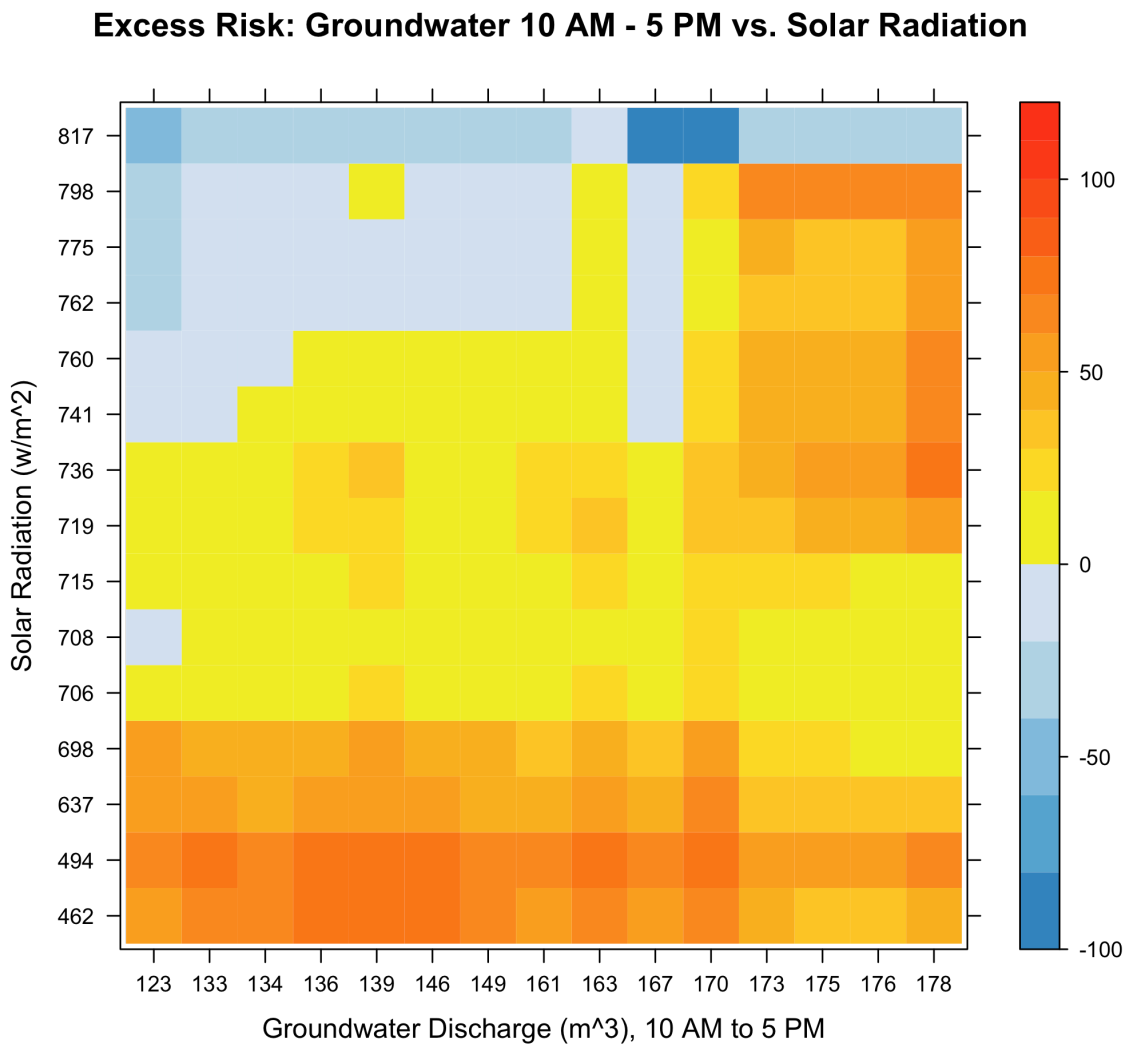


Figure 3: Level plot illustrating excess risk levels of HCGI for a variety of cutoff points for solar radiation and groundwater discharge from 10 AM to 5 PM.



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CHAPTER 5: CONCLUSIONS

5.1 Heterogeneity

After completing the above investigations and examining the prior research on the subject, one striking theme emerges. Research concerning the adverse health effects of exposure to recreational water is dominated by heterogeneity. Although regulations promulgated by the government to protect the health of swimmers seem to work in many circumstances, it is difficult to craft regulations that work optimally at every beach. Sources of variability in the results of findings in this field include the types of beaches being studied, which indicator organisms are being used, the conditions under which the indicator organisms are being used, the sources of contamination on the beach, and types of individuals who swim at the beach, among others.

The type of beach being studied can have a substantial impact on how well indicator organism levels predict illness. Some beaches have a point source or focal point for fecal contamination. Other beaches do not have a point source, meaning that the beach is only indirectly contaminated with fecal material. Relationships between indicator organisms and disease at non-point source beaches (e.g. Mission Bay CA) have been found to be much weaker than those at point source beaches (Colford 2007). In a review of 27 studies conducted by Wade et al., most studies showed increasing risk of illness with greater levels of fecal indicator bacteria (Wade 2003). However, most of these beaches had known point sources of human sewage contaminating the beach. At Mission Bay, the source of feces was thought to be mainly birds, which could explain the lack of an association between the indicator bacteria examined and illness (Gruber 2005). Results such as these indicate that a "one size fits all" approach to regulating beach safety may not always succeed.

Beyond the differences in non-point vs. point source beaches, climate and water temperature have a strong influence on the flora present in the water. Tropical beaches are known to have vastly different microbial makeups than beaches in more temperate locations. In fact, after the lawsuit filed against the EPA, one of the new requirements was that the EPA would complete epidemiologic studies at a tropical beach and at a temperate beach affected by urban runoff by the end of 2010. A study by Fleisher and Sinigalliano et al. was completed at a subtropical marine site with no known point source of fecal material. The results were similar to those found at Mission Bay, the previously mentioned non-point source beach. No relationship was found between indicator bacteria levels and gastrointestinal illness in these settings (Sinigalliano et al. 2010). However, in this study, swimmers were somewhat more likely to experience GI illness and AFRI than non-swimmers, although the results were not statistically significant. However, skin conditions were more likely to occur in swimmers than in non-swimmers (Fleisher 2010). Even though indicators of fecal contamination and regulatory guidelines were initially formed to address gastrointestinal illness, indicators did not perform as anticipated at either non-point source beach.

The results presented here further support the idea that indicators perform very differently under different circumstances. For instance, indicator organism concentrations and groundwater flow were both better predictors of illness when solar radiation levels were much lower.

Indicators could be useful on days when fecal indicator organisms and pathogens are likely to persist (e.g. lower solar inactivation), although they may perform much worse when high levels of solar inactivation are present. In addition, when groundwater flow was high at Avalon (akin to a point source) the indicators showed stronger associations with illness. However, when groundwater flux levels were lower, the association between indicators and the risk of illness decreased. Groundwater, in this circumstance, could be argued to be a contributing factor to a "point source" for fecal contamination. However, on low groundwater flow days, indicators persisted in the water at levels similar to high groundwater flow days. In addition, the number of days when the level of *Enterococcus* exceeded the 104 cfu per 100 mL threshold was roughly evenly distributed between low and high groundwater flow days.

Yet another example of heterogeneity in indicator performance lies in the types of individuals who frequent the beach. Local residents are thought to have substantially greater immunity to infection than visitors for several reasons. First, local residents may swim more frequently in recreational waters located close by, and may thereby have developed immunity to the local microorganisms. Second, the local community generates the fecal material that eventually may end up contaminating the beaches and might be expected to be far more resistant to the local pathogens than tourists and visitors to the area. Tourists may have a strong immunity built up against pathogens common at their home locations, but may have little to no protection against different strains of the pathogens, including those found at the beaches they are visiting. When heterogeneity in reported odds ratios was examined between studies included in the systematic review and meta analysis in Chapter 2, it was found that in marine water settings, studies that adjusted for visitor and native status found very different results than studies that did not account for visitor and native status.

These sources of heterogeneity mean that crafting a new set of regulatory standards that works well at all beaches in the U.S. will be an extremely difficult task. A "one size fits all approach," like ones taken in the past, may have the same problem of working at certain beaches, but failing to work at other beaches.

5.2 Multiple Testing

Given that heterogeneity can play such an important role in determining the conditions under which indicator organism based standards can be useful, a related problem is multiple testing. In order to find the perfect conditions under which the indicators work, it is tempting to examine the data in hundreds, or even thousands of different ways in order to find "significant" associations between indicator organism levels and risk of illness. This same problem threatened the validity of findings from genetics studies, in which thousands of genes are examined to determine if there are associations between certain genes and diseases. It is inevitable that, by chance alone, one exposure out of hundreds will be significantly related to an outcome. However, scientific progress is based on being able to repeat results across multiple studies, not "cherry pick" results out of a single dataset. Thus, several hypotheses that are biologically plausible should be tested in conjunction, in order to determine how robust the relationships are between exposure and disease.

Two approaches were used in this dissertation. The first was examination of dose response effects. It is quite easy to find one or more results that are statistically significant if enough tests are run, but finding a biologically plausible trend as concentrations of indicators increase is much less likely to occur by chance. The figures showing the excess risk values for a variety of cutoffs help paint a picture- even though not all of the excess risk values were statistically significant, the fact that there were biologically plausible patterns helps strengthen the argument that solar radiation mediates the relationship between indicators and groundwater flow with illness in swimmers.

In addition to finding evidence of a dose response, it is reassuring to know that the AUC values demonstrating improved prediction of illness when solar radiation and indicator concentrations were used together remained present even when cross-validation was used. By randomly splitting the data and only predicting outcomes on observations that the model did not use for its initial fit, the investigator can avoid the pitfalls of multiple hypothesis testing and worry less about "overfitting" the data. Combining cross-validation with dose-response patterns gives a layered sense of security- in order for a result to be taken seriously, it would have to overcome two sets of challenges rather than just one.

As multiple testing increases, investigators should focus on multiple methods to prove or disprove their hypotheses. Another step taken in Chapter 4 was to plot the incidence of illness among swimmers under different conditions. Seeing peaks in illness occurring roughly two days after swimming at the beach is much more convincing than having a flat line for disease incidence across all days. A peak in illness two days after exposure suggests that there is an infectious process initiated by swimming at the beach. Adding this argument to the puzzle bolsters the plausibility of the results.

A number of studies of recreational water have used adjustments for multiple testing (Griffith 2006, Craig 2002, Noble 2004), although many studies did not report if they did or did not use these methods. Improvements in computing and statistical packages have made methods like these more accessible to researchers, and these methods should increased use in future studies.

5.3 Swimmer vs. Swimmer Comparisons

Many of the analyses in this dissertation focus on comparing swimmers to other swimmers, rather than swimmers to non-swimmers. This approach was taken because swimming and non-swimming populations are often quite different. Swimmers could be more physically fit or healthier, for example, when compared to non-swimmers. In order to obtain less biased results, swimmers who were exposed to high indicator organism concentrations were compared to swimmers exposed to lower indicator organism concentrations. An interesting feature of this comparison is its similarity to a randomized controlled trial (RCT). In an RCT, individuals are typically randomized to various exposures, and then their outcomes are measured at the end of the study. When indicator organism concentration levels are the exposure of interest, the exposure status is essentially blinded to swimmers. This is because indicator concentration levels are invisible to the naked eye, and swimmers cannot know what their exposure status is. When

they enter the water, swimmers are receiving an unknown, "random" dose of indicator concentrations. Indicator concentration levels cannot be measured without laboratory equipment.

A potential confounder of the indicator-disease relationship would have to be causally related to both indicator and disease level. However, it is difficult to conceive of a variable that might influence both the indicator concentration level in the water as well as a swimmer's health status days later. Sewage discharge onto the beach would be expected to influence indicator organism levels as well as subsequent health outcomes, but the very goal of using indicators is to indicate when sewage is present- thus, sewage is not so much a confounder but a variable that is closely tied to the exposure of interest. Other traditional confounders, such as gender, socio-economic status, and age, are unlikely to predict the concentration of indicator organisms in the water, and thus adjustment for these variables is unnecessary. Nevertheless, all odds ratios calculated were adjusted for the traditional set of confounders (enumerated above). The results of these adjusted OR's parallel the results found by the excess risk calculations, which did not adjust for confounders. This suggests that these variables do not confound the relationship between indicator and illness, or if they do, they do so in very low amounts.

When the adjusted and unadjusted odds ratios were compared, the findings were very similar. For example, in Table 2 of Chapter 4, the adjusted OR when the groundwater flow was above the median for HCGI was 1.54 [1.07, 2.2], while the unadjusted OR was 1.6 [1.14, 2,23]. When the groundwater flow was below the median, the adjusted and unadjusted OR's were 0.76 [0.48, 1.21] and 0.93 [0.65, 1.33], respectively. The results are consistently statistically significant or not significant, though the point estimates differ slightly. The question arises, which OR should be calculated and reported? A common assumption is that the adjusted results are less biased, but this is not always the case. Adjusting for variables that are not confounders, or even potential confounders, tends to reduce the power of a study to detect a difference. In the worst case scenario, adjustment could induce bias, especially if the variable adjusted for does not cause the exposure and outcome, but rather is *a cause of* the exposure and outcome. An example of this situation is conducting a study in a hospital, with the intent of applying the results to the general populace- a harmful exposure like smoking and conditions like pneumonia might increase the chances of being admitted into a hospital, but individuals in a hospital are very different than the general populace. Findings based on hospitalized individuals are not likely to be identical, or even similar to, results that were generated from a study based on smokers and individuals who contracted pneumonia in the general populace.

5.4 Randomized Controlled Trials (RCT's) Vs. Observational Studies

One study design that avoids many issues of confounding is the randomized controlled trial (RCT). While RCT's are quite useful in a number of circumstances (drug trials, etc.), RCT's are not necessarily superior to observational studies with respect to indicator organism levels in recreational water and illness. Many well run RCT's have been done (Jones 1991, Fleisher 1993, Kay 1994, Fleisher 1996, Fleisher 1998, Wiedenmann 2005, Fleisher 2010, Sinigalliano 2010), but one limitation that they all share is that the swimmers' behavior is not likely to be the same as that of a normal person swimming at a beach. Exposure was very controlled and thus quite artificial in each study. For example, all swimmers in the study conducted by Fleisher et al. in

2010 were required to spend 15 minutes in knee deep water, and to submerge their head three times during that interval. While some swimmers may normally choose to interact with the water in that way, it is likely that most do not. Observational studies allow swimmers to behave any way they wish, and thus results from an observational study are more representative of what happens in the "real world." Generalizability of swimmer risk from indicator organism exposure as found in an observational study may be more plausible than a study where swimmers are instructed to follow a given protocol. Of course, there are advantages inherent in conducting an RCT- not only is exposure measured more exactly, but potential sources of biases are likely very minimal. Thus, findings are likely to be internally valid, but it is questionable whether or not the study findings would have external validity.

5.5 Future Directions

The future for developing improved recreational water quality regulations looks quite optimistic. New regulations are about to be formulated by next year (2012), and will likely involve the use of more rapid methods of measuring indicators. The use of rapidly measured indicators will overcome the key concern regarding delayed beach advisory warnings, which are likely to be useless if posted the next day. As technology and research further the field, it is possible that quite sophisticated methods may be employed to allow both flexibility and timely beach advisories. For instance, rather than advise that beaches be closed based on a binary cutoff for an indicator, an online tool might be developed. Beach regulators may be able to input the weather conditions at their beach, an indicator organism concentration, and information about point sources or other relevant features into an online website hosted by the EPA. The website might then take the information and feed it to a sophisticated statistical model that calculates the excess risk associated with swimming when those conditions exist- if the excess risk is greater than 19 GI cases per 1,000 swimmers, the website might advise the regulator to close the beach. This would provide a flexible means of regulating beaches. If solar inactivation was quite high, but indicators levels were higher than the standard by a small amount, the risk might not be great enough to warrant closing the beach. Conversely, if indicator levels were slightly lower than the regulatory standard, but there was little to no solar radiation or other inhibitory environmental conditions were present, then it might be better to close the beach. This approach would also allow the EPA the flexibility to update the model as new study results or data become available. Beach regulators would also not have to use and rely on complex statistical models.

The above situation would be ideal, and it is clear that there is great room for improvement upon current methods. The findings of this dissertation suggest some of the likely candidates for improvement: use of multiple indicators (biological and physical), flexible modeling approaches to predict illness, approaches to prevent overfitting and false positives, examination of potential sources of sewage transport into marine waters, and examination of other symptoms besides just GI cases could help inform beach regulators when beaches are safe or unsafe.

5.6 References

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